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TALANTA REVIEW*

SEPARATION OF TRACE ELEMENTS IN SOLID SAMPLES BY FORMATION OF VOLATILE INORGANIC COMPOUNDS

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Summary—Methods for the separation and determination of trace elements by volatilization and by gas chromatography are reviewed. Examples are given of typical separations and apparatus suitable for inorganic gas chromatography is described.

Trends in the development of analytical methods for inorganic substances are the determination of very low concentrations of elements, the simultaneous determination of many elements (multielement analysis), the determination of different species of the overall concentration of an element and increasing precision and accuracy.

High-purity substances used in optical waveguides, metals and semiconductor materials, should have concentrations of trace elements which are often lower than the detection limits of the analytical methods available. Very low concentrations are often responsible for large effects, especially in biochemical systems.

Complete analysis of a system requires the determination of many elements. The simplest cases are high-purity materials, for which the influence of an element on the conductivity or light absorption can easily be estimated. Much more complicated are biochemical or environmental systems, where the effect of one element depends on the concentration of other elements present.

In many analytical problems it is important to know the species or the phase (for example, aerosol or gas phase) of an element. Since the common methods of molecular analysis are often restricted to higher concentrations it is necessary to obtain indirect information about the species by its chemical behaviour during separation.

It is necessary in all analytical fields to increase accuracy and precision. For example, the laws against environmental hazards require more precise determinations.

These four requirements must have a considerable impact on future trends in analytical research. Since systematic errors may be due to the interference of other elements, one possibility of increasing accuracy is to carry out a complete separation which allows comparison with a standard. If we compare instrumental analyses and combined systems in which instrumental determination is preceded by separation, then the main drawback of instrumental analysis is interference from other elements, while that of combined systems is contamination from reagents and losses due to adsorption.

Any separation method suitable for trace elements should minimize contamination and loss. The contamination problem can be reduced by working in closed systems and using purified reagents. The loss of trace elements can be avoided by using small surface areas and high temperature. We have found that the general requirements for separation procedures in ultratrace analysis are met by using the gas phase.[1–17] The gases used (*e.g.*, Ar, N_2, O_2, Cl_2, HCl, CCl_4) can be highly purified with respect to the trace elements usually determined. The surface area can be kept small and the whole separation carried out in one container. Losses of trace elements at high temperatures can be neglected. In experiments on separation of only a few atoms per hr of element 104, as its tetrachloride, it has been found[18,19] that at high temperatures no losses due to adsorption occur. Furthermore, it is easy to purify all containers since pretreatment at high temperatures with gases such as Cl_2 will clean the surface completely, but caution has to be exercised with elements which diffuse through quartz.

In Table 1 gas-phase separations are compared with the most important alternative separation method, extraction. More than 50% of all preconcentration methods depend on extraction, whereas only about 7% are carried out in the gas phase.[20] The table differentiates between a single-stage and a multistage

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Table 1. Comparison of extraction and gas phase separation in trace element analysis

	Separation factor	Number of elements or problems	Technology	Time	Detection limit
Extraction	Medium	Not limited	Simple	Fusion takes time, whereas single extraction is fast	Limited by purity of reagents, and lowest possible volume for sample dissolution and extraction
Extraction chromatography	High		Complicated	~0.5 hr	
Volatilization	Medium	Limited	Complicated	~2 hr	In many cases only limited by the detector sensitivity
Gas chromatography	High	Not limited	Complicated	~0.5 hr	

separation, *viz.* extraction and partition chromatography, or volatilization and gas chromatography. Volatilization is a much more complex process than extraction, since the equilibria in extraction, *e.g.*, from an aqueous into an organic phase, can be described by a distribution coefficient, whereas volatilization from a solid sample involves the conversion of a non-volatile into a volatile compound, transport to the surface of the sample, and desorption or evaporation. For many samples volatilization is a direct process since they are present in the solid phase and a fusion is necessary. In gas-phase reactions the same factors are involved as in extraction, namely the composition of the two phases, but in addition temperature plays an important role.[21]

The enrichment factor obtained in volatilization is much higher than that obtained in extraction. For example, if a trace element is volatilized from a 1-g sample, the total amount of the trace element can be used for the determination. For the dissolution of a 1-g sample at least 10 ml of aqueous phase are necessary, and for the extraction 2 ml of organic phase. If 50 μl are used in an absorption spectrophotometer, a factor of 40 is lost compared with the gas-phase separation.[22] The comparison between extraction chromatography and gas chromatography is more complicated. In both cases high enrichment factors are necessary, since the capacity of the stationary phase is limited.

This survey deals principally with solid samples which can be treated in four ways: (*a*) by direct analysis, (*b*) by hybrid techniques, (*c*) by volatilization, (*d*) by fusion and dissolution.

The most commonly used method is fusion, followed by direct analysis. In atomic spectroscopy the process of atomization (flame, plasma, or electrothermal heating) is a separation process, with the drawback that it cannot be regulated. Even more serious are the matrix effects, which permit direct analysis only in special cases or with the use of very similar reference standards. Therefore, hybrid methods[23] in which breakdown and vaporization of the sample take place in a separate device are preferable. Because it is not possible with this technique to run a lengthy temperature programme with different reactive gases, complete separation involving volatilization and detection is preferable. However, thermodynamic and kinetic factors rule out direct analysis of most solid samples.

The choice of volatilization or gas chromatography for a separation depends on the analytical problem and the detection system available. Figure 1 illustrates the relation between gas chromatography and volatilization. With solid samples, gas chromatography can only be a secondary process after the volatilization which gives the first enrichment. Ideal gas chromatography is not possible with amounts higher than 3 μg.[17] In gas chromatography a non-selective detector can be used, whereas volatilization requires a specific detector. For analysis of solutions it is time-consum-

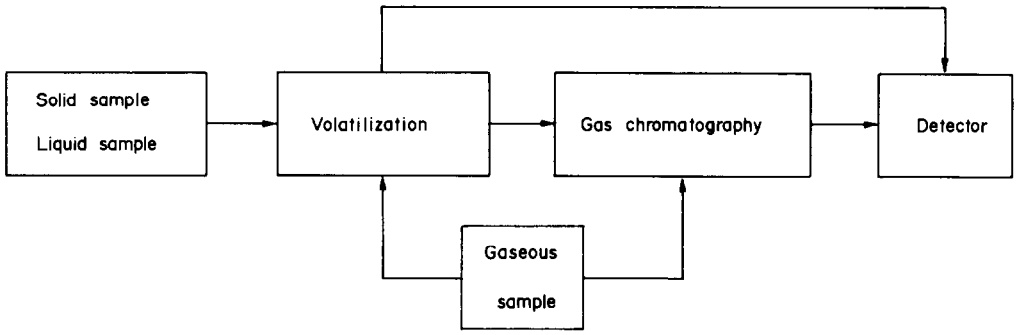

Fig. 1. Relationship between volatilization and gas chromatography.

ing to evaporate the solvent before the separation step, so extraction or precipitation is preferable.

An important reason for choosing a gas-phase separation is the variety of detection systems available. For example, in atomic spectroscopy the formation of gaseous compounds precedes atomization and is often subject to interference by other elements if it involves volatilization of a solvent. It is therefore advantageous to use a gas-generation system that preferentially volatilizes from solution a compound of the element of interest. For these reasons, a gaseous system is more free from interferences than a solution system. The use of an MIP (microwave-induced plasma) is much easier, *e.g.*, with gases. In many cases a chlorine atmosphere in a plasma or d.c. arc is advantageous[24,25] and its possible influence must at least be taken into acount.[26,27]

VOLATILIZATION

Volatilization can be used as a separation step with both liquid and solid samples. In this review the discussion is restricted to solid samples or those samples in which a solid residue is analysed after evaporation of the liquid matrix. Volatilization from liquids is used in the case of hydride generation and more recently in nickel carbonyl formation.[28]

Apart from a few isolated instances such as evolution of H_2S, SiF_4, H_2SiF_6 and SnI_4, volatilization was introduced into analytical chemistry mainly by Geilmann and co-workers. Table 2 summarizes examples characterized by the volatilization of one very volatile element from a non-volatile matrix. Very low concentrations have been determined only in the last few examples. Geilmann defined this method of separation as "evaporation" analysis, since an element is evaporated out of the matrix. We have tried to develop this method to separate as many elements as possible by using reactions which lead to volatile compounds. We use the term "volatilization" when the volatile compound is formed in the first step. This development is facilitated by the use of radioactive tracers and high-resolution γ-spectroscopy.

Table 3 summarizes the elements which form molecules that volatile below 1000°. The temperature is limited because most parts of the volatilization apparatus are made from fused silica. The elements in parentheses form volatile compounds only under special conditions, *e.g.*, molybdenum can be transported in an oxygen stream in the presence of water. Table 3 is not complete, since metal chelates and organometallic compounds have been intentionally omitted. In ultratrace analysis, organic compounds are less advantageous because of incomplete formation and owing to decomposition and adsorption on the vessel walls.[41]

The construction of volatilization apparatus depends to some extent on the methods of separation

Table 2. Typical examples for the separation of trace elements by evaporation from solid samples

Matrix	Element	Temperature, °C	Yield, %	Amount	Gas stream	Reference
ZnO	Cd	300/750	100	2–3000 ng	H_2/N_2	29
Compounds	Zn	1000/1200	100	μg–mg	H_2	30
Al, Ga, In	Zn	1000/1150	91.0	20–1000 ng	H_2	31
Bauxite	Be	1000	100	5–100 ng	O_2	32
Bauxite	Zn	1150	100	30 μg	H_2	33
Solid samples	Tl	1000	100	2–1000 ng	H_2	34
Cu	Se	1000	96.7	2 μg	—	35
Cu	Se	1150	100	1 ng	O_2	36
Al	Cd, Zn	600–700	100	20 pg	H_2/Ar	37
Aerosol	Cd	600–700	100	0.15–0.25 μg	H_2/N_2	38
Rock	Bi, Cd, Tl	1000–1200	95–100	10–100 pg	H_2/N_2	39
Plant	Pb	1000	100	ng–μg	H_2	40
Metals, Si, SiO_2	B	190	100	ng–μg	HF and H_2O	5

Table 3. Suitable inorganic volatile compounds for the concentration of trace elements

Type of compound	Elements which form suitable volatile compounds (25–1000°C)
Chlorides	Ti, Zr, Hf, V, Nb, Ta, (Cr), Mo, W, Tc, Re, Mn, Fe, Ru, Os, Au, Zn, Cd, Hg, Al, Ga, In, Tl, Si, Ge, Sn, Pb, P, As, Sb, Bi, S, Se, Te, Po, Ce
Fluorides	Ti, Zr, Hf, V, Nb, Ta, Mo, W, Tc, Re, Ru, Os, Rh, Ir, Hg, Si, Ge, Sn, P, As, Sb, Bi, S, Se, Te
Oxides	S, Se, Te, Po, (As), Tc, Re, Ru, Os, (Mo, W), Zn, Cd, Hg, (Ir)
$AlCl_3$-complexes	Lanthanides, actinides, Ca, Sr, Ba, Ra, Fe, Co, Ni, Cu, Pd, Pa, Mo
Elements	Noble gases, halogens, O, S, Se, Te, Po, N, P, As, Sb, Bi, Sn, Pb, Tl, Zn, Cd, Hg, (Li, Na, K, Rb, Cs, Fr)
Hydrides	As, Se, Sn, Pb, Te, Sb, Bi

and subsequent detection used. Several different types of separation method are possible. (1) A gas may react with only the trace element to form volatile compounds, while the matrix remains non-volatile. (2) A reactive gas forms a volatile compound with the matrix, while the trace elements remain non-volatile. (3) The gas reacts with both matrix and trace elements to form volatile compounds. (4) The gas reacts with both matrix and trace elements, but only the latter form volatile compounds. (5) A solid substance is used for forming volatile compounds.

In practice, trace and matrix elements may behave in a manner intermediate between the extremes formulated above. The examples quoted in Table 2 are characteristic of the first types although no reactive gases are used (except for some reductions with hydrogen). In the first type it is possible that part of the trace elements will not react because they are occluded. The second type is suitable for matrix elements which are volatile in an oxygen stream, *e.g.* Li, Na, K, Cs, Cd, Tl and Pb. In the third type no separation occurs during reaction and volatilization, but may be achieved in subsequent temperature-programmed fractional distillation or desorption. An advantage is that reaction is complete, so no trace elements are occluded. Applications are in the analysis of high-purity metals, *e.g.*, of Al, Nb and Ta by chlorination of the matrix and trace elements. The fourth type is more attractive than the first, but there are few cases to which it may be applied. The fifth type is exemplified by the addition of sodium borohydride ($NaBH_4$) for the formation of volatile hydrides, but the disadvantage is that contamination is greater than that encountered in reactions with the gas.

Volatilization of trace elements

Figures 2[39,42] and 3[43] illustrate two experimental arrangements suitable for separations of the first four types. The apparatus consists of three parts: (*a*) gas regulation and purification, (*b*) oven with temperature programming and control and (*c*) condensation or adsorption with temperature regulation. There may be a fraction-sampling device, depending on the nature of the detection system. Suitable gases include inert gases, *e.g.*, N_2, He, Ar, reactive gases, *e.g.*, H_2O, O_2, H_2 and halogenating gases, *e.g.*, CCl_4, Cl_2, HCl, Br_2, BBr_3, F_2, HF and complexing gases, *e.g.*, $AlCl_3$. When a complexing gas is used, the advantage of low contamination by the reagent is lost, since it is diffi-

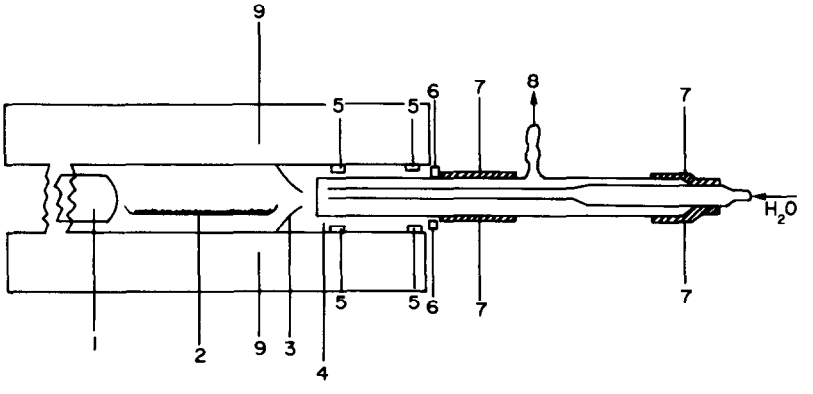

Fig. 2. Arrangement for the evaporation of elements from solid samples: 1, diffusion device; 2, boat; 3, nozzle; 4, cold finger; 5, holdfast; 6, holdfast spacer; 7, rubber tube; 8, pump; 9, oven. (By permission of the copyright holders, Springer-Verlag, Berlin).

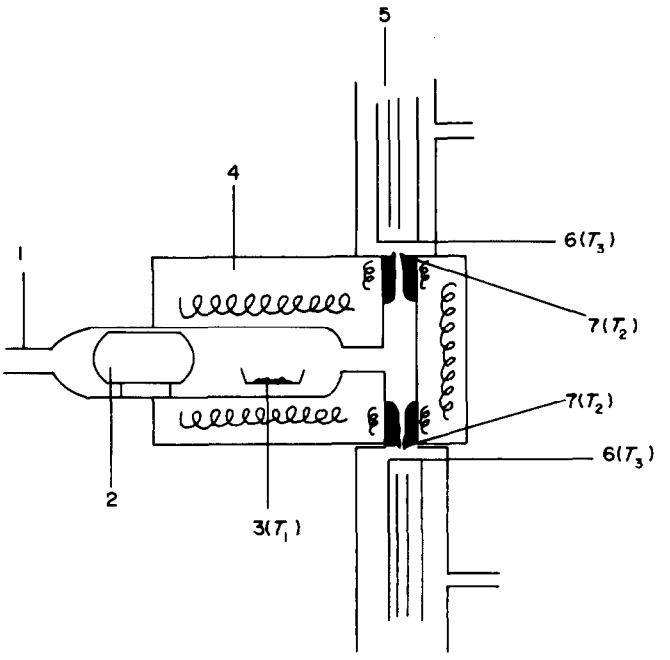

Fig. 3. Experimental arrangement of the volatilization device:[43] 1, input of reactive or inert gas; 2, diffusion device (quartz) to prevent back-diffusion; 3, sample (T_1 = volatilization temperature); 4, oven; 5, cold finger; 6, graphite plate (T_3 = 15°); 7, quartz capillary (T_2 = T_1 + 100°).

cult to purify it sufficiently. The constancy of gas flow-rate is not important, but high purity of the gas is often essential. In particular, the presence of O_2 or H_2O in chlorinating gases or in inert gases, even at low concentrations, can lead to the formation of non-volatile oxides or rather involatile oxychlorides, so that no separation is possible. It is therefore necessary to purify the gases or to add a scavenger for the interfering impurity. Oxygen can be removed, for example, with a molecular sieve cooled by liquid nitrogen. Reactive gases are more effectively purified by the addition of a scavenger, *e.g.*, carbon tetrachloride is decomposed at above 500°, forming radicals which scavenge oxygen. If boron tribromide is added to bromine, the oxygen in the bromine is scavenged. A very effective way of eliminating oxygen is the use of mercury in aluminium containers. With this system and a low gas velocity it is possible to purify inert gases to an oxygen level lower than 10^{-23} Pa.[44,45] It is important to purify the gases from the elements to be determined. Since most metals are retained on aerosol paricles, a filter must be used.

If more complex mixtures must be resolved, the oven should be equipped with a temperature programme and a facility for changing gas streams. If the apparatus is to be used for only one problem, *e.g.*, the evaporation of cadmium from various matrices, then the temperature programme may be omitted. The gas velocity should be high, so that there is no back-diffusion of the volatile trace elements into the cold zone. If volatilization is to be used, the trace element should be either completely volatilized or a reproducible amount evaporated. The best way to monitor this and to optimize the separation is to use radionuclides, either by activating the sample or by adding tracers. In the latter case, the added radionuclide should be in the same chemical state as the trace element in the sample. In some cases, neither activation nor addition of a radionuclide will make it possible to determine low concentrations of the elements. In such cases the investigation requires a second analytical determination to find the volatilization yield. An additional check for the volatilization yield can be made with reference materials.

The volatilization depends on the amount of sample, the temperature and the amount of the trace element relative to matrix elements or compounds which interfere. The amount of sample determines the time of volatilization. In theory, there is no limit to the amount of sample which can be used (we have used up to 5 g of sample), but for fast volatilization the surface area exposed to the carrier gas should be high and the adsorption enthalpy of the volatilized elements low. At the time of separation the grain size of the sample should be as small as possible. The temperature should never be so high that the sample melts, since most of the volatile compounds would dissolve in the molten sample. One special modification is that of surface analysis, in which a melt extracts the trace element out of the surface layer and the liquid layer is then volatilized together with the trace elements. This method has been used for the surface analysis of tantalum, with indium as the liquid phase.[46]

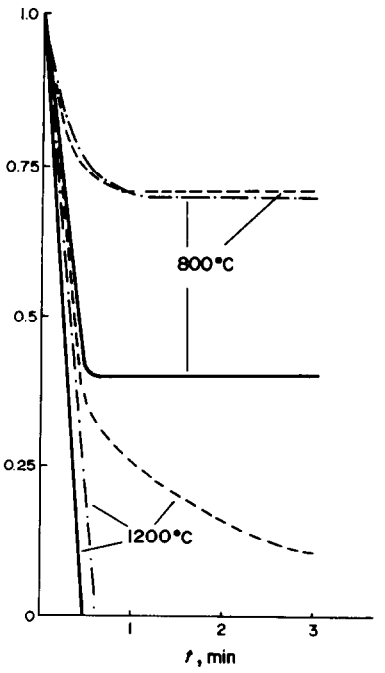

Fig. 4. Release of Pt, Ir, Os and Re from Au: (——) Re, (—·—·—) Os, (– – – –) Ir. (By permission of the copyright holders, Springer-Verlag, Berlin).

The relationship of volatilization to temperature is more complicated than would be expected because in addition to evaporation, diffusion and reaction processes take place. For example, a reaction with the main component in the sample might lead to a difference in the temperature at which trace elements are released. In Fig. 4[1,17] an example is shown in which platinum, osmium, iridium and rhenium are released from gold at different temperatures. The interesting fact is that only a certain fraction of the element is released at any given temperature and is not increased by prolonging the heating. There is no reasonable explanation for this phenomenon, but in trace analysis such anomalous effects are often encountered.

Figure 5[1] shows that the release of zinc is concentration-dependent. At the two highest concentrations of 215 and 187 μg/g, part of the zinc is released at a lower temperature. This behaviour may be interpreted by assuming that part is volatilized, while the remainder forms a compound which is not volatile.

A crucial part of separation by volatilization is the collection of the volatilized trace elements by condensation or adsorption. There is also the possibility of an on-line determination of the volatile compounds (see later). The yield of condensate and the reproducibility depend on the diameter of the capillary (see Fig. 3), the gas velocity, the distance from the capillary to the collector plate, the shape of the collector plate, the temperature in the capillary, and the temperature and coating of the collection surface. Collection on the surface of a tube or in a liquid is also possible.

The geometrical parameters can be optimized for all problems, but the temperature and stationary phase on the collection plate vary for different elements or compounds. One problem is to avoid the condensation of the trace element in the capillary. This may be achieved either by inserting the collection plate into the oven (Fig. 2) and cooling, with the attendant disadvantage of a temperature gradient on the collection plate, or by heating the capillary (Fig. 3). Heating of the capillary allows the temperature gradient to be optimized by choice of the capillary temperature, because the temperature T_1 of volatilization and T_2 of condensation are determined by physical or chemical requirements. The space between the capillary and the tube itself should be filled with quartz powder to provide a high heat-capacity at the end of the capillary, where cooling from the collection plate is possible.

The use of different coatings on the collection surface may give selectivity for certain trace compounds. For example, zirconium chloride is retained on a coating of sodium chloride even at a temperature of 400–500°.

Additional heating can be done with a heating coil between the quartz tube and the capillary (Fig. 6),[19] but because of the small space available it is not possible to reach very high temperatures. Calculations on the distribution of the trace compounds on the collection surface are difficult since most of the diffusion coefficients are unknown. Therefore, we have measured some of the distributions by using radionuclides. With iodine it is possible to deposit more than 90% of the trace compounds on a spot 8 mm in diameter and with cadmium on a 4-mm spot.[43] The distribution of the iodine is Gaussian, so most of the iodine is within a 3-mm circle.

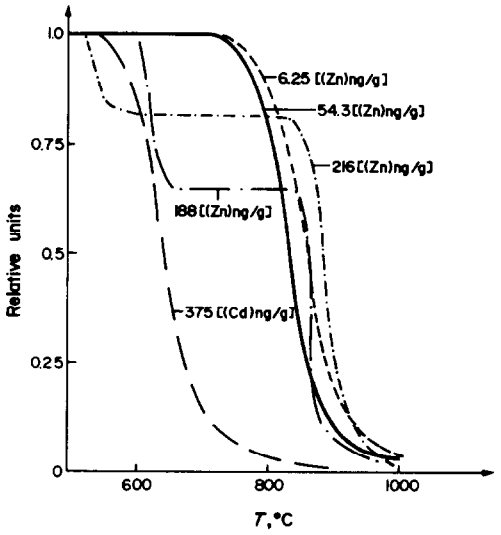

Fig. 5. Release of Cd and Zn from Al as a function of temperature. (By permission of the copyright holders, Springer-Verlag, Berlin).

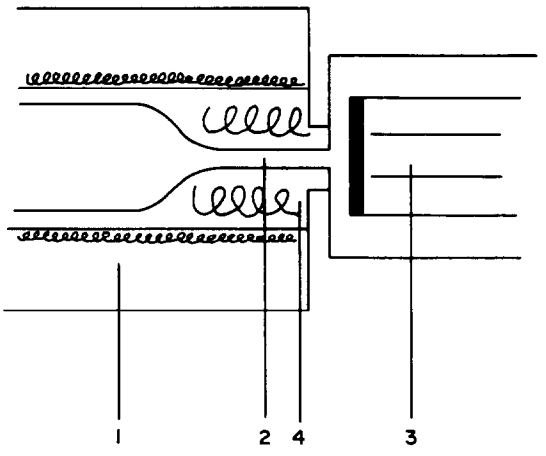

Fig. 6. Arrangement for heating capillary: 1, oven; 2, capillary, 3, cold finger; 4, additional heating.

The simplest example of volatilization as a separation method is the volatilization of one element. When only one element is to be separated, careful temperature programming must be used. This is particularly so in the case of chlorides, because many elements form volatile chlorides. In the case of the pure elements in a stream of inert gas, or hydrogen, or sometimes oxygen, it is possible to separate the element to be determined, either alone or at least accompanied only by elements which do not interfere.

A critical factor is the collection of the volatile species. Figure 7 shows an example where selenium is determined in a copper matrix.[36] The selenium dioxide is trapped in a U-tube by condensation with oxygen. In a gas stream there is always the possibility of the formation of aerosols which are unlikely to be adsorbed, owing to their slow diffusion.

Table 4 lists those elements which can be volatilized either directly or as oxides. These elements are more suitable for separation by volatilization than those which form volatile chlorides. A number of these elements are important in environmental problems, *e.g.*, Se, As, Cd, Pb and Hg. The high volatility of these elements is often the reason for losses in the fusion of samples, and therefore it is advantageous to use this volatility for their separation from solid samples. A difference in the volatility of the oxides or elements can be used for the separation of two volatile elements: thus Geilmann and Hepp[29] have shown that it is possible to separate cadmium from zinc. Cadmium oxide is reduced in a hydrogen stream at temperatures below 300°, whereas zinc oxide is not reduced at temperatures below 360°. It is therefore possible to reduce the cadmium oxide first and then to evaporate the cadmium at a temperature at which the zinc oxide is not volatile: the zinc oxide can then be reduced and the zinc volatilized. Geilmann and Hepp have separated cadmium in this way from a zinc oxide matrix and have separated cadmium and zinc from pure aluminium.

Volatilization of the matrix

In some cases all the trace elements are volatilized at the same time or temperature. An example[3] is that of aluminium, which forms aluminium chloride at about 500° with chlorinating agents. This is similar to most metals which form volatile chlorides, so that a separation requires several temperature-programmed volatilizations.[3] When pure aluminium is chlorinated with hydrogen chloride, most of the trace elements are evaporated at about the same temperature of 450°. Even sodium is partly evaporated because it forms a volatile complex ($NaAlCl_4$). In a second step the condensed chlorides may be volatilized in a stream of argon or argon/HCl. The trace elements can thus be separated from the matrix ($AlCl_3$), but the behaviour is complicated by the formation of complexes of $AlCl_3$ and $ZnCl_2$. This complication can be avoided by first forming aluminium trifluoride (AlF_3), which is non-volatile and subsequently reacting it with hydrogen chloride.

A less complicated case is volatilization of the matrix alone. This is only possible when the matrix is much more volatile than the trace elements. This is seldom the case for chlorides, but is possible for elements or oxides and is more attractive when the sample contains only one major matrix element or compound. To this category belong reagents such as

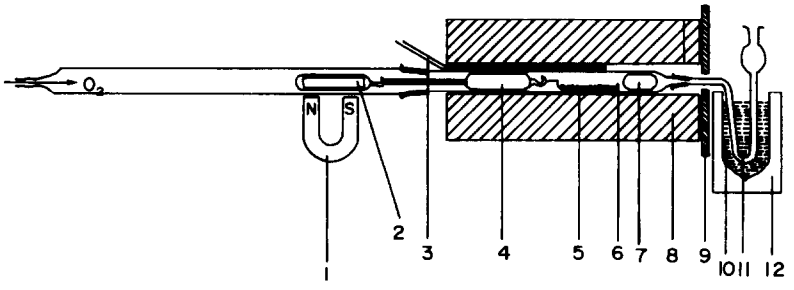

Fig. 7. Experimental arrangement for separation of Se as SeO_2 from copper, using an O_2 stream (ng-range);[36] 1, magnet; 2, magnet follower; 3, thermocouple; 4, quartz device for preventing back-diffusion; 5, boat (corundum) with sample; 6, fusion tube with quartz ground joint; 7, damping body of quartz; 8, oven; 9, asbestos plate; 10, liquid nitrogen; 11, quartz U-shaped trap; 12, Dewar flask. (By permission of the copyright holders, Springer-Verlag, Berlin).

Table 4. Elements which are volatile in an inert gas stream or oxygen stream

Element	m.p., °C	b.p., °C	Oxide	m.p., °C	b.p., °C
Li	179	1317	Li_2O	1700	subl 1000
Na	98	892	Na_2O	—	subl 1275
			Na_2O_2	d 460	
K	64	774	K_2O	d 350	
			K_2O_2	490	d
Cs	29	690	Cs_2O	d 400	
Rb	39	688	Rb_2O	d 400	
			Rb_2O_2	570	d 1011
B	2070 ± 25	subl 2550	H_3BO_3	169	300
Tc	—	—	Tc_2O_7	—	—
Re	3180	5627	Re_2O_7	—	subl 250
Os	3000 ± 10	5000	OsO_4	39	130
Ru	2250	3900	RuO_4	26	d 108
Ir	—	—	—	transported in an O_2 stream	
Pt	—	—	—		
Zn	419	907	ZnO	1975	
Cd	320	765	CdO	d 900	subl 1559
Hg	−39	356	Hg_2O	d 100	
Ga	30	2403	Ga_2O		subl 500
In	156	2000	In_2O_3		subl vac 565–700
			In_2O		
Tl	303	1457	Tl_2O_3	717	d
Sn	231	2260	SnO	d 1080	subl 1800–1900
			SnO_2	1127	
Pb	327	1744	PbO	888	
Mg	651	1107	MgO	2800	3600 (not possible)
P	44	280	P_2O_5	580	subl 300
As	d 358		As_2O_3	subl 193	457
Sb	650	1380	Sb_2O_3		subl 1550
Bi	271	1560	Bi_2O_3	860	—
S	112	444	SO_2	72	10
Se	217	685	SeO_2	—	subl 340
Te	452	1390	TeO_2	733	1245
Po	254	962	PoO_2	d 500	—

HF, HCl, HNO_3 as well as important products such as $AsCl_3$, $SiCl_4$, $SnCl_4$, PCl_3, and $TiCl_4$.[48,49]

We have studied the possibilities of using matrix volatilization in the analysis of optical waveguide materials. The trace elements of interest are those absorbing light in the range 900–1000 nm, *e.g.*, Cu, Cr, Co, Fe, Mn, Ni and V. Table 5[5] summarizes the compounds investigated and the conditions for matrix volatilization. The waveguide materials are striking examples of the superiority of volatilization analysis.

In addition, a number of chemicals used for MOS products and in semiconductor production can be analysed by using volatilization, *e.g.*, NH_4F, PbF_2, CdS, CdSe, CdTe, GaAs, Si and In.[50] Its use in the investigation of refractory materials and uranium should also be mentioned.

The situation is more complicated when the matrix consists of several elements or compounds. For example, the main constituents in biological, geological and water samples are alkali metals, alkaline earth metals, iron and aluminium in the form of silicates, chlorides and phosphates. For these complex matrices the anionic composition is important when a separation by volatilization is considered. The volatilization of alkali metals from biological materials is interesting since these metals interfere in the determination of many other elements. We have found that in biological materials it is possible to evaporate about 90% of the alkali metals. The residual amount depends on the anions present in the sample. It is possible to evaporate the carbonates, nitrates, sulphates and even halides, but it is not possible to evaporate the phosphates or silicates. Biological systems are investigated in more detail with respect to the very volatile elements, *e.g.*, As, Se and Cd, which can be evaporated in an oxygen[51] or hydrogen[52] stream. For a number of other elements volatilization as the chlorides is possible.

The determination of anions by use of volatilization

For the determination of O, S, C and N in metals, vacuum extraction at high temperature has long been used. A more recent development is the determination of halides by volatilization at high temperatures. Farzaneh and Troll have determined fluoride in rocks by pyrohydrolysis[53,54] with the formation of H_2SiF_6 which is collected in sodium hydroxide solution. Burguera and Townshend[55] have used the formation of SiF_4 for the same purpose. The disadvantage of these methods is the need to add reagents in order to form the volatile compounds. Such methods are not suitable for really low concentrations of fluoride. Chlor-

Table 5. Waveguide materials investigated by the Merck company, and the volatilization conditions

Material	Separation step	Gas/liquid	Flow-rate, *l./hr*	Temperature, °C	Heating rate, *deg/min*	Duration, *min*	Weight of sample, *mg*	Remarks
Li_2CO_3	Matrix volatilization	Argon	15	900	6	180	15–45	Attacks the tube very strongly
Na_2CO_3	Matrix volatilization	Argon	15	950	60	120	40–60	Attacks the tube very strongly
K_2CO_3	Matrix volatilization	Argon	15	950	60	120	10–25	Attacks the tube very strongly
Cs_2CO_3	Matrix volatilization	Argon	15	900	20	90	10–60	Attacks the tube very strongly
$CaCO_3$	Trace element volatilization	Argon/ CCl_4/Cl_2	10	900	60	120	60–110	Attacks the tube very strongly
$NaNO_3$*	Matrix volatilization	Argon	15	850	60	60	10–15	Attacks the tube very strongly
B_2O_3	Matrix volatilization	Ar/H_2O	—	130	2	150	50–120	120 ml H_2O needed
H_3BO_3	Matrix volatilization	Ar/H_2O	—	130	2	150	50–110	120 ml of H_2O needed
SiO_2	Fusion	HF	—	140	3	150	50–125	80–100 ml of HF needed
PbO	Matrix volatilization	Argon	10	950	60	30	105–165	
CdS†	(a) Oxidation	O_2/Ar	10	950	60	30	20–55	
	(b) Reduction and matrix volatilization	H_2	12	560	20	60		
			3	600				

* $NaNO_3$ *p.a.* grade.
† CdS suprapur grade.

ide is determined in rocks[56] by a similar method with hydrogen chloride. Evans and Moore[57] used a combustion–ion chromatographic determination of chloride in silicate rocks but did not show whether the method was applicable in the nanogram range. Because of inhomogeneities in the anion distribution it is difficult to prepare appropriate standards.

We have used the formation of volatile chromyl chloride in order to determine hydrogen chloride in air.[58] The volatile CrO_2Cl_2 is decomposed in a graphite tube and the chromium determined by atomic-absorption spectrophotometry.

Application of volatilization in radioecology (plutonium and americium)

A number of radionuclides important in radioecology are suitable for an enrichment technique using volatilization. Examples are ^{210}Pb, ^{210}Po, ^{222}Rn (for ^{226}Ra), ^{129}I, ^{239}Pu, ^{241}Am and ^{240}Pu. One of the important analyses in radioecology is the determination of very low amounts of plutonium (10^{-15} g) in soils, plants, air and water, since it is necessary to obtain background values for the ubiquitous plutonium and to obtain information about the transportation of plutonium in ecological systems. The classical method for the separation of plutonium is a combination of precipitation, extraction and ion-exchange, with the last step being electrolysis on a disc followed by α-spectroscopy. This procedure is time-consuming and cannot be automated. It is possible to separate very low concentrations of plutonium from relatively high amounts of sample material by a combination of differential reaction rates, fractional distillation and chromatography. Figure 8 shows the process schematically. In the first step, plutonium is volatilized together with aluminium chloride in a stream of chlorine and carbon tetrachloride. A number of other elements, including alkali metals, are also volatilized, but they are deposited in the temperature gradient in different zones. The main interference with plutonium is from $FeCl_3$, $MnCl_2$, $ZnCl_2$ and $PbCl_2$. In the second step, the temperatures at different points in the oven are changed (as is shown in Fig. 8) and the chlorine supply is cut off. Under these conditions, $AlCl_3$ and $FeCl_3$ are evaporated and at higher temperatures $ZnCl_2$ and $PbCl_2$. $PuCl_3$, $AmCl_3$ and $MnCl_2$ are left. The principle of this separation is that Pu is deposited as $PuCl_3$ and may only be transported as $PuCl_4$ by oxidizing chlorinating agents. In the third step, in which a stream of chlorine is used, $PuCl_4$ and $AmCl_3$ are evaporated and then deposited at different temperatures on a disc. This analysis is faster than the classical method and can be fully automated. This example is characteristic of the complex reactions which take place at high temperature and are not yet fully understood. In order to predict such separations it is necessary to discover more about complex formation in the gaseous phase, *e.g.*, of $AlCl_3$ with other chlorides.

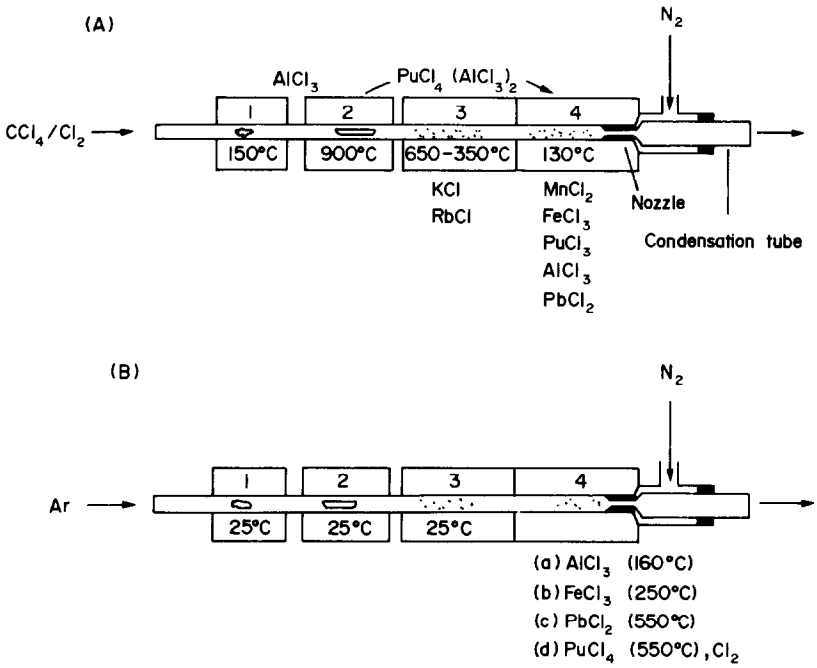

Fig. 8. Procedure for the separation of plutonium from soil with $AlCl_3$ as carrier gas: (A) volatilization of the chlorides and transport with $AlCl_3$; (B) separation of the chlorides by sublimation at different temperatures.

Figure 9[59] illustrates the differing behaviour of plutonium on volatilization in streams of HCl, Cl_2 and CCl_4/Cl_2.

GAS CHROMATOGRAPHY OF INORGANIC COMPOUNDS

Inorganic gas chromatography is in a state of development far behind that of organic gas chromatography. The reasons are the non-availability of commercial gas chromatographs working at above 500°, the difficulty in finding suitable instrumentation for handling highly corrosive gases, and the superiority of other methods of elemental analysis. This last reason is no longer important since in ultratrace analysis new methods have to be developed. In those cases where volatilization does not result in a separation permitting determination without interference, it is necessary to carry out a gas chromatographic separation. There are several reasons for the choice of gas chromatography as a separation method.

(1) In gas chromatography, separation is almost complete so there is no interference from other elements and this means that a real comparison between standard and sample can be made.

(2) When gas chromatography is used it is possible to use a scanning spectrometer and to determine several elements in one microsample.

(3) When an element is characterized by its retention volume or retention time it is possible to use a non-selective detector.

(4) It is possible to analyse for several different species derived from one element.

Table 6 compares organic with inorganic gas chromatography. Inorganic gas chromatography can be defined as the chromatography of volatile inorganic compounds and that means that metal chelates are not included. Gas-phase separation of chelate compounds or metal organic compounds will not be dealt with in this review. Also omitted is the separation of low-boiling gases such as O_2, N_2, CO and CO_2. The literature on such separations up to 1972 has been reviewed by Rüssel and Tölg.[60]

The main difference between inorganic and organic gas chromatography is that the latter determines compounds, while the former is used mainly for the determination of elements. The element should form a compound suitable for gas chromatographic separation. In some cases, *e.g.*, air analysis, volatile compounds are separated directly and it is important that no reactions should occur on the column which would destroy information about their nature. A second feature is the use of a reactive mobile phase. Reactive gases influence the separation in three ways. (1) Non-volatile compounds [*e.g.*, $LaCl_3(AlCl_3)_4 \rightarrow LaCl_3 + 4AlCl_3$], formed by decomposition of volatile compounds on the surface of the stationary phase can react again with the reactive gas to form a volatile compound. (2) The properties of the stationary phase may be changed by adsorption of the reactive mobile phase. (3) The reactive gas can be self-purifying, reacting with impurities in it, so that interfering reactions

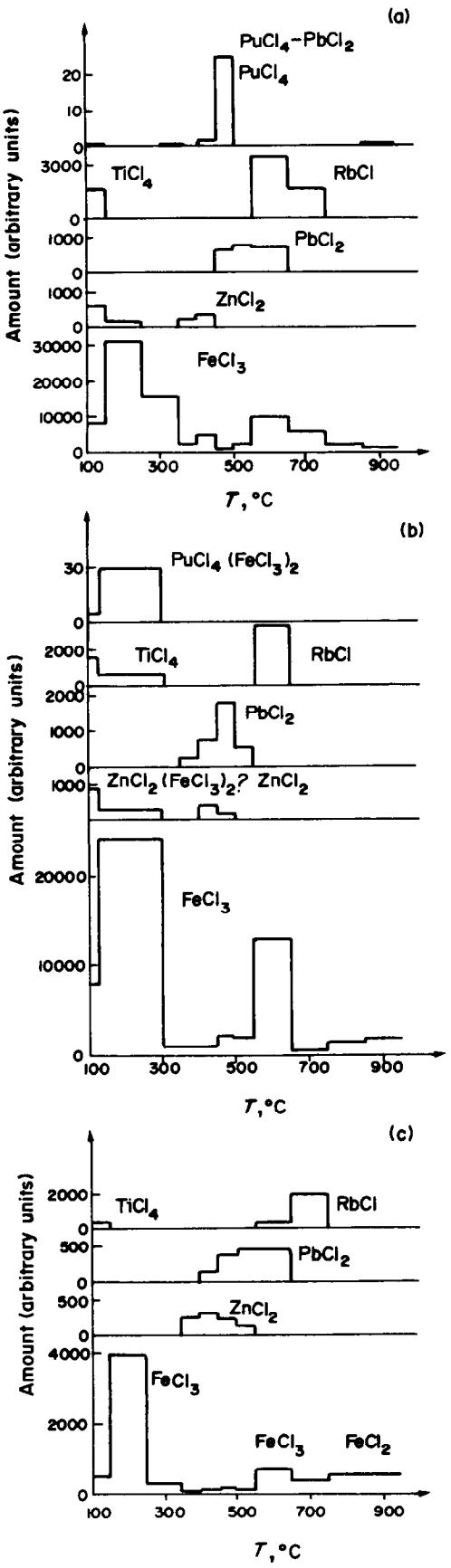

Fig. 9. Sublimation of chlorides as a function of temperature: (a) using N_2/CCl_4 as reactive gas, (b) using Cl_2/CCl_4 as reactive gas. (Reprinted from *Anal. Chem.*, 1980, **52**, 620, by permission of the copyright holders, the American Chemical Society).

are avoided. In particular, BBr_3 and CCl_4 may be used as scavengers for H_2O or O_2. Further differences from organic gas chromatography are the high temperatures and the wide range of temperatures used. This means that the materials of construction for an inorganic gas chromatography must be different from those of a commercially available apparatus and that the properties of the stationary phase may change during the course of a separation.

Table 7 gives a compilation of typical inorganic gas chromatographic separations used for trace element analysis. The separation of volatile hydrides has been investigated.[69,75,76] In most cases the hydrides were generated from aqueous solution by the addition of sodium borohydride. A Poropak Q column was used[69] for the separation of GeH_4, AsH_3, SnH_4 and SbH_3. The oven was temperature-programmed at 8°/min from 75 to 120°. The formation of hydrides in aqueous solution is influenced by the presence of other elements, so this method is limited by interferences. It does not make sense to use a very selective and sensitive gas chromatograph in conjunction with hydride evolution, which is subject to interference by many elements.[82] The formation of hydrides from solid substances is complicated, since the hydrides are decomposed at high temperatures, but high temperatures are necessary for diffusion. We have some preliminary results for the reaction of H_2 with Se and As in biological samples.[52] Part of the Se and As forms hydrides so that analysis is possible. For the detection of hydrides after separation, flame ionization, atomic absorption, mass spectrometry and a katharometer were used. In the last case, the hydrogen produced by decomposition of the hydrides in a furnace at 1000° is detected.

The chemical group most extensively studied for inorganic gas chromatography is the chlorides. The bromides and iodides are often likely to decompose at high temperatures, and the fluorides have high boiling points and are reactive, so quartz columns are unsuitable. A typical separation of chlorides is shown in Fig. 10.[7] Complete separation is not necessary when a specific detector is used. It can be seen that uranium gave a broad peak owing to its high amount (>100 mg) and that bismuth gave two peaks due to $BiCl_3$ and $BiCl_x$. Under certain conditions two compounds may be formed, and this is undesirable in an analysis. The sequence of elution of the chlorides (valid for all compounds) is not dependent on the heats of evaporation (vapour pressure or boiling point) in ideal chromatography, but on the heats of adsorption. However, in many cases, the boiling points give a guide to the sequence.

Table 6. Comparison of organic and inorganic gas chromatography

	Organic gas chromatography	Inorganic gas chromatography
Temperature range	Up to 400°C	Up to 1000°C
Separation mechanism	Distribution, adsorption	Distribution, adsorption (with complex formation, transport reaction)
Stationary phase	—	Change of stationary phase as a function of temperature
Mobile phase	Inert	Reactive gases: reactions with the analyte and the stationary phase
Detector	Often non-selective	Selective and placed outside of the column
Identification	Often retention time	Often by spectroscopic data

Table 7. Separation of trace elements by inorganic gas chromatography

Type of compound	Stationary phase	Elements which are separated	Detection	References
Fluorides	PTFE Kel-F	Te, I, Mo, Np, Tc, Pu, Sb, Nb, Ru	ECD	61, 62, 63
Chlorides	Fused salts as $InCl_3$–TlCl	Zr, Ti, Ta, Sn, Sb, Nb, Mo, Hg, Fe, As, Al	FPD	64
Chlorides	Graphite LiCl-KCl	Nb, Ta, Zr, Hf	FPD	65
Chlorides	Silicone oil DC 550, *etc.*	Si, Ge, P, As, Sn	TCD	66
Fluorides	PTFE	Mo, W, Ta, Nb, Sb, As	TCD	67
Bromides	Fused salts	Sn, P, Ti, As, Al, Fe,	TCD	
Iodides		Si, Ge, Ta, Nb	FPD	68
Chlorides	Quartz, graphite NaCl, KCl, $SrCl_2$ YCl_3, *etc.*	Nb, Mo, Tc Zr, Te	Radioactivity	8
Chlorides	Quartz, graphite NaCl, KCl, $SrCl_2$ YCl_3, *etc.*	Re, Ta, Tl, Ga, Cd Pb, In, Sn, Po, Bi Sb, Pa, Hf, Zr, Os Te, Tc	Radioactivity	13, 14
Bromides	Quartz NaBr, KBr, *etc.*	Sn, Sb, Nb, Te Bi, Mo, Zr, In, Tc	Radioactivity	16
Oxides	Quartz	Ru, Te, Re, Os, Ir	Radioactivity	11
Hydrides	Porapak Q	Ge, As, Sn, Sb	FID	69
$AlCl_3$-complexes	Glass	Lanthanides	Radioactivity	70
$AlCl_3$-complexes	Glass	Actinides	Radioactivity	22
$AlCl_3$-complexes	Quartz, NaCl	Lanthanides	Radioactivity	71
Chlorides	Quartz	Lanthanides	Radioactivity	72
Chlorides	Kel-F	Si, P, S, Ti, V, Cr, Ga, Ge, As, Mo, Sn, Sb	Gas density balance	73
Chlorides	$MgCl_2$, KCl, $CaCl_2$, NaCl, LiCl, $BaCl_2$	Bi, Be, In, Sn, Zn, Tl, Pb, Cd, Sn, Sb	Electrical conductivity detector	74
Hydrides	Porapak PS	Sn, Sb	Radioactivity	75
Hydrides	Silica gel	As, Ge, Sb	PCD	76
Chlorides	Sil-O-gel brick	Ge, Sn, As, Fe	Radioactivity	77
Chlorides	$InCl_3$ $InCl_3$–NaCl	Nb	FPD	78
Metals	Charcoal	Mg, Cd, Zn	Pneumatic detector	79
Chlorides	Chromosorb W/ halocarbon	Sn, As, Ge	TCD	80
Chlorides	Silica gel	Hf, Zr, Ca, Mn	Radioactivity/MIP	17
Carbonyls	Squalane, Apiezon L	Fe, Cr, Mo, W	TCD	81

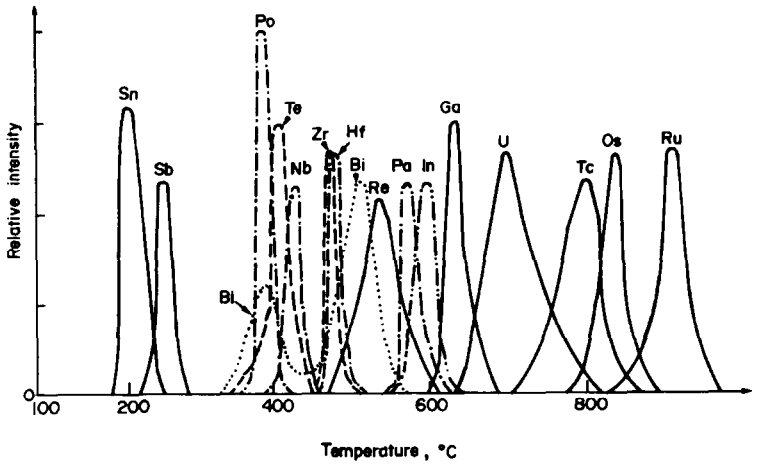

Fig. 10. Temperature-programmed gas chromatography (stationary phase NaCl, heating rate 2°/min, mean carrier gas flow 0.8 m/min). (By permission of the copyright holders).

Figure 11 shows the separation of volatile oxides.[11] In addition to the elements giving typical volatile oxides such as osmium and ruthenium, there are also elements, such as iridium, which are transported in an oxygen stream by reaction with the mobile phase. When water is present, molybdenum is transported as H_2MoO_4. When a potassium chromate filter is inserted into the column, technetium and rhenium are retained as rhenate and technetate. These are methods for gaining more selectivity. The separation of rare earths with $AlCl_3$ is listed in Table 8. This shows the interesting possibility of forming highly volatile compounds from non-volatile chlorides. From the point of view of trace element analysis the use of $AlCl_3$ would contaminate the sample with most of the common trace elements, and $AlCl_3$ cannot be purified because of its property of complex formation.

Another group of compounds not suitable for trace element analysis is the metal carbonyls. Pommier and Guiochon have separated Fe, Cr, Mo and W as carbonyls on squalane, but no analytical application has been reported.[81]

High-temperature gas chromatograph for inorganic compounds

Several problems have to be solved for gas chromatography to be applied to inorganic compounds. For the volatilization of many inorganic compounds, temperatures up to 1200 K are necessary. Since most inorganic compounds react with metal surfaces at high temperatures, all contact with metal or similar reactive materials has to be avoided. Stationary phases have to be found which are stable up to the high temperatures needed.

The chromatographic column can be made of fused quartz and one end serves as an injection area ("on-column injection"), so the use of fittings or unions is avoided, as shown[15] in Fig. 12. The elements or compounds under investigation are in contact only with quartz, which is chemically inactive. Two methods of separation are available, direct evaporation and intermediate trapping. The column section and experimental arrangements for both methods are shown in Fig. 13. In direct evaporation (13a), the sample is introduced into the evaporation zone, which can be

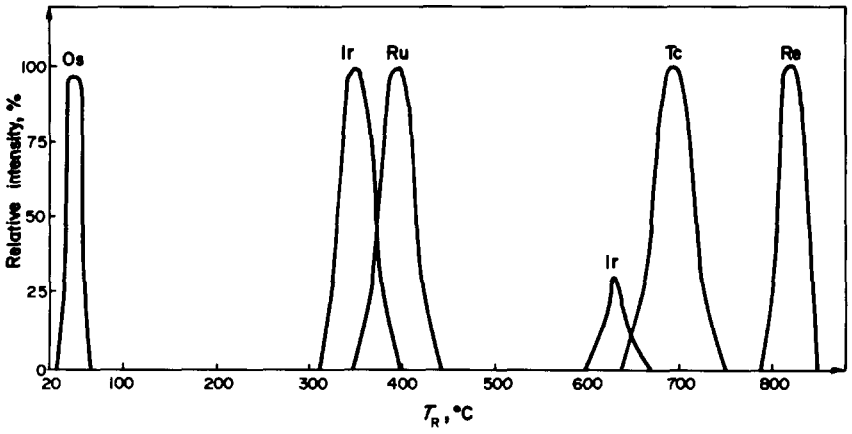

Fig. 11. Chromatogram from a temperature-programmed experiment with O_2 as reactive gas, heating rate 20°/min. (By permission of the copyright holders, Pergamon Press).

Table 8. Stationary phases for inorganic gas chromatography

Stationary phase	m.p. or b.p., °C	Elements which are separated	Characterization	References
Silicone oil DC 550 on celite 545	—	$SiCl_4$, $GeCl_4$, $SnCl_4$, $TeCl_4$		74
PEG 1500 on celite 545	—	$SiCl_4$, $GeCl_4$, $SnCl_4$, $TiCl_4$		74
Silicone oil on Chromosorb P	—	N_2, O_2		cited in 60
OV 17 + QF 1 on Chromosorb W-HP	—	MeHg, EtHg, MeHgCl, $HgCl_2$		83
Molecular sieve	—	N_2, O_2, CO, CO_2, N_2O,	5 A 60–80 mesh	84
Graphite	—	$NbCl_5$, $TcCl_4$, $TeCl_4$,	10–1000 m^2/g	13, 14
Quartz		$ZrCl_4$, $SbCl_3$, $BiCl_3$, InCl, $PaCl_5$, *etc.*		
Silica gel	—	$HfCl_4$, $ZrCl_4$	300 m^2/g	17
NaCl, KCl, CaCl, $MgCl_2$, YCl_3, *etc.*		$NbCl_5$, $TeCl_5$, $ZrCl_4$, $TeCl_4$, InCl, $PbCl_2$, *etc.*	0.5 m^2/g	13, 14
NaBr, KBr, CsBr $CaBr_2$		$ZrBr_4$, $NbBr_5$, $MoBr_4$, $TeBr_4$, $InBr_3$, $SnBr_4$	Adsorption chromatography	16
$MgCl_2$/KCl on Chromosorb WAW	423	$BiCl_3$, $BeCl_2$, $InCl_2$, $SnCl_2$, $ZnCl_2$, TlCl, *etc.*	—	74
$KAlI_4$	350	SnI_4, PI_3, TiI_4	—	68
KBr/AgBr	300	$SnBr_4$, $AsBr_3$, $TiBr_4$	—	68
$NaFeCl_4$	158	$SnCl_4$, $TiCl_4$, $AsCl_3$		64
KF/HF on Al_2O_3	—	Hydrocarbons	60–80 mesh	85
CdF_2 on Al_2O_3	—	Olefins, aromatics	—	86
H_3PO_4 on carbon black	—	SO_2, H_2S, CH_3SH, CH_3SCH_3	90 m^2/g	87
$MnCl_2$, $CoCl_2$, $ZnCl_2$ on silica gel	—	Benzene, pentane, 1-pentene, n-hexane, *etc.*	—	88
NaOH on Al_2O_3	—	Toluene, benzene, ethylbenzene	—	89
Graphite	—	$NbCl_5$, $ZrCl_4$, $TeCl_4$, *etc.*	—	90
$HfCl_4$ on graphite	—	ICl	—	90

heated with a small oven (10 cm length) to temperatures higher than 1300 K. The sample may be contained in a small porcelain or quartz boat or injected directly with a syringe. This method is useful if the conditions in the reaction zone can be chosen, so that the evaporation/desorption or reaction with the reactive component of the carrier gas is sufficiently fast for a narrow sample input profile to be obtained. In intermediate trapping (13b), the difference from direct evaporation is that the volatile compounds are not directly flushed into the chromatographic section of the column, but are first adsorbed at 20° on a short

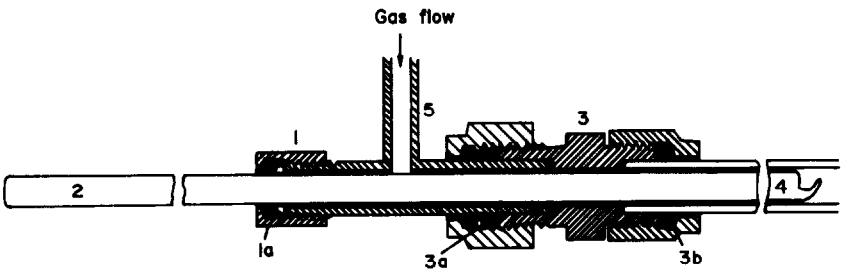

Fig. 12. Sample inlet (brass); 1, screw joint with fitting (1a, rubber or graphite gasket); 2, quartz rod with hook; 3, Swagelok union (3a, brass fitting; 3b, hard rubber fitting); 4, quartz column. (By permission of the copyright holders, Springer-Verlag, Vienna).

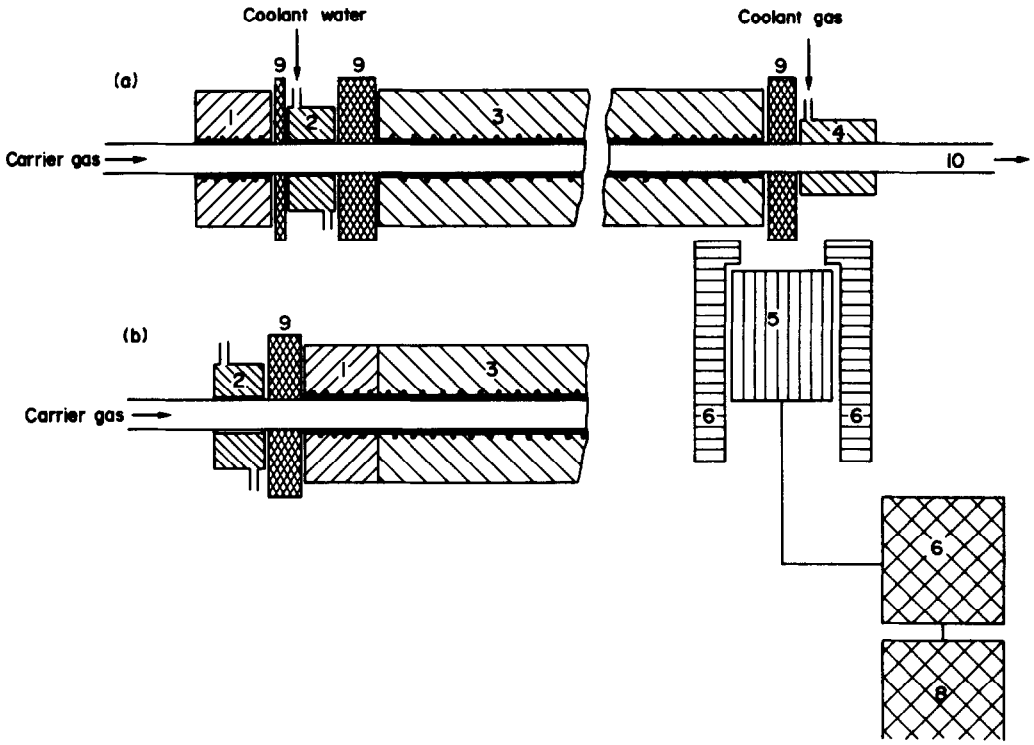

Fig. 13. Column heating, cooling and detector sections, (a) for experiments with intermediate trapping, (b) for experiments without intermediate trapping: 1, injection port oven; 2, water cooling for intermediate trapping; 3, column oven, 4, gas cooling for detector section; 5, detector with shielding; 6, 7, single- or multichannel analyser with data storage and print-out (8); 9, isolating ceramic ring; 10, quartz tube. (By permission of the copyright holders, Springer-Verlag, Vienna).

section of the column, which acts as a trap. To start the separation, the column is moved so that the trapping section is in the column oven, which is kept at the temperature required for the chromatography. If a temperature programme is used in which the initial temperature is such that all components are trapped at the beginning of the column, the arrangement in Fig. 13a may be used, which simplifies the experiments considerably. Both ovens are electrically heated and consist of 1-mm Kantal wire wound on ceramic tubing. The evaporation oven is 100 mm long and the column oven 830 mm. The temperature distribution inside the ceramic tube is sufficiently uniform for chromatographic experiments.

Special problems arise when fluorides are being separated, because there is no material which is completely inert to all inorganic fluorides. Nickel, monel metal, aluminium and polymers such as Kel-F powder are reasonably resistant.[61]

Influence of the mobile phase

In inorganic gas chromatography the mobile phase is not only a carrier gas, but can also influence the separation. One important point is that the decomposition of a chloride, leading to a non-volatile chloride adsorbed on the stationary phase, results in the irreversible loss of trace elements, while in the presence of

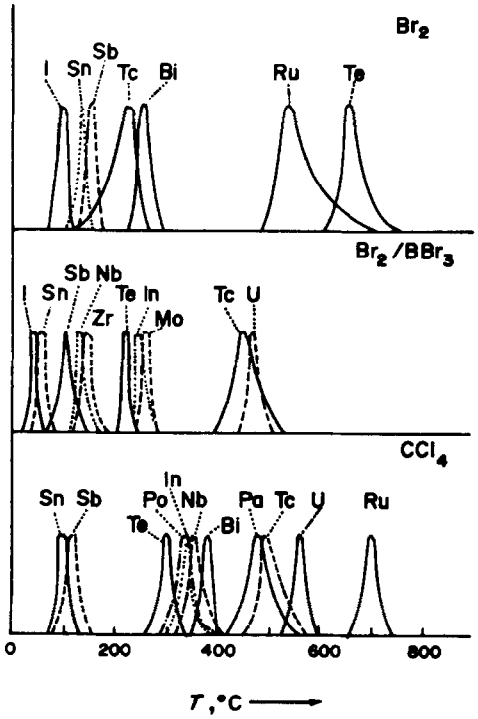

Fig. 14. Temperature-programmed separation on silica with different reactive gas mixtures. (By permission of the copyright holders, Springer-Verlag, Vienna).

the reactive gas a volatile compound can be reformed. This is one of the advantages of the use of inorganic compounds rather than metal organic compounds, which are not easily reformed. Owing to the high excess of reactive gas, the equilibrium favours the metal chloride with the higher valency, which is usually more volatile. A second important point is that the reactive gas can be self-purifying as already mentioned. Molecules such as CCl_4 or BBr_3 decompose at a certain temperature and form radicals, which can react with O_2 or H_2O, thus scavenging these impurities. This is important because the formation of oxyhalides must be avoided. Careful purification of the gas before its admission to the gas chromatograph is, nevertheless, still necessary. Juvet and Fisher[67] used ClF_3 for the deactivation of moisture and organic materials in the column before the analysis of fluorides.

A third effect of the mobile phase is an alteration of the surface of the stationary phase. Figure 14 shows a separation of bromides on a quartz surface and it can be seen that in the case of Tc and Te the retention time is changed by the addition of BBr_3.[9] This might be due to the formation of complexes of BBr_3 with the surface or, more probably, by the scavenging effect of BBr_3 and the elimination of oxygen. When a real equilibrium is involved, as in the case of the separation of $AlCl_3$ complexes of rare earths, the partial pressure of the mobile phase influences the separation. In most other cases a partial pressure of about 1 mmHg of the reactive gas in argon or nitrogen is sufficient for the separation. Sometimes the separation can be carried out in an inert gas stream.

Influence of the stationary phase

Table 8 lists the stationary phases used in inorganic gas chromatography. They fall into five groups. The first group includes stationary phases common in organic gas chromatography, *e.g.*, silicone oil and squalane. They are mainly used for separations of low boiling gases such as CO and H_2, or $SiCl_4$, $GeCl_4$, $SnCl_4$, $TiCl_4$, or mercury compounds. Conditioning of the stationary phase with the same mobile phase is always necessary before separation when the surface is changed by a reactive gas. In addition, it is sometimes necessary to run two or three separations of the trace elements before the first quantitative determination is carried out. The second group consists of quartz, graphite, or molecular sieves. It also contains Al_2O_3 and silica gel, which together with activated carbon have the highest surface areas. The third group comprises metal salts, the salt chosen depending on the volatile species being separated. In the case of chlorides, chloride salts are used as the stationary phase to avoid the complication of reaction with a stationary phase and the formation of new compounds. For adsorption chromatography a stationary phase with a very high boiling point is used, and for the separation complex formation with a solid phase is used. The fourth group consists of liquid stationary phases such as fused metal salts of low melting point, usually as eutectic mixtures. The fifth group consists of supports such as silica gel, graphite, or modified Al_2O_3 coated with inorganic salts so as to exploit the high surface area and the advantage of adsorption chromatography.

Although up to now there has been no direct information about the structures of surface complexes, we have shown by the determination of thermodynamic data that there is evidence for the following simplified picture of the chemisorbed state in adsorbent–adsorbate systems. The interaction between the chemisorbed molecules and the surface is, in principle, a Lewis acid–base interaction. This is the main type of interaction between surface and adsorbed molecule. Van der Waals forces may take part in the adsorbent–adsorbate interaction, but cannot explain the observed adsorption enthalpies. The chemisorbed molecules act as Lewis acids and the surface adsorption sites act as Lewis bases. Surface sites may act as uni- or multidentate ligands, depending on the chemisorbed molecule. There is no evidence as to whether a single surface site may act both as a uni- and as a multidentate ligand, or can play only the one role or the other. The adsorbed molecules have limited degrees of freedom [rotational, vibrational and translational (from site to site)]. The restriction of the rotational, vibrational and translational movements depends on the strength of the chemisorptive bond.

Giddings[91] laid the basis for a theoretical treatment of gas–solid chromatography. The coefficient of adsorption/desorption exchange, C_K, is much smaller than the term for resistance to mass transfer in the liquid phase, C_L, which it replaces in the van Deemter equation, and hence better resolution is obtained. Furthermore, in trace element separation the losses in the liquid phase are higher owing to impurities and irreversible adsorption on the support. In gas–solid chromatography it is possible to clean the surface by suitable treatment with a reactive gas at high temperature. Di Corcia *et al.*[92] showed that by treatment at 900° with a hydrogen stream, separations on graphitized carbon were considerably improved. Several factors influence the separation properties of a graphite surface. Graphite has a surface structure completely different from that of quartz or ionic chlorides. The layer lattice structure of graphite favours absorption of volatile chlorides rather than adsorption. At higher temperatures graphite may have a reducing effect on the chlorides of elements with easily accessible lower oxidation states, such as molybdenum.

Dependence of the separation on temperature

Gas chromatographic separations can be carried out either isothermally or with temperature programming. In Fig. 15 the isothermal separation of some elements at different temperatures is shown.[7] At the first three temperatures the retention time decreases continuously as expected. At 700° niobium shows a behaviour completely different from that at 800°, as

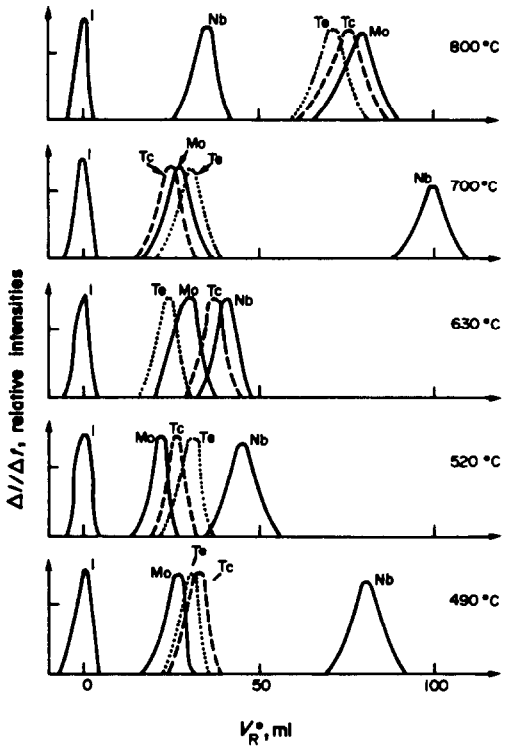

Fig. 15. Some gas chromatographic separations of inorganic chlorides with KCl as stationary phase at different temperatures. (By permission of the copyright holders).

do tellurium, technetium and molybdenum. This is due to the change from adsorption to distribution chromatography, since potassium chloride melts at 770°. Temperature-programmed separation is generally superior to isothermal separation. In temperature-programmed gas chromatography we used an increase of 2–24 deg/min. The errors in retention times are somewhat larger than those obtained in isothermal gas chromatography. In temperature-programmed gas chromatography it is very difficult to achieve an absolutely temperature-independent gas flow-rate. For optimization of a separation both temperature and gas flow should be programmed.

Optimization of a gas chromatographic separation

In optimization of inorganic gas chromatography the three important parameters are the reactive gas, the temperature and the stationary phase. They determine the optimization of the thermodynamic and not the kinetic behaviour. Separation between two components is widest when the retention ratio $r_{2,1}$ is high. The ratio is defined in terms of the retention times t_1 and t_2 or of ΔH, ΔS and T:

$$r_{2,1} = \frac{t_2}{t_1} = \exp\left[\frac{(\Delta S_2 - \Delta S_1)}{R} - \frac{(\Delta H_2 - \Delta H_1)}{RT}\right]$$

ΔH and ΔS depend on the nature of the stationary phase and therefore an optimization with respect to ΔH, ΔS and T is possible, if these values are known. We have carried out determinations of these values for most of the known chlorides at different stationary phases. It is interesting that compounds of similar structure show similar enthalpies and entropies, so by gas chromatography some information about the structure of the gaseous species and perhaps of the surface complexes can be obtained. Figure 16 shows the retention ratio as a function of temperature and different stationary phases for $MoCl_5$, $TeCl_4$ and $NbCl_5$. A high retention ratio means a satisfactory separation. Thus CsCl should be used as stationary phase at a relatively low temperature, and this would give a separation between Mo and Te, but not between Mo and Nb, for which graphite could be used at higher temperatures. Hence two columns, one coated with CsCl and one with graphite could be used to solve this separation problem at different temperatures.

Dependence of the separation on the amounting material

Successful gas chromatography is only possible when true adsorption takes place and not condensation. Figure 17 shows plots[93] of the enthalpy and of adsorption entropy of $NbCl_5$ on quartz against adsorption density and it can be seen that over an adsorption range of about 10 orders of magnitude ΔH and ΔS are constant and then ΔH decreases and ΔS increases over the adsorption range where the monolayer is becoming complete (about 75% of the surface is covered) and the formation of a second layer begins, *i.e.*, the adsorption is followed by condensation, and the curve becomes linear again at about -14 kJ/mole for ΔH and $14\,\text{cal.deg}^{-1}.\text{mole}^{-1}$ for ΔS. For many chlorides and stationary phases a correlation can be found between the adsorption and evaporation enthalpies. We have investigated the separation of $HfCl_4$ and $ZrCl_4$ as a function of the amount of the two components, and found that separation under ideal conditions is possible up to about 3 μg. At higher concentrations fractional distillation and not chromatography is the separation process, though this does not mean that a separation is impossible (as demonstrated in Fig. 14) for 100 mg of uranium.

Optimization of kinetic parameters

The chromatographic resolution is given by the distance between peaks (determined by ΔH, ΔS and T) and the peak width.[40] The plate number ($n = t_{dr}^2/\sigma^2$) is often used (where t_{dr} = overall retention time and σ = standard deviation of t_{dr}), because it is a useful expression for column performance and resolution. The use of plate numbers implies that the simplifying assumption that the peaks are Gaussian is justified because the peaks are symmetric with very little tailing or fronting, and is also necessary because exact determination of peak shape is quite difficult. The plate number of a chromatographic separation depends on several factors. The sample input profile,

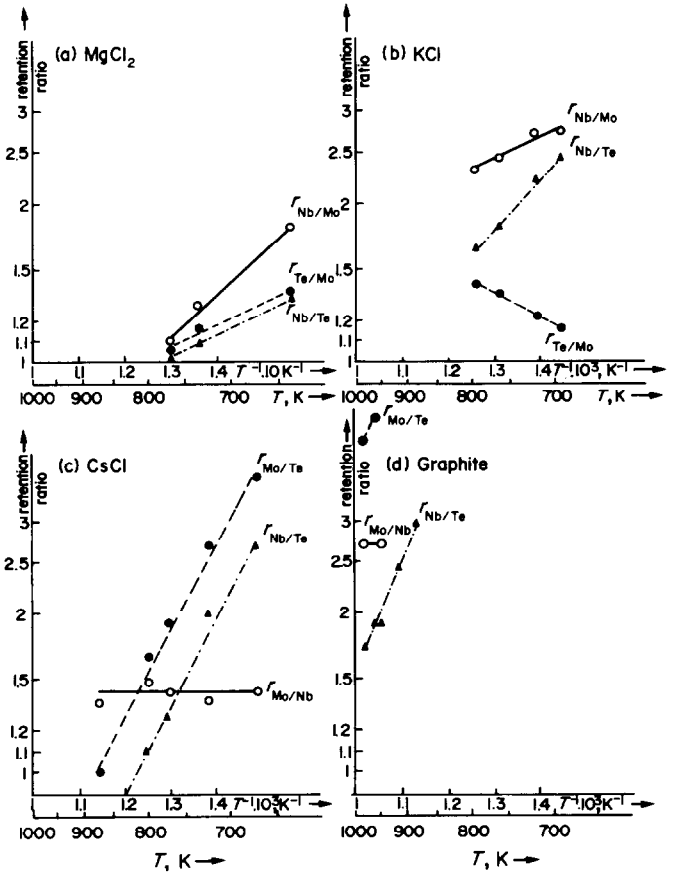

Fig. 16. Retention of Nb, Mo and Te chlorides as a function of the inverse of the temperature (ln r *vs.* T^{-1}) for different stationary phases: (a) $MgCl_2$, (b) KCl, (c) CsCl, (d) graphite.

ideally infinitely narrow, in reality contributes considerably to the final peak width, especially in trace element analysis, where the sample is introduced by rapidly heating a small column section in which the synthesized compounds have been trapped at room temperature. The time taken to heat the samples from room temperature to a temperature necessary for volatilization ranges from 10 to 30 sec.

Owing to the high temperature, the small particle size and the low flow velocity, radial diffusion is fast compared with the other processes determining the adsorption and desorption rates. Therefore, the peak shape (or relative variance RV), given by

$$\mathrm{RV} \equiv \frac{\sigma^2}{t_{\mathrm{dr}}^2} \equiv \frac{1}{n}$$

depends mainly on longitudinal diffusional band-spreading and the desorption and adsorption rates. Under these conditions the RV of the peak can be calculated from the equation:

$$\frac{1}{n} \equiv \mathrm{RV} = \frac{1}{x}\left[\frac{2\gamma D}{u} + \frac{2qu}{(1+q)^2 k_{\mathrm{d}}}\right] \qquad (1)$$

where k_{d} is the adsorption rate constant (dimensions $1/t$), and q the capacity factor or distribution ratio:

$$q = K_1 \frac{v_{\mathrm{s}}}{v_{\mathrm{m}}} = K_2 \frac{a_{\mathrm{s}}}{v_{\mathrm{m}}}$$

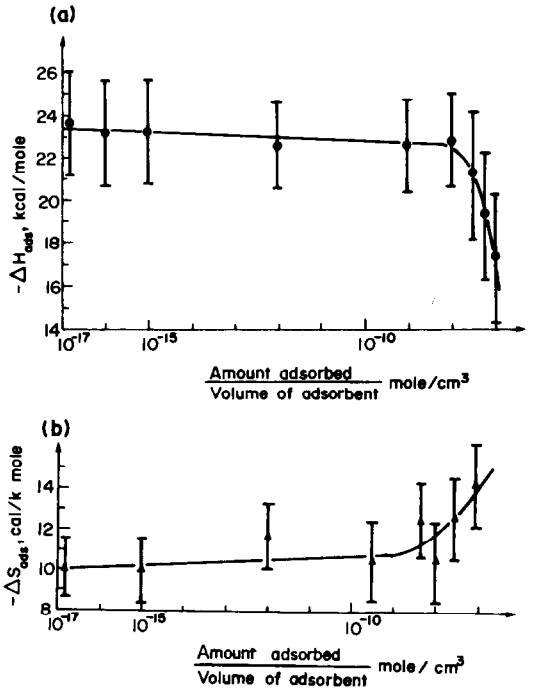

Fig. 17. (a) Adsorption enthalpy of $NbCl_5$ on quartz as a function of concentration, (b) adsorption entropy of $NbCl_5$ on quartz as a function of concentration. (By permission of the copyright holders).

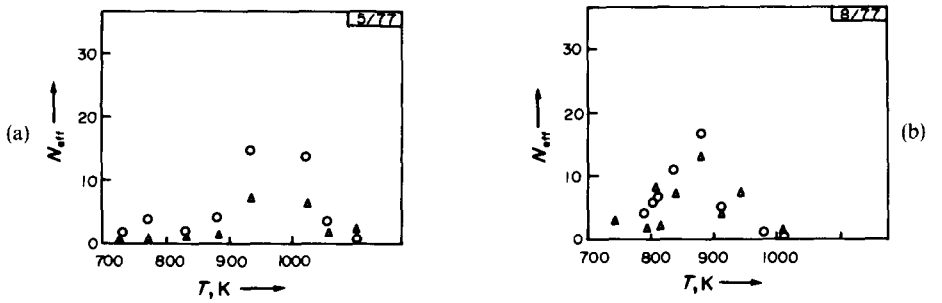

Fig. 18. Effective plate number as a function of temperature. Column 75 × 8 cm, particle size 25 mesh; △ tellurium chloride, ○ niobium chloride, □ molybdenum chloride; (a) NaCl surface, 2 ml/min N_2 (100 mmHg CCl_4), (b) graphite surface, 2 ml/min N_2 (100 mmHg CCl_4).

v_s and v_m being the volumes of solid and mobile phase per unit length of column, a_s the surface area per unit column length and K_1 and K_2 equilibrium constants. For K_1 the concentration in the stationary phase is expressed as concentration per unit volume of solid and for K_2 as concentration per unit area of solid phase. Equation (1) can be derived from the expressions[94] for σ and by omitting the pore-diffusion terms and taking account of the fact that the surface concentration in gas–liquid chromatography can be substituted by the surface concentration in adsorption chromatography, if the effective film thickness is set equal to unity and the equilibrium in the stationary phase is reached instantaneously. By use of equation (1), the desorption rate constants can be calculated from the experimental data, with a gas diffusion coefficient estimated from molecular kinetics.

A comparison between the adsorption enthalpies and the activation energies of desorption calculated from the temperature dependence of the retention volumes and the desorption rate constants, shows that the values are quite similar, which is an indication that no specific interaction between adsorbate and adsorbent occurs.

More information about the efficiency of a separation than that given by the theoretical plate number n can be obtained from the effective plate number N_{eff}.

$$N_{eff} = \left(\frac{t_{dr} - t_d}{\sigma_t}\right) = \left(\frac{t_r}{\sigma}\right) \qquad (2)$$

where t_d = desorption time and t_r = retention time. The relation between N_{eff} and n is given by:

$$N_{eff} = N/(1 + t_d/t_r)^2. \qquad (3)$$

As t_r decreases rapidly with increasing temperature, N_{eff} will be small at temperatures where $t_r \ll t_d$. At low temperatures where $t_r \gg t_d$, n is small owing to the low desorption rate constants and therefore N_{eff} is small. Consequently N_{eff} has a maximum at temperatures where n is large (fast establishment of desorption equilibrium) and $t_r \geqslant t_d$. Unfortunately, these two conditions are in conflict since a temperature rise increases the desorption rate and decreases the retention time. Figure 18 shows the temperature dependence of N_{eff} for two different experimental conditions. There is always a definite maximum, but it is of differing height and at different temperatures because of the different interactions determining the adsorption equilibrium and the rate of equilibrium establishment. Experimental results have shown that if capillary columns are used (instead of a column of 8 mm bore) the peak width is considerably reduced. The plate numbers are increased from about 20 to 1000. With this arrangement, even $ZrCl_4$ and $HfCl_4$[17] and the adjacent lanthanide chlorides are separable (Fig. 19).[71,72]

Gas chromatography of inorganic anions

Anions cannot be separated directly by gas chromatography, but compounds with highly electronegative elements such as HF, HCl, HBr and H_2S can be separated. The separation of sulphur compounds at the ng/l. level in air has been investigated by Bruner *et al.*,[87] who used graphitized carbon black treated with 0.5% H_3PO_4 and 0.3% Dexsil. These authors also separated HCl and HF on Graphon (graphitized carbon) treated with benzophenone and a polyfluoro ether, but unfortunately gave no details of amounts. Compounds of high polarity are difficult to handle by gas chromatography at low concentrations, since the chance of loss at an activated site is high. A survey of the chromatography of gaseous compounds of environmental interest was made by Fishbein.[95] The separation of very low concentrations of polar compounds such as HF and HCl and of anions such as Cl^-, F^-, SO_4^{2-} and PO_4^{3-} is made possible by use of derivatives.[96] Since the derivative is always an organic compound, the gas chromatography presents no new aspects or problems.

THERMOCHROMATOGRAPHY

Thermochromatography is a separation method which uses a thermal gradient in a column. The method was introduced in organic gas chromatography by Zhukhovitskii and Turkeltaub.[97] They used a moving gradient and obtained better resolution, but the method has never gained importance. It was used mainly in radiochemical separations, but

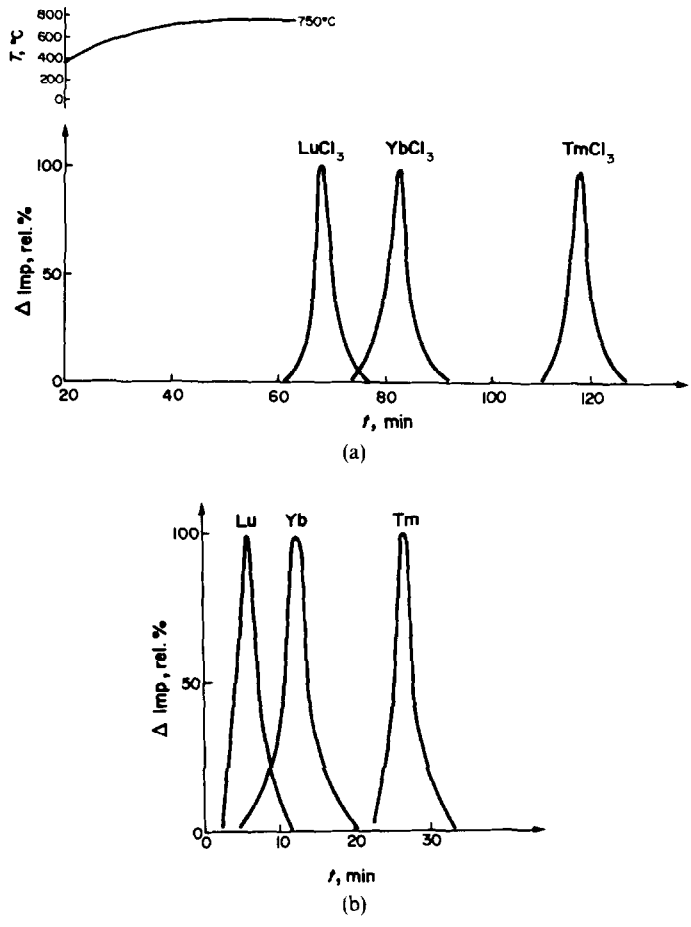

Fig. 19. (a) Separation of $LuCl_3$, $YbCl_3$ and $TmCl_3$: gas flow Cl_2 1 l./hr; temperature 20–750°; length 1 m; bore 8 mm; packing quartz powder, particle size 0.1–0.2 mm. (b) Separation of $LuCl_3$, $YbCl_3$ and $TmCl_3$ as $AlCl_3$ complexes: gas flow $N_2/AlCl_3$ 0.7 l./hr; temperature 200° length 1 m; bore 4 mm; packing quartz powder, particle size 0.2–0.28 mm.

with a static gradient in order to deposit certain elements at certain positions in the column. The possibilities of thermochromatography in trace element analysis have never been closely investigated. A separation, not as a function of time as in normal gas chromatography, but as a function of distance (from the starting point) should have advantages such as the ability to determine elements at any convenient time and with different detectors. A column with a thermal gradient can be used directly after sample volatilization in an oven since the time of volatilization does not affect the position at which the element or compound is deposited. However, the position in the tube is time-dependent until the final distribution is reached. The advantage of this method is that the selectivity can be increased by subsequent heating of certain restricted zones and desorption of the element into a detector. In this way, one element after another can be introduced into the detector. The temperature at the final positions of the elements lies always between the boiling and melting points of the appropriate compound. The disadvantage of this method is that the deposition zones are rather broad. There is an experimental difficulty in the evaporation of the compounds into the detection system. The separation between the zones must be wide enough to avoid the evaporation of two adjacent zones at the same time. The selectivity can be further increased by combining a thermal gradient with the use of different stationary phases. The resolution is improved if an element which forms stable compounds at a certain temperature is deposited in a narrow zone coated with the appropriate stationary phase. For the detection it is necessary to decompose the compound formed. In Table 9 a number of characteristic thermochromatographic separations are listed.

SPECIATION

It is becoming more and more evident that in the study of environmental pollution from metal compounds it is insufficient to determine only the total concentration of the metal. The chemical form of the pollutant is also important. For gaseous samples (air)

Table 9. Thermochromatographic separations of elements or compounds

Elements or compounds which are separated	Column surface	Temperature gradient, °C	References
$ZrCl_4$, $RuCl_4$, $NbCl_5$, $MoCl_5$, *etc.*	Quartz coated with different chlorides	900–25	10
Cf, Es, Fm, Md	Titanium	1700–800	98
I, At, Hg	Silver-coated quartz	1000–100	99
I, At, Hg, Po, Cd, In, Sb, Te, Tl, Pb, Bi, Sn in H_2 stream	Quartz	1000–25	100
Ir, Re, Os; O_2 stream	Quartz	1000–25	101
YBr_3, $ZnBr_2$, Ga_2Br_6, $NbBr_5$, $TeBr_5$, $ZrBr_4$, $HfBr_4$, $AsBr_3$, $GeBr_4$	Quartz	1000–25	102
CaCl, $CeCl_3$, $CeCl_4$, $ZrCl_4$, $TeCl_4$, $TcCl_4$	Quartz	1000–25	103
Ir, Os, Re, Ru, Tc in O_2 stream	Quartz	1000–25	12
$ZrCl_4$, $HfCl_4$, $NbCl_5$, $TeCl_5$, $TcCl_4$, $ReCl_3$, $RuCl_x$, $OsCl_4$, $CdCl_2$, InCl, $SnCl_2$, $SbCl_3$, $SbCl_5$, $BiCl_3$, $TeCl_4$	Quartz, $BeCl_2$, NaCl, KCl, CaCl	1000–25	104
$ZrBr_4$, $NbBr_5$, $TcBr_5$, $CdBr_2$, InBr, $SnBr_2$, $SnBr_4$, $SbBr_3$, $BiBr_3$, $TeBr_4$	Quartz, NaBr, KBr, CsBr	1000–25	16
$OsCl_4$, $ReCl_6$, $AuCl_3$, $PtCl_4$	Quartz	1000–25	105

and liquid samples (water) a number of methods such as gas chromatography[106–110] coupled with atomic-absorption spectrometry, atomic fluorescence, atomic emission, or extraction have been applied. For solid samples it is much more difficult, since the normal procedure of producing a solution may change the chemical state.

For solid samples it may be possible to exploit the different extraction behaviour of different trace compounds. A second possibility is to use the selective volatilization of the element in different chemical states. We have found that only part of the arsenic in a sample is released as arsine in a stream of hydrogen. In this case it is not the species present which is important, but the rate of formation of a volatile compound.[111] The release of the gaseous compound depends on the diffusion and desorption of the various species in which the element is present. Even if a reactive gas is used as mobile phase and new compounds are formed with the elements, it has to be assumed that the original compounds will react in different ways and at different temperatures. This means that the release of the element during volatilization will at least partly reflect the pattern of the compounds. If it is only the total concentration of the element which is required, this effect is undesirable and may result in part of the element not being released. For example, selenium in biological samples can be evaporated as Se or SeO_2 but not as SeO_4^{2-}. On the other hand, when information about the species is desirable, the effect might be used for an indirect determination of the different species. This experimental procedure is used in thermal analysis, when a temperature gradient is used for the analysis of compounds on the macro scale, *e.g.*, the water content of salts. It has not been investigated on the micro scale, but in some cases may prove to be the only method of obtaining information. This might be the case for biochemical problems, such as the analysis of aerosols.

Three approaches have been used. The first is direct injection into the graphite tube of an atomic-absorption spectrophotometer with continuous signal recording of the output for an element. In some cases the pattern of formation of the free atoms allows conclusions to be drawn about the original species present. One problem is that the time-dependent signal is subject to interference by all other elements present and in many cases a quantitative measurement is not possible. The second, and more flexible, system is one in which the volatilization and atomization sections are separated[112,113] (Fig. 20). This arrangement permits the use of different temperatures in the two sections, but it is not possible to use the most suitable reactive gas for the volatilization. However, it is possible to determine more than one element in a sample by using a temperature programme. The most promising approach with great flexibility and applicability is the third, involving a complete separation of the detection and volatilization steps. In this case it is possible to use different reactive gases and a slow temperature programme for the separation. With one device a great number of samples can be treated without using the detection system. Especially interesting are the compounds of toxic elements such as As. We have begun an investigation[111] of the behaviour of

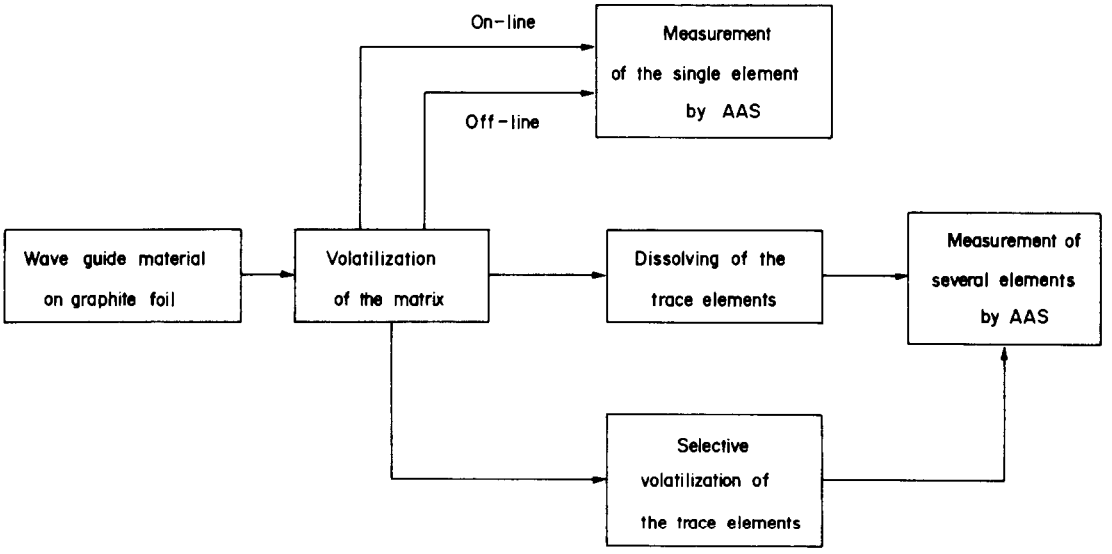

Fig. 20. Scheme for matrix volatilization and coupling with AAS.

arsenic compounds in a stream of hydrogen and the results show that differentiation is possible.

COUPLING OF VOLATILIZATION OR GAS CHROMATOGRAPHY TO DETECTION SYSTEMS

As shown in Fig. 21 it is possible to couple inorganic gas chromatography or volatilization with a number of different detection systems.[5] In gas chromatography the detection system must be suitable for on-line coupling, whereas in volatilization off-line coupling is also possible. The most familiar combination in organic gas chromatography is with mass spectrometry (GC/MS). This has not been used in inorganic gas chromatography (with analytical aspects) because of technological problems due to the corrosive nature of the reactive gases and the high temperatures involved, but in principle it should be possible to solve these problems. For on-line detection spectroscopic methods such as atomic absorption, atomic emission, or atomic fluorescence are possible, as is the measurement of radioactivity. For X-ray fluorescence and electroanalytical methods an off-line method is preferable. There is no direct experience with electroanalytical methods, but when an element is collected on a suitable collector and is suitable for an electroanalytical determination, no problems should arise. The choice of on-line and off-line coupling may be decided by the detection limit when very low concentrations must be determined. Any on-line coupling will result in a broadening of the detection signal which increases the detection limit.

Figure 22 shows[5] the different possibilities for coupling volatilization or gas chromatography with atomic-absorption spectrometry. A direct combination of volatilization with atomic spectroscopy is only possible when the volatile compound is released rapidly. If the amount of sample is large, volatilization takes too long and a very broad peak is observed. Therefore, this method should only be used in exceptional cases such as the direct volatilization of Hg. The off-line mode has the disadvantage that only one element can be determined at a time. This could be dealt with by selective desorption or decomposition before the atomic-absorption measurement or by using a gas chromatographic separation, but the AAS light source would have to be changed between successive admissions of species. It would be easier to use emission spectroscopy with a simultaneous detec-

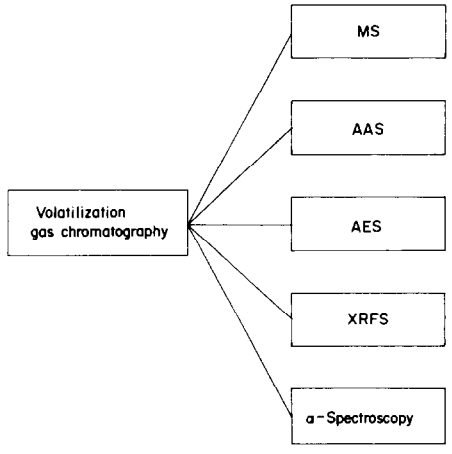

Fig. 21. Coupling of the techniques of volatilization and gas chromatography with various detector systems. (By permission of the copyright holders, Springer-Verlag, Berlin).

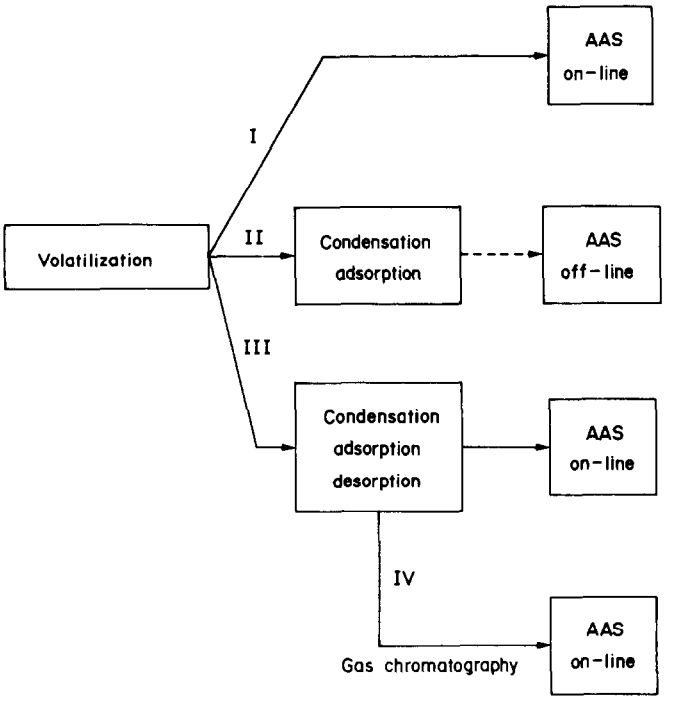

Fig. 22. Schematic presentation of the possibilities of coupling AAS with volatilization. (By permission of the copyright holders, Springer-Verlag, Berlin).

tion system. For all off-line measurements a collection system is necessary and three methods are available; collection on a plate or disc, collection in a capillary tube and collection in a liquid. In the first two cases, the elements collected can be dissolved and determined by classical methods. The disc is preferable for radioactivity counting, X-ray fluorescence of thin layers and for atomic spectroscopy (with thin foils suitable for introduction into an oven). The capillary tube is preferable for direct use in an oven and, in certain cases, together with a temperature gradient for selective desorption. Collection in a liquid is only possible with very volatile compounds, because it is impossible to heat the end of the capillary to prevent condensation of high-boiling components. We have measured distributions in tubes and on plates and found that there is incomplete condensation, depending on the flow-rate and the distance from the end of the capillary. One method of collecting very small amounts of volatile species such as selenium or selenium dioxide is to carry out a co-condensation with an inert gas. Meyer *et al.*[35] have used this principle in the condensation of Se. Optimization of these methods is only possible with the aid of radionuclides. A future development for X-ray fluorescence might be collection on a thin band which is moved slowly at the end of the capillary. For off-line determination after gas chromatography, this might be preferable to the use of a sample changer with plates.

When the volatilization or gas chromatography is coupled with spectroscopic detectors, some of the problems associated with liquid or solid samples do not arise, but the formation of the gaseous compound is always subject to matrix interference. Further, the gaseous compound is introduced into the detection system, no evaporation energy is needed, so no quenching effects occur, and a microwave-induced plasma can be used instead of an inductively coupled plasma for the emission spectroscopy.

CONCLUSIONS

Volatilization and inorganic gas chromatography are separation and enrichment methods for inorganic trace analysis which have not been widely used. This is partly due to technological difficulties and the lack of commercially-produced equipment. Also, the behaviour of many elements and compounds at high temperatures is not known. There has not been much demand for this type of analysis, but the need for the determination of very low concentrations and for multielement analysis requires the development of new methods, and these gas-phase methods have a number of advantages which make them worth further investigation.

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DETERMINATION OF TRACES OF TELLURIUM IN HEAT-RESISTING ALLOYS BY GRAPHITE-FURNACE ATOMIC-ABSORPTION SPECTROMETRY AFTER CO-PRECIPITATION WITH ARSENIC

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Summary—A simple analytical method has been developed for the determination of traces of Te in complex heat-resisting alloys by graphite-furnace atomic-absorption spectrometry. Nickel-base and cobalt-base heat-resisting alloys are dissolved in concentrated hydrochloric and hydrofluoric acids plus 30% hydrogen peroxide. Tellurium is separated from the matrix by co-precipitation with As and dissolved in nitric acid. Memory effects are eliminated by the oxidation of Te(IV) to Te(VI). Standard solutions for the calibration are prepared by the procedure used for the sample solution. The detection limit for Te is 0.05 ppm in the alloy.

Heat-resisting alloys are used, in jet engines or industrial gas turbines, under severe circumstances where high strength at high temperature is needed. Tellurium is known to have, even at trace concentrations, deleterious effects on the rupture life and elongation of Ni-base heat-resisting alloys.[1,2] The maximum allowable concentration of Te in Ni-alloy castings is given as 0.5 ppm in the SAE Aerospace Material Specification 2280.[3]

Traces of Te in heat-resisting alloys have been determined by neutron-activation analysis,[4] X-ray spectrometry,[5] emission spectrography[6] and graphite-furnace atomic-absorption spectrometry.[7–9] The first three methods[4–6] require preliminary separation of Te from the matrix. Direct determination of Te in solutions of Ni-base alloys by graphite-furnace atomic-absorption spectrometry[7] needs well-characterized standard alloys or standard solutions. Dulski and Bixler[8] also used this method, with a single-point standard addition for calibration. Traces of Te have been directly atomized from chips of Ni-base alloy[9] with commercial graphite furnaces. This method is superior to the solution method[7] in needing only a small sample, giving no molecular absorption and only a small risk of contamination, and saving analysis time because dissolution and preatomization heating are not required. However, it necessitates standard alloys for the calibration and is less accurate because the commercial graphite furnace is exclusively designed for liquid samples.

Attempts were made to determine traces of Te in solutions of heat-resisting alloys directly by graphite-furnace atomic-absorption spectrometry using the calibration curve method. The results were, however, unsatisfactory because the background absorption exceeded the correction capacity of the instrument.

Various separation methods for traces of Te have been reported.[10] Of these, co-precipitation of Te with arsenic as carrier[11–13] is simple and gives a high recovery for traces of Te. This method is selective and may separate traces of Te from large quantities of complex heat-resisting alloy in a single separation. We have combined it with graphite-furnace atomic-absorption spectrometry to obtain a simple analytical method for the determination of traces of Te in heat-resisting alloys. This method is suitable for routine analysis of large numbers of samples and applicable to a wide variety of complex samples, owing to the selectivity of the separation.

EXPERIMENTAL

Apparatus

A Perkin–Elmer Model 703 atomic-absorption spectrophotometer, equipped with a Model HGA-2100 graphite furnace and a deuterium background-corrector, was used. The light-source for Te determination was a Perkin–Elmer electrodeless discharge lamp (EDL) operated at 9 W with a Perkin–Elmer EDL power supply. The analytical wavelength used was 214.28 nm. The spectral band-width was 0.2 nm. The test solution (20 μl) was injected into the graphite furnace with a Perkin–Elmer Model AS-1 autosampling system. A part of the controller circuit was modified to proceed automatically with the high-temperature cleaning cycle immediately after the atomization cycle. The duration of the high-temperature cleaning cycle can be varied, up to about 15 sec. Absorbance was recorded with a Hitachi Model 056 recorder. A thermocouple or an optical pyrometer was used for measuring the temperature of the furnace. Argon was used as purge-gas.

Reagents and solutions

Tellurium stock standard solution (Te 1 mg/ml) was prepared by dissolving "Specpure" Te metal in the minimum of concentrated nitric acid, evaporating to dryness twice

with concentrated hydrochloric acid, and diluting with 0.3*M* hydrochloric acid. Working standard solutions were prepared daily from the stock standard solution.

Phosphinic acid (50%) was used as reducing agent. The other reagents were of analytical-reagent grade. Standard solutions of various metals were prepared by dissolving the high-purity metals in appropriate acids. An As^{3+} solution (As 1 mg/ml) was prepared by dissolving 0.660 g of arsenic (III) oxide in 10 ml of 5% potassium hydroxide solution and diluting to 500 ml with distilled water.

Heat-resisting alloys

Table 1 summarizes the chemical composition of the Ni-base and Co-base heat-resisting alloys used, listing the alloying elements present at the 0.1% level or above. Standard heat-resisting alloys containing known concentrations of Te were not available.

Procedure

Weigh 0.5 g of chips of a heat-resisting alloy into a 100-ml Teflon beaker. Add 10 ml of concentrated hydrochloric acid and 5 ml of concentrated hydrofluoric acid. Cover the beaker and heat the solution on a hot-plate. Add 10–15 ml of 30% hydrogen peroxide little by little and decompose it by prolonged heating. After complete dissolution of the sample, evaporate the solution to 15 ml. Add 4 ml of As^{3+} solution and 25 ml of concentrated hydrochloric acid, heat the solution to 90–100° and add 6 ml of 50% phosphinic acid (total volume: 50 ml). Continue the heating for 20 min, then allow the solution to stand for at least 5 hr at room temperature. Filter off the precipitate on a 9-cm Toyo Roshi No. 5B filter paper (4-μm average pore size, equivalent to Whatman No. 40 paper) and wash it thoroughly with 1*M* hydrochloric acid and distilled water. Dissolve the precipitate in 6 ml of hot 8*M* nitric acid added in several portions, followed by 1 ml of hot concentrated nitric acid. Wash the beaker and paper with distilled water, collecting the washings in a 25-ml standard flask. Add 2 ml of 0.1*N* potassium permanganate, and dilute to the mark with distilled water.

Prepare standard solutions for the calibration in the following manner. Add graded volumes of the working standard Te solutions to the mixtures of 30 ml of concentrated hydrochloric acid and 2–3 ml of concentrated hydrofluoric acid, dilute to 40 ml, add 4 ml of As^{3+} solution and heat. Add 6 ml of 50% phosphinic acid, then follow the same procedure as for the samples. Transfer the solutions into polystyrene cups on the turn-table of the instrument. Push the start button of the auto-sampling system, with the programme set as summarized in Table 2. Interrupt the flow of purge-gas during the atomization. Measure the absorbance of Te. Prepare a calibration curve and calculate the concentration of Te in the sample.

Table 2. Operating parameters

Drying temperature	80°C
Drying time	30 sec
Ashing temperature	500°C
Ashing time	30 sec
Atomization temperature	2550°C
Atomization time	4 sec
High-temperature cleaning	2500°C
High-temperature cleaning time	3 sec

RESULTS AND DISCUSSION

Direct determination of Te in alloy solutions

Nickel-base heat-resisting alloys were dissolved in concentrated nitric acid and concentrated hydrofluoric acid, which are widely used for the dissolution of such alloys. This combination of acids was used only for the direct determinations. The concentrations of Te and matrices used were 0.005–0.025 μg/ml and 10–20 mg/ml, respectively. For the 10-mg/ml matrix concentration, 0.5 g of alloy was dissolved in 5 ml of concentrated nitric acid and 2 ml of concentrated hydrofluoric acid, and the solution was diluted to 50 ml with water. Optimum heating temperatures and times were selected. Because of the matrix effects, Ni and Cr were added to the standard solutions for the calibration, for matrix-matching, in an attempt to determine directly traces of Te in solutions of Ni-base heat-resisting alloys by the calibration curve method, but the accuracy and precision by this method were unsatisfactory. Even if the beam of the deuterium lamp was carefully aligned with that of the electrodeless discharge lamp and all conditions were optimized, the large background absorption due to the salts from the complex matrices could not be compensated for by the background corrector. The direct method was found applicable only when matrix-

Table 1. Chemical composition of Ni-base and Co-base heat-resisting alloys used (% w/w)

Alloy	C	Al	Si	Ti	Cr	Mn	Fe	Co	Ni	Zr	Nb	Mo	Ta	W
NBS SRM 349*		1.2		3.1	19.5			14.0	57.2			4.0		
IN-792		3.4		3.9	12.6			9.1	61.0			1.8	4.1	4.0
IN-738 LC		3.4		3.4	15.8	0.1	0.2	8.3	61.6	0.1	0.8	1.8	1.7	2.6
MAR-M 246		5.5		1.5	10.1		0.3	9.9	57.7			2.6	1.5	10.9
Udimet CO263		0.5		2.2	20.1		0.3	19.9	50.9			5.8		0.2
JAERI-R 9†		0.3	0.3		21.5	0.3	17.6	1.2	49.3			9.1		0.6
René 95	0.2	3.5		2.5	14.0			8.0	61.0		3.5	3.5		3.5
TM-47	0.1	3.6		3.6	12.8			9.2	60.1				2.6	8.0
NBS SRM 168§					20.3	1.5	3.4	41.2	20.3		3.0	4.0	1.0	4.0
Modified S-816					20.2	0.9	0.4	45.6	20.0		4.0	4.1		3.7
WI-52					21.2	0.3	2.0	62.5	0.8		1.8			10.4
X-45			1.0		25.6	0.7	1.1	53.2	10.5					7.3

* Waspaloy.
† Hastelloy X.
§ Modified S-816.

matched standard solutions or standard alloys containing a known concentration of tellurium were used. The direct method is, however, impractical as a routine method because such standard alloys or solutions are difficult to obtain or prepare, and a separate set is needed for each alloy matrix encountered.

Co-precipitation of Te with As

It is generally necessary to separate Te from the matrix of heat-resisting alloys before graphite-furnace atomic-absorption spectrometry. Co-precipitation of Te with As was used as the separation method. A preliminary study showed that reduction with tin(II) chloride and subsequent co-precipitation with As carrier was not suitable for graphite-furnace atomic-absorption spectrometry. The small quantities of tin which remained owing to incomplete washing of the precipitate suppressed the Te signal. Phosphinic acid (50%) was found suitable as a reducing agent.

Co-precipitation conditions. Heat-resisting alloys (0.5 g) were dissolved in the mixture of concentrated hydrochloric acid, concentrated hydrofluoric acid and 30% hydrogen peroxide. The best conditions for the co-precipitation of traces of Te with As were established by experiments with a Ni-base heat-resisting alloy, René 95. For convenience, the volume of the solution was adjusted to 50 ml at the co-precipitation step. The quantity of Te added was 1.25 μg. The effect of acids on the co-precipitation was studied. The recovery of Te was almost constant (89–95%) when 1–10 ml of concentrated hydrofluoric acid was present in the 50 ml of solution. The recovery was calculated from the ratio of the absorbance for the test solution to the absorbance for a reference solution containing 1.25 μg of Te, 4 mg of As, 6 ml of 8*M* nitric acid, 1 ml of concentrated nitric acid and 2 ml of 0.1*N* potassium permanganate in 25 ml. The presence of 10 ml of concentrated hydrofluoric acid in the reference solution lowered its absorbance by about 10%, however.

The effect of hydrochloric acid concentration in the 50 ml of alloy solution was investigated from 3.0 to 8.6*M*. The recovery of Te was 89–93% over the concentration range 5.0–8.6*M*. The quantity of As^{3+} added was varied from 1 to 4 mg, and the recovery of Te was 95% for 3–4 mg of As^{3+}. Varying the volume of 50% phosphinic acid added between 2 and 8 ml had no effect on the recovery of Te, and 6 ml of 50% phosphinic acid was chosen as optimal. The recovery of Te was 95–98% when the solution was heated for 20–80 min at 90–100°, but the standing time at room temperature had a definite effect on the recovery. It was necessary to stand the solution for at least 5 hr, and preferably overnight, to obtain a high and constant recovery (90–91%) of Te. The standing time affects the completeness of reduction and/or coagulation of the precipitate. The bulk of the precipitate of Te and As was dissolved with 8*M* nitric acid and the rest with concentrated nitric acid. The volumes used were chosen by considering the minimum needed for dissolution of the precipitate, and the effect on the life of the graphite furnace. As a result, 6 ml of hot 8*M* nitric acid and 1 ml of hot concentrated nitric acid were used.

Memory effects. When elemental Te co-precipitated with As was dissolved in the 8*M* and concentrated nitric acid, memory effects were found. These effects were independent of the presence of As and the kind of reducing agent used. Figure 1 shows the recorder traces of the signals from TeO_3^{2-} and TeO_4^{2-} (1.25 μg of Te) dissolved in 2.5*M* nitric acid. Tellurium(IV) shows memory effects, but Te(VI) does not. It was confirmed by spectrophotometry with thiourea[14] that Te(IV) is obtained by the dissolution of elemental Te in the 8*M* and concentrated nitric acid. The reason why Te(IV) shows memory effects is not apparent. These effects were not eliminated by a prolonged high-temperature cleaning cycle. To eliminate the memory effects, the Te(IV) should either be oxidized to Te(VI) or extracted into an organic solvent to change the atomization mechanism of the Te. Oxidation of Te(IV) to Te(VI) was examined, and the memory effects were found to decrease on addition of 0.1*N* potassium permanganate, 2 ml of the permanganate solution eliminating the memory effects completely. According to the equation,

$$5Te^{4+} + 2Mn^{7+} \longrightarrow 5Te^{6+} + 2Mn^{2+}$$

only 0.2 μl of 0.1*N* permanganate would be needed to oxidize 1.25 μg of Te, and the rest of the 2 ml was presumably involved in oxidation of As(III) to As(V).

Effect of diverse elements. The effect of the alloying elements in the Ni-base and Co-base heat-resisting alloys on the co-precipitation was studied and, if they were co-precipitated with As, their effect on the atomic absorption of Te was also examined. The concentration of Te used was 0.025 μg/ml. The concentrations of the elements tested were 2 and 10 mg/ml, corresponding to 8×10^4- and 4×10^5-fold ratio,

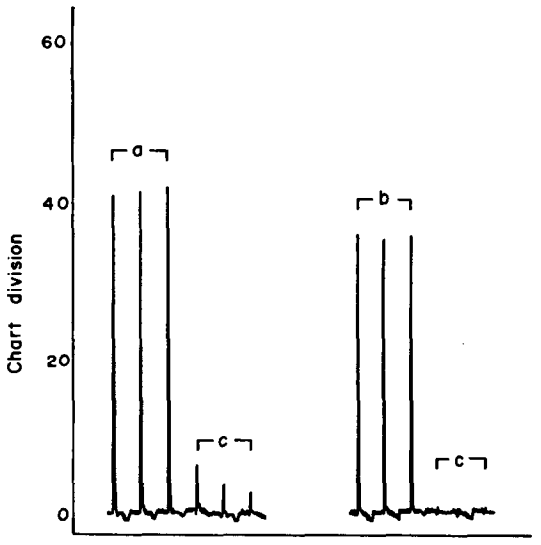

Fig. 1. Recorder traces of signals from Te(IV) and Te(VI). a, Te(IV); b, Te(VI); c, blank.

Table 3. Effect of diverse elements on co-precipitation and atomic absorption of Te

Element mg/ml	Relative absorbance*											
	Al	Ti	Cr	Mn	Fe	Co	Ni	Zr	Nb	Mo	Ta	W
2	1.00	1.00	0.98	0.99	1.01	1.00	1.03	1.01	1.01	1.01	1.02	0.99
10	0.97	0.98	0.96	0.99	0.99	1.00	1.00	0.99	1.00	1.02	0.95	1.01

* Absorbance of Te co-precipitated with As in matrix-free solution = 1.00.

Table 4. Determination of traces of Te in spiked Ni-base and Co-base heat-resisting alloys*

Alloy	0.50 ppm			2.50 ppm		
	$\bar{x}$	s	$100s/\bar{x}$,%	$\bar{x}$	s	$100s/\bar{x}$, %
NBS SRM 349	0.55	0.011	2.0	2.57	0.032	1.3
IN-792	0.52	0.021	4.0	2.61	0.056	2.1
IN-738 LC	0.51	0.012	2.3	2.60	0.042	1.6
MAR-M 246	0.51	0.014	2.8	2.53	0.034	1.3
Udimet CO263	0.50	0.019	3.9	2.62	0.027	1.0
JAERI-R 9	0.48	0.015	3.1	2.54	0.031	1.2
René 95	0.49	0.021	4.3	2.55	0.038	1.5
TM-47	0.53	0.016	3.0	2.49	0.036	1.5
NBS SRM 168	0.50	0.013	2.7	2.50	0.024	0.98
Modified S-816	0.53	0.013	2.4	2.50	0.023	0.92
WI-52	0.50	0.010	2.1	2.40	0.020	0.84
X-45	0.52	0.019	3.6	2.50	0.025	1.0

* Te < 0.05 ppm in unspiked samples.

respectively, to the concentration of Te. Table 3 shows the results. The precipitate was washed with 10% hydrofluoric acid, if necessary, before the washing with 1*M* hydrochloric acid and distilled water. When the effect of 10 mg/ml of Fe was studied, 8 ml of 50% phosphinic acid was used. As shown, the elements tested do not interfere with the co-precipitation and atomic absorption of Te, over the concentration range studied. It has been reported that small quantities of Se, Sn, Sb and Bi, which may co-precipitate with As, do not interfere with the atomic absorption of Te,[12] and in any case these elements present only as trace impurities in heat-resisting alloys.

Determination of Te in heat-resisting alloys

The proposed method was applied to the determination of traces of Te in a wide variety of practical Ni-base and Co-base heat-resisting alloys. The detection limit, defined as the concentration of Te which produces an absorbance twice the fluctuation of the base-line, was 0.05 ppm of Te in the sample. Table 4 summarizes the results of analysis of heat-resisting alloys for traces of Te by co-precipitation and graphite-furnace atomic-absorption spectrometry. The concentrations of Te found were all less than 0.05 ppm. To establish the validity of the method, known concentrations (equivalent to 0.50 and 2.50 ppm in the alloy) of Te were added to the alloy solutions and the total Te was determined. The average value ($\bar{x}$), standard deviation (σ) and relative standard deviation ($100\sigma/\bar{x}$) were obtained from 10 repetitive measurements of the absorbance of Te in the same solution. The concentrations of Te found agreed well with those added. It is concluded that the inter-element effects of the alloying elements of heat-resisting alloys are negligible. Analysis of 10 separately weighed samples of René 95, spiked with 0.50 ppm of Te, gave $\bar{x} = 0.50_4$ ppm, $s = 0.013$ ppm, $100s/\bar{x} = 2.5\%$.

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DETERMINATION OF SULPHUR FUNCTIONS WITH IODOSO-COMPOUNDS

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Summary—Iodoso derivatives, *e.g.*, *m*- and *p*-chlorophenyl, *m*- and *p*-methylphenyl and *p*-nitrophenyl-iodosoacetates and *o*-diacetoxyiodobenzoate, have been thoroughly investigated as oxidants and are proposed as analytical reagents for the determination of thiols, thioureas, isothiocyanates, organic sulphides, disulphides, xanthates, dithiocarbamates, amines and alcohols in aqueous and non-aqueous (acetic acid, acetonitrile, dimethylformamide) media. The error of the determination of thiols is 0.2% thioureas 0.5%, organic sulphides and disulphides 0.4%, isothiocyanates 0.4%, dithiocarbamates 0.4%, and xanthates 0.5%. All the methods are precise to 0.2–0.5%. It is also shown that the properties of the *o*-derivatives differ markedly from those of the *m*- and *p*-derivatives. The *o*-derivatives contain a 1-substituted 1,2-benzoiodoxilin-3-one ring system. The stability of this system is apparently associated with the size of the five-membered ring; other compounds in which iodine is apparently part of larger rings are less stable. *o*-Diacetoxyiodobenzoate appears to be the best analytical reagent of the iodoso series. The *m*- and *p*-iodosobenzoates are found unsuitable for quantitative oxidation of cysteine.

Among all the halogen compounds, iodoso-type compounds are peculiar to iodine. Similar compounds of other halogens either do not exist or are too unstable to be used as analytical reagents. The peculiarity of iodoso compounds lies in the fact that the iodine, even though in a positive oxidation state, must be linked to a carbon atom attached to a double bond. No stable compounds of positive iodine are known containing comparatively electropositive saturated aliphatic radicals.

Hellerman *et al.*[1,2] devised a method for determining cysteine with *o*-iodosobenzoate at pH 7, but when this was applied to enzymes, inactivation occurred. Hellerman concluded that this inactivation could result from competitive inhibition by the benzoate moiety rather than from any effect of –SH groups.

In the course of a systematic study of the determination of sulphur functions, we have considered the behaviour of a series of iodoso compounds. We have found that *m*- and *p*-derivatives of iodosobenzoates oxidize thiols quantitatively to the corresponding disulphides but do not oxidize cysteine to cystine stoichiometrically. *o*-Diacetoxyiodobenzoate[3] appears to be the best analytical reagent of the iodoso series because it possesses the qualities of *o*-iodosobenzoate[1,2] and of all the *m*- and *p*-derivatives of the phenyliodosoacetate series. It can be used in aqueous (pH 7) as well as non-aqueous media, *e.g.*, acetic acid, methanol, acetonitrile and dimethylformamide.

EXPERIMENTAL

Reagents

Preparation of o-diacetoxyiodobenzoic acid. A mixture of 70 ml of 30% hydrogen peroxide and 305 ml of acetic anhydride was stirred for 4 hr at 4°. *o*-Iodobenzoic acid (60.7 g) was then added to the solution, which was stirred for a further hour and kept overnight. Some *o*-diacetoxyiodobenzoic acid separated out and was filtered off. The filtrate, on concentration to small volume under reduced pressure (70 mmHg), yielded a second crop. The combined needle-shaped crystals were washed with ether and dried (over sodium hydroxide) in a vacuum desiccator; yield 63.0 g, m.p. 206°.

An aqueous *o*-diacetoxyiodobenzoate solution (0.05*M*) was prepared by adding a slight excess of 1*M* potassium hydroxide to the solid reagent and diluting to volume. It was standardized iodimetrically and a 0.01*N* solution was made by dilution.

Non-aqueous *o*-diacetoxyiodobenzoate solutions (0.05*M*) were prepared by dissolving the solid in glacial acetic acid, acetonitrile or dimethylformamide, and were standardized iodimetrically. The *p*- and *m*-methyliodosoacetates,[4] and *m*- and *p*-chlorophenyliodosoacetates[5] were prepared through the corresponding iodo-compounds,[6] and *p*-nitrophenyliodosoacetate[7] was prepared from the corresponding dichloride by reaction with lead acetate.

While all the procedures for preparing iodoso-compounds were found to be satisfactory, that of Pausacker[5] was found to be the simplest and most convenient. The products were recrystallized from anhydrous acetic acid and were freed from the solvent with dry air under suction, and were stored in a vacuum desiccator. The purity was determined by dissolving known weights in acetic acid (5–10 ml), adding 20 ml of 10% potassium iodide solution, diluting to 100 ml with water, and titrating the liberated iodine with thiosulphate. Samples of 99.5% purity could easily be prepared.

Stability of the stock solutions

The solutions are remarkably stable when kept in amber-coloured bottles, their strengths remaining unchanged for more than a month. Even in clear glass bottles, solutions are stable for up to 7 days. It may be pointed out that solutions of dichloramine-T and iodobenzene dichloride deteriorate quickly.

Test materials

Most of the thiols were gifts from Evans Chemetics, New

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York. Diethyldithiocarbamate was a commercial product, the rest were prepared by the usual methods.[8,9]

Xanthates were prepared from the corresponding sodium or potassium alkoxide by reaction with carbon disulphide. The products were recrystallized by dissolution in acetone, filtration and precipitation by addition of petroleum ether.[10]

Amines, commercial grade, were distilled before use. All other chemicals used were of reagent grade.

Direct procedure for thiols in partially non-aqueous media

Thiols are quantitatively oxidized to their disulphides according to the equation:

$$2RSH + \xrightarrow{R'\text{-}IO} RSSR + I\text{-}R' + H_2O$$

where R′ = substituted benzoate moiety of the iodoso-derivative.

A sample containing 0.1–0.5 meq of thiol was weighed into a 150-ml Erlenmeyer flask containing 50 ml of distilled water. If insoluble in water, the sample was dissolved in 10–15 ml of acetic acid, acetonitrile or dimethylformamide and before titration a roughly equal volume of water was added, to make its concentration roughly 40–60% v/v. (Less water could be used if the sample is partially precipitated, but at higher acetic acid concentrations the reaction is slow and the end-point is not so sharp.) About 50 mg of potassium iodide and 1 ml of 1% starch solution were added and the solution was titrated with a 0.01*N* solution of any of the iodoso-compounds.

For potentiometric titrations, in a 100-ml beaker was used, with a modified calomel or antimony electrode as reference and a bright platinum were as indicator electrode.

Indirect procedure for thioureas

Thioureas are quantitatively oxidized to the corresponding urea and sulphate:

$$RNHCSNH_2 + 4R'\text{-}IO + H_2O \rightarrow RNHCONH_2 + H_2SO_4 + 4I\text{-}R'$$

where R = alkyl or aryl group, R′ = benzoate moiety of the iodoso-derivative.

Thiosemicarbazide reacts as follows:

$$NH_2CSNHNH_2 + 5R'\text{-}IO + 6H_2O \rightarrow N_2 + HCN + 5I\text{-}R' + H_2SO_4 + H_2O$$

A portion of sample containing 0.01–0.1 meq of thiourea (dissolved in water or acetic acid or acetonitrile) was pipetted into a 250-ml Erlenmeyer flask. If an organic solvent was used, enough water was added to make the organic solvent concentration 30–80% v/v. A measured and excessive volume of 0.05*M* iodoso-compound solution was added, then 30 min later a known and excessive volume of 0.05 *M* ascorbic acid (which reacts with the iodoso compound in a 1:1 molar ratio) or 0.05*M* mercaptoacetic acid and 3 ml of 1*M* sulphuric acid were added and after 30 sec the residual ascorbic acid or mercaptoacetic acid was titrated with 0.04*N* iodine, with starch as indicator. The ascorbic acid could also be titrated with 2,6-dichlorophenol–indophenol solution. A blank determination was also done.

Procedure for isothiocyanates

Organic isothiocyanates are quantitatively converted into the corresponding thioureas with n-butylamine in dimethylformamide at room temperature:

$$RNCS + C_4H_9NH_2 \rightarrow RNHCSNHC_4H_9$$

An aliquot (2–5 ml) of dimethylformamide solution containing 2–18 mg of organic isothiocyanate was taken in a 100-ml iodine flask and 3 ml of n-butylamine (approximately 0.1*M* solution in dimethylformamide) were added. The solution was diluted to 10 ml with the solvent and mixed. After 10 min to ensure completion of the reaction, about 50% excess of 0.05*M* iodoso compound solution was added, with enough water and acetic acid to make the acid concentration 30–80%. After 30 min the determination was completed in the same way as for thioureas.

Procedure for dithiocarbamates and xanthates

In acetonitrile medium dithiocarbamates and xanthates are quantitatively oxidized according to the equations

$$2RR'CSS^- \rightarrow (RR'CS.S)_2 + 2e$$

$$2ROCSS^- \rightarrow (ROCS.S^-)_2 + 2e$$

A sample containing 0.1–1.0 meq of dithiocarbamate or xanthate was dissolved in 20 ml of acetonitrile. About 35 ml of methanol and 50 mg of potassium iodide were added. The flask was stoppered and the reactants were mixed. The mixture was titrated with 0.05*N* iodoso-compound solution, to the appearance of the iodine colour. Methanol should be 60–80% of the total solvent at the end-point, or the reaction will be too slow.

Procedure for amines

In acetonitrile medium, primary and secondary amines are quantitatively converted by excess of carbon disulphide into the corresponding mono- or dialkyldithiocarbamate

$$2RNH_2 + CS_2 \rightarrow RNHCS.SNH_2R$$

$$2RR''NH + CS_2 \rightarrow RR'NCSSNH_2RR''$$

which can be titrated as just described.

The sample (10–40 mg), weighed directly or in an aliquot of amine solution in acetonitrile, was placed in a glass-stoppered flask containing about 20 ml of acetonitrile and 2–5 ml of carbon disulphide were added to it. The reactants were mixed, cooled to room temperature, and titrated as above.

Procedure for alcohols

About 5 ml of the alcohol were mixed with 3 g of powdered potassium hydroxide in a 50-ml ground-glass-joint flask fitted with a water condenser, and refluxed for 15 min. The liquid was then cooled and the supernatant clear liquid was decanted into a dry flask and chilled. About 4 ml of carbon disulphide were added from a tap-funnel during 5 min with vigorous stirring of the mixture. The heavy yellow precipitate was filtered off on sintered glass and washed with 25-ml portions of anhydrous diethyl ether.

The crude product was dissolved in the minimum of hot acetone. The solution was cooled and anhydrous diethyl ether was added to precipitate the product. The xanthate was filtered off, washed with ether, dried in a vacuum desiccator, then determined as above.

Procedure for disulphides and sulphides

Disulphides are oxidized stoichiometrically to the α-sulphones in dilute acetic acid

$$RSSR + 4OI\text{-}R' \xrightarrow{H_2O} RSO_2RSO_2 + 4I\text{-}R'$$

Organic sulphides are oxidized to sulphoxides

$$RSR + OI\text{-}R' \xrightarrow{H_2O} RSOR + I\text{-}R'.$$

A sample containing 0.1–0.5 mmole of sulphide or 0.02–0.08 mmole of disulphide was weighed into a 250-ml Erlenmeyer flask and dissolved in 15–20 ml of glacial acetic acid. About 50% excess of 0.05*M* iodoso-compound solution was added along with enough distilled water to make the acid concentration 30–80% v/v (the reactions are slow in anhydrous acetic acid and catalysed moderately by water; less water was used if the sample precipitated). The mixture was left for at least 30 min (50 min in the case of methionine and overnight for cystine) then a measured and

Table 1. Determination of thiols with iodoso compounds in acetic acid medium (expressed as % recovery)

Compound	Present methods[a]						Comparison method
	o-DIB	*m*-MPIA	*p*-MPIA	*m*-CPIA	*p*-CPIA	*p*-NPIA	
Ethane thiol	100.1	—	—	99.6	100.2	—	99.8[b]
Glutathione	98.0	98.2	98.3	—	—	98.4	98.1[c]
p-Chlorobenzenethiol	96.2	96.4	—	95.9	—	96.3	96.1[d]
2-Mercaptopropionic acid	96.6	—	96.9	—	96.8	—	96.6[e]
p-Toluene thiol	98.5	98.3	—	98.2	—	98.6	98.4[f]
2-Diethylaminoethanethiol hydrochloride	98.1	98.4	98.1	—	98.0	—	98.2[g]
1-Propane thiol	94.8	94.7	94.6	94.2	—	94.2	94.5[h]
Toluene-α-thiol	98.3	98.5	—	98.5	98.4	—	98.2[i]
Mercaptoacetic acid	80.0	80.2	80.1	—	—	80.1	80.3[j]
Thiobenzoic acid	95.3	—	—	94.8	94.9	—	95.0[b]
2-Mercaptobenzothiozole	98.0	—	98.2	—	—	98.1	97.8[d]
1-Butane thiol	90.3	90.4	—	—	98.7	98.8	99.3[e]
2-Mercaptoethanol	99.5	99.6	99.6	99.1	—	—	99.3[e]
Allyl thiol	93.4	—	—	93.1	93.0	—	93.2[f]
p-Chlorotoluene-α-thiol	98.4	98.9	98.6	—	—	98.6	98.7[g]
2-Butane thiol	97.2	—	—	97.2	97.7	97.6	97.4[h]
Mercaptoethylammonium chloride	97.4	—	97.2	—	—	—	97.5[c]
Methyl-3-mercaptopropionate	—	96.2	—	95.9	—	—	96.0[d]
2-Naphthalene thiol	—	99.6	99.5	—	100.1	100.1	99.8[e]
Benzene thiol	98.7	—	98.8	99.2	99.3	—	90.0[f]
m-Mercaptobenzoic acid	—	93.2	—	93.4	—	93.2	93.0[h]
m-Chlorothiophenol	99.5	—	99.9	99.8	—	99.8	99.7[i]
o-Toluene thiol	—	98.8	99.1	99.2	—	—	99.0[b]
o-Chlorobenzene thiol	98.1	—	98.5	—	98.1	98.0	98.3[b]
Glycerol-1-thiol	79.2	—	79.3	—	—	—	79.0[d]
Cyclohexane thiol	—	95.5	—	95.4	95.4	—	95.7[d]

[a], Mean of 10 determinations; *o*-DIB = *o*-diacetoxyiodobenzoate, *p*- & *m*-MPIA = methylphenyliodosoacetate; *m*- & *p*-CPIA = chlorophenyliodosoacetate; *p*-NPIA = nitrophenyliodosoacetate; [b], iodimetry;[11] [c], iodimetry;[12] [d], ICI method;[13] [e], acetylation;[14] [f], Cu^{2+} titration;[15] [g], Hg^{2+} titration;[16] [h], alkalimetry;[17,18] [i], nitrite titration;[19] [j], Pb^{4+} titration.[20,21]

excessive volume of 0.05*M* ascorbic or mercaptoacetic acid was added along with 3 ml of 1*M* sulphuric acid. The mixture was then titrated with 0.05*N* iodine, with starch as indicator. Excess of ascorbic acid can also be titrated with 2,6-dichlorophenolindophenol. A blank determination was run separately.

RESULTS

Results for the determination of several thiols (Table 1) show that the iodoso-compound methods in non-aqueous media are generally sufficiently accurate, but cysteine and 3-mercaptopropionic, *o*-mercaptobenzoic and mercaptosuccinic acid are oxidized beyond the disulphide stage in an unaccountable fashion and hence the method is not recommended for them. 1-Butane thiol gave incomplete reaction with *o*-DIB and *m*-MPIA. Results for thioureas (Table 2) showed excellent recovery, especially when dilute sol-

Table 2. Determination of thioureas with iodoso compounds (% recovery)

Compound	Present methods[a]						Comparison method
	o-DIB	*m*-MPIA	*p*-MPIA	*m*-CPIA	*p*-CPIA	*p*-NPIA	
Thiourea	99.7	—	100.1	—	99.6	99.8	99.9[b]
Thiosemicarbazide	99.3	99.8	—	99.9	—	—	99.6[c]
Phenyl thiourea	99.7	99.8	99.8	—	—	100.4	110
Phenyl thiourea	99.7	99.8	99.8	—	—	100.4	110.1[d]
o-Tolyl thiourea	96.8	—	97.5	—	97.4	—	97.2[e]
p-Tolyl thiourea	—	98.2	—	98.8	98.3	—	98.5[f]
Allyl thiourea	96.6	96.4	—	96.5	—	96.5	96.1[g]
p-Bromophenyl thiourea	—	95.6	95.7	—	95.6	—	96.0[h]
m-Chlorophenyl thiourea	98.2	—	97.7	—	97.7	—	97.9[i]
p-Anisyl thiourea	99.9	99.9	—	100.6	—	99.9	100.4[f]
m-Anisyl thiourea	—	—	97.0	97.0	—	97.8	97.4[c]
n-Butyl thiourea	98.7	—	98.7	—	99.4	—	99.0[b]
n-Amyl thiourea	99.6	99.1	—	—	99.0	—	99.2[g]
o-Ethoxyphenyl thiourea	—	—	98.0	98.0	—	98.6	98.2[e]
Methyl thiourea	98.2	—	98.0	—	98.4	—	98.1[d]

[a], Average of 10 determinations; [b], hypoiodite;[22] [c], nitrite titration;[23] [d], bromimetry;[24] [e], chloramine-T;[25] [f], iodine monochloride;[26] [g], iodine trichloride;[26] [h], cerimetry;[27] [i], non-aqueous cerimetry.[28]

Table 3. Determination of xanthates and dithiocarbamates in non-aqueous media

Sample	Recovery %				Comparison method
	o-DIB	*m*-MPIA	*p*-CPIA	*p*-NPIA	
Ethyl xanthate	90.2	—	90.4	—	90.0[a]
1-Propyl xanthate	—	97.8	—	98.0	98.1[a]
1-Butyl xanthate	94.8	—	94.4	—	94.6[a]
Isopropyl xanthate	78.1	—	78.0	78.0	78.2[a]
Allyl xanthate	—	94.4	—	94.8	94.5[a]
Isoamyl xanthate	95.5	—	95.2	—	95.0[a]
1-Octyl xanthate	—	97.0	—	97.1	97.3[a]
Diethyldithiocarbamate	75.9	—	76.0	—	77.3[b]
Di-n-butyldithiocarbamate	91.5	91.3	—	91.0	91.4[b]
Diamyldithiocarbamate	—	91.8	92.2	92.0	92.1[b]
Di-isopropyldithiocarbamate	87.2	—	87.5	—	87.0[b]
Phenylethyldithiocarbamate	—	80.2	—	80.4	80.1

* Average of 10 determinations

[a], Ag^+ titration;[29] [b], iodimetry;[30] [c], total sulphur analysis gives higher results owing to the presence of thiourea disulphides.

utions were used, but diphenylthiourea does not react stoichiometrically. Table 3 gives the results for xanthates and dithiocarbamates in non-aqueous media, and Table 4 those for disulphides. Table 5 gives results for mercaptans and derivatives of dithiocarbonic acid in the presence of foreign substances, including compounds that interfere in other methods and compounds that contain sulphur functions. In the determinations of thioureas, large amounts of glycine, alanine, formic acid, dextrose and urea do not inter-

Table 4. Determination of disulphides with iodoso compounds

Sample	Recovery, %*						
	o-DIB	*m*-MPIA	*p*-MPIA	*m*-CPIA	*p*-CPIA	*p*-NPIA	Bromimetry[31]
Cystine	99.0	—	99.5	—	99.6	99.4	99.3
Di-n-butyldisulphide	97.3	97.4	—	96.4	—	96.4	96.9
Di-n-benzyldisulphide	99.7	99.7	—	100.6	—	99.8	100.2
Di-n-pentyldisulphide	98.4	—	97.8	—	97.8	—	98.1
Di-n-propyldisulphide	94.7	94.8	—	94.3	—	94.3	94.6
Diphenyldisulphide	95.4	95.3	95.9	—	95.9	—	95.7

* Average of 10 determinations.

Table 5. Determination of thiols and xanthates in presence of foreign substances

Sample	Oxidant	Substance added	Molar ratio added substance: sample	Recovery, %*
Mercaptopropionic acid	*m*-DIB	Potassium cyanide	83:1	100.7
	m-MPIA	Diethylsulphoxide	314:1	100.0
	p-NPIA	Diethylsulphide	14:1	100.0
Potassium n-butylxanthate	*o*-DIB	Acrylonitrile	60:1	99.7
	p-MPIA	Diacetone alcohol	7:1	100.2
	p-CPIA	Dimethylsulphoxide	38:1	99.0
2-Mercaptoethanol	*p*-MPIA	Acetone	9:1	99.9
	m-MPIA	Thiophene	10:1	100.5
	m-CPIA	Dibenzyldisulphide	70:1	100.0
	p-CPIA	Alanine	69:1	100.1
	p-NPIA	Formic acid	90:1	100.5
Potassium allylxanthate	*o*-DIB	Carbon disulphide	58:1	101.2
	m-MPIA	Cystine	27:1	100.1
	p-NPIA	Glycine	34:1	100.0
	m-CPIA	Alanine	44:1	100.0
	p-CPIA	Thiophene	19:1	101.8
2-Mercaptopropinoic acid	*o*-DIB	Dextrose	170:1	100.2
	p-NPIA	Carbon disulphide	105:1	100.2
	m-CPIA	Methylacrylate	10:1	101.0
	p-MPIA	Acrylonitrile	14:1	99.7

* Average of 4 determinations; takes into account previously determined purity of the sample.

Table 6. Determination of isothiocyanates, amines, alcohols and sulphides with iodoso compounds

Sample	Oxidant	Range of sample, *mg*	Recovery, %
Isothiocyanates			
Methyl	*p*-MPIA	2.01–5.99	100.2
Ethyl	*m*-CPIA	8.00–12.00	100.1
n-Propyl	*p*-NPIA	15.01–39.92	100.0
Isopropyl	*p*-CPIA	4.01–6.02	100.4
n-Butyl	*m*-MPIA	8.02–19.94	100.1
Isobutyl	*m*-MPIA	24.94–34.92	99.9
Phenyl	*p*-CPIA	14.97–39.92	99.8
Amines			
n-Propyl	*m*-MPIA	10.04–40.19	100.2
Isobutyl	*p*-DIBA	9.97–32.94	100.2
n-Butyl	*p*-NPIA	9.98–40.15	100.4
Monoethanol	*m*-CPIA	10.03–40.12	100.4
Diethyl	*p*-MPIA	10.05–39.87	100.4
Diethanol	*p*-NPIA	9.94–40.08	100.3
Di-n-butyl	*o*-DIP	10.02–40.11	100.5
Piperidine	*p*-CPIA	9.98–99.84	100.5
Alcohols			
Ethyl	*m*-MPIA	2.62–9.09	99.8
Octyl	*p*-CPIA	5.35–13.82	99.6
Allyl	*p*-NPIA	2.33–7.21	99.5
Isoamyl	*m*-CPIA	4.39–10.45	99.7
n-Butyl	*p*-MPIA	3.02–8.64	99.6
Sulphides			
Dibenzyl	*m*-MPIA	2.10–15.27	103.9
Methyl-n-propyl	*p*-CPIA	8.02–20.38	101.9
Methionine	*p*-NPIA	5.99–30.06	99.4
Di-n-butyl	*m*-CPIA	5.99–24.95	99.9

fere but organic sulphides and disulphides do. In the determination of sulphides and disulphides, alanine, glycine, dextrose, and formic, cinnamic and formic acid can be tolerated.

Results for determination of alcohols by xanthate formation, isothiocyanate by thiourea formation amines by dithiocarbamate formation and of sulphides are summarized in Table 6. As a check, sulphides and disulphides were also determined by total sulphur analysis and by the method of Siggia and Edsberg.[31]

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THE PROTONATED 4,4′-DIAMINOTRIPHENYLMETHYL CATION AS A REAGENT AND CYCLOHEXANONE AS AN ABSORBANT FOR SULPHUR DIOXIDE DETERMINATION

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Summary—Protonated 4,4′-diaminotriphenylmethyl cation, DATM, has been investigated as a colorimetric reagent for the determination of sulphur dioxide. The bisulphite addition compound with formaldehyde alkylates a primary arylamine group in the protonated reagent to re-establish resonance, and hence produces a colour that is proportional in intensity to the concentration of either bisulphite or formaldehyde (the other being in excess). The large bathochromic spectral shift results in a negligible blank correction. Cyclohexanone in aqueous solution has been found to stabilize bisulphite solutions for up to 6 hr. The stabilization by cyclohexanone has been compared with stabilization by tetrachloromercurate(II). The Beer-Lambert law is obeyed with good precision for both sulphite and formaldehyde determination.

The reaction product of bisulphite with formaldehyde in acid aqueous solution combines readily with protonated primary arylamine groups to form aminomethylsulphonates. The reaction regenerates the absorption in the visible region, which had been suppressed by protonation of the primary amine groups. Protonated pararosaniline is a versatile reagent for the determination of either sulphur dioxide[1–4] or formaldehyde[5]. Reactions which produce sulphite or formaldehyde quantitatively form the basis for analytical methods for sulphate aerosols (reduction to sulphite)[6] and ozone (formaldehyde formed from the ozonolysis product of the alkene eugenol).[7] A fundamental study of the primary arylamine–bisulphite–formaldehyde reaction was made by Nauman *et al.*[8] who studied similarities in the reactions of pararosaniline and aniline with bisulphite and formaldehyde. A significant contribution to the methods for sulphur dioxide was made by West and Gaeke,[9] who stabilized sulphur dioxide in sample solutions with tetrachloromercurate(II).

Two reagents of simpler structure than pararosaniline have been proposed for the determination of sulphur dioxide: *p*-aminoazobenzene[10] and *p*-nitroaniline.[11] In common with pararosaniline, these two reagents produce appreciable blanks. 4-Nitro-1,2-diaminobenzene[12] was proposed as a reagent with negligible blank correction, but gives lower sensitivity than pararosaniline.

The protonated 4,4′-diaminotriphenylmethyl cation, [DATM, benzylidenebis(4-aminophenyl) cation] has been synthesized and investigated as a reagent for sulphur dioxide. The possible stabilizing effect of cyclohexanone on aqueous solutions of sulphur dioxide (bisulphite) has also been investigated. As observed for the analogous triphenylmethane dyes, Malachite Green (which has *N*,*N*-dimethylamino groups in the *para*-position of two of the three phenyl rings), and Crystal Violet (which has *N*,*N*-dimethylamino groups on all three of the phenyl rings), two electron-donating amino-nitrogen atoms participate in a more linear resonating structure than three such nitrogen atoms do and hence produce a greater bathochromic shift from the faint yellow colour of the protonated reagents. The farther the shift toward the red, the less the absorption peaks in the ultraviolet region interfere to produce a blank correction at the absorbance maximum.

EXPERIMENTAL

Demineralized water was used for preparing all reagent solutions and for making dilutions. Chemicals used were reagent grade or the purest grade available.

Reagent[13]

Bis(4-amino)triphenylmethane, the leuco-base of the reagent, was prepared by heating a mixture of 27.9 g (0.3 mole) of aniline, 10.6 g (0.1 mole) of benzaldehyde, and 24 g (0.2 mole) of concentrated hydrochloric acid for 20 hr with vigorous stirring under reflux. The end of the condenser was closed with a plug of glass wool to prevent excessive oxidation of the aldehyde during the reaction period. The resulting mixture was treated with 12 g of sodium carbonate and the water removed with a rotary evaporator to leave a dark blue, tarry solid. The product was washed several times by decantation with water to remove excess of sodium carbonate.

The leuco-base so prepared (about 0.05 mole) was dissolved in 300 ml of water containing 20 ml of concentrated hydrochloric acid. Ice was added to bring the volume to 400 ml and the temperature to 0°. To the well-stirred solution was added, in one portion, 12.0 g (0.05 mole) of lead dioxide, and the stirring continued for 4 hr. An aqueous

solution containing 25 g of sodium sulphate was added to precipitate the reduced lead(II) as lead sulphate. Any unreacted lead dioxide and the precipitated lead sulphate were removed by filtration. On addition of 15 g of sodium carbonate to the filtrate, the carbinol form of the dye was precipitated and, after collection, was washed several times with water and dried. NMR (D_2O/d_6-DMSO) gave 4.75 (s, NH_2, 4H), 7.1–7.4 (m, aromatic, 13H). CMR (d_6-DMSO) gave 156.29, 146.43, 138.93, 137.26, 129.80, 128.96, 128.33, 126.15, 118.26.

The carbinol form of the dye was purified by dissolving it in chloroform and passing the solution through a column of basic alumina. A brown substance, thought to be an oxidation product, was removed from the column by washing with chloroform. The purified carbinol was eluted with 95% ethanol, dried, and converted into the protonated 4,4′-diaminotriphenylmethyl cation by treatment with hydrochloric acid. The λ_{max} of the cation is 560 nm and the molar absorptivity, ϵ, is 3.0×10^4 l.mole^{-1}.cm^{-1}.

Solutions

Reagent solution. Dissolve 0.5 g of DATM in 20.0 ml of concentrated hydrochloric acid and sufficient water to make 100.0 ml of solution.

Formaldehyde solution. Mix 2.0 ml of 37% aqueous formaldehyde solution and sufficient 0.005*M* hydrochloric acid to make 100.0 ml of solution.

Hydrochloric acid, 0.005M. Dilute 0.41 ml of concentrated hydrochloric acid to 1 litre.

Standard bisulphite solution for HSO_3^- calibration curves. Dissolve 0.128 g of sodium bisulphite, $NaHSO_3$, in water and dilute to exactly 1000 ml. This solution contains 104 ppm bisulphite ion, HSO_3^-, and must be prepared fresh as needed.

Cyclohexanone solution. Dissolve 25 ml of reagent grade cyclohexanone in water and dilute to 1000 ml.

Tetrachloromercurate(II) solution. Dissolve 27.2 g of mercury(II) chloride, (0.1 mole) in 20.0 ml of concentrated hydrochloric acid (0.24 mole) and water, and dilute to 500.0 ml.

Bisulphite solution for formaldehyde determination. Dissolve 0.50 g of sodium bisulphite, $NaHSO_3$, in water and dilute to 100.0 ml to make a 0.048*M* solution.

Calibration graphs

Three calibration graphs were prepared: for formaldehyde with excess of bisulphite in aqueous cyclohexanone solution; for formaldehyde with excess of bisulphite in aqueous tetrachloromercurate(II) solution; formaldehyde in aqueous cyclohexanone with excess of bisulphite present.

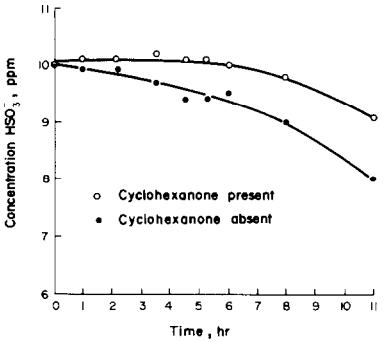

Fig. 2. Effect of cyclohexanone on the stability of 10-ppm bisulphite solutions.

The data shown in Fig. 1 represent two series of solutions. For each concentration on the line labelled HSO_3^-–cyclohexanone, 2.0 ml of cyclohexanone solution and the appropriate volume of freshly prepared standard bisulphite solution were diluted to 100.0 ml. For each determination, 10.0 ml of the bisulphite–cyclohexanone solution so prepared plus 1.0 ml each of the formaldehyde solution and the DATM reagent solution were mixed, heated at 50° for 30 min, and cooled, and then the absorbance was measured. Five determinations were made at each concentration.

For each concentration on the line labelled HSO_3^-–$[HgCl_4]^{2-}$ in Fig. 1, the appropriate volume of freshly prepared standard bisulphite solution was added to 50.0 ml of tetrachloromercurate(II) solution and sufficient water was added to bring the volume to 100.0 ml. For each determination, 10.0 ml of this bisulphite–tetrachloromercurate(II) solution plus 1.0 ml each of the formaldehyde solution and the DATM reagent solution were mixed, heated at 50° for 30 min, and cooled, and then the absorbance was measured. Five determinations were made at each concentration.

Figure 2 shows the stabilizing effect of cyclohexanone on 10-ppm bisulphite solution compared to 10-ppm bisulphite solution without cyclohexanone, for periods up to 11 hr before addition of formaldehyde and DATM reagent solutions.

The data shown in Fig. 3 show the response of the DATM reagent to formaldehyde in the presence of excess of bisulphite. The appropriate volume of formaldehyde solution at each concentration was diluted to 100.0 ml with 0.005*M* hydrochloric acid. For each determination, 10.0 ml of the properly diluted formaldehyde solution plus 1.0 ml

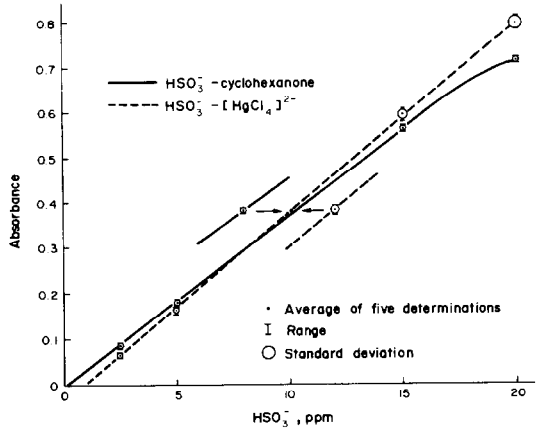

Fig. 1. Calibration plots for bisulphite–cyclohexanone solutions and bisulphite–tetrachloromercurate(II) solutions.

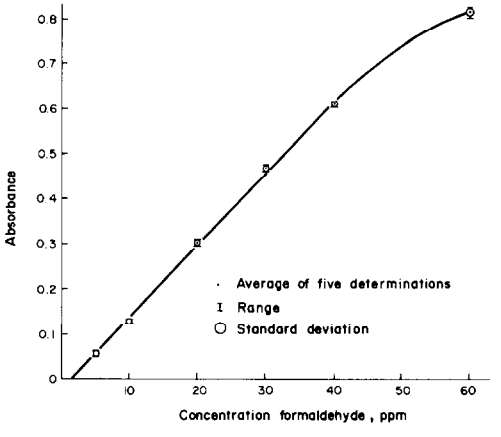

Fig. 3. Calibration curve for formaldehyde solutions in the presence of excess of bisulphite.

Table 1. Comparison of absorption maxima and molar absorptivities of reagents for sulphur dioxide*

Reagent or product	λ_{max}, nm	ϵ, $l.mole^{-1}.cm^{-1}$	Reference
Pararosaniline	544	1.4×10^4	9
Protonated pararosaniline	—	—	
Pararosaniline *N*-methylsulphate	560	3.0×10^4	14
p-Aminoazobenzene	386	—	15
Protonated *p*-aminoazobenzene	—	—	
p-Aminoazobenzene *N*-methylsulphonate	505	—	10
p-Nitroaniline	380	1.4×10^4	11
Protonated *p*-nitroaniline	267	8.3×10^3	11
p-Nitroaniline *N*-methylsulphonate	387	1.9×10^4	11
4-Nitro-1,2-diaminobenzene	406	5.6×10^3	12
Protonated 4-nitro-1,2-diaminobenzene	367	6.1×10^3	12
4-Nitro-1,2-diaminobenzene *N*-methylsulphonate	475	4.5×10^3	12
DATM	560	3.0×10^4	13
Protonated DATM	405†	—	13
DATM *N*-methylsulphonate	585	3.5×10^4	13

* Quoted from, or derived from, data presented in the references listed.
† λ_{max} of a barely distinguishable peak of low absorbance.

each of the 0.048*M* bisulphite solution and 1.0 ml of DATM reagent solution were mixed, heated at 50° for 30 min, and cooled, and then the absorbance was measured. Five determinations were made at each concentration.

RESULTS AND DISCUSSION

The violet-blue dye cation was decolorized in 0.2*M* hydrochloric acid solution, with formation of the diprotonated species. Only a small barely distinguishable peak was observed, at 405 nm. The blank correction was negligible. No reaction appeared to occur with formaldehyde alone, but with addition of bisulphite an aminomethylsulphonate group was formed and a blue-violet chromogen, $\lambda_{max} = 585$ nm, was reconstituted. According to Pate *et al.*[14] in their study of the corresponding pararosaniline reagent, in the presence of excess of formaldehyde only one bisulphite ion is required to re-establish resonance and colour in one dye cation. The reconstituted chromogen absorbs at a longer wavelength than the non-alkylated dye: 560 nm for the unprotonated dye cation and 585 nm for the aminomethylsulphonate derivative. For pararosaniline, the corresponding wavelengths are 544 nm and 560 nm, respectively. The bathochromic shift is thus greater by 9 nm for the more nearly linear harmonic oscillator DATM chromogen described here, than the shift observed with pararosaniline. The greater bathochromic shift is partially responsible for the lower blank, which is the principle advantage of the DATM reagent. The absorption maxima, along with the molar absorptivities that were available or calculable from the articles which described the previously proposed reagents, are included in Table 1 for comparison purposes. The molar absorptivities correspond approximately to the relative sensitivities of the reagents, although relative reactivities must also be taken into account.

Cyclohexanone stabilizes aqueous solutions of bisulphite for periods up to 6 hr (Fig. 2), after which slight progressive decomposition occurs. Without cyclohexanone present, decomposition (probably aerial oxidation to sulphate) starts to occur in a very short time. Cyclohexanone does not stabilize bisulphite nearly as efficiently as does tetrachloromercurate(II), which is effective for periods up to 2 weeks or more, but for collection periods up to 6 hr cyclohexanone solution is as efficient as tetrachloromercurate(II) as an absorbant for sulphur dioxide and far less of a toxic hazard than the mercury solution. The stabilizing reaction of bisulphite with cyclohexanone produces a substituted hydroxymethylsulphonate addition compound. Steric considerations, as observed with space-filling molecular models, suggest that the addition product will be less stable if the alkyl groups of the ketone are bulky. Cyclopentanone and cyclohexanone, of all the readily available water-soluble ketones, offer the minimum steric hindrance to formation of the hydroxymethylsulphonate addition product.

The calibration plot obtained with cyclohexanone as the stabilizing agent (Fig. 1) conformed closely to the Beer-Lambert law and the standard deviations for the individual concentration determinations were small. The calibration data obtained with tetrachloromercurate(II) as the stabilizing agent (also Fig. 1) produced a straight line that had a small intercept on the concentration axis and larger standard deviations for the individual concentration determinations.

The alkylation reaction can also be applied with good precision to the determination of formaldehyde, or substances which produce formaldehyde, as shown in Fig. 3.

Interferences (especially nitrogen oxides) have been thoroughly studied for the bisulphite–formaldehyde alkylation of primary amine reagents of the type investigated here. Nitrogen dioxide interference has been effectively eliminated by the use of sulphamic acid,[16] a strong acid which serves the same purpose as hydrochloric acid and which destroys the nitrous

acid formed along with nitric acid by the hydrolysis of the nitrogen dioxide.

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DETERMINATION OF ASCORBIC ACID WITH *o*-IODOSOBENZOATE

ANALYSIS OF MIXTURES OF ASCORBIC ACID WITH METHIONINE AND CYSTEINE OR GLUTATHIONE

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Summary—Ascorbic acid has been determined in pure solutions, pharmaceutical preparations, foodstuffs and biological fluids by titration with *o*-iodosobenzoate, with visual or photometric detection of the end-point, with leuco-2,6-dichlorophenolindophenol plus potassium iodide as indicator. Cysteine and glutathione, which interfere quantitatively, are masked by cyanoethylation; the cyanoethylated product and methionine have been determined with *o*-iodosobenzoate in the presence of acidified potassium bromide, with Methyl Red as indicator. Procedures are given for the analysis of mixtures of ascorbic acid with sulphur-containing amino-acids.

Oxidation of ascorbic acid with 2,6-dichlorophenolindophenol is extensively used for its determination,[1,2] but the value of this method is limited because the dye reacts with other reductants, *e.g.*, cysteine and glutathione, which are often present in biological fluids,[3] or with iron(II), which is sometimes found in foodstuffs.[4,5] Titration with *N*-bromosuccinimide has been claimed to be free from the shortcomings of the indophenol method,[6,7] but only the freshly prepared reagent should be used. Cysteine and glutathione also react with *N*-bromosuccinimide and vitiate the analysis.[8,9] Standard copper(II),[10] thallium(III),[11] hexacyanoferrate(III)[12] and iodine or iodate solutions[13,14] are more stable than *N*-bromosuccinimide but otherwise there seems to be no implicit advantage in using these reagents.

A new method for determination of ascorbic acid in pure solutions, pharmaceutical preparations, biological fluids and edible fruits is described here based on oxidation of ascorbic acid with *o*-iodosobenzoate which has already been studied kinetically.[15]

Ascorbic acid is titrated with standardized *o*-iodosobenzoate at neutral pH, with leuco-2,6-dichlorophenolindophenol plus potassium iodide as indicator; the end-point, which is shown by the appearance of the blue colour of the oxidized form of the indicator, is sharp, reproducible and stable. The reaction of *o*-iodosobenzoate with ascorbic acid at pH 7 is fast but that with iodide to yield iodine is slow, and it is the iodine produced that oxidizes the indicator. Therefore it is necessary to titrate the sample solution slowly in order to locate the correct end-point. However, a rapid titration can be used for orientation, followed by another in which all but about 1 ml of the titrant volume used in the first titration is added quickly and the titration is completed slowly. Starch–iodide mixture can also be used as indicator, but with dilute titrant solutions (0.001*M* or less) the end-point colour is harder to detect than that from the indophenol. For solutions containing only 10–40 μg of ascorbic acid the end-point should be detected photometrically. Iron(II) gives positive errors if the leuco-indophenol is used as indicator, presumably because it reduces the quinone dye, but starch–iodide indicator gives a stable and correct end-point. With dilute titrant solutions an indicator blank correction is necessary.

The redox potential of *o*-iodosobenzoate at 25° was found to be 1.21 V at pH 1, 1.08 V at pH 2, 0.53 V at pH 4 and 0.48 V at pH 7.

Mixtures of ascorbic acid with cysteine or glutathione have been analysed by first titrating the sum, according to the thiol compounds reacting

$$2RSH + OIC_6H_4CO_2^- \rightarrow RSSR + IC_6H_4CO_2^- + H_2O,$$

then determining the ascorbic acid alone in a second aliquot after reaction with acrylonitrile in phosphate buffer (pH 7), the thiol compounds undergoing quantitative cyanoethylation to yield thio-ethers which are inert to *o*-iodosobenzoate:

$$RSH + CH_2{=}CHCN \rightarrow RSCH_2CH_2CN$$

Cysteine or glutathione is obtained by difference. Bisulphite can be masked similarly:

$$HSO_3^- + CH_2{=}CHCN \rightarrow {}^-O_3SCH_2CH_2CN$$

In mixtures of ascorbic acid with methionine and cysteine or glutathione, the ascorbic acid and thiol contents are determined as before; methionine does not interfere. Then a third aliquot is cyanoethylated and titrated with *o*-iodosobenzoate after addition of acidified potassium bromide and Methyl Red. The

bromine formed in the titration oxidizes ascorbic acid to dehydroascorbic acid, whereas methionine and the cyanoethylated cysteine or glutathione are oxidized to their sulphoxides:

$$2HBr + OIC_6H_4CO_2^- \rightarrow Br_2 + H_2O + IC_6H_4CO_2^-$$

$$CH_3SCH_2CH_2CH(NH_2)CO_2H + Br_2 + H_2O \rightarrow CH_3SOCH_2CH_2CH(NH_2)CO_2H + 2HBr$$

$$RSCH_2CHCN + Br_2 + H_2O \rightarrow RSOCH_2CH_2CN + 2HBr$$

The methionine content is obtained by subtracting from the *o*-iodosobenzoate consumption for the third aliquot, the sum of the consumption for ascorbic acid and twice that for the cysteine or glutathione.

EXPERIMENTAL

Reagents

*o-Iodosobenzoate solution, 0.005*M. Prepared by dissolving 1.32 g of *o*-iodosobenzoic acid in 55 ml of 0.01*M* potassium hydroxide and diluting to 1 litre with water, standardized iodometrically[16] and diluted as required.

Phosphate buffer, pH 7. Prepared by dissolving 117.7 g of K_2HPO_4 and 44.1 g of KH_2PO_4 in 1 litre of water.

Leuco-2,6-dichlorophenolindophenol indicator solution. To 20 ml of 0.05% solution of the sodium salt of the indicator add about 5 ml of phosphate buffer and bleach the blue colour by dropwise addition of 0.05% ascorbic acid solution. The reduced indicator oxidizes when exposed to the air and it is necessary to decolorize the solution with ascorbic acid before use.

Methyl Red solution. A 0.04% solution in 95% ethanol.

Procedures

Determination of ascorbic acid. A solution containing 0.2–15.0 mg of ascorbic acid is mixed with 5 ml of phosphate buffer, 5–10 ml of water, 1 ml of indophenol indicator and about 50 mg of potassium iodide, and titrated with 0.001*M* *o*-iodosobenzoate when the sample solution contains up to 3 mg of ascorbic acid and with 0.005*M* titrant for larger amounts. The titration must be done slowly (5 ml of titrant per min) with swirling for 10 sec between addition of drops near the end-point (shown by the vivid blue colour of the oxidized indicator).

When the sample contains iron(II), 5 ml of 3% acetic acid, solution 5–10 ml of water, about 50 mg of potassium iodide and 1 ml of 0.5% starch are added, and the solution is titrated till the appearance of a blue colour.

Photometric determination of microgram amounts of ascorbic acid. Sample solution (10 ml or less) containing 9–45 μg of ascorbic acid is taken in a 200-ml titration cell and treated with 20 ml of phosphate buffer, 1 ml of indophenol indicator and 50 mg of potassium iodide, and made up to 100 ml with water and slowly titrated spectrophometrically at 600 nm with $5 \times 10^{-5}M$ *o*-iodosobenzoate. An indicator blank is also determined.

Determination of ascorbic acid in the presence of cysteine or glutathione. A known volume of sample is treated with 5 ml of phosphate buffer, 5–10 ml of water, 1 ml of indophenol indicator and 50 mg of potassium iodide, and titrated with *o*-iodosobenzoate, the concentration of titrant depending on the total titrand concentration.

To determine ascorbic acid alone, a known volume of sample is mixed with 5 ml of phosphate buffer, 5–10 ml of water and 0.5 ml of acrylonitrile. The flask is stoppered, shaken for 1 min and left for 15 min. Then 50 mg of potassium iodide and 1 ml of indophenol indicator are added and the ascorbic acid is titrated with *o*-iodosobenzoate.

Analysis of mixtures of ascorbic acid, methionine and cysteine or glutathione. First ascorbic acid plus cysteine or glutathione is determined as before, then the ascorbic acid alone is titrated in a second aliquot after cyanoethylation of the sulphydryl compound. To determine the total of the three components, a third aliquot of mixture is cyanoethyl-

Table 1. Determination of ascorbic acid

Ascorbic acid taken, *mg*	Indicator	Ascorbic acid found,* *mg*			
		Present method	Relative std. devn., %	In titn.	NBS titn.
0.172	InH	0.170	0.30	0.172	0.175
0.229†	St	0.233	0.20	0.229	0.232
0.327	InH	0.328	—	0.328	0.331
0.530§	St	0.529	0.32	0.532	0.530
0.612	inH	0.610	0.22	0.611	0.615
0.821‡	St	0.825	—	0.820	0.824
1.00	St	1.02	0.10	0.999	1.02
2.11	InH	2.13	—	—	2.15
3.01‡	St	3.00	0.13	—	3.02
4.90	St	4.88	—	—	4.93
5.24	InH	5.25	0.12	—	5.27
8.55	InH	8.54	—	—	8.56
9.75§	St	9.77	0.10	—	9.78
13.65	InH	13.63	0.11	—	13.66
15.32	St	15.34	0.10	—	15.31

* Average of six determinations.
† Plus 0.39 mg of iron(II).
§ Plus 0.82 mg of iron(II).
‡ Plus 0.98 mg of iron(II).
Abbreviations: In = 2,6-dichlorophenolindophenol;[19] NBS = *N*-bromosuccinimide;[6] InH = leuco-2,6-dichlorophenolindophenol; St = starch.

Table 2. Photometric titration of ascorbic acid

Ascorbic acid taken, μg	Ascorbic acid found, μg	Coefficient of variation, %
9.1	9.2	2.0
20.3	20.7	1.9
26.9	26.4	1.8
33.6	30.0	1.6
39.0	38.3	1.8
44.8	44.0	1.7

ated, then treated with 5 ml of 0.1*M* sulphuric acid, 50 mg of potassium bromide and 2 or 3 drops of Methyl Red indicator, and titrated with 0.005*M* *o*-iodosobenzoate, until the Methyl Red is bleached. An indicator blank correction must be applied.

Applications of the method

Determination of ascorbic acid in pharmaceutical preparations. A finely ground tablet or a whole capsule is stirred with 30 ml of water for about 15 min. The residual solid is filtered off on a Whatman No. 42 paper and washed with water. The filtrate is made up to such a volume that 5 ml contains 1–10 mg of ascorbic acid. Injections are diluted directly. A suitable volume is titrated with 0.005*M* *o*-iodosobenzoate as described.

Determination of ascorbic acid in foodstuffs. Fruit juice is mixed with 5% metaphosphoric acid to precipitate proteins. The acidified juice is filtered, diluted so that 5 ml of solution contains 1–10 mg of ascorbic acid, and titrated with 0.005*M* *o*-iodosobenzoate, with starch–iodide as indicator. If bisulphite has been added as a preservative, mix 10 ml of fruit juice or squash with 5 ml of pH-7 phosphate buffer and 1 ml of acrylonitrite. After 5 min add 5 ml of 3% acetic acid, 20 ml of water, 50 mg of potassium iodide and 1 ml of starch solution, and titrate.

Determination of ascorbic acid in blood and plasma. Five ml of whole blood or plasma are added dropwise with shaking to 5 ml of 2% trichloroacetic acid solution, in a 25-ml centrifuge tube, stirred with a thin glass rod, allowed to stand for 5 min, and then centrifuged. The supernatant solution is separated and 5 ml of it are mixed with 3 ml of phosphate buffer, 0.2 ml of indophenol indicator and 50 mg of potassium iodide in a 25-ml Erlenmeyer flask, and titrated with 0.0001*M* *o*-iodosobenzoate to a distinct blue colour.

Determination of ascorbic acid in urine. Fifty ml of urine (preferably freshly passed) are mixed well with 5 ml of 2% metaphosphoric acid solution, and 10 ml of this acidulated sample are added to 5 ml of phosphate buffer, 0.5 ml of indophenol indicator and 50 mg of potassium iodide. The ascorbic acid is titrated with 0.001*M* *o*-iodosobenzoate to the appearance of a blue colour.

RESULTS AND DISCUSSION

The titration of ascorbic acid with *o*-iodosobenzoate is accurate in phosphate buffer of pH 6–7.5 (Table 1). The oxidation of iodide by *o*-iodosobenzoate at pH 5–6 is faster than at pH 7, but the oxidized indophenol indicator will be in its pink acid form and will give a less distinct end-point.

The following compounds in up to tenfold molar ratio to ascorbic acid do not interfere, whichever indicator is used: glucose, fructose, lactose, maltose, sucrose, starch, thiamine hydrochloride, riboflavin, citric, oxalic, formic, tartaric and maleic acids, glycine, alanine, tyrosine, tryptophan, methionine, cysteine, biotin, serine, proline, oxidized glutathione and urea.

Substances which interfere even when present in only small amounts include thiourea, thiosulphate and sulphide. Thiols also react with *o*-iodosobenzoate but can be eliminated by cyanoethylation as can bisulphite. Sodium hydroxide, sodium methoxide or benzyltrimethylammonium hydroxide, which are ex-

Table 3. Analysis of mixtures of ascorbic acid with methionine, cysteine or glutathione (means of 6 determinations)

Ascorbic acid, *mg*		Methionine, *mg*		Cysteine, *mg*		Glutathione, *mg*	
Taken*	Found	Taken†	Found	Taken§	Found	Taken‡	Found
0.82	0.80	0.78	0.81	1.03	1.04	—	—
1.30	1.29	1.29	1.30	—	—	3.30	3.32
2.92	2.91	2.50	2.49	3.49	3.53	—	—
3.84	3.86	3.62	3.60	—	—	4.98	4.96
4.31	4.33	3.47	3.50	3.51	3.55	—	—
3.26	3.28	2.09	2.07	—	—	5.63	5.60
2.70	2.73	1.38	1.42	1.73	1.70	—	—
1.03	1.02	0.75	0.74	—	—	7.59	7.63
0.88	0.85	—	—	5.21	5.24	—	—
1.37	1.39	—	—	3.94	3.96	—	—
2.59	2.62	—	—	2.73	2.70	—	—
4.21	4.22	—	—	1.08	1.07	—	—
5.82	5.80	—	—	—	—	3.18	3.20
3.72	3.75	—	—	—	—	4.73	4.71
2.60	2.58	—	—	—	—	5.92	5.95
0.92	0.93	—	—	—	—	7.13	7.10

* Standardized with 2,6-dichlorophenolindophenol.[19]
† Standardized with chloramine-T.[20]
§ Standardized by *o*-hydroxymercuribenzoate.[21]
‡ Standardized by chloramine-T.[22]

Table 4. Determination of ascorbic acid in fruits

Fruit	Ascorbic acid found, *mg*/100 *g**	
	Present method	NBS
Orange	81.5	82.0
Lemon (yellow)	176.1	178.2
Amla (green)	609	612
Amla (yellow-green)	593	591
Tomato (red)	23.2	22.9

* Average of four determinations.

Table 5. Determination of ascorbic acid in pharmaceutical preparations

Sample	Vitamin C, *mg**		
	Manufacturer's specification	Present method	NBS titration
Tablets			
Sorvicin (East India)	500	483	485
Chewcee (Lederle)	500	505	506
Ascorbic acid (Cadila)	500	498	497
Suckcee (IDPL)	500	492	490
Celin (Glaxo)	500	502	500
Citravite (Pharmed)	500	496	492
Cecon (Abott)	500	509	507
Capsules			
Cebexin (IDPL)	500	505	503
Becosules (Pfizer)	300	298	302
Becadexamin (Glaxo)	30	29	31
Injections			
Redoxon (Roche)	500	504	506
Calcium-Sandoz (Sandoz)	500	501	498

* Average of three determinations.

The excipients in the capsules are nicotinamide, thiamine hydrochloride, ribovflavine, calcium pantothenate, pyridoxine hydrochloride, folic acid, cyanocobalamin, ferrous fumarate, *etc.* Those in the injections are methyl- and propylparaben and calcium gluconogalactogluconate.

tensively used as catalysts in cyanoethylation,[17,18] have been found to destroy ascorbic acid and should, therefore, not be used. In phosphate buffer of pH 6–7.5 the cyanoethylation is complete and there is no effect on ascorbic acid.

Results for the photometric titration of microgram amounts of ascorbic acid are given in Table 2. In Table 3, the results are given for the analysis of mixtures of ascorbic acid with methionine and cysteine or glutathione, made from solutions of the components. Results for the determination of ascorbic acid in fruit juices and pharmaceutical preparations are presented in Tables 4 and 5 respectively. Most vitamin C capsules gave coloured solutions but the leuco-indophenol furnished a sharp end-point (greenish-blue in yellow solutions, violet in red solutions and vivid blue in colourless solutions). The exactness of this end-point

Table 6. Determination of ascorbic acid in biological fluids

Sample	Ascorbic acid, *mg*/*ml**	
	Present method	NBS titration
Urine	2.78	2.81
	5.29	5.30
	6.77	6.75
	9.30	9.28
	12.65	12.69
Blood and plasma	0.88	0.87
	0.98	0.99
	1.62	1.60
	1.94	1.92
	2.32	2.35

* Average of three determinations.

was verified by titrating known amounts of ascorbic acid in presence of coloured materials, *e.g.*, Methyl Red, eosin and picric acid, the results agreeing with the theoretical to within 0.5%.

Results for the determination of ascorbic acid in urine and total blood and plasma are given in Table 6 and compared with those found by the previously checked *N*-bromosuccinimide method.[7]

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SHORT COMMUNICATIONS

DIRECT OXIDIMETRIC DETERMINATION OF THIOCARBONATE SULPHUR WITH FERRICYANIDE, USING IRON(II)–DIMETHYLGLYOXIME OR SODIUM NITROPRUSSIDE AS INDICATOR

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(*Received* 24 *July* 1980, *Revised* 2 *June* 1981. *Accepted* 10 *June* 1981)

Summary—Direct determination of 15–50 mg of thiocarbonate sulphur can be achieved by a one-step titration with potassium ferricyanide, using iron(II)–dimethylglyoxime or sodium nitroprusside as indicator. Only one sulphur atom of the thiocarbonate ion undergoes oxidation to elemental sulphur, the other two separating as carbon disulphide.

Our current spectrophotometric studies on trithiocarbamate complexes of metals have required standardization of the ligand solution, which can be done by determining the thiocarbonate sulphur. A number of indirect methods have been reported for this, including gravimetric determination as thallium(I) trithiocarbamate,[1] and indirect titration[2,3] with iodine, iodate, chloramine-T, ferricyanide, permanganate or vanadate. In the ferricyanide method[3] an aliquot of sample containing not more than 17 mg of thiocarbonate sulphur was heated with a measured excess of 5*M* ferricyanide solution for 15–20 min at 60°. The mixture was then cooled to room temperature and the excess of ferricyanide determined by adding potassium iodide and sulphuric acid, and titrating with thiosulphate. The trithiocarbonate was oxidized completely to sulphate. We have now found a direct method for determination of 15–50 mg of thiocarbonate sulphur by titration with ferricyanide, using iron (II)–dimethylglyoxime or sodium nitroprusside as indicator.

EXPERIMENTAL

Reagents

Potassium ferricyanide, 0.10M. Prepared by dissolving the analytical reagent grade material in demineralized water, and standardized with sodium thiosulphate solution. Titrants of lower concentrations were prepared by dilution and standardized in the same manner.

Iron(II)–dimethylglyoxime indicator. Made by mixing 10 ml of 0.02*M* ferrous sulphate, 50 ml of saturated dimethylglyoxime solution in ethanol and 5 ml of concentrated ammonia solution.

Sodium nitroprusside indicator, 1% aqueous solution. Freshly prepared.

Potassium thiocarbonate, 1M. Prepared and standardized as reported earlier[3] and diluted as required.

Ammonia–ammonium chloride buffer, pH 9.4. Made by dissolving 50 g of ammonium chloride and 70 ml of concentrated ammonia solution in water and diluting to 1 litre.

Procedures

A. To a solution containing 15–50 mg of thiocarbonate sulphur (equivalent to 5–17 mg of oxidizable sulphur, *i.e.*, one third of the total sulphur), add 10 ml of pH 9.4 buffer and dilute to about 30 ml. Add 2 drops of iron(II)–dimethylglyoxime indicator and titrate with 0.05*M* potassium ferricyanide until the indicator complex changes from cherry red to yellow.

B. To a solution containing 15–50 mg of thiocarbonate sulphur, add 1 ml of sodium nitroprusside solution and 10 ml of pH 9.4 buffer and titrate with 0.1*M* potassium ferricyanide until the purple colour disappears or changes to pale yellow. Any pH in the range 8–10 is suitable.

DISCUSSION AND RESULTS

The results obtained showed that under the reaction conditions used, one of the three sulphur atoms in the trithiocarbonate ion is oxidized to elemental sulphur, the other two sulphur atoms separating as carbon disulphide. This stoichiometry is supported by amperometric studies[4] of the potassium thiocarbonate–ferricyanide reaction. Also, Charlot[5] found that sulphide was oxidized to elemental sulphur by ferricyanide, when iron(II)–dimethylglyoxime was used as indicator. The reaction between thiocarbonate and ferricyanide can be expressed as:

$$CS_3^{2-} + 2Fe(CN)_6^{3-} \rightarrow 2Fe(CN)_6^{4-} + CS_2 + S$$

1 ml of 0.10*M* $K_3Fe(CN)_6$

$\equiv$ 9.32 mg of K_2CS_3 $\equiv$ 1.603 mg of S

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Formation of carbon disulphide was confirmed by the acetone test.[6]

The results are quantitative for 15–50 mg of thiocarbonate sulphur (equivalent to 5–17 mg of oxidizable sulphur) with a relative error of less than 0.1%. With larger amounts of trithiocarbonate the carbon disulphide formed slowly reacts with the free alkali to re-form thiocarbonate, resulting in positive errors.

$$6OH^- + 3CS_2 \rightarrow 2CS_3^{2-} + CO_3^{2-} + 3H_2O$$

Sulphide interferes seriously, since it is also oxidized by ferricyanide. However, potassium thiocarbonate solutions free from sulphide can be easily prepared, and stored for long periods of time at pH $\geqslant$ 8.0 without undergoing any decomposition.

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BACTERIAL ELECTRODE FOR L-ARGININE

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Summary—*Streptococcus lactis* bacterial cells are employed for the conversion of L-arginine into ammonia. An ammonia gas-electrode is used as a detector. The combination of bacterial action and the gas electrode responds linearly to L-arginine over the concentration range $8.0 \times 10^{-6} - 1.0 \times 10^{-3} M$, with a slope of 59.0 mV/decade and is selective with respect to other L-amino acids.

Enzymes coupled with an appropriate electrode are increasingly used in analysis. Progress in this field[1,2] has been delayed because the enzymes have to be isolated and purified. Furthermore, many enzymes which seem to be of analytical use are still not commercially available. Living micro-organisms, such as bacterial cells, have been used instead of enzymes.[1,3–5] *Streptococcus faecium* has been employed at the surface of an ammonia gas-sensing electrode[3] for the determination of L-arginine in the presence of a few amino-acids. However, this bacterial gas-electrode combination has certain disadvantages as an electrode, such as a slow response time of 20 min, and it is also not truly selective for L-arginine relative to glutamine, asparagine and urea.

This paper describes a bacterial sensor in which the living organisms, such as *Streptococcus lactis*, are employed in suspension. The sensor gives a selective to 18 other L-amino-acids. An ammonia gas-electrode was used to determine the amount of ammonia generated by the following micro-organism metabolic sequence of L-arginine:[6]

$$\text{L-Arginine} \xrightarrow[\text{deaminase}]{\text{arginine}} \text{citrulline} + NH_3$$

$$\text{citrulline} + H_3PO_4 \xrightarrow[\text{transcarbamylase}]{\text{ornithine}} \text{ornithine} + \text{carbamoyl phosphate}$$

$$\text{carbamoyl phosphate} + \text{ADP} \xrightarrow[\text{kinase}]{\text{carbamate}} \text{carbamic acid} + \text{ATP}$$

$$\text{carbamic acid} \longrightarrow CO_2 + NH_3$$

EXPERIMENTAL

Apparatus

An Orion model 95–10 gas-sensing combination electrode was used. Potentiometric measurements were made with a Radiometer PHM 64 research pH-meter connected to a Radiometer Servograph Pen-Drive REA 310 strip-chart reconder with a high sensitivity unit REA 112. Measurements were made in a cell kept at 30.0 ± 0.1° in a Haake model E1-V water-bath.

Reagents

All solutions were prepared with distilled water. L-Arginine and all other amino-acids, in the purest form available, were obtained from Sigma Chemicals Co., and were of analytical grade. *Streptococcus lactis* No. 19435 was obtained from the American Type Culture Collection, Rockville, Maryland. Todd-Hewitt broth and thioglycollate medium were purchased from Difco Lab., Michigan.

Procedure

The *Streptococcus lactis* strain was maintained (at 4°) in thioglycollate medium with excess of calcium carbonate added,[7] and transferred to fresh medium every 2 weeks. For experimental purposes *S. lactis* was grown aerobically in Todd-Hewitt broth for ~2 days at 37°. The bacteria cells were collected by centrifugation at room temperature, washed 3 times with 0.1*M* phosphate buffer (pH 7.80) and stored in the 0.1*M* phosphate buffer solution at 4° for at least 12 hr before use.

Approximately 3×10^{11} cells were washed twice with 0.1*M* phosphate buffer solution (pH = 7.80) and suspended in 5.00 ml of 0.1*M* phosphate buffer (pH = 7.80) in a temperature-controlled cell at 30°. They were kept in suspension by magnetic stirring. The ammonia electrode was preconditioned by soaking in buffer for several hours and was stored in 0.1*M* phosphate buffer (pH = 7.80) when not in use. It was immersed in 5.00 ml of phosphate buffer solution and calibration curves were constructed by adding successive portions of freshly prepared amino-acid solutions ($1.000 \times 10^{-2} M$). After the cells had been used they were washed 3 times and stored in 0.1*M* phosphate buffer (pH = 7.80) at 4°.

RESULTS AND DISCUSSION

The potentiometric response of the bacteria–ammonia electrode system at different ages to L-arginine is shown in Fig. 1. Within the L-arginine concentration range from $8 \times 10^{-6} M$ to $10^{-3} M$ the electrode system showed a linear response with a slope of 59.0 ± 3 mV/decade when new (Fig. 1). After 11 days the response slope was still the same (58.7 mV/decade) but the upper limit of the linear range had decreased to $4 \times 10^{-4} M$ L-arginine (Fig. 1). However, by the 20th day the response slope had dropped to 49 mV/decade and the linear range had narrowed to 1.0×10^{-5}–$1.6 \times 10^{-4} M$ for the same cell crop. The response time was 6–8 min when the bacteria–electrode system was new and was not dependent on the L-arginine concentration. However, the response time increased to ~12 min after 11 days and to ~18 min after 20 days (Fig. 1).

About $\sim 3 \times 10^{11}$ cells were used in 5 ml of buffer solution. Use of a smaller number of cells decreases the linear range of the calibration curve, whereas doubling the number stated has no influence on the linear range.

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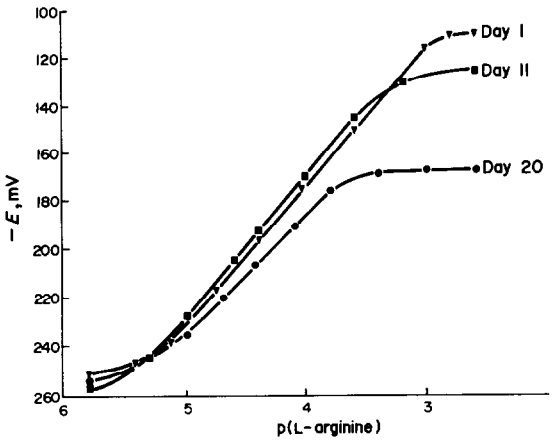

Fig. 1. Calibration curves for the bacteria–ammonia electrode combination when new, after 11 days and after 20 days; $T = 30°$, pH = 7.80, 0.10M phosphate buffer.

The specificity of the bacteria–ammonia electrode system was studied by measuring the electrode response to various amino-acids in 0.10M phosphate buffer (pH = 7.80) at 30°, up to a concentration of $10^{-3}M$ amino-acid. Negligible or no response was found to L-glutamine, L-asparagine, L-lysine, L-histidine, L-leucine, L-alanine, L-aspartic acid, L-valine, L-serine, L-glutamic acid, L-methionine, L-cysteine, L-proline, L-isoleucine, L-threonine, hydroxy-L-proline and L-citrulline. There was also no response to urea.

The response of the cell-electrode system was tested over a certain pH range (Fig. 2), since the bacteria (*S. lactis*) used can grow at a pH value of ~6–9.2,[8] and Todd-Hewitt broth, pH = 7.8, is recommended as nutrient. The highest pH would be the most favourable for the ammonia gas-electrode, but the linear range of the calibration curve at pH 8.20 (Fig. 2) was already narrowed to 8×10^{-6}–$8 \times 10^{-5}M$. Furthermore, the linear range was longest at pH = 7.80, with a reasonable slope (59.0 mv/decade), so pH = 7.80 was used in subsequent studies.

The use of Tris-HCl buffer instead of phosphate buffer caused only a small shift and a slightly lower slope (56.0 mV/decade, Fig. 2) of the calibration curve. Thus Tris-HCl buffer can be used in place of phosphate buffer.

Three calibration curves at three different temperatures are shown in Fig. 3. These temperatures were chosen because the optimum growth temperature for *S. lactis* is given as 30°. The slope of the calibration curve is highest at 37°, but the linear range is shorter than at 30°, while at 25° both the linear range and slope are smaller.

The response time of the bacterial electrode system (Fig. 4) is faster at higher temperatures, although the difference between response at 37° and 30° is small (~1 min). Therefore, a temperature of 30° was used, with pH 7.80 phosphate buffer in subsequent studies.

As the response time more than tripled when the bacteria were held on the electrode by means of a dialysis membrane, the cells were suspended in the buffer solution. The same effect has been found with other biological electrodes.[4] However, Rechnitz *et al.*[3] reported a bacterial electrode (*S. faecium*) for L-arginine, in which the bacteria were kept on the gas electrode and not suspended in the buffer, and the response time was 20 min. The linear range of our *S. lactis*-electrode system is significantly longer than that of the Rechnitz electrode. Furthermore, much smaller L-arginine concentrations can be determined with this electrode, though slightly higher L-arginine concentrations could be determined with the *S. faecium* electrode. The slope obtained from our electrode is higher than that from the Rechnitz electrode (52.5 mV/decade); both electrodes have the same lifetime. Our electrode is much more selective for L-arginine.

Reproducibility studies on a known amount of L-arginine in the presence of $1.00 \times 10^{-4}M$ concen-

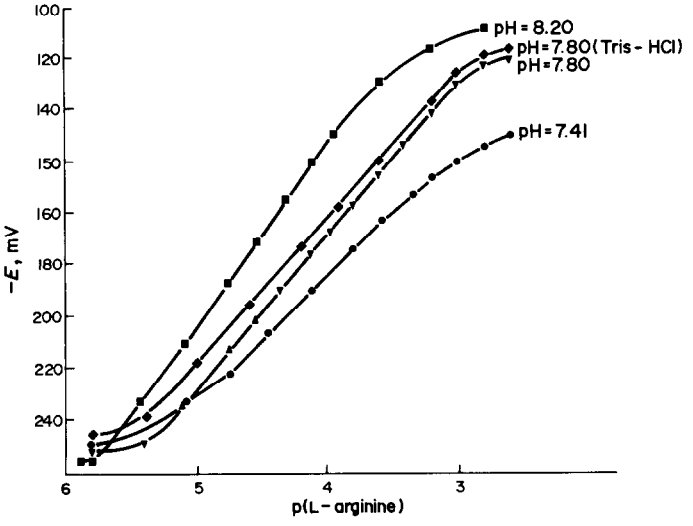

Fig. 2. Effect of pH on the response of the bacteria–ammonia electrode combination, $T = 30°$, 0.10M phosphate and Tris-HCl buffers.

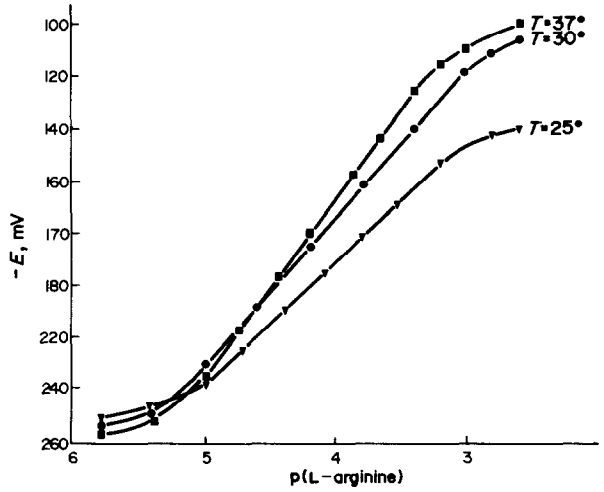

Fig. 3. Calibration curves for the bacteria–ammonia electrode combination at 37°, 30° and 25°, pH = 7.80, 0.10*M* phosphate buffer.

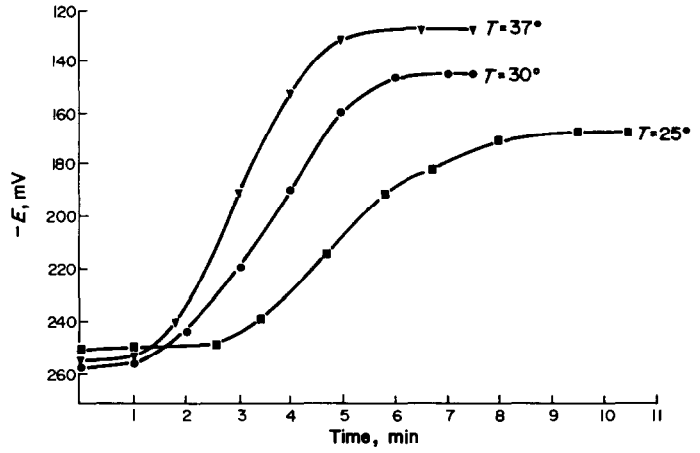

Fig. 4. Response curves at three temperatures. L-Arginine = $3.0 \times 10^{-4}M$, pH = 7.80, 0.10*M* phosphate buffer.

Table 1. Determination of a known amount of L-arginine in the presence of a mixture of other L-amino-acids and urea (see text), measured in 0.10*M* phosphate buffer at pH 7.80 (5 replicates)

L-Arginine, μg		
Taken	found	Std. devn.
52.5	52.5	3.3
169.8	169.7	8.2

trations of each of the other L-amino acids and urea are shown in Table 1. The L-amino-acid mixture shows a background ammonia concentration of $2.6 \times 10^{-4}M$, as measured with the ammonia electrode. Therefore, this blank ammonia concentration was taken into account in the L-arginine determinations. The results of Table 1 indicate that sufficient accuracy and precision can be obtained with the bacterial ammonia gas-electrode system. A possible use for the system would be the determination of L-arginine in body fluids and tissues.[9]

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ESTIMATION OF IRON WITH SALICYLALDEHYDE HYDRAZONE

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Summary—Iron is determined by precipitation as the reddish orange ferrous complex of salicylaldehyde hydrazone (SH) in the pH range 10.0–11.0. With ferric iron an unstable complex is formed which changes readily to the stable ferrous complex. The iron complex has a metal–ligand ratio of 1:2, contradicting the 1:3 ratio reported earlier. The reagent can be used for separation of iron from several ions.

The use of salicylaldehyde hydrazone (SH), one of the condensation products of salicylaldehyde and hydrazine, for the gravimetric estimation of copper and nickel has been reported.[1,2] In the present study it was observed that under suitable conditions SH forms a reddish orange iron(II) complex irrespective of the original oxidation state of the iron. It is stable in presence of tartrate, citrate and ammonium salts, but is partly soluble in EDTA and completely soluble in potassium cyanide solution and acetic or mineral acid. The ferrous complex is very stable and permits the separation of iron from a large number of ions by use of sodium potassium tartrate as masking agent.

The difficulty experienced in isolation of pure reagent (SH), free from salicylaldazine (SA), was overcome by following the recommendations reported by Okafor,[3] and later employed by us in the isolation of pure hydrazones of substituted salicylaldehydes,[4] *viz.* (*i*) the salicylaldehyde has to be added to the hydrazine, and slightly more hydrazine than required for a 1:1 molar ratio has to be used in order to avoid any formation of the azine; (*ii*) fairly dilute ethanolic solutions of both reactants should be used; (*iii*) the salicylaldehyde solution has to be added slowly and with stirring; (*iv*) the reaction is exothermic, so no heating is necessary.

EXPERIMENTAL

Pure SH free from SA was prepared by slowly adding 0.1 mole (12.6 g) of salicylaldehyde to slightly more than 0.1 mole (5.0 g) of hydrazine hydrate, both diluted with ethanol, shaking thoroughly after each addition. On cooling, creamy white flakes separated, which on recrystallization from methanol yielded shining white plates with m.p. 97.5°.

The reagent was used as a 1% solution in 1% aqueous potassium hydroxide solution. The iron solutions were prepared from ferrous ammonium sulphate and ferric alum and standardized by the oxine method.[5] Analytical-reagent grade chemicals were used whenever possible.

Excess of reagent solution was added to slightly ammoniacal ferrous ammonium sulphate or ferric alum solution containing sodium potassium tartrate. The precipitate formed was collected, washed, and dried at 110°. The dried product was decomposed by heating with nitric acid and then fumed with sulphuric acid, and the iron content determined by the oxine method.[5] The ligand content of the product was determined by adding hydrochloric acid and titrating with potassium periodate in presence of iodine monochloride.[6] Analysis gave 82.7% ligand, 16.9% iron. $(C_7H_7ON_2)_2Fe$ requires 82.86% ligand, 17.14% iron.

The complex is stable up to 200° and can be dried to constant weight at 110–160°.

The freshly precipitated complex is appreciably soluble in benzene, chloroform and carbon tetrachloride, but when dried is almost completely insoluble in these solvents. The complex is soluble in excess of ammonia or strong alkalis as well as in acids.

Determination of iron(II) or (III)

The sample solution, containing 5–30 mg of iron(II) or (III) and 10 ml of 10% sodium potassium tartrate solution is adjusted to pH 10.0–11.0 with ammonia, diluted to 125 ml and warmed to 70–80°. The iron is precipitated by addition of excess of reagent solution. The precipitate is digested for 30 min on a water-bath, cooled, filtered off, washed with water and dried at 110–130° to constant weight. The results are given in Table 1.

Determination in presence of interfering species

Mn^{2+}, Co^{2+}, UO_2^{2+}, Hg^{2+}, Zn^{2+}, Pd^{2+}, Ag^{+}, Cd^{2+}, Ni^{2+} and Cu^{2+} interfere. However, silver can be eliminated beforehand by precipitation as sulphide or chloride, and palladium, mercury and cadmium by precipitation as sulphide. Mercury(II) interference can also be eliminated by complexing it with 2–3 g of potassium iodide in alkaline medium (tolerance limit 50 mg). Nickel and copper can be precipitated with salicylaldehyde hydrazone from 0.1*M* potassium hydroxide medium containing 4–5 g of sodium potassium tartrate; the iron complex is not precipitated. The precipitate is washed first with 1.0% potassium hydroxide solution, then with hot water; the pH of the combined filtrate and washings is then lowered to 10.0–11.0 with acetic acid to precipitate the iron. Cobalt, zinc and manganese require preliminary separation of the iron by double precipitation of the hydroxide under conditions precluding their co-precipitation. Uranium also requires a preliminary separation of the iron. Results for determination of iron in presence of masking agents and other ions are given in Table 2.

Table 1. Estimation of iron(II and III) with SH

Fe(II or III) taken, *mg*	Fe(II) found, *mg*	Fe(III) found, *mg*
5.0	4.97	4.99
10.0	9.98	10.02
15.0	14.94	14.99
20.0	20.04	19.93
25.0	25.05	24.98
30.0	29.93	29.98

Table 2. Estimation of 20.0 mg of iron(II) with SH in presence of masking agent and diverse ions

Fe(II) found, *mg*	Species added
19.93	Sodium citrate (2 g)
20.04	Sodium thiosulphate (200 mg)
19.95	Sodium oxalate (100 mg)
20.07	Ammonium sulphate (50 mg)
19.93	Mg^{2+} + Be^{2+} (20 mg each)
19.95	Al^{3+} + Cr^{3+} (20 mg each)
20.07	Cu^{2+} (20 mg)*
19.90	Ni^{2+} (20 mg)*

* Separated as described in text.

Table 3. Analysis of alloys

Alloy	Iron present, %	Iron found, %
Nilo 'K' Wire	53.2	53.0
BCS 235/18/8 Stainless steel	68.4	68.7
BCS 241/1 Stainless steel	71.0	71.2

Analysis of ferrous alloys

The sample is dissolved in dilute nitric acid and the insoluble material filtered off. The filtrate is evaporated with sulphuric acid to fumes of sulphur trioxide. The residue is diluted with water and hydrogen sulphide is passed through the solution. The precipitated sulphides were filtered off and washed with water. The hydrogen sulphide is boiled out of the filtrate, which is then oxidized with nitric acid and the iron is precipitated as ferric hydroxide with ammonia in presence of ammonium chloride, and the iron is estimated as already described. Results are given in Table 3.

RESULTS AND DISCUSSION

Salicyladehyde hydrazone (SH) precipitates iron quantitatively as a reddish orange ferrous complex $(C_7H_7ON_2)_2Fe$ in the pH range 10.0–11.0, irrespective of whether ferrous or ferric iron is initially present, because the reagent itself is a good reducing agent and reduces the ferric iron as reported earlier.[1,2] The reagent is unstable in presence of oxidizing agents, and is converted into the more stable salicylaldazine (SA), another condensation product of salicylaldehyde and hydrazine.

Ray[7] isolated SH for use as a spectrophotometric reagent for copper and iron *etc.*, and found a metal–ligand ratio of 1:1 for copper and 1:3 for iron.[8,9] Our earlier study indicated a metal–ligand ratio of 1:2 for the copper complex,[1,2] which was later supported by the infrared and microanalysis study made by Okafor.[3] The present study indicates the same metal–ligand ratio (1:2) for the iron complex as well.

The conflict between our findings and those of Ray *et al.*[7–9] may arise from the difficulty of ensuring that the reagent is free from salicylaldazine. The yellowish colour of their reagent and its solution in acetone, the use of ferric perchlorate (an oxidizing agent) for preparation of the metal complex, and the low pH used indicate that there was probably partial conversion of SH into SA, resulting in formation of an altogether different complex species. Their report[9] that the reddish brown colour of their complex faded on standing in contact with the aqueous phase, and that an extremely large excess (260-fold) of reagent was needed for full colour development further indicates that a very weak and unstable SH complex of iron(III) might have been formed, which then changed to the stable iron(II) complex.

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DETERMINATION OF THE FATTY-ACID COMPOSITION OF SOYBEAN OIL BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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Summary—A method for determining the fatty-acid composition of soybean oil by using high-pressure liquid chromatography (HPLC) is discussed and compared with the determination using gas chromatography. The oil is saponified and an aliquot is treated directly to form the *p*-bromophenacyl esters of the fatty acids, which are separated by gradient-elution HPLC. It is shown that glycerol does not interfere with the esterification, thus obviating the solvent extraction previously used to isolate fatty acids from biological samples.

The analytical determination of fatty-acid compositions in polyunsaturated vegetable oils is important in nutritional labelling. The methods now in use involve gas chromatography.[1–3] However, problems still exist with this method. Artifact formation during esterification of fatty acids with boron trifluoride in methanol,[4] geometric isomerization and thermal degradation,[5] double-bond migration,[6,7] and irreversible retention of polyunsaturated fatty esters[8–10] have been the major problems reported. The thermal lability of the unsaturated esters is a major cause of these problems. Difficulties are also encountered with high molecular-weight esters, because their volatility is low.

In recent years high-pressure liquid chromatography (HPLC) has become a popular technique for the separation of thermally labile compounds. While HPLC methods have been developed for separating lipid materials, one of the major problems with these methods is the use of refractive-index detection, which is not suitable for trace analysis.

Ultraviolet detection is the most commonly used in HPLC for microanalysis. Triglycerides do not absorb strongly at the wavelengths commonly used (254 or 280 nm). Therefore, ultraviolet absorbing derivatives must be prepared. The most widely used derivatives have been phenacyl and substituted phenacyl esters. Borch[11] and Jordi[12] demonstrated the usefulness of HPLC, with ultraviolet detection at 254 nm, for separating these esters. Octadecylsilane-bonded silica-packed columns were used with gradient-elution liquid chromatography to obtain the separations. Separation time was 3–4 hr per sample.

The acid composition of bacterial samples,[13–16] fingertips,[17] and river water[18] was determined by methods based on these separations. Fatty acids found in human blood plasma[19] and butter[20] were analysed by use of an octylsilane-bonded silica column. In these methods, the fatty acids were isolated from the saponification mixture by solvent extraction before conversion into suitable derivatives. This procedure minimized potential interference.

In this paper, a method of determining the fatty acid composition of soybean oil by using high-pressure liquid chromatography is discussed. The soybean oil is saponified, then, without solvent extraction, the resulting acids are esterified to form the *p*-bromophenacyl esters, with 18-crown-6 as the catalyst. The esters are analysed quantitatively on an octylsilane-bonded silica column. A similar esterification procedure and HPLC separation was used by Tweeten and Wetzel[21] to determine fatty-acid compositions in grain and feed extracts. They chromatographed the esters isocratically on an octyldecylsilane-bonded silica column with a methanol–water mobile phase. Palmitic and oleic esters were not separated with this

system. In the method reported here, an acetonitrile–water gradient was employed, which separated the palmitic and oleic esters with a total separation time of 1 hr per sample. To assess the efficacy of this method, determinations of fatty acid composition by HPLC and by gas chromatography were compared, and the results found in reasonable agreement. In addition, a separate experiment was conducted to determine whether glycerol, a by-product of the soybean oil, interferes with the esterification. Because it does not, the need for solvent extraction is obviated.

MATERIALS AND METHODS

Standard preparation

A stock solution of fatty acids was prepared by dissolving 0.015 g each of palmitic, oleic, linoleic, and linolenic acids and 0.001 g each of arachidic and behenic acids in 10 ml of chloroform. A 0.3-ml aliquot of this solution was transferred to a 3.5-ml reaction vial and evaporated to dryness under nitrogen. As an internal standard, 0.15 ml of a lauric acid stock solution (0.02 g of lauric acid dissolved in 10 ml of methanol) was added to the vial. The acids were neutralized with 0.1*M* methanolic potassium hydroxide to a phenolphthalein end-point. The solution was evaporated to dryness under nitrogen and the residue was esterified (see below). The concentration of the standard esters was in the 300-μg/ml range (20 μg/ml for arachidic and behenic acids).

Sample preparation

A 0.2-g sample of soybean oil (Standard Food Grade; iodine value 130) was saponified with 50 ml of 0.5*M* methanolic potassium hydroxide by refluxing for 10 min. A 0.5-ml aliquot of the cooled solution was transferred to a 3.5-ml reaction vial and spiked with 0.15 ml of lauric acid internal standard solution. The solution was neutralized with 0.5*M* methanolic hydrochloric acid to a phenolphthalein end-point and evaporated to dryness under nitrogen. The residue was esterified according to the following procedure.

Esterification procedure[22,23]

A Reacti-Therm Heating/Stirring Module (Pierce Chemical Co.) was used. To each of the standard and sample residues, in the 3.5 ml screw-cap septum vials, 1 ml of the alkylating reagent (0.1*M* α-*p*-dibromacetophenone/0.005*M* 18-crown-6, Aldrich Chemical Co.) and 2 ml of acetonitrile (Burdick and Jackson Laboratories) were added. The vials were sealed, and heated at 80° for 30 min with constant stirring. The solutions were filtered through a 0.5 μm Fluoropore filter (Millipore Corp.) before HPLC analysis.

HPLC analysis

The *p*-bromophenacyl esters were separated by use of a Waters Associates ALC/GPC 244 liquid chromatograph equipped with a 6000A pump and a 660 solvent programmer. A Hibar-11 column with Li Chrosorb RP-8 packing (Merck; 10 μm particle size, 250 mm long, 4.6 mm internal diameter) was used for the separation. Chromatograms were recorded on a Sigma 10 Chromatography Data Station (Perkin-Elmer Corp.).

HPLC-grade water was obtained from a Milli-Q water purification system (Millipore Corp.). The acetonitrile used was HPLC grade from Burdick and Jackson Laboratories. The gradient-elution conditions employed were: 70% acetonitrile/water to 100% acetonitrile at 2 ml/min over a 50-min period with a concave gradient (curve 7 on the solvent programmer). Detection was at 254 nm. Peaks were identified from relative retention times measured with respect to the laurate internal standard peak. Measurement of peak height was used for quantification. Relative response factors were determined for each ester. Ester concentrations were converted into acid concentrations before normalization calculations were performed.

Glycerol interference experiment

Because glycerol was the major by-product in the soybean oil saponification mixture, an experiment was performed to determine whether glycerol interfered with the esterification. A standard solution of fatty acids with a composition typical of soybean oil was spiked with glycerol at two concentration levels and esterified. The esters were separated by HPLC. Peak heights for the spiked and unspiked esters were then compared.

Gas chromatography (GLC)

The fatty-acid composition of the soybean oil sample was also determined by a standard gas chromatography method. Methyl esters of soybean oil fatty acids were prepared according to A.O.C.S. Official Method Ce 2-66.[2] The fatty-acid composition was determined according to A.O.C.S. Official Method Ce-1-62.[2]

RESULTS AND DISCUSSION

The peak heights for *p*-bromophenacyl esters are given in Table 1. As can be seen from the data, the additions of glycerol did not appear to cause interference.

Accurate neutralization of the fatty-acid solutions was necessary to obtain high esterification yields. This proved to be the most difficult step. Lauric acid was used as an internal standard so that the sample esterification yields could be monitored relative to a single

Table 1. Peak heights of *p*-bromophenacyl esters with and without glycerol in the esterification step

Component	Concentration of ester, μg/ml	No glycerol, mm	Glycerol 189 μg/ml, mm	Glycerol 594 μg/ml, mm
Palmitate	90	38.2	40.2	39.7
Stearate	64	14.8	15.4	15.9
Oleate	258	54.3	55.1	54.3
Elaidate	74	18.3	18.9	18.3
Linoleate	804	144.4	149.8	147.4
Linoelaidate	77	15.0	15.2	14.9
Linolenate	135	9.5	9.8	9.5

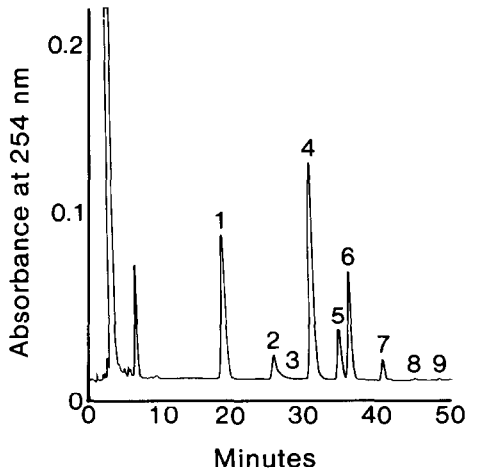

Fig. 1. HPLC chromatogram of *p*-bromophenacyl esters of soybean oil fatty acids on a Li Chrosorb RP-8 column. Peak identification: 1, laurate (internal standard); 2, linolenate; 3, γ-linolenate; 4, linoleate; 5, palmitate; 6, oleate; 7, stearate; 8, arachidate; 9, behenate.

standard. The peak height ratio (sample/standard) for the laurate ester was 1.02.

For each ester, a plot of peak height *vs.* concentration of ester was linear over the concentration range 10–600 μg/ml. Correlation coefficients for the major esters were palmitate 0.9971; stearate 0.9927; oleate 0.9994; linoleate 0.9995; linolenate 0.9989.

Figure 1 shows a chromatogram of the HPLC separation of the *p*-bromophenacyl esters of soybean-oil fatty acids. Quantitative results are given in Table 2. The results are in reasonable agreement except for those obtained with γ-linolenic acid, which was not sufficiently resolved from linolenic acid for accurate quantification.

CONCLUSIONS

A method for the determination of the fatty-acid composition of soybean oil by HPLC has been developed. Separation times are less than 1 hr per sample on the octylsilane-bonded silica column. This is a significant improvement over the 3–4 hr separation time required for the octadecylsilane-bonded silica columns. The esterification is not significantly affected by glycerol, and this allows omission of the solvent extraction step used previously to isolate the fatty acids from biological samples. Preliminary results indicate that the HPLC method can be used as an alternative approach to the determination of fatty-acid composition of soybean oil, with accuracy and precision comparable to that of GLC. In this work, the problems encountered with GLC methods [4–10] were not observed. HPLC would be the preferred approach in situations where these problems arise.

Table 2. Fatty-acid composition of soybean oil by HPLC and GLC

Component	HPLC, %*	GC, %*†
Palmitic	10.38 ± 0.18	9.65 ± 0.04
Stearic	4.33 ± 0.13	4.08 ± 0.02
Oleic	23.18 ± 0.14	23.90 ± 0.04
Linoleic	53.66 ± 0.29	55.01 ± 0.03
Linolenic	6.16 ± 0.11	6.36 ± 0.02
γ-Linolenic	1.68 ± 0.05	0.59 ± 0.01
Arachidic	0.36 ± 0.05	
Behenic	0.25 ± 0.04	

* Average of three determinations ± standard deviation.
† Two minor components were unidentified: content 0.42 ± 0.01%.

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TITRATION OF THIACETAZONE AND ISONIAZID WITH SODIUM METHOXIDE IN NON-AQUEOUS MEDIUM

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Summary—Thiacetazone and isoniazid dissolved in dimethylformamide can be accurately titrated with sodium methoxide in benzene–methanol mixture, with *o*-nitroaniline as indicator. Sodium 4-aminosalicylate does not interfere.

Iodometric determination of thiacetazone[1] is rather sensitive to variation in the amount of reagents, reaction time and temperature. Iodometry[2] based on the oxidation of thiacetazone to the semicarbazone is preferable to the method involving oxidation to the thiadiazole with ferric chloride in sulphuric acid medium, because of its practicality. Oxidation with alkaline hydrogen peroxide[3] is rather time-consuming. Gravimetric estimation[4] as the 2,4-dinitrophenylhydrazone gives low recovery but the argentimetric method[5,6] is satisfactory. Other methods include acidimetric titration[7], alkalimetric determination of acetic acid formed by hydrolysis,[8] and mercurimetric[9] and spectrophotometric determinations. Thiacetazone can be titrated in dimethylformamide medium with sodium methoxide solution in benzene–methanol, *o*-nitroaniline being used as indicator. This method offers the advantage over acidimetric determination[7] that it is unaffected by sodium 4-aminosalicylate, but isoniazid interferes quantitatively. Determination of isoniazid in this way is better than the alkalimetric determination[10] in diethylamine medium.

The end-points are sharp and stable for more than 5 min.

EXPERIMENTAL

Procedure

A weighed quantity of thiacetazone or isoniazid is dissolved in 25 ml of dimethylformamide. *o*-Nitroaniline (2 drops of 1% solution in benzene) is added and the solution is titrated with sodium methoxide solution in benzene–methanol (4:1) to a distinct orange colour. Blanks are run on the same volume of solvent, and their value deducted, For a sharp end-point the molarity of the sodium methoxide (0.05, 0.075 and 0.1*M* for thiacetazone, the binary mixture and isoniazid respectively) should be chosen so that the volume used in the titration does not exceed 8 ml.

RESULTS

The mean recovery for 49.9 mg of thiacetazone was 49.95 mg, standard deviation 0.14 mg (five replicates). The corresponding results for isoniazid (50.0 mg) were 49.8 mg and 0.13 mg.

When x mg of a mixture of pure thiacetazone (y mg) and isoniazid were titrated with V ml of sodium methoxide (molarity M), and the results were evaluated by means of the equation

$$y = \frac{236x - 32332VM}{99}$$

the recovery of both drugs was in the range 99.5–100.5% (15 replicates).

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A DIRECT TITRIMETRIC METHOD FOR THE MICRODETERMINATION OF SOME PHENOTHIAZINE DERIVATIVES IN PHARMACEUTICAL PREPARATIONS

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Summary—A micro method has been developed for the determination of some phenothiazine derivatives in pure form and in pharmaceutical preparations, 1–5 mg of sample being titrated directly with 0.02*M* *N*-bromosuccinimide, with Methyl Red as indicator. The error does not exceed ±1%.

Phenothiazine derivatives are used variously as antipsychotropic, anticholinergic and antihistaminic drugs,[1,2] and methods for their assay are important. Kasakalian and McGlotten[3] have reported determination by anodic oxidation of about 1.5 mg of chlorpromazine. Blažek[4] has used an amperometric method for the determination of 50 mg of chlorpromazine. Porter[5] has determined microgram quantities of chlorpromazine polarographically with 0.5*M* hydrochloric acid as supporting electrolyte. The pharmacology and assay of phenothiazine and prochlorperazine have been discussed.[6–9] Some new phenothiazine derivatives such as triflupromazine and trifluperazine have been estimated by Schrine.[10]

In this paper we describe a quick and convenient method for the microdetermination of phenothiazine derivatives with *N*-bromosuccinimide (NBS), a well-known brominating and oxidizing agent.[11,12]

EXPERIMENTAL

Sample solutions

These were prepared by dissolving 25 mg of sample in distilled water and diluting to volume in a 25-ml standard flask.

N-*Bromosuccinimide solution*, 0.02M

The reagent (0.3560 g) was dissolved in the minimum of warm distilled water, made up to 100 ml with cold distilled water, and standardized iodometrically.[13,14]

Procedure

A portion of solution, containing 1–5 mg of drug, was placed in a 100-ml titration flask, and 6 ml of 2*M* hydrochloric acid and 2 drops of 0.04% Methyl Red indicator solution were added. The mixture was titrated with 0.02*M* NBS until the indicator was decolorized. The excess of NBS was back-titrated iodometrically. The same volume of NBS was then titrated iodometrically under identical conditions. The amount of drug was calculated from the difference between volumes of NBS consumed in the blank and the determination.

$$\text{mg of drug} = \frac{(B - A)MN}{n}$$

where A = volume of NBS consumed by the blank (ml); B = volume of NBS consumed by the drug (ml); M = molecular weight of the drug; N = molar concentration of NBS; n = moles of NBS consumed per mole of the sample.

RESULTS AND DISCUSSION

The precision is shown in Table 1, and the accuracy (established by recovery tests based on determination of the drug content of spiked and unspiked samples) in Table 2.

The effects of variables such as temperature, acidity, NBS concentration and amount of sample were studied.

Temperatures above 26° tend to cause inaccurate results and create difficulty in detecting the end-point, because of decomposition of NBS at higher temperature. Room temperature (~25°) is the most suitable.

The reaction of phenothiazine derivatives with NBS at pH 4–5 is not quantitative and gives variable results. An approximately 0.08–0.15*M* hydrochloric acid medium gives consistent and accurate results.

Table 1. Microdetermination of phenothiazine derivatives (1–5 mg) with NBS (10 variates)

Sample	Coefficient of variation, %
Chlorpromazine hydrochloride	0.3
Prochlorperazine di(hydrogen maleate)	0.8
Promethazine hydrochloride	0.3
Triflupromazine hydrochloride	0.4
Trifluperazine dihydrochloride	0.4

The best recovery was obtained with 0.02*M* NBS, which should be prepared just before use and kept in a cool dark place. Because the Methyl Red reacts with the reagent, the amount of indicator taken should be kept constant and only a small quantity (2 drops) should be used. Excipients do not interfere. The method is applicable to sample weights ranging from 1 to 20 mg.

Table 2. Results obtained by the standard-addition method

Preparation	Added, *mg*	Found, *mg*	Recovery, %
Chlorpromazine hydrochloride			
(*a*) Largactil tablets*	18.0	17.9	99.4
	36.0	35.8	99.4
	45.0	44.9	99.8
(*b*) Widactil tablets†	18.0	17.9	99.4
	36.0	35.7	99.2
	45.0	44.8	99.6
(*c*) Ingatron tablets§	18.0	18.1	100.6
	36.0	36.2	100.6
	45.0	45.2	100.4
Prochlorperazine di(hydrogen maleate)			
(*a*) Stemetil tablets*	18.0	17.9	99.4
	36.0	35.7	99.2
	54.0	53.8	99.6
(*b*) Stemetil injection*	18.0	17.9	99.4
	36.0	35.8	99.4
	54.0	53.6	99.3
Promethazine hydrochloride			
(*a*) Phenergan tablets*	18.0	18.1	100.6
	36.0	36.1	100.3
	45.0	45.2	100.4
(*b*) Phenergan injection*	18.0	18.1	100.6
	36.0	36.2	100.6
	45.0	45.2	100.4
Triflupromazine hydrochloride			
Siquil tablets‡	18.0	17.9	99.4
	36.0	35.7	99.2
	54.0	53.7	99.4
Trifluperazine dihydrochloride			
(*a*) Eskazine tablets‖	18.0	18.1	100.6
	36.0	36.1	100.3
	45.0	45.3	100.7
(*b*) Eskazine injection‖	18.0	17.9	99.4
	36.0	35.8	99.4
	45.0	44.8	99.6
(*c*) Fluozine tablets¶	18.0	18.1	100.6
	36.0	35.9	99.7
	45.0	44.9	99.8
(*d*) Ifizine tablets†	18.0	17.9	99.4
	36.0	35.8	99.4
	45.0	44.8	99.6

* May & Baker.
† Unique.
‡ Sarabhai.
§ Inga.
‖ Smith, Kline & French (Stelazine).
¶ Medicare.

The value of n in the calculation equation is 20 for all the drugs tested, except prochlorperazine di(hydrogen maleate), for which it is 28 (the maleate consumes the extra NBS[15]).

Although its mechanism is not clear, the reaction could be useful for quality control. The method has not been tested in the presence of degradation products of the drugs, so it is not known whether it is suitable for metabolic studies. In the case of prochlorperazine di(hydrogen maleate), any maleic acid present, owing to poor preparation, will cause a positive error.

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DETERMINATION OF TRACE QUANTITIES OF SELENIUM BY INDIRECT ATOMIC-ABSORPTION SPECTROPHOTOMETRY

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Summary—The selenium(IV)–iodide interaction in acid medium, leading to the liberation of iodine, has been utilized for the indirect determination of selenium by atomic-absorption spectrophotometry (AAS). The iodine is extracted into benzene and subsequently reductively stripped into an aqueous solution of ascorbic acid. After extraction of the resulting iodide as tris(1,10-phenanthroline)cadmium(II) iodide into nitrobenzene, the cadmium content of the organic extract is determined by AAS. Beer's law is applicable up to 0.75 ppm of selenium. The few interferences are readily overcome. The chemical yield in the system is about 80% overall.

Direct methods for the determination of traces of selenium by atomic-absorption spectrophotometry (AAS) are not satisfactory because of the strong absorption by flame gases and extraneous matter at the wavelength (196.0 nm) of the selenium resonance line,[1] and because selenium dioxide sublimes. These methods have been improved to a great extent by using the hydrogen selenide generation techniques,[2–5] but these are not well suited for routine analysis.

Lau and Lott have reported a sensitive indirect AAS method for the determination of selenium, based on the reaction of palladium(II) with piazselenols[6] (sensitivity 16 ng/ml), but the method is critically pH-dependent and rather time-consuming because of the nature of the chemical reactions involved.

The reaction of selenium(IV) with acidified iodide to liberate iodine is well known. In the present study the feasibility of using this reaction for determining selenium by recovering the iodine completely, reducing it to iodide and adapting the indirect AAS procedure for the determination of iodide reported by Yamamoto *et al.*,[7] has been examined.

EXPERIMENTAL

Apparatus

A Carl Zeiss PMQ II spectrophotometer with FA-2 flame unit provided with a 5-cm long air–acetylene burner head and a d.c. power supply to provide a stabilized current in steps from 3 to 60 mA to a demountable Westinghouse cadmium hollow-cathode lamp.

Reagents

All reagents were of analytical grade.

Standard selenium(IV) solution (2.5 ppm). Prepare a 1000-ppm solution of selenium(IV) by dissolving 0.3482 g of pure selenium dioxide in water and making up to 250 ml with water. Dilute this solution to obtain the 2.5-ppm selenium standard.

Cadmium phenanthroline sulphate (CPS) solution, 2.5 mM. Dissolve 0.3226 g of $3CdSO_4.8H_2O$ and 0.9208 g of 1,10-phenanthroline hydrate in water and dilute to 500 ml.

Procedure

Transfer an aliquot of sample solution not exceeding 12.5 ml in volume and containing up to 12.5 μg of selenium(IV) into a 60-ml separating funnel. Add 5 ml of 5*N* sulphuric acid and 2.5 ml of 1% potassium iodide solution and dilute to 20 ml. Shake this for 2–3 min with 5 ml of benzene. Allow the phases to separate and discard the aqueous phase. Wash the organic extract with three 15-ml portions of distilled water, then shake it gently with 5 ml of 0.05% ascorbic acid solution for about 1 min. Allow the phases to separate, transfer the aqueous phase into another 60-ml separating funnel, wash the organic phase with two 5-ml portions of water and add the washings to the aqueous phase. To the combined aqueous extracts add 2.5 ml of 2.5 m*M* CPS solution and extract the $Cd(Phen)_3^{2+}$–$2I^-$ ion-pair into 5 ml of nitrobenzene by shaking the solution for 2–3 min. Prepare a reagent blank similarly.

Aspirate the nitrobenzene extracts into the air–acetylene flame and determine their cadmium content, using the following instrumental parameters: lamp current 10 mA; wavelength 228.8 nm; air flow-rate 6.3 l./min; acetylene flow-rate 0.6 l./min. Use the blank to set the read-out to zero absorbance. Prepare a calibration graph covering the range from 2.51 to 5.0 μg of selenium(IV).

RESULTS AND DISCUSSION

Benzene was chosen for extraction of the iodine liberated by the reaction of selenium(IV) with iodide in acid medium, because (*i*) the distribution coefficient for iodine between benzene and water is high, and (*ii*) it is easier to wash an extract if the wash-liquid has

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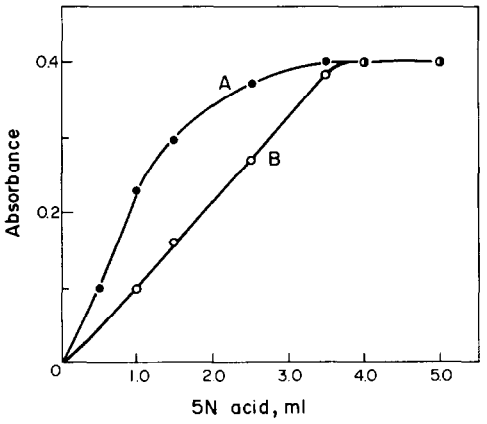

Fig. 1. Effect of acidity: 10 μg of Se(IV) + x ml of 5N acid (A, HCl; B, H_2SO_4) + 2 ml of 1% KI solution in 20 ml. The iodine liberated was extracted into 5 ml of benzene. The rest of the procedure was that given in the text.

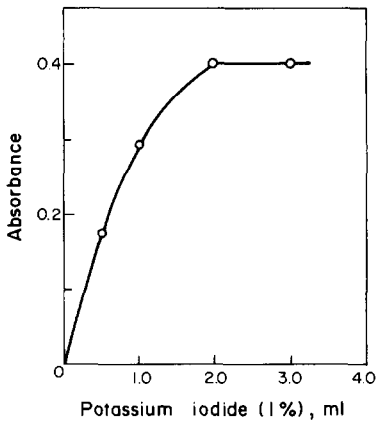

Fig. 2. Effect of potassium iodide concentration: as in Fig. 1 with 5 ml of 5M HCl and x ml of 1% KI solution.

the higher density. The iodine was quantitatively stripped with ascorbic acid solution and the iodide produced was determined by extracting it as the tris(1,10-phenanthroline)cadmium(II) iodide complex into nitrobenzene and measuring the cadmium content of the extract by atomic-absorption spectrophotometry.[7]

Figures 1 and 2 show the influence of acidity and concentration of potassium iodide on the reaction of selenium(IV) with iodide and the extraction of iodine into benzene. From these experiments, overall concentrations of 1.2M sulphuric acid and 7.5 mM potassium iodide were chosen as optimum.

Mixing the phases for 2–3 min was found to be sufficient for quantitative extraction of the iodine liberated. Longer mixing caused the iodide in the aqueous phase to undergo aerial oxidation, resulting in high blank values. Slight variations in the volume of the aqueous phase was found not to affect the selenium recovery.

Beer's law and precision

Linear calibration curves passing through the origin were obtained for selenium in the range 0–15 μg. The relative standard deviation (21 determinations) was 2%.

Effect of diverse ions

A number of cations and anions were examined for interference in the procedure, when 1 mg of the ion was present along with 5 μg of selenium. Table 1 gives the results. Interfering ions are underlined.

Ag, Tl(I), Bi and Pb were precipitated as their iodides and dispersed in the organic phase. Their interference was eliminated by repeatedly washing the benzene extract with a solution of citric acid till all the precipitate disappeared from the organic phase. Fe(III) and Sb(V) interfered by oxidizing iodide to iodine and were masked by fluoride before the addition of potassium iodide. VO_3^- was reduced with Fe(II) and the Fe(III) formed was masked with fluoride. As(V) was masked with molybdate. NO_2^- was destroyed by the addition of sulphamic acid. Sn(II) and As(III) interference is not of any consequence, as they are oxidized during the wet digestion step used for decomposition of samples.

Chemical yield

Theoretically, 1 μg of selenium should produce 2.84

Table 1. Interference studies

Group	Ion
I	Ag^+, Cu^{2+}
II	Be^{2+}, Mg^{2+} Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}
III	Tl^+, Al^{3+}, BO_3^{3-}
IV	Pb^{2+}, Sn^{2+}
V	Bi^{3+}, Sb^{5+}, NO_2^-, AsO_2^-, AsO_3^-, VO_3^-, PO_4^{3-}
VI	TeO_3^{2-}, MoO_4^{2-}, WO_4^{2-}, $Cr_2O_7^{2-}$
VII	Mn^{2+}, ClO_4^-, Br^-
VIII	Co^{2+}, Fe^{2+}, Ni^{2+}, Fe^{3+}
Miscellaneous: citrate, tartrate, fluoride, EDTA	

μg of cadmium in the final solution, but in practice it was found to yield only 2.3 μg, a yield of about 80%. Examination of the various steps showed that the phase-volume ratios used, combined with the various washing steps and other experimental conditions, would account for the loss. Thus in the extraction of iodine, the side-reaction with the excess of iodide in the aqueous phase (to form the tri-iodide ion), together with the phase-volume ratio results in 94% extraction. The yield is diminished by 2% by the water-washes. In the extraction of the cadmium complex the distribution coefficient (established by radiotracer experiments) is quite low, and this, combined with the rather large phase-volume ratio ($V_{aq}:V_{org} = 3.5$), results in an extraction yield of only 90% in this step. The overall yield should thus be $92 \times 90/100 = 83\%$, in fairly good agreement with experiment. However, provided the volumes used are not too different from those specified, the loss will be compensated for in the calibration procedure.

If repetitive washing with citrate solution is needed for removal of interference of silver *etc.*, the recovery will be correspondingly lower and a similarly treated set of standards should be run or the standard-addition method used.

Applications

The ability of the method for analysis of plant materials was studied, with use of nitric acid–perchloric acid mixtures for attacking the samples.[8] Cane sheath and neem leaf samples (5 g) were used and the final acid digest was heated with concentrated hydrochloric acid to reduce any selenate to selenite. The solutions were finally made up to 100 ml and 5- and 10-ml aliquots were analysed. The samples did not contain any selenium, and recoveries were determined by the addition of 50 and 100 μg of selenium to the plant samples before the decomposition. The results were all satisfactory. When these experiments were repeated with 1.0 and 2.5 μg of selenium as spikes, the recoveries were 80–120% at the 1-μg level and 96–120% at the 2.5-μg level (per 5 g of sample).

CONCLUSIONS

The method is superior to the indirect method based on the determination of palladium, reported by Lau and Lott,[6] in that it is more rapid and less rigorous in its experimental conditions. It is also three times as sensitive, because cadmium gives higher sensitivity than palladium in AAS.

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ANALYTICAL DATA

IONIZATION CONSTANTS AND FLUORESCENCE EXCITATION AND EMISSION SPECTRA OF THE ISOMERIC BENZO[*a*]PYRENE PHENOLS

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Summary—The acid dissociation constants, for the ground and lowest excited singlet states of the benzo[*a*]pyrene phenols, are reported, and correlated with current thought on carcinogen activation. The corrected fluorescence excitation and emission spectra of these compounds and their anions are recorded. The shifts caused by ring hydroxylation of the parent compound, and the relative spectral band intensities for each phenol are compared to those of pyrene, in a brief assessment of spectral transition polarization in the phenols.

This study is an extension of our earlier work which described both the ground and excited state protolylic equilibria of two mono- and three dihydroxy benzo-[*a*]pyrenes.[1] The ground and excited state ionization constants for all the isomeric benzo[*a*]pyrene phenols have not previously been investigated, and should be of use to workers who employ fluorescence analysis for these metabolites of benzo[*a*]pyrene.

EXPERIMENTAL

Materials

The twelve monohydroxy benzo[*a*]pyrenes, 1-OH-BP–12-OH-BP, were supplied by J. N. Keith, ITT Research Institute, Chicago. These compounds were available to us in lots of 5 mg or less. All were used without further purification but their purity was established by comparing the absorption and corrected fluorescence excitation spectra of their dilute ethanolic solutions, and from their fluorescence emission, and by HPLC. Stock ethanolic solutions (*ca.* $1.0 \times 10^{-6} M$) of these agents were prepared fresh weekly and stored in the dark at 10°. Daily purity checks were conducted to ensure that no decomposition had occurred. Solid samples were kept in a freezer at −10°. Basic solutions were prepared by dilution of carbonate-free sodium hydroxide with distilled demineralized water. Similar water was used to make acidic solutions from Baker reagent-grade sulphuric acid, and these were standardized by means of the corrected Hammett acidity scale of Jorgenson and Hartter.[2] Solutions for intermediate pH values were prepared by dilution of sulphuric acid or sodium hydroxide solution. Buffer solutions were not used, because of their possible interference in fluorimetric titrimetry.[3]

Methods

Solutions for the fluorimetric study of acid–base character were prepared by adding 100 μl of stock ethanolic hydrocarbon solution to 2 ml of acid or base which had been pipetted into a 2-ml cuvette. The mixed solvent had no apparent effect on the spectra. After addition of the hydrocarbon, each solution was mixed rapidly with a Teflon paddle. The spectrum of each solution was recorded within 1 min. All acid–base studies were conducted in triplicate. Values of pK_a and pK_a^* were obtained graphically from plots of fluorescence excitation and emission intensity respectively, *vs.* pH or Hammett acidity.

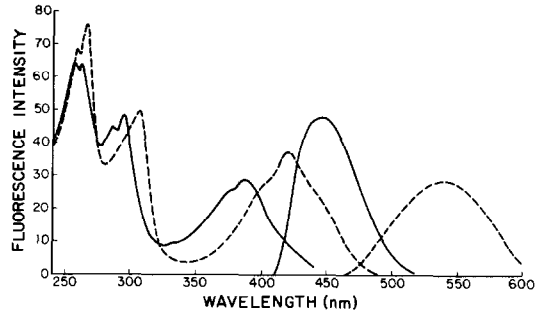
Apparatus

Corrected excitation and emission fluorescence spectra were obtained with a Perkin–Elmer MPF-44A spectrofluorimeter with 1-cm quartz cells. Spectra were corrected for the wavelength response of the light-source, photomultiplier tube, and optical system by means of a microprocessor. Electronic absorption spectra were taken in 1-cm silica cells on a Cary 118 spectrophotometer. All spectral measurements were made at room temperature, 24 ± 2.0°. A modular Altec HPLC system with a single-piston pump, a 254-nm single-wavelength detector, a μ-Bondpak C-18 column and a CO:PELL ODS pre-column eluted with methanol:water (7:3) was used to check the hydrocarbon purity. Microlitre portions of ethanolic hydrocarbon solutions were delivered with a 100-μl syringe (Precision Sampling Corp.). A Corning Model 12 pH-meter with a Sensorex 5300 C silver–silver chloride–glass combination pH-electrode was used.

RESULTS

The corrected fluorescence excitation and emission spectra of the neutral and anionic species of each of the twelve monohydroxybenzo[*a*]pyrenes, are presented in Figures 1–12.

The fluorescence excitation of the twelve phenols was quenched as the solution acidity was decreased from 7 to 12. In contrast, that from the twelve phenolate anions increased over the same pH range. The effects were monitored at the respective emission maxima. The ground-state acid dissociation constants were determined from the mid-point of quenching, enhancement or both, depending on the severity of spectral band overlap between the two species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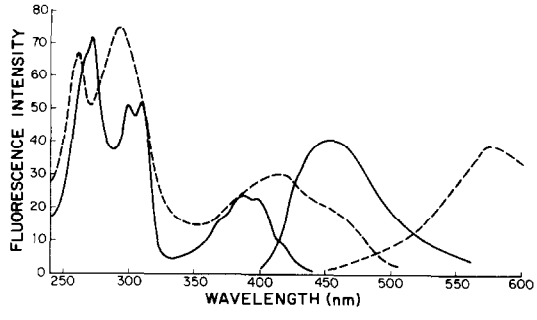
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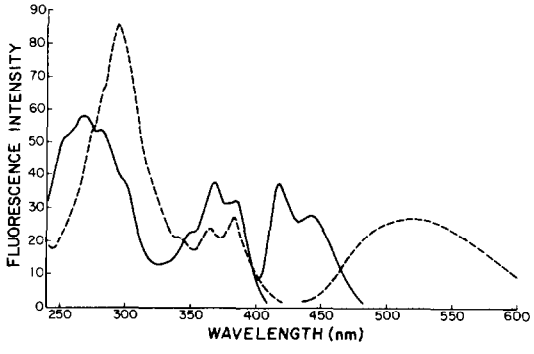
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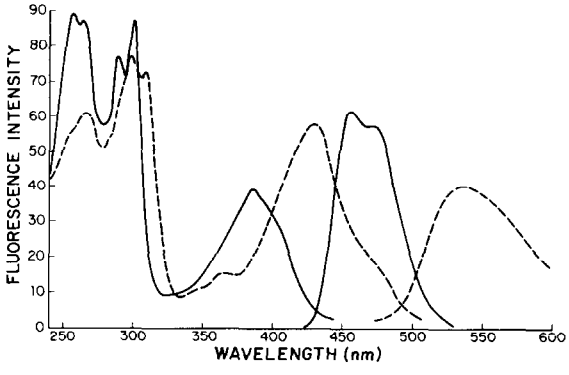
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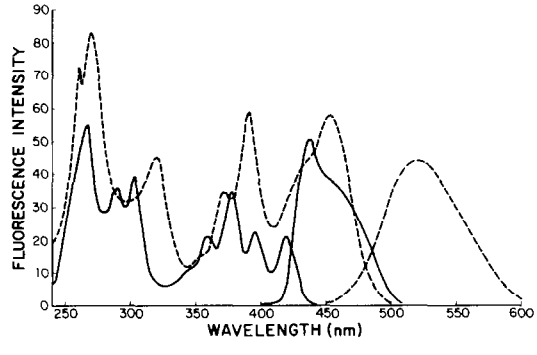
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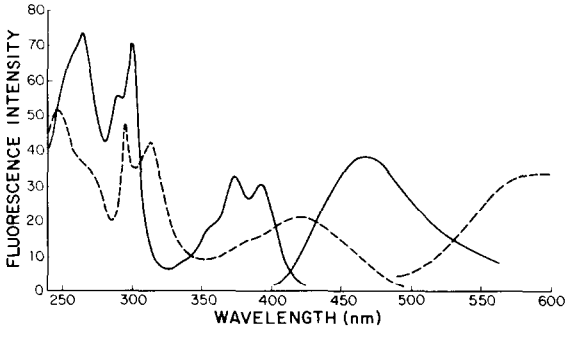
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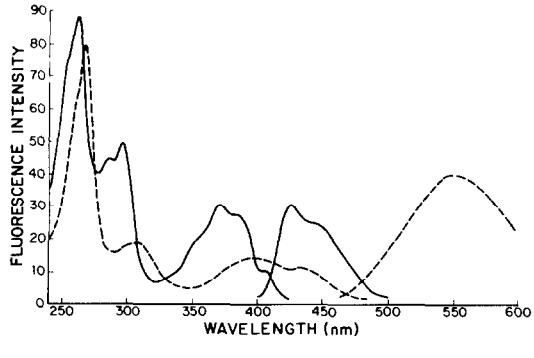
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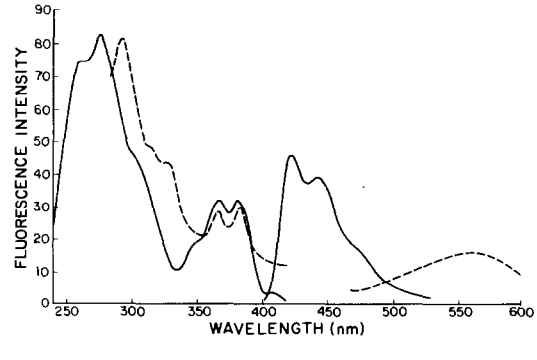
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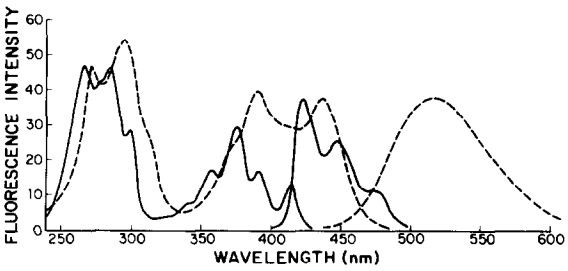
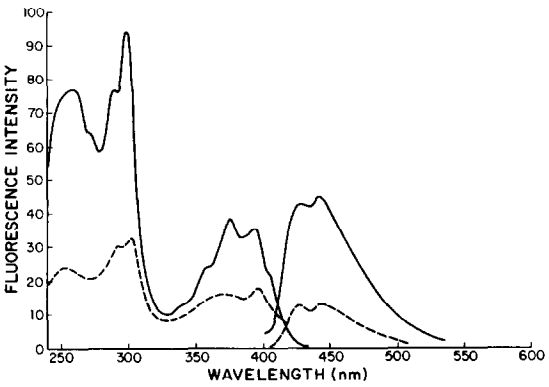
WAVELENGTH (nm)

(8)



(9)



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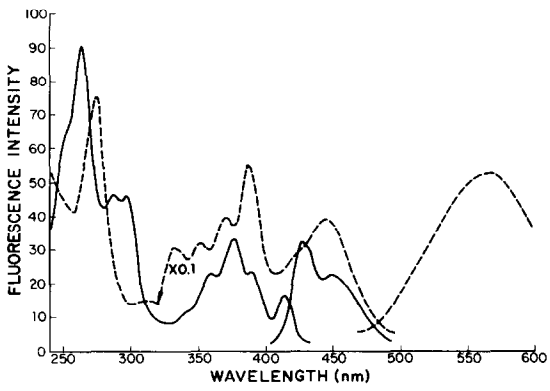

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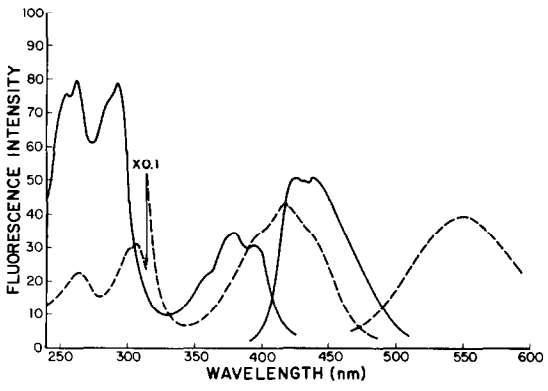

(12)

Figs. 1–12. The corrected fluorescence excitation and emission spectra of the isometric benzo[*a*]pyrene phenols (——) and phenolate anions (---) in numerical order of OH substitution position.

(Table 1). Formation of the anion resulted in a shift of the entire spectrum to longer wavelengths (red shift) relative to the spectrum for the neutral molecule. The shift was accompanied by some loss in vibrational fine structure for all the compounds except 2-, 8- and 10-OH-BP. For 2-OH-BP the spectral bands in the wavelength region 330–410 nm demonstrated a slight shift to shorter wavelengths (blue shift) when compared with those for the phenol, while those in the wavelength region 240–330 nm demonstrated a red shift. For 8-OH-BP, formation of the anion resulted in quenching of the band system between 340 and 410 nm. However, the shorter wavelength bands demonstrated a large red shift. The compound 10-OH-BP gave a very slight red slight red shift of the entire excitation spectrum upon formation of the anion.

The fluorescence emission from the phenols was quenched as the solution acidity was varied from $H_o - 1.57$ to pH 7.0. Corresponding to the disappearance of the shorter wavelength (and usually structured) emission bands of the phenols, was the appearance of a new diffuse emission band at much longer wavelengths, belonging to the anions. The exception was 10-OH-BP, which did not give a fluorescent anion. Both the disappearance of the neutral species and the appearance of the anion were monitored at the excitation maxima of band system 2 (370–390 mm), belonging to the neutral molecule. The excited-state acid dissociation constants were determined from the mid-point of both the disappearance and appearance of this spectral band for all the compounds except 10-OH-BP, for which only the disappearance of the emission from the neutral species was plotted.

The band structures remained unchanged throughout each acid–base reaction, showing that no decomposition was occurring.

Table 2 shows the difference in excitation energy shifts between BP and its hydroxyl derivatives. For spectral band systems 1 and 4 (400–425 and 255–280

Table 1. Acid dissociation constants for the ground (pK_a) and lowest excited singlet states (pK_a^*) for the monohydroxy derivatives of benzo[*a*]pyrene (BP)

BP	pK_a	Wavelength, *nm*	pK_a^*	Wavelength, *nm*
1-OH	9.0	375, 430	2.0	447, 540
2-OH	9.3	370	3.2	414, 520
3-OH	8.6†	380, 450	4.3†	436, 520
4-OH	9.4	260	1.6	425, 550
5-OH	10.8	300	1.0	457, 576
6-OH	9.2	350	1.0	443, 535
7-OH	9.2	425	−0.4	460, 590
8-OH	10.2	385	1.6	422, 555
9-OH	9.5†	375, 440	5.8†§	424, 520
10-OH	11.1	395	0.0	428
11-OH	10.2	380, 430	2.3	428, 565
12-OH	9.0	430	1.6	428, 540

† From Ref. 1.

§ This value replaces the one reported in reference 1, which we found to be in error.

Table 2. Fluorescence excitation spectral energy shifts caused by ring hydroxylation of benzo[*a*]pyrene

Compound	$\Delta\nu = \nu_{BP,max} - \nu_{-OH,max}$, $cm^{-1} \times 10^{-4}$ 1[a]	2[b]	3[c]	4[d]
1-OH-BP	0.088	0.14	0.011	0
2-OH-BP	0.013	0.015	0.022	0.12
3-OH-BP	0.12	0.094	0.099	0.121
4-OH-BP	0.031	0.051	0	0.014
5-OH-BP	0.025	0.086	0.034	0
6-OH-BP	0.070[e]	0.14	0.034	0
7-OH-BP	0.060[e]	0.072	0.045	0.028
8-OH-BP	0.013	0.0070	0.045	0.17
9-OH-PH	0.10	0.079	0.067	0.18
10-OH-BP	0.031	0.079	0.067	0.15
11-OH-BP	0.084	0.093	0.045	0.028
12-OH-BP	0.040[e]	0.086	0.011	0

[a]Corresponds to the bands lying at 400–425 nm in Figs 1–12; [b]corresponds to the bands lying at 370–390 nm; [c] corresponds to the bands lying at 290–310 nm; [d]corresponds to the bands lying at 255–280 nm; [e]from the estimated band maxima.

mm respectively), substitution in the 1-, 12-, 5- or 6-position gives a smaller $\Delta\nu$ value than for band systems 2 and 3 (370–390 and 290–310 nm respectively). In contrast, substitution in the 3-, 8- or 9-position gives a greater $\Delta\nu$ for band systems 1 and 4 than for 2 and 3.

The band shapes and intensities of the spectra for the neutral species shown in Figs. 1–12 bear a much closer resemblance to those for both BP and pyrene than to those for the polyacenes or polyphenes such as anthracene or benz[*a*]anthracene, except for 1,2-benztetraphene (1,2-benz-benz[*a*]anthracene) which has an absorption spectrum[4] almost identical in structure to that of BP.

The relative spectral band intensities generally follow the pattern band 2 > band 1 and band 4 > band 3. Exceptions to this are observed only for bands 3 and 4, where for 7-, 9- and 12-OH-BP band 3 = band 4, and for 10-OH-BP band 3 > band 4. No mirror image relationships were discernible between the fluorescence excitation and emission spectra for any of the compounds examined.

DISCUSSION

Protolytic equilibria

The red shift in both the excitation and emission spectra of the twelve isomeric phenols from formation of the phenolates and their increased acidity in the lowest excited singlet state, are characteristic of molecules bearing electron-donating substitutes.[1] Both the red shifts and the relative differences in acidity of the hydroxyl groups, in the ground and lowest excited singlet states, are indicative of the degree to which intramolecular charge-transfer occurs. The pK_a values in Table 1 differ by as much as 2.5. The differences become more interesting when the comparative magnitudes of these values are examined for the compounds suspected of undergoing metabolic conversion to form active carcinogens. Figure 13 shows that the positions which possess the greatest relative basicity are those comprising the "bay region"[5] and Pullman's "K region".[6] The greater basicity of the hydroxyl groups when substituted in these regions, the 4-, 5-, 8-, 10- and 11-positions, indicates a localization of charge near these carbon atoms. Current theory on carcinogen activation implicates both the "bay" and "K" regions as primary sites of metabolic epoxidation.[6] A correlation between epoxide metabolites and the π-bond densities of some aromatic hydrocarbons has indicated that metabolism of these aromatic hydrocarbons to the epoxide occurs by way of electrophilic attack on a π-bond.[7,8] More recent work indicates that metabolic epoxidation occurs at an electron-rich region at or near the "bay region".[1] Calculations have been employed to show that the sites on BP most reactive toward metabolic epoxidation are the 4–5, 7–8 and 9–10 π-bonds.[9] These three bonds and to a lesser degree the 11–12 π-bond are believed to exist as partially localized ethylenic π-bonds.[9] Therefore, the correlation between the pK_a values in Table 1 and current work on the activation of aromatic hydrocarbons indicates that simple acid–base studies can be employed to predict and verify regions of π-electron localization, and perhaps metabolic attack, on suspected carcinogens that demonstrate protolytic activity.

A similar correlation was not observed for the pK_a^* values. Thermal relaxation processes which occur before the molecule enters the lowest excited singlet state may account for this. Solvent-cage reorganization presumably alters the molecular dipole moment relative to that in the ground state. This, in turn, alters the interaction of the electronic vector of the hydroxyl groups with the excited-state dipole, so that position-by-position it differs from that of the ground state. The lack of correlation between pK_a^* values and the "bay" and "K" regions, in contrast to that observed for the ground-state constants, implies that in the lowest excited singlet state these molecules do not possess these regions. However, if we presume that the pK_a^* values are also indicative of charge localization regions in the BP phenols, then Table 1 indicates two such regions centered on the 2- and 3-position and on the 9-position. It is interesting to note that a straight line connecting the "bay" and "K" regions, if pivoted counterclockwise, would also connect the 9-position with the 2- and 3-positions (Fig. 13). Perhaps these molecules, when in the lowest excited singlet state, possess different electron-rich reactive regions from those for the ground state.

Assessment of polarization direction

The band systems we label 1–4 correspond to the α, p, β and β' bands, respectively, for the parent com-

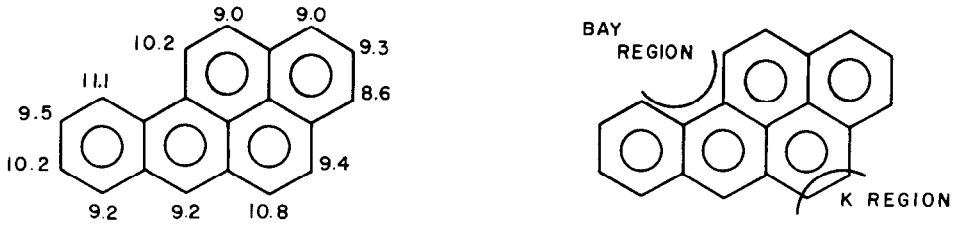

Fig. 13. The pK_a values at each hydroxy position and the most reactive regions on BP.

pound, BP, as designated by Clar.[10] The α, ρ and β designations correspond to Platt's $'L_b$, $'L_a$ and 'B spectral band designations for the catacondensed aromatics.[11] The subscripts a and b refer to polarization of the molecule perpendicular and parallel to its long axis, respectively. For pyrene, a pericondensed aromatic, the meaning of the subscripts is reversed so that a and b refer to polarization parallel and perpendicular to the long axis, respectively.[4,12] The shifts presented in Table 2 indicate that band systems 1 and 4 are long-axis (l) polarized while 2 and 3 are short-axis (s) polarized *i.e.* l, s, s, l, for bands 1–4.

However, this result is contrary to that expected from comparing the relative intensities of bands 1 and 2 or of bands 3 and 4. The lower intensity of band 1 relative to band 2 and the generally lower intensity of band 3 relative to band 4 indicate that the direction of polarization should be s, l, s, l for bands 1–4, respectively. The latter assignment is the same as that determined for the first four transitions of pyrene, $'L_b \leftarrow 'A$, $'L_a \leftarrow 'A$, $'B_b \leftarrow 'A$ and $'B_a \leftarrow 'A$, respectively.[12] The disagreement between the spectral shifts reported in Table 2 and the relative band intensities (Figs 1–12) may indicate that the first two transitions of BP are not purely long- or short-axis polarized. This possibility has been shown to exist for benz[*a*]anthracene.[12] The third and fourth transitions are clearly like those in pyrene and should have the same polarization direction and band assignments.

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ANNOTATIONS

THE POSSIBILITY AND ACCURACY OF POTENTIOMETRIC EQUILIBRIUM STUDIES AT VERY HIGH LIGAND TO METAL CONCENTRATION RATIOS

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Summary—The possibility and accuracy of potentiometric equilibrium studies are analysed by use of the rules of the implicit function-systems for the mass-balance equations. The conditions necessary for the use of potentiometry at very high ligand to metal concentration ratios are briefly outlined. The effect of the total ligand (glycine) concentration on the measured pK is studied experimentally and interpreted in terms of the change in the difference in diffusion potential between the reference half-cell and the solution.

For our NMR relaxation studies on transition metal complexes in solution,[1–3] knowledge of the composition and stability of the complexes formed is a prerequisite. The equilibrium relations of the complexes of vanadium(IV) and chromium(II) with nitrogen-donor ligands have had to be studied at high ligand excess, to avoid hydrolysis of the metal ions.

It seems almost a general belief that the use of pH-titration methods should be restricted to comparatively low (from 1:1 to ~20:1) ligand to metal concentration ratios. The work of Ciavatta *et al.*,[4] who studied the nickel(II)–glycine system in 1M glycine medium, is an exception.

Several papers[5–8] deal with the theory and accuracy of the pH-titration determination of protonation and stability constants, but none deals with the use of the method at high ligand excess. This and our own needs led us to examine the situation. The results are summarized in this paper.

MATHEMATICAL CONSIDERATIONS

For simplicity, a system containing only HA and MA species besides the components H, A and M is analysed, for which the mass-balances are

$$T_H = [HA] + [H] - [OH]$$
$$= \beta_{HA}[H][A] + [H] - K_w/[H]$$
$$T_A = [HA] + [MA] + [A]$$
$$= \beta_{HA}[H][A] + \beta_{MA}[M][A] + [A]$$
$$T_M = [M] + [MA] = \beta_{MA}[M][A] + [M]$$

where

$$\beta_{HA} = [HA]/[H][A]$$

and

$$\beta_{MA} = [MA]/[M][A].$$

T_H, T_A and T_M are the total concentrations of proton, ligand and metal ion respectively. The criteria for the applicability of pH titration are the $\partial \log \beta_{MA}/\partial pH$ and $\partial \log \beta_{MA}/\partial T_H$ derivatives, which express the effect of unit error in pH or T_H on the accuracy of $\log \beta_{MA}$. These derivatives may be obtained by applying the rule of differentiation of implicit-function systems to the mass-balance equations, as done earlier for homogeneous equilibrium systems.[9,10]

$$\frac{\partial \log \beta_{MA}}{\partial pH} = -\frac{[H] + [OH]}{\bar{n}(1-\bar{n})T_M} - \left(1 + \frac{[H] + [OH]}{[HA]}\right) \times \left(1 + \frac{[A]}{\bar{n}(1-\bar{n})T_M}\right) \quad (1)$$

$$\frac{\partial \log \beta_{MA}}{\partial T_H} = -\frac{0.4343}{[HA]} - \frac{0.4343(T_A - \bar{n}T_M)}{\bar{n}(1-\bar{n})[HA]T_M} \quad (2)$$

where $\bar{n} = [MA]/T_M$. (The details of the mathematically simple but rather lengthy derivation are available for the interested reader on request.)

Representative results of calculations based on these equations are illustrated in Figs. 1–3. Figure 1 shows the $\partial \log \beta_{MA}/\partial pH$ functions for $\log \beta_{HA} = 12$, $\log \beta_{MA} = 8$ and $T_M = 0.001M$, at different T_A/T_M ratios. It is seen that increasing the T_A/T_M ratio shifts the measurable region of complex formation towards lower pH-values, but the experimental data between

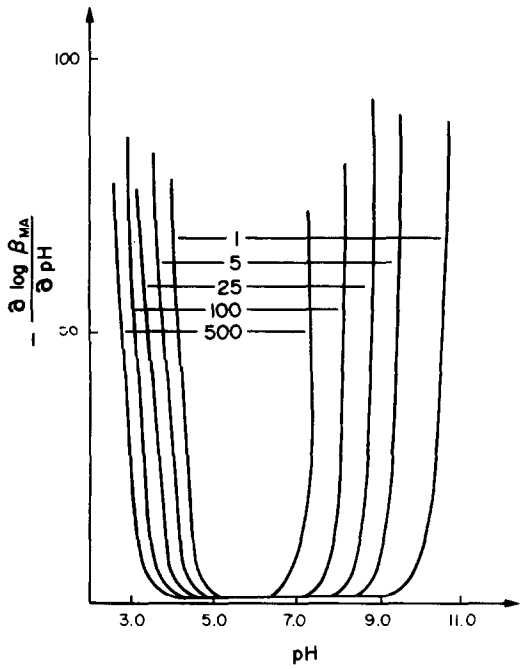

Fig. 1. $\partial \log \beta_{MA}/\partial pH$ as a function of pH at different T_A/T_M concentration ratios: $\log \beta_{HA} = 12$, $\log \beta_{MA} = 8$, $T_M = 0.001M$. The numbers on the arrows are the T_A/T_M ratios.

pH 4 and 6 may be evaluated for $\log \beta_{MA}$ even with 500-fold ligand excess.

The $\partial \log \beta_{MA}/\partial pH$ function for various $\log \beta_{HA}$ values, a fixed value for the equilibrium constant for $HA + M \rightleftharpoons MA + H$, and a 250-fold ligand excess is shown in Fig. 2.

It is seen that the extremely high ligand excess can only be used if $\log \beta_{HA} > 7$, *i.e.*, with ligands containing aliphatic amine or phenolic hydroxyl groups.

Figure 3 shows that the ligand excess has no significant effect on the $\partial \log \beta_{MA}/\partial T_H$ function, *i.e.*, the error in the volume or concentration of the titrant is almost the same at low and high ligand to metal concentration ratio.

On the basis of these results, the following conclusions can be drawn.

(1) The ligand used to make up the solutions to be titrated should be in a single stoichiometric form H_jA. Thus, even if there is a small error (some tenths of 1%) in the initial total ligand (T_A^0) and proton (T_H^0) concentrations, if this error is comparable with T_M^0 (the initial total metal concentration), their difference ($T_H^0 - jT_A^0$) should be negligible in comparison with the total metal concentration. If a metal–ammine complex is to be studied for example, the initial solution should be made up with ammonium chloride rather than from aqueous ammonia solution and hydrochloric acid.

(2) The complex formation should take place in a pH-region where protonation of the ligand does not have a significant buffer effect.

Besides these conclusions drawn from the mathematical analysis, the following chemically evident considerations should be regarded.

(1) The ligand should be extremely pure, since a very small percentage of complex-forming impurities may cause a completely erroneous result.

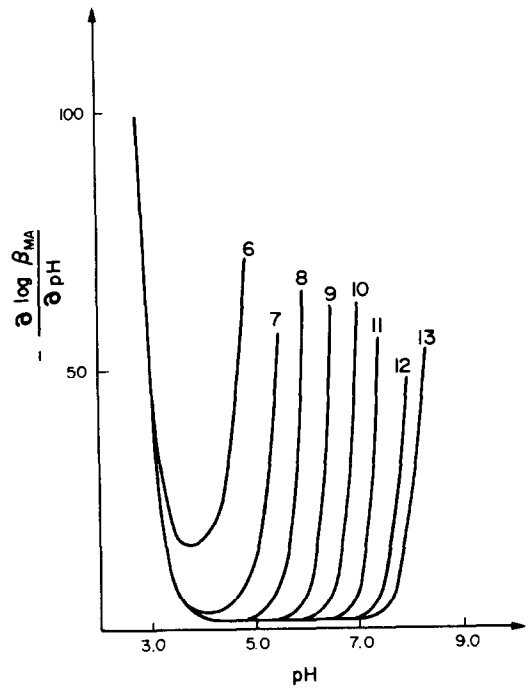

Fig. 2. $\partial \log \beta_{MA}/\partial pH$ for 250-fold ligand excess and different $\log \beta_{HA}$ values. The equilibrium constant for $HA + M \rightleftharpoons MA + H$ is the same (10^{-4}) in all cases.

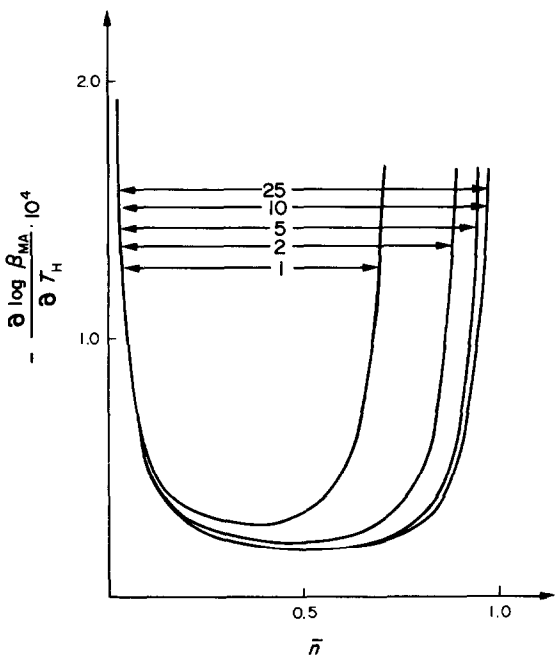

Fig. 3. $\partial \log \beta_{MA}/\partial T_H$ as a function of $\bar{n}$ at different T_A/T_M concentration ratios.

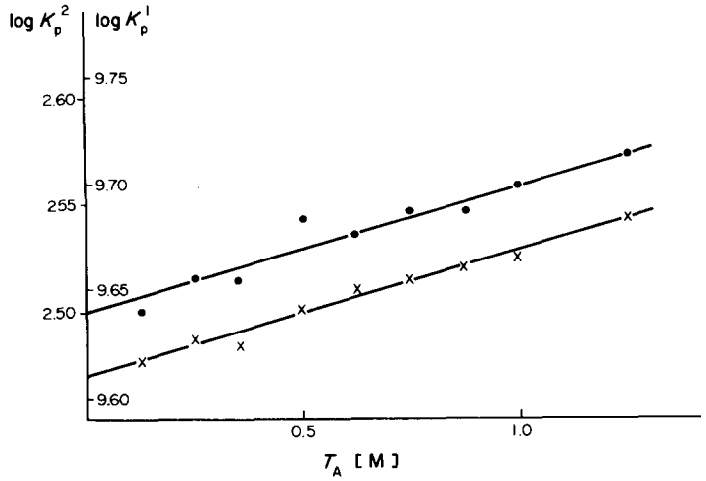

Fig. 4. The protonation constants of glycine as a function of the total glycine concentration. $I = 1M$ $NaClO_4$; 25°C.

(2) At high ligand excess, the ligand concentration may become comparable with the concentration of electrolyte added to ensure constant ionic strength, so the constancy of the standard state may be questionable.

These conditions have been investigated by measuring the pK values of glycine in 1M sodium perchlorate at total glycine concentrations up to 1.25M.

EXPERIMENTAL

Determination of the protonation constants of glycine

The titrations were at 25 ± 0.1°, with a Radiometer PHM-52 type pH-meter and GK 2301 combined electrode filled with saturated sodium chloride solution instead of potassium chloride solution in the calomel section. The glycine (Reanal) was recrystallized twice from aqueous ethanol. The sodium perchlorate was prepared from Merck p.a. sodium hydroxide and perchloric acid. The electrode system was calibrated for $-\log[H^+]$, according to Irving *et al.*,[6] from the perchloric acid-sodium hydroxide titration curve in 1M sodium perchlorate medium.

Table 1. Composition of the solutions studied for the determination of the protonation constants of glycine, and the result of the pH-titration investigation

T_H, M	T_A, M	log K_p^1	pH-range	log K_p^2	pH-range
0.1746	0.1249	9.640	8.11–9.05	2.477	2.69–3.92
0.2995	0.2498	9.655	8.31–8.76	2.486	3.10–4.24
0.3974	0.3477	9.654	8.13–8.85	2.484	3.30–4.41
0.5492	0.4995	9.683	7.76–8.70	2.500	3.45–4.54
0.6741	0.6244	9.675	8.00–8.43	2.510	3.57–4.14
0.7990	0.7493	9.686	7.58–8.59	2.514	3.66–4.85
0.9239	0.8742	9.686	8.08–8.47	2.520	3.74–4.81
1.0488	0.9991	9.699	7.91–8.52	2.524	3.79–4.90
1.2987	1.2490	9.713	7.66–8.44	2.533	3.91–4.98

$$K_p^1 = \frac{[HA^\pm]}{[H^+][A^-]}; \quad K_p^2 = \frac{[H_2A^+]}{[H^+][HA^\pm]}.$$

The standard deviation of individual log K_p values is less than 0.003 in all cases.

RESULTS

The initial concentrations of the 25-ml samples are collected in Table 1, together with the pH-ranges which were measured and used to calculate the two protonation constants. The dependence of the constants on the total glycine concentration is shown in Fig. 4.

The standard deviation (there was no bias) of the constants showed that any acid–base impurity in the glycine was below 0.01%, otherwise it would not have been possible to obtain self-consistent pK values from the pH-ranges far away from the half-neutralization points. It is seen, moreover, that both protonation constants change in the same way as a function of the total glycine concentration. There may be two reasons for a change in the observed pK:

(*i*) a change in the activity coefficients, *i.e.*, the standard state is no longer constant;

(*ii*) a change in the diffusion potential between the calomel electrode (filled with saturated sodium chloride solution) and the solution studied, as a consequence of the change in the total glycine concentration.

In case (*i*) the two pK values would change differently, because the change in charge in the two processes are basically different:

$$H^+ + A^- \rightleftharpoons HA^\pm$$
$$HA^\pm + H^+ \rightleftharpoons H_2A^+$$

A completely different dependence of the two pK values on the ionic strength was found earlier.[11]

In case (*ii*), however, parallel changes would be expected and were found. Thus it may be concluded that in the case of glycine there is no change in the

standard state even if the concentrations of the supporting electrolyte and the ligand are comparable.

Change in the diffusion potential should be taken into account, however, when the metal complexes are studied. The simplest way to do this is to use the pK values extrapolated to $T_A \rightarrow 0$, and to correct the measured pH with an appropriate factor read directly from Fig. 4. This procedure was used during the pH-titration study of the VO^{2+}–glycine system, the result of which will be given in a succeeding paper.

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COPPER(I) ACTIVITY OF THE COPPER(II) ION-SELECTIVE ELECTRODE

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Summary—The Orion copper(II) ion-selective electrode responds well to copper(II) ions in aqueous medium. However, in the presence of acetonitrile and copper(I) ions, it can behave as a copper(I) ion-selective electrode with Nernstian behaviour.

The Orion cupric ion-selective electrode seems to be well suited to the determination of stability constants of copper(II) complexes,[1] as long as the ligands are not too negatively charged.[2] Hence this electrode was used in an investigation of complex formation in aqueous solution by 5-sulphonatoisatin β-thiosemicarbazone.[3,4] However, the parameters pCu, pH and pL could not be combined into stability constants. The thiosemicarbazone reduced copper(II) to copper(I). An assumption that the electrode responded to free copper(I) concentration seemed to fit with the data. This agreed with theoretical considerations[2,5] which showed that the solid-state copper electrode is essentially a copper(I)-sensitive electrode. Experimental confirmation of the behaviour of the electrode towards copper(I) was sought by using acetonitrile as ligand, since this is known to stabilize copper(I).

EXPERIMENTAL

Materials

Potassium nitrate ("AnalaR") was recrystallized from water. Acetonitrile ("Ajax Unichrom") was used without further purification. $Cu(CH_3CN)_4ClO_4$ was synthesized by adding sodium perchlorate to an equilibrated mixture of copper(II) nitrate and copper metal in acetonitrile.[6] The white precipitate was recrystallized from acetonitrile.

Instrumentation and measurements

The pCu values were determined with the electrode assembly Cu-electrode (Orion 94-29A) ‖ solution | 1.8*M* KNO_3 + 1.8*M* KCl | 3.5 *M* KCl | calomel. A glass electrode (EIL all-purpose standard) was used for pH measurements,[7] with the same (external) reference electrode. The electrodes were connected to an Orion 801A pH-meter through an Orion 605 manual electrode switch.

The measurements were carried out in aqueous 0.15*M* potassium nitrate at 37° with constant stirring. High-purity nitrogen was bubbled through a solution of cuprous chloride in 1*M* hydrochloric acid, then 2*M* potassium hydroxide and water [where appropriate, containing copper(I) and acetonitrile]. The copper electrode was routinely calibrated overnight with $10^{-4}M$ copper nitrate in 0.15*M* potassium nitrate at pH 4. The Nernst equation

$$E = E^0 + \frac{RT}{nF} \ln[Cu^{2+}]$$

correlates the measured potential E with the copper(II) concentration. E^0 was obtained from

$$E^0 = E + (S/2)\,pCu^{2+}$$

with $pCu^{2+} = -\log[Cu^{2+}]$ and $S = 61.6$ mV. It varied from 295 to 300 mV. The difference between the E values for 10^{-4} and $10^{-2}M$ copper nitrate was 61.0 mV, which is near to the expected 61.6 mV. Hence, there was no interference[5] from the trace of chloride leaking out of the salt bridge. Additionally, alkalimetric titrations of solutions containing copper(II) and excess of histidine or glycine were performed to check the response of the electrode (stability constants for the copper histidine and glycine complexes were taken from Stünzi and Perrin[8]). From pH 2.5 to 10 and for pCu^{2+} values between 4 and 17.5, the experimental and calculated pCu^{2+} values agreed to within 0.07. (The pH-dependence of the cupric ion-selective electrode seems to be less serious than suggested by Sekerka and Lechner.[9])

In agreement with Westall *et al.*,[5] interference lowered the measured potential in copper(II) solutions with acetonitrile concentrations above 0.9*M*.

RESULTS AND DISCUSSION

The behaviour of the Orion cupric ion-selective electrode towards copper(I) ions was investigated. Titrations were carried out in aqueous solutions containing acetonitrile. To solutions 0.2–2*M* in acetonitrile [$I = 0.15M$ (KNO_3), 37°, pH 4] were added increments of a freshly prepared solution of $Cu(CH_3CN)_4ClO_4$ (0.1*M*) in acetonitrile. The potential became stable within 1–10 min. The corresponding pCu^+ values were calculated from the total acetonitrile concentrations and from the stability constants for the copper(I) acetonitrile complexes: $\log K_1 = 3.28$, $\log \beta_2 = 4.35$ and $\log \beta_3 = 4.39$.[10] The stability constants (measured in 0.2*M* $NaNO_3$ medium at 20°) were used without correction for the change in experimental conditions. Thus, $pCu^+ = -\log[Cu^+]$ was obtained from the equation

$$[Cu^+] = [Cu^+]_{tot}/(1 + K_1[L] + \beta_2[L]^2 + \beta_3[L]^3)$$

where $[Cu^+]$ and $[L]$ are the free copper and acetonitrile concentrations and $[Cu^+]_{tot}$ is the total copper concentration. Assuming Nernstian behaviour ($S = 61.6$ mV) an $E^0(Cu^+)$ value was calculated:

$$E^0(Cu^+) = E + S pCu^+$$

Table 1. Titration of 50 ml of 0.38*M* acetonitrile with 0.1*M* $Cu(CH_3CN)_4ClO_4$ in acetonitrile [I = 0.15*M*(KNO_3), 37.0°, pH 4]

ml added,	$[CH_3CN]$, *M*	$[Cu^+]_{tot}$, m*M*	pCu^+*	*E*, *mV*	$E^0(Cu^+)$, *mV*
0.05	0.40	0.10	7.78	210.5	690.9
0.10	0.42	0.20	7.52	227.2	691.7
0.20	0.45	0.39	7.30	240.7	691.7
0.40	0.53	0.78	7.14	249.9	691.3
0.80	0.67	1.54	7.09	253.1	691.1

* Calculated (see text).

The $E^0(Cu^+)$ values were reasonably constant throughout a titration (Table 1), indicating Nernstian response of the copper electrode to copper(I) ion.

In order to make the result independent of the reference electrode, the difference between $E^0(Cu^+)$ and $E^0(Cu^{2+})$ is used. Table 2 gives the summary of the experiments done. At acetonitrile concentrations between 0.1 and 0.3*M* the readings were unstable and could only be read within ±3 mV. The decrease in the potentials with time was indicative of oxidation and/or disproportionation. Oxidation was evident from the increase of the pH values. The stability of the system improved at 0.4*M* acetonitrile concentration.

For acetonitrile concentrations between 0.4 and 2*M*, the result was

$$E^0(Cu^+) = E^0(Cu^{2+}) + 399 \text{ mV}$$

The response was good down to $10^{-4}M$ total copper concentration and was still satisfactory at $10^{-5}M$ (Table 2).

If the measured potentials have already been converted into "pCu^{2+}" values, the pCu^+ values are obtained from

$$pCu^+ = \tfrac{1}{2}\text{"}pCu^{2+}\text{"} + 6.48$$

For solutions containing Cu^{2+} and Cu^+ a mixed potential would be expected:

$$E = E^0(Cu^+) + S\log([Cu^+] + K[Cu^{2+}]^{\frac{1}{2}})$$

with $\log K = -6.48$, where $\log K$ is the selectivity constant. The Orion cupric ion selective electrode is hence essentially a copper(I) electrode. This is consistent with its composition: a silver-contacted Ag_2S–Cu_2S membrane.[11] A similar selectivity $\log K = -5.9$)[12] has been found for a copper electrode with a Cu_2S membrane. It was assumed that the sensitivity to Cu^{2+} was the result of the exchange reaction

$$Cu^{2+} + Cu_2S = CuS + 2\,Cu^+$$

Thus, $\log K$ was calculated to be -6.6,[12] and -6.2.[13] This suggests that the Orion electrode responds to Cu^{2+} by the same exchange reaction.

Addition of copper(II) at the end of the titrations did not change the observed potential significantly although a change of 18 mV would have been expected for $pCu^+ \simeq 7.8$ and $[Cu^{2+}] = 2mM$. This insensitivity towards Cu^{2+} is surprising in view of the generally accepted assumption that acetonitrile does not complex copper(II). It is, however, in agreement with the observation that with low concentrations of Cu^{2+} in aqueous acetonitrile the potentials are too small. This "interference" with the Ag/Ag_2S–Cu_2S electrode has not yet been explained. (For the graphite-contacted Ag_2S–CuS electrode, however, the mechanism of interference is discussed in refs. 5 and 14.)

Table 2. Summary of experiments done

$[Cu^+]_{tot}$*, m*M*	$[CH_3CN]$, *M*	δE^0†, *mV*	pCu^+	δE^0† at $10^{-5}M$ Cu^+
0.6–1.3	0.1–0.3	387	6.5	349
0.1–1.5	0.4–0.7	396	7.1–7.8	—
0.06–1.1	1.0–1.1	399	7.8–8.8	396§
0.08–1.9	1.0–1.2	402	7.7–8.8	390
0.08–2.5	2.0–2.2	398	8.2–9.5	403
30#	2.0	401	6.9	—

* Readings considered reliable.
† $\delta E^0 = E^0(Cu^+) - E^0(Cu^{2+})$.
§ $2 \times 10^{-5}M$.
# $Cu(NO_3)_2$ and Cu in the titration vessel.

Conclusion

Under conditions where copper(I) is stable, the Orion cupric ion selective electrode behaves as a copper(I) electrode. The response is Nernstian within the experimental precision. Hence, this electrode appears to be suitable for studying the stability of copper(I) complexes with ligands that preferentially stabilize this ion (*e.g.*, sulphur-containing ligands).[15]

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HYDRAZONES AS ANALYTICAL REAGENTS: A REVIEW

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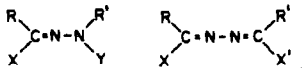
Summary—Applications of hydrazones in inorganic analysis since 1950 are reviewed.

Hydrazones are azomethines characterized by the grouping >C=N–N<. They are distinguished from other members of this class (imines, oximes, *etc.*) by the presence of the two interlinked nitrogen atoms. The hydrazone grouping occurs in organic compounds of the types:



(I) (II)

where R and R′ = H, Alk, Ar, RCO, Ht (heterocyclic group); Y = H, Alk, Ar, Ht, RCO; X and X′ = H, Alk, Ar, Ht, Hal, OR″, SR, CN, SO_2R, NO_2, NHNR″R‴, N = NR″, COOR″, CONR″R‴. The general name "hydrazone" is used for all the compounds having structure (I). Compounds of type (II) are termed "azines".

Nomenclature

Hydrazones are usually named after the carbonyl compounds from which they are derived; thus benzaldehyde and phenylhydrazine give benzaldehyde phenylhydrazone. The name originally used was benzylidenephenylhydrazine. Some authors have recently reverted to this system, which is, however, cumbersome when applied to more complex hydrazones. Bishydrazones of α-diketones are commonly called osazones. The nomenclature commonly used in the literature is often not in accord with IUPAC rules.

Preparation

Hydrazones, in general, are prepared by refluxing the stoichiometric amounts of the appropriate hydrazine and aldehyde or ketone dissolved in a suitable solvent. The compound usually crystallizes out on cooling. Detailed accounts of their preparations are given in a recent review.[1] Many hydrazones are now commercially available.

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Non-analytical applications

Many of the physiologically active hydrazones find application[2] in the treatment of diseases such as tuberculosis, leprosy and mental disorder, and aroylhydrazones (III) are also reported to possess tuberculostatic activity.[3,4] This is attributed to the formation of stable chelates with transition metals present in the cell.

R–CH=N–NH–CO–R′
(III)

Thus many vital enzymatic reactions catalysed by these transition metals cannot take place[5–7] in presence of hydrazones. Hydrazones also act as herbicides, insecticides, nematocides, rodenticides and plant-growth regulators. They show spasmolytic activity, hypotensive action and activity against leukaemia, sarcomas and other malignant neoplasms. Hydrazones are used as plasticizers and stabilizers for polymers and as polymerization initiators, antioxidants, *etc.* Hydrazones of 2-methylphthalazone[8] are effective sterilants for houseflies. 3-*N*-Methyl-*N*-(4-chloro-1-phthalazinyl) and 3-*N*-methyl-*N*-(4-oxo-1-phthalazinyl) hydrazones possess anthelmintic activity.[9]

ANALYTICAL APPLICATIONS

In analytical chemistry, the formation of hydrazones is extensively used in the detection, determination and isolation of compounds containing the carbonyl group. Photometric methods for determining aldehydes and ketones are based on their reaction with 2,4-dinitrophenylhydrazine to form the corresponding hydrazones.[10,11]

Spectrophotometric applications

Biscyclohexanone oxalyldihydrazone was one of the earliest used hydrazones for the spectrophotometric determination of copper. It gives a blue colour with traces of copper and is used for determination of copper in paper pulp products,[12] human serum,[13]

steel,[14,15] plants,[16,17] non-ferrous metals and alloys[18–20] and cadmium sulphide.[21] Ethylidene oxalyldihydrazone[22] has been used to determine copper in zinc and its alloys. A similar type of reagent, bis(ethylacetoacetate) oxalyldihydrazone,[23] is very selective and sensitive for copper. Oxalyldihydrazone has been used in presence of acetaldehyde for determination of copper.[24,25]

Lions and Martin[26] introduced pyridine-2-aldehyde 2-pyridylhydrazone (PAPH) and similar types of hydrazones and have made detailed physico-chemical investigations on their metal complexes.[27–29] This has opened a wide field for their use in analytical chemistry, which began in 1963 when Cameron *et al.*[30] published a survey of the visible spectra of aqueous solutions containing metal ions and PAPH. They stated that PAPH should be useful as a colorimetric reagent and as an acid–base indicator. PAPH has been used for the determination of copper in foodstuffs.[31] Quddus and Bell[32] have studied the complexes formed between PAPH and Zn, Cd, Fe(II), Cu(II), Ni(II), Mn(II) and Pd. Distribution ratios of the complexes between chloroform and buffer solutions of various pH values have been determined. The sensitivity is increased by extraction. The analogous picolinaldehyde 2-pyridylhydrazone reacts with palladium(II)[33–35] and forms a ternary complex with cobalt(II) and eosin.[36]

2,2′-Bipyridyl 2-pyridylhydrazone (BPPH)[37] reacts with cobalt(II) to form an orange complex (λ_{max} 480 nm) which shows a bathochromic shift on addition of perchloric acid (λ_{max} 500 nm), giving a pink complex which is stable even in 50% perchloric acid medium. This reaction has been utilized in the spectrophotometric determination of cyanocobalamine[38] and of cobalt in sea-water and brine.[39] BPPH also reacts with iron(II),[40] zinc[41] and cadmium.[42]

2-Benzoylpyridine 2-pyridylhydrazone reacts with Fe(II), Co(II), Ni and Zn.[43] The reagent has also been sulphonated[44] and used for the preconcentration of trace metal ions by combined complexation and anion-exchange.[45] Asuero[46] introduced biacetyl bis(2-pyridyl)hydrazone as an analytical reagent and its reactions with several metal ions have been reported. Benzil mono(2-pyridylhydrazone)[47] reacts selectively with cobalt(II) in acid medium. The corresponding bishydrazone and the mono and bis(2-pyridylhydrazone)s of 2,2′-pyridil react with iron(II), copper(I), cobalt(II) and nickel.[48]

Two new pyridylhydrazones[49] have been prepared from quinoline-2-aldehyde and phenanthridine-6-aldehyde (PDAPH) and the latter has been used for the spectrophotometric determination of zinc.[50] Other pyridylhydrazones investigated include those of bi-2-quinolyl ketone for [Pd(II)][51] and biacetyl (monohydrazone) [for Co(II)].[52]

In investigation of the effect of substitution of a more extended π-system in the hydrazine moiety of nitrogen-heterocycle hydrazones, a new heterocyclic hydrazone, pyridine-2-aldehyde 2-quinolylhydrazone (PAQH) was introduced by Heit and Ryan.[53] Cobalt and nickel were selectively determined with it spectrophotometrically.[54] In the presence of thioglycollic acid, only the nickel complex is extractable into chloroform. The reagent can also be used for the determination of nickel in sea-water.[55] Prior concentration of the metal is not necessary. The reagent also complexes iron(II), copper(II)[53,56] and palladium(II).[57–59] Quinoline-2-aldehyde 2-quinolylhydrazone has been used for the determination of copper in sea-water[60,61] and tap-water.[62]

Other quinolylhydrazones investigated include those of 6-methylpicolinaldehyde [Pd(II)],[63] phenylpyruvic acid [Cu(II)][64], 2,2′-bipyridyl [V(V),[65] Zn,[66] Co(II),[67] Pd(II),[68,69] Rh(III)[70] and Fe(II), Ni, Cu(II), Cd, Hg(II)[71]], benzothiazole-2-aldehyde [Pd(II),[72] Cu(II)[73]], biacetyl (monohydrazone) [Co(II), Cu(II)],[74] benzil (monohydrazone) [Cu(II)][75], 2,2′-pyridil (bishydrazone) [Co(II),[76] Pd(II),[77] Cu(II), Zn, Cd, Rh(III), Hg(II)[78]], and monohydrazone [Zn, Cd, Hg(II),[78] Cu(II),[79] Rh(III),[80] Pd(II),[81] Fe(II),[82] Co(II), Ni[83]].

Frei *et al.*[84] have studied the extraction properties of chelates of Ni, Cu(II), Co(III), Fe(II), Zn and Cd with PAQH. Of the three organic solvents investigated (benzene, isoamyl alcohol and methyl isobutyl ketone) the last two gave solutions suitable for direct aspiration into the flame (for atomic-absorption spectroscopy) after an equal volume of ethanol had been added. The charged cobalt complex is believed to be extractable, under suitable conditions, as an ion-pair. The other extractable compounds are 1:2 (metal:hydrazone) chelates.

Singh and co-workers[71,85–89] introduced 2,2′-bipyridyl 2-pyrimidylhydrazone as an analytical reagent for V(V),[85] Co(II),[86] Zn,[87] Fe(II),[88] Pd(II)[89] and Ni, Cu(II), Cd and Rh(III)[71] and claimed it had advantages over the pyridyl and quinolylhydrazones.

A series of 2-benzothiazolylhydrazones have been described [benzothiazole-2-aldehyde for Cu(II), Co(II), Ni;[90] thiophene-2-aldehyde for Cu(II);[91] 5-chlorothiophene-2-aldehyde for Co(II),[92] Cu(II);[93] 2-furfural for Cu(II), Ag, Co(II), Hg(II), Ni, Zn;[94] 5-methylfurfural for Co(II);[95] 1-naphthaldehyde for Cu(II);[89] biacetylmonoxime for Pd(II);[96] 2-hydroxy-1-naphthaldehyde for Cu(II);[97] pyridine-2-aldehyde for Cu(II), Ni, Co(II), Pd(II), Fe(III);[53] pyruvaldehyde for Cd].[98] Libergott *et al.*[99] used isatin-2-benzothiazolylhydrazone for the determination of lead in plastic milk cartons.

Salicylaldehyde isonicotinoylhydrazone[100] was used for the spectrophotometric determination of gallium and indium at pH 6–6.5 (λ_{max} 390 and 380 nm). These complexes are extractable into pentanol. Since the aluminium complex is not extractable, it does not interfere. 2-Hydroxy-1-naphthaldehyde isonicotinoylhydrazone[101] has been used for the colorimetric determination of iron(II) and (III) in presence of each

other and other metals, and applied to various pharmaceuticals, and for Mo(VI) in steels.[102]

The complexes formed by vanadium(V) in acidic 50% aqueous ethanol medium with acetone isonicotinoylhydrazone and with 4-hydroxybenzaldehyde isonicotinoylhydrazone have been examined,[103] and used for spectrophotometric determination of vanadium. The 2-hydroxy isomer has been used for Al,[104] and Zn, Co(II), Ni and Mn(II).[105] The gossypol derivative reacts with U(VI)[106] and Ti(IV).[107] *o*-Hydroxybenzaldehyde benzoylhydrazone reacts with zinc and manganese(II);[105] the hydrazone of isonicotinic acid and naphthyl methyl ketone reacts with titanium(IV).[108]

A number of simple hydrazones have been examined: of salicylaldehyde for Fe(III),[109] Co(II),[109] Cu(II),[110] Pd(II),[111] Os(VIII);[111] of *o*-hydroxyacetophenone for Ni;[112] of 6-methylpicolinaldehyde for Cu(I);[113] of 2-benzoylpyridine for Fe(II);[114] of biacetylazine for Cu(I);[115] of bis(6-methyl-2-pyridyl)glyoxal dihydrazone for Cu(I).[116]

Recently Schilt *et al.*[117–119] have introduced a large number of ferroin-type hydrazones and tabulated properties of their complexes with Cu(I), Fe(II), Co(II) and Ni.

Several new hydrazones are prepared *in situ* and applied for the spectrophotometric determination of metal ions. The product (λ_{max} 490 nm) which results from the interaction of biacetyl, hydrazine and iron(II) has been applied to the colorimetric estimation of iron.[120,121] The interferences are the same as in the determination of iron(II) with 1,10-phenanthroline and 2,2′-bipyridyl, but the method is cheap and simple. Mohr's salt behaves differently from ferrous sulphate, owing to the reaction of the ammonium ion with the biacetyl. Zinc, cadmium and aluminium can be tolerated in large amounts, and addition of EDTA and citrate masks many possible interferents. Iron(III) can be determined after reduction to iron(II). The complex can also be extracted into nitrobenzene.

The coloured complex formed by Fe(II) and α-pyridyldihydrazone (used as such or synthesized *in situ*), has been investigated.[122] The complex can be extracted into nitrobenzene and its absorbance measured at 486 nm. Mn(II), Al and Cd do not interfere but ions which complex Fe(II) interfere seriously. Other ferroin-type reagents used for traces of iron[123] are 2,2′-bipyridylglyoxal dihydrazone, biacetyl dihydrazone and phenyl 2-pyridyl ketone hydrazone.

The bis(phenylhydrazone) of oxamide is oxidized by Cu(II), Hg(II), Fe(III), $Fe(CN)_6^{3-}$ or chlorine water to give violet or blue species extractable into chloroform. Based on this phenomenon, photometric methods have been developed[124] for the microdetermination of these ions or chlorine and also for determination of the hydrazones by means of ferricyanide. Bahr *et al.*[125] used biacetyl di(thiobenzoylhydrazone) for the photometric determination of copper.

The bis(4-hydroxybenzoylhydrazone)s[126] of glyoxal, methylglyoxal and dimethylglyoxal form coloured chelates with several cations. The glyoxal derivative has been proposed as a reagent for the determination of calcium and cadmium (in the presence of cyanide or citrate, respectively, when other cations are present).

Miscellaneous reagents include 2,2′-bipyridyl phenylhydrazone for Pd(II);[127] pyridoin phenylhydrazone for Cu(I)[128] and Pd(II);[129] picolinaldehyde 4-nitrophenylhydrazone for Pd(II);[130] biacetylmonoxime *p*-nitrophenylhydrazone for Co(II);[131,132] pyridine-2-carbaldehyde 2-hydroxybenzoylhydrazone for Ni and Zn,[133] and for Fe(II)[134] and V(V);[135] benzil bis(2-hydroxybenzoylhydrazone) for Ti(IV);[136] salicylaldehyde benzoylhydrazone for Cu(II) and Pd(II);[137] picolinaldehyde 1-thionaphthylhydrazone[138] and biacetyl bis(thiobenzoylhydrazone)[125] for Cu(II); the 2-(2-hydroxyphenylhydrazone) of ethyl 2,3-dihydroxybutyrate for Co(II);[139] anthranilic acid isopropylidenehydrazide for V(V);[140,141] 2-methylisonicotinic acid salicylidenehydrazide for Ti(IV);[142] 6-methyl-2-pyridylaldehyde 2-quinolylhydrazide for Cu(II)[143] and di-2-pyridyl ketone 2-furancarbothiohydrazone[144] for Cu(II), Ni, Co(II), Fe(II) and Re(VII).

Fluorimetric applications

The fluorescence of the zinc complex formed at pH 8 with PAQH in chloroform has been measured at 535 nm with excitation at 485 nm, to determine 0.026–0.31 ppm of zinc.[145] The main interference is from Co(II), Cd, Cu(II), Hg(II), Fe(II), Ni, CN^- and SCN^-; in their absence the accuracy is high.

Organothiophosphorus pesticides have been determined by fluorimetry *in situ* after TLC separation on silica gel.[146] The process involves bromination and spraying with a mixture of Mn(II) and salicyl-2-aldehyde 2-quinolylhydrazone. A direct relationship exists between the sensitivity and the number and oxidation state of the sulphur atoms in the pesticides.

The fluorescence of the isonicotinic acid hydrazones of a number of carbonyl compounds (2-hydroxy-1-naphthaldehyde, salicylaldehyde, 2-hydroxy-*m*-tolualdehyde, 3-hydroxy-*p*-tolualdehyde, 4-hydroxy-*m*-tolualdehyde, 3-chloro-2-hydroxybenzaldehyde, 5- chloro-2-hydroxybenzaldehyde and 2-hydroxyacetophenone) has been examined.[147] In the presence of aluminium, these hydrazones give a yellowish-green fluorescence in an acetate buffer, while under similar conditions the parent carbonyls exhibit only feeble fluorescence. The fluorescence intensity of the hydrazone of 2-hydroxy-1-naphthaldehyde (in the presence of aluminium) is particularly strong, and the aldehyde is found to be a good reagent for the fluorimetric determination of 0.1–1 ppm of isonicotinic acid hydrazide.

Four derivatives of 2-hydroxy-1-naphthaldehyde hydrazone have been synthesized, and the fluorescence due to the reaction between these hydrazones and various metal ions indicates[148] that they are use-

ful for the detection of aluminium. 2-Hydroxy-1-naphthaldehyde benzoylhydrazone gives the strongest fluorescence. A fluorimetric method for the determination of 0.1–1 ppm of aluminium with this reagent at pH 4.6 (acetate buffer) in a mixed solvent medium of methanol and dimethylformamide has been established.

In alkaline medium (preferably 0.1*M* potassium hydroxide), calcium forms a fluorescent 1:1 complex with 8-hydroxyquinaldehyde 8-quinolylhydrazone.[149] The method has been used for determination of down to 0.1 ppm of calcium in potassium chloride and methyltrichlorosilane. The fluorescence is measured at about 510 nm (with excitation at around 420 nm). Ivanova *et al.*[150] used this reagent for fluorimetric determination of traces of calcium in alkali-metal chlorides and iodides.

The zinc, aluminium, scandium and gallium complexes of β-resorcylaldehyde acetylhydrazone[151] exhibit blue fluorescence. The fluorescence intensity of the scandium complex is appreciable (excitation at 406 nm). The reaction is used for the determination of scandium (1–18 μg) in acetate buffer medium (pH 6). Chromium, nickel, cobalt and iron(III) interfere by decreasing the fluorescence intensity and zinc and aluminium by increasing it.

The most sensitive reagent for the fluorimetric determination of aluminium is salicylaldehyde formylhydrazone,[152] but the fluorescence intensity takes at least 7 min to become stable; 0.03–0.88 ppm of aluminium can be determined with a relative error of $< 1\%$.

Taniguchi *et al.*[153] used 2-hydroxy-1-naphthaldehyde benzoylhydrazone for the fluorimetric titration of copper(II). The 2-hydroxy-1-naphthylmethylene hydrazide of 4-methoxybenzoic acid was used by Dolgorev *et al.*[154] for the fluorimetric determination of scandium in rare-earth oxides. Recently bipyridylglyoxal diphenylhydrazone was used for the fluorimetric determination of gold(III).[155]

Heterocyclic hydrazones of *o*-hydroxyaldehydes have been studied[156] as reagents. They are promising for Zn, Al, Ga, In, Sc, *etc.* Salicylaldehyde 2-quinolylhydrazone (SAQH) and β-resorcylaldehyde 2-quinolylhydrazone (RAQH) are suitable for the fluorimetric determination of zinc in 80% ethanol and tris-buffer (pH 6.9–7.2). Salicylaldehyde 2-pyridylhydrazone (SAPH), β-resorcylaldehyde 2-pyridylhydrazone (RAPH) and 2-hydroxy-1-naphthaldehyde 2-pyridylhydrazone (HNAPH) are suitable for the fluorimetric determination of aluminium in 70–80% ethanol and acetate buffer (pH 4.5). HNAPH is also used for the fluorimetric determination of gallium, indium and scandium in 70% ethanol and acetate buffer (pH 3.9–4.5).

The bis(4-hydroxybenzoylhydrazone)s of glyoxal, methylglyoxal and dimethylglyoxal form coloured chelates with several cations.[126] The chelates formed are fluorescent, the fluorescence from the lanthanum chelates being the most intense.

Gravimetric applications

An ethanolic solution of resacetophenone phenylhydrazone[157] quantitatively precipitates copper from ammoniacal solutions without rigid control of the experimental conditions; up to 32 mg can be accurately determined in the presence of cadmium.

Of eight phenylhydrazone derivatives of phenolic aldehydes and ketones, salicylaldehyde phenylhydrazone[158] was found to be the best gravimetric reagent for copper(II). A 10% ethanolic solution of the reagent was used and the precipitate was ignited to the oxide. Copper(II) in amounts of 25–60 mg was determined with an error of $< 0.1\%$ in the presence of 100 mg of cadmium.

o-Hydroxyacetophenone phenylhydrazone[159] was found to be the best among the various hydrazones tested for palladium(II). It is possible to determine 15–47 mg of palladium; the precipitate is ignited to the metal.

Schwartz and Dickmann[160] used biacetyl bis(salicylohydrazone) and biacetyl bis(5-bromosalicylohyrazone) for the gravimetric determination of zinc and cadmium. With the former, 7–98 mg of zinc and 12–116 mg of cadmium can be determined, but only 40 mg of zinc with the bromo-derivative. Rastogi *et al.*[161] used benzoyl salicylal hydrazone, *i.e.*, benzoic acid salicylidenehydrazide (in acetone) for the gravimetric determination of copper(II).

Patal *et al.* used the benzoyl[162] and salicyl[163] hydrazones of 2-hydroxyiminoacetoanilide and 2-hydroxyiminoaceto-*o*-tuluidine for the gravimetric estimation of palladium(II) in the presence of copper(II) and nickel(II). The pH range for precipitation is very wide. Salicylaldehyde hydrazone[164] was used for the gravimetric determination of 5–30 mg of copper or nickel at pH 10.5–13. Prior separation of silver as its chloride and uranium as peruranate is necessary. Copper can be determined in the presence of nickel at pH 11–12 in the presence of EDTA.

Potentiometric studies

The stability constants of the complexes formed by pyridine-2-aldehyde 2-pyridylhydrazone with copper, zinc, cadmium, manganese, iron and nickel have been reported.[165] The formation and deprotonation equilibria[166] have been studied for iron(II) complexes of the three terdentate chelating agents, 6-methylpyridine-2-aldehyde 2-pyridylhydrazone, pyridine-2-aldehyde 2-pyrimidylhydrazone and pyridine-2-aldehyde 3'-methyl-2'-pyrazinylhydrazone. The respective stability constants were log β_1: 6.3, 6.0, 7.9; log β_2: 12.6, 14.0, 15.6; the acid dissociation constants of the respective protonated bis-complexes were pk_1: 6.28, 4.56, 4.12; pk_2: 7.95, 6.09, 5.61.

The acid dissociation constants for β-resorcylaldehyde acetylhydrazone in 40% aqueous ethanol have been measured[151] potentiometrically and spectrophotometrically. The stability constants of the 1:1 scandium complex has been determined.

The stability constants of the bivalent metal complexes with salicylaldehyde hydrazone,[167] which have been determined potentiometrically in 75% aqueous dioxan, follow the order $UO_2^{2+} > Pb^{2+} > Co^{2+} > Zn^{2+}$; for those with *o*-hydroxyacetophenone hydrazone in 50% aqueous dioxan the order is $UO_2^{2+} > Cu^{2+} > Co^{2+} > Ni^{2+}$.

The chelates of pyridine-2-aldehyde 2-benzothiazolylhydrazone and pyridine-2-aldehyde 2-quinolylhydrazone[53] have been studied potentiometrically in 1:1 dioxan–water media. The ligands react with iron(II), nickel, zinc and cadmium to form octahedral complexes. The titration curves for these reactions show only one break, which corresponds to the neutralization of perchloric acid plus two equivalents of hydrogen-ion per mole of metal added and is indicative of the formation of the bis-chelates. The copper reaction, in which both mono and bis-chelates are found, is described in detail.

Applications as indicators

The complexes of bivalent Cu, Zn, Cd, Fe and Ni with pyridine-2-aldehyde 2-pyridylhydrazone (PAPH) have been used as acid–base indicators[168] in titration of weak and strong acids and bases. Based on the extraction of their intensely coloured deprotonated forms into organic solvents, the copper, nickel and iron complexes have been used as extraction indicators[169] in titrations of strong acids and bases.

The zinc and cadmium complexes of 2,2′-bipyridyl 2-pyridylhydrazone have been proposed as indicators in acid–base titrations[170] in aqueous solution, the apparent p*K* values being 8.5 and 9.5, respectively. The indicators give sharp end-points and have proved to be similar in behaviour to phenolphthalein.

Chugreeva[171] suggested the use of the *p*-nitrophenyl- and 2,4-dinitrophenylosazones of dihydroxytartaric acid and the 2,4-dinitrophenylhydrazone of acetone, which behave as acid–base indicators at pH 11.5–13.5 and may be used in solutions with high concentrations of salts, ethanol and protein in the temperature range 0–80°, and also[172] for the determination of free sodium hydroxide in mixtures with alkali-metal carbonates, aqueous ammonia, potassium cyanide, sodium phenoxide, sulphacetamide and sulphapyridine sodium.

Some *p*-nitrophenylhydrazones have proved to be better indicators because of their high stability and readily observable colour changes. Studies have been made of the change of the absorption spectra with pH, the useful pH-ranges, and stability of the colours of the *p*-nitrophenylhydrazones derived from crotonaldehyde and acraldehyde,[173] furfuraldehyde and some furfuraldehyde derivatives,[174] benzaldehyde and its 4-nitro-4-hydroxy-, 2,4-dihydroxy- and 4-dimethylamino- derivatives among others[175] and the *p*-nitro and 2,4-dinitrophenylhydrazones derived from 4′-nitroacetophenone.[176]

The potassium salt of benzaldehyde *p*-nitrophenylhydrazone[177] has also been studied. Likewise, the phenylhydrazones of pyridine 2-aldehyde and pyridine-4-aldehyde, when used as indicators for titration of a base with an acid, change from colourless to yellow, and are suitable for use in spectrophotometric titrations.[178]

The *p*-nitrophenylhydrazone of pyridine-2-aldehyde has recently been proposed as an indicator[179] for colorimetric pH measurements. Katiyar *et al.*[180] have proposed *N*-isonicotinoyl *N*′-salicylidenehydrazine as a metallochromic indicator for iron.

Applications as spot-test reagents

Singh[71] has used 2,2′-bipyridyl 2-quinolylhydrazone and 2,2′-bipyridyl 2-pyrimidylhydrazone as spot-test reagents for the detection of iron, cobalt and copper.

p-Dimethylaminobenzaldehyde isonicotinoylhydrazone forms an intensely orange–yellow precipitate with mercury(I or II) in slightly acidic, neutral or slightly alkaline medium.[181] The *p*-diethylaminobenzaldehyde compound behaves similarly but with lower sensitivity for mercury(II).

Libergott *et al.*[182] used pyruvylidine-2-hydrazinobenzothiazole in benzene for the selective detection of cadmium, an orange colour being developed with an alkaline test solution. The test is highly selective in presence of cyanide, the tetracyanocadmate complex being demasked with formaldehyde.

Sawicki *et al.*[183] used benzaldehyde 2-benzothiazolylhydrazone for the detection of nitrite in presence of *p*-aminobenzene.

An ethanolic solution of biacetylmonoxime benzothiazolylhydrazone[184] gives a red–violet product (extractable into chloroform) with palladium(II) in acidic or alkaline medium. Cyanide interferes in acidic and cobalt in alkaline medium.

If one drop of 0.05% acetophenone *m*-nitrophenylhydrazone solution and 1 ml of 0.05*M* sodium hydroxide are mixed with 5 μg of Mg^{2+}, a rose–violet precipitate is formed.[171]

Anand *et al.* used the 2,4-dinitrophenylhydrazones of biacetylmonoxime,[185] 4-methylpentane-2,3-dione-2-oxime[186] and hydroxyiminoacetophenone[187] and the *p*-nitrophenylhydrazones of pyruvaldoxime,[188] 4-methylpentane-2,3-dione-2-oxime,[186] hydroxyiminoacetophenone[187] and 3-hydroxyiminopentane-2-one[189] for selective and sensitive detection of cobalt. Feigl *et al.*[190] also used the *p*-nitrophenylhydrazone of biacetylmonoxime (cobaltone I) for the detection of cobalt.

Other applications of hydrazones

Copper(II) (1–16 μg) has been determined by amperometric titration[191] in 0.15*M* sodium acetate (containing gelatine) as supporting electrolyte, with ethanolic resacetophenone phenylhydrazone solution. There is no interference from a 10-fold ratio (to Cu) of Ni, Zn or Cd. Iron(II) interferes, but an amount equal to that of copper can be tolerated if sodium fluoride is added as masking agent. The method has been used

in analysing nickel–silver. Recently Jain *et al.*[192] used ethyl cyanoglyoxalate 2-carboxyphenylhydrazone for the selective amperometric determination of thorium.

The complex of cobalt(III) with pyridine-2-aldehyde 2-pyridylhydrazone (PAPH) has been used in the nephelometric determination[193] of silver and mercury(II). The procedure is based on adduct formation of these ions with $Co(PAPH)_2^+$, in which the two unco-ordinated (imino) nitrogen atoms are peripheral and interact with the silver or mercury(II). The adducts formed correspond to the formulae $[Co(PAPH)_2]ClO_4.AgNO_3$ and $[Co(PAPH)_2].ClO_4.Hg(NO_3)_2$.

Pyridine-2-aldehyde 2-quinolylhydrazone (PAQH) has been used as detection agent for cobalt, nickel and copper after their TLC separation.[194] Frei *et al.*[195] have separated the PAQH complexes of Fe, Ni, Cu and Co chromatographically and determined them semiquantitatively in the presence of up to 10-fold amounts of interfering metal ions. Diffuse reflectance spectroscopy,[196] and ring-oven separation and circular chromatography[197] have been used for the same purpose.

Biacetyl bis(thiobenzoyl) hydrazone and pentane-2,3-dione bis(4-methoxythiobenzoyl) hydrazone[198] form coloured complexes with a large number of metal ions. Most of the complexes can be extracted into chloroform containing pyridine or into isobutyl methyl ketone in the presence of tetrahydrofuran and may be separated by TLC.

Conclusions

These reagents are in general useful for determination of a limited number of metals (Co, Cu, Cd, Zn, Ni, Mn, Fe, V, Hg, Mo, Ti, U, Pd, Re, Rh, Os) but are highly selective for virtually only cobalt, thanks to its ability to form inert complexes.

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SPECTROPHOTOMETRIC DETERMINATION OF SILVER WITH 2-(3,5-DIBROMO-2-PYRIDYLAZO)-5-DIETHYL-AMINOPHENOL IN THE PRESENCE OF ANIONIC SURFACTANT

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Summary—A sensitive and selective spectrophotometric method for silver has been established by reacting silver(I) with 2-(3,5-dibromo-2-pyridylazo)-5-diethylaminophenol (3,5-diBr-PADAP) in the presence of an anionic surfactant, sodium lauryl sulphate. The molar absorptivity is 7.7×10^4 l.mole^{-1}.cm^{-1} at 570 nm. The molar ratio of silver to 3,5-diBr-PADAP is 1:2. Beer's law is obeyed from 0.1 to 1 ppm of silver. With EDTA as masking agent, common ions do not interfere. The method has been applied to the determination of silver in waste water.

In continuation of investigations on the use of anionic surfactants in the spectrophotometric determination of silver, the colour reactions of silver with some pyridylazo compounds in the presence of anionic surfactants have been studied, and it has been found that the silver complex formed with 2-(3,5-dibromo-2-pyridylazo)-5-diethylaminophenol (3,5-diBr-PADAP) in the presence of sodium lauryl sulphate (SLS) gives a higher sensitivity than the 2-(5-chloro-2-pyridylazo)-5-dimethylaminoaniline complex studied previously.[1] Its molar absorptivity is 7.7×10^4 l.mole^{-1}.cm^{-1} at 570 nm, and it is one of the most sensitive colour reactions known for silver. With EDTA as masking agent at pH 5, this reaction is specific for silver. This paper reports the investigation of optimum conditions for the reaction and a simple and sensitive method for the determination of silver in waste-water.

EXPERIMENTAL

Reagents

Silver standard solution, 1 mg/ml. Dissolve 0.158 g of silver nitrate in 100 ml of 0.1*M* nitric acid, and store in a brown flask. Dilute the solution as required.

3,5-DiBr-PADAP ethanolic solution, 5×10^{-4}M. Dissolve 0.020 g of 2-(3,5-dibromo-2-pyridylazo)-5-diethylaminophenol in 100 ml of absolute ethanol.

The 3,5-diBr-PADAP was synthesized according to Johnson and Florence's procedure[2] with minor modifications, namely, use of 3,5-dibromo-2-aminopyridine instead of 5-bromo-2-aminopyridine, sodium ethoxide in absolute ethanol instead of sodium amide in toluene, butyl nitrite instead of isopentyl nitrite, and recrystallization from 95% ethanol instead of aqueous ethanol (1:1). Yield, *ca.* 30%. Orange-red needles, m.p. 159–160°. Analysis gave, C 42.3%, H 3.7%, N 13.1%, Br 37.7%: $C_{15}H_{16}N_4OBr_2$ requires C 42.11%, H 3.77%, N 13.09%, Br 37.33%.

SLS solution, 1%. Dissolve 1 g of sodium lauryl sulphate in 100 ml of water.

Buffer solution, pH 5. Adjust 0.1*M* acetic acid with 0.1*M* sodium acetate to pH 5, using a pH-meter.

EDTA solution, 0.1M. Dissolve 3.7 g of disodium ethylenediaminetetra-acetate dihydrate in 100 ml of water.

Potassium citrate solution, 5%.

Sodium thiosulphate solution, 7%.

Unless otherwise stated, all reagents used were of analytical-reagent grade, and demineralized water was used throughout.

RESULTS AND DISCUSSION

In a search for a more sensitive reagent for silver, we synthesized twelve pyridylazo compounds as listed in Table 1, and compared the characteristics of their reactions with silver in the presence of an anionic surfactant, sodium lauryl sulphate. From Table 1, it can be seen that reagent IV is the most sensitive. Its sensitivity is higher than that of two analogous compounds containing only one halogen atom substituted in the pyridine ring, *viz.* reagents V and VI; several of the sulphonated reagents do not produce a colour at all with silver.

Spectral characteristics

The absorption spectra of 3,5-diBr-PADAP alone, of 3,5-diBr-PADAP–SLS and of 3,5-diBr-PADAP–SLS–Ag in aqueous solutions at pH from 2 to 11 have been studied. The spectra at pH 5 are shown in Fig. 1. Aqueous solutions of 3,5-diBr-PADAP over the pH-range given above are all orange-yellow and exhibit an absorption maximum within the range 455–465nm (curve A in Fig. 1). The addition of SLS to 3,5-diBr-PADAP causes no significant shift in the wavelength of maximum absorption of the 3,5-diBr-PADAP (curve B in Fig. 1). The ternary system, 3,5-diBr-PADAP–SLS–Ag, forms a red product and exhibits two absorption peaks at 530 and 560 nm (curve C in Fig. 1). The optimal wavelength for measurement of the silver complex is found to be 570 nm, against a reagent blank. However, in the absence of SLS the silver and 3,5-diBr-PADAP produce no colour change, which shows that the anionic surfactant is indispensable in the colour reaction.

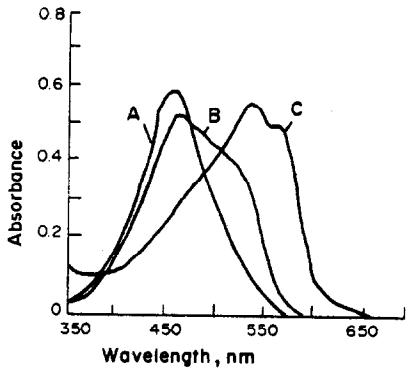


Fig. 1. Absorption spectra. A—3,5-diBr-PADAP ($1.5 \times 10^{-5}M$). B—3,5-diBr-PADAP ($1.5 \times 10^{-5}M$)-SLS (0.08%). C—3,5-diBr-PADAP ($1.5 \times 10^{-5}M$)-SLS (0.08%)-Ag^{+} (excess). (pH 5, 1-cm cells, against water).

Effect of pH

Because 3,5-diBr-PADAP produces colour reactions with a number of common metal ions, it was necessary to find a suitable masking agent and an optimum pH-value for the colour reaction of silver and 3,5-diBr-PADAP to be usable for practical determinations. Hence the colour reaction characteristics of silver and 3,5-diBr-PADAP at various pH-values in the presence and absence of EDTA were studied. It was found that the absorbance of the silver complex in the absence of EDTA remains constant at pH 3–9, but it decreases to different extents after addition of EDTA. Fortunately, at pH 5 and in the presence of EDTA, the absorbance of the silver complex remains essentially the same as in the absence of EDTA, while the absorbance of the reagent blank is lower. Hence pH 5 and EDTA as masking agent are chosen for the determination of silver.

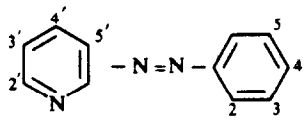
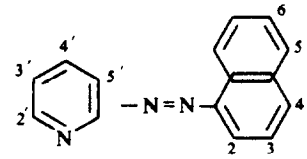
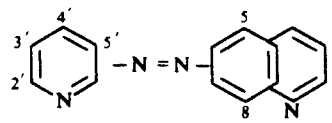
Effect of reagent concentration

The chosen final concentrations of the reagents ($3.5–4.5 \times 10^{-5}M$ 3,5-diBr-PADAP, 0.05–0.1% SLS, 0–0.01M EDTA and 0–1% potassium citrate) are those giving maximum absorbance.

Effect of time and temperature

The colour stability was studied at 25° by measuring the absorbance at regular time intervals. Maximum absorbance was attained after 2 min in the absence of EDTA, or after 15 min in its presence, and then remained constant in both cases for at least 6 hr.

Table 1. Reaction characteristics of silver with some pyridylazo compounds in the presence of sodium lauryl sulphate

Reagent*	pH	Reagent λ_{max}, *nm*	Complex λ_{max}, *nm*	Wavelength for measurement, against reagent blank, *nm*	Molar absorptivity, 10^4.l.mole^{-1}.cm^{-1}
					
I 3-Cl,2-NH_2,4-$N(CH_3)_2$	10	470	500	530	6.7
II 3′-Cl,2,4-diamino,5-CH_3	7	470	520	520	3.9
III 3′,5′-dibromo,2,4-diamino	10	495	500	530	2.5
IV 3′,5′-dibromo,2-OH,4-$N(C_2H_5)_2$	5	460	530	570	7.7
V 3′-Cl,2-OH,4-$N(C_2H_5)_2$	5	442	545	547	5.0
VI 3′-Br,2-OH,4-$N(C_2H_5)_2$	5	490	549	560	5.3
VII 3′-Cl,2-CH_3,4-$N(C_2H_5)_2$	5	490	505	560	1.4
					
VIII 3′Cl,4,5-diamino	11	540	580	620	2.0
IX 3′-Cl,2-NH_2,6-SO_3H	6–11	No colour reaction			
X 3′-Cl,2-OH,3,6-di-SO_3H	4–9.5	No colour reaction			
					
XI 3′-Cl,8-OH	7.5	450	510	510	1.1
XII 3′-Cl,5-SO_3H,8-OH	4–9.5	No colour reaction			

* The syntheses of the reagents except No. IV (3,5-diBr-PADAP) will be published elsewhere.

The absorbance of the silver complex varies with temperature; raising the temperature decreases the absorbance and *vice versa*, but the measured absorbance is always the same at the same temperature irrespective of whether the solution has been heated or cooled to that temperature. It seems likely that the decrease in absorbance with increase in temperature arises from dissociation of the complex. Therefore, all absorbance measurements should be made, as far as possible, at the same temperature.

Beer's law

Under the optimized conditions established, a linear calibration graph was obtained for 0.1–1.0 ppm of silver. The apparent molar absorptivity was found to be 7.7×10^4 l.mole^{-1}.cm^{-1} at 570 nm.

Composition of the complex

The nature of the complex was investigated by the continuous variation and molar-ratio methods at a definite SLS concentration. The molar ratio of silver to 3,5-diBr-PADAP in the complex was found to be 1:2.

Effect of diverse ions

The selectivity of the method was investigated by the determination of 0.5 ppm of silver in the presence of a number of other ions. An ion was considered to interfere if the absorbance obtained differed by more than $\pm 2\%$ from that for silver alone. The following ions, added as nitrate or sulphate, cause no interference at the M:Ag weight ratio given: Al(III) $\leqslant$ 400, Bi(III) $\leqslant$ 100, Cd(II) $\leqslant$ 200, Cu(II) $\leqslant$ 200, Co(II) $\leqslant$ 20, Cr(III) $\leqslant$ 50, Pb(II) $\leqslant$ 200, Cr(VI) $\leqslant$ 10, Fe(II) $\leqslant$ 100, Fe(III) $\leqslant$ 200, Ni(II) $\leqslant$ 100, La(III) $\leqslant$ 200, Y(III) $\leqslant$ 200, Mn(II) $\leqslant$ 200, Hg(II) $\leqslant$ 200, V(V) $\leqslant$ 100, Th(IV) $\leqslant$ 100, W(VI) $\leqslant$ 100, Zn(II) $\leqslant$ 400, In(III) $\leqslant$ 20, Ca(II) $\leqslant$ 500, Sr(II) $\leqslant$ 200, Ba(II) $\leqslant$ 100, Mg(II) $\leqslant$ 400 and alkali metals $\geqslant$ 1000. Among anions, phosphate, acetate, fluoride, perchlorate, carbonate, sulphate, nitrite, hexametaphosphate and oxalate (added as the alkali metal salts) do not interfere even when their weight is at least 400 times that of silver, but chloride, iodide, bromide, cyanide, thiocyanate and thiosulphate interfere seriously.

Reactions of 3,5-diBr-PADAP with some other metal ions

With many metal ions, 3,5-diBr-PADAP forms coloured products which are extractable into organic solvents. We expected that other ions besides silver could also form water-soluble complexes in the presence of anionic surfactant and 3,5-diBr-PADAP, and this proved to be so. The characteristics of some of these metal complexes are summarized in Table 2. We expect that these reactions may be used for spectrophotometric determinations without extraction, and we are continuing these studies.

Application

The proposed method has been applied to the determination of silver in the waste-water from photographic film factories. By fuming the sample with nitric and sulphuric acids, interfering substances such as chloride, bromide, thiosulphate, organic compounds, *etc.* can be driven off.

Procedure

Take a sample containing less than 25 μg of silver in ~20 ml in a 50-ml beaker. Add 5 drops of 30% hydrogen peroxide, 1 ml of nitric acid (1 + 1) and 1 ml of sulphuric acid (1 + 1). Evaporate to fuming, and heat for 2–3 min more. Cool to room temperature, add 1 ml of nitric acid (1 + 1) and carefully rinse the beaker wall with about 5 ml of water. Warm the solution to dissolve any residue, and then transfer into a 25-ml standard flask. Add 1 ml of 5% potassium citrate solution, 1 drop of phenophthalein indicator solution, and then 4*M* sodium hydroxide dropwise until the solution turns red, then nitric acid (1 + 5) dropwise to discharge the colour. Finally, add 2 ml of pH-5 buffer, 1 ml of 0.1*M* EDTA, 2 ml of 1%

Table 2. Reaction characteristics of some metals with 3,5-diBr-PADAP in the presence of sodium lauryl sulphate

Metal	pH	λ_{max} (against water), *nm*		Wavelength of measurement (against reagent blank), *nm*	Molar absorptivity, 10^4 *l.mole*$^{-1}$.*cm*$^{-1}$
Ni(II)	5	530	575	575	1.2
Cu(II)	5	530	580	580	1.0
Fe(III)	5	530	595	595	0.9
Cd(II)	9	530	580	580	1.5
Mn(II)	8.5	570		575	1.2
Zn(II)	8.5	545		570	1.6
Co(II)	8.5	570	600	570	0.97
Hg(II)	8.5	545		550	1.2
Bi(III)	5	560	600	600	0.73
Pb(II)	8	580		580	0.6
Y(III)	8.5	570	600	570	0.82
In(III)	5	550	580	580	0.92

Table 3. Comparison of analytical results for samples

Sample	AAS method, *ppm*	Proposed method, *ppm*
1	0.40	0.39
2	0.75	0.79
3	0.86	0.84
4	1.7	1.56
5	5.2	4.98

SLS solution and 2 ml of $5 \times 10^{-4}M$ 3,5-diBr-PADAP in ethanol, and dilute to the mark with water. Mix and let stand for 20 min at room temperature. Measure the absorbance at 570 nm in a 1.0-cm cell, against a reagent blank prepared by adding 2 drops of 7% sodium thiosulphate solution to the rest of the test solution and mixing to discharge the colour.

Prepare the calibration curve by taking a series of 20-ml portions of water to which 1, 5, 10, 15, 20 and 25 μg of silver (as standard solution) have been added, and treat according to the method above.

Results

Recovery studies were performed with different amounts of silver, and the recoveries were found to be >90%.

The analytical results obtained by the recommended method agreed well with those from atomic-absorption spectrophotometry (Table 3).

Eight replicate portions of a waste-water sample containing silver were analysed individually by the recommended procedure and gave an average value of 0.77 ppm with a standard deviation of 0.02 ppm.

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SELECTION OF THE COUNTER-CATION IN THE SOLVENT EXTRACTION OF ANIONIC CHELATES: SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNTS OF COBALT WITH 2-NITROSO-1-NAPHTHOL-4-SULPHONIC ACID AND TETRABUTYLAMMONIUM ION

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Summary—The importance of selecting the most suitable counter-cation in the solvent extraction–spectrophotometric determination of an anionic metal chelate containing a sulphonic acid group is demonstrated and discussed. In the case of cobalt and 2-nitroso-1-naphthol-4-sulphonic acid (nitroso-NW acid), the most suitable counter-cation is the tetrabutylammonium ion (Bu_4N^+): in this case only the ion-pair of the anionic cobalt chelate is extracted into chloroform and the excess of the nitroso-NW acid remains in the aqueous phase. The absorption maximum of the chelate in chloroform is at 307 nm, at which the molar absorptivity is 6.5×10^4 l.mole^{-1}.cm^{-1}. The absorbance of the reagent blank at 307 nm is less than 0.010. By use of nitroso-NW acid and Bu_4N^+, trace amounts of cobalt may be determined in nickel salts and in iron and steel samples.

Many studies of solvent extraction with long-chain amines or quaternary ammonium salts have been made since Ziegler *et al.* reported the extraction of an anionic chelate of iron(III) and 7-iodo-8-hydroxyquinoline-5-sulphonic acid with tributylammonium ion into amyl alcohol.[1] In almost all of the studies reported to date, preference has been shown for counter-cations which are also extractable, as a result of which the excess of reagent is extracted. In spectrophotometric methods the reagent blank will then be larger if there is appreciable overlap of the absorption spectra of the species extracted. The sensitivity and precision will suffer accordingly.

Ishibashi and Kohara studied the extraction of cobalt with nitroso-NW acid and Amberlite LA-2 into toluene.[2] The absorbance was measured at 530 nm, at which the molar absorptivity was low: 1.76×10^4 l.mole^{-1}.cm^{-1}. Adam and Přibil studied the extraction of cobalt with nitroso-R acid and trioctylmethylammonium chloride into chloroform.[3] The absorbance in this case was measured at 500 nm. In these methods, as the excess of reagent was co-extracted with the chelate, it was impossible to measure the absorbance at the wavelength of maximum absorption of the chelate, where the absorbance of the reagent blank was very large, and the absorbance measurements were seriously disturbed by the co-extracted anions.[3]

In this work, several extraction equilibrium constants have been determined, and conditions have been found for the selective extraction of the anionic chelate of cobalt(II) and 2-nitroso-1-naphthol-4-sulphonic acid (nitroso-NW acid). A highly sensitive spectrophotometric method for cobalt has been developed and applied to the determination of cobalt in practical samples.

EXPERIMENTAL

Apparatus

A Shimadzu UV 300 recording spectrophotometer and a Shimadzu QV-50 spectrophotometer were used with 10-mm quartz cells.

Reagents

2-Nitroso-1-naphthol-4-sulphonic acid. The reagent was obtained by nitrosation of the parent compound in aqueous solution with sodium nitrite.[4,5] The crude nitroso compound was recrystallized twice from dilute hydrochloric acid solution. Aqueous solutions were used.

Cobalt(II) solution. Solutions of cobalt chloride hexahydrate were standardized by EDTA titration.

Quaternary ammonium, tetraphenylarsonium and tetraphenylphosphonium chloride solutions. Commercially available salts were dried under reduced pressure (about 5 mmHg) and at 50–60° to constant weight, and were dissolved in distilled water.

Buffer solutions. Phosphate buffer solution (0.5*M*, pH = 8) and trisodium citrate solution (2*M* or 1.5*M*) were used.

Wash solution. Ammonium sulphate (0.05*M*)—ammonia (0.1*M*) buffer solution containing 0.002*M* EDTA.

Chloroform. Used without further purification, and saturated with water by shaking with water before use.

All reagents used, except nitroso-NW acid, were of analytical reagent grade.

Table 1. Extraction constants (log K_{ex}) for the ion-association complexes

	Cl^-	Br^-	I^-	HR^-	R^{2-}	CoR_3^{3-}
Ph_4As^+	1.31	2.70	4.74			
Ph_4P^+	1.18	2.56	4.78			
$DoTMA^+$	1.05	1.91	3.55			14.57
$DeTMA^+$	−0.31	0.82	2.26			
Bu_4N^+	0.07	1.24	3.06	2.45	3.45	13.19
$Zeph^+$	4.67	5.88	7.44	7.17	12.73	25.91

H_2R: nitroso-NW acid; Ph_4As^+: tetraphenylarsonium ion; Ph_4P^+: tetraphenylphosphonium ion; $DoTMA^+$: dodecyltrimethylammonium ion; $DeTMA^+$: decyltrimethylammonium ion; Bu_4N^+: tetrabutylammonium ion; $Zeph^+$: tetradecyldimethylbenzylammonium ion.

General procedure

A 50-ml portion of the aqueous solution of quaternary ammonium, tetraphenylarsonium or tetraphenylphosphonium chloride ($C^+.Cl^-$, $10^{-4}M$) was transferred to a separatory funnel and the solution of the sodium salt of the anion (A^-) was added in 10–100-fold molar excess with respect to the $C^+.Cl^-$. This solution was shaken with 50 ml of chloroform. The separated organic phase was shaken with a fresh solution of anion, A^-, containing a 10–100-fold molar excess of the cation (C^+). Then 5-ml portions of the organic phase and of an aqueous solution containing various amounts of the anion (A^-) were shaken in 25-ml stoppered test-tubes for about 30 min to ensure extraction equilibrium was reached. After phase separation, the concentrations of quaternary ammonium, tetraphenylarsonium, or tetraphenylphosphonium salt in the organic and in the aqueous phases were determined with potassium tetrabromophenolphthalein ethyl ester according to Tsubouchi.[6]

From the concentrations of C^+ in the aqueous and organic phases, the distribution ratio was calculated, the values of D^{-1} were plotted against the values of $[A^-]^{-1}$, and values of K_{ex} were calculated from the slopes. The results obtained for several cations and univalent anions are shown in Table 1.

RESULTS

Determination of the extraction constants of univalent–univalent ion-pairs

An extraction system in which a univalent cation (C^+) and a univalent anion (A^-) form only one kind of ion-pair (C^+A^-) in the aqueous phase, and in which the extracted ion-pair does not dissociate or aggregate, involves the following equilibria:

$$C^+ + A^- \rightleftharpoons C^+.A^-; \quad K_A = [C^+.A^-]/[C^+][A^-] \tag{1}$$

$$C^+.A^- \rightleftharpoons (C^+.A^-)_{org}; \quad K_D = [C^+.A^-]_{org}/[C^+.A^-] \tag{2}$$

where the subscript org refers to the organic phase, and absence of a subscript indicates the aqueous phase.

The distribution ratio (D) of C^+ between the aqueous and organic phases, and the extraction constant (K_{ex}) refer to

$$D = [C^+.A^-]_{org}/([C^+] + [C^+.A^-]) \tag{3}$$

$$K_{ex} = [C^+.A^-]_{org}/[C^+][A^-] = K_D K_A \tag{4}$$

From equations (1)–(4), the following equation can be derived:

$$D^{-1} = K_D^{-1} + (K_{ex}[A^-])^{-1}. \tag{5}$$

Plots of D^{-1} against $[A^-]^{-1}$ should be linear; from the intercept on the y-axis and the slope, K_D and K_{ex} can be calculated.

In the general case, for formation and extraction of a single species $C_n^+.A^{n-}$, we can write

$$K_{form} = [C_n^+.A^{n-}]/[C^+]^n[A^{n-}] \tag{6}$$

$$K_D = [C_n^+.A^{n-}]_0/[C_n^+.A^{n-}] \tag{7}$$

$$D = n[C_n^+.A^{n-}]_0/([C^+] + n[C_n^+.A^{n-}]) \tag{8}$$

$$K_{ex} = [C_n^+.A^{n-}]_0/[C^+]^n[A^{n-}] = K_{form}.K_D \tag{9}$$

$$\frac{1}{D} = \frac{1}{K_D} + \frac{1}{nK_{ex}[C^+]^{n-1}[A^{n-}]}. \tag{10}$$

Equation (10) is rather intractable from a practical point of view, but from equation (9) we can deduce

$$\log[C^+] = \frac{1}{n}\log\frac{[C_n^+.A^{n-}]_0}{[A^{n-}]} - \frac{1}{n}\log K_{ex} \tag{11}$$

Hence when the total concentrations of the anion species in the two phases are equal, $\log[C^+]$ is equal to $1/n \log K_{ex}$ (Table 2). In a practical sense, it is useful to compare the values of $1/n \log K_{ex}$ for the various systems, as shown in Fig. 1.

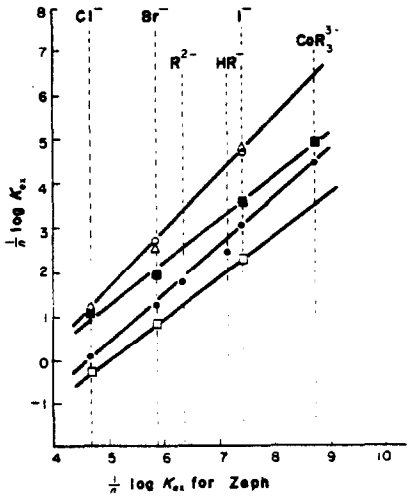

Fig. 1. Plots of $1/n \log K_{ex}$ for different cations against $1/n \log K_{ex}$ for the tetradecyldimethylbenzylammonium cation (Zeph). Cations: (○) tetraphenylarsonium; (△) tetraphenylphosphonium; (■) dodecyltrimethylammonium; (●) tetrabutylammonium; (□) decyltrimethylammonium; solvent, chloroform.

Table 2. Extraction constants calculated for CoR_3^{3-}, HR^- and R^{2-} ions and optimal concentrations of counter-cations

	$\frac{1}{3}\log K_{ex(CoR_3^{3-})}$	$\frac{1}{2}\log K_{ex(R^{2-})}$	$\log K_{ex(HR^-)}$	Optimal conc., M
Ph_4As^+	6.4	3.3	4.4	10^{-5}
Ph_4P^+	6.4	3.3	4.4	10^{-5}
$DoTMA^+$	4.8 (4.9)	2.5	3.3	2×10^{-4}
$DeTMA^+$	3.5	1.3	2.0	4×10^{-3}
Bu_4N^+	4.5 (4.4)	1.9 (1.7)	2.7 (2.5)	6×10^{-4} (8×10^{-4})

The log K_{ex} values in parentheses are those determined experimentally.

Determination of the extraction constants for a univalent cation and multivalent anion association complex

Constants for the complexes with multivalent anions could not be determined by using equation (5). Accordingly, log K_{ex} was evaluated for R^{2-} and the cobalt complex anion (CoR_3^{3-}) by assuming that the concentration of the ion-association complex in the aqueous phase was negligibly small compared with the total concentrations of the reactants in the aqueous phase and that the species extracted into chloroform did not dissociate. The results obtained for tetradecyldimethylbenzylammonium (zephiramine; $Zeph^+$), dodecyltrimethylammonium and tetrabutylammonium ions are also shown in Table 1.

Selection of the most suitable cation for extracting the anionic chelate

The values of $1/n \log K_{ex}$ were plotted against the corresponding values for the zephiramine systems, as shown in Fig. 1. The points for a given counter-ion lie on a straight line, from which the extraction constants for other anions can be calculated if the corresponding constants for the zephiramine complexes are known.

The slopes of the plots for the tetraphenylarsonium and tetraphenylphosphonium systems were the highest and those for the dodecyltrimethylammonium and decyltrimethylammonium systems were the lowest. In Table 2, the estimated log K_{ex} values are shown. In Fig. 2, the curve of per cent extraction for the anionic cobalt chelate and for the chelating agent (R^{2-} and HR^-) against log $[C^+]$, calculated from log K_{ex}, are shown for the case of the tetraphenylarsonium cation. In Fig. 2, a value on the abscissa, at which the per cent extraction of the anion is 50%, corresponds to the value of $(\log K_{ex})/n$. It can be seen that the nitroso-NW acid in the R^{2-} form is less easily extracted than HR^- or the anionic complex. The optimal concentration of the counter-cation, at which as much of the chelate anion as possible and as little of the reagent as possible are extracted into the organic phase is intermediate between the values of $\frac{1}{3}\log K_{ex(CoR_3^{3-})}$ and $\frac{1}{2}\log K_{ex(R^{2-})}$, is shown in Table 2.

This difference between $\frac{1}{3}\log K_{ex(CoR_3^{3-})}$ and $\frac{1}{2}\log K_{ex(R^{2-})}$ was greatest for the tetraphenylarsonium and tetraphenylphosphonium cations, which should therefore give the most efficient extraction of the anionic chelate coupled with minimum co-extraction of the chelating agent, R^{2-}. However, as the extractability of

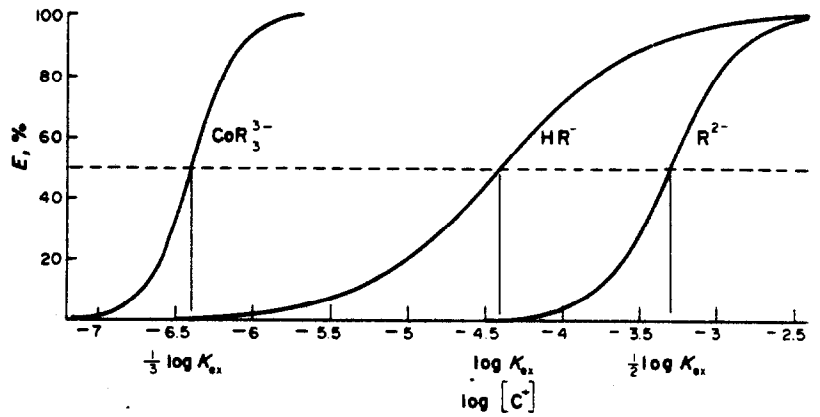

Fig. 2. Percentage extraction of HR^-, R^{2-} and CoR_3^{3-} as a function of the concentration of cation. H_2R: nitroso-NW acid; C^+, tetraphenylarsonium ion.

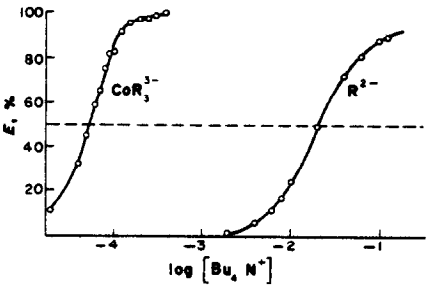

Fig. 3. Percentage extraction of R^{2-} and CoR_3^{3-} as a function of the initial concentration of tetrabutylammonium ion in the aqueous phase. H_2R: nitroso-NW acid, $8 \times 10^{-5}M$; CoR_3^{3-}, $1 \times 10^{-5}M$; pH 8.6.

Table 3. Tolerable concentrations of other ions

Ion	Maximum tolerable concentration, M
Na^+, K^+, Cl^-	1*
citrate	0.5
SO_4^{2-}, PO_4^{3-}	0.1
Br^-, NO_3^-	0.1
Fe^{3+}, Ni^{2+}, Cu^{2+}	0.02
Mn^{2+}, Cr^{3+} Al^{3+}, Cd^{2+}, Pb^{2+} Zn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, I^-	10^{-3}*
V(V), Mo(VI), W(VI)	10^{-4}*
Sn(IV)	10^{-5}*

* Maximum tested.

the tetraphenylarsonium and tetraphenylphosphonium ions is very large, the optimal concentration must be very low and is about $10^{-5}M$, as shown in Table 2, which is the same order as that of the anionic chelate. From a practical point of view it is not convenient to work with too low a concentration of the counter-cation.

The tetrabutylammonium cation shows a relatively large difference between $\frac{1}{3}$ log $K_{ex(CoR_3^{3-})}$ and $\frac{1}{2}$ log $K_{ex(R^{2-})}$. Figure 3 shows the variation in extraction (as %) for the anionic cobalt chelate and the chelating agent (R^{2-}), for the case where tetrabutylammonium ion is the counter-cation. As expected from Table 2 and can be seen in Fig. 3, when the extraction is carried out in the presence of the tetrabutylammonium ion at a concentration of about $10^{-3}M$, the anionic cobalt chelate can be extracted completely into chloroform, while the excess of the chelating agent in the form of R^{2-} is not extracted at all. Tetrabutylammonium ion is therefore recommended as the most useful counter-cation for this extraction of cobalt.

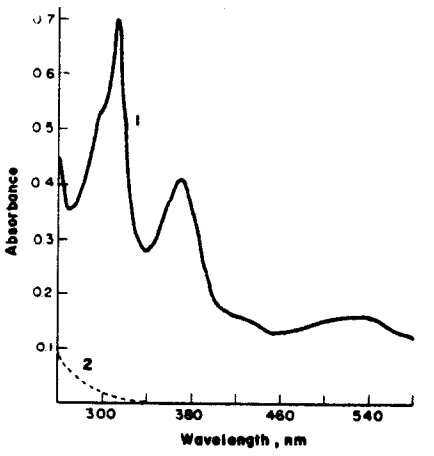

Fig. 4. Absorption spectra of the tetrabutylammonium ion-association complex of the cobalt–nitroso-NW acid anionic species, in chloroform.

Nitroso-NW acid, $2 \times 10^{-4}M$; tetrabutylammonium chloride, $10^{-3}M$; (1) [cobalt] = $1 \times 10^{-5}M$; (2) reagent blank; chloroform reference; pH = 8.6.

Solvent extraction–spectrophotometric determination of cobalt with nitroso-NW acid and tetrabutylammonium ion (Bu_4N^+)

Procedure. Pipette up to 5 ml of sample solution into a stoppered test-tube, and dilute to 5 ml with distilled water. Add 1 ml of trisodium citrate solution (2*M*) or 1.5 ml of 1.5*M* solution. Add 1 ml of nitroso-NW acid solution ($2 \times 10^{-2}M$), mix and allow to stand for 10 min. Add 1 ml of sulphuric acid (1 + 2) and 0.5 ml of $Bu_4N^+.Cl^-$ solution (0.01*M*). Shake with 5 ml of chloroform for 10 min. Discard the aqueous phase, and shake the organic phase with 5 ml of washing solution for about 5 min. After the phases have separated, measure the absorbance of the organic phase at 307 nm.

Absorption spectra and calibration graph. Figure 4 shows the absorption spectra for the cobalt complex and for the reagent blank. The linear range for the calibration plots was 0–$1.5 \times 10^{-5}M$ and the molar absorptivity was 6.5×10^4 l.mole^{-1}.cm^{-1} at 307 nm, with the absorbance of the reagent blank being about 0.003.

Effect of other ions. Nitroso-NW acid also reacts with iron(II), iron(III), copper and nickel ions to form coloured complexes which are extracted to some extent into chloroform with Bu_4N^+ and thus cause positive errors. It was for this reason that citrate buffer is used, since it also acts as a complexing agent.

The calibration graph is linear and nickel, copper(II) and iron(III) at concentrations of 0.02*M* do not interfere. As shown in Table 3, other metal and non-metal ions commonly present in practical samples do not interfere.

Determination of cobalt in pure nickel salts. Trace amounts of cobalt in commercially available nickel salts were determined. The nickel salts were dissolved in distilled water and portions of the sample solutions were used. The results obtained are shown in Table 4. The recovery tests were also good.

Determination of cobalt in iron and steel samples. Micro amounts of cobalt in standard iron and steel samples were determined by the following procedure.

Weigh accurately about 0.1 g of the iron or steel sample and dissolved it in 5 ml of hydrochloric acid

Table 4. Determination of cobalt in nickel salts

Sample	Supplier	Grade*	Sample solution: Concentration, g/100 ml	Sample solution: Volume taken, ml	Cobalt added, μg	Absorbance†	Cobalt: Found, μg	Cobalt: Recovery, μg	Cobalt, %
$NiCl_2 . 6H_2O$	A	GR (<0.1%)	0.2344	5		0.071 ± 0.003	0.32		0.0028
				5	0.57	0.190 ± 0.003	0.87	0.55	
	B	E (<0.3%)	0.2922	5		0.059 ± 0.002	0.27		0.0018
				5	0.57	0.185 ± 0.002	0.84	0.57	
$Ni(NO_3)_2 . 6H_2O$	A	GR (<0.1%)	0.1200	5		0.655 ± 0.008	2.98		0.050
				5	0.57	0.779 ± 0.003	3.54	0.56	
	B	GR (<0.1%)	0.1177	3		0.421 ± 0.004	1.92		0.054
				3	1.15	0.689 ± 0.003	3.13	1.21	
$NiSO_4 . 6H_2O$	A	GR (<0.1%)	0.1585	3		0.273 ± 0.002	1.24		0.026
				3	1.15	0.541 ± 0.001	2.46	1.22	
$NiSO_4(NH_4)_2SO_4 . 6H_2O$	A	GR (<0.1%)	0.1265	5		0.808 ± 0.002	3.68		0.058
				5	0.57	0.932 ± 0.008	4.24	0.56	

* GR, guaranteed reagent; E, extra-pure reagent. The value in parentheses is the cobalt content indicated in the guarantee certificate or specification.
† Reference, reagent blank; 10-mm path-length. Average of 3 determinations.

Table 5. Determination of cobalt in standard iron and steel samples

Sample*	Sample solution: Weight of sample, g/100 ml	Sample solution: Volume of solution taken, ml	Absorbance†	Cobalt content, %
NBS 55e	0.1011	3	0.048 ± 0.002	0.0072
		5	0.079 ± 0.003	0.0071
NBS 19g	0.1009	3	0.075 ± 0.001	0.0113
		5	0.125 ± 0.001	0.0113
NBS 126b	0.1126	3	0.239 ± 0.002	0.0323
		5	0.397 ± 0.001	0.0322
NBS 101e	0.0307	5	0.615 ± 0.003	0.183

* Main components: NBS55e, Co: 0.007; Cu: 0.065; Ni: 0.038; Cr: 0.006; Mn: 0.035; Sn: 0.007; Al: 0.002%. NBS19g, Co: 0.012; Cu: 0.093; Ni: 0.066; Cr: 0.374; Mn: 0.554; Sn: 0.008; Al: 0.031%. NBS126b, Co: 0.032; Cu: 0.082; Ni: 3.599; Cr: 0.066; Mn: 0.380%. NBS101e, Co: 0.18; Cu: 0.359; Ni: 9.48; Cr: 17.98; Mn: 1.77; Sn: 0.020%.

† Reference, reagent blank; 10-mm path-length. Average of 3 determinations.

(1 + 2) and 4 ml of hydrogen peroxide (about 30%). Evaporate the resulting solution to about 1 ml and add 0.5 ml of sulphuric acid (1 + 2), then dilute with distilled water to 100 ml. Take a suitable size of aliquot for the cobalt determination.

The results obtained for some standard steels are shown in Table 5. The cobalt contents obtained by the recommended procedure are all in good agreement with the certificate values for cobalt.

DISCUSSION

Many studies have been carried out on the solvent extraction of anionic chelates. In such extraction systems, long-chain amines or alkyl ammonium ions have usually been preferred, though these compounds do not necessarily give the best results. The most undesirable effect is the increase in the reagent blank owing to the co-extraction of the excess of the reagent, which often leads to disadvantages in the spectrophotometric analysis as follows: (1) interference from other anions such as halogen ions is more likely, and (2) the absorbance of the reagent blank becomes rather large. This study has shown that by using a suitable counter-cation it is possible to extract only the anionic chelate into the organic phase, and thus to overcome the two problems just mentioned.

In the proposed solvent extraction–spectrophotometric determination of cobalt with nitroso-NW acid, the tetrabutylammonium ion was found to be the most useful counter-cation. As it extracts very little of the excess of nitroso-NW acid into chloroform, and the measurement can be made at the maximum absorption wavelength (307 nm). As the reagent blank at this optimum wavelength is very small, long-path cells (10 cm) can also be used, giving a ten-fold increase in sensitivity. In such cases, cobalt at concentrations of 0–1.5 × $10^{-6}M$ could be determined.

By using the method recommended in this study, it is possible to determine trace amounts of cobalt in nickel salts and in samples of iron and steel.

As discussed in this study, the selection of the most suitable counter-cation is very important in such solvent extraction–spectrophotometric determinations, and in some of the extraction systems studied earlier the most suitable counter-cation may not always have been chosen.

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ANALYTICAL REACTIONS OF SUBSTITUTED PYRIMIDINES

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Summary—Analytical aspects of the chemistry of substituted pyrimidines are reviewed.

Pyrimidine can be considered as derived from pyridine. It is a weaker base (pK_a 1.3) than the related compounds pyridazine, pyridine and imidazole. Considering the distribution of π-electron densities and the resonance hybrids representing the pyrimidine and pyridine molecules, it is inferred that the C_5 position of pyrimidine should correspond to C_3 in pyridine and be the most susceptible to electrophilic attack. Similarly, positions 2, 4 and 6 in pyrimidine should correspond to 2 and 4 in pyridine. Substituents at these positions should have comparable reactivities.[1]

Pyrimidine (pK_a 1.3)

Pyridazine (pK_a 2.33)

Pyridine (pK_a 5.23)

Imidazole (pK_a 7.2)

Pyrimidine derivatives and compounds in which the pyrimidine ring is a part of a more complex system were amongst the first compounds to be synthesized. They are vital compounds, widely distributed in living organisms. The chemistry[2–8] and the biochemical aspects[9–14] of the pyrimidines have been extensively reviewed. Recently, the stereochemistry of metal–pyrimidine complexes has also been reviewed.[15] The compounds have long been extensively used for analytical work, and these aspects are reviewed in this article, together with methods for analysis of the compounds themselves.

Barbituric acids

Their determination and applications have been reviewed.[16] The electrochemical methods are more sensitive than colorimetric and titrimetric methods for their determination. Differential pulse polarography has been used for determination of phenobarbital in blood (detection limit 0.1 μg/ml),[17] and traces of barbituric and 2-thiobarbituric acids,[18] and polarography for phenobarbital and methyl phenobarbital in human plasma (detection limit 3.5–5.5 μg/ml).[19] Diallyl and isopropyl barbituric acids can be potentiometrically titrated,[20] by using bromination reactions, the titrant being lead tetra-acetate in presence of excess of bromide. Phenobarbital, amobarbital, barbital, allyl isobutyl barbituric acid, pentobarbital and secobarbital can be determined by differentiating nonaqueous titration in methyl isobutyl ketone with sodium methoxide in benzene–methanol mixture.[21]

Phenobarbital, amobarbital and secobarbital have been similarly determined in tetramethylurea[22] and sulpholane[23] media with tetrabutylammonium hydroxide, the recovery for 0.1–0.3 mmole of compound being 97.5–104.4% and 99–101%, respectively. In 3-methyloxazolidin-2-one medium, barbitone, amylobarbitone and quinalbarbitone can also be titrated.[24] Barbituric acid in acetic acid medium can be titrated[25] potentiometrically with mercury(I) acetate. A conductometric method for the determination of alo-, amo- and phenobarbital in pharmaceuticals has been reported.[26] Barbituric acid derivatives can be determined amperometrically[27] and coulometrically[28] in aqueous and water–acetone media. Sodium barbiturate has been used to give a colour reaction with the product of reaction between chloramine-T and cyanide or thiocyanate.[29] 2-Thiobarbituric acid has been used for amperometric titration of copper.[30]

Violuric acids

Applications of violuric acids as analytical reagents have been reviewed by Singh *et al.*[31] Little work of analytical interest has since been reported.

Dilituric acids

Dilituric acid was first prepared in 1845.[32] Many of its salts were examined[32,33] but up to 1940 almost no work except its examination as a precipitant for or-

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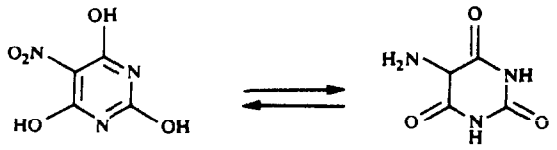
ganic and inorganic bases[34] was reported.



Dilituric acid (5-nitrobarbituric acid)

The nearly white crystalline acid gives a yellow solution in water (λ_{max} at 218 and 318 nm, shoulder at 237 nm). The peaks at 218 and 318 nm have been attributed to primary and secondary ionization of the acid respectively.[35] The acid decomposes explosively at about 185° on heating.[35] The optical properties of the dilituratcs of primary and secondary amines and amino-acids have been utilized for identification of these compounds;[36–38] alkaloids have been similarly characterized.[39] Dilituric acid has been used for gravimetric determination of potassium[40,41] and indirect colorimetric determination of potassium[42,43] and of magnesium[44] from the absorbance of the residual dilituric acid after precipitation of the cation.

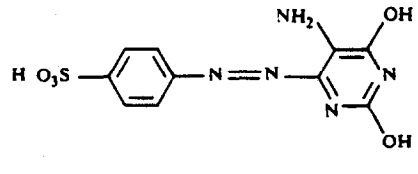
Copper, nickel, cadmium and cobalt have been determined gravimetrically[45] and lead, potassium and magnesium thermogravimetrically.[46] The dilituratcs of the alkali metals,[47] silver, lead, thorium and manganese[48] have also been examined thermogravimetrically. Their thermal stability is adequate for gravimetric determinations but the lithium, sodium and silver salts are too soluble for gravimetric purposes. The other metals can be determined with an average deviation of 0.5%. Rubidium and caesium can be determined by X-ray fluorescence.[49] The solubilities and thermolysis curves of ethylenediamine, polymethylenediamine and quinine dilituratcs have been examined.[50] Ethylenediamine and quinine can be determined gravimetrically, but the solubilities of the polymethylenediamine dilituratcs increase with increasing number of carbon atoms in the chain. Trimethylenediamine dilituratc is an exception, however, being more soluble than expected, presumably because of the internal hydrogen-bonding possible in this polymethylenediamine.[51] The crystalline nature of these organic salt dilituratcs makes it easy to identify them by X-ray powder diffraction methods.[52]

Nutiu and co-workers[53,54] synthesized the 2-thio derivative of dilituric acid and studied its analytical potentialities. It gives precipitates with alkali and alkaline earth metals. The precipitates dissolve in alkaline medium, giving intense yellow colours. The compound is not very suitable for gravimetric determination, errors being as high as 2.5%, but the intense yellow colour of the potassium salt in alkaline medium is suitable for spectrophotometric determination at 410 or 420 nm.

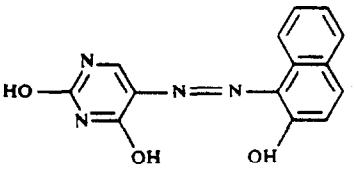
Uracils

X-Ray diffraction studies[55] have shown that the arrangement of atoms in crystalline uracil (2,4-dihydroxypyrimidine) is analogous to that in pyridone. Complexation of palladium(II) with uracil,[56,57] 2-thiouracil[57] and 6-methyl-2-thiouracil[57] has been investigated photometrically. All the complexes have maximum absorption at 380 nm and are suitable for determination of palladium in acidic medium (pH < 2.0). Platinum and gold interfere. Uracil forms a 1:1 metal:ligand complex, the other two form 1:2 complexes.[57]

5-Aminouracil forms a red complex with Ru(III) at pH 2.9–4.0 (λ_{max} 480 nm, ϵ 7.6×10^3 $l.mole^{-1}.cm^{-1}$) on heating for 1 hr on a steam-bath, and a red complex with Os(VIII) in alkaline medium, without heating.[58] Its azo-dye (I) is used as an indicator in chelatometric titrations[58] of Zn, Cd and Hg. 1-(2,4-Dihydroxy-5-pyrimidylazo)-2-naphthol (II), obtained by coupling β-naphthol with diazotized 5-aminouracil, finds use as an indicator[58] in titration of MoO_4^{2-}, WO_4^{2-} and PO_4^{3-} with lead nitrate.



(I)



(II)

5,6-Diaminouracil (λ_{max} 255–265 nm, $\epsilon = 2 \times 10^4$ $l.mole^{-1}.cm^{-1}$) has been used for the spectrophotometric determination of Ru(III) and Os(VIII)[59,60] but tolerance for other platinum metals in these determinations is poor.

6-Aminouracil can be hydrolysed by hydrochloric or *p*-toluenesulphonic acid to barbituric acid, which in turn can be condensed with Ehrlich's reagent to give a benzylidene compound. This reaction has been used to determine the uracil colorimetrically.[61]

Metal complexes of thiouracils are used for determination of the ligands. 5-Iodo-6-benzyl- and 5-butyl-6-methyl-2-thiouracil can. be potentiometrically titrated with mercury(II) acetate.[62] 2-Thiouracil, 6-methyl-2-thiouracil and 2,4-dithiouracil can be similarly determined with silver nitrate.[63]

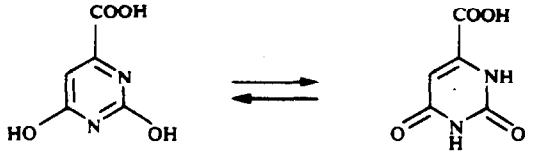
Ružička and Lyčka[64] prepared 1,3-dimethyl-5-nitroso-6-aminouracil and characterized its salts with alkali metals, Cu(II), Ag(I), Hg(I), Hg(II), Ni(II) and Pd(II). Copper and nickel were determined gravi-

metrically and polarographically. 1,3-Dimethyl-4,5-diaminouracil (DAL)[65] is a very selective and sensitive colorimetric reagent for Co(II) in alkaline medium (phosphate buffer, pH 11.2), but Fe(III), Cu(II) and Ag(I) interfere at fairly low levels.[65]

Au(III) is colorimetrically determined with 5-(*p*-ethoxyanilino)-5,6-dihydrouracil[66] at pH 6.0–6.5. The complex (λ_{max} 520 nm) is extractable into a 1:1 mixture of chloroform and toluene.

Orotic acids

Orotic acid (2,6-dihydroxypyrimidine-4-carboxylic acid, **III**) was first studied analytically by Selleri and Caldini.[67] They determined sodium and potassium gravimetrically (at pH 6.5–8.5 and 3° in ~80% methanol or ethanol medium), the reagents being the highly soluble ammonium or substituted ammonium salts of orotic acid. Potassium can be determined in presence of NH_4^+, Li^+, Cu^{2+}, alkaline earth metals, Al^{3+}, Fe^{3+} and Ni^{2+}. Babbie and Wagner[68] used *N,N*-dimethylethanolammonium orotate for the gravimetric determination of rubidium and caesium at pH 6.8. The error obtained was within ±0.5% but the precipitation mixture had to be kept in the refrigerator for 2 hr before filtration. Several substituted orotic acids (as the *N,N*-dimethylethanolammonium salts) were extensively studied[69] as potential reagents for precipitation of alkali metals, but it appeared that substitution generally results in an increase in the solubility of the alkali metal salts. However, it was found that uracil-3-acetic acid was selective for lithium, 5-ethylorotic acid for sodium and 5-methyluracil-3-acetic acid for potassium.



(III)

Orotic acid forms 1:1 complexes with copper(II) at pH 3–8,[70] with cobalt(II) at pH 6–10[71] and zinc at pH 6–9.[72] Nickel and cadmium also react. 2-Thioorotic acid has also been examined.[73,74] It reacts with 17 metals in ammoniacal medium, but with only a few at pH < 3, and gives better sensitivity than orotic acid.[73] It has been used as developing reagent in the paper chromatographic separation of metal ions present in alloys.[74] Thallium(I) can be determined amperometrically or gravimetrically[74] without interference from Cu(II), Co(II) and Ni(II).

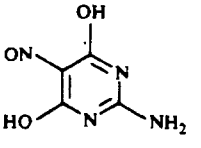
Pandey *et al.*[75–77] have examined the complexes of 2-thio-orotic acid with Ag(I), Cu(I), Ti(I), Hg(I), Zn(II), Cd(II), Hg(II), Fe(II), Co(II), Ni(II), Pd(0,II,IV), Pt(0,II,IV) and Rh(I,III).

Other pyrimidinols

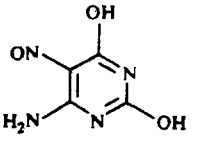
Many other types of hydroxypyrimidines (pyrimidinols) also find wide use as analytical reagents. 2,4,5-Trihydroxypyrimidine[78] (isobarbituric acid) undergoes reactions similar to those of 2,3-dihydroxypyridine[79,80] and reacts with iron(III) to form blue 1:2 (λ_{max} 590 nm, ϵ 600) and red 1:3 (λ_{max} 500 nm, ϵ 4.8×10^3) complexes at pH 2.0–4.5 and 4.6–11.0, respectively. The complexes can be used as acid–base indicators. Iron is determined spectrophotometrically as the red complex. The Ru(III)–4,5-diamino-6-pyrimidinol complex (λ_{max} 530 nm, ϵ 6.5×10^3) formed in acidic medium is suitable for spectrophotometric determination of the metal,[81] but the other platinum metals interfere and a preliminary separation is necessary. The ligand forms a highly unstable brown complex (λ_{max} 470 nm) with Os(VIII),[82] but the addition of mercury(II) perchlorate to this complex gives a pink colour (λ_{max} 500 nm; Os:Hg:ligand = 1:2:2) which can be employed to determine osmium at pH 8.5–11.0.

2,4,5-Triamino-6-pyrimidinol is used to determine Ru(III) and Os(VIII) photometrically.[83,84] It reacts with ninhydrin[85] to give a purple complex (λ_{max} 555 nm) that can be used to determine it in plants.

The complexation reactions of Fe(II), Co(II), Ru(III) and Rh(III) with 2-amino-5-nitroso-4,6-pyrimidinediol (**IV**) and 6-amino-5-nitroso-2,4-pyrimidinediol (**V**) have been studied spectrophotometrically and used in determination of these metals.[86–88] For ruthenium, reagent (**V**) is more sensitive and selective than (**IV**) but for determination of iron (**IV**) is superior.[86,87]



(IV)



(V)

4-Amino-5-nitroso-2,6-pyrimidinediol forms complexes with Fe(II), Co(II), Ru(III) and Rh(III)[88–90] and can be used as an indicator for EDTA titration of iron.[90] The cobalt complex is inert and does not decompose when the solution is made 6*N* with respect to hydrochloric, perchloric or sulphuric acid, making the reagent very selective for cobalt. Generally, nitrosopyrimidinols have been found to be more sensitive than nitrosophenols for cobalt, iron and ruthenium. 4-Amino-5-nitroso-2,6-pyrimidinediol is the most sensitive for cobalt.

Iron(II) gives a 1:4 complex with 2,6-diamino-5-nitroso-4-pyrimidinol (λ_{max} 653, ϵ 2.0×10^4) which is used for spectrophotometric determination of iron.[91]

2,4-Diamino-5-nitroso-6-pyrimidinol forms a bluish green complex (λ_{max} 650 nm) with iron(II) and is used for the photometric determination of 0.9–3.7 ppm of the metal at pH 7.0–8.5 or as indicator in the EDTA titration.[92] It is also used for spectrophotometric determination of Ru(III) and Rh(III).[59,88] This reagent and 2,6-diamino-4-pyrimidinol are fairly sensitive for nitrite detection[93] by diazotization and coupling to

azo dyes. The closely related 2,4-diamino-6-pyrimidinol has long been used for this purpose.[94]

Complexation with 2-mercapto-5-(4-methoxybenzyl)-4,6-pyrimidinediol has been used for potentiometric, conductometric and amperometric titration of gold (III) in hydrochloric acid medium.[95] 4,5-Diamino-2-mercapto-6-pyrimidinol acts as a very selective and sensitive reagent for detection and determination of osmium(VIII) in 1.0–6.0*M* sodium hydroxide.[96] The red 1:2 (Os:ligand) complex (λ_{max} 520 nm, ϵ 2.7 × 10^4) obeys Beer's law up to 8.0 ppm metal concentration. The iron and platinum group metals do not interfere. Ruthenium(III) forms a red 1:2 complex (λ_{max} 540 nm, ϵ 9.6 × 10^3) on heating at pH 2.2–3.3, which is also used for determination of the metal, but the method is not very selective. Both the Ru and Os complexes are cationic and not extractable into organic solvents. 4,5-Diamino-2-methyl-4-pyrimidinol also gives complexes with ruthenium[59] and osmium,[60] suitable for their spectrophotometric determination.

Cu(II), Fe(II), Co(II), Ni(II), Ru(III), Rh(III), Pd(II) and Os(VIII) form complexes with 4-amino-2-mercapto-5-nitroso-6-pyrimidinol, suitable for their spectrophotometric determination.[97–101] The complexes are not extractable into organic solvents, contain the metal and the ligand in 1:1–1:3 ratio and conform to Beer's law. A comparative study with 4-amino-2-methyl-5-nitroso-6-pyrimidinol shows that the thiol derivative is more sensitive (ϵ 6.1 × 10^3–4.8 × 10^4) and forms the metal complexes at higher pH. On the other hand, the methyl derivative is a more selective reagent, particularly for determination of iron.

In a recent study, the blue water-soluble Fe(II)–1,3-dimethyl-5-nitroso-2-thio-oxoperhydropyrimidine-4,6-dione complex (λ_{max} 630 nm; metal–ligand 1:3) has been found suitable for the spectrophotometric determination[102] of iron at pH 5.5–6.8. Large amounts of Cu(II) and Co(II) do not interfere. 6-Amino-2-benzylthio-5-nitroso-3,4-dihydropyrimidine[103] is also used for determination of iron(II) at pH 5–6.5.

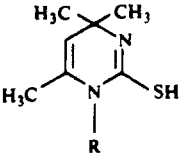
Pyrimidinethiols

Pyrimidine-2-thiol, 2,4-diaminopyrimidine-6-thiol, 2,6-diaminopyrimidine-2-thiol and 2,5-diaminopyrimidine-2,6-dithiol have been synthesized and explored for their analytical utility.[104,105] They give many useful reactions with metal ions—the most significant (sensitivity given in parentheses, ppm) are those of pyrimidine-2-thiol with Pd (1), Cu (2), Hg^+ (2), Bi (2) and Cd (10); 2,4-diaminopyrimidine-6-thiol with Pd (1) and Co (2); 2,6-diaminopyrimidine-2-thiol with Co (1) and Pd (2) and 2,5-diaminopyrimidine-2,6-dithiol with Se^{4+} (0.4) and Bi (5). The introduction of the 2-amino group does not significantly affect the reactivity, but a thiol group at C_6 has a stronger reducing character than at C_2.

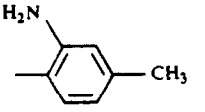
Chan[106] used 4,5-diamino-6-pyrimidinethiol for spectrophotometric determination of Se(IV) at pH 1.2–2.5 or still lower. The yellow complex formed (λ_{max} 380 nm, ϵ 1.92 × 10^4) conforms to Beer's law from 0.1 to 2.5 ppm of selenium. The absorbance is measured within 30 min of addition of the reagent to avoid precipitation of elemental selenium. Though Bi(III) and Te(IV) interfere, the presence of a 50-fold amount of transition metals does not affect the determination. The method is used to determine traces of Se in semiconductors.

Alkyl and aryl substituted 4,4,6-trimethyl-1H,4H-pyrimidine-2-thiols (**VI–XVII**)[107] react selectively with palladium(II), forming yellow complexes extractable into non-polar solvents.

(**VI**) R = H; (**VII**) R = C_2H_5; (**VIII**) R = C_3H_7;
(**IX**) R = n-C_4H_9; (**X**) = R = C_2H_5;
(**XI**) R = $C_6H_4OCH_3$ (anisyl)
(**XII**) R = *p*-$C_6H_4NO_2$; (**XIII**) R = C_6H_{11} (cyclohexyl); (**XIV**) R = $C_{10}H_7$(α-naphthyl);

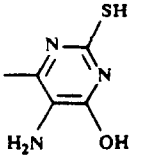


(**XV**) R =



(**XVI**) R = *p*-$C_6H_4OC_2H_5$ (phenacyl);

(**XVII**) R =



By extractive procedures, microgram amounts of Pd can be photometrically determined[108–113] in presence of milligram amounts of many metal ions, including platinum group metals.

The abovementioned pyrimidine-2-thiols except (**XV**) are also used for the spectrophotometric determination of osmium.[112–116] With the α-naphthyl derivative (**XIV**) palladium and osmium can each be determined in presence of large amounts of the other, because at acidity lower than 4*M* (hydrochloric acid)

osmium cannot be extracted with a chloroform solution of the thiol, but palladium can be completely extracted. Simultaneous determination, without prior separation of the metals, is also reported.

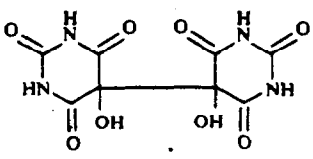
Bismuth(III) and tellurium(IV) react with the thiol (VI) forming orange-red (λ_{max} 500 nm, ϵ 1.27 × 10^4) and yellow (λ_{max} 385 nm, ϵ 1.26 × 10^4) complexes[117] respectively. The other derivatives (VII–XII) do not undergo complexation with these metals. It appears that steric effects become operative because Bi and Te are larger than Pd. The orange-red bismuth complex formed in 1.5–3.0*M* perchloric acid is extracted into chloroform and the metal determined accurately in the concentration range 4.0–14.6 ppm. The tellurium complex (not extractable) is also used in determination of 1.5–7.0 ppm of the metal in 1.8–2.5*M* perchloric acid medium.

Palladium(II) and osmium(VIII) react with 1-amino-, 1-anilino- and 1-(2',4'-dinitroanilino)-4,4,6-trimethyl-1H,4H-2-pyrimidinethiols in 1:2 metal–ligand ratio, forming coloured complexes extractable into non-polar solvents.[118] The ligands are very selective and the two metals can be determined in presence of large amounts of several ions, including Fe(III), Co(II), Ni(II), Ru(III), Rh(III), Ir(III) and Pt(IV). The anilino and 2',4'-dinitroanilino derivatives form two coloured complexes with ruthenium(III), iridium(III), rhodium(III) and platinum(IV), depending upon the acidity of the solution.[119–121] The yellow complexes formed in acidic medium (pH < 3) are anionic and may be assigned structures of the type $[ML_2Cl_2]^-$ (for the Ru, Rh and Ir-complexes) and $[MLCl_4]^-$ (for Pt), if LH represents the bidentate ligand. The extractable complexes formed at pH > 3 may be regarded as ML_3 (for Rh, Ru and Ir) and ML_2Cl_2 (for Pt). If reduction of Pt(IV) to Pt(II) occurs during the complexation with thiol, the probable structures will be $[PtLCl_2]^-$ and PtL_2 for the yellow and the extractable complex respectively. Possibilities for use of these complexes for analytical purposes have been explored.

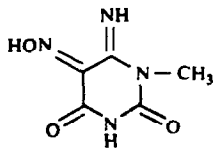
Alloxans

The reactions of alloxan (the degradation product of uric acid) with Ag(I), Hg(II), Pb(II), Fe(II) and Fe(III) were reported[122] in 1940. Alloxan was subsequently used for the colorimetric determination of thiophene[123] and identification of amino-acids[124] after their chromatographic separation. It is, however, less sensitive than ninhydrin for this purpose.

Alloxan is reduced to alloxantin (XVIII) by hydrogen sulphide. The reaction of iron(II) with alloxantin to form dihydroxydiferrodialloxantin has been used[125] to detect iron (limit of identification 1.7 μg).



(XVIII)



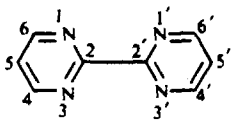
(XIX)

The ligand forms a yellow complex with molybdate[126] (λ_{max} 400 nm) at pH 3.3–3.9, which obeys Beer's law up to 25 ppm of Mo. Up to at least a 10-fold amount of tungstate, 40-fold amount of Zn(II), Co(II) or Ni(II) and 20-fold amount of Mn(II) will not interfere, but Cr(III), oxalate, tartrate, citrate and vanadate should be absent. The thiosemicarbazone of alloxan has also been prepared and used[127] for the detection of Cu(II), Cu(I), Ag(I), Hg(II) and Ni(II).

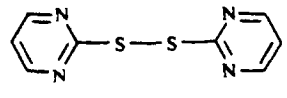
1,3-Dimethyl-4-imino-5-oximino alloxan (XIX) finds use as a reagent for spectrophotometric determination of copper[129] (λ_{max} 382 nm, 1:1 complex at pH 7–9.5) and gravimetric determination of palladium.[129] Fe(II), Co(II), Ni(II) and Pd(II) interfere in the copper determination. The red palladium complex is precipitated quantitatively at pH 1–3 and dried at 100–110°. It may be dissolved in formamide and the metal determined colorimetrically. Cu(II), Au(III), Co(II), Ni(II), Os(VIII), Ir(IV) and Pt(IV) interfere.

Bipyrimidines

Bly and Mellon prepared[130] 2,2'-bipyrimidine (m.p. 113–115°, p*K* 0.6) and studied[131] its complexation reactions with 30 metals, those with Cu(I) and Fe(II) being particularly useful. The absorption characteristics of the Fe(II)-complex (λ_{max} 490 nm, ϵ 5.0 × 10^3, stability constant 3.4 × 10^7) are similar to those of Fe(II) complexes with 2,2'-bipyridyl and 1,10-phenanthroline. The complex is insensitive to pH in the range 2–6, contains metal and the ligand in 1:3 ratio (dominant species) and conforms to Beer's law from 1 to 10 ppm of iron. Many ions, but not Cu(I), do not interfere.

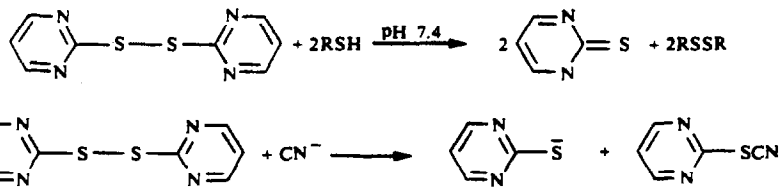


Bipyrimidine



Dithiodipyrimidine

2,2'-Dithiodipyrimidine is used to determine the thiol group[132] and cyanide ion[133] on the basis of the reactions:



The absorbance of the thiol is measured photometrically and related to the concentration of RSH or CN^-.

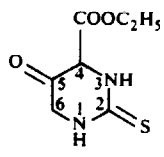
Other substituted pyrimidines

The complexation of Fe(II), Co(II), Ru(III) and Ir(III) with 5-nitroso-2,4,6-triaminopyrimidine has been studied.[134] The ligand is particularly useful for sensitive determination of ruthenium without interference from 10-fold amounts of other platinum metals. 2,4,5,6-Tetra-aminopyrimidine is also used for ruthenium and osmium determination.[135]

2-Amino-6-methylthio-4-pyrimidine carboxylic acid and 6-methoxy-2-methylthio-4-pyrimidine carboxylic acid[136] have been used for photometric determination of Ag(I)[137] and Mn(VII),[138] respectively. The silver complex (λ_{max} 375 nm, ϵ 2.09 × 10^3), obeys Beer's law up to 5 × $10^{-4}M$ Ag. However, many cations partially destroy the complex or are precipitated and adsorb the colour. The manganese determination is based on reduction of permanganate to green manganate (in alkaline medium) by 6-methoxy-2-methylthiopyrimidine-6-carboxylic acid, and measurement of the absorbance of the Mn(VI) at 580 nm (ϵ 1.48 × 10^3). Metal ions do not interfere unless they precipitate as hydroxides and adsorb some of the manganese.

The fluorescence intensity of 4,6-bis(methylthio)-5-aminopyrimidine decreases in proportion to the amount of osmium tetroxide added to it.[139] At liquid-nitrogen temperature, as little as 10 ng of OsO_4 per ml can be detected. The intensity is not affected by variation in pH from 3 to 12. In determination of OsO_4 at the 1-ppm level, 10 ppm of Ir(III), 1 ppm of Fe(III) and 1 ppm of Pd(II) do not interfere. The metal:ligand ratio is 2:3. The decrease in fluorescence is attributed to complexation, with the first site of reaction at the NH_2 group.

Sheppard and Brigham[140] have synthesized 2-thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine (**XX**) and its metal complexation reactions have been investigated.[140,141] The reagent is suitable for silver(I) in acidic medium (detection limit 1 part in 10^6) and employed in its colorimetric determination.[141] For 20 μg of Ag(I), the tolerance limits (in mg) for other metals are: 2.0 for Fe(III); 1.5 for Zn(II), Cu(II), Pb(II), Mn(II) and Ni(II); 1.0 for Cd(II); 0.5 for Co(II).



(XX)

Conclusions

In general, this class of reagents tends to be versatile in the sense of a wide range of application, but to suffer from a lack of selectivity in many of the uses. A few of the reagents, however, do show considerable promise.

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EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF NON-IONIC SURFACTANTS IN WATER

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Summary—The solvent extraction of non-ionic surfactants with potassium chloride and tetrabromophenolphthalein ethyl ester potassium salt has been studied, and a method developed for determining trace amounts of non-ionic surfactants in water spectrophotometrically. Various non-ionic surfactants of general type $RO(CH_2CH_2O)_nH$ (where R is an alkyl or alkylphenyl group and n is the number-average degree of polymerization) are extracted quantitatively into *o*-dichlorobenzene from the water sample, and reacted in the organic phase with tetrabromophenolphthalein ethyl ester potassium salt to give a coloured product, the absorbance of which is measured at 620 nm.

Non-ionic surfactants, $RO(CH_2CH_2O)_nH$ are widely used in industrial and domestic detergents and it is increasingly necessary to determine these surfactants in water. This can be done at trace levels with potassium picrate as reagent and 1,2-dichloroethane as extracting solvent.[1,2] The reaction is based on coordination of the polyether chain to the potassium cation, in much the same way as potassium ions form complexes with crown ethers.[3] The complexed potassium ion is extracted as its ion-pair with picrate, which is the anionic chromophore measured in the spectrophotometric determination.

In our investigation tetrabromophenolphthalein ethyl ester potassium salt (TBPE-K) was used instead of potassium picrate to improve the extractability and sensitivity. The molar absorptivity of the TBPE complex was 5–6 times that of the picrate complex.

EXPERIMENTAL

Apparatus

A Hitachi Perkin–Elmer model 139 spectrophotometer and a Hitachi EPS-3T recording spectrophotometer were used, with glass cells of 10 mm path-length.

Reagents

Tetrabromophenolphthalein ethyl ester potassium salt solution. Dissolve 0.0525 g of TBPE-K in 50 ml of ethanol to make a $1.5 \times 10^{-3}M$ solution.

Potassium chloride solution (2*M*).

Buffer solution (*pH 7.8*). Prepared by mixing 1*M* potassium dihydrogen phosphate and 1*M* dipotassium hydrogen phosphate.

Polyoxyethylenealkyl(aryl) ethers. The non-ionic surfactants shown in Table 1 were examined. They were dried under reduced pressure (2–3 mmHg) at 50°, and known weights were dissolved in water.

Preliminary tests

The surfactant solution ($\leq$3 ml) was placed in a stoppered 25-ml test-tube, buffer solution ($\leq$1 ml), potassium chloride solution (2 ml) and reagent solution ($\leq$1 ml) were added, and the solution was made up to 7 ml with distilled water, mixed well and shaken vigorously (mechanical shaker) with 5 ml of 1,2-dichloroethane added by pipette. The absorbance of the organic phase was measured at 609 nm.

RESULTS AND DISCUSSION

In the preliminary tests, the absorption spectrum of the TBPE complex in dichloroethane was found to have its absorption maximum at 609 nm, which was therefore chosen for the measurement (Fig. 1).

The pH was adjusted with the phosphate buffer. The absorbance increased with the increase of pH, but the blank value rose abruptly at pH > 8, so then the measurements were made at pH 7.8.

When the amount of $1.5 \times 10^{-3}M$ TBPE-K was varied from 0.1 to 1 ml, the absorbance difference between a blank and 0.02 mg of Triton X-100 increased gradually with increase in the amount of reagent. Hence 0.7 ml was chosen as the volume of reagent, because it gave a relatively low blank absorbance (<0.05) and the absorbance difference was large and almost the same as with 0.8 ml of reagent.

The volume of 2*M* potassium chloride was selected as 2 ml, because the absorbance was constant with 2 ml or more of this reagent.

The shaking time needed is 15 min for complete extraction, and the phase separation takes 5 min.

Procedure 1

From these experiments procedure 1 was devised, the preliminary test procedure being followed, with the reagent volumes, *etc.*, quoted above. The sample solution taken ($\leq$3 ml) should contain 1–50 μg of non-ionic surfactant.

Table 1. Non-ionic surfactants of the type $RO(CH_2CH_2O)_nH$ examined

R	n or $\bar{n}$	Designations of the products	Suppliers
$C_{12}H_{25}$—	4(*n*)	Extra pure	Nikko Chemicals Co. Ltd.
$C_{12}H_{25}$—	6(*n*)	Extra pure	Nikko Chemicals Co. Ltd.
$C_{12}H_{25}$—	8(*n*)	Extra pure	Nikko Chemicals Co. Ltd.
$C_{12}H_{25}$—	4.6	Emulgen 106	Kao Soap Co. Ltd.
$C_{12}H_{25}$—	13.7	Emulgen 120	Kao Soap Co. Ltd.
$C_{12}H_{25}$—	18.5	Emulgen 147	Kao Soap Co. Ltd.
$C_{18}H_{35}$—	13	Emulgen 420	Kao Soap Co. Ltd.
$C_{18}H_{35}$—	26	Emulgen 430	Kao Soap Co. Ltd.
n-C_9H_{19}	4	Antarox CO-430	GAF Corporation
n-C_9H_{19}—⬡—	6	Antarox CO-530	GAF Corporation
n-C_9H_{19}—⬡—	9	Antarox CO-630	GAF Corporation
n-C_9H_{19}—⬡—	10.5	Antarox CO-710	GAF Corporation
n-C_9H_{19}—⬡—	30	Antarox CO-880	GAF Corporation
n-C_9H_{19}—⬡—	8	Emulgen 909	Kao Soap Co. Ltd.
n-C_9H_{19}—⬡—	17	Emulgen 920	Kao Soap Co. Ltd.
n-C_9H_{19}—⬡—	50	Emulgen 950	Kao Soap Co. Ltd.
n-C_9H_{19}—⬡—	86	Emulgen 985	Kao Soap Co. Ltd.
$CH_3C(CH_3)_2CH_2C(CH_3)_2$—	10	Triton X-100	Rohm & Haas Company
$CH_3C(CH_3)_2CH_2C(CH_3)_2$—	40	Triton X-405	Rohm & Haas Company
H—	8.8		Nakarai Chemicals Ltd.
H—	13.3		Nakarai Chemicals Ltd.
H—	22.3		Nakarai Chemicals Ltd.

Calibration graphs and molar absorptivities

The calibration graph obtained for Triton X-100 (1–50 μg) by procedure 1 was linear and passed through the origin. The apparent molar absorptivity was 6.7×10^4 l.mole^{-1}.cm^{-1}, the molar concentration being calculated on the basis of $n = 10$. This molar absorptivity is over 5 times that for the potassium picrate system (1.2×10^4 l.mole^{-1}.cm^{-1}).

The molar absorptivities for several kinds of non-ionic surfactants were determined from the slopes of the calibration curves; the molar concentrations were calculated by using the degree of polymerization, n, or the number-average degree of polymerization, $\bar{n}$, shown in Table 1. The molar absorptivities increase linearly with n and $\bar{n}$ (Fig. 2). The relation between molar absorptivity and $\bar{n}$ is linear for $\bar{n} > 8$. Poly(ethylene glycol) derivatives having no surface activity give values not on the straight line, and give lower molar absorptivities. On the other hand, the values for non-ionic surfactants used commercially all lie on the straight line. This implies that the non-ionic surfactants which have the same polyoxyethylene chain length can catch the same amount of potassium ion, regardless of the kind of alkyl or alkylaryl groups, and the longer the polyoxyethylene chain, the more the amount of potassium combined. The pure $C_{12}H_{25}O$–compounds ($n = 6$ and 8) have higher molar absorptivities than the corresponding commercial products, and the molar absorptivity of the $C_{12}H_{25}O(CH_2CH_2O)_8H$–TBPE complex is 7.8×10^4 l.mole^{-1}.cm^{-1}, whereas that of the TBPE–Zephir-

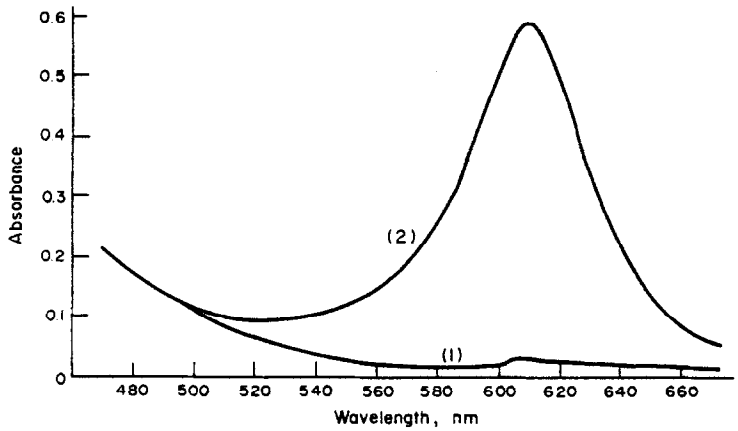

Fig. 1. Absorption spectra: (1) reagent blank; (2) $C_{12}H_{25}O(CH_2CH_2O)_8H$ (extra pure), 20 μg; solvent 1,2-dichloroethane; measured against solvent.

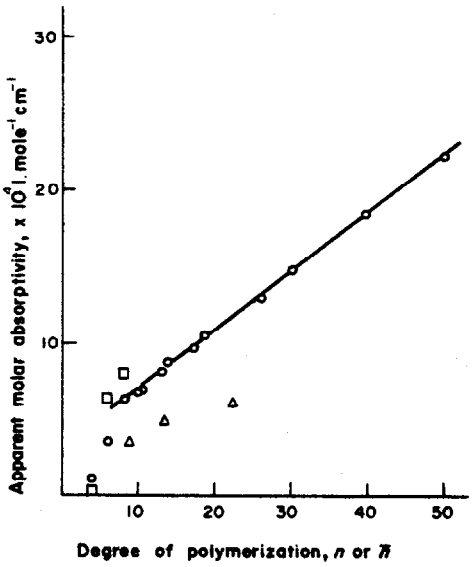

Fig. 2. Relationship between apparent molar absorptivity and degree of polymerization: ○, number-average degree of polymerization ($\bar{n}$); □, pure substance (degree of polymerization n); △, poly(ethylene glycol).

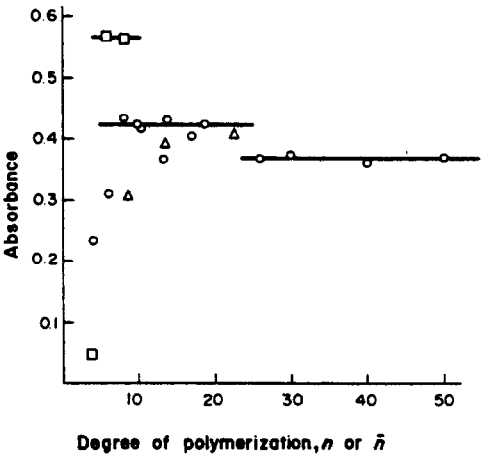

Fig. 3. Absorbances for 20 μg of surfactants: ○, number-average degree of polymerization ($\bar{n}$); □, pure substance (degree of polymerization n); △, poly(ethylene glycol), reference: reagent blank.

amine complex is 9×10^4 l.mole^{-1}.cm^{-1}.[4] Therefore, one mole of the pure surfactant ($n = 12$) or the commercial product ($n = 16$) would combine with one mole of potassium, and when n is 36–40, with two moles of potassium. It is known[5] that polyoxyethylene derivatives can form spirals which can trap potassium ions.

The molar absorptivities of the commercial products vary with the type of surfactant, making it seem that minute amounts of the commercial products in water could not be determined by this method. However, the number-average degree of polymerization of the commercial products is mainly 8–15, and the absorbance of a definite *weight* of the products is almost constant, about 0.042 per μg (Fig. 3), and the method is usable.

Application to water samples

The non-ionic surfactant content of water is usually so small that it must be concentrated into a small volume of organic solvent from a large volume of water sample, if our method is to be used. 1,2-Dichloroethane is not suitable as the extraction solvent, because of its solubility at large water:solvent phase-volume ratios; *o*-dichlorobenzene was chosen instead because it scarcely dissolves in water.

$C_{12}H_{25}O(CH_2CH_2O)_8H$, Triton X-100 and $C_9H_{19}O(CH_2CH_2O)_{17}H$ were determined by procedure 1, with dichloroethane and *o*-dichlorobenzene as solvents. The results are shown in Table 2. The absorbance maximum is at 620 nm for the *o*-dichlorobenzene system.

To concentrate the surfactant into 5 ml of *o*-dichlorobenzene from 200 ml of water sample, the procedure is as follows.

Procedure 2

Take 200 ml of water sample in a 250-ml separatory funnel and add 2 ml of 1*M* phosphate buffer (pH 7.8), 2 ml of 0.5*M* potassium sulphate, 20 ml of 4*M* potassium chloride as salting-out agent and 4 ml of 1.5×10^{-3}*M* reagent and mix well. Pipette 5 ml of *o*-dichlorobenzene into the funnel and shake it horizontally in the shaker for at least 30 min and then let stand for 10 min. Measure the absorbance of the organic phase at 620 nm.

Interferences. Among the ions generally present in natural water, Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} and Cl^- do not affect the determination. The interference of iron and aluminium is eliminated by adding 1 ml of 0.2*M* EDTA. However, large amounts of anionic surfactant disturb the determination, because of extraction of the ion-pair between the anionic surfactant and the cationic potassium complex with the non-ionic surfactant, causing negative errors. Hence elimination of the anionic surfactant is necessary

Table 2. The absorbances for 20 μg of non-ionic surfactant in dichloroethane (609 nm) and *o*-dichlorobenzene (620 nm) by procedure 1

Solvent	Absorbance*		
	$C_{12}H_{25}O(CH_2CH_2O)_8H$	Triton X-100	C_9H_{19}—⬡—$O(CH_2CH_2O)_{17}H$
Dichloroethane	0.565	0.420	0.404
o-Dichlorobenzene	0.561	0.438	0.415

* Reference: reagent blank.

Table 3. The absorbances of 20 μg of non-ionic surfactants by procedures 1 and 3

Surfactant	Absorbance* Procedure 1	Procedure 3
$C_{12}H_{25}O(CH_2CH_2O)_8H$ (extra pure)	0.561	0.559
Triton X-100	0.420	0.420
C_9H_{19}—⬡—$O(CH_2CH_2O)_8H$	0.436	0.436
C_9H_{19}—⬡—$O(CH_2CH_2O)_{17}H$	0.404	0.404
C_9H_{19}—⬡—$O(CH_2CH_2O)_{50}H$	0.368	0.237
C_9H_{19}—⬡—$O(CH_2CH_2O)_{86}H$	0.362	0.019

* Reference: reagent blank.

before determination of the non-ionic surfactant in water. *o*-Dichlorobenzene (5 ml) will give preferential extraction of the non-ionic surfactant, leaving the anionic surfactant in the water phase. The *o*-dichlorobenzene extract can then be analysed.

The procedure is as follows.

Procedure 3

Add 1 ml of 0.2*M* EDTA, 1 ml of 0.2*M* sodium sulphate and 5 ml of *o*-dichlorobenzene to 200 ml of water sample in a 250-ml separatory funnel and shake for 10 min on the shaker and let stand for 10 min. Transfer the organic phase into a stoppered 25-ml test-tube and add 1 ml of the 1*M* phosphate buffer (pH 7.8), 2 ml of 2*M* potassium chloride, 0.7 ml of the reagent solution and 4.3 ml of distilled water. Shake the test-tube on the shaker for 15 min and let stand for 5 min. Measure the absorbance of the organic solvent at 620 nm.

By this procedure anionic surfactants at concentrations below a few ppm do not disturb the determination. Various kinds of non-ionic surfactants were tested by procedures 1 and 3, and the results are shown in Table 3. The absorbances by the two procedures are almost the same as each other when n is 8–17, and the average absorbance is about 0.42 for 20 μg. When n is 50 or 86, the absorbances are low, especially for procedure 3, because of the low extractability of the surfactants into *o*-dichlorobenzene.

Table 4. Determination of non-ionic surfactant in river water

Sample	Pre-treatment	Absorbance*	Surfactant† ng/ml	Recovery, %
Asahi River§	(1)	0.012	4	96
	(4)	0.014	4	94
Zasu River‡	(1)	0.462	83	96
	(2)	0.377	68	100
	(3)	0.401	73	96
	(4)	0.129	24	93

Recovery was determined from results for addition of 2.5 or 5 μg pure surfactant (n = 8) to 200 ml of sample solution.

* Mean of three determinations. Reference: reagent blank.

† Obtained from the calibration curve made by using pure surfactant (n = 8).

§ Sampled on 3 March 1980. The concentration of anionic surfactant determined immediately after sampling was 32 ng/ml. The Asahi River is one of the three largest rivers in Okayama Prefecture.

‡ Sampled on 3 March 1980. The concentration of anionic surfactant determined immediately after sampling was 750 ng/ml. The Zasu River is one of the branches of the Asahi River and domestic waste-water flows into it.

Determination of non-ionic surfactant in river water

Procedure 3 was applied to 200-ml samples of river water; (1) immediately after sampling, (2) after storage of the sample in a glass bottle for 24 hr, (3) after filtration through No. 5C filter paper (Toyo Roshi Co. Ltd.), (4) after filtration through a membrane filter (0.45 μm), and recovery tests were done by adding 2.5 or 5 μg of pure surfactant (n = 8) to the sample solution. The results are shown in Table 4. The amount of non-ionic surfactant was decreased by treatments (2), (3) and (4). This implies that the non-ionic surfactant is adsorbed on the filter paper or on the wall of the vessel on standing, and/or on some microparticles suspended in the water. Therefore, the determination should be done immediately after the sampling, without filtration. The method is accurate enough according to the recovery tests (about 95% recovery).

CONCLUSION

The polyoxyethylene chain in non-ionic surfactants can trap potassium ion to form a complex cation which can be extracted into dichloroethane or *o*-dichlorobenzene with TBPE as counter-ion, TBPE providing a sensitive anionic chromophore for subsequent spectrophotometric determination.

As anionic surfactants disturb the determination, non-ionic surfactants are separated from them by extraction into *o*-dichlorobenzene, the anionic surfactants remaining in the water phase, but this separation will not deal with more than a few ppm of anionic surfactants, and, unfortunately, these are widely used and generally present at about ten times the level of the non-ionic surfactants. Therefore, this method cannot be applied if the water sample contains more than a few ppm of anionic surfactants.

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ELECTROTHERMAL ATOMIC-ABSORPTION DETERMINATION OF VANADIUM

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Summary—Factors such as sample nature, matrix and heating programme have been found to influence both the sensitivity and precision of the determination of vanadium by electrothermal AAS. The reciprocal sensitivity, detection limit and precision (RSD) are 55 pg, 86 pg and 4%, respectively for aqueous solutions and 88 pg, 80 pg and 4% for organic solutions. Only tungsten and nitric acid have been found to interfere appreciably. Moderately concentrated sodium chloride solutions ($\leq 6\%$) can be analysed for vanadium (≥ 0.05 μg/ml) without background correction, as can the acid digests of phosphate rocks and crude petroleum samples.

The determination of vanadium by atomic-absorption spectrometry (AAS) can be considered as one of the earliest applications of the method using the fuel-rich oxy–acetylene flame.[1,2] The use of the nitrous oxide–acetylene flame[3,4] improved the performance to a level which compared favourably with that of X-ray fluorescence.[5] The flame AAS determination of vanadium suffers from chemical and spectral interferences which may be suppressed by the addition of aluminium[6] or iron[4] or the simultaneous nebulization of a reducing agent with the test solution[7] (by means of a T-tube). West *et al.*[8] determined vanadium indirectly by converting it into phosphovanadomolybdic acid and measuring the molybdenum by AAS. The sensitivity for vanadium can be increased for both the air–acetylene and nitrous oxide–acetylene flame systems by addition of organic solvents to the sample solutions. The determination of vanadium in organic solutions is affected by the compound used for the preparation of the standard solutions.[10] This effect was said to be removed by the addition of halogens to the organic solution,[11] but this treatment was later claimed to be inefficient.[12] Plasma jets have been used for the atomization of vanadium but the high temperature leads to low populations of atoms in the ground state compared with those in the excited state.[13] Solid samples have been directly analysed for vanadium with the aid of cathodic sputtering[14] and laser atomization.[15]

Electrothermal atomization (ETA) was first used by Donega and Burgess[16] for the AAS determination of vanadium with a graphite furnace. They used a graphite boat in a closed chamber with quartz windows and a gas pressure of 1–300 mmHg (H_2, N_2 or Ar). The sensitivity of the method was 0.6 ng. Graphite-furnace atomization was evaluated for use in the AAS analysis of petroleum and its products for vanadium and proved to be useful, because the method involves only simple dilution of the samples with organic solvents, followed by injection into the furnace.[17–19] A detection limit of 6.8 pg was obtainable with this method.[20] The method has also been used for the determination of the element in sea-water,[21,22] titanium dioxide,[23] carbonate rocks,[24] steels[25] and biological materials.[26] Several authors[27–29] have discussed the use of graphite furnaces coated with pyrolytic graphite or with metal carbides, for the atomization of vanadium. It has been shown that improved sensitivity and better graphite-tube performance can be obtained with the coated-tube furnaces. Nitric acid has a perceptible suppression effect in the AAS determination of vanadium with graphite furnaces, and this is attributed to the co-ordinative ability of the oxovanadium species in acidic solutions.[30] The addition of complexing agents or ammonia is useful in overcoming this suppression.[30]

It is the aim of the present work to study the determination of vanadium with a graphite-tube furnace and to apply the method to the analysis of samples having various matrix compositions.

EXPERIMENTAL

Apparatus

All measurements were performed with a Varian–Techtron model CRA-63 graphite furnace mounted in a Varian–Techtron AA-5 atomic-absorption spectrophotometer, as described earlier.[31] The operating conditions of the furnace are shown in Table 1. In all experiments a lamp current of 10 mA and a spectral bandpass of 0.2 nm were used but no scale expansion or damping. The 318.5 nm line from a Varian–Techtron hollow-cathode lamp was employed.

The voltage–time combinations used in drying and ashing were varied according to the composition of the sample solution injected. Unless otherwise stated, the atomization voltage was 9 V applied for 5–6 sec. The aqueous solutions were injected with an Excalibur "Autopette", and an

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Table 1. Operating conditions for the CRA-63

Solution	Drying		Ashing		Atomization	
	Voltage, *V*	Time, *sec*	Voltage, *V*	Time, *sec*	Voltage, *V*	Time, *sec*
Aqueous	4.5	30	6.0	25	9.0	5–6
Phosphate rock digest	4.0	30	7.0	25		
Sodium chloride	3.0	40	8.5	25		
Crude oil and xylene	2.5	40	6.0	35		

SGE 10-μl syringe was used for the injection of organic solutions.

Reagents

All reagents were of analytical-reagent grade. Demineralized distilled water was used for the preparation of solutions, dilution and final rinsing of the glassware. A BDH 1-mg/ml standard AAS vanadium solution was used as stock solution, and diluted to 50 μg/ml (weekly) and further to 5 μg/ml as required. The solutions analysed were made up to contain 5% hydrochloric acid (*i.e.*, 5 ml of the concentrated acid per 100 ml). Series of standard solutions were prepared in 2–6% sodium chloride. An organic vanadium standard solution (250 μg/ml) was prepared by dissolving the appropriate weight of bis(1-phenyl-1,3-butadione)oxovanadium according to Pradhan.[32] Two dilutions of this solution (25 and 2.5 μg/ml) were made with xylene immediately before analysis.

Sample preparation

The phosphate rock samples were digested as described previously.[31] Samples of crude oils were obtained from two oil fields in Iraq, namely, Khanakeen (sp.gr. 0.825) and Kirkuk (sp.gr. 0.954). The samples were diluted with xylene in the ratio 1:15–1:30, depending on the vanadium concentration levels. Both standard-addition and direct calibration methods were employed.

RESULTS AND DISCUSSION

Calibration

The analytical results from the calibration graphs are summarized in Table 2. The calibration graphs show a linear relationship between the absolute mass of the injected analyte and the measured absorbance, and were constructed from the results obtained by injecting different volumes of the same solution, as reported previously.[33]

Performance of the tube furnace

The tube furnace functions efficiently for 40–50 firings, and gives an average reciprocal sensitivity of 120 pg and a precision (RSD) of 3% at the 0.4-μg/ml level. After about 80 firings the precision deteriorates, as indicated in Fig. 1.

Table 2. Analytical results obtained for the ETA–AA of vanadium

Parameter	Aqueous solution	Organic solution
Upper linear boundary of the calibration graph, *ng*	9.0	3.0
Reciprocal sensitivity, *pg*	55	88
Detection limit (S/N = 2), *pg*	86	80
Relative std. devn., (8 results) %:		
for 0.2 μg/ml	4	4
for 1.5 μg/ml	2	—
for 1.0 μg/ml	—	3

With more than 40 firings, the sensitivity and precision also strongly depend on the temperature of the tube at the moment of injection, being superior when the injection is done at a tube temperature not below ~70°. These results may be explained by assuming that on prolonged firing the furnace coating deteriorates and becomes porous so that part of the sample (~30%) becomes irreversibly adsorbed. To avoid this behaviour the injection should be made at temperatures not below 70° because the solution then dries on contact with the tube surface and does not penetrate into the pores.

As the tube ages the absorption peak profile changes. The time for the first appearance of the signal remains the same for the whole of the tube life, but the peak maximum is gradually delayed, *i.e.*, the atomization time, τ_1, increases with tube age (Fig. 2). Sturgeon and Chakrabarti[27] showed that for uncoated tubes the atomization times for Mo and V

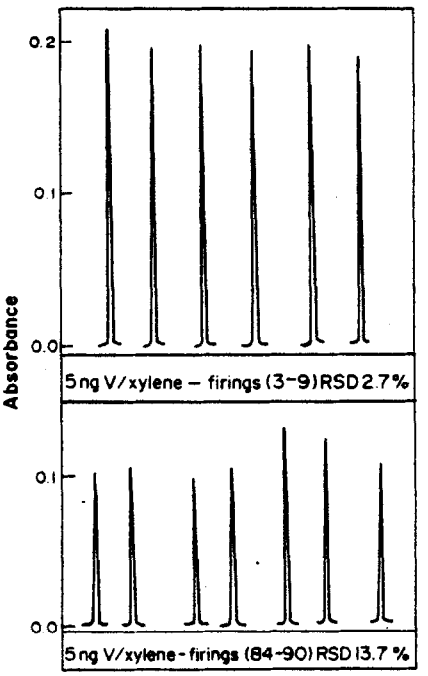

Fig. 1. Performance of a CRA-63 tube-furnace at two different stages of its life.

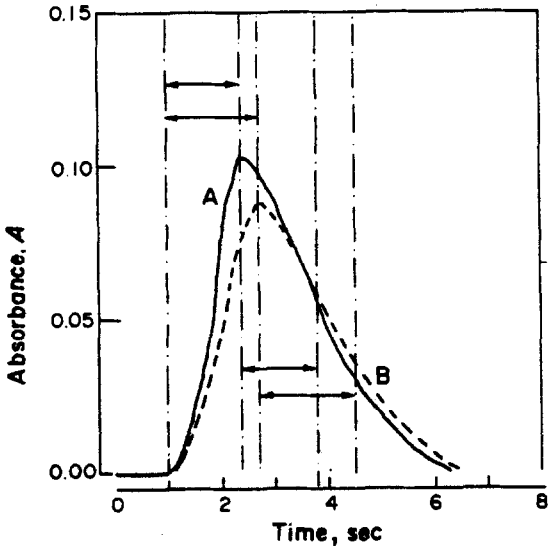

Fig. 2. Atomic-absorption signals of 2.0 ng of vanadium atomized at firing No. 23(A) and at firing No. 59(B).

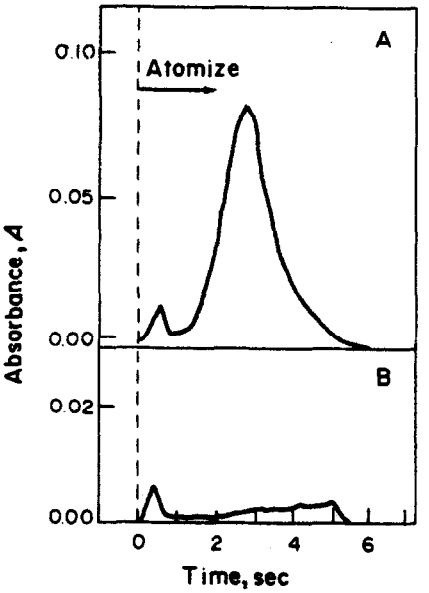

Fig. 4. Absorption signals of 1.0 ng of vanadium in the presence of 2.0 μg of calcium.

are longer than in the case of pyrolytically graphite-coated tubes. In this work, the gradual increase in atomization time indicates a gradual destruction of the pyrolytic graphite coating of the tube because of the high temperature used (2600°). The increase in the atomization time, τ_1, with tube age is accompanied by a corresponding increase in the residence time of the atoms, τ_2, so the ratio τ_1/τ_2 is almost constant. The constancy of the appearance time and τ_1/τ_2 suggests that the mechanism of vanadium atom formation does not change throughout the tube life.

Interference studies

The common elements interfering in the AAS determination of vanadium include those forming refractory compounds at high temperatures, such as Al, Ba, Mo, U and W, as well as Ca, Fe and Mg. Figure 3 summarizes the effects of increasing concentrations of the interfering elements on the AA signals obtained for a constant concentration of vanadium. It is clear that the element can be determined in the presence of ≤500-fold amount of Al, Mo and U, ≤1000-fold amount of Ca, Mg and Ba and ≤200-fold amount of Fe. The observations on the interference of Fe are in agreement with a recent report.[30] The determination of vanadium is difficult when more than 100-fold amount of W is present. This may be attributed to the formation of thermally stable species in the V–W–C system similar to those reported for Mo,[34] which are not decomposed under the heating conditions used. However, other investigators[29] have attributed this behaviour to the mechanical loss of analyte in the stable particles of tungsten formed at high temperatures. For Ca and Mg (≥1000-fold amount) the mechanism of the interference is different from that described above. At the beginning of the atomization stage a small but sharp absorption peak is noticed which decays rapidly and is followed by the rise of the AA peak of vanadium. With increasing concentrations of Ca or Mg, the small initial peak is

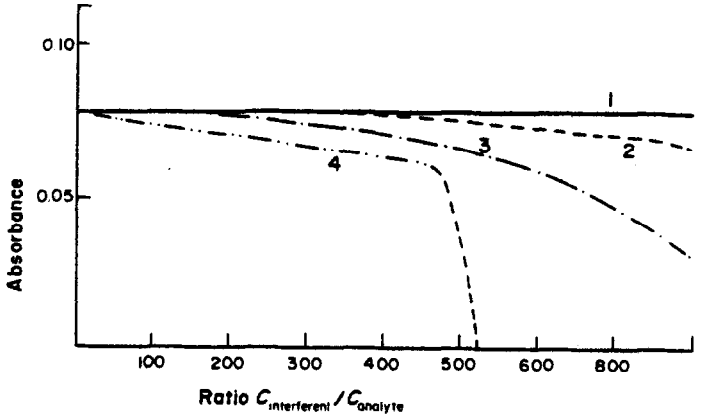

Fig. 3. Interference effects on the AA of vanadium, (1) Mg, Ca, Na and Ba; (2) Al, Mo and U; (3) Fe; (4) W.

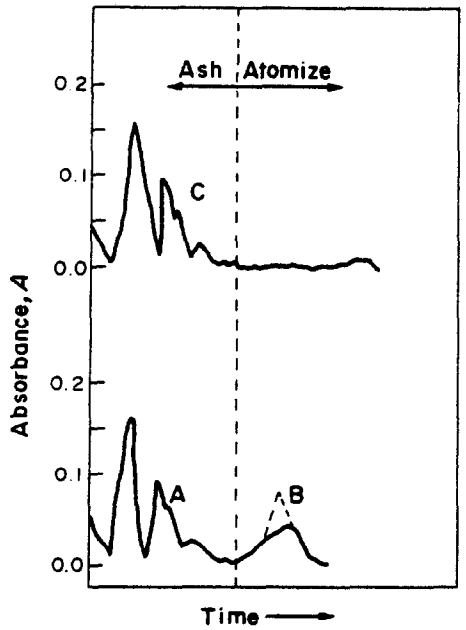

Fig. 5. Ashing and atomization signals of (A) brine diluted five times + 0.5 ng of vanadium; (B) 1.0 ng of vanadium; and (C) as for (A) but measured with a hydrogen lamp.

enlarged and examination of it by use of a hydrogen lamp shows it to be background absorption due to Ca or Mg molecular species, and making a negligible contribution to the AA peak of vanadium (Fig. 4). The nature of the electrothermal atomization of the element allows complete resolution of the background peak from the AA peak, because time is needed for the atomizer to attain the appearance temperature for vanadium (2200 ± 30 K).

Effect of sodium chloride

The determination of vanadium in sea-water and brines is important and so the ETA of vanadium in the presence of high concentrations (2–6%) of sodium chloride was investigated. The relatively high ashing temperatures possible in the ETA of vanadium eliminate these high concentrations of the salt during the ashing stage without any loss of the analyte. Incomplete ashing, however, results in a sharp, irreproducible peak appearing at the start of the atomization stage, before the AA peak of vanadium. This is due to the signal from the NaCl remaining in the furnace. The absorption traces obtained under the optimum heating conditions (Table 1), with a diluted brine sample to which known amounts of vanadium had been added, are shown in Fig. 5, which also shows the absorption signals obtained with a continuum source. It is clear that vanadium at the 0.1-μg/ml level can be determined in sodium chloride solutions of up to 6% concentration, without background correction.

Interference of acids

The effects of hydrochloric, nitric and phosphoric acids on the determination of vanadium have been investigated. The first two are the reagents usually used in the digestion of geological samples, while the third is the predominant species appearing in the acid digests of phosphate rocks. The AA signal is stable for a wide range of hydrochloric acid concentrations so there is no risk in using the acid for making up solutions to be analysed for vanadium. When a series of solutions, in which the concentration of nitric acid is increasing, is atomized, the AA signals for vanadium decrease correspondingly and this observation is in agreement with previous reports.[30] In the presence of increasing concentrations of phosphoric acid the AA signal of vanadium is slightly suppressed, a behaviour which may be attributed to the formation of stable vanadium phosphates.

In order to study the analysis of the phosphate rock digests, synthetic samples were made up containing the two major constituents, calcium and phosphate, in appropriate amounts together with vanadium. The results are interesting. The background peak due to calcium disappears and the suppression of the AA signal by the phosphoric acid is reduced. This shows the masking effect of the acid on calcium and the releasing action of calcium for vanadium in phosphoric acid medium. The most probable reason for these effects is the formation of calcium phosphate.

Determination of vanadium in phosphate rocks

Five phosphate rock samples have been analysed. Two were local standards with certified vanadium contents and three were analysed for the first time. The procedure of bracketing the unknown AA measurements with those of the standards is necessary

Table 3. Vanadium content of Iraqi phosphate rocks

Sample	Vanadium content, μg/g		
		Values found	
	Certified value	Std. addn.	Calibration graph
Standard phosphate rock, PH1	188	196 ± 4*	193 ± 6*
Standard phosphate rock, PH3	100	96 ± 5*	95 ± 4*
Core phosphate	—	46	45
Fine grain phosphate	—	366	362
Umm-Alradhuma phosphate	—	235	239

* Average of 8 determinations ± standard deviation.

Table 4. Vanadium content of Iraqi crude oils*

Sample	Vanadium content, μg/g		Deviation %
	Standard addition	Direct calibration	
Kirkuk crude oil	24.7	24.2	2
Khanakeen crude oil	15.9	16.5	3

* Recovery of vanadium added, 97.7%, RSD ± 3%.

to compensate for the variation in sensitivity resulting from the graphite-tube degradation. The method of standard additions was used to overcome matrix effects. The results are shown in Table 3. It can be seen that the standard-additions method gives values in agreement with those from the calibration graphs. These findings demonstrate the reliability of direct analysis of the acid digests of the phosphate rocks, *i.e.*, there is no need to use the standard-additions method in this case. The absorption traces show only the AA peaks of vanadium and a background correction is not necessary. In the standard-additions method it is obviously essential that all the measurements lie in the linear part of the calibration graph.

Determination of vanadium in crude oils

The crude oils supplied from the two fields have different vanadium contents, which reflect the mineralogy of the areas. The standard-addition and calibration-graph results are in agreement. The absorption profiles were checked by use of the hydrogen lamp and the removal of the matrix during the ashing stage was shown to be complete. This makes the use of background correction unnecessary. The results are listed in Table 4.

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A POTASSIUM-SELECTIVE ELECTRODE WITH SOLID INTERNAL CONTACT

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Summary—The performance characteristics of potassium-selective electrodes made with valinomycin membranes plasticized with dioctyl adipate or sebacate, and with a solid silver contact or an internal solution, have been critically examined. The choice of electrode depends on a number of factors, including the interfering ion(s) present. The electrodes can be used for determination of potassium in natural waters.

The promising results obtained for calcium[1] and nitrate[2,3] selective electrodes with PVC membranes and solid internal contact have stimulated further investigations for the potassium selective electrode. The active membrane component used throughout this work was valinomycin, and the plasticizers were dioctyl adipate (DOA) or dioctyl sebacate (DOS) which had been proved to be effective[4,5] in similar electrodes.

Although several studies have been published concerning the mechanism of functioning of electrodes with solid contacts,[6,7] we thought it interesting to make a comparative study of some electrode characteristics, using sensors with a solid silver contact and with an internal solution. Among the parameters investigated were stability of the electrode potential, range of linear response, selectivity and dynamic parameters. Two practical analytical applications of the electrode were also developed.

EXPERIMENTAL

Electrodes and apparatus

The plastic PVC membranes containing valinomycin (Sigma, U.S.A.) and di-n-octyl adipate (DOA) or di-n-octyl sebacate (DOS) as plasticizers were prepared according to Fiedler and Růžička.[4] The construction of the exchangeable electrode tip is shown in Fig. 1. The plastic membrane (4), 5 mm diameter, is placed on the convex silver disc (3) and fixed with a threaded brass ring (2). The body (1) is machined from polypropylene. Unless otherwise indicated, 0.01*M* potassium chloride was used as the internal solution in the inner reference electrode and for electrode conditioning between measurements.

A double-junction reference electrode (Orion model 90-02) was used with a salt bridge filled with 10% ammonium nitrate solution. This system has been proved to have the lowest liquid-junction potential, in good agreement with the values calculated on the basis of the Henderson equation. The calculated differences in liquid-junction potential between 10^{-4} and $10^{-2}M$ potassium chloride and bridging solution containing various electrolytes were subtracted from the experimentally measured electrode slopes (Table 1). The resulting slopes were relatively constant and close to the Nernstian value. This was of importance when the electrode characteristics were measured in media of varied ionic strength.

The potential measurements were taken with a digital potentiometer (Orion model 701). The flame photometer used for comparative measurements was a "Flaphkol" (Zeiss, Jena).

RESULTS AND DISCUSSION

Measuring range, sensitivity and potential stability

Electrodes with a solid contact and those with an internal solution were systematically checked over a period of 6 months for response slope and linear range, in solutions of pure potassium chloride. For both types of electrode the slopes at 25° were found to be 56.5 ± 0.5 and 56.0 ± 0.5 mV/decade, respectively. No systematic drift of these values was observed during the lifetime of the electrodes. The estimated temperature coefficient in the range 25–50° was 0.47 mV/deg.

For both types of electrode the response range was linear down to pK 5.0, irrespective of the plasticizers used for making the membranes.

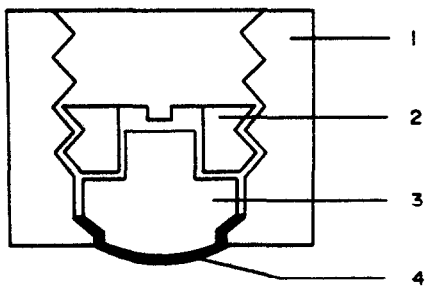

Fig. 1. Scheme of the electrode construction with the solid silver contact and PVC membrane: 1—polypropylene body, 2—brass ring, 3—silver contact, 4—PVC membrane.

Table 1. Effect of liquid-junction potential on the electrode response in the range from 10^{-4} to $10^{-2}M$ potassium chloride; electrode with DOA plasticizer and internal reference solution

Bridging electrolyte	Electrode response, mV/pK	
	Measured	Corrected for liquid-junction potential
$1M$ LiCl	75.5	56.0
$0.1M$ NaCl	67.0	56.0
$2M$ NaCl	66.0	53.9
$1M$ $NaNO_3$	67.0	56.7
$1.9M$ NH_4Cl	57.5	56.4
10% NH_4NO_3 ($1.2M$)	56.0	56.8

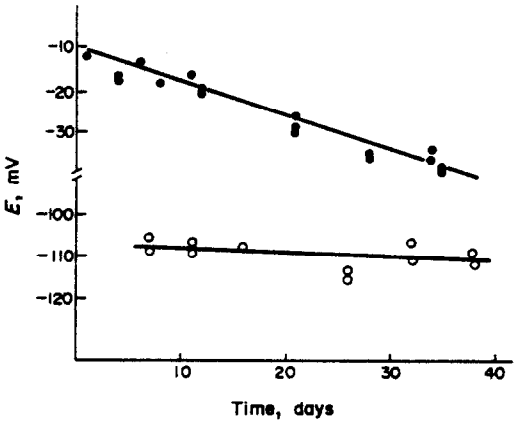

Fig. 2. Long-term potential drift for solid-contact potassium electrode (○) and electrode with internal solution (●).

On the other hand, differences were observed in the stability of the potential readings in spite of the fact that the membranes for both types of electrode were cut out of the same master membrane and were identically conditioned. For the DOA membranes the electrode with internal solution exhibited a regular shift of the potential reading at the rate of approx. 0.8 mV/day (Fig. 2). The potential of the electrode with solid contact, except for random variation over a range of a few millivolts, was constant during at least 6 weeks. This behaviour is significantly dependent on the plasticizer used. For the DOS membranes, the potential of both types of electrode decreased by 1.7 mV/day during two weeks but then became stable. Between measurements the electrodes were kept in $10^{-2}M$ potassium chloride.

Selectivity and interferences

The selectivity coefficients of the potassium electrodes were determined in mixed solutions[8] containing other alkali-metal cations, or ammonium or calcium ions, added as the chlorides. The concentrations used were $0.1M$ except for rubidium and caesium, for which, because of their greater interference, only $0.001M$ solutions were used. The selectivity coefficients, calculated from the intersections of the extrapolated linear portions of the calibration curves, were practically constant over a period of six months for the membranes containing DOA (Table 2). For the DOS membranes the selectivity coefficients had nearly the same values as for the DOA electrode with calomel paste as the internal reference system and were in good agreement with the data reported by Schindler *et al.*[7]

Because the effect of lipophilic anions on the response of the neutral carrier electrodes has been discussed widely in the literature,[9,10] calibration with potassium chloride, nitrate and thiocyanate was performed in the pK range from 1 to 6. In the case of DOS membranes both types of electrodes give similar deviation from linearity at concentrations above $10^{-2}M$.

These deviations amounted to 25 and 35 mV for the solid-contact and internal-solution electrodes, respectively, at a concentration corresponding to pK 1.2. Greater differences in the electrode response were observed for the DOA membranes. In potassium chloride and nitrate media the potential response was linear up to pK 1.2, but in the thiocyanate solutions the deviation for the solid-contact electrode was only 7 mV at pK 1.2, whereas for the internal-solution electrode at the same concentration the deviation was 39 mV, with the change from cationic to anionic function at pK 2 (Fig. 3). For DOS electrodes the change in function occurred at pK 2.5.

Table 2. Logarithms of selectivity coefficients for potassium membrane electrodes investigated (DOA membrane; measurements at different times after electrode construction)

Interfering ion	Electrode with internal solution		Solid-contact electrode		Values from Fiedler and Růžička[4]
	1 month	6 months	1 month	6 months	
Na^+	−4.40	−4.26	−4.28	−4.36	−4.22
Li^+	−4.42	−4.16	−4.40	−4.36	−3.62
NH_4^+	−1.94	−2.12	−2.10	−1.94	−1.88
Cs^+	−0.55	−0.52	−0.42	−0.42	−0.33
Rb^+	+0.30		+0.36		+0.67
Ca^{2+}	−4.14	−3.80	−4.04	−3.96	−4.31

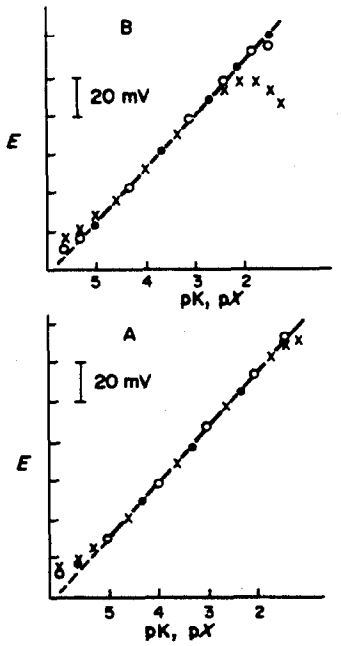

Fig. 3. Anionic interferences for solid-contact (A) and internal-solution (B) potassium-selective electrodes with membrane containing DOA as plasticizer: ●—Cl^-, ○—NO_3^-, ×—SCN^-.

The increasing anionic response of the electrode is related to penetration of the membrane phase by thiocyanate ions and the more advantageous behaviour of the solid-contact electrode is probably connected with the properties of the membrane–silver contact at the interface. For quantitative treatment of production of the membrane potential in the presence of an anion which penetrates the membrane, the processes on both the internal and external interfaces[11] must be considered. The steady-state concentration of an anion is connected with these processes, and the transport of a mobile anion will result in the anionic response characteristics of the electrode. For a solid internal contact the anion transport is limited to the membrane phase only. Therefore a relatively short time is needed for establishing the concentration of interfering anions in the membrane and the changes in potassium-ion activity are dominant among the potential-forming processes, and result in decrease of the anionic function of the electrode. The differences between electrodes made with different plasticizers may be attributed to differences in the mobilities of the anions in the membrane.

Dynamic characteristics

The response time for a given change in the potassium-ion activity also depends on the plasticizers used for making the membranes. For a potassium-ion activity change from 10^{-3} to $2 \times 10^{-2}M$ the potential difference between the 1st and 3rd, or 1st and 15th minute was measured, for solutions containing only a potassium salt or also a sodium or calcium salt (Table 3). The potential differences were somewhat smaller for electrodes with internal solution. The 15-min period for the test was chosen arbitrarily as being convenient for practical purposes. True equilibrium is not necessarily established in this time.

The potential differences were more significant, *i.e.*,

Table 3. Changes in electrode potential (mV) between 1st and 3rd and between 1st and 15th min after change of potassium concentration from 10^{-3} to $0.02M$ (DOS membrane; internal solution $0.01M$ KCl)

Interfering ion in external solution	Between 1st and 3rd min		Between 1st and 15th min	
	Internal solution	Solid contact	Internal solution	Solid contact
—	0.1	0.2	0.1	0.3
$1M$ Na^+	0.2	0.4	0.3	0.9
$1M$ Ca^{2+}	0.2	0.4	0.4	1.3

Table 4. Changes in electrode potential (mV) between 1st and 15th min after change of potassium-ion concentration from 10^{-3} to $0.02M$ (DOA membrane; different internal solutions)

Interfering ion in external solution	Internal solutions				Solid-contact electrode
	$0.01M$ KCl	$1M$ KCl	$1M$ NaCl	$1M$ $CaCl_2$	
—	1.2	0.1	0.2	0.2	1.0
$1M$ Li^+	5.6				1.7
$0.1M$ Na^+*	4.4				1.0
$1M$ Na^+	6.0	1.2	3.5	3.2	2.4
$1M$ Ca^{2+}	6.3	3.2	4.0	3.1	3.0

* Change of potassium ion concentration from 10^{-4} to $2 \times 10^{-3}M$.

Table 5. Potassium determination in river water by the multiple standard addition technique

Sample	Found by flame photometry, *mg/l.*	Number of measurements	Found by potentiometry: Graphical interpretation *mg/l.*	Graphical interpretation Error, %	Numerical interpretation *mg/l.*	Numerical interpretation Error, %
I	1.60	3	1.59	−0.6	1.58	−1.2
II	3.70	3	3.68	−0.5	4.03	+8.9
III	6.50	4	6.84	+5.3	6.09	−6.3

the electrode reacted more slowly to concentration changes, when the membrane contained DOA (Table 4). When 0.01*M* potassium chloride was used as internal solution the response was practically the same as for the solid-contact electrode, but larger than for the corresponding systems with DOS in the membrane. Much faster response was observed when the internal solution was more concentrated, provided the external solution concentration was greater than 0.01*M*. When the potassium chloride concentration is 0.01*M* on both sides of the membrane an equilibrium concentration of potassium ions is established within the membrane. Increasing the potassium ion concentration in the external solution causes diffusion of potassium ions, owing to the concentration gradient. When potassium ions reach the internal membrane–solution interface, they influence the equilibrium there, which results in slow changes in the electrode potential. When the internal solution concentration is greater than that of the external solution the concentration gradient is in the opposite direction and the membrane potential changes only at the external interface. In such a case the increase in potassium ion concentration in the membrane reduces the linear range of electrode response, the lower limit being pK 4.5.

Because interfering elements may also change the dynamic characteristics of the potential response[12] the effect of lithium, sodium and calcium was investigated (Table 4). In these measurements, increasing the concentration of the internal electrolyte solution gave more rapid equilibration of the potential readings (Fig. 4). The behaviour of the 1*M* potassium chloride electrode was nearly the same as that of the solid-contact electrode (Fig. 4), for which the potential at the internal membrane interface could be taken as constant.

The time needed to establish a constant potential reading mostly depends on the diffusion in the membrane but not in the aqueous phase.[13] Therefore the phenomena observed may be interpreted as follows. When present at high concentration, the interfering ions penetrate the membrane until a steady state is reached inside it, giving rise to a stable potential. The diffusion of potassium ions is disturbed by the other ions and therefore the final response is attained more slowly. This effect is successfully eliminated by using an internal solid contact or a concentrated internal solution.

Determination of potassium in river water

The potassium electrode with a solid contact and the DOA membrane was used for determination of potassium in river samples. A 50-ml sample was used, made 0.1*M* in sodium chloride. The multiple standard-addition method was used, with addition of at least eight 0.5-ml portions of 0.001*M* potassium chloride. After each addition the potential was recorded when the drift in reading was less than 0.2 mV/min. The results (Table 5) were evaluated graphically and also numerically with the program CALIB,[14] but the graphical method gave better agreement with the flame photometric determinations.

The possibility of using the electrode as a sensor in flow-injection analysis[15] was also tested. The same electrode was used in a specially designed electrode cell (Fig. 5). A 145-μl sample of river water was injected into a stream of 0.1*M* sodium chloride flowing at 8 ml/min. The reproducibility of the recorded peaks was better than 0.5%. The results were 3.3% lower than the flame-photometric values.

To obtain good results in the flow-injection analysis it was necessary to keep the dynamic characteristics of the detector as contant as possible. However, during the first few days of use of the electrode, some peak-height changes were observed (Fig. 6), the peaks becoming smaller with increase in electrode-condi-

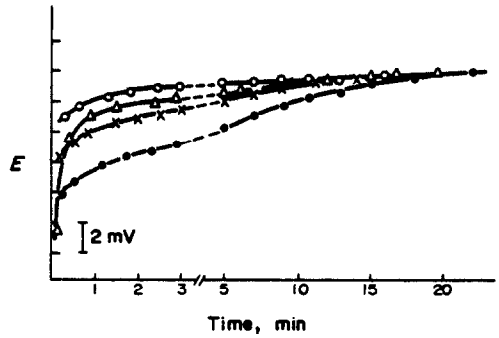

Fig. 4. Potential changes for potassium electrodes with solid contact (Δ) and different internal solutions (●—10^{-2}*M* KCl, ○—1*M* KCl, ×—1*M* NaCl) for the potassium-ion concentration change from 10^{-3}*M* to 0.02*M* in presence of 1*M* NaCl.

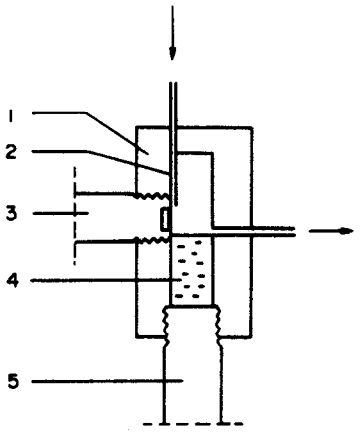

Fig. 5. Electrode cell for flow-injection analysis with potentiometric detection: 1—cell body (Perspex), 2—Teflon tube, 1-mm bore, 3—sensing electrode, 4—solution, 5—reference electrode (Ag/AgCl).

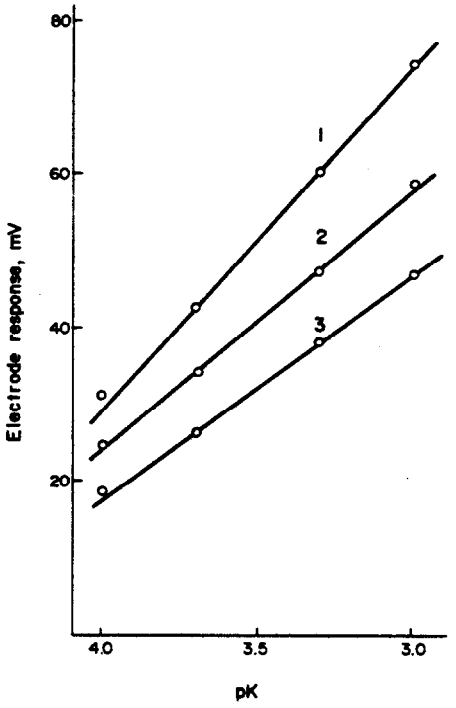

Fig. 6. Calibration curves for the flow-injection analysis with potassium electrodes conditioned for different time periods: 1—non-conditioned, 2—conditioned for 2 days, 3—conditioned for 3 days.

tioning time, which suggests that the response time for the electrode increases. This may be due to increase in the thickness of the hydrophilic gel layer and to a growing contribution of the diffusion of ions through the layer. Similar facts have been observed for the plastic nitrate-selective electrode.[16]

CONCLUSIONS

Investigation has shown that both the solid-contact and internal-solution potassium-selective electrodes have similar characteristics. The behaviour of the solid-contact electrodes depends on the plasticizer used for the membrane preparation and may sometimes be more advantageous than that of the internal-solution electrode. For di-n-octyl adipate membranes the solid-contact electrode gives a more stable response, eliminates anionic interferences and improves the dynamic characteristics, especially in the presence of interfering ions. The electrodes described have been successfully used for determination of potassium in natural waters.

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EXTRACTION OF URANIUM(IV) FROM PHOSPHORIC ACID SOLUTIONS WITH 1-PHENYL-3-METHYL-4-BENZOYLPYRAZOLONE-5 (PMBP)

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Summary—The extraction of uranium(IV) from phosphoric acid solutions with PMBP and PMBP–TOPO mixtures has been studied. The synergic extraction with PMBP–TOPO is more effective than the simple chelate extraction with PMBP and both systems are more effective than the synergic extraction of uranium(VI) with DEHPA–TOPO. It is established that the complexes extracted are $U(PMBP)_4$ and $U(PMBP)_4 \cdot TOPO$ for the chelate and synergic extraction respectively. The most probable uranium(VI) species in the aqueous phase (2.9–6.33*M* H_3PO_4) is the neutral complex $U(H_5P_2O_8)_4$. Analytical methods suitable for determination of uranium in phosphoric acid solutions have been developed. The highest sensitivity is achieved by combining the synergic extraction with the uranium(IV)–arsenazo III colour reaction.

The present paper deals with determination of the uranium content of phosphoric acid solutions by extraction photometric methods. Several techniques for this have been studied.[1] Among them is a method for low uranium content (0.02–0.2 g/l.) based on synergic extraction of uranium(VI) with di(2-ethylhexyl)phosphoric acid (DEHPA) and tri-n-octylphosphine oxide (TOPO), stripping as uranium(IV) with 8.6*M* phosphoric acid containing 20 g of iron(II) per litre and final determination with arsenazo III in acetate buffer. The calibration graph is linear but depends on phosphoric acid concentration. A similar method has been proposed in which tributyl phosphate is used instead of TOPO.[2] The TOPO system is the more effective.

The extraction of uranium(IV) with 1-phenyl-3-methyl-4-benzoylpyrazolone-5 (PMBP) has not been studied, but by analogy with the extraction behaviour of the actinide elements,[3,4] a high degree of extraction would be expected. On the other hand, the synergic extraction of uranium(IV) with β-diketones (other than PMBP) and neutral organophosphorus compounds has been investigated.[5,6] The greatest synergic effect was obtained with the relatively most acidic β-diketone, thenoyltrifluoroacetone (TTA) and the most basic neutral reagent TOPO. PMBP is a stronger acid than TTA, so it should give a larger synergic effect, and this system should therefore be worth investigation.

The extraction of uranium(VI) from phosphoric acid media with organic solutions of PMBP is of no practical importance, because of the high extractability of iron(III).[3,4] The iron(III) must be reduced to iron(II), and the uranium(VI) is simultaneously reduced to uranium(IV).[7]

EXPERIMENTAL

Reagents

Stock uranium(IV) solutions were prepared in the following manner. Uranium(VI) was reduced with zinc in 1–2 *M* hydrochloric acid, the reaction being stopped when a dark colour appeared [U(III)]; the excess of reductant was filtered off and after dilution of the filtrate to give a hydrochloric acid concentration of about 0.2*M*, uranium (IV) phosphate was precipitated with 1–2 ml of concentrated phosphoric acid. The precipitate was filtered off, washed with water until free from chloride, dissolved in concentrated phosphoric acid and diluted to the desired volume. The stock solution was about 0.01*M* in U(IV) and 5*M* in phosphoric acid. It was stable for at least a month. It was standardized by potentiometric titration with potassium dichromate. The uranium(VI) content was less than 0.3% of the total uranium concentration. Working solutions were prepared by dilution of the stock solution with the required quantities of phosphoric acid and water. The acidity was determined by potentiometric titration with sodium hydroxide.

Organic solutions of the extractants were made by dissolving weighed amounts in toluene. The PMBP was synthesized at the Institute of General and Inorganic Chemistry, BAS, Sofia, Bulgaria.

Procedures

Uranium determination in organic extracts. An aliquot of organic extract containing less than 35 μg of uranium is evaporated and mineralized with 1–2 ml of concentrated perchloric acid and 4–6 drops of 30% hydrogen peroxide in a sand-bath until a moist residue remains. The residue is dissolved at room temperature with 10 ml of a 0.5-mg/ml solution of arsenazo III in 7*M* nitric acid (prepared from the concentrated acid presaturated with urea) and the absorbance is measured at 655 nm against a reagent blank. If the sample contains phosphorus, as in the synergic extraction, aluminium nitrate must be added to the reagent solution. The uranium(VI) calibration standards and the samples must have a similar phosphorus content, which should have a molar concentration that is between $\frac{1}{3}$ and $\frac{2}{3}$ of that of the added aluminium.

An alternative is to determine the metal as uranium(IV). An aliquot of the organic extract containing less than 12 μg of uranium is decomposed as before. The moist residue obtained is dissolved with 5 ml of concentrated hydrochloric acid at room temperature, the uranium is reduced with 0.5 ± 0.02 g of medium-sized zinc granules, and 0.5 ml of 0.5% arsenazo III solution is added. The absorbance is measured at 665 nm against a reagent blank. If the sample contains phosphorus, the moist residue must be dissolved with 5 ml of 0.1*M* aluminium chloride solution in concentrated hydrochloric acid. The phosphorus content permissible is 0.05–0.35 mmole. The calibration standards and samples should not have a phosphorus content that is outside these limits.

Uranium determination in aqueous phase. An aliquot of the aqueous phase is appropriately diluted to known volume and a fraction of the resulting solution (containing up to 40 μg of uranium and 1–2 mmole of phosphoric acid) is transferred to a 10-ml standard flask. The uranium is oxidized by addition of 1% potassium permanganate solution dropwise until a pink colour appears, then the excess of oxidant is eliminated by dropwise addition of 1% ascorbic acid solution. Finally, 6 ml of 10*M* nitric acid–0.5*M* aluminium nitrate solution presaturated with urea) and 1 ml of 0.5% arsenazo III solution are added in succession and the mixture is diluted to the mark. The absorbance is measured at 655 nm against a reagent blank.

PMBP determination in the aqueous phase. An excess of copper sulphate solution is added, the solution is adjusted to pH 5, and the copper–PMBP complex is extracted into methyl isobutyl ketone. The copper in the extract is then determined by atomic-absorption spectrometry and the PMBP concentration calculated from it.[8]

Extraction tests

These were performed under the following conditions—aqueous:organic phase ratio 2:1; temperature 25 ± 1°; contact time 5 min at 200 strokes/min (this is long enough for extraction equilibrium to be attained.). The uranium concentration was determined in the phase with the lower uranium content, by the arsenazo III method. The PMBP left in the aqueous phase was determined.

RESULTS AND DISCUSSION

PMBP species

The possible association of PMBP in the organic phase was studied by distribution measurements. The distribution coefficients (D_{HA}) were obtained at various PMBP concentrations up to 0.75*M* and fixed phosphoric acid content in the aqueous phase. The D_{HA} values were 1450 ± 80, 1080 ± 50 and 125 ± 10 for 3.24, 4.82 and 8.92*M* phosphoric acid respectively, independent of the PMBP concentration up to the highest level tested. These results show that no association occurs.

In slightly polar solvents PMBP exists in enolic form and can undergo one dissociation and two protonation reactions in the aqueous phase.[3,4] At constant PMBP concentration in the organic phase, the inverse of the distribution coefficient can be expressed by the equation:

$$\frac{1}{D_{HA}} = \frac{1}{K_r} + \frac{K_d}{K_r[H^+]} + \frac{K_{p1}[H^+]}{K_r} + \frac{K_{p2}[H^+]^2}{K_2} \quad (1)$$

where K_r, K_d, K_{p1} and K_{p2} are the apparent equilibrium constants for the partition of neutral molecules, the dissociation and the two protonation constants respectively. Thus, the different processes can be studied by measuring the D_{HA} values at different phosphoric acid concentrations and solving equation (1). The hydrogen-ion concentrations were calculated according to Elmore *et al.*[9] The mean D_{HA} values and the corresponding experimental conditions are given in Table 1.

A statistical test of equation (1) indicated that the significant terms are those corresponding to the partition of neutral molecules and to the second protonation. Therefore, the best fit is obtained with the expression

$$\frac{1}{D_{HA}} = \frac{1}{K_r} + \frac{K_{p2}[H^+]^2}{K_r} \quad (2)$$

where $K_r = 1690 \pm 40$ and $K_{p2} = 0.32 \pm 0.02$.

These results indicate that the predominant PMBP species in the aqueous phase are the neutral molecules and the doubly protonated cation H_2PMBP^{2+}.

Another possible reaction is the association between PMBP and TOPO in the organic phase, but the D_{HA} values showed no dependence on TOPO concentration up to 0.25*M*. The values obtained were 1450 ± 60 and 790 ± 50 for 3.02 and 5.84*M* phosphoric acid respectively. The PMBP concentration in the organic phase was fixed at 0.50*M*.

The enolic form of PMBP is the active one in extraction processes. This form passes into the keto-

Table 1. Distribution coefficient values for PMBP (D_{HA}) at various phosphoric acid concentrations

No.	Total H_3PO_4 conc., *M*	$[H^+]$	D_{HA}	
			Experimental	Calc. by eq. (2)
1	0.98	0.16	1750	1680
2	1.97	0.35	1590	1630
3	2.96	0.62	1500	1510
4	3.91	0.98	1340	1300
5	4.91	1.45	1040	1010
6	5.85	1.99	780	750
7	6.84	2.61	480	530

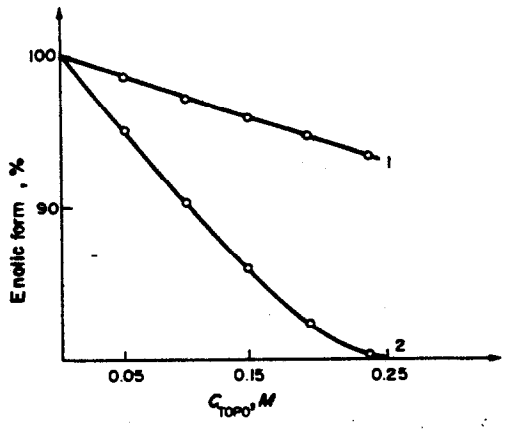

Fig. 1. Influence of TOPO content on the enolic form concentration. (1) 0.50*M* PMBP and (2) 0.25*M* PMBP.

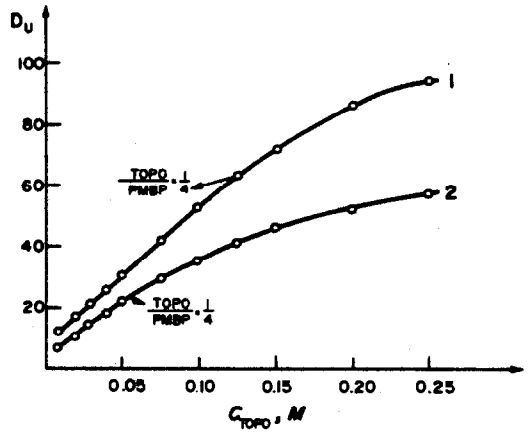

Fig. 2. Influence of TOPO concentration on uranium(IV) extraction. (1) 0.50*M* PMBP and (2) 0.25*M* PMBP.

nic form as the solvent polarity is increased. In consequence, the addition of TOPO to the organic solution leads to a shift to the ketonic PMBP, and so the extraction of uranium should be affected. The influence of TOPO addition on the tautomeric equilibrium was studied photometrically by measuring the absorbance of the yellow enolic form in the visible region, where the ketonic form does not absorb. The variation in the fraction in the enolic form as a function of the amount of TOPO added, at two fixed PMBP concentrations, is given in Fig. 1. The results confirm that TOPO causes conversion into the ketonic form, the extent of conversion increasing if the total PMBP concentration is decreased. This must be taken into account in interpretation of the extraction data.

Extractable uranium(IV) complexes

The influence of PMBP concentration on the uranium distribution coefficient (D_U) was investigated and the stoichiometry of the complexes extracted was determined by plotting log D_U *vs.* log [PMBP] for different phosphoric acid concentrations. The graphs were linear, fitting the empirical equations:

(*a*) for 4.40*M* H_3PO_4

$$\log D_U = 2.13 + 4.03 \log[\text{PMBP}] \quad (3)$$

(*b*) for 5.35*M* H_3PO_4

$$\log D_U = 1.32 + 3.84 \log[\text{PMBP}] \quad (4)$$

If TOPO is present, corrections must be made for the decrease in enolic form concentration ($PMBP_e$). The D_U values and the corresponding enolic form concentrations give the empirical equation

$$log\ D_U = 3.84 + 3.89 \log[\text{PMBP}_e] \quad (5)$$

for 4.24*M* phosphoric acid. The results all suggest that four PMBP molecules are present in the complexes extracted. Moreover, the addition of TOPO enhances the uranium(IV) extraction, *i.e.*, a synergic effect is observed. The synergic extraction of uranium(IV) can take place by formation of a ternary complex. This process was studied by examining the influence of the TOPO concentration on the D_U values for constant phosphoric acid and PMBP concentrations. The results are given in Table 2 and Fig. 2. From the latter, a decrease in the synergic effect at a

Table 2. Uranium distribution as a function of TOPO concentration at 0.25 and 0.50*M* PMBP

		0.25*M* PMBP			0.50*M* PMBP		
No.	TOPO conc., *M*	[$PMBP_e$]	D_U exp.	D_U calc. eq.(7)	[$PMBP_e$]	D_U exp.	D_U calc. eq. (10)
1	0.010	0.2472	7.2	5.4	0.4985	12.2	13.2
2	0.020	0.2448	11.3	10.2	0.4970	17.7	19.5
3	0.030	0.2425	14.7	14.7	0.4955	21.5	23.8
4	0.040	0.2403	18.4	18.4	0.4945	25.8	28.2
5	0.050	0.2380	21.6	22.7	0.4930	30.8	32.3
6	0.075	0.2332	28.7	30.9	0.4895	42.9	42.2
7	0.100	0.2262	35.0	37.1	0.4865	52.7	51.8
8	0.125	0.2205	41.2	41.8	0.4830	62.7	60.6
9	0.150	0.2148	46.9	45.1	0.4795	71.9	68.8
10	0.200	0.2057	52.1	50.7	0.4730	85.7	84.1
11	0.250	0.2010	57.9	57.4	0.4665	93.7	97.4

molar ratio TOPO:PMBP > 1.4 is observed. In this region, the negative effect of the reduced enol concentration is not fully compensated by the ternary complex formation.

The number of TOPO molecules in the ternary complex cannot be accurately determined by a simple log–log plot, because of the decreased enol concentration. This problem was solved by use of the expression

$$D_U = C[\text{PMBP}_e]^4[\text{TOPO}] \quad (6)$$

where C is a composite term including the apparent equilibrium constant for the synergic extraction and the complex formation in the aqueous phase. This term may be considered constant at fixed phosphoric acid concentration.

Equation (6) was evaluated from the experimental data (see Table 2), with $[\text{PMBP}_e]^4[\text{TOPO}]$ as the independent variable. The resulting equations are:

(*a*) for 0.25*M* PMBP

$$D_U = 1.41 \times 10^5[\text{PMBP}_e]^4[\text{TOPO}] \quad (7)$$

(*b*) for 0.50*M* PMBP

$$D_U = 8.74 \times 10^3[\text{PMBP}_e]^4[\text{TOPO}] \quad (8)$$

The empirical equation (7) fits the experimental data well, but equation (8) does not. In the latter case, a new model allowing for co-existence of the ternary complex and the U(IV)–PMBP complex in the organic phase was proposed, giving

$$D_U = C_1[\text{PMBP}_e]^4 + C_2[\text{PMBP}_e]^4[\text{TOPO}] \quad (9)$$

where C_1 and C_2 include the apparent equilibrium constants for complex formation in the aqueous phase and the chelate and synergic extraction respectively. These two terms may be considered as constants at fixed aqueous phase composition. The empirical equation obtained is

$$D_U = 170[\text{PMBP}_e]^4 + 7550[\text{PMBP}_e]^4[\text{TOPO}] \quad (10)$$

Equation (10) fits the experimental data. The D_U values calculated from equations (7) and (10) are given in Table 2.

From the results it follows that the extractable complexes of uranium(IV) are $U(PMBP)_4$ and $U(PMBP)_4 \cdot TOPO$ for the chelate and synergic extraction respectively. In the latter case, both complexes can co-exist, if the PMBP concentration is high enough to give significant uranium(IV) extraction by itself.

Extraction curves

The plots of $\log D_U$ *vs.* $\log C_{H_3PO_4}$ for chelate and synergic extraction were linear, and described by the equations

(*a*) for chelate extraction

$$\log D_U = 5.07 - 6.56 \log C_{H_3PO_4} \quad (11)$$

(*b*) for synergic extraction

$$\log D_U = 5.27 - 6.44 \log C_{H_3PO_4} \quad (12)$$

where $C_{H_3PO_4}$ is the total phosphoric acid concentration in the aqueous phase.

The chelate extraction data were used in investigation of the complex formation in the aqueous phase. The phosphoric acid species were calculated according to Elmore *et al.*[9] and all possible reactions were tested. No satisfactory fit was obtained but the most significant uranium(IV) complexes in 2.9–6.33*M* phosphoric acid were $(U(H_3P_2O_8)_{4-n}(HPO_4)_n^{n-}$, where $n = 1$–4. The predominant species is the neutral complex $U(H_3P_2O_8)_4$. The difficulty in interpreting the data arose from uncertainties in the concentrations of the phosphoric acid species, and the fact that no convincing activity corrections could be made.

Equations (3) and (11) indicate that higher chelate extraction efficiency may be expected at lower phosphoric acid concentrations and higher PMBP concentrations. For example, at 2.84*M* phosphoric acid and 0.72*M* PMBP (20% w/v), the D_U value was 445 ± 85, high enough to guarantee quantitative extraction. The phosphoric acid concentration must be at least 2.5*M*, however, for the uranium(IV) to be stable.[7] On the other hand, quantitative synergic extraction at the mean phosphoric acid concentration in wet-process acid (4.3*M*) may be obtained if higher concentrations of the extractants are used. Thus $D_U = 420 \pm 80$ was obtained by extracting from 4.32*M* phosphoric acid with 0.64*M* PMBP and 0.16*M* TOPO.

Uranium determination

During these studies, no significant extraction of phosphoric acid was observed. Hence the addition of aluminium salts in the photometric determination is necessary only for the analysis of the synergic extraction extracts, because of the presence of TOPO.

Most common substances accompanying wet-process phosphoric acid do not interfere (up to the highest concentrations tested) in uranium determination by the four possible combinations of extraction systems and colour reactions. The substances and highest concentrations tested (in g/l.) were calcium 8; aluminium 8; titanium(IV) 0.1; vanadium(IV) 0.2; rare-earth elements (Ce and Y) 0.05; thorium 0.05; sulphate 30; free sulphuric acid 15; fluorosilicic acid 30.

The behaviour of iron required special attention. Both uranium(VI) and iron(III) ions were effectively reduced by the addition of zinc powder, to extractable uranium(IV) and non-extractable iron(II). To prevent iron oxidation and co-extraction, it was necessary to maintain reducing conditions during the extraction process. This was achieved by adding iron powder before the extractant. The iron-powder reaction is slower than that with zinc and continues during the separation process. In this way, an iron content of up to 8 g/l. in the phosphoric acid is prevented from interfering.

The extraction procedures developed are as follows.

Chelate extraction. Two ml of the phosphoric acid solution (~4.3*M*) are placed in a 10-ml extraction tube and diluted with 1 ml of water, then 10–15 mg of zinc powder are added, and when the hydrogen evolu-

Table 3. Analysis of phosphoric acid solutions

No.	Uranium content, *mg/l**	Chelate extraction		Synergic extraction	
		U(IV)–arsIII	U(VI)–arsIII	U(IV)–arsIII	U(VI)–arsIII
1	5.8	—	—	5.9 ± 0.3	—
2	24.0	23.6 ± 1.0	23.4 ± 1.1	24.0 ± 0.9	23.4 ± 1.0
3	47.0	47.0 ± 1.4	48.0 ± 1.6	47.2 ± 1.2	48.2 ± 1.3
4	140	—	145 ± 4	—	140 ± 4

* From weight of U_3O_8 standard taken.

tion diminishes, 5–10 mg of iron powder are added and the uranium is extracted by shaking with 3 ml of 20% w/v PMBP in toluene for 5 min. The uranium is determined with arsenazo III (without addition of aluminium).

Synergic extraction. Three ml of the phosphoric acid solution (~4.3*M*) are placed in a 10-ml extraction tube and reduced as above, then the uranium is extracted by shaking with 3 ml of 0.64*M* PMBP–0.16*M* TOPO (in toluene) for 5 min. The uranium is determined by the arsenazo III method in presence of aluminium ions.

Finally, four phosphoric acid samples with known uranium contents were analyzed by the methods developed. The results are given in Table 3 and illustrate the suitability of the methods for uranium determination in this kind of sample. The combination of synergic extraction with the uranium(IV)–arsenazo III colour reaction allows the determination of very low uranium contents.

Conclusions

The methods developed are suitable for determination of uranium in phosphoric acid solutions. The highest sensitivity is obtained by combination of the synergic extraction with the uranium(IV)–arsenazo III reaction. The synergic extraction of uranium(IV) with PMBP and TOPO is more effective that the simple chelate extraction of uranium(IV) with PMBP, and both systems are superior to the synergic extraction of uranium(VI) with DEHPA–TOPO.[10]

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EVALUATION OF DIFFERENTIAL PULSE ANODIC-STRIPPING VOLTAMMETRY AT A STATIONARY MERCURY-FILM ELECTRODE WITH STIRRED SOLUTION

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Summary—Differential pulse anodic-stripping voltammetry at a stationary mercury-film electrode with the solution stirred during the deposition step has been investigated. The sensitivity achieved by using such a simple set-up is similar to that obtained with a mercury-film rotating disk electrode. The effects of various experimental parameters on the peak current are described. Lead and cadmium were used as test systems, and gave detection limits of around $1 \times 10^{-10} M$ with 5-min deposition times.

A thin mercury-film electrode (TMFE) deposited on a glassy carbon substrate has been shown to be the most sensitive electrode used in anodic-stripping voltammetry (ASV) for trace metals analysis.[1] Studies to improve the ASV response usually involve comparison of different excitation waveforms employed in the stripping step (*e.g.*, differential pulse *vs.* linear scan[1]), or of different electrode configurations (*e.g.*, TMFE *vs.* hanging mercury drop[2]) or of different substrates (glassy carbon *vs.* impregnated graphite[3]). While considerable research effort has gone into these aspects, little attention has been paid to the choice of different convective modes that may be employed during the deposition (preconcentration) step.

The deposition step in ASV is usually facilitated by convective transport of the metal ions to the electrode surface. The overall sensitivity and precision are largely dependent on the effectiveness and reproducibility of the hydrodynamics, which controls the amount of the metal plated during the deposition step. Most work with the TMFE (*e.g.*, references 4 and 5) has involved the use of a rotating disk electrode to improve the rate of transport of ions. Other modes of mass-transport employed in ASV have included flowing solutions (used mainly in on-line analysis[6]), and stirred solutions (used mainly in conjunction with the hanging mercury drop electrode[7]). One of the major practical restrictions of the TMFE is the apparent need for a rotating disk assembly; the high price of such an assembly (around $2000) often precludes the adoption of this technique. The use of a rotating disk assembly also has some limitations in ultratrace analysis: the location of the motor and electrical connections above the cell significantly enhance the contamination risk.[4] Some researchers have employed a stirred solution in conjunction with ASV at a TMFE,[8] instead of a rotating electrode, but no comprehensive comparison has yet been published of ASV at a stationary TMFE in a stirred solution with ASV at a rotating disk electrode.

This report describes the results of such a comparison.

Conventional stirring apparatus, using, for example a magnetic stirring bar, is cheaper than a rotating disk assembly by a factor of 15–20, and is available in most laboratories. The motion produced by stirring was one of the earliest modes of transporting electroactive species to electrode surfaces.[9] This approach is now used mainly in dissolved oxygen meters[10] or in ASV at the hanging mercury drop electrode,[7] but has not become widespread for other applications. Recently we have exploited it for sensitive voltammetric measurements based on hydrodynamic modulation procedures.[11] In such measurements the detectability is limited by the noise level associated with the stirrer. However, this is not the case with ASV, which is an indirect technique where the actual measurement (stripping) step is usually performed on a quiescent solution. Overall, the combination of the efficient stirring mass-transport (during the deposition step) with the sensitive differential pulse approach (during the stripping step) results in extremely high sensitivity, obtainable with relatively simple instrumentation.

EXPERIMENTAL

Apparatus and instrumentation

A 200-ml (6.5-cm diameter, 7.5-cm high) Pyrex glass cell with a four-hole "Plexiglas" cover was employed. The working electrode was a mercury thin film deposited on a 0.75-cm diameter glassy carbon disk. The Ag/AgCl (satd. KCl) reference electrode and the spectroscopic graphite counter-electrode were supported in two of the holes in the cover. The working electrode was mounted on a rotating

disk assembly, allowing direct comparison of the stirred-solution ASV experiments with those of rotating-electrode ASV. A 3.5-cm long Teflon-coated stirrer bar was placed on the cell bottom, exactly below the centre of the working electrode. The cell was located in a reproducible position on a variable-speed magnetic stirrer (Sargent-Welch, No. 76490). A paper towel was placed between the cell and the stirrer to reduce changes in the solution temperature due to heat from the motor. The arbitrary scale of the stirrer controller was calibrated for the empty cell with a digital tachometer. The stirring settings 4–10 yielded stirrer speeds of 340, 425, 515, 660, 830, 1020 and 1165 rpm, respectively, with a reproducibility of about 3%. Accurate calibration was not feasible when the cell contained a sample solution, as the water–air interface interfered in the optical detection system of the tachometer. Since the viscosity of the solution may affect the stirring-rate, settings are reported throughout this paper. An EG&G Princeton Applied Research model 364 polarograph was used as a three-electrode potentiostat. Voltamperograms were displayed on a strip-chart recorder.

Reagents

All solutions were prepared from analytical-grade chemicals and demineralized water. Stock solutions, 2.5mM, of various metal nitrates were prepared and stored in polyethylene bottles. Portions of these solutions were diluted as required for standard-addition purposes. The mercury plating solution, 2.5mM mercuric nitrate, was similarly prepared. All samples were prepared in 0.1M potassium nitrate supporting electrolyte.

Procedure

The glassy carbon electrode was polished, at the start of the work, with 0.1-μm alumina slurry until a mirror-like finish was obtained. The ASV data were obtained by co-depositing the mercury film and the trace metals on the working electrode in the following manner. The sample and 4 ml of the mercuric solution (total volume, 200 ml) were introduced into the cell and deaerated, then the nitrogen delivery tube was raised above the solution, and a potential of -1.0 V was applied at the working electrode while the solution was stirred (stirrer setting, 7). After 6 min, the potential was switched to 0.0 V and held there for 2 min. The electrode was then ready for use in an analytical run. Background and sample determinations were carried out successively by applying the plating potential for a selected time suitable for the concentration levels concerned. The stirring was then stopped, and after a 10-sec rest period the metals were stripped from the mercury film by applying a differential pulse anodic potential-ramp with a 2 mV/sec scan-rate and 50 mV amplitude. The scan was terminated at 0.0 V and after 30 sec the system was ready for the next plating–stripping cycle. The mercury film was removed at the end of the day by wiping the electrode face with a soft tissue wetted with 2M nitric acid, and the electrode was rinsed with demineralized water.

RESULTS AND DISCUSSION

The equation for the differential pulse anodic-stripping peak-current at a TMFE is given[12] by $i_p = -0.138\, q/t$, where t is the pulse duration, and q the total charge passed during deposition, equal to $i_L t_{dep}$, where i_L is the limiting current for the deposition of the metal in question, and t_{dep} the deposition time. If the deposition potential is in the mass-transport-controlled region, then under forced-convection conditions i_L is given[13] by $i_L = nFAC_bM$, where n, F, A, and C_b have their usual meanings in ASV work, and M is the mass-transport coefficient, which is proportional to the flux of the ion at the electrode surface. M has the general forms $K\omega^x$ and $K'U^{x'}$ for a rotating TMFE and for a stationary TMFE in a stirred solution, respectively (ω is the rotation speed, U the stirring rate, and K, K', x, and x' are constants, characteristic of the hydrodynamics and electrode configuration). Combining these equations leads to the following expression for the ratio of the peak-currents at the rotating TMFE and at the stationary TMFE with stirred solution,

$$\frac{i_{p,\,\text{rotation}}}{i_{p,\,\text{stirring}}} = \frac{M_{\text{rotation}}}{M_{\text{stirring}}} = \frac{K\omega^x}{K'U^{x'}}$$

which means that under the same experimental conditions the peak current ratio is equal to the ratio of the concentration gradients (*i.e.*, fluxes) during the deposition step.

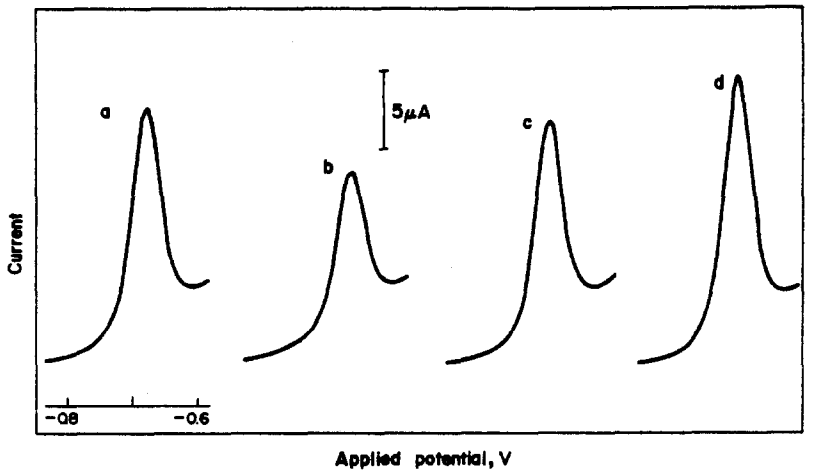

Fig. 1. Voltamperograms for $5.6 \times 10^{-6}M$ cadmium in 0.1M potassium nitrate, obtained with a stationary electrode in a stirred solution (a) and with a rotated electrode (b–d). Stirrer setting, (a): 8. Rotation speeds, 400 (b), 900 (c), and 1600 (d) rpm. Deposition step, 3 min at -1.0 V. Differential pulse amplitude, 50 mV. Scan-rate, 2 mV/sec.

Figure 1 compares differential pulse anodic-stripping peak-currents for the stationary electrode with stirred solution (a) and for the rotating electrode (b–d), obtained for $5.6 \times 10^{-8}M$ cadmium under otherwise similar experimental conditions. The peak-currents are of the same order of magnitude. The peak-current for the stationary TMFE with stirring at about 830 rpm is quite close to that for the rotating TMFE at 900 rpm and is 47% larger than that for the rotating TMFE at 400 rpm. As $i_{p,\,rotation}$ increases proportionately to the square-root of the rotational speed,[12] the peak-height at 1600 rpm is 15% larger than that for the stirred solution stationary TMFE. These results indicate that magnetic stirring does produce significant angular motion of the solution near the surface of the stationary disk electrode, resulting in similar flux values, M, and hence in similar sensitivities for the two methods.

Similar limits of detection would therefore also be expected, since the stripping step in both cases is usually carried out under the same conditions (stationary disk and quiescent solution), and thus background currents and noise levels are likely to be similar. Figure 2 illustrates the detectability which can be obtained with stirred solution (during the deposition) and a stationary TMFE; it shows a differential pulse anodic-stripping voltamperogram for $9 \times 10^{-10}M$ (0.1 ng/ml) cadmium and $6 \times 10^{-9}M$ (1.2 ng/ml) lead in $0.1M$ potassium nitrate solution, obtained by employing 5-min deposition. If the limit of detection is defined as the concentration giving a signal equal to the noise, its value for cadmium is around $1 \times 10^{-10}M$ (0.011 ng/ml). Even better detectability would be obtainable by using longer deposition periods. When stirring was used during the stripping step, the noise level was significantly larger, obscuring the peak currents of interest and raising the limits of detection.

Table 1 shows the dependence of the cadmium peak current on the stirrer setting. Increasing the stirring rate increases the peak current, but a maximum is reached at about stirrer setting 9 (about 1000 rpm). In addition, some difficulties occur at high stirring speeds because of vortex formation and frothing of the solution, producing tiny gas bubbles that cling to the mercury film. This problem gets worse the nearer the electrode is to the surface of the solution: though the distance between bottom of the beaker and electrode surface (in the range 1.5–3.5 cm) has no significant effect on the peak-current, interference due to gas bubbles is observed when this distance is larger than 2 cm (for the apparatus used). For these reasons stirring settings of 7 and 8 (about 660–830 rpm) and a distance of 1.5 cm between the disk surface and the bottom of the cell have been used throughout and are recommended for similar experimental conditions (different conditions, such as the size of the magnetic stirrer, may affect the efficiency of convective-transport).

Table 1. Dependence of peak-current or stirrer setting*

Stirrer setting	Approximate speed, *rpm*†	i_p, μA
4	340	6.1
5	425	6.5
6	515	8.0
7	660	8.4
8	830	9.4
9	1020	10.6
10	1165	10.8

* $5.6 \times 10^{-8}M$ Cd^{2+} in $0.1M$ KNO_3; deposition step, 2 min at -1.0 V; differential pulse amplitude, 50 mV; scan-rate, 2 mV/sec.
† Measured for an empty cell.

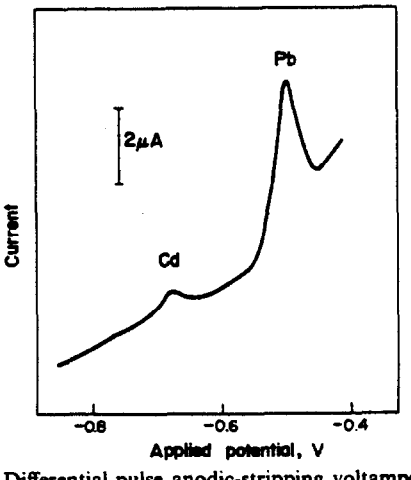

Fig. 2. Differential pulse anodic-stripping voltamperogram for $9 \times 10^{-10}M$ cadmium and $6 \times 10^{-9}M$ lead in $0.1M$ potassium nitrate. Deposition step, 5 min at -1.1 V. Stirrer setting, scan-rate and pulse amplitude, as in Fig. 1 (a).

The plot of peak-current *vs.* deposition period is linear for periods ranging from 1 to 7 min, with a slope of 2.2 μA/min for a $4.5 \times 10^{-8}M$ cadmium solution, but on extrapolation the straight line does not give $i_p = 0$ at $t_{dep} = 0$. The intercept, 1.05 μA, is attributed to plating-out of the metal ions during the slow scan from the deposition potential to the peak potential,[14] and to some of the metal ions stripped from the electrode during the pulse being replated into the mercury film in the waiting time between pulses.[12]

The precision was estimated by 10 successive measurements on a $1.3 \times 10^{-7}M$ lead solution over a 60-min period [conditions: 1-min deposition at -0.8 V; stirrer setting, scan-rate and pulse amplitude as in Fig. 1 (a)]. The mean peak-current was 17.5 μA with a range of 17.2–17.8 μA. The relative standard deviation over the complete series was 1.1%. The precision obtained compares favourably with that reported for the TMFE with other modes of convective transport.[14] This indicates that any heating effects of the stirrer motor have negligible influence on the precision. Over a 4-hr period of continuous operation with stirring, peak-currents for metal ions at the ng/ml level were found to decrease by about

15%. Such changes might be expected as a result of the gradual deterioration of the mercury film[15] or adsorption of the trace metals onto the cell and the electrode.[16] Mechanical stress is expected to cause less deterioration of the stationary TFME than of the rotating electrode, but on the other hand, the heat produced by the stirrer motor may affect the stability of response. The temperature was found to increase by 3–4° over the 4-hr period of continuous stirring, and the temperature coefficient of the diffusion coefficient is generally about 2–3% per degree.[17] Since the standard-addition method is generally used for calibration in ASV, these slow temperature changes do not affect the accuracy of the results, and if necessary the changes can be minimized by using electronically-generated stirring (*e.g.*, EG&G Model 305 stirrer) or thermostatic control of the cell assembly. In view of the results presented here, and the simplicity of the instrumentation and its operation, stripping analysis at a stationary TMFE with stirred solution may be considered as a rival approach to ASV at the rotating TMFE.

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SPECTROPHOTOMETRIC DETERMINATION OF IRON AND REDUCING AGENTS WITH PPTS, A NEW WATER-SOLUBLE FERROIN-TYPE CHROMOGEN OF SUPERIOR SENSITIVITY

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Summary—A sulphonated derivative of 3-(4-phenyl-2-pyridyl)-5-phenyl-1,2,4-triazine has been prepared, its structure identified, and its chelation products with iron(II) and copper(I) identified and characterized. The water-soluble compound, referred to as PPTS, has been applied to the determination of iron in various types of samples and the spectrophotometric determination of trace quantities of certain reductants.

The most sensitive chromogenic reagent of the ferroin-type for iron found to date is 3-(4-phenyl-2-pyridyl)-5-phenyl-1,2,4-triazine.[1] Analytical applications for this reagent, however, are limited to non-aqueous solutions and extraction procedures because of its low solubility in aqueous solutions. To obtain a more useful water-soluble derivative of this compound we have prepared and characterized a sulphonated product which we refer to as PPTS. The structure of this compound and the stoichiometries of its iron(II) and copper(I) chelates are reported together with recommended procedures and results for the use of PPTS to determine iron in a variety of samples. Spectrophotometric determination of various reducing agents, based upon the reduction of iron(III) in the presence of PPTS, is also described.

EXPERIMENTAL

Reagents

The parent compound 3-(4-phenyl-2-pyridyl)-5-phenyl-1,2,4-triazine (PPT) was synthesized as described by Case[2] and sulphonated by the method of Cryberg and Diehl.[3] The sulphonated product was isolated as the ammonium salt, recrystallized from distilled water, and dried in air. Analysis gave C 44.3%, H 4.5%, N 15.2%, S 12.0%, H_2O 6.4%; $C_{20}H_{12}N_4(SO_3NH_4)_2.2H_2O$ requires C 44.44% H 4.47%, N 15.55%, S 11.86%, H_2O 6.7%. PPTS is also commercially available from the G. Frederick Smith Chemical Company (Item No. 728).

A 0.010*M* solution of PPTS [ammonium 3-(4-phenyl-2-pyridyl)-5-phenyl-1,2,4-triazine-disulphonate dihydrate] was prepared by dissolving 0.54 g of the reagent in 100 ml of distilled water.

Standard solutions of iron and copper were prepared from weighed amounts of the pure metals (electrolytic) dissolved in hydrochloric or nitric acid and diluted to known volumes with distilled water.

A 10% solution of hydroxylamine hydrochloride was prepared by dissolving 100 g in 900 ml of distilled water. Traces of iron and copper were removed by extraction with isoamyl alcohol.[4]

For the spectrophotometric determination of reducing agents, composite reagent solution was freshly prepared as needed. The concentrations of the components were $2.0 \times 10^{-3}M$ PPTS, $2.0 \times 10^{-4}M$ ferric chloride and $2.0 \times 10^{-3}M$ hydrochloric acid.

Buffer solutions were prepared for the pH range 4–6 by adding glacial acetic acid to 1*M* ammonium acetate, and for the range 8–10 by adding concentrated ammonia solution to 1*M* ammonium chloride. The pH 7 buffer was 1*M* ammonium acetate.

The standard reference materials (SRMs) used in testing the recommended procedure for the determination of iron in plant material were obtained from the U.S. National Bureau of Standards. The synthetic sea-water sample had known concentrations of iron, sodium chloride (30 g/l.) and sodium fluoride (0.022 g/l.).

Apparatus

Spectra and absorbances were recorded with a Cary Model 14 spectrophotometer (visible region), a Sargent-Welch Model 3-200 spectrophotometer (infrared), and a JEOL Model PFT 100 spectrometer [proton magnetic resonance (PMR)].

Determination of iron in plant matter

Transfer an accurately weighed 1-g sample of the plant material into either a 125-ml conical flask or a porcelain crucible, depending on whether wet oxidation or dry ashing is to be used. For wet oxidation use either of the procedures described on pages 75 and 76 in Ref. 5. For refractory substances the procedure on page 76 (with nitric, sulphuric and perchloric acids) is recommended. To dry ash the sample, first heat the crucible and contents at low temperature to char the sample, then at higher temperature to drive off most of the carbon, and finally at 800–900° to ash the sample completely. Cool to room temperature, moisten the ash with 1–3 drops of concentrated nitric acid, evaporate to dryness, and again ignite at 800–900° for 1 hr. Cool, dissolve the ash in 3 ml of 6*M* hydrochloric acid and transfer the solution quantitatively to a 100-ml standard flask. Wash the crucible with another 3-ml portion of 6*M* hydrochloric acid and rinse into the same flask. Dilute to volume with distilled water.

Pipette a 10-ml aliquot of the sample solution into a 25-ml standard flask and add 1 ml of 0.1*M* PPTS, 5 ml of

10% hydroxylamine hydrochloride solution, and 5 ml of 5*M* ammonium acetate (to adjust to pH 5–7). Dilute to volume with distilled water, and measure the absorbance at 565 nm. Also measure the absorbances of a blank and standards carried through the complete procedure (including the ashing or wet oxidation procedure used). Correct the absorbances for the blank and determine the concentration of iron in the sample from the data obtained for the standards.

Determination of iron in sea-water or potable water

Pipette a 50.0-ml sample of the water into a 125-ml glass-stoppered conical flask. Add by pipette 1.00 ml of 0.01*M* PPTS, 1.00 ml of 10% hydroxylamine hydrochloride solution and 2.00 ml of 5*M* ammonium acetate. Stopper the flask, heat the contents to 90–100°, and set aside to cool to room temperature. Measure the absorbance at 565 nm, using a 10-cm cell for ng/ml levels of iron. Carry a blank and one or more standards through the same procedure to deduce the unknown iron concentration.

Spectrophotometric determination of reductants

Into a glass-stoppered flask pipette 5.00 ml of the composite reagent solution and 5.00 ml of reductant sample solution. After the predetermined reaction time, measure the absorbance of the solution at 565 nm against a reagent blank (prepared at the same time as the test solution). Determine the concentration of the reductant by using a calibration curve obtained with standards treated in the same way as the unknown.

RESULTS AND DISCUSSION

The structure of PPTS, deduced from elemental and spectral analysis of the sulphonated product, is depicted in Fig. 1. The infrared and PMR spectra indicated that the phenyl groups were sulphonated in the *para* position, as found for other phenyl-substituted ferroin-type reagents.[6]

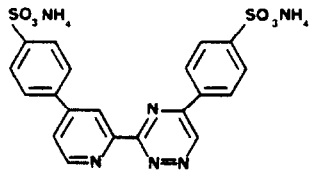

Fig. 1. Structure of PPTS.

The stoichiometry and formation constants for iron(II) and copper(I) chelates of PPTS in aqueous solutions at pH 7 were determined by a modification[7] of the mole-ratio method. A ligand to metal ratio of 3:1 and a conditional formation constant of 4.4×10^{15} were found for the iron(II) chelate. For the copper(I) chelate a ratio of 2:1 and a formation constant of 6.5×10^{9} were obtained. Unfortunately, the relative stabilities of the PPTS and PPT chelates cannot be directly compared because the formation constants of the PPT chelates are not determinable for aqueous solution owing to solubility limitations. However, PPTS forms much weaker complexes than 2,2′-bipyridyl,[8] presumably because of the electron-withdrawing effect of the sulphonate groups.

The spectral data for the iron(II) and copper(I) chelates of PPTS are compiled in Table 1 together with data for comparison with the PPT chelates. Since the PPT chelates are insoluble in water, the absorption characteristics were determined as a function of ethanol content of the solution. Thus the chelates of PPT and PPTS can be compared for similar conditions. Clearly, sulphonation has little effect on the molar absorptivity of the iron(II) chelate but an appreciable

Table 1. Molar absorptivities of PPTS and PPT complexes in aqueous and ethanolic solutions (pH 7)

Complex	λ_{max}, nm	Ethanol, % v/v			
		0	40	60	80
$Fe^{II}(PPTS)_3$	565	3.32×10^4	3.45×10^4	3.45×10^4	3.50×10^4
$Fe^{II}(PPT)_3$	561	—	3.46×10^4	3.42×10^4	3.42×10^4
$Cu^{I}(PPTS)_2$	454	1.08×10^4	8.9×10^{3}*	—	—
$Cu^{I}(PPT)_2$	495	—	1.05×10^4	—	—

* Wavelength of maximum absorbance shifted to 491 nm.

Table 2. Effect of various ions on the determination of 0.84 ppm iron

Ions tolerated at 100 ppm:					
Al^{3+}	K^+	NH_4^+	CO_3^{2-}	IO_3^-	Acetate
As^{3+}	Li^+	Pb^{2+}	Cl^-	NO_2^-	Trichloroacetate
Ba^{2+}	Mg^{2+}	Sr^{2+}	ClO_4^-	NO_3^-	Borate
Bi^{3+}	Mn^{2+}	Br^-	F^-	SO_4^{2-}	Citrate
Ca^{2+}	Na^+	BrO_3^-	I^-	SCN^-	Oxalate
					Tartrate

Interfering ions (tolerance levels in ppm):					
Cd^{2+}	(30)	Ni^{2+}	(0.9)	CN^-	(0.7)
Co^{2+}	(5)	Sn^{4+}	(5)	PO_4^{3-}	(60)
Cu^+	(0.3)	V^{4+}	(50)	$P_2O_7^{4-}$	(13)
Cr^{3+}	(26)	Zn^{2+}	(50)		

Table 3. Determination of iron in various samples

Sample	Decomposition method	Number of replicates	Iron, *ppm* Present	Found	Relative standard deviation, %
Spinach (SRM 1570)	$HNO_3 + HClO_4$	6	550 ± 20	556	1.9
	Dry ashing	5		522	2.6
Pine needles (SRM 1575)	$HNO_3 + HClO_4$	6	200 ± 10	174	3.3
	Dry ashing	6		174	0.5
	$HNO_3 + H_2SO_4 + HClO_4$	2		194	3.3
Orchard leaves (SRM 1571)	$HNO_3 + HClO_4$	3	300 ± 20	255	1.8
	$HNO_3 + H_2SO_4 + HClO_4$	3		271	2.1
	Dry ashing	2		285	3.0
Tomato leaves (SRM 1573)	$HNO_3 + HClO_4$	3	690 ± 25	575	1.7
	$HNO_3 + H_2SO_4 + HClO_4$	2		597	0.0
	Dry ashing	2		604	1.8
DeKalb municipal water	None	8	0.0488*	0.0484	1.2
Sea-water (synthetic)	None	6	0.0297	0.0296	1.0

* Average of 4 determinations (R.S.D. = 1.3%) with bathophenanthroline as chromogen and extraction into isoamyl alcohol.[11]

effect on that of the copper(I) chelate. Similar effects were observed with sulphonation of 2,4-BDTP, PDT, PPDT and 2,6-BDTP,[6] all closely related to PPT. The high sensitivities of PPT and these chromogens for iron determination are thus retained when they are converted into water-soluble derivatives by sulphonation.

Interference studies, are summarized in Table 2. A test ion was deemed to interfere if it caused an error of ±3% in the determination of 0.84 ppm of iron. Only copper and chromium gave high results, because they formed coloured products with PPTS. The other metal ion interferences were due to competitive complexation, giving rise to incomplete chelation of iron and low results, though there was no spectral interference. Anion interference was due to competitive complexation of the iron by the anion. Cyanide interference can be easily avoided by acidification and

Table 4. Spectrophotometric determination of selected reducing agents, based on reduction of iron(III) to produce $Fe^{II}(PPTS)_3$

Reductant	Reaction time, *min*	Slope of calibration curve* Theory†	Observed
L-Ascorbic acid	60	0.0664 ($n = 2$)	0.027
	180		0.044
L-Cysteine	30	0.0332 ($n = 1$)	0.033
	110		0.034
Hydroquinone	60	0.0664 ($n = 2$)	0.032
	240		0.032
Hydroxylamine	60	0.0664 ($n = 2$)	0.072
	240		0.073
$NaHSO_3$	60	0.0664 ($n = 2$)	0.021
	180		0.023
$Na_4Fe(CN)_6$	60	0.0332 ($n = 1$)	0.034
	180		0.034
Thioglycollic acid	15	0.0332 ($n = 1$)	0.008
	240		0.012

* Absorbance at 565 nm (for 1 cm path-length) plotted *vs.* concentration (in μmole/l.).

† Assumes quantitative reduction of iron(III) to iron(II) (where n is the number of electrons transferred per molecule of reductant) and complete formation of an equivalent amount of $Fe^{II}(PPTS)_3$.

boiling before addition of PPTS and final adjustment to pH 7.

To evaluate its practical effectiveness, PPTS was used for determination of iron in a variety of samples. The results, complied in Table 3, provide several interesting conclusions. PPTS is seen to be highly sensitive and to give good precision and accuracy, but the recovery of iron is clearly dependent on the sample decomposition method used. Complete recovery was obtained more often when wet oxidation with the nitric–sulphuric–perchloric acid mixture was used. Furthermore, sample treatment and operator dexterity were less critical for the wet oxidation than for the dry ashing procedure. Finally, it was disturbing not to be able to reproduce the result reported by NBS for Tomato Leaves SRM 1573. We obtained essentially the same result with 1,10-phenanthroline in place of PPTS and are inclined to believe that our result adequately approximates the "true" iron content of SRM 1573.

The use of ferroin chromogens for the determination of reducing agents[8–10] is based on the reaction of the reductant with iron(III) and determination of the iron(II) produced. We therefore examined the usefulness of PPTS for determining trace amounts of certain reductants of biological or chemical interest. The results are summarized in Table 4. Only cysteine and ferrocyanide gave reasonably rapid and stoichiometric results. The other reductants tested proved slower and less complete in their reactions with iron(III) at room temperature but gave linear empirical calibration curves suitable for use if a strict time-schedule was kept. Colour formation was much faster at elevated temperatures (60–90°), so a heating step should enhance the sensitivity and shorten the reaction time. The reagent-blank absorbance increased slowly with time, more rapidly on heating, but the increase was reproducible and small relative to the absorbance for a sample. Uric acid and 2-thioethylamine, though not included in Table 4, were also found to reduce iron(III) on heating, so they too should be determinable by a proper modification of the procedure. It also appears probable that differences in reaction rates could be used for distinguishing between two or more reductants in a mixture, enabling multicomponent determinations to be made. Such applications and the development of optimum procedures are commended to those who recognize the merits of PPTS and its analytical potential.

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SHORT COMMUNICATIONS

AN ENZYMATIC METHOD FOR TLC DETECTION AND DETERMINATION OF FENITROTHION IN WATER

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Summary—A simple enzymatic method is described for field TLC detection and determination of fenitrothion as fenitrooxon in water, with pig liver acetone powder as enzyme source. By this method, fenitrothion can be detected as fenitrooxon at ng levels and amounts ranging from 5 to 50 ng can be estimated.

The cholinesterase (ChE) inhibition technique is promising and practical for the detection and determination of organophosphorus pesticides.[1,2] An enzymatic method for TLC determination of parathion as paraoxon, with use of raw rat liver as the enzyme source, was reported by Nandakumar *et al.*[3,4] but can be used only in the laboratory. Bhaskar and Nandakumar[5,6] find that pig liver acetone powder is more advantageous than raw live sources, owing to its easy procurement and instant use. Hence, this is used as the enzyme source in the present method, which is suitable for field detection and determination of fenitrothion by thin-layer chromatography. The procedure takes only 1 hr and may find application in evaluation of fenitrothion residues in contaminated waters in remote places, rivers or lakes.

EXPERIMENTAL

Reagents

All chemicals were analytical grade. Fenitrothion (99% pure) was used for preparing acetone solutions of various concentrations. Pig liver acetone powder (Sigma Chemical Co., U.S.A.) was homogenized in water (20–28°) with a mortar and pestle to give a 1% suspension, filtered through four layers of cheesecloth and used immediately as enzyme source. 1-Naphthyl acetate solution in acetone, 0.5%, and *p*-nitrobenzenediazonium fluoroborate solution in acetone, 0.4%, were prepared.

TLC plates (20 × 10 cm) coated with a 450-μm thick layer of silica gel G were prepared as reported earlier[3] and dried before use.

Thin-layer chromatography

Different concentrations of standard pesticide solutions (1–10 μl) in acetone were spotted on a dried TLC plate with a graduated glass capillary. The plate was exposed to evenly distributed bromine vapour[3] for complete oxidation of fenitrothion to fenitrooxon. The plate was removed and exposed to air for 5 min to volatilize the bromine. An acetone:hexane (1:9 v/v) solvent system was employed. After the chromatography, the plate was dried. The pig liver acetone powder suspension was uniformly sprayed over the plate, thoroughly wetting the gel. About 5–10 ml of suspension is required for a 20 × 10 cm TLC plate. The plate was kept in a moist atmosphere for 10 min, the moist atmosphere being generated as follows. A glass tank (20 × 30 × 10 cm) was fitted with a raised platform inside to carry the TLC plate. Boiling water was poured into the tank to a level below the raised platform, and the tank was closed with a lid. This generated a sufficiently moist atmosphere for enzyme incubation to occur within 5–10 min. The air temperature from the raised platform to the top of the TLC plate ranged from 45 to 35°.

The plate was then sprayed with 1-naphthyl acetate solution in acetone and was replaced in the moist atmosphere for 2 min. It was next sprayed uniformly with *p*-nitrobenzenediazonium fluoroborate in acetone. The white spots that appeared on the orange-red background were marked.

The fenitrothion (as fenitrooxon) was estimated by both the area measurement and area weight methods previously reported.[3]

Residue estimation

A litre of water uncontaminated with organophosphorus pesticides was mixed with different amounts (Table 2) of fenitrothion in 1 ml of acetone and a drop of Tween 80 (for uniform mixing). After 48 hr, the sample was transferred to a separatory funnel and 100 ml of hexane were added. The mixture was shaken vigorously for 2 min. The layers were allowed to separate and the aqueous layer was run into another separatory funnel and shaken vigorously with another 100 ml of hexane for 2 min. The aqueous layer was discarded. The combined hexane extracts were filtered through a column (150 × 24 mm) of anhydrous sodium sulphate into a Petri dish and left to evaporate in the heat of the sun. The residue extract was appropriately diluted with acetone and the fenitrothion determined as described above.

RESULTS AND DISCUSSION

The ChE inhibition zones were determined by the area measurement and area weight methods. The results (Table 1) showed that fenitrothion can be estimated at the nanogram level by this method, the minimum detectable quantity being 1 ng.

Table 1. Relationship of ChE inhibition with fenitrothion concentration as determined by area measurement method and area weight method

Fenitrothion, *ng*	Area measurement method		Area weight method*	
	Inhibition zone, mm^2	ChE inhibition, %*	Inhibition zone *mg*	ChE inhibition, %*
5	18	8 ± 0.1	4	8 ± 0.5
10	31	14 ± 0.5	7	13.5 ± 1
20	78	32 ± 1	15	29 ± 1
30	108	48 ± 1	25	48 ± 2
40	148	67 ± 1.5	33	63.5 ± 2
50	184	82 ± 2	40	77 ± 3.5

* Values are means of 6 observations ± range.

Table 2. Estimation of fenitrothion residues as fenitrooxon in fortified water samples

Fenitrothion added, *μg*	Fenitrothion recovered as fenitrooxon, *μg*	
	Area measurement method	Area weight method
5	5 ± 0.5*	5 ± 0.9
10	10 ± 0.7	10 ± 0.6
15	14 ± 0.9	15 ± 0.9
20	19 ± 0.3	19 ± 1.4
25	24 ± 1.1	24 ± 0.8

* Values are means of 6 observations ± range.

The method can be used for field analysis of fenitrothion residues in water. The water for the moist atmosphere for enzyme incubation can be boiled on a portable stove, and in hot climates the hexane can be evaporated by the heat of the sun. In cold climates the hexane can be evaporated by placing the Petri dish on a metal plate previously warmed to about 40–45°. Table 2 shows that the results for residue analysis are satisfactory.

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SYNTHESIS AND ANALYTICAL PROPERTIES OF DI(2-PYRIDYL)-*N*,*N*-DI[(8-QUINOLYL)AMINO]METHANE

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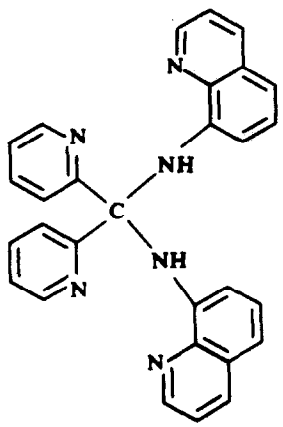
Summary—The synthesis and preliminary analytical assessment of di(2-pyridyl)-*N*,*N*-di[(8-quinolyl)-amino]methane is reported. It shows some potential as a reagent for iron(II).

Schiff's bases derived from 8-aminoquinoline have been synthesized by Lions and co-workers,[1-3] and the chelates of these compounds with iron(II) and some other metal ions have been isolated. The compound 8-picolylideneaminoquinoline, formed by condensation of 8-aminoquinoline with pyridine-2-aldehyde in the form of its chelate with iron(II) has been used for the extraction of perchlorate into nitrobenzene.[4]

This paper describes a new reagent obtained by reaction of 8-aminoquinoline with di-2-pyridyl ketone in a 2:1 ratio, to yield di(2-pyridyl)-*N*,*N*-di[(8-quinolyl)amino]methane, DPQAM, and not the Schiff's base.



EXPERIMENTAL

Synthesis of DPQAM

A solution of 8-aminoquinoline (2.8 g) and di-2-pyridyl ketone (2.5 g) in ethanol (15 ml) was heated under reflux for 4 hr, and then cooled to room temperature. The yellow precipitate was collected and washed with hot ethanol, and dried at 100° for 10–12 hr (m.p. 225–227°, yield 60%). Changing the reactant ratio did not affect the synthesis.

Elemental analysis gave C 76.8%, H 4.8%, N 18.9%. $C_{29}H_{22}N_6$ requires C 76.65%, H 4.83%, N 18.52%. Formation of a Schiff's base would give $C_{20}H_{14}N_4$ which requires C 77.39%, H 4.54%, N 18.06%. The molecular weight found cryoscopically was 440 ± 40. $C_{29}H_{22}N_6$ requires 454, and $C_{20}H_{14}N_4$ 310.

The infrared spectrum of DPQAM in $CHCl_3$-*d* showed the stretching vibrations of the NH groups at 3495 and 3355 cm^{-1}, but none due to a C=N group. Bands characteristic of the quinoline ring were found at 1592, 1579, 1569, 1508, 1477, 1429, 1378, 1337, 1098, 820 and 795 cm^{-1} (in KBr). In contrast, 1-benzylideneaminoaphthalene (synthesized for comparison) showed the C=N stretching band at 1629 cm^{-1}, but no bands in the NH stretching region.

The NMR spectrum in $CHCl_3$-*d* showed two multiplets at 6.8–8.2 and 8.7–9.0 ppm, due to the heterocyclic rings, and a peak at 9.9 ppm which was attributed to the –NH– group; this peak disappeared on addition of deuterium oxide. Integration of the peaks confirmed the structure.

The mass spectrum gave molecular ions at m/e = 454 (corresponding to the parent compound), 310 (Schiff's base) and 144 (8-aminoquinoline). These results confirmed the proposed structure and partial fragmentation into the Schiff's base and 8-aminoquinoline.

Physical properties

Solutions of DPQAM in DMF, ethanol or DMF–water mixtures are stable for at least a week.

The absorption spectra of DPQAM show bathochromic shifts in acid and basic media. The reagent shows three ionization steps. Since the molecule is symmetrical and the distance between the dissociable protons is relatively great, the values of the ionization constants for corresponding groups in the two halves would be expected to lie close together, and be indistinguishable by spectrometry. The pK values of the first and second ionization steps were calculated from the variation of absorbance with pH, at different wavelengths, by the Stenstrom and Goldsmith[5] and Sommer[6] methods; the mean values found were 2.48 and 3.83. For the calculation of the third pK value, in highly basic medium, the acidity function[7] H_- was used: $pK = H_- + \log [HL]/L^-]$; when $[HL] = [L^-]$, $H_- = pK$. Values of H_- have been tabulated by Schwarzenbach and Sulzberger[8] for known concentrations of sodium hydroxide. The absorbance at 275 nm was plotted against H_-, and the inflection point calculated. The value obtained was 13.6.

Spectrophotometric study of reactions with metal ions

The reactions of 40 cations with DPQAM were tested at different pH values. The most sensitive were those of iron(II) (green colour), cobalt (II) (yellow) and nickel(II) (yellow). For the spectrophotometric study of the most interesting reactions, the samples were prepared in 25-ml standard flasks with 1–8 ppm of metal ion, 5 ml of 0.1% DPQAM solution in dimethylformamide, 5 ml of buffer solution and dilution with distilled water. The absorbance

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Table 1. Characteristics of DPQAM complexes

Ion	Optimum pH	λ_{max}, nm	ϵ, $l.mole^{-1}.cm^{-1}$	M:R
Co(II)	1.0–7.5	380	8.2×10^3	1:2
Ni(II)	2.5–6.5	365	2.9×10^4	1:2
Fe(II)	3.5–6.5	685	9.4×10^3	1:2

was measured at 350–700 nm against a reagent blank. The most important results are summarized in Table 1.

DISCUSSION

From the experimental evidence the structure of DPQAM was deduced and it was concluded that the Schiff's base is not formed. However, when 1-naphthylamine reacts with benzaldehyde, the Schiff's base is formed very easily. This difference in behaviour may be attributed to the effect of the heterocyclic nitrogen atoms, resulting in formation of intramolecular hydrogen bonding between the NH groups and the N atoms of the pyridine rings, which stabilizes the structure sufficiently for this compound to remain unaltered in acid and alkaline media.

The first ($K_1 = K_2$) and second ($K_3 = K_4$) pairs of dissociation constants found must be due to the four protonated heterocyclic nitrogen atoms, so the neutral compound acts as a polyacidic base. The first pK value (2.48) may be attributed to the protonated pyridine rings. That their basicity is less than that of the dipyridyl ketone is presumably due to hydrogen bonding. The second pK value of 3.83 is attributed to the protonated nitrogen atoms of the quinoline rings, and is similar to that reported (3.56) for protonated 8-aminoquinoline.[9] The third value (13.6) is due to deprotonation of the NH-groups, and is similar to that for other compounds with the same group.[10]

The stoichiometric ratios found for the complexes of iron(II), cobalt(II) and nickel(II) show that each half of the molecule behaves independently, and this may be attributed to internal steric hindrance.

Compounds of this type have not previously been used as analytical reagents. DPQAM is a promising reagent for iron(II); the green 1:2 complex is extracted into chloroform in the presence of perchlorate or iodide; this extraction is very selective, and the partition coefficient very favourable. Although the sensitivity of the iron(II) reaction is of the same order as that of related reagents which also form green chelates, the selectivity of DPQAM is greater.[11]

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POLAROGRAPHY OF AN ACIDIC DEGRADATION PRODUCT FROM CEPHALEXIN

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Summary—2-Hydroxy-3-phenyl-6-methylpyrazine is identified as the product obtained by acidic degradation of cephalexin in the presence of formaldehyde. In 5*M* hydrochloric acid this product gives a well-defined reduction wave with a half-wave potential of −0.45 V *vs.* SCE. The wave is irreversible and diffusion-controlled. The diffusion current shows a linear relation with the cephalexin concentration and can be used for determination of cephalexin in plasma.

The β-lactam rings of cephalosporins and penicillins readily hydrolyse. The opening of the β-lactam ring of penicillins gives the penicilloates, but the analogous cephalosporoates are unstable and undergo further reaction. Cephalosporins with a substituted 3-methyl group undergo polarographic reduction,[1,2] but those with an unsubstituted 3-methyl group and no other reducible group do not give a peak at the dropping mercury electrode, though some of their degradation products act as depolarizers.[3,4]

In previous papers[5,6] we have reported polarographic methods for cephradine and cephalexin, based on the electroactivity of an acidic degradation product. The present paper reports on the electroactive degradation product from cephalexin obtained by acidic hydrolysis with 5.0*M* hydrochloric acid containing 1% of formaldehyde, and on a polarographic method for cephalexin in plasma.

EXPERIMENTAL

Reagents

Cephalexin (100% chromatographically pure, 99.0% activity) was obtained from Benguerel Laboratories, Santiago, Chile. All other chemicals were analytical grade.

Sørensen citrate buffer, pH 5.0, was prepared by dissolving 21 g of citric acid monohydrate in 200 ml of 1*M* sodium hydroxide and diluting to 1 litre. For preliminary work, Clark–Lubs buffers were used for pH 1–2, McIlvaine buffers for pH 2–8 and Sørensen buffers for pH 9–11.

Apparatus

The apparatus was that used in our work on flucloxacillin.[7] The operating conditions were: imposed drop-time 1 sec; scan-rate 10 mV/sec; voltage range 0.50 V; current range 5–15 μA full-scale; capillary characteristics $m^{2/3}t^{1/6} = 0.693$ mg$^{2/3}$.sec$^{1/6}$. The water-jacketed polarographic cell was kept at 25°. Solutions for polarography were deoxygenated with nitrogen which had previously been passed through a vanadium(II) scrubber.

Procedure

Cephalexin solution was hydrolysed in presence and absence of formaldehyde, at various acidities and for various times. The hydrolysed cephalexin solution was adjusted to pH 4.0 with sodium hydroxide, buffered with the pH-5.0 citrate solution, and extracted repeatedly with ethyl acetate. The combined organic extract was evaporated to dryness and the crude product was recrystallized from ethyl acetate. The product had an uncorrected m.p. of 206–208°. Analysis gave C 70.7%, H 5.5%, N 15.2%; $C_{11}H_{10}H_2O$ requires C 70.95%, H 5.41%, N 15.04%. The product had the following spectral characteristics: visible spectrum, λ_{max} (5.0*M* hydrochloric acid) 375 nm; infrared, ν (KBr disc) 2800 cm^{-1} (OH), 1650 cm^{-1} (amide), 1617, 1295, 750 and 690 cm^{-1}; NMR, δ($CDCl_3$, TMS external standard), 2.36 (3 Hs); 7.42 (4 Hm); 13.45 (1 Hs).

2-Hydroxy-3-phenyl-6-methylpyrazine was synthesized according to Barbhaiya.[8] Its elemental analysis, spectral characteristics and half-wave potential were identical with those of the degradation product, and a mixed melting point showed no depression.

For calibration 1.0–5.0-mg amounts of cephalexin were dissolved in 0.2 ml of plasma and diluted to 15 ml with 1% formaldehyde solution in 5.0*M* hydrochloric acid. For recovery tests, 2.0-mg cephalexin calibration solutions were used. These samples were hydrolysed by heating at 80° for 60 min in a constant temperature bath.

RESULTS AND DISCUSSION

The acidic hydrolysis of cephalexin in presence of formaldehyde yields a degradation product which has been isolated and identified as 2-hydroxy-3-phenyl-6-methyl pyrazine. An identical product from neutral hydrolysis of cephalexin was reported by Fogg.[9] We have obtained the same compound from the acidic hydrolysis of ampicillin.[10]

2-Hydroxy-3-phenyl-6-methylpyrazine exhibits a well-defined polarographic wave with a half-wave potential of −0.45 V *vs.* SCE in 5.0*M* hydrochloric acid. The half-wave potential is strongly dependent on pH ($\Delta E_{\frac{1}{2}}/\Delta pH = -0.068$ V, from pH 1 to pH 9).

The wave appears to be diffusion-controlled and the limiting current is proportional to the concentration of the depolarizer, as predicted by the Ilkovič equation. This linear relation can be used for determination of cephalexin in plasma. The compound, in hydrochloric acid medium, also shows a sharp absorbance maximum at 375 nm; the molar absorptivity is pH-dependent. We have used both properties to study the effect of acid concentration on the hydrolysis of cephalexin (Fig. 1). Figure 2 shows the rate of

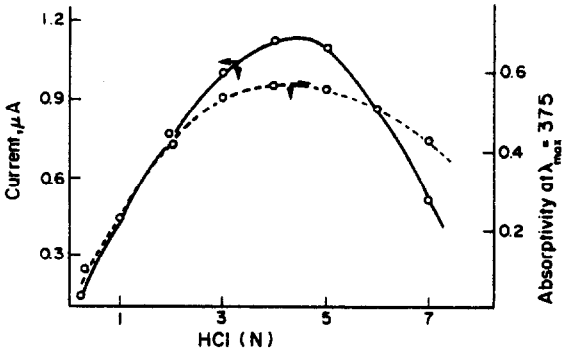

Fig. 1. Effect of acidity on diffusion current (——) and UV absorption at 375 nm (-----) of hydrolysed cephalexin.

formation of 2-hydroxy-3-phenyl-6-methylpyrazine at 60° and 80°. At 40° the reaction takes 180 min to reach completion. The hydrolysis product obtained in the absence of formaldehyde (compound A) cannot be isolated by the procedure used for the product (B) obtained in presence of formaldehyde. Product A gives two polarographic waves (at $E_{\frac{1}{2}}$ −0.50 and −0.78 V) but B gives only one wave ($E_{\frac{1}{2}}$ = −0.45 V). The identity of product A is being sought through synthesis of various substituted pyrazines.

The hydrolysis in presence of formaldehyde practically doubles the sensitivity for cephalexin.

The effect of different amounts of formaldehyde on the polarography of the pyrazine derivative is shown in Table 1: no suppressive action on the polarographic wave height was found.

There is an optimum formaldehyde concentration for the hydrolysis reaction (Table 2). This effect was observed for all the cephalexin concentrations tested (0.50–6.00 mg/ml). The use of more than 1% of formaldehyde decreases the degree of degradation of the cephalexin. Hence the recommended hydrolysis conditions are those used for the calibration. The calibration graph is linear, the equation being: $i_d(\mu A) = 1.18 \times 10^3 C - 0.032$ where C is the cephalexin concentration (mole/l.); the correlation coefficient is 0.991. The recovery for 2 mg of cephalexin was 98%, standard deviation 2% (7 replicates). The lower limit of detection was 40 μg of cephalexin per ml of plasma. This sensitivity is not adequate, because in man, after a 500-mg dose the peak antibiotic concentration is about 15 μg/ml.[11,12] The sensitivity might be improved by using differential pulse polarography, which is not available to us. Our earlier polarographic method for cephalexin was based on incomplete hydrolysis, and the method given here is better. The major advantage of the proposed method is that no extraction or deproteination is needed.

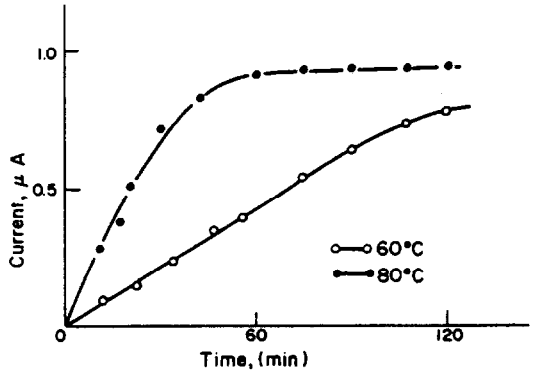

Fig. 2. Diffusion current after hydrolysis of cephalexin in 5.0*M* HCl, as a function of hydrolysis time and temperature.

Table 1. Influence of formaldehyde on the diffusion current of 2-hydroxy-3-phenyl-6-methylpyrazine (0.5 mg/ml in 5*M* HCl)

Formaldehyde, %	i_d, μA
0.5	11.8
1.0	11.7
1.5	12.0
2.0	11.8
3.0	12.0
5.0	11.8

Table 2. Influence of formaldehyde on the diffusion current of hydrolysed cephalexin solutions (1.0 mg/ml in 5*M* HCl)

Formaldehyde, %	i_d, μA
0.5	3.0
1.0	3.5_5
1.5	3.3
2.0	3.1
3.0	2.6_5

Acknowledgements—We are very grateful for support for this work from the Servicio de Desarrollo Científico, Artístico y de Cooperación Internacional. Also, we are indebted to Benguerel Laboratories, Santiago, Chile and Dr. O. Miranda for providing the cephalexin standard. Thanks are due to Professor Renato V. González for assistance in rewriting our paper.

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A COMPOUND OF IRON(II), PHENYLBIGUANIDE AND CYANIDE

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Summary—A spectrophotometric investigation has been made of the red water-soluble complex of phenylbiguanide with iron(II) in cyanide medium. The complex is found to have maximum absorbance at 520 nm. Use of the extension of the Asmus method to $M_rL_mL'_n$ complexes and of Koch and Ackermann's method suggests the formula $[Fe(phenylbiguanide)(CN_4)]^{2-}$ for the red compound. Anlytical procedures for the detection of iron and cyanide are proposed.

In a previous paper on the reactivity of phenylbiguanide with metal ions, the formation of a new coloured compound with iron(II) in cyanide medium was reported.[1] Its nature and properties have now been established by means of a spectrophotometric investigation in the visible region, and analytical procedures for the determination of iron and cyanide are proposed.

The co-ordination sphere of the metal centre in Fe(II)–cyanide–ligand ternary complexes is octahedral, and the ligand often contains nitrogen as donor-atom. Most iron(II) complexes are high-spin and kinetically labile. A few ligands, such as cyanide, interact strongly with this metal centre to force spin-pairing, giving the kinetically inert configuration, where activation energies are high.

The great number of physical and chemical studies done, especially since 1970, shows the interest in these compounds. References to formation constants or analytical applications are very few, however.

EXPERIMENTAL

Reagents

Phenylbiguanide hydrochloride was prepared according to Cohn[2] and purified as described before.[1] Analysis gave C 45.0%, H 5.5%, N 32.9%, Cl 16.6%; $C_8H_{13}N_5Cl$ requires C 44.97%, H 5.66%, N 32.78%, 16.59%. All chemicals used were of analytical grade.

Procedures

These are indicated in the text.

RESULTS AND DISCUSSION

Iron(II) reacts with phenylbiguanide in cyanide medium to form a soluble red compound. The compound is also obtained, in presence of excess of phenylbiguanide, from iron(III), probably because of reduction to iron(II) by the reagent. In the presence of oxidizing or iron(III)-complexing agents, the reaction is partially or totally suppressed. In the presence of reductants, the reaction is quantitative and more rapid. There is no reaction with ferrocyanide or ferricyanide. The product is retained on an anion-exchange column (Dowex 1, OH-form), suggesting the existence of an anionic Fe(II) complex. Similar red compounds have been obtained with biguanide and ethylenedibiguanide.[3]

Iron(II) solutions containing excess of phenylbiguanide and cyanide at pH >10 exhibit a characteristic spectrum (Fig. 1) with a band due to charge transfer ($t_{2g} \rightarrow \pi^*$) from iron to phenylbiguanide; this assignment is based on the high molar absorptivity and comparison with the spectra of similar compounds.[3]

The absorption spectrum changes with pH (Fig. 2). The band at 520 nm increases with pH, while the band at 420 nm decreases. The maximum colour development is at pH 11.2–11.7; with further increase in pH the absorbance falls rapidly. At pH >11 the reproducibility of the results is poor, owing to precipitation of iron(III) hydroxide because of atmospheric oxidation.

Phenylbiguanide must be present before the iron(II) and cyanide solutions are added if the colour is to be developed without precipitation of iron(III) hydroxide. For the highest reproducibility, it is best to add the cyanide to the mixture of iron(II) and phenylbiguanide, then adjust the pH with sodium hydroxide. The maximum absorbance is reached in an hour and the colour is stable for at least 6 hr, then gradually fades; the total decomposition of the compound requires more than six months. Temperature does not much affect the absorbance and boiling for an hour is necessary for the solution to decolorize completely.

The wavelength of the absorbance maximum is independent of the ligand concentration. Large excesses of the ligands are needed, however, with the molar ratios cyanide/Fe(II) and phenylbiguanide/Fe(II) >60 and >150, respectively.

Coleman's method[4] was applied to solutions having molar ratios of phenylbiguanide/Fe(II) >0.5 and of cyanide/Fe(II) >6 [lower molar ratios could not be

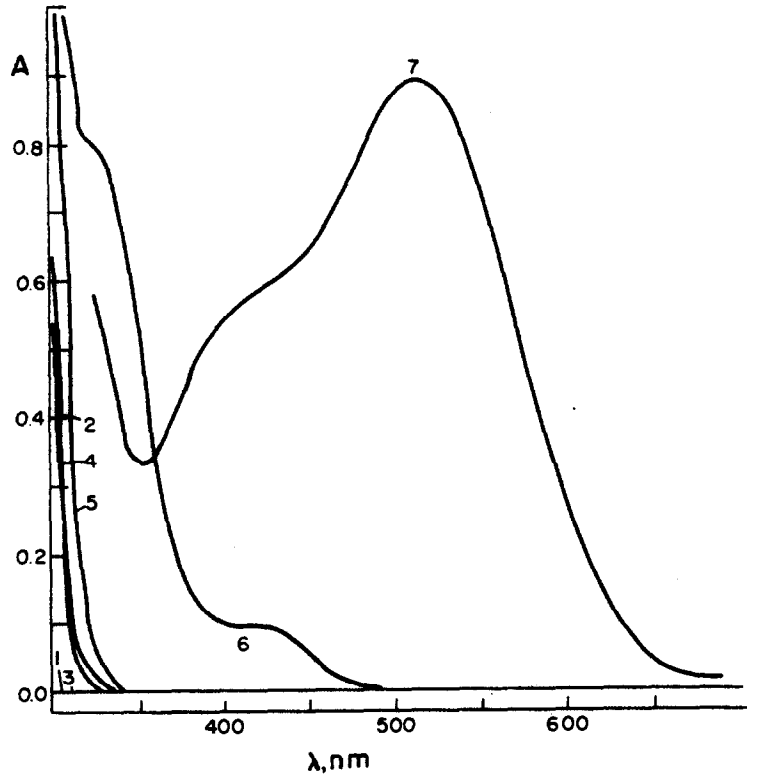

Fig. 1. Absorption spectra: Fe(II) $1 \times 10^{-3}M$, phenylbiguanide $2.5 \times 10^{-3}M$, CN^- $5 \times 10^{-3}M$; 1-cm cells. (1) Fe(II) (acid medium, $2 \times 10^{-4}M$ H_2SO_4); (2) phenylbiguanide; (3) CN^-; (4) Fe(II)–phenylbiguanide (acid medium, $2 \times 10^{-4}M$ H_2SO_4); (5) phenylbiguanide–CN^-; (6) Fe(II)–CN^-; (7) Fe(II)–phenylbiguanide–CN^-.

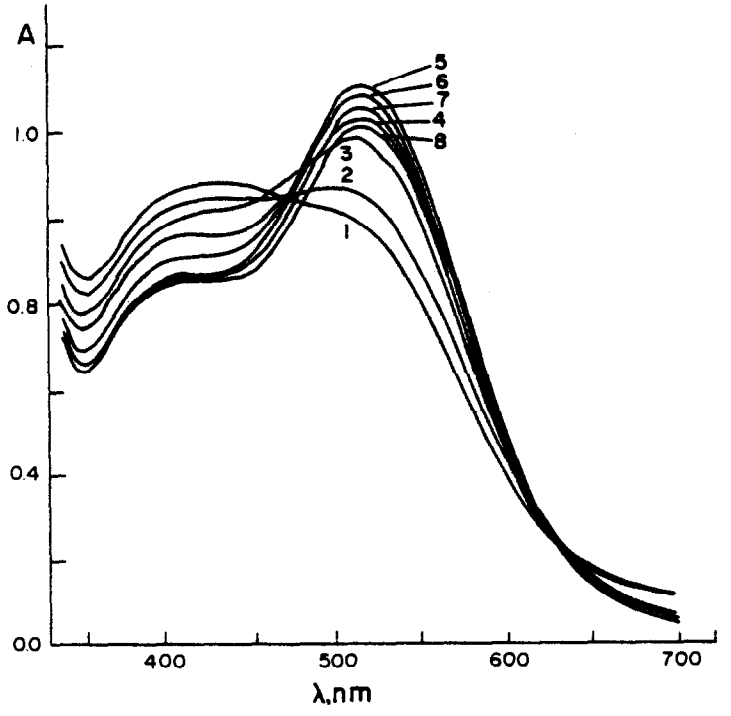

Fig. 2. Absorption spectra at various pH values [Fe(II) $1 \times 10^{-3}M$, phenylbiguanide $8 \times 10^{-3}M$, CN^- $2 \times 10^{-2}M$, 1-cm cells]. (1) 9.94; (2) 10.14; (3) 10.35; (4) 10.74; (5) 11.37; (6) 12.22; (7) 12.7; (8) 12.9.

investigated because of irreproducibility caused by formation of iron(III) hydroxide]. The results indicate that the absorbance at wavelengths longer than 480 nm is due to a single species, the red complex. The absorbance between 400 and 480 nm is also due to the red species when the solution is saturated with both ligands, but if the solution is saturated with cyanide and the molar ratio cyanide phenylbiguanide/Fe(II) is varied between 0.5 and 75 two distinct absorbing species are present. Solutions saturated with phenylbiguanide and with molar ratios cyanide/Fe(II) between 6 and 30 show the presence of three distinct absorbing species. In both cases, one of these species must be the red complex and the other(s) probably other cyanide complexes of iron.

The extension of the Asmus straight line method to $M_sL_mL'_n$ complexes[5] was applied to the absorbance at 520 nm of the solutions used for the Coleman method (Fig. 3). Linear functions were obtained that indicated 1:1:1 ratio of phenylbiguanide to cyanide to an iron(II)-complex in the red compound, but gave no information about the nature of the iron(II) complex concerned. The method due to Koch and Ackermann[6] was therefore applied to the absorbance at 520 nm of solutions at pH between 10 and 11.2 and with ionic strength 0.01M. From the general equilibrium

$$ML_{m-r}L'_{n-r'+s} + rH_aL + r'H_bL' \rightleftharpoons ML_mL'_n + sH_bL' + qH^+$$

where $H_aL = PhBH_2^+$, $H_bL' = HCN$ and $ML_mL'_n$ is the red compound, linear functions were obtained for the sets of values $r = 1$, $r' = 1$, $s = 0$, $q = 2$ and $r = 1$, $r' = 0$, $s = 0$, $q = 1$. The molar absorptivities calculated for $ML_mL'_n$ from the two functions are 1740 and 1818 l.mole^{-1}.cm^{-1}, respectively, and the value obtained from the calibration curve is 1838 l.mole^{-1}.cm^{-1}, which means that the second set of values for r, r' and q is the more probable, suggesting that uncharged phenylbiguanide is added to an iron(II)–cyanide complex to form the red compound.

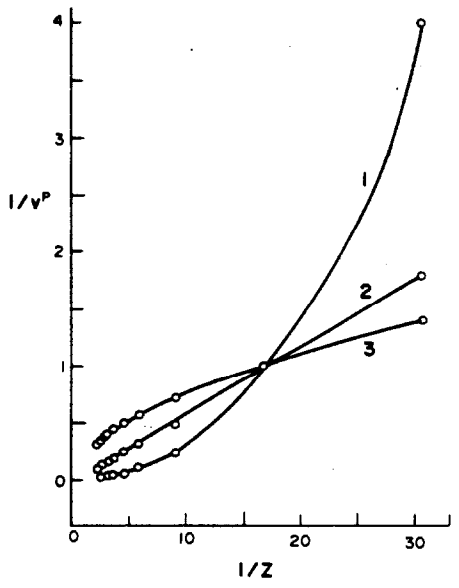

Fig. 3. Asmus method [v ml of 1.5 × 10^{-3}M Fe(II) diluted to 50 ml; concentrations of phenylbiguanide and cyanide 1 × 10^{-2}M; pH 10.6; 4-cm cells, p = (1) 2; (2) 1; (3) ½.

From the anionic nature of the complex, the neutral and bidentate character of the phenylbiguanide ligand and the tendency of iron(II) to form octahedral complexes, together with knowledge of similar compounds,[7] the formula [Fe(phenylbiguanide)$(CN)_4$]$^{2-}$ is proposed for the red compound. The precursor species $ML_{m-r}L'_{n-r'+s}$ would thus be $[Fe(CN)_4(H_2O)_2]^{2-}$, the reaction involving replacement of the two molecules of water by a molecule of phenylbiguanide; a similar reaction has been suggested with histidine as the replacement ligand.[8] That the precursor is no ferrocyanide is shown by the fact that ferrocyanide will not react with phenylbiguanide.

The development of analytical procedures based on the formation of the red complex has been studied. The detection of iron and cyanide is rapid and simple, the dilution limits being 1:8 × 10^5 and 1:2 × 10^5, respectively. Heat and alkaline conditions make the reaction instantaneous. Ag^+, Hg^{2+}, Cd^{2+}, Zn^{2+}, F^-, tartrate, SCN^-, CH_3COO^-, SO_4^{2-}, AsO_3^{3-}, AsO_4^{3-}, Cl^-, ClO_4^-, NO_3^- and NO_2^- in 100-fold molar ratio to iron do not interfere in the detection of iron at the 100-ppm level. The following species interfere at the indicated molar ratios with respect to iron: Cu^{2+} and Co^{2+}, 50; Ni^{2+} and $C_2O_4^{2-}$, 25; Au^{3+}, Mn^{2+} and $S_2O_8^{2-}$, 10; Pd^{2+} and PO_4^{3-}, 5; EDTA, CO_3^{2-} and S^{2-}, <1. F^-, tartrate, PO_4^{3-}, SCN^-, CH_3COO^-, CO_3^{2-}, SO_4^{2-}, BO_3^-, AsO_4^{3-}, Cl^-, ClO_4^- and NO_3^- in 100-fold molar ratio do not interfere in detection of cyanide at the 50-ppm level. The following species interfere at the indicated molar ratios with respect to cyanide: Mn^{2+} and AsO_3^{3-}, 75; Th^{4+}, Zr^{4+} and NO_2^-, 50; $C_2O_4^{2-}$, 40; $S_2O_8^{2-}$, 10; Cd^{2+}, Pd^{2+} and Au^{3+}, 2; Ag^+, Hg^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, EDTA and S^{2-}, <1.

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SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNTS OF IRON(III) BY EXTRACTION OF THE MIXED-LIGAND IRON–FLUORIDE–PURPURIN COMPLEX

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Summary—The characteristics of the mixed-ligand iron(III)–fluoride–purpurin complex, including optimum conditions of formation and extraction into methyl isobutyl ketone are described. A procedure for determination of trace amounts of iron in fluoride medium ($\geq 0.5M$) with purpurin (1,2,4-trihydroxyanthraquinone) in methyl isobutyl ketone is given. The method is suitable for determining iron in the presence of large amounts of aluminium, cyanide, phosphate and nickel.

Most spectrophotometric methods for iron are based on complexes of iron(II) (*e.g.*, references 1–7), and comparatively few are based on iron(III) complexes.

We have developed a new iron reagent group, typified by purpurin (1,2,4-trihydroxyanthraquinone) which forms an ion-association complex with iron(III) and fluoride, that can be extracted into methyl isobutyl ketone (MIBK). The complex has a molar absorptivity of 4.6×10^4 l.mole^{-1}.cm^{-1}. The possibility of determining trace amounts of iron in the presence of large concentrations of fluoride is an attractive feature of the method.

EXPERIMENTAL

Reagents

Purpurin solution. A $4 \times 10^{-4}M$ solution was prepared by dissolving 0.1024 g of the Merck product and diluting to 1 litre with methyl isobutyl ketone. More dilute solutions were prepared from this.

Iron(III) solution. Prepared from $Fe(NO_3)_3.9H_2O$ and standardized gravimetrically. A $10^{-4}M$ solution was prepared by dilution.

Buffer. A $2.0M$ fluoride buffer prepared by dissolving 74.0 g of ammonium fluoride and enough ammonia to give pH 8.1, in 1 litre of water. This solution must be made iron-free by shaking it with successive portions of purpurin solution in MIBK until the organic phase is yellow-orange. Any purpurin left in the aqueous phase is extracted with MIBK.

All other chemicals were reagent-grade and were checked for iron contamination.

Procedure

Samples were prepared in 100-ml separatory funnels by taking 20 ml of aqueous iron(III) solution containing less than 10 μg of iron, 20 ml of pH 8.1 buffer solution ($2M$ fluoride) and 10 ml of $2 \times 10^{-4}M$ reagent solution in MIBK, and shaken vigorously for 10 min. The phases were separated and the organic phase was centrifuged. Its absorbance was then measured at 595 nm against a reagent blank similarly prepared.

RESULTS AND DISCUSSION

Absorption spectrum of the complex

Equal volumes of $8 \times 10^{-5}M$ purpurin (in MIBK) and $2 \times 10^{-5}M$ iron(III) (at pH 8.1 in $1.0M$ ammonium fluoride) were shaken together for 1 hr, and the absorption spectrum of the organic phase was measured, with MIBK as reference. Figure 1 shows the absorption spectra of the complex and the reagent. The absorption spectrum obtained in the same way but with ammonium chloride solution at pH 8.1 instead of the fluoride is practically the same as the spectrum of the purpurin solution. These facts can only be interpreted as indicating the formation of an iron(III)–fluoride–purpurin complex.

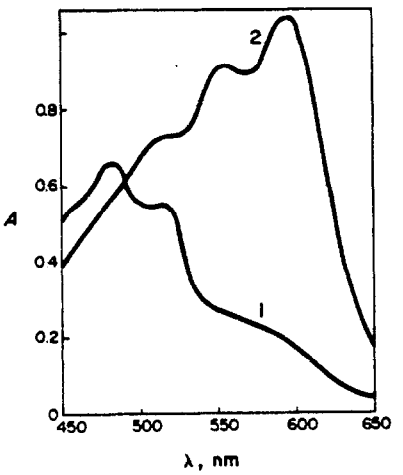

Fig. 1. Absorption spectra of purpurin in MIBK (1) and the iron(III)-fluoride-purpurin complex in MIBK (2).

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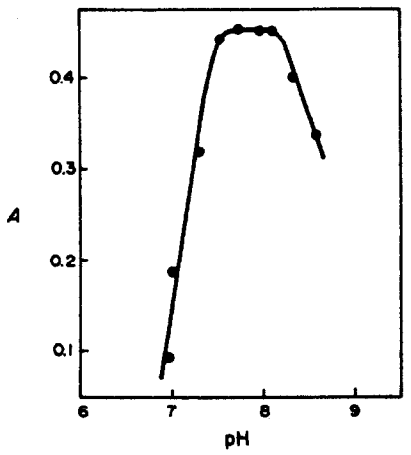

Fig. 2. Effect of pH on the formation and extraction of the mixed-ligand complex.

Effect of pH and fluoride concentration

The influence of pH was investigated for a $10^{-5}M$ iron(III) solution in $1M$ ammonium fluoride and a $2 \times 10^{-4}M$ purpurin solution in MIBK. The phase-volume ratio was 1:1 and the shaking time 30 min. Fig. 2 shows the results. The absorbance increases with pH and is maximal at pH 7.7–8.2.

The influence of fluoride concentration was investigated with $2 \times 10^{-5}M$ iron(III) and $2 \times 10^{-4}M$ purpurin at pH 8.1, obtained by addition of x ml of ammonium fluoride/ammonia buffer and $(10 - x)$ ml of ammonium chloride/ammonia buffer (both of pH 8.1) so that the ionic strength was always the same. The results are plotted in Fig. 3, which shows that the extraction of the violet complex is dependent on the fluoride concentration, becoming constant at $[F^-] \geq 0.5M$. The large concentration of fluoride necessary for complete complex formation indicates that the system would be suitable for determining very small amounts of iron in fluoride and also that rigorous control of the fluoride concentration (for $[F^-] \geq 0.5M$) is not necessary.

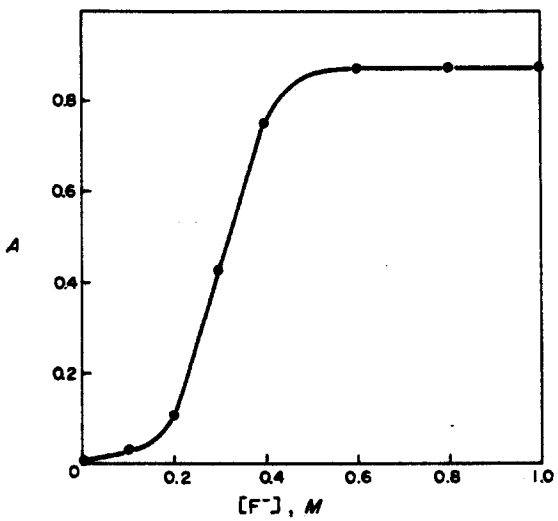

Fig. 3. Effect of fluoride concentration on the formation and extraction of the mixed-ligand complex.

Stoichiometry

The metal–purpurin ratio for the violet iron(III) complex, at pH 8.1 and phase ratio 1:1, was determined by the Job and the Yoe and Jones methods. The fluoride concentration was kept at $1.0M$. The stoichiometry was found to be 1:3 metal–purpurin by both methods.

The metal–fluoride ratio in the violet complex was determined as follows. The violet complex in MIBK was washed twice with ammonium chloride/ammonia buffer (pH 8.1) and dried with anhydrous sodium sulphate, then stripped with dilute hydrochloric acid. The purpurin remained in organic phase. The aqueous phase was made up to standard volume and the iron was determined by atomic absorption, and the fluoride spectrophotometrically by the Ce(III)–alizarin complexan method.[8] The metal–fluoride ratio was found to be 1:1. These results show that the stoichiometry of the mixed-ligand iron(III)–fluoride–purpurin complex is 1:1:3. As the complex is extractable it must be neutral, so we conclude that the third purpurin ligand is neutral and forms an adduct of the type $FeFl_2.LH$ where LH is purpurin.

Extraction constant

The extraction constant, at two different pH values, was determined by our previously published method[9] extended to cover study of mixed-ligand complexes.[10]

The results for pH 7.87 and 8.15, $2 \times 10^{-5}M$ iron(III) and $1.0M$ fluoride gave a molar absorptivity for the violet complex of 4.6×10^4 l. mole^{-1}.cm^{-1}. The extraction constant was found to be log $K_e^* = 15.11$ and 15.53 at pH 7.87 and 8.15 respectively.

If the iron(III) extraction is regarded as quantitative when the degree of extraction of the metal, α_e, is $\geq 99\%$, the expression

$$\alpha_e = \frac{[ML_3]_0}{[M]_w + [ML_3]_0} = \frac{K_e^*(C_L - 3[ML_3]_0)^3}{1 + K_e^*(C_L - 3[ML_3]_0)^3}$$

allows calculation of the maximum concentration of iron that can be extracted quantitatively for a particular analytical concentration of purpurin; *e.g.*, if $C_L = 2 \times 10^{-4}M$ up to 3 ppm of iron can be quantitatively extracted.

Spectrophotometric characteristics

The effect of phase-volume ratio was studied for the optimal conditions. The absorbance of the organic phase was practically constant for the range 1:1–4:1 aqueous phase/organic phase. We chose the 4:1 ratio as being satisfactory, and found that Beer's law is obeyed, at this phase ratio, for 0.025–0.250 ppm of iron in the aqueous phase. A shaking time of 10 min is sufficient for equilibrium to be reached.

The absorbance of the violet complex at 595 nm is constant within 1% over a period of 1 hr, and within 3% over a period of 5 hr.

Table 1. Interference of foreign ions in the determination of 0.14 ppm of iron

Ion	Tolerable concentration of foreign ion, *ppm*
Phosphate	600
Cyanide	500
Silver, beryllium, magnesium, molybdenum(VI), uranium(VI), vanadium(V), tungsten(VI)	100
Cadmium, copper(II), mercury(II) nickel, palladium, zinc	100*
Aluminium	50
Chromium(III), yttrium	25
Cerium	10
Manganese(II)	10*
Lead	6
Calcium	2
Cobalt(II)	2*
Bismuth	1
Indium	0.5
Tin(II)	0.1

* Cyanide (20 mg) added.

The standard deviation is 1.4×10^{-3} ppm for 0.14 ppm of iron(III).

Interferences

Foreign ions were added to a 0.14 ppm iron solution, and the colour was developed by the usual procedure. Cations were added up to 100 ppm concentration and anions up to 1000 ppm. The tolerance limit was taken as the concentration that did not cause more than $\pm 2\%$ change in the absorbance of the 0.14-ppm iron solution. The results are summarized in Table 1.

The interference of cadmium, cobalt(II), copper(II), mercury(II), manganese(II), nickel, palladium and zinc can be eliminated by adding 20 mg of cyanide.

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ANALYTICAL DATA

A STUDY OF THE STABILITY OF ALKALINE-EARTH METAL COMPLEXES WITH FLUORIDE AND CHLORIDE IONS AT VARIOUS TEMPERATURES BY POTENTIOMETRY WITH ION-SELECTIVE ELECTRODES

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Summary—The complexes of the alkaline earth metals with fluoride and chloride were studied over the temperature range 15–85°. The stability constants of the MX^+ complexes were determined by potentiometry with fluoride and chloride ion-selective electrodes and the appropriate thermodynamic functions (ΔH^0_{298}, ΔS^0_{298} and ΔG^0_{298}) were calculated.

Study of the fluoride and chloride complexes of the alkaline-earth metals is especially important for geochemists, as in the theory of migration of fluoride ions in various types of sodium chloride solutions with high concentrations of sodium and alkaline earth ions, at common and elevated temperatures, the fluoride is assumed to be transported in the form of these complexes. Complexes containing fluoride and chloride ions may also play an important role in the transport of metals in hydrothermal solutions in the earth's crust, especially at higher temperatures.

The most suitable method for the determination of the stability constants of these complexes is potentiometry with ion-selective electrodes (ISEs). Both fluoride and chloride ISEs exhibit good reproducibility and potential stability, and can be used at temperatures up to about 100°.

So far, the stability constants of these complexes have been measured[1–14] at temperatures from 0 to 60°. However, this temperature interval can readily be extended up to nearly 100°. The stability constant values at temperatures higher than 100°, which are especially important in modelling geochemical transport and precipitation processes, are obtained either by extrapolation from the data obtained for lower temperatures, or by solubility measurements in autoclaves. In this way, for example, the stability constants for the MgF^+ and CaF^+ complexes were obtained[15] for temperatures of 200° and 260° ($K_{MgF^+} = 70.9 \pm 5.1$ for 200° and 268 ± 75 for 260°, and $K_{CaF^+} = 27.6 \pm 3.7$ for 200° and 43.8 ± 6.9 for 260°).

EXPERIMENTAL

Chemicals

Stock 1*M* solutions of the alkaline-earth metal nitrates (Mg, Ca, Sr) were prepared from the solid *p.a.* substances (Lachema, Czechoslovakia) and standardized by EDTA titration. Because of its low solubility, solid barium nitrate was weighed and added directly to the solutions to be measured. Stock 0.2*M* solutions of sodium chloride and fluoride were also prepared from the *p.a.* substances (Lachema, Czechoslovakia).

The ionic strength of all test solutions was adjusted with 2*M* sodium nitrate, prepared from the solid substance freed from heavy metals by ion-exchange (Dowex 50W, Na^+-form) and recrystallized. In the study of the CaF^+ complex, the ionic strength was adjusted with a solution of similarly purified potassium nitrate.

Because of the large temperature ranged used, expansion of the solutions could not be neglected and the concentrations were therefore expressed in terms of molality. The weight of water per litre of each standard solution was found and the test solutions were diluted with distilled water to contain 100 g of water. The solutions used for calibration of the ISEs, with molalities of 4×10^{-3}, 1×10^{-3}, 4×10^{-4} and 5×10^{-5}, were prepared in the same manner. The pH of all the solutions was between 5 and 6 and was not further adjusted.

The reference-electrode liquid bridge was filled with 3*M* potassium nitrate (2·5*M* for tests at 15°).

Apparatus

Radiometer equipment was used, consisting of a PHM-64 pH-meter, fluoride and chloride ISEs (F1052F

and F1012Cl, respectively), a K 401 reference electrode with a liquid bridge (precision of potential-reading, 0.1 mV), a PHM-62 pH-meter with a G 2040B glass electrode (precision of pH-reading, 0.01), a TTA 60 stirrer and an REC 61/REA 100 recorder, used for monitoring the ISE potential stabilization. The test solutions were placed in 100-ml polyethylene bottles held in a U-10 ultrathermostat (VEB Prüfgeräte Werke, GDR), and solutions with a volume of less than 10 ml were placed in a V 533 vessel with external thermostatic jacket, in which the temperature was maintained within $\pm 0.1°$ ($\pm 0.2°$ for 85°).

All calculations were done on a Hewlett-Packard HP-67 programmable calculator with our own programs (calculation of liquid-junction potential, non-linear regression, *etc.*).

Procedure

The test solutions were prepared with an ionic strength of 1*m* ($NaNO_3$), fluoride or chloride molality of $10^{-3}m$ ($10^{-4}m$ for the CaF^+ work because of the poor solubility of CaF_2) and alkaline-earth metal molality of 0.05, 0.1, 0.2 and 0.3*m*. Simultaneously, the same number of solutions containing only sodium fluoride or chloride, at the same molalities and ionic strengths, were prepared.

Before and after each series of measurements, the slope of the potential *vs.* concentration graph was checked and the ISE sensitivity determined. The ISE potential-drift was suppressed as much as possible, by measuring in immediate succession solutions containing and not containing a test metal ion and using these potentials to calculate ΔE. For each measurement, a fresh solution from the polyethylene bottle was employed.

Calculations

The stability constant of complex MX^+ is expressed by

$$\beta = \frac{[MX^+]}{[M^+][X^-]} \tag{1}$$

Under the given experimental conditions, when the total metal concentration $c_M \gg [MX^+]$, equation (1) can be rewritten in the form

$$\beta = \frac{c_x - [X^-]}{c_M[X^-]} = \frac{1}{c_M}\left\{\frac{c_x}{[X^-]} - 1\right\} \tag{2}$$

Equation (2) contains a single unknown, $[X^-]$, which can be found from the ISE calibration curve. However, because the absolute potential varies with time (in contrast to the relatively stable slope), it is better to find the ratio $c_x/[X^-]$ from two successive measurements in the absence (potential E_0) and presence (potential E_1) of the metal ion:

$$E_0 = E^0 - S \log c_x + \Delta\phi_0$$
$$E_1 = E^0 - S \log[X^-] + \Delta\phi_1 \tag{3}$$

when the E^0 value can be assumed to be constant; S is the response slope and $\Delta\phi$ is the liquid-junction potential between the test solution and the potassium nitrate solution in the liquid bridge, which can be estimated from the Henderson equation[16] (the other liquid-junction potentials also have non-zero values, but are constant). For the ratio $c_x/[X^-]$ it then holds that

$$\frac{c_X}{[X^-]} = 10^{\Delta E'/S} \tag{4}$$

where

$$\Delta E' = E_1 - E_0 + \Delta\phi_0 - \Delta\phi_1 \tag{5}$$

The resultant relationship for the stability constant of complex MX^+ is thus

$$\beta = \frac{1}{c_M}(10^{\Delta E'/S} - 1) \tag{6}$$

Random results were excluded from the results by using the *T*-test;[17] the remaining results were treated statistically and are given in Tables 1 and 2.

The temperature dependence of an equilibrium constant can be expressed by various equations, as described in any

Table 1. The stability constants of the chloride complexes

Complex	T, °C	$\bar{\beta}$	s^*_{β}	$\log \bar{\beta}$
$MgCl^+$	15	0.72	0.08	−0.14
	25	0.77	0.23	−0.13
	45	0.83	0.04	−0.08
	65	0.96	0.09	−0.02
	85	1.27	0.14	0.10
$CaCl^+$	15	0.54	0.09	−0.28
	25	0.68	0.08	−0.17
	45	0.63	0.07	−0.20
	65	0.82	0.04	−0.09
	85	0.98	0.09	−0.01
$SrCl^+$	15	0.56	0.05	−0.25
	25	0.54	0.08	−0.27
	45	0.89	0.05	−0.05
	65	1.01	0.08	0.00
	85	0.95	0.10	−0.03
$BaCl^+$	15	0.25	0.07	−0.61
	25	0.34	0.07	−0.48
	45	0.66	0.18	−0.20
	65	0.71	0.16	−0.16
	85	0.70	0.18	−0.18

* Ten replicates.

Table 2. The stability constants of the fluoride complexes

Complex	T, °C	$\bar{\beta}$	s^*_{β}	$\log \bar{\beta}$
MgF^+	15	20.3	1.6	1.31
	25	22.5	1.9	1.35
	45	27.8	2.1	1.44
	65	34.6	3.2	1.54
	85	43.9	4.3	1.64
CaF^+	15	4.62	0.23	0.66
	25	4.81	0.27	0.68
	45	5.83	0.30	0.76
	65	7.58	0.44	0.88
	85	9.72	0.71	0.99
SrF^+	15	1.44	0.15	0.16
	25	1.37	0.08	0.14
	45	1.76	0.11	0.25
	65	2.14	0.16	0.33
	85	2.72	0.29	0.43
BaF^+	15	0.58	0.08	−0.24
	25	0.67	0.12	−0.18
	45	0.95	0.10	−0.03
	65	1.27	0.03	0.11
	85	1.57	0.11	0.19

* Ten replicates.

Table 3. Dependence of the log β values on temperature in the interval 288–358 K (rounded off to two decimals after calculation)

Complex	log β
$MgCl^+$	$\log \beta = -33.14658 + 8.21289 \times 10^{-5}T^2 - 7.60913 \times 10^{-2}T + 8.49545 \ln T$
$CaCl^+$	$\log \beta = -130.00903 + 1.63105 \times 10^{-4}T^2 - 0.19682T + 30.53397 \ln T$
$SrCl^+$	$\log \beta = 241.02305 - 3.67544 \times 10^{-4}T^2 + 0.42395T - 58.78936 \ln T$
$BaCl^+$	$\log \beta = -33.02600 - 1.55733 \times 10^{-4}T^2 + 9.73845 \times 10^{-2}T + 3.05025 \ln T$
MgF^+	$\log \beta = 13.49190 - 6.01329 \times 10^{-6}T^2 + 1.78561 \times 10^{-2}T - 2.97146 \ln T$
CaF^+	$\log \beta = -60.86936 + 2.89712 \times 10^{-5}T^{-2} - 1.12161 \times 10^{-2}T + 10.81822 \ln T$
SrF^+	$\log \beta = -108.33374 + 4.31140 \times 10^{-5}T^{-2} - 3.13507 \times 10^{-2}T + 19.83241 \ln T$
BaF^+	$\log \beta = 85.42184 - 1.29908 \times 10^{-4}T^2 + 0.15601T - 21.15952 \ln T$

textbook on physical chemistry. The most advantageous form for our purposes was

$$\log \beta = a + bT^{\pm 2} + cT + d \ln T, \quad (7)$$

where the minus sign in the exponent of the second term better satisfied the dependence for the CaF^+ and SrF^+ complexes. Coefficients a, b, c and d in equation (7) can be determined by the least-squares method, by solving the normal equations for multiple regression. The regression curves for the given temperature interval, 15–85°C, are given in Table 3 and in Figs. 1 and 2. In view of the small data set and the considerable scatter of the values (especially with the chloride complexes), extrapolation toward temperatures higher than 85°C must be cautious, chiefly with the $SrCl^+$ and $BaCl^+$ complexes, with which the change in the slope of the regression curve around 80°C is probably caused by the poor precision of the last term. Linear regression was also tested but gave correlation coefficients substantially less than unity.

From log β and its temperature dependence, the values of the thermodynamic functions ΔH^0, ΔS^0 and ΔG^0 can be found for a temperature of 25° (298.15 K), namely, the standard Gibbs free energy change

$$\Delta G^0_{298} = -2.303RT \log \beta \quad (T = 298.15\,\text{K}), \quad (8)$$

the standard enthalpy change,

$$\Delta H^0_{298} = 2.303RT^2 \frac{\partial \log \beta}{\partial T} \quad (T = 298.15\,\text{K}), \quad (9)$$

where

$$\frac{\partial \log \beta}{\partial T} = \left\{2bT + c + \frac{d}{T}\right\}$$

or

$$\frac{\partial \log \beta}{\partial T} = \left\{-\frac{2b}{T^3} + c + \frac{d}{T}\right\} \quad (10)$$

and the standard entropy change

$$\Delta S^0_{298} = 2.303RT\left(\frac{\partial \log \beta}{\partial T}\right)_{298} + 2.303 \log \beta. \quad (11)$$

The calculated values are given in Table 4.

DISCUSSION

Chloride complexes

The stability of the complexes decreases in the order $Mg^{2+} > Ca^{2+} \sim Sr^{2+} > Ba^{2+}$, *i.e.*, with increasing cation radius. In view of the low values of the stability constants (the potential changes were often less than 0.5 mV), the agreement with the literature data can be considered good. The somewhat lower values compared with those of Šůcha *et al.*[9]

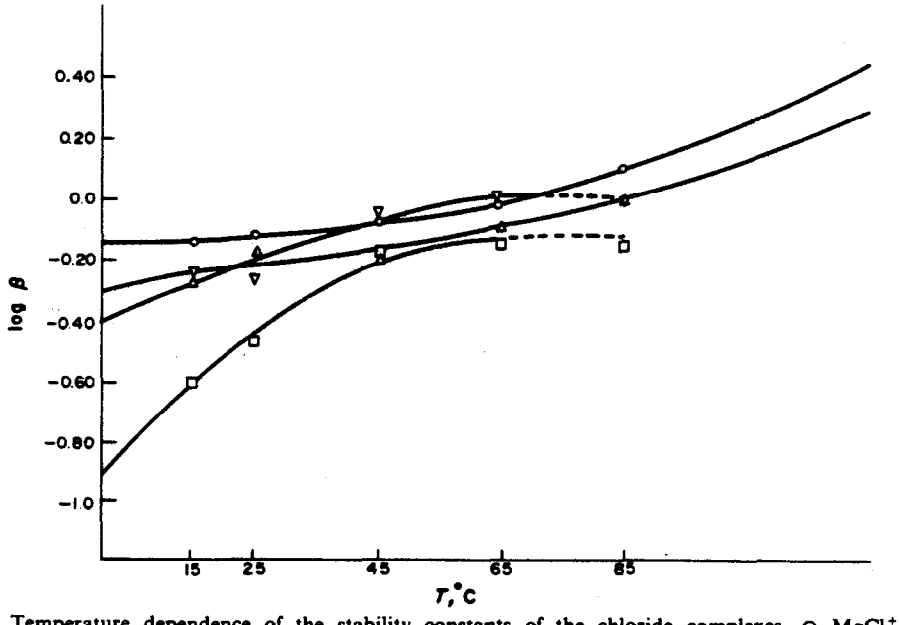

Fig. 1. Temperature dependence of the stability constants of the chloride complexes. ○ $MgCl^+$. △ $CaCl^+$, ▽ $SrCl^+$, □ $BaCl^+$.

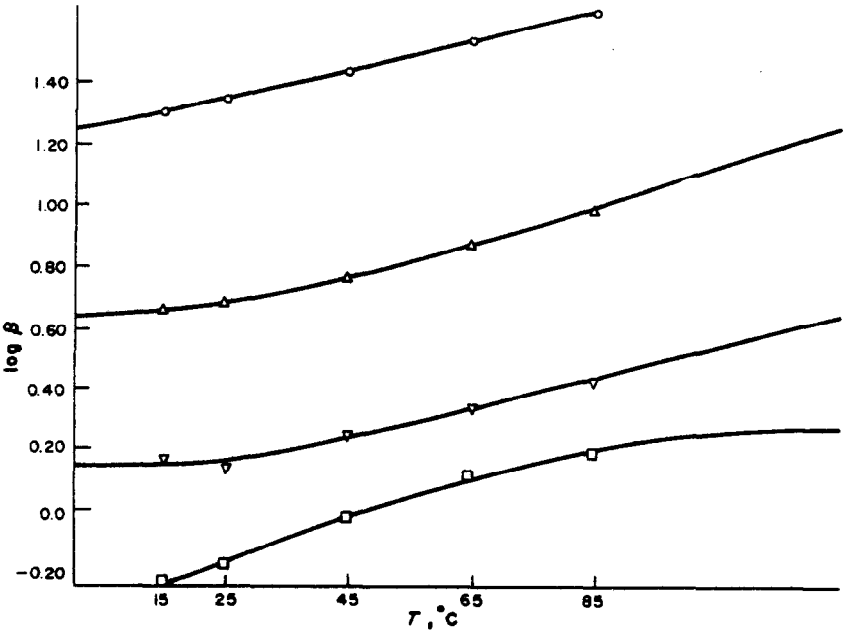

Fig. 2. Temperature dependence of the stability constants of the fluoride complexes. ○ MgF^+, △ CaF^+, ▽ SrF^+, □ BaF^+.

may be due to differences in the purity of the chemicals used.

However, the thermodynamic functions reported for the present work are different from those of Šůcha *et al.*[9] In view of the wider temperature interval studied in the present work and of the differentiation of the log β *vs.* T dependence performed here (compared with simple differences used in ref. 9) we consider the present values to be more reliable.

Fluoride complexes

The stability of the fluoride complexes depends on the cation radius analogously to that of the chloride complexes, but the dependence is more pronounced. The temperature dependence indicates a substantial increase in the stability of the MgF^+ and CaF^+ complexes with increasing temperature. The dependence can reliably be extrapolated to temperatures above 100° for all the complexes studied. The present stability constant values are somewhat higher than the average of the literature values, but are within their variance. The thermodynamic functions are also in satisfactory agreement with the literature.[1,7]

Table 4. Standard thermodynamic functions for the reactions at 298.15 K

Complex	ΔH^0_{298}, $kcal.mole^{-1}$	ΔS^0_{298}, $cal.mole^{-1}.K^{-1}$	ΔG^0_{298}, $kcal.mole^{-1}$
$MgCl^+$	0.56	1.3	0.18
$CaCl^+$	1.16	2.9	0.30
$SrCl^+$	3.09	9.4	0.28
$BaCl^+$	6.00	18.0	0.62
MgF^+	1.75	12.1	−1.84
CaF^+	1.30	7.5	−0.93
SrF^+	1.07	4.4	−0.23
BaF^+	3.08	9.6	0.23

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ADSORPTION OF THE SILVER ION ON SODA-GLASS—I

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Summary—A study of the adsorption of silver ions on soda-glass, using a radiochemical technique, has been made, and the effects of time and temperature investigated. A simple kinetic treatment allows estimation of the rate constants for the adsorption and desorption processes.

The adsorption of cations on glassware is of obvious importance in analytical chemistry, particularly when solutions of low concentration are involved. The present study of the adsorption of silver ions on soda-glass follows our previous observations of silver loss, in a study of the solubility of silver chloride in methanol + water mixtures.[1] It was decided to investigate in detail the adsorption of silver from aqueous solution, and a radiochemical technique was developed, with ^{110m}Ag and an NaI(Tl) well-type crystal. The technique has been used to measure the amount of silver adsorbed per unit area of glass from a 0·5-mg/l. (0.5 ppm) silver nitrate solution, for a range of contact times, at 298 K. The concentration of 0.5 mg/l. was chosen, as being in the same range as that used in earlier studies of silver adsorption,[2–5] so that a direct comparison could be made. The effects of varying temperature and initial concentration, and of heat-treatment of the glass, were also investigated.

EXPERIMENTAL

The essential features of the technique involve the labelling of a standard silver nitrate solution with ^{110m}Ag (a β- and γ-emitter with a half-life of 253 days), to give a solution of specific activity of silver of approximately 20 μCi/mg, which is then left in contact with a glass surface for a given period of time. The amount of silver adsorbed per unit area of the surface is then determined by gamma scintillation-counting with an NaI(Tl) well-type crystal. The following procedure was adopted.

For each measurement, a 3-ml portion of the labelled standard silver nitrate solution was placed in a 10-ml soda-glass vial. The surface area of the glass in contact with the solution, calculated from the dimensions of the vial, was 8.9 cm^2. The vials were wrapped in aluminium foil to minimize exposure to light and then kept in a thermostatic water-bath for a predetermined time (the contact time). The vials were then transferred to the counting equipment, care being taken not to tilt them. After the count-rate had been measured, the solution was removed from the vial by suction through a drawn-out glass tube designed to give rapid removal with minimum disturbance of the solution. Doubly demineralized water was then added to the vial, the entire contact area being covered. The water was left in contact with the glass, without agitation, for 5 sec and then sucked out. This washing procedure was carried out ten times, and further washing did not remove significant quantities of silver from the glass. The activity of the empty vial was then counted and by a comparison of this with that of the standard solution, the amount of silver adsorbed was calculated. Correction for the decay of ^{110m}Ag was unnecessary, as the standard solution and the adsorbed silver were counted at virtually the same time.

Pretreatment of the glass vials with solvents or detergents was avoided because of the risk of modifying the surface. Before use, each vial was half-filled with doubly demineralized water, capped, and shaken gently for 15 sec, then emptied and placed upside down on absorbent paper to air-dry for 24 hr.

Analytical-reagent grade silver nitrate was used without further purification. The silver-110 m was obtained from the Radiochemical Centre, Amersham, as silver nitrate in nitric acid solution.

Further work has shown that the extent of adsorption of silver depends on the pH of the solution. In the present work the pH was monitored and was about 5.3 throughout. All labelled standard silver nitrate solutions were made up in new graduated flasks and allowed to stand for seven days before use. This allowed a correction to be made for the silver adsorbed on the graduated flask, if necessary.

RESULTS AND DISCUSSION

The technique involves washing the vial ten times with 3-ml portions of demineralized water, each wash being of 5 sec duration. The effectiveness of the washing procedure was checked by measuring the amount of silver remaining on the glass after each wash. The behaviour, for a 4-day initial contact time, is illustrated in Fig. 1. It is concluded that all the silver nitrate solution, and any "loosely" adsorbed silver is removed by the ten consecutive washes; the silver remaining after ten washes is referred to as "firmly" adsorbed silver. The dependence of the amount of this firmly adsorbed silver on contact time at 298 K is shown in Figs. 2 and 3, for an initial silver nitrate concentration of 0.5 mg/l.

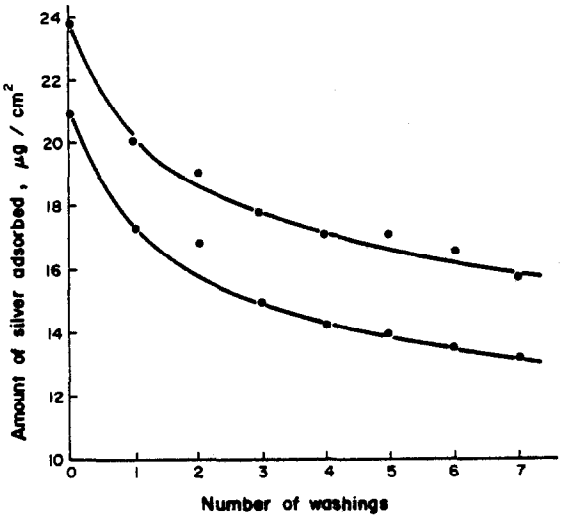

Fig. 1. Effect of successive washings on silver adsorbed after a 4-day contact time with $AgNO_3$ solution of initial concentration 0.5 mg/l., at 298 K.

Measurements were carried out in duplicate, and the difficulty in obtaining reproducible results can be gauged from the graphs. There is rapid initial adsorption in the first few minutes, followed by a much slower process which becomes almost complete after about 40 days. This may be compared with the results of West *et al.*[4] who found that maximum losses from 0.05- and 1.0-mg/l. solutions occurred during a 10–30 day period. A 4-day contact time was used for most of the present studies, for convenience, and so that the rapid initial adsorption was complete. However, the firmly adsorbed silver is not the equilibrium amount, and a much longer contact time, >40 days, is necessary to obtain this value.

The effect of increasing the initial concentration and the temperature is illustrated in Fig. 4 for a 4-day contact time. The extent of adsorption for a given concentration increases as the temperature is increased, but it is difficult to draw any further conclusions from the temperature dependence since the adsorption has not reached equilibrium after only four days. However, the extent of adsorption is clearly large enough to have serious consequences in trace analysis for silver.

The effect of heat pretreatment of the vials has also been studied. The results are presented in Table 1, and it is seen that heating in an oven at 200° for 1½ hr leads to an approximate doubling of the amount of silver adsorbed. Flaming the glass before contact results in an even larger increase. Hensley *et al.*[2] obtained a pronounced increase in adsorption with similar heat pretreatment. The mechanism of the increase is far from clear. Stress tests on the untreated vials indicated that while the outer surfaces showed a small level of residual stress, the inner surfaces were virtually stress-free.*

The present study indicates that the adsorption takes place by two processes, one occurring rapidly and reaching equilibrium at 298 K in approximately 10 min, while the other occurs relatively slowly and requires about 40 days to reach equilibrium. It seems likely that the firmly held silver is chemisorbed, although we recognize that some penetration of the glass surface, *i.e.*, absorption, may occur and that the radiochemical technique can only monitor the total silver held by the glass. Nevertheless, we may apply the following kinetic treatment to the long-term adsorption process.

*We are indebted to Dr. E. J. Hearn, City of Birmingham Polytechnic, for carrying out these tests.

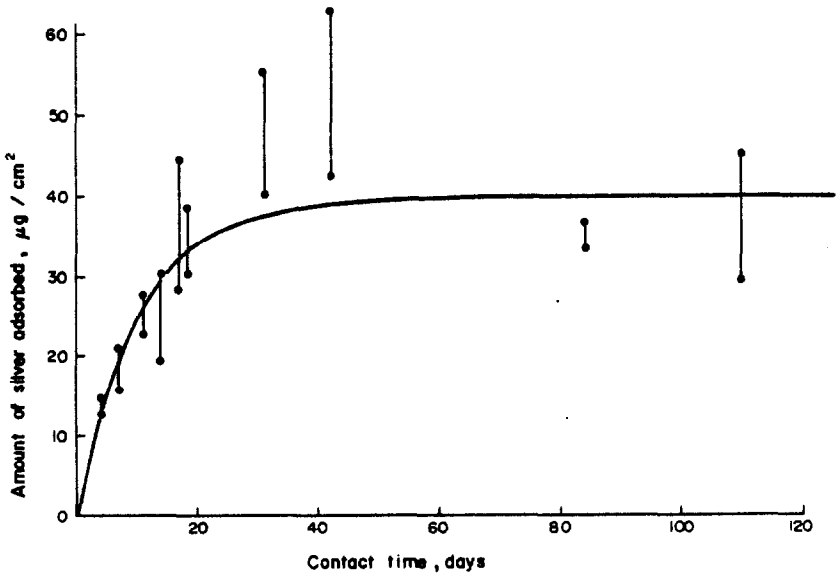

Fig. 2. Dependence of the amount of adsorbed silver on extended contact time at 298 K: initial concentration of $AgNO_3$ solution 0.5 mg/l.

Table 1. Effect of heat pretreatment of glass vials

Treatment of vial	Amount of Ag held, $\mu g/cm^3$ (4-day contact time; 298 K)	
Flamed to redness in bunsen burner	35.0	23.2
Heated in oven at 200°C for 1½ hr	23.1	25.8
No heat treatment	10.4	18.3

If we assume that the process can be described by the equilibrium

$$\text{Ag(solution)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Ag(glass surface)} \qquad (1)$$

and that the adsorption and desorption processes are first order in silver, we obtain the equation

$$\ln\left(\frac{x_{eq} - x}{x_{eq}}\right) = -(k_1 + k_2)t. \qquad (2)$$

In this equation, x is the concentration of adsorbed silver at time t, x_{eq} is the equilibrium concentration of

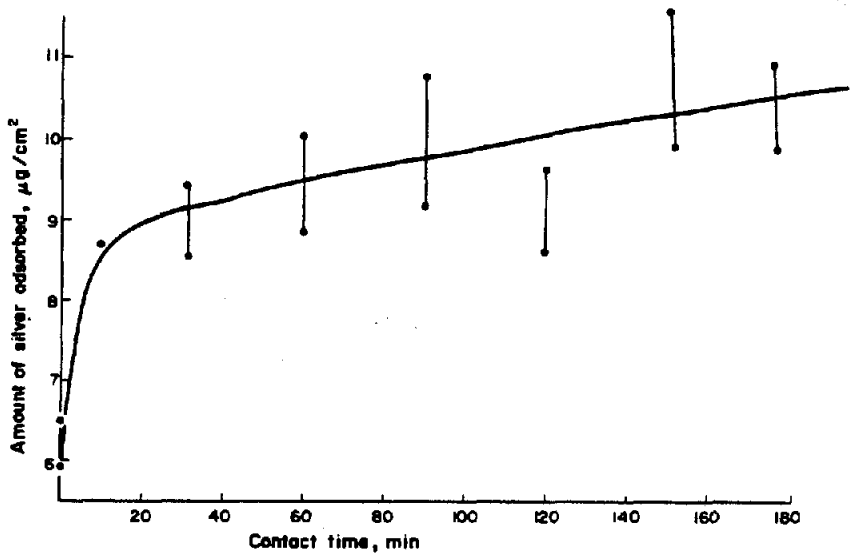

Fig. 3. Dependence of the amount of adsorbed silver on short-term contact time at 298 K: initial concentration of $AgNO_3$ solution 0.5 mg/l.

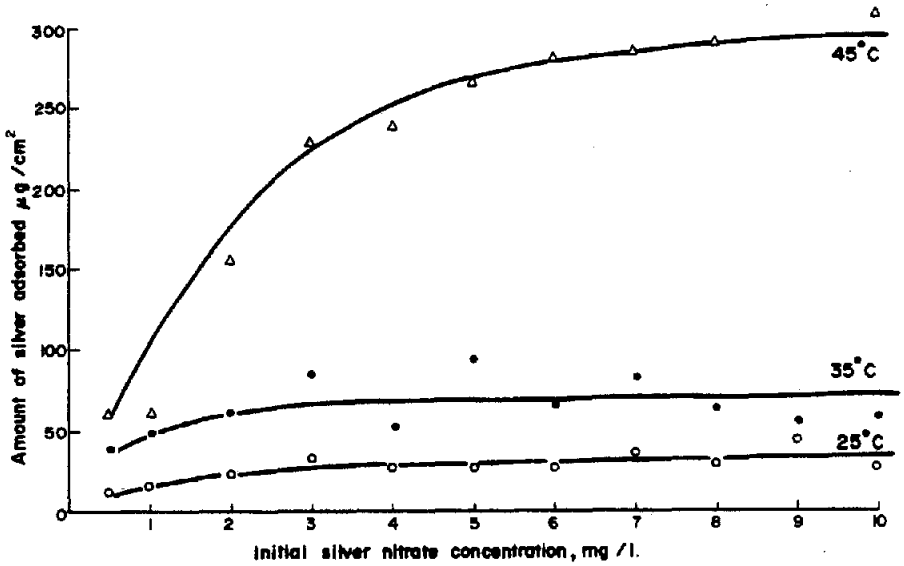

Fig. 4. Dependence of the amount of adsorbed silver on the initial concentration of $AgNO_3$, after a 4-day contact time.

adsorbed silver, and k_1 and k_2 are the rate constants for the adsorption and desorption processes. The equilibrium concentration x_{eq} may be related to the initial concentration a (converted into the same units as x, by using the known volume of solution and surface contact area), by $x_{eq} = k_1 a/(k_1 + k_2)$. From Fig. 2, we adopt the value 40×10^{-6} mg/cm^2 for x_{eq} and calculate the value 170 mg/cm^2 for a. Rearrangement of equation (2) gives

$$x = x_{eq}\{1 - \exp[-(k_1 + k_2)t]\} \quad (3)$$

and comparison with Fig. 2 yields values of 0.023 and 0.076 days^{-1} for k_1 and k_2 respectively, at 298 K. The curve in Fig. 2 is calculated from equation (3) by using these values of k_1 and k_2, and is a fair representation of the experimental data. Although the estimates of k_1 and k_2 are very approximate, it is noteworthy that k_2 is appreciably larger than k_1 and that less than a third of the silver originally in solution is transferred to the surface at equilibrium. Furthermore, if we assume that the silver is adsorbed as Ag^+, with an effective radius of 1.26 Å, we may estimate the fraction of the surface covered at equilibrium. It is estimated that the actual available surface area is greater than the measured geometric surface by a factor of at least 2.* If this factor is taken into account then the fraction of the surface covered at equilibrium for an initial concentration of 0.5 ppm is around 10% at 298 K.

As mentioned earlier, there is a strong indication that the extent of adsorption varies with the pH of the solution and further studies are in progress.

*We are indebted to Dr. B. Evans of Messrs. Pilkington Bros. Ltd, for this estimate.

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ANNOTATION

THE THEORETICAL BASIS OF MERCURIMETRY IN NON-AQUEOUS SOLUTIONS

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Summary—The methods for thermodynamic calculations of the equilibria in solutions of mercury salts and complexes are presented. The calculations of equilibrium constants in non-aqueous solvents are based on the transfer activity coefficients of mercury ions from water to the non-aqueous solvent. The dismutation and precipitation reaction constants are calculated, and the redox potentials of mercury systems are measured. Examples of analytical use of the thermodynamic functions of mercury salt solvation are given in the text.

The complexation and precipitation reactions of mercury are used for both inorganic and organic analysis; the use of non-aqueous media increases the rates of these analytical reactions.[1] However, undesirable complications such as solvolysis and dismutation can change the rates and cause errors in the results.

The basis for the use of mercury reactions in non-aqueous solvents and for prediction of the necessary analytical conditions is the thermodynamics of transfer of mercury ions from water to the non-aqueous solvent. According to IUPAC recommendations,[2] the transfer activity coefficient $\gamma_t(i)_{W\to S}$ characterizes the Gibbs free energy change $\Delta G_t(i)_{W\to S}$ for transfer of ion (i) from water (W) to non-aqueous solvent (S). The value of $\gamma_t(Hg_n^{2+})_{W\to S}$ can be calculated from the standard potentials for the corresponding systems:

$$\Delta G_t(Hg_n^{2+})_{W\to S} = RT \ln \gamma_t(Hg_n^{2+})_{W\to S} = zF({}^{W}E^0_{Hg_n^{2+}/Hg} - {}^{S}E^0_{Hg_n^{2+}/Hg}) \quad (1)$$

The redox potentials and transfer activity coefficients for mercury systems

The values of $\log \gamma_t(Hg_n^{2+})_{W\to S}$ are calculated from polarographic data (Strehlow's non-thermodynamic hypothesis[3]) and potentiometric data.[4] The redox and half-wave potentials and the transfer activity coefficients calculated from them are given in Table 1. Discrepancies between our values and those published by others will not be discussed here. They may be due, at least in part, to the use of very different solution conditions, and may also be due to traces of water (known to have strong effects in this context), but in any case the discrepancies do not affect the general sense of the discussion.

In protic solvents mercury salts are solvated more slowly than in water: this is the reason for the rapid oxymercuration reactions in alcohols and acetic acid.[5,6] In these solvents the oxidizing capacity of mercury salts increases: in acetic acid mercury(II) perchlorate quickly oxidizes the dihydroxybenzenes[7] and inorganic ions such as Fe^{2+}, Sn^{2+}, Cu^{+}, $Fe(CN)_6^{4-}$, $S_2O_3^{2-}$. In dipolar aprotic media the reducing capacity of mercury(I) salts increases.

The transfer activity coefficients of mercury ions can indicate the thermodynamic possibility of analytical reactions and allow calculation of the corresponding equilibrium constants. We will demonstrate this for some reactions of mercury.

Formation of insoluble mercury(I) salts

The solubility products of Hg_2L_2 in water and in a non-aqueous solvent are related by the equation:

$$(pK_{s_{Hg_2L_2}})^S = (pK_{s_{Hg_2L_2}})^W - \log \gamma_t(Hg_2^{2+})_{W\to S} - 2 \log \gamma_t(L^-)_{W\to S}. \quad (2)$$

The pK_s values in non-aqueous media are calculated from $\log \gamma_t(Hg_2^{2+})$ (Table 1) and $\log \gamma_t(L^-)_{W\to S}$. In most cases, although mercury ions are strongly solvated by dipolar aprotic solvents, the solubility of Hg_2L_2 is lower in these media because of the much lower dielectric constant. Some exceptions (*e.g.*, Hg_2I_2 in DMSO) are explained by the existence of weak anion solvation by hydrogen-bonding to solvent molecules. The solubility of the mercury(I) halides is levelled by their solvation in dipolar aprotic media

Table 1. The standard (E^0) and half-wave ($E_{\frac{1}{2}}$) potentials, dismutation constants (pK) and transfer activity coefficients ($p\gamma_t$) of mercury salts (25°C) from water to organic solvent

Method	Quantity measured	Solvents					
		H_2O	MeOH	EtOH	PrOH	BuOH	AmOH
Potentiometry,	$E^0_{Hg(II)/Hg(I)}$, V	0.907	1.000	1.025	1.041	1.053	1.067
	$E^0_{Hg(I)/Hg(0)}$, V	0.792	0.902	0.925	0.937	0.949	0.975
$\mu \rightarrow 0$	pK_d	1.94	1.66	1.69	1.76	1.76	1.53
	$\log \gamma_t Hg_2(ClO_4)_{2w\rightarrow s}$	0	1.25	1.51	1.65	1.79	2.08
	$\log \gamma_t Hg(ClO_4)_{2w\rightarrow s}$	0	1.18	1.42	1.70	1.78	2.00
		H_2O	MeOH	EtOH	PrOH	EG	PC
Polarography, in 1M $LiClO_4$	$E_{\frac{1}{2}}$, $Hg_2^{2+} \rightarrow Hg$, V	0.427	0.497	0.520	0.550	0.225	0.460
	$E_{\frac{1}{2}}$, $Hg^{2+} \rightarrow Hg$, V	0.457	0.510	0.540	0.570	0.250	0.540
	pK_d	1.03	0.68	0.68	0.67	0.84	2.75
	$\log \gamma_t(Hg_2^{2+})_{w\rightarrow s}$	0	2.1	3.1	4.2	−6.8	2.8
						−5.6[a]	3.4[b]
	$\log \gamma_t(Hg^{2+})_{w\rightarrow s}$	0	1.8	2.8	3.8	−7.0	2.8
						−6.8[a]	
		AN	DMSO	DMF	AC	NM	AcOH
	$E_{\frac{1}{2}}$, $Hg_2^{2+} \rightarrow Hg$, V	0.690	0.170	0.210	0.460	0.630	0.576
	$E_{\frac{1}{2}}$, $Hg^{2+} \rightarrow Hg$, V	0.550	0.120	0.160	0.500	0.590	0.630
	pK_d	−4.82	−1.72	−1.72	1.37	−1.37	0.34
	$\log \gamma_t(Hg_2^{2+})_{w\rightarrow s}$	8.9	−8.7	−7.4	1.1	6.9	4.8
		8.4[c]	−15.2[d]	−11.0[e]			
	$\log \gamma_t(Hg^{2+})_{w\rightarrow s}$	3.1	−11.4	−10.0	1.5	4.5	4.2
		9.0[c]	−13.9[d]	−12.4[e]			

MeOH—methanol, EtOH—ethanol, PrOH—propanol, BuOH—butanol, AmOH—pentanol, EG—ethylene glycol, PC—propylene carbonate, AN—acetonitrile, DMSO—dimethylsulphoxide, DMF—dimethylformamide, AC—acetone, NM—nitromethane, AcOH—acetic acid.

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Table 2. The transfer activity coefficients of anions and the solubility products of mercury(I) salts in non-aqueous solvents (25°)

Solvent*	$\log \gamma_t(L^-)$† $_{w\rightarrow s}$					$pK_{s_{Hg_2L_2}}$				
	Cl^-	Br^-	I^-	SCN^-	OAc^-	Cl^-	Br^-	I^-	SCN^-	OAc^-
H_2O	0	0	0	0	0	17.9	22.3	28.3	19.5	14.7
EG	4.9	4.5	3.6	4.0	—	20.9	24.5	28.7	20.7	—
PC	8.8	6.9	4.5	3.2	—	36.6	37.2	38.4	27.0	—
AN	8.0	6.3	2.3	0.8	10.7	42.0	43.8	41.8	30.0	45.0
DMSO	8.7	6.6	4.0	3.7	9.4	26.6	26.8	27.6	18.2	24.8
DMF	9.7	7.6	4.4	4.1	12.1	29.9	30.1	29.7	20.3	31.5
DMA	10.5	8.3	4.7	4.4	18.1	27.9	27.9	26.7	17.3	29.9
P	7.1	5.2	4.8	3.1	—	20.3	20.9	26.1	13.9	—
NMP	10.8	8.5	5.4	4.5	—	27.0	26.8	26.6	16.5	—
NM	8.2	6.2	5.3	3.8	11.4	41.2	41.6	45.8	34.0	41.4

* DMA—dimethylacetamide, P—pyrrolidone, NMP—*N*-methylpyrrolidone; other designations as in Table 1.

† Reference 2.

(Table 2). For mercurometric titration of halide ion mixtures, water is the best medium, but thiocyanate cannot be differentiated from chloride or bromide because of the small difference in pK_s and for this determination DMSO or DMF should be used. The lower limit of individual halide determinations is lower in acetonitrile (AN) medium than in other solvents.

Dismutation reactions and stability of mercury(I) solutions

The stability of the Hg(I) salts used in analytical chemistry depends on the solvolysis and dismutation reactions. The first may be avoided by keeping the pH below 2; the second takes place if the Hg(II) formed gives stable solvates with solvent molecules. For soluble and dissociated Hg(I) salts (nitrate, perchlorate) the dismutation constant K_d can be determined from the standard potentials (approximately the half-wave potentials):

$$\ln K_d = \frac{zF}{RT}(E^0_{Hg_2^{2+}/Hg} - E^0_{Hg^{2+}/Hg}). \quad (3)$$

Mercury(I) perchlorate is stable in water, less stable in alcohols, acetic acid and acetone (Table 1). In dipolar aprotic solvents [acetonitrile (AN), nitromethane (NM), dimethylsulphoxide (DMSO) and dimethylformamide (DMF)] the concentration of Hg(I) decreases continually because of the dismutation.

Redox reactions

Protic solvents enable mercury salts to be used in redox titrations: these solvents weakly solvate the mercury reagents, and are readily obtainable and easily purified. Some examples are presented in Fig. 1. The potential-jump at the end-point can be increased by addition of mercury-complexing reagents:[8] thus addition of thiocyanate or iodide increases the jumps in the titration curves for Fe^{3+} and Cr^{3+}:

$$2Fe^{3+} + Hg_2^{2+} + 8SCN^- \rightarrow 2Fe^{2+} + 2Hg(SCN)_4^{2-} \quad (4)$$

$$CrO_2^- + 3Fe(CN)_6^{3-} + 4OH^- \rightarrow CrO_4^{2-} + 3Fe(CN)_6^{4-} + 2H_2O \quad (5)$$

$$2Fe(CN)_6^{3-} + Hg_2^{2+} + 8I^- \rightarrow 2HgI_4^{2-} + 2Fe(CN)_6^{4-} \quad (6)$$

The choice of solvent for mercury reactions

The very low solvation of electrophilic reagents (mercury salts) is thermodynamically favourable for the electrophilic reactions of organic compounds with Hg(II) or cationic complexes HgL^+. This is observed for mercury salts in alcohols, acetic acid, nitromethane or propylene carbonate (PC); the HgL^+ complexes are weakly solvated in protic media.[9]

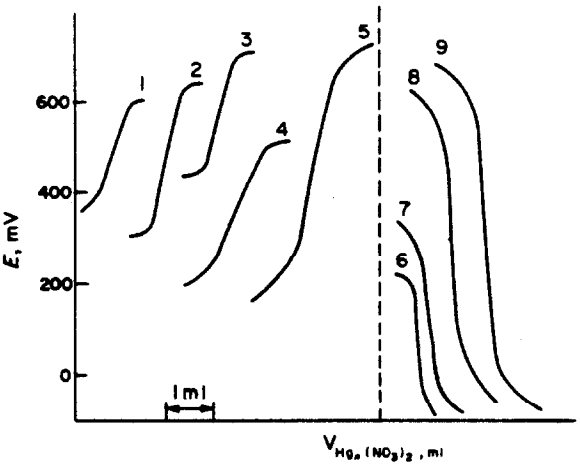

Fig. 1. The potentiometric curves for titration of inorganic salts with (1–5) $Hg(NO_3)_2$ in acetic acid and (6–9) $Hg_2(NO_3)_2$ in water: 1—$SnCl_2$, 2—$FeCl_2$, 3-CuCl, 4—$K_4[Fe(CN)_6]$, 5—$Na_2S_2O_3$, 6—$K_3[Fe(CN)_6]$, 7—$FeCl_3$, 8—$KMnO_4$, 9—$K_2Cr_2O_7$.

In contrast, dipolar aprotic solvents are better media for the reactions of anionic mercury(II) complexes.

Thus, the thermodynamic transfer function data for mercury ions and complexes allow prediction of their analytical reactions and permit the corresponding equilibrium constant calculations.

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LETTER TO THE EDITOR

FLOW-INJECTION ANALYSIS AND ITS EARLY HISTORY

Sir,

Referring to the recent article by Stewart,[1] we wish to point out that we have repeatedly suggested in the past[2-4] that the term Flow-Injection Analysis be used exclusively for methods which involve the following three requirements:

(a) sample injection;
(b) controlled dispersion of the injected sample zone;
(c) reproducible timing.

Such a definition avoids any confusion such as may arise from Stewart's paper. Indeed, from Stewart's interpretation of FIA it might be wondered whether the method was not already conceived by Skeggs in 1957, because in Fig. 1 of Stewart's paper, depicting Skeggs's analyser, a section between the debubbler and the flow cell accommodates an unsegmented continuously flowing stream of liquid which alternately carries discrete samples and wash solution with the purpose of a subsequent detection of analyte. In fact Stewart's definition is so broad that it perfectly well accommodates even liquid chromatography!

The purpose of writing this comment is, however, not to create a discussion on semantics, but to draw your readers' attention to the key role of the concept of controlled dispersion of the sample zone in understanding how and why FIA works. Not until this was established and the theory behind it became outlined, was the road towards development of various FIA techniques open, and the borderline between the prehistory of FIA and its present - still early - stage of development, crossed.

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ORGANIC COMPLEXING AGENTS IN ATOMIC-ABSORPTION SPECTROMETRY—A REVIEW

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Summary—The influence of organic complexing agents on the determination of a particular element by atomic-absorption spectrometry (AAS) with flame or electrothermal atomization may increase the sensitivity and selectivity. The effect of the structure and properties of the organic ligand in connection with the particular atomization mechanism is discussed.

Organic analytical reagents can exhibit various effects in AAS: (*i*) the degree of atomization of elements is changed, depending on the mode and mechanism of atomization, and the efficiency of free atom formation is sometimes increased; (*ii*) the reagent may suppress or decrease interference from accompanying elements during the AAS determination of a particular analyte; (*iii*) preconcentration can be achieved by extraction of metal chelates or ion-association complexes into non-aqueous solvents, the extracts then being nebulized into flames or treated by flameless techniques. When an organic solvent is used, it also can have an effect on the AAS behaviour of a particular system.[1–9]

In general, the mechanisms by which complexing agents affect the AAS behaviour of metals are rather complicated and not yet fully understood, but it can be taken that they are mainly concerned with formation and evaporation of aerosol droplets, the thermal decomposition of the solid species, and the atomization process.

AAS IN PREMIXED FLAMES

In flames, the very fine aerosol droplets of the nebulized solution are desolvated, the resulting solid particles are thermally decomposed and the gaseous metal atoms formed may be further involved in secondary chemical reactions. The atom concentration in the flame is predominantly controlled by the concentration of the particular element in the test solution.[10] All these steps can be influenced by the presence of complexing agents, even the nebulization process being affected by resulting changes in the viscosity and surface tension of the solution. However, there is no direct relation between the stability of a metal complex in solution and the effect of the ligand on the determination of the metal by AAS. For one thing, there will always be an excess of complexing agent present, and its behaviour in the flame may also have important effects. Thus data for complex equilibria in solution, such as the stoichiometry and stability of the complexes, are of little help in predicting the behaviour of a particular complexation system in AAS.

Formation of metal atoms in flames

During the pre-atomization steps, such as the melting, volatilization and partial thermal decomposition of the solid particles, not only the oxides but also sulphides, carbides, nitrides and volatile metal complexes may be formed, these being subsequently decomposed if they are not thermally stable at the actual flame temperature. Free atoms appear by dissociation or reduction of the compounds present in the gaseous state after volatilization. The spatial distribution of free atoms in the flame (lateral flame profile) depends on the temperature and composition of the flame, and hence on the fuel: support gas ratio. The number of free analyte atoms is considerably decreased if ions or thermally stable compounds are formed or if the analyte is dispersed in some kind of non-volatile matrix which does not decompose at the flame temperature. On the other hand, the number of free atoms may be increased if readily decomposed volatile compounds are formed, or the analyte is dispersed in a volatile matrix, or high-temperature flames are used.

At flame temperatures, the compounds present in the gaseous state are generally considered to be the monoxides (although other oxides may exist in some cases) and compounds with halides, *etc.* (X) or ligands (L), which dissociate according to the equilibria

$$MO \rightleftharpoons M + O$$
$$MX_x \rightleftharpoons M + xX$$
$$M_yL_z \rightleftharpoons yM + zL$$

Organic ligands may be thermally decomposed, of course, but some of the other species may be produced in the process. The partial pressure of oxygen in the flame depends on the flame stoichiometry and controls the dissociation of the oxides.[11–14]

Reduction of oxides is the second important process leading to analyte atoms in flames and is provided by

carbon-containing radicals in the interconal zone of the flame, with production of carbon monoxide.

$$MO + C = M + CO$$

This reaction is highly exothermic since the heat of formation of carbon monoxide (1014 kJ/mole) is larger than that of most monoxides ($\leq$836 kJ/mole). Thus, carbon is able to reduce all monoxides at 2773 K.[15]

In acetylene–air flames (2550 K), CO, H and C_2-radicals provide reduction of monoxides,[16,17] but oxides with a dissociation energy $<$533 kJ/mole will be atomized at this temperature anyway. The absorbance signal of cadmium and lead is independent of the flame composition, whereas Cu, Zn, Ni, Co, In and Ga need fuel-lean flames, Fe, Ca and Mn stoichiometric flames, and Cr, Mo and Sn fuel-rich flames. The optimal flame composition between fuel-lean and fuel-rich correlates with the dissociation energy of the monoxide. A reducing atmosphere is especially suitable for elements with thermally stable oxides.[18] In the acetylene–nitrous oxide flame (2950 K) highly reducing C-, C_2-, and CH-radicals are assumed to be present.[11,19] This flame is especially used for Be, Sc, Mo, Al, Si, V, Ti, Y, Ge, Zr, W, B, Hf, Re, Tc, Nb, La, U and lanthanides, which all form stable monoxides. The red interconal reaction zone of the fuel-rich flame, which contains CN- and C_2-radicals, is most effective in this case.[20] Carbon radicals can reduce the metal monoxide to the metal or form a carbide in the solid phase as well. The absorbance signal of the element is increased if the metal or carbide formed is more volatile than the corresponding oxide (*e.g.*, Be, Al, Sc, Y, La). On the other hand, a decreased volatility in the order oxide > metal > carbide causes a decrease in the absorbance signal (*e.g.*, Mo, Ti, W, Si, V, B). Zr, Hf, Nb, Ta, and Th all give non-volatile species and their absorbance signals are not influenced by reduction in the solid state but rather by the formation of polynuclear compounds during evaporation of aqueous solutions.[21,22]

Effect of organic ligands with N, and N and O donor atoms, in flames

Aliphatic amines such as trimethylamine, diethylamine and methylamine decrease the absorbance of various elements in acetylene–air flames (*e.g.*, Fe, Ni, Co). For cobalt the absorbance is drastically lowered if a solution containing 6% triethylamine and ethanol is nebulized. A lesser effect is observed for dimethylamine and pyridine, but ethylamine or aniline has no effect. Such effects arise from insufficient dissociation of metal complexes into atoms at the particular flame temperature. In the hotter acetylene–nitrous oxide flame, the behaviour of these elements is normal and there is no effect by the amines.[23] A similar small negative effect on the absorbance signal of silver in low-temperature flames (coal gas–air) is caused by the presence of methylamine, ethylenediamine or ammonia. EDTA has no effect.[24] In contrast, the same amines increase the degree of atomization for zirconium in acetylene–nitrous oxide flames, because mononuclear complexes are formed which are more volatile and more easily atomized than the corresponding oxide. A similar effect is observed for titanium and hafnium.[25] The chelates of Cu, Ni and Pb with 8-hydroxyquinoline or salicylaldoxime produce slightly lower absorbances than the analogous chelates with cupferron, diethyldithiocarbamate or dithizone in acetylene–air flames.[26] The absorbance for calcium in the presence of 8-hydroxyquinoline or EDTA in propane–air flames (2200 K)[27,28] is lower than that for acetylene–air flames (2550 K), because the lower flame temperature is not sufficient to produce full dissociation of the calcium chelates. On the other hand, magnesium gives higher absorbance in the presence of 8-hydroxyquinoline in propane–air flames.[29] This metal chelate evidently decomposes even at moderate temperature, since most of the magnesium atoms appear close to the burner slot and their concentration rapidly decreases with distance above it. Opposite effects are also observed for titanium and molybdenum in acetylene–nitrous oxide flames, the absorbance of titanium in the presence of cupferron being double that in the presence of 8-hydroxyquinoline,[30] whereas for molybdenum the absorbance is higher in the presence of 8-hydroxyquinoline than of cupferron.[31,32] For cobalt complexes with ligands binding through both oxygen and nitrogen as donor atoms, *e.g.*, Co(III) EDTA, the distribution of free atoms in acetylene–air flames is the same as for complexes containing only oxygen donor atoms, *e.g.*, Co(III)(oxalate)$_3$.[33,34]

Effect of organic ligands with oxygen donor atoms, in flames

For titanium the yield of free atoms in acetylene–nitrous oxide flames is increased in the presence of formic, acetic, propionic and butyric acids but decreased in the presence of oxalic, citric and ascorbic acids.[30,35,36] The absorbance decrease in the presence of the last three acids probably arises from reduction in the solid phase to titanium metal or carbide which will not evaporate or decompose even at high flame temperature. The absorbance increase caused by the first four acids is due to faster evaporation of the more volatile Ti(IV) complexes in the flame, so that the slow reduction step is hindered.

It has been shown for titanium, that the presence of *o*- or *peri*-dihydroxy groups or *o*-hydroxycarboxylic groups on the aromatic nucleus, which is obligatory for stepwise formation of soluble coloured complexes in solutions, is not essential for the organic ligand to enhance the atomization process in acetylene–nitrous oxide flames. For example, 1,5-naphthalenedisulphonic acid, which does not form Ti(IV) complexes in solution, has the same effect on the behaviour in the flame[25] chromotropic acid, which forms very stable complexes of Ti(IV) in solution. What is essential, is the presence of one or more sulphonic groups in the

molecule. Thus naphthalenesulphonic acids and 5-sulphosalicylic acid have been used with success. Phenolic complexing agents without sulphonic groups have a negligible effect on the absorbance, and aliphatic ligands (*e.g.*, oxalic, ascorbic or citric acid) can decrease the atomization yield for titanium.[36] It has also been shown that a large excess of 5-sulphosalicylic acid, chromotropic acid or a number of further sulphonated aromatic derivatives increases the absorbance signal of titanium and also of molybdenum in the presence of K^+ or Na^+.[36,37] However, if the concentration of aromatic sulphonic acids is low, the absorbance for Ti and Mo in acetylene–nitrous oxide flames decreases, since the non-volatile carbide or metal is formed in the condensed phase. If a large excess of the sulphonated compounds and Na^+ or K^+ is present, sodium or potassium sulphate is formed in the solid particles in the flame and can convert the metal or carbide into the monoxide (or Mo into MoS_2). These products finally dissociate after evaporation or are reduced to atoms in the gaseous state, and the absorbance signal from the flame again increases.[36,37]

Tungsten and vanadium behave similarly in the presence of sulphonated aromatic compounds and K^+ or Na^+, but with little or no change in sensitivity. In both cases, the metallic or carbide forms are less volatile than the corresponding oxides, so their formation in the solid phase by reaction with carbon from the destroyed aromatic compound will cause a considerable decrease in signal, in the absence of large amounts of the reagent and K^+ or Na^+. However the simultaneous presence of sulphonic acid and Na^+ or K^+ enables the formation of alkali-metal sulphates at high flame temperature, which oxidize the non-volatile forms of the metals to the more volatile and more easily dissociating oxides and the full absorbance signals are restored.[38]

In general, elements can be divided into three groups with respect to the volatility of the monoxide (O), metal (M) and carbide (C) in the acetylene–nitrous oxide flame.[21] For the first group, *e.g.*, Be, Al, Sc, Y, La, the volatility increases in the order O < M < C. For the second group (*e.g.*, Mo, Ti, W, Si, V, B) the order of volatility is C < M < O, and for the third group (*e.g.*, Nb, Ta, Zr, Hf, U) all forms are non-volatile. In the presence of 5-sulphosalicylic acid, the absorbance signal of Al and Be increases by 15 and 8% respectively, because the monoxides are reduced to the more volatile metals or carbides in the solid phase by the reagent. A similar sensitivity increase is observed for calcium.[38] Formation of the less volatile metal and carbide in the flame depresses the sensitivity of AAS for the second group of elements in the presence of aromatic compounds. This interference can be removed if the metal and carbide are converted into the more volatile oxide, *e.g.*, by an alkali-metal sulphate formed as discussed above. Elements of the third group produce polynuclear species during the oxidation and reduction processes in the flame. These compounds are non-volatile and hinder the evaporation and atomization of the elements. In the presence of 5-sulphosalicylic acid, the absorbance signal of tantalum is almost completely depressed, and that of uranium decreases by 85%.[38]

The absorbance signal of calcium in the acetylene–air flame is also influenced by the presence in the nebulized solution of various ligands containing oxygen donor atoms. Citric or tartaric acid or triethanolamine will decrease the signal. The absorbance signal for manganese in an acetylene–air flame is increased slightly more by the presence of cupferron in the test solution, than of other complexing agents such as diethyldithiocarbamate, 8-hydroxyquinoline or thenoyltrifluoroacetone.[26] No effect of organic complexing agents on the copper signal from acetylene–air, propane–air or hydrogen–air flames is observed (the signal comes from the easily formed and dissociated oxide). The signal from cobalt is only slightly enhanced by cupferron, thenoyltrifluoroacetone or 8-hydroxyquinoline, but is enhanced even less in the presence of acetylacetone, *viz.* by 6% in acetylene–air or by 3% in hydrogen–air flames respectively, relative to the signal from methanolic cobalt acetate solution.[40]

Organic ligands containing carbon donor atoms, in flames

If metallocenes of Zr, Hf, Nb and Ta are nebulized, the atomization yield and sensitivity of determination are considerably increased. Refractory oxides are not formed when metallocenes containing M–C bonds decompose.[41,42] The signal for the cyclopentadiene complex of titanium (Cp_2TiCl_2) is >22 times that for an equivalent amount of the cupferron complex. Similarly, the absorbance signal for Cp_2ZrCl_2 in 0.14*M* hydrochloric acid is 5% higher than that for $ZrOCl_2$ in an acetylene–nitrous oxide flame.[43] The absorbance signal of molybdenum is lower for the binuclear sandwich complex $[MoCp(CO)_3]_2$ than for the carbonyl complex $Mo(CO)_6$ in an acetylene–air flame. This is explained by the formation of non-volatile molybdenum carbide from the binuclear species because of the shorter Mo–C bonds in the sandwich species, *i.e.*, there is a shorter distance between Mo and C in the condensed phase during evaporation.[15]

Tetramethyl-lead gives a lower absorbance signal for lead than that from lead nitrate or the 8-hydroxyquinoline chelate in a propane–air flame.[44] In contrast, the absorbance signal from CH_3HgCl is 50% larger than that from aqueous mercury(II) chloride solution in an acetylene–air flame. The same increase is produced if the mercury(II) chloride solution is made in 1:1 v/v methanol–water, but the signal for CH_3HgCl is not changed under these conditions.[45]

Organic ligands containing sulphur donor atoms, in flames

For copper and cobalt the atomization yield and the absorbance in acetylene–air flames for solutions of

the diethyldithiocarbamate and pyrrolidinedithiocarbamate do not differ from those for the complexes of organic ligands containing oxygen and nitrogen as donor atoms.[39,40] On the other hand, the absorbance signal for molybdenum is much larger for the 3,4-toluenedithiol complex than for the 8-hydroxyquinoline, cupferron and acetylacetone complexes.[32] Little attention has been paid to the effect of aromatic compounds containing chelating sulphur donor atoms on the behaviour of various elements in flames. The formation of a more volatile covalent sulphide during reductive decomposition in the flame would be interesting for the AAS determination of elements which tend to form the refractory oxide, carbide or metal in the condensed phase in flames, since it should considerably increase the sensitivity.

Summary of the effect of organic reagents in flames

The advantageous effects are as follows.

(*i*) Formation of metal species that are more volatile and more easily evaporated and dissociated than the corresponding monoxides in particular flames.

(*ii*) Atoms formed by decomposition of non-hydrated metal chelates with organic ligands are protected by the reducing atmosphere.

(*iii*) The non-selective reduction of oxides by carbon and the decomposition products of organic compounds in flames gives products in the condensed or the gaseous phase which can change the properties of a particular flame.[46]

(*iv*) The flame temperature can be increased by exothermic reactions and increased heat of combustion in the presence of organic ligands, especially aromatic and cyclic compounds.[47] The organic species burn exothermically in the flame and metal atoms are liberated more easily from the refractory oxides.

The effects are disadvantageous under the following circumstances.

(*i*) The analyte is reduced to the metal or metal carbide in the condensed phase in a particular flame if the volatility of the species decreases in the order oxide > metal > carbide.[21,22,37,38]

(*ii*) There is insufficient dissociation of the particular analyte complex at the given flame temperature.

(*iii*) If the metal chelates are not sufficiently volatile or are decomposed to species not able to dissociate in the condensed phase.

Elimination of interferences in flame AAS by use of organic ligands

The interfering elements usually hinder evaporation of the analyte or give thermally stable ternary species which do not dissociate at the particular flame temperature.

The interferences in AAS determination of the alkaline-earth elements are removed by means of various complexing agents which form chelates with the interferent or the analyte or both. 8-Hydroxyquinoline has been used to eliminate the interference of Ba,[48] Be,[49] Cr,[50] Co[51] and Fe[52] in the determination of calcium and magnesium.[27,53–59] 5-Sulphosalicylic acid behaves as a universal agent for removing interferences in the determination of Mg and Ca,[27,56] Cr, Al and Fe,[60] Ti[36] and Mo.[37] Similarly EDTA is suitable for removing interferences in the determination of Mg and Ca,[27,61–66] and Cu, Mn and Ni.[67]

Complexing agents are sometimes used together with releasing agents (an excess of a metal salt which displaces the analyte from non-volatile binary and ternary species in the condensed phase in flames), *e.g.*, 8-hydroxyquinoline + Ca^{2+} for magnesium determination, and EDTA + La^{3+}, EDTA + Sr^{2+}, or 5-sulphosalicylic acid + La^{3+} for the AAS of magnesium and calcium, especially in acetylene–air flames.[27,56–58,62,64–66]

The elimination of interferences by addition of complexing agents can be explained as due to formation of complexes with the analyte or the interferent or both, which protects the analyte from interactions with the interferent and hinders the formation of thermally stable species.[41,58] However, the stability of the chelates in solution seems not to be the decisive factor, since weak complexing agents such as glucose, sucrose, ethylene glycol, glycerol, mannitol are also effective in the AAS determination of Ca, Sr[68–70] and Mg.[66] Metals such as Ca, Mg, Al, Ti and Fe, *etc.* when bound as stable EDTA chelates at the stage at which particles are formed in the flame, are protected from the formation of non-volatile species such as $Ca_3(PO_4)_2$, $CaO.Al_2O_3$, $CaO.TiO_2$, $2CaO.Fe_2O_3$, *etc.*[47] That a fixed or nearly stoichiometric molar ratio of interferent to organic ligand is necessary for active protection of the analyte, proves the prior existence of complexes with the interferent.[27,53,56] On the other hand, the previous chelation of the analyte with the protecting agent is also probable, *e.g.*, a small concentration of 5-sulphosalicylic acid is sufficient to remove the influence of a large excess of silicate during the AAS determination of Mg and Ca.[27,56] However, if 5-sulphosalicylic acid or another aromatic is used, the reduction of the analyte or interferent complexes to the metal or carbide in the solid particles in the flame cannot be neglected. In general, the reduction of analyte and/or interferent to the metal or carbide prevents the formation of a mixed thermostable oxide and thus the organic reagent contributes to the elimination of interferences.[58,68]

Another explanation can be offered for the effect of organic reagents. The formation of metal atoms from chelates is frequently an exothermic process because of the heat of combustion of the organic species, whereas the production of metal atoms from inorganic species is almost always endothermic. Thus there could be an explosive disintegration of a droplet containing an organic species, when the organic moiety is burned in the flame. Thus, the analyte and interferent may be separately reduced to metal atoms without close contact with each other, with no thermally stable ternary species being formed.[53] This explanation, however, would mean that organic spe-

cies not containing any complexing groups would have similar effects, which does not seem to be true.

PRINCIPLES OF ELECTROTHERMAL ATOMIZATION (ETA)

The form and size of the absorbance pulse depends on the kinetics of the atomization processes and the time taken for the analyte and its atoms to be completely removed from the atomizer. As in flames, salt dehydration, decomposition and thermal dissociation and reduction of oxides take place. In contrast to flames, however, the temperature at which the various steps take place, *i.e.*, drying, salt decomposition and atomization, can be easily programmed and the form and size of the absorbance peak influenced in this way. Free atoms are produced by electrothermal atomization, especially with carbon atomizers, according to the schemes:[71,72]

$$M_xO_y(s, l) \rightleftharpoons M_xO_y(g) \rightarrow xM(g) + yO(g)$$

or

$$M_xO_y(s, l) + yC \rightarrow xM(s, l) + yCO(g)$$

$$x \downarrow\uparrow \searrow$$

$$\frac{x}{2} M_2(g) \longrightarrow xM(g)$$

V, Ca, Mg, Cd, Zn and Mn atomize through dissociation of the monoxides in the gaseous phase. For Pb, Cu, Co, Ni and Sn the oxides are reduced by graphite to metal atoms or diatomic molecules, which evaporate and dissociate in the gaseous phase. Mo, Cr and Fe behave similarly but the metal atoms are evaporated. The behaviour of elements depends on the actual temperature changes.[71,72] The atomization schemes given above were deduced from the appearance time of particular atoms and the dependence of the absorption signal on time, for various atomizer temperatures. Sometimes atomization occurs without oxide forming:

$$MX_y(s, l) = MX_y(g) \rightarrow M(g) + yX(g)$$

as happens for Fe(III), Cd and Zn halides.[72] In the ETA methods, the absorbance signal is measured during a period of rapid temperature change, and the signal depends not only on the completeness and the kinetics of evaporation, but also on the temperature at which the evaporation of analyte begins. Incomplete evaporation may arise from carbide formation by interaction of analyte with the surface of a graphite atomizer. Some elements form non-volatile thermally stable carbides even at high temperatures (3000 K), which hinders total evaporation of the element. Use of graphite tubes with their surfaces covered by pyrolytic graphite or with a carbidized surface (Ta, Nb, Zr, W, Ti, Mo carbides) prevents or diminishes the formation of carbides.[73–80] Metallic atomizers made from tungsten,[81–84] molybdenum[85] or tantalum foil[86–89] are also suitable. In this way, the sensitivity for the AAS of lanthanides, uranium and yttrium can be made two orders of magnitude better than that for atomization from graphitic surfaces.[86,90] All organic materials which decompose to carbon at elevated temperature will promote the formation of non-volatile metallic carbides and produce a decrease in the absorbance signal. Accompanying elements can considerably influence or change the mechanism of atomization of a particular analyte. The analyte atoms can interact with the interferent in the gas phase, forming non-dissociated molecules, which lowers the absorbance signal of the analyte. This especially takes place if both the analyte and the interferent evaporate simultaneously, *i.e.*, the interference is proportional to the extent of overlap of the evaporation curves of both reactants and to the bond strength between the two species in the product. The effect of the interferent can be prevented by using a precarbidized atomizer, since the evaporation intervals are then generally separated.[91–93] The analyte can also be released from the product with the interferent, if another metal is present that forms a stronger bond than the analyte with the interferent.[91]

Organic reagents in graphite atomizers

Metal chelates with different organic ligands evaporate and decompose at different temperatures. Volatilization of a chelate at a temperature lower than that of atomization produces loss of the analyte and decrease of the absorbance signal. If the species formed during decomposition of metal complexes is not an oxide, the absorbance signal will be different from that from atomization of the oxide. The elemental carbon formed during decomposition of an organic complexing agent assists in reducing the metal oxide to free gaseous atoms, but the effect will be offset if a non-volatile metal carbide is formed during the decomposition. The volatilization of metal chelates, *e.g.*, of Cu or Co, at low temperatures has not been observed to occur in the graphite atomizer. This phenomenon, however, takes place during the AAS of the metal chelates of Cu, Co, Fe(III), Mn, Ni and Zn heated in a tantalum boat in acetylene–air flames.[39,40,94] There is no influence of 8-hydroxyquinoline, dithizone, diethyldithiocarbamate, 1-pyrrolidinedithiocarbamate, cupferron or thenoyltrifluoroacetone on the absorbance signal of Zn, Cd, Ag, Pb and Cu, during atomization from a graphite filament, compared with the signal for the corresponding inorganic salts.[95] The decomposition of the Cd, Co, Cu, Fe(III), Ni and Pb chelates of pyrrolidinedithiocarbamate at 473–573 K in argon produces the metal sulphides. If oxygen is also present in the argon, a mixture of sulphides and oxides is formed and no elemental carbon appears from the organic species, as it does in pure argon.[96] The decomposition of the Ni and Cu chelates of organic ligands involving M–N, M–O and M–S bonds, such as α-furildioxime, dimethylglyoxime, cuproine, bathocuproine, acetylacetone, 8-hydroxyquinoline, dithizone, pyrrolidinedithiocarbamate,

diethyldithiocarbamate, leads to the metal, metal monoxide and metal sulphide, or a mixture of metal and metal sulphide, in an atmosphere of argon.[97] The absorbance signal for copper increases by 10% relative to that for the nitrate if its chelates with organic ligands having S-donor atoms (*e.g.*, pyrrolidinedithiocarbamate, diethyldithiocarbamate) are injected in methanolic solution into the atomizer. Even larger differences are observed if chlorinated solvents are used.[39,97] For cobalt, a 20% absorbance increase is observed (relative to results for cobalt acetate) in the presence of chelating agents containing N, S or O donor atoms, e.g., acetylacetone, thenoyltrifluoroacetone, cupferron, 8-hydroxyquinoline, 8-mercaptoquinoline, diethyldithiocarbamate, pyrrolidinedithiocarbamate or 1-(2-pyridylazo)naphthol. The increase probably arises from the additional reduction of CoO by the elemental carbon resulting from the decomposed reagent.[40] As much as a 40% increase in the absorbance signal for nickel is observed for ETA of the α-furildioxime or dimethylglyoxime chelates applied as solutions in 1:4 chloroform/methyl isobutyl ketone mixture.[97]

Similar absorbance increases (by 10 and 15% respectively) appear for copper and cadmium atomized in a graphite cup in the presence of EDTA, pyrrolidinedithiocarbamate or diethyldithiocarbamate, and there is a 250% increase for lead.[98] The enhancement effect of ascorbic acid on the absorbance signal of Pb, Cu or Ga is explained by the presence of elemental carbon after its decomposition.[99] Depression of the absorbance signals may also be caused by organic ligands, however. The absorbance of iron decreases if its 1-phenyl-1,3-butenedione chelate is analysed, especially if chlorinated solvents are used. Double peaks may appear, and the atomization is considerably slower in reaching the maximal value at 2300°C. This effect has not been observed for atomization from a tantalum surface. The presence of carbon and halogen is responsible for the occurrence of the second peak. It comes from the formation of a thermally more stable compound which needs a higher temperature for its decomposition. On a carbon surface the formation of a reactive compound is assumed, such as $C_n^+X^-.3X_2$ (where X is halogen) released from halogenated solvents at elevated temperature. This then interacts with iron(III) chloride, forming a new species $C_n^+.FeCl_4^-.3FeCl_3$. If the excess of halogen is freed from the graphite surface, it may react with the iron of the sample, producing relatively volatile iron(III) chloride. A somewhat different mechanism was indicated for the effect of halogenated solvents on the atomization of other metals.[8]

The formation of a low-volatility carbide is probably the reason for depression of the absorbance signals in particular cases such as the AAS determination of Ni and V in lubricating oils, or for Ni and V in the presence of benzoylacetone (M–O bonds), dithizone (M–S bonds) and porphyrin (M–N bonds) in the presence of heavy Conostan oil. Porphyrin gives the largest negative effect because of easy carbonitride formation.[91]

The free atom yield as well as the sensitivity of AAS determination is sometimes decreased by the presence of nitric acid. This is due to destruction of the pyrolytic surface of the graphite furnace and the simultaneous introduction of the metal into the graphitic structure. The presence of nitric acid vapour or its decomposition products at 1000 K can also cause this effect. For nickel the interference of even 2*M* nitric acid is removed if previously the chelate with dimethylglyoxime, $Ni(LH)_2$ has been formed.[100] Vanadium as VO_3^- at pH 8 does not interact with nitrate, but VO_2^+ and VO^{2+} do in acid solutions.[100] The interfering effect of chloride in the AAS determination of Pb, Cu, and Mn is removed by various organic complexing agents such as ascorbic, maleic, adipic, glutaric or tartaric acid.[91,101–104] If pure metal-ion solutions are applied in the presence of excess of chloride, metal atoms in the gaseous phase are formed by dissociation of the corresponding chloride, but non-absorbing volatile metal monochloride species are also formed, which has a depressive effect on the absorbance peak. For Mn and Cu, however, it seems that the large depressive effect of excess of chloride is due to hindrance of the atomization through oxide formation and dissociation at the particular furnace temperature. Organic acids, *e.g.*, ascorbic or oxalic acid, added to the sample solutions applied, act as a flux, preventing the dried sodium chloride crystals in the furnace from lowering the surface tension, and the improved thermal contact of matrix and furnace accelerates the pyrolysis and the decomposition of the matrix.[104]

Organic reagents in metallic atomizers

When metallic atomizers are used in AAS, the effect of elemental carbon from decomposed organic species becomes more evident, and the dissociation of oxides is the predominant mechanism during atomization. If vanadium is heated in a tungsten atomizer in the presence of thiocyanate or organic reagents, a low-volatility carbide is formed during the decomposition (charring) step at 573 K and the absorbance signal at 2473 K decreases. Because of this, the charring step was omitted in the presence of thiocyanate, *N*-benzoyl-*N*-phenylhydroxylamine, diethyldithiocarbamate or 8-hydroxyquinoline.[84] In the presence of ligands containing sulphur as donor atom, the metal sulphide is formed rather than the oxide in metallic atomizers, and this readily dissociates. Thus antimony atoms appear in the gas phase in a molybdenum microtube atomizer at lower temperature (1483 K) in the presence of thiourea than from the pure trioxide (1913 K) and the absorbance peak height is also larger in the presence of thiourea.[85]

The heats of formation, $H_f^0 = 176$ kJ/mole for the sulphide and 706 kJ/mole for antimony trioxide, correlate with this fact. Similar effects are observed for As,[105] Bi[106] and Pb.[107] For lead, the absorbance

peak maximum is usually at 1683 K but in the presence of thiourea it occurs at 1400 K (with argon at 400 ml/min and hydrogen at 100 ml/min as purge gas). The lead atoms are formed from PbS ($H_f^0 = 94$ kJ/mole) and the interference of sodium chloride in the determination of lead is prevented.[107] On the other hand, lead gives different forms of absorbance peak at different temperatures in the presence of diethyldithiocarbamate, but similar atomization profiles are obtained from various lead species in the presence of thiourea.[107] A considerably lower absorbance signal also results for tin in a molybdenum microtube atomizer in the presence of thiocyanate, thiourea or diethyldithiocarbamate in comparison with that for pure tin(IV) tetrachloride, probably because of tin sulphide formation[108] In the presence of *N*-benzoyl-*N*-phenylhydroxylamine or 8-hydroxyquinoline the atomization profiles for tin are different but the peak heights practically identical. This comes perhaps from hydrogen being premixed with the shielding argon gas. Various absorbance peak profiles result from the different thermal stabilities of tin compounds.[108]

Summary of effects of organic reagents in ETA

(*i*) Carbon resulting during pyrolysis of the organic reagent enhances the reduction of the metal monoxide to produce free atoms in the gas phase during the atomization step.

(*ii*) The character of the metal–donor atom bonds and the stability and properties of the metal complexes formed with organic reagents control the preatomization processes (formation of oxide, carbide, nitride, sulphide or volatile metal chelate).

(*iii*) The analyte may be reduced to thermally stable carbide during thermal decomposition in the presence of organic reagents.

(*iv*) Volatile metal chelates may be formed with organic reagents, and the metal species disappear before the atomization step proper.

(*v*) If a solution of a metal chelate in an organic solvent is applied, the organic solvent can interact with the graphite surface and be released at higher temperatures, and then interfere with the metal atomization (multiple peak formation).

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CONCENTRATION OF TRACE METALS FROM SEA-WATER BY COMPLEXATION WITH 8-HYDROXYQUINOLINE AND ADSORPTION ON C_{18}-BONDED SILICA GEL

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Summary—A reversed-phase liquid chromatographic technique based on a combination of multielement chelation by 8-hydroxyquinoline with subsequent adsorption on C_{18}-bonded silica gel is described for the concentration of Cd, Zn, Cu, Ni, Co, Mn and Fe from sea-water. Enrichment factors of 50–100 are readily obtained following elution of the absorbate with methanol to provide a matrix-free concentrate suitable for graphite-furnace atomic-absorption analysis. Quantitative recovery of these elements from near-shore samples of sea-water is demonstrated and the accuracy and precision of the technique are discussed.

Analysis of sea-water for heavy metals currently requires concentration and/or matrix separation prior to determination by most instrumental methods of analysis. A number of techniques have been successfully used for this purpose, including co-precipitation and co-crystallization,[1,2] chelation and solvent extraction,[3–6] chelating ion-exchange resins,[5,7–9] and electrolytic concentration.[10] By far the most widely used procedure is based on the formation of metal dithiocarbamate complexes and their extraction into an organic solvent.[11]

Recent studies have indicated that the true concentrations of heavy metals in open ocean water are much lower than previously thought, *e.g.*, Cd 0.2 mg/l., Cu 80 ng/l., Zn 4 ng/l.,[12] Pb 3.3 ng/l.,[13] necessitating substantial preconcentration before analysis.

Because extractions from large volumes are impractical, both from theoretical and physical considerations, there is an upper limit to the preconcentration factor which can be achieved with such single-stage separations. Again, although the application of chelating resins, such as Chelex-100,[5,8] to the analysis of sea-water permits higher concentration factors to be attained, the method is time-consuming (sample flow-rates 1–2 ml/min) and removal of significant amounts of calcium and magnesium from the resin before elution of the trace metals requires careful washing procedures.[5,14] Hence other methods must be sought.

In a preliminary report from this laboratory,[15] a method based on the formation of metal–8-hydroxyquinoline complexes and their subsequent adsorption onto C_{18}-bonded silica gel was described for the preconcentration of Cd, Zn, Cu, Pb, Ni, Mn and Fe from sea-water. Encouraged by the initial success of this approach, we report here a detailed investigation of the applicability of this method to the determination of Cd, Pb, Cu, Mn, Fe, Ni, Cr and Co in samples of near-shore sea-water.

EXPERIMENTAL

Apparatus

All atomic-absorption analyses were carried out with a Perkin–Elmer HGA-2200 heated graphite atomizer. Sample aliquots of 10 or 20 μl were delivered to the furnace with a Perkin–Elmer AS-1 autosampler and absorbance peak heights were recorded on a fast-response Speed Servo II strip-chart recorder (Esterline Corp., Indiannapolis, Ind., U.S.A.). Continuum-source background correction was used. A detailed description of the instrumentation has already been given.[5]

Figure 1 shows the preconcentration apparatus, consisting of a 1-litre polypropylene reservoir connected by a short segment of Bev-A-line IV tubing (Cole Parmer Co., Chicago, Ill.) to a borosilicate glass column in which approximately 600 mg of C_{18}-bonded silica gel (Bondapak, Porasil B, 37–75 μm, Waters Associates, Milford, MA) was supported by a coarse sintered-glass frit.

Reagents

The C_{18}-bonded silica gel was used without further purification. All acids, solvents and other reagents were purified as previously reported.[5] Subsurface samples of near-shore Atlantic sea-water (salinity 29.5‰) were collected during late autumn off the coast of Nova Scotia, filtered through a 0.45-μm membrane filter, acidified to pH 1.6 with nitric acid and stored in precleaned polypropylene bottles.

Procedure

Sample preparation was done in a clean-laboratory equipped with laminar-flow benches and fume cupboards providing a class 100 working environment.

The glass column was slurry-loaded with 600 mg of the C_{18} gel suspended in redistilled methanol. Three 10-ml

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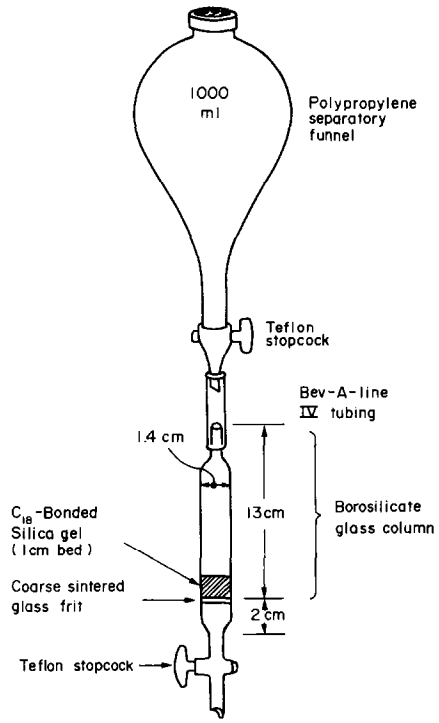

Fig. 1. Preconcentration apparatus.

portions of methanol were then passed through the bed under gravity flow to effect clean-up. Before passage of the last 200–300 μl of the methanol, distilled, demineralized water (DMW) was carefully added in small (100–200 μl) portions, thereby changing the solvent from methanol to water, in the manner of a gradient elution, without disturbing the resin bed. Following complete switch to the aqueous system, the column was washed with 5 bed-volumes of DMW and left covered with 4–5 ml of DMW.

A 500-ml sea-water sample was loaded into the reservoir and 0.5 ml of 5% purified[5] 8-hydroxyquinoline solution was added. The pH was adjusted to 8.9 with ammonia solution and the sample was drawn through the C_{18} bed with a water-aspirator, at a typical flow-rate of about 20 ml/min. Visual observation confirmed that the metal chelates were adsorbed on the top layer of the bed to form a thin green band.

Following passage of the sample, the column was washed free from sea-water with 10 ml of pH-8.9 DMW containing 50 μg of oxine per ml. The adsorbed metal chelates of 8-hydroxyquinoline were then eluted from the column with 5 ml of methanol. This eluate was either collected in a 10-ml standard flask and made up to volume with 2% v/v nitric acid or collected in a "Vycor" crucible and evaporated to dryness in the presence of 200 μl of concentrated nitric acid and 500 μl of 60% perchloric acid (this being repeated until a clear, colourless solution was obtained) and thereafter diluted to 10.0 ml with 1% v/v nitric acid. These procedures provide a theoretical concentration factor of 50.

Blanks were determined by applying the procedure to 40 ml of DMW (containing the same absolute amount of 8-hydroxyquinoline as the sample) instead of the sea-water sample.

Between runs, the column was reslurried to ensure proper packing of the bed, thoroughly flushed with methanol and changed over to the aqueous system.

The concentrates were analysed by graphite-furnace atomic-absorption, with calibration by means of solutions of appropriate metal standards in 1% v/v nitric acid as well as by the addition of metal spikes to aliquots of the concentrate in order to effect a matrix match.

RESULTS AND DISCUSSION

The method described for the preconcentration of trace metals from sea-water is essentially reversed-phase liquid column adsorption chromatography (RPLC). The application of this technique to the separation and concentration of trace amounts of heavy metals from a variety of matrices has recently been reviewed by Schwedt.[16] In RPLC, the stationary phase (in this case the C_{18} silica gel) is, by definition, less polar than the mobile phase (here, sea-water). Its effectiveness as a trace enrichment method in the present case lies in the fact that manipulation of secondary chemical equilibria in the aqueous mobile phase (formation of metal chelates) permits concentration of these hydrophobic, less polar complexes, at the head of the column. Furthermore, the weak surface attraction energies of the non-polar stationary phase promote rapid mobile-phase equilibration on the column during sample enrichment and elution, as well as gradient regeneration.[17]

Reversed-phase techniques are versatile methods for trace enrichment and have been used on several occasions to separate and/or preconcentrate heavy metals from a variety of matrices.[18–21] Applications of these techniques to sea-water analysis have been undertaken in both this laboratory[15] and, more recently, by Mills and Quinn.[22] The latter authors utilized prepacked C_{18} SEP-PAK cartridges (Waters Associates, Milford, MA) to collect both dissolved organic material and copper organometallic complexes from samples of near-shore sea-water. The use of SEP-PAK cartridges may prove very convenient for this application.

As 8-hydroxyquinoline is capable of forming insoluble metal complexes with more than 25 elements, advantage may be taken of its non-specific reactivity to complex simultaneously a number of metals present in sea-water. With suitable adjustment of pH and oxine concentration, chelation of the matrix elements (Ca and Mg) can be minimized relative to that of the transition metals.[23]

Of the elements selected for study, manganese forms the weakest complex with 8-hydroxyquinoline (log $\beta_2 = 17.5$).[24] Initial studies[15] indicated that manganese could be quantitatively recovered from sea-water at pH 8.9 with negligible recovery of calcium and magnesium.

The recommended pH range for operation of bonded-phase silica gels is 1–9.[25] Siloxane bonds are attacked by hydroxide in aqueous solution and the silica substrate must be "protected" by a high density of bonded organic phase in order to avoid dissolution. Although no deleterious effects on the performance of the columns were noted even after extended use at pH 8.9, care was taken to reslurry and repack

Table 1. Absolute blanks

Element	Blank, *ng** Methanolic concentrate	Acid decomposed concentrate
Cd	<0.1	<0.1
Zn	14 ± 8†	19 ± 14†
Cu	23 ± 8	16 ± 11†
Mn	<1	<1
Fe	37 ± 4	88 ± 19
Ni	<6	<6
Co	<1	<1
Pb	1 ± 1†	10 ± 6†

*Mean and standard deviation for 12 determinations.
†Skew distribution.

the bed after elution of each sample, because of the possibility of the silica dissolving under these conditions which might cause shifting and channelling of the bed.

Analytical blanks. Absolute blanks are presented in Table 1. Data are given for both the direct procedure, wherein the methanolic concentrates were directly analysed, and also following total acid decomposition of the blank eluate with dissolution of the residue in 1% v/v nitric acid. Blank values for the latter procedure are significantly higher for lead and iron, reflecting the consequences of increased sample manipulation. Furthermore, the precision of the blank determinations for acid decomposition procedures is seriously poorer than that for direct analysis of the methanolic concentrates.

Whereas reagent impurities can account for the blank values obtained for Cd, Mn, Ni, Co and Pb,[5] the C_{18}-bonded silica gel is probably the source of the high levels of Zn, Cu and Fe. Semiquantitative d.c. arc spectrographic analysis of this material revealed the presence of substantial concentrations of iron (0.06% w/w); lower concentrations of copper (0.01% w/w) and zinc (0.01% w/w) were also present. Even slight leaching of the silica substrate at high pH during each sample run could easily account for the high blank from these elements.

The low analytical blanks obtained in this study make the technique attractive for sea-water analysis. This is a significant advantage over use of activated charcoal[23] as the adsorption agent for oxine complexes and organometallic species.[22]

Recovery of spikes. The efficiency of recovery of spikes added to both DMW and sea-water samples is shown in Table 2. With the exception of cobalt, all elements of interest could be quantitatively recovered from DMW. From sea-water media, however, recovery of lead was erratic, varying from 17 to 88%. As a consequence, the technique could not be used for the determination of lead in sea-water. The reproducibility of the technique was slightly poorer for all elements when sea-water was analysed and an isolated check for chromium indicated that recovery of this element was less than 10%.

Recovery of spikes from sea-water did not vary over the range of sample flow-rates 8–35 ml/min.

Analytical results. Results for the analysis of two near-shore sea-water samples are given in Tables 3 and 4. The samples were filtered during collection and

Table 2. Recovery of metal spikes

Element	Recovery, %* Demineralized water	Sea-water
Cd	94 ± 10	108 ± 18
Zn	102 ± 13	97 ± 12
Cu	104 ± 4	91 ± 12
Mn	95 ± 5	99 ± 7
Fe	98 ± 7	104 ± 8
Ni	105 ± 11	99 ± 20
Co	65 ± 5	67 ± 9
Pb	94 ± 5	17–88

* Mean and standard deviation for 4 DMW samples and 10 sea-water samples (sample preconcentration 50–100-fold).

Table 3. Analysis of sea-water sample 1

Element	Concentration, *ng/ml* C_{18} concentration*	Accepted value†
Cd	0.030 ± 0.002	0.027 ± 0.003
Zn	0.30 ± 0.07	0.41 ± 0.05
Cu	0.97 ± 0.02	0.96 ± 0.04
Mn	0.78 ± 0.02	0.68 ± 0.05
Fe	0.9 ± 0.1	1.03 ± 0.04
Ni	0.26 ± 0.01	0.31 ± 0.04
Co	0.019§	0.015 ± 0.007

* Mean and standard deviation for triplicate analyses.
† Values accepted by this laboratory following extensive analysis by several methods.[6]
§ Single determination.

Table 4. Analysis of sea-water sample 2

Element	Concentration, *ng/ml* C_{18} concentration*	Accepted value†
Cd	0.031 ± 0.002	0.025 ± 0.001
Zn	0.4 ± 0.1	0.28 ± 0.01
Cu	0.11 ± 0.02	0.13 ± 0.01
Mn	0.98 ± 0.05	1.06 ± 0.02
Fe	7.7 ± 0.5	6.9 ± 0.2
Ni	0.46 ± 0.06	0.39 ± 0.01
Co	0.017 ± 0.001	0.017 ± 0.002

*† See footnotes to Table 3.

acidified to pH 1.6 with nitric acid for storage purposes.

Each sample was analysed in triplicate following concentration (50-fold) from 500-ml portions of sea-water. As a result of the lower blanks obtained by direct analysis of the methanolic concentrates (Table 1), all analyses were completed in this manner. Calibration against metal standards in 1% nitric acid or in 1% v/v nitric acid in 50% aqueous methanol was not successful, as the presence of oxine in the solution (0.3 mg/ml in the methanolic concentrates) produced significant positive interferences (signal enhancements of up to 100%). Calibration was achieved by spiking a sample of the concentrate with the element of interest, thereby obtaining an exact matrix match (standard addition method).

Apart from the oxine, no other matrix constituents were present. Rejection of calcium and magnesium was excellent, there being less than 1 μg/ml (Ca) and approximately 20 μg/ml (Mg) in the concentrates.

In calculation of the results account was taken of the 65% recovery of cobalt. For all other elements, quantitative recovery was assumed (Table 2).

Data obtained by using the C_{18} concentration procedure are compared with "accepted" values for these samples (Tables 3 and 4). The "accepted" values were obtained by exhaustive analysis by several different techniques,[6] including isotope-dilution spark-source mass spectrometry[26] and are believed to be quite reliable.

Application of the *t*-test (at the 95% confidence level) shows no significant difference between the results obtained by the C_{18} adsorption procedure and the accepted values.

The precision of analysis averages 10% RSD (range of RSD being 4–25%) for the two samples and is generally worse for those elements for which blanks were relatively large (zinc and copper).

CONCLUSIONS

The ability of 8-hydroxyquinoline to form chelates with a large number of elements, coupled with their non-selective adsorption onto C_{18}-bonded silica gel, provides a versatile, multielement concentration technique for trace metals in sea-water.

The C_{18}-bonded gel may be reused repeatedly under the conditions cited in these experiments, thereby maintaining low operating costs. Additionally, all reagents are easily purified. The relatively fast sample processing (up to 35 ml/min) and minimum of sample manipulation permit the rapid attainment of high concentration factors,[15] contributing to its potential use as an on-site preconcentration procedure.

Blank levels ultimately determine practical detection limits, and these can potentially be lowered by synthesis of a C_{18}-bonded phase with high-purity silica gel as the substrate, and perhaps by operating the column at lower pH (to minimize hydrolysis of the silica).

RPLC with C_{18}-bonded silica gel may also prove very useful for preconcentrating trace organics from sea-water, including that portion binding trace metals, thereby permitting speciation studies. A recent study by Mills and Quinn attests to the feasibility of this application.[22]

Future application of liquid column chromatography to the concentration of trace elements from sea-water could prove more rewarding if the chelation were performed within a column bed containing immobilized 8-hydroxyquinoline. Work is in progress in this direction.

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ION-EXCHANGER COLORIMETRY—VIII

MICRODETERMINATION OF COPPER IN NATURAL WATERS

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Summary—A microdetermination method for copper by ion-exchanger colorimetry has been developed. The porphyrin α,β,γ,δ-tetrakis(4-*N*-methylpyridyl)porphine was used as the colour reagent. After the complexation at pH 4.5 and 40°, the surplus ligand was converted into the protonated species by addition of sulphuric acid. The complex and the ligand were easily sorbed on the cation-exchange resin, Dowex 50W-X2-H^+ (100–200 mesh). The resin-phase absorbances of the complex species at 424 nm and of the resin background at 510 nm were used for the measurements. There were no interferences by the foreign ions expected to be present in natural waters, at up to 1000 times the concentration of copper. With a 1-litre sample the detection limit is 0.072 μg/l. It is possible to determine copper at μg/l. or lower levels in natural waters.

Porphyrins have high sensitivity for some transition metals and have been employed for their direct determination at μg/l. levels. An absorption band at about 400 nm, called the Soret band, is used for the analysis.[1,2] The molar absorptivity of the Soret band is of the order of 10^5 l.mole^{-1}.cm^{-1}. The method for determination of copper with a porphyrin which has pyridinium groups, α,β,γ,δ-tetrakis(4-*N*-methylpyridyl)porphine [T(4-MPy)P], has been reported by Ishii and Koh.[3] The complexation of T(4-MPy)P with copper(II) is accelerated remarkably by a small amount of a reducing agent such as hydroxylamine, which overcomes the general low reactivity of porphyrins with metal ions. After the complexation is complete, the spectral absorption of the ligand at the Soret band can be eliminated by addition of acid. This method has high selectivity for copper(II).

We have combined use of this highly sensitive reagent with ion-exchanger colorimetry, based on direct measurement of the resin-phase absorbance after sorption of the coloured complex,[4,5] for determination of copper(II) in natural waters at μg/l. or lower levels.

EXPERIMENTAL

Reagents

All chemicals used were of analytical grade and demineralized water was used.

Standard copper(II) stock solution (1000 ppm). Prepared by dissolving 2.68 g of cupric chloride dihydrate in 10 ml of concentrated hydrochloric acid and diluting to 1 litre with water. The concentration was standardized by EDTA titration with PAN as indicator.

T(4-MPy)P stock solution (10^{-4}M). Prepared by dissolving 0.0319 g of T(4-MPy)P tetra-*p*-toluenesulphonate (Dojin Pharmaceutical Laboratories) in water and diluting to 250 ml. A working solution was freshly prepared by 10-fold dilution.

Dowex 50W-X2-H^+ (100–200 mesh). Conditioned, air-dried, and stored in a polyethylene container.

Apparatus

A Hitachi recording spectrophotometer (Model EPS-2U) was used for measurements of absorption spectra both in the solution and resin phases. The absorbance measurements were made with air as reference, with a Hirama single-beam spectrophotometer, Model 6B, which can measure absorbance in the range 0–2.5.

Procedure for the determination of copper

To a nearly neutral 200-ml sample containing 0.1–1.2 μg of copper were added 10 ml of 0.5% hydroxylamine hydrochloride solution, 2 ml of 5*M* acetate buffer (pH 4.5), and 4 ml of 1×10^{-5}*M* T(4-MPy)P. The solution was stood for about 5 min at 40°, then 30 ml of sulphuric acid (1 + 1) and 0.50 g of Dowex 50W-X2 resin were added. The mixture was stirred for 30 min, the coloured resin collected and the resin slurry transferred to a 1-mm quartz cell. The absorbances at 424 and 510 nm were measured.

For the determination of lower levels of copper in water, a 1-litre water sample can be used. To a nearly neutral 1-litre sample containing 0.1–1.2 μg of copper, five times the amounts of the reagents given above were added, except for the colour reagent and the resin, the amounts of which were the same as for a 200-ml sample.

When natural waters were sampled, 10 ml of concentrated hydrochloric acid were added per litre of sample immediately after filtration of the sample through a Whatman glass fibre filter, GF/D. The sample solutions were stored in polyethylene containers.

Absorbance measurements

The net absorbance of the copper(II)–T(4-MPy)P complex sorbed on the resin, at the maximum absorption wavelength (424 nm), $A_{RC(424)}$, is obtained from the equations

$$A_{RC(424)} = A_{(424)} - A_{(510)} - A_R^* \quad \text{(for sample)}$$

$$A_R^* = A_{(424)} - A_{(510)} \quad \text{(for blank)}$$

where A is the overall absorbance at the wavelength indicated by subscript and A_R^* is the difference between the resin background absorbances at 424 and 510 nm.

RESULTS AND DISCUSSION

Absorption spectra in the resin phase

T(4-MPy)P instantaneously forms a 1:1 complex with copper at pH 4.5 in the presence of hydroxylamine.[3] Once formed, the complex is very stable and is not decomposed by the addition of strong acids. At pH above 4.5, both the complex and the reagent have a charge of 4+ and show the Soret band at almost the same wavelength (426 nm). When enough sulphuric acid is added to the solution after the complex formation, the free reagent is doubly protonated to form a species with a charge of 6+ because $pKa_1 = 0.8$ and $pKa_2 = 2.06$ for the protonated species;[6] the absorption maximum of the reagent Soret band then shows a 20-nm shift to longer wavelength. We believe that the function of the hydroxylamine is to reduce the copper(II) to copper(I) which is then complexed rapidly and oxidized back to copper(II) in the complex. Cation-exchange resin can easily sorb the copper complex as well as the free ligand, because of their high positive charges. The sorbed complexes give absorption spectra similar to those for aqueous solution. As shown in Fig. 1, the separation of the Soret band of these two species in the resin phase (30 nm) is better than that for solution. The larger separation of the electronic states may be caused by interaction between these species and the resin skeleton. In the conventional solution method,[3] the decrease in absorbance of the free ligand at 446 nm is measured, because of its high sensitivity; the apparent molar absorptivity is 2.46×10^5 l.mole^{-1}.cm^{-1}. In ion-exchanger colorimetry, however, the utilization of the absorption band of the free ligand is undesirable from the point of reproducibility. The absorbance at the absorption maximum of the complex (424 nm) and that in a range where only the resin absorbs light (510 nm), are used.

Optimization of conditions

T(4-MPy)P concentration. The contribution of the absorbance of the protonated free ligand at 424 nm cannot be neglected. Excess of the reagent makes the background absorbance (A_R^*) large. The addition of 4

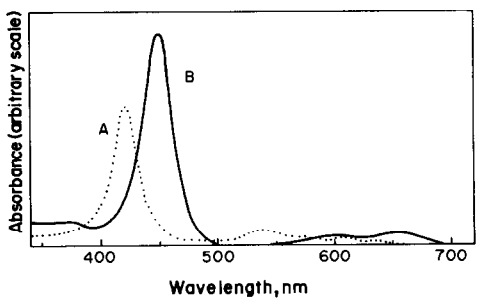

Fig. 1. Absorption spectra of T(4-MPy)P and its copper(II) complex in the cation-exchange resin phase. Resin Dowex 50W-X2-H$^+$, 100–200 mesh. Cell path-length 1 mm. A: Cu(II)–T(4-MPy)P complex; B: T(4-MPy)P.

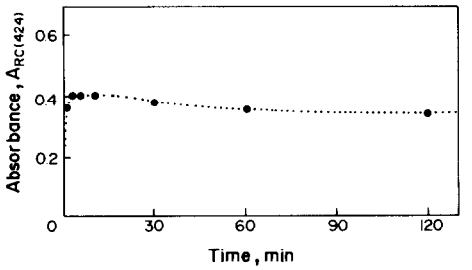

Fig. 2. Standing time (at 40°) for complex formation. Solution 200 ml, 5-μg/l Cu(II). Resin Dowex 50W-X2-H$^+$, 100–200 mesh, 0.50 g.

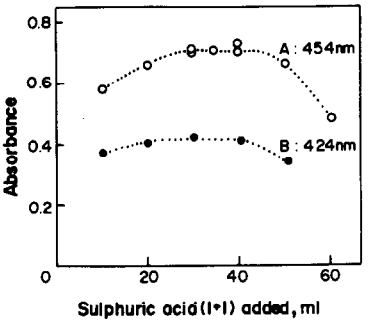

Fig. 3. Effect of concentration of acid on colour development. Solution 200 ml + 2 ml of $1 \times 10^{-5}M$ T(4-MPy)P; A: no Cu(II); B: 0.5 mg/l Cu(II). Resin Dowex 50W-X2-H$^+$, 100–200 mesh 0.50 g. Stirring time 30 min. Temperature 40°.

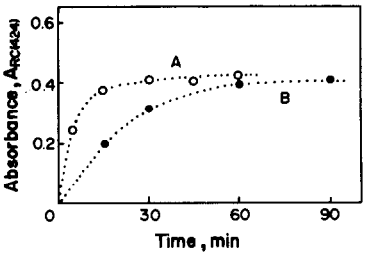

Fig. 4. Time dependence of colour development. Solution A: 200 ml, 5-μg/l Cu(II). B: 1 litre, 1-μg/l Cu(II). Resin Dowex 50W-X2-H$^+$, 100–200 mesh, 0.50 g. Temperature 40°.

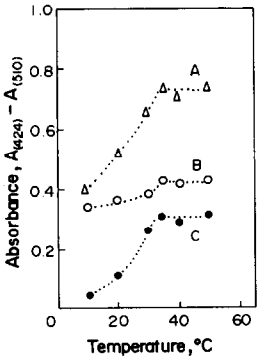

Fig. 5. Effect of temperature on colour development. Solution 200 ml, 4-μg/l Cu(II). Resin Dowex 50W-X2-H$^+$, 100–200 mesh, 0.50 g. Stirring time 30 min. A: Total absorbance; B: blank; C: net absorbance of the complex.

Table 1. Reproducibility of measurements, and detection limit

Sample volume, *ml*	Cu added, *μg*	*n*	$A_{RC(424)}$	A_R^*†	Detection limit, *μg/l.*
200	0	10		0.277 ± 0.0088	0.44
200	1	5	0.400 ± 0.014		
1000	0	5		0.301 ± 0.0056	0.072
1000	0.5	5	0.162 ± 0.0098		

† $A_R^* = A_{(424)} - A_{(510)}$ (for blank).

ml of $1 \times 10^{-5} M$ T(4-MPy)P solution is adequate for 1.2 μg of copper and 0.50 g of the resin.

pH and hydroxylamine concentration. On the basis of the results obtained by Ishii and Koh,[3] the pH of the solution was maintained at 4.5 with acetate buffer during the complex formation, and 0.5 ml of 2.5% hydroxylamine hydrochloride was added per 100 ml of sample solution.

Time for complex formation. At 40° the complex was formed completely in 2 min. Keeping at this temperature for more than 15 min lowered the absorbance of the complex because of adsorption on the vessel wall (Fig. 2).

Concentration of sulphuric acid. After the complex formation, the free ligand should be protonated by addition of sulphuric acid. With increasing amounts of sulphuric acid added to a solution containing only the ligand, the absorbance at 454 nm first increased and then (because of competition between protons and protonated ligand for sorption on the resin) decreased (Fig. 3). When sulphuric acid was added to a solution containing only the complex, the absorbance at 424 nm showed similar behaviour (Fig. 3). The addition of acid prevents adsorption of the complex on the surface of the glassware. In the standard procedure, 15 ml of sulphuric acid (1 + 1) were added per 100 ml of sample solution.

Stirring time. The effect of the stirring time on sorption of the complex species on the resin is shown in Fig. 4. The colour development was almost complete within 20 min for 200-ml water samples. As the ligand is a macromolecule (effective cross-sectional area 1 nm^2),[7] the rate of diffusion into the resin may be fairly slow. The stirring time for 200-ml water samples was fixed at 30 min.

It took longer for 1-litre water samples to complete the colour development (over 1 hr). The stirring time was fixed at 30 min with a view to more rapid determination of copper, but the calibration curve is still linear. If higher sensitivity is necessary, stirring for 60 min should be selected.

Effect of temperature. The absorbance was independent of the temperature used if this was higher than 35°. With use of temperatures lower than 35°, the absorbance was lower (for fixed stirring time), because of the lower rate of sorption of the complex on the resin (Fig. 5).

Table 2. Effects of foreign ions on the determination of copper*

Ion	Concentration, *ppm*	$A_{RC(424)}$	Relative error, %
Cu^{2+}	0.005	0.402	—
Mg^{2+}	5	0.409	+2
	50	0.395	−2
Al^{3+}	50	0.416	+3
Ca^{2+}	5	0.423	+5
	50	0.386	−4
Fe^{3+}	0.5	0.404	0
	5	0.429	+4
Mn^{2+}	0.5	0.419	+4
	5	0.387	−4
Zn^{2+}	0.5	0.402	0
	5	0.428	+6

* Sample: 200 ml. Resin: Dowex 50W-X2-H^+, 100–200 mesh, 0.50 g.

Calibration

The calibration curves were reasonably linear and could be expressed by the equations $A_{RC(424)} = 0.0800C$ for 200-ml samples, and $A_{RC(424)} = 0.313C$ for 1-litre samples in the linear ranges, where C is the concentration of copper in the sample solution, in μg/l. The sensitivity for 1-litre samples is about 4 times that for 200-ml samples, and about 80 times that for the conventional method.[3] Different lots of Dowex 50W-X2 resin gave different sensitivity and therefore the calibration equations must be checked for the reagents (and instrumentation) used.

Precision and detection limit

Precision was measured with 200-ml sample solutions containing 1.0 μg of copper, and 1-litre sample containing 0.50 μg of copper. For 5 determinations, the relative standard deviations were 3.5 and 6.0%, respectively (Table 1).

The relative detection limits, defined in the previous paper,[5] are also shown in Table 1.

Effects of foreign ions

In Table 2, the effects of various foreign ions are listed. Almost all those tested did not cause more than 5% error. The absorbance decreased when the concentration of sodium chloride was higher than 3%. The sorption of the complex species on the resin may decrease by competition between sodium ions and the sample species. When copper in sea-water is deter-

Table 3. Determination of copper in natural waters*

Sea-water: Shikanoshima, Fukuoka					
Cu added, μg	0	0.10	0.20	0.30	0.40
$A_{RC(424)}$	0.065	0.094	0.125	0.141	0.197
Cu found, μg	0.20	0.29	0.38	0.43	0.60
River water: Umi River, Fukuoka					
Cu added, μg	0	0.25	0.50	0.75	
$A_{RC(424)}$	0.135	0.213	0.283	0.359	
Cu found, μg	0.48	0.74	0.98	1.25	

* Sample: 1 litre. Resin: Dowex 50W-X2-H^+, 100–200 mesh, 0.50 g.

mined, the calibration curve should be prepared with solutions of the same saline concentration as the sample, or the standard-addition method should be used.

Determination of copper in sea-water and river water

The method was applied to the determination of copper in natural waters. The results are shown in Table 3. For sea-water, the calibration curve was prepared by using sample solution from which trace elements had been stripped by passage of the sample through a column of chelating resin.[8] Recovery of the added copper was almost complete. The concentration of copper found was 0.18 ± 0.03 μg/l. For the surface sea-waters in the western area of the North Pacific Ocean the average content of total soluble copper is 0.64 ± 0.21 μg/l. and more than 70% of this is bound to organic matter. These organometallic compounds are not easily decomposed when samples are stored under acidic conditions.[9,10] Hence the present method can be used for determining the concentration of soluble copper in inorganic forms in sea-water. Recovery was almost complete for the river water. The concentration of copper found was 0.49 ± 0.01 μg/l.

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STUDIES ON CHELATING RESINS—I

GENERAL EQUATION FOR THE CALCULATION OF THE PROTONATION CONSTANTS OF CHELATING RESINS

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Summary—A general equation has been derived which describes the protonation equilibria of basic groups attached to an insoluble polymer matrix. The derivation is based on the application of the charge and mass-balance equations. When the relationship obtained is applied to water-soluble ligands, it reduces to the well-known Schwarzenbach equation for titration of polybasic acids.

For titration of a fully protonated N-basic acid with a strong base, the equation given by Schwarzenbach[1] can be written in the following form,† for constant ionic strength, I:

$$\sum_{j=0}^{N} (\alpha^* - N + j)\beta'_j[H^+]^j = 0 \qquad (1)$$

where

$$\alpha^* = \alpha + \frac{[H^+] - [OH^-]}{C_L}$$

$$\alpha = \frac{[\text{strong base}]}{C_L}$$

C_L is the molal total analytical concentration of all forms of the N-basic acid in the equilibrium solution, and

$$\beta'_j = \Pi K'_j$$

where K'_j is the jth protonation constant at ionic strength I:

$$K'_j = \frac{[H_jL]}{[H_{j-1}L][H^+]}$$

If L is the anion of a monobasic acid ($N = 1$) and $C_L \geqslant 10^{-3} m$, then when the pH is between 5 and 9 equation (1) reduces to:

$$(\alpha - 1) + K'\alpha[H^+] = 0 \qquad (2)$$

or

$$\log K' = \text{pH} + \log \frac{1 - \alpha}{\alpha} \qquad (3)$$

This equation does not apply, however, to polyelectrolytes or to conjugate bases fixed to insoluble matrices. When equation (3) is used for evaluation of the titration curves of such ligands, the calculated protonation constant vary significantly with the degree of titration as well as with the ionic strength of the solution.[2,3]

Several attempts have been made to extend the validity of this equation to polyelectrolyte systems. These approaches fall into two classes. Those of the first type are empirical, utilizing several arbitrary constants,[4] and the second class comprises those which are based on thermodynamic principles, such as the equation derived by Chatterjee and Marinsky,[5] which, however, only describes single-step protonation reactions.

We present here a general equation for resin protonation equilibria, which can also be applied to overlapping protonation processes.

THEORY

In our discussion the protonation will be treated as a heterogeneous chemical reaction between the components of two phases in contact: the solution phase and the resin phase.

The components of the solution phase are water molecules, protons and their counter-ions, and the electrolyte, $G^+ G^-$, used to adjust the ionic strength.

The resin phase is considered as a concentrated aqueous solution of electrolytes in which the concentrations of the components are experimentally accessible quantities.

The resin phase consists of the following components.

(1) Basic groups.
(2) Conjugate acid groups formed in the process of protonation.
(3) Counter-ions.
(4) Water molecules.

† Schwarzenbach's symbols have been replaced as follows (his symbols are given first), $m = N$, $a = \alpha$, $a^* = \alpha^*$, $Z_t = C_L$) $\bar{K}^H_{Hjz} = \beta'_j$, and his equation has been multiplied by -1. Molal concentrations are used here because the system is heterogeneous.

The resin phase can be separated from the solution phase by centrifugation.

In course of the protonation the total mass of the basic and conjugate acid groups remains the same, but their proportion as well as the quantity of the counter-ions and the water will change.

Molal concentrations will be used for components (1)–(3), the solvent medium being the water in the resin phase.

The protonation equilibria

For aqueous solutions the protonation reaction can be written as:

$$L^{n-} + H^+ = HL^{(n-1)-} \tag{4}$$

where L represents a base and HL its conjugated acid.

The protonation constant is defined as the thermodynamic equilibrium constant for this reaction where a represents activity:

$$K = \frac{a_{HL}}{a_L a_{H^+}} \tag{5}$$

According to the principles of electroneutrality an equivalent amount of counter-ions G^+ or G^- should also be present in the system, according to whether L is an anion, a neutral species or a cation, and under certain circumstances must be taken into account in the equilibria.

Protonation in a homogeneous system. When the base and the protons are present in the same phase, then equation (5) will apply irrespective of whether the base is L^-, L or L^+, since the counter-ions are not involved in the equilibrium, and simply cancel if included in the equation.

Protonation in a heterogeneous system. If the base and the protons are present in different phases, then the protonation is a heterogeneous reaction and the principles of electroneutrality apply to both phases.

Suppose the base is attached to an insoluble matrix (*e.g.*, is in a resin phase) and the protons are in the solution phase. We will denote resin-phase components by bars.

Consider first the protonation of a negatively charged base, $\overline{L}^-$, taking the counter-ions into account:

$$\overline{L}^- + \overline{G}^+ + H^+ + G^- = \overline{HL} + G^+ + G^- \tag{6}$$

The protonation constant is:

$$\overline{K}_{L^-} = \frac{\overline{a}_{HL} a_{G^+}}{\overline{a}_{L^-} \overline{a}_{G^+} a_{H^+}} \tag{7}$$

If the base is neutral, then the reaction is:

$$\overline{L} + H^+ + G^- = \overline{HL}^+ + \overline{G}^- \tag{8}$$

and the protonation constant is:

$$\overline{K}_L = \frac{\overline{a}_{HL^+} \overline{a}_{G^-}}{\overline{a}_L a_{G^-} a_{H^+}} \tag{9}$$

Finally, if the base has a positive charge:

$$\overline{L}^+ + \overline{G}^- + H^+ + G^- = \overline{HL}^{2+} + 2\overline{G}^- \tag{10}$$

and the protonation constant is

$$\overline{K}_{L^+} = \frac{\overline{a}_{HL^{2+}} \overline{a}_{G^-}}{\overline{a}_{L^+} a_{G^-} a_{H^+}} \tag{11}$$

Activity of the hydrogen ions in the resin phase

Consider the protonation process as a homogeneous reaction between the components of the resin phase:

$$\overline{L} + \overline{H}^+ = \overline{HL} \tag{12}$$

As mentioned earlier, if the protonation reaction takes place in a homogeneous system, then the counter-ions cancel in the equilibrium expression, so in this case

$$\overline{K}_L = \frac{\overline{a}_{HL}}{\overline{a}_L \overline{a}_{H^+}} \tag{13}$$

From a comparison of equations (7), (9) and (13), the activity of the protons in the resin phase can be expressed by

$$\overline{a}_{H^+} = a_{H^+} \left(\frac{\overline{a}_G}{a_G} \right)^{\pm 1} \tag{14}$$

where the sign of the exponent is positive when the counter-ion is a univalent cation, and negative when the counter-ion is a univalent anion.

Equation (14) can also be obtained if it is supposed that the protons in reaction (6) originate from the solution phase, in which case the process proceeds by transfer of protons from the solution phase into the resin phase:

$$H^+ \rightarrow \overline{H}^+$$

To preserve electroneutrality, however, this proton transfer should be accompanied by transfer of the counter-cations of the base anion, from the resin into the solution phase ($\overline{G}^+ \rightarrow \overline{G}^-$), or transfer of the counter-anions of the protons from the solution into the resin phase ($G^- \rightarrow \overline{G}^-$).

The overall processes can therefore be written as:

$$H^+ + \overline{G}^+ \rightleftharpoons \overline{H}^+ + G^+$$

$$K = \frac{\overline{a}_{H^+} \cdot a_{G^+}}{a_H \cdot \overline{a}_{G^+}}$$

and

$$H^+ + G^- \rightleftharpoons \overline{H}^+ + \overline{G}^-$$

$$K = \frac{\overline{a}_{H^+} \overline{a}_{G^-}}{a_{H^+} a_{G^-}}$$

If the standard chemical potential of the electrolyte components in the resin and the solution phases are

defined in exactly the same way, the thermodynamic equilibrium constants for both reactions above should be equal to unity, and the activity of the protons in the resin phase is then given by equation (14).

Calculation of the protonation constant for single-step protonation processes

From equations (13) and (14)

$$\log \bar{K}_L = \mathrm{pH} \pm \log \frac{a_G}{\bar{a}_G} + \log \frac{\bar{a}_{HL}}{\bar{a}_L} \qquad (15)$$

where the $\pm$ sign has the same meaning as in equation (14).

From the definition of activity, equation (15) can be written as

$$\log \bar{K}_L = \mathrm{pH} \pm \log \frac{a_G}{(G)} + \log \frac{(HL)}{(L)} + \log \frac{\bar{\gamma}_{HL}}{\bar{\gamma}_L(\bar{\gamma}_G)^{\pm 1}} \qquad (16)$$

where (L), (HL) and (G) are the concentrations in the resin in terms of mole per kg of water in the resin phase, and $\bar{\gamma}_L$, $\bar{\gamma}_{HL}$ and $\bar{\gamma}_G$ are the respective activity coefficients. These latter, experimentally non-accessible quantities, can be lumped in a $\bar{K}_\gamma$ term:

$$\bar{K}_\gamma = \frac{\bar{\gamma}_{HL}}{\bar{\gamma}_L(\bar{\gamma}_G)^{\pm 1}} \qquad (17)$$

$\bar{K}_\gamma$ can be considered as a measure of the strength of the interactions in the resin phase. The directly measurable and calculable quantities in equation (16) are the following.

(1) The pH of the equilibrium solution phase.

(2) The activity of the counter-ions in the solution phase, a_G.

(3) (G), the molality of the counter-ions in the resin phase. This term should be considered as the sum of the chemically bound and invasive amounts of the counter-ions, divided by the water content of the resin phase:

$$(G) = \frac{\bar{G}_K + \bar{G}_I}{\overline{H_2O}} \qquad (18)$$

where $\bar{G}_K$ is the amount of chemically bound counter-ions, mmole per g of dry resin; $\bar{G}_I$ is the amount of the electrolyte (in the resin phase) which has migrated from the equilibrium solution phase by the so-called electrolyte invasion process, mmole per g of dry resin, and $\overline{H_2O}$ is the water content of the resin, g per g of dry resin.

(4) The proton activity in the resin phase, $\bar{a}_H$, cannot be measured directly, nor calculated from experimentally accessible quantities, because $\bar{\gamma}_G$, the activity coefficient of the counter-ions, is not susceptible to direct measurement, but if $\bar{\gamma}_G$ is lumped into the $\bar{K}_\gamma$ term (H^+) can be redefined as a mixed proton activity in the resin phase, which can be calculated from the measurable quantities given above, as follows:

$$(H^+) = a_H \left(\frac{(G)}{a_G}\right)^{\pm 1} \qquad (19)$$

In what follows, this (H^+), defined by equation (19) will be called the calculated proton activity in the resin phase, and will also be used to express the concentration of the protons in the resin phase (as is commonly done in the treatments of complex equilibria, where $[H^+]$ is used in the sense of a_H) in the charge-balance equations.

Then $\overline{pH}$ for the resin phase can be written as:

$$\overline{pH} = \mathrm{pH} \pm \log \frac{a_G}{(G)} \qquad (20)$$

where $\mathrm{pH} = -\log a_H$.

In equations (19) and (20) the same remarks apply for the $\pm$ sign as for equation (14).

(5) (HL)/(L), the concentration ratio of the protonated and non-protonated forms in the resin phase, can be calculated from charge and mass-balance equations, as follows.

(*a*) If the base is an anion, the charge-balance equation is

$$(L^-) = \frac{\bar{G}_K^+}{\overline{H_2O}} + (H^+) - (OH^-) \qquad (21)$$

where (OH^-) is the activity of the hydroxide ions in the resin phase, calculated from the ionic product of water.

The mass-balance equation, which gives $\bar{C}_L$, the analytical concentration of the functional groups in the resin phase, is

$$\bar{C}_L = \frac{Q}{\overline{H_2O}} = (L^-) + (HL) \qquad (22)$$

where Q is the experimentally measurable capacity of the resin, mmole of functional group per g of dry resin, in uncharged (hydrogen) from.

With the help of the charge and mass-balance equations the mole fraction of the non-protonated base in the resin phase can be expressed as:

$$\bar{\alpha}^* = \frac{(L^-)}{\bar{C}_L} = \frac{\bar{G}_K^+}{Q} + \frac{(H^+) - (OH^-)}{\bar{C}_L} \qquad (23)$$

From equations (22) and (23):

$$1 - \bar{\alpha}^* = \frac{(HL)}{\bar{C}_L} \qquad (24)$$

and from equations (23) and (24) the sought-for ratio is

$$\frac{(HL)}{(L)} = \frac{1 - \bar{\alpha}^*}{\bar{\alpha}^*} \qquad (25)$$

(*b*) If the base is uncharged, then the charge and

mass-balance equations are

$$(HL^+) = \frac{\overline{G}_K^-}{\overline{H_2O}} + (OH^-) - (H^+) \quad (26)$$

$$\bar{C}_L = (L) + (HL^+) = \frac{Q}{\overline{H_2O}} \quad (27)$$

and the mole fraction of the non-protonated base is

$$\bar{\alpha}^* = \frac{(L)}{\bar{C}_L} = 1 - \frac{\overline{G}_K^-}{Q} + \frac{(H^+) - (OH^-)}{\bar{C}_L} \quad (28)$$

From equations (21)–(23) and (26)–(28) the logarithm of the protonation constant for single-step processes is

$$\log K = \overline{pH} + \log \frac{1 - \bar{\alpha}^*}{\bar{\alpha}} + \log \bar{K}_\gamma \quad (29)$$

For water-soluble monomers equation (29) reduces to equation (3), *i.e.*, the following identities exist:

$$pH = \overline{pH},$$
$$\bar{\alpha}^* = \alpha, \quad (I = \text{const.})$$
$$\bar{K}_\gamma = 1.$$

General equation for description of the protonation equilibria

For the derivation of a general equation the concepts defined by equations (17)–(19) will be used.

(*a*) Let the non-protonated base be an anion, $\overline{L}^{N-}$. The first step of protonation is

$$\overline{L}^{N-} + N\overline{G}^+ + H^+ = \overline{HL}^{(N-1)-} + (N-1)\overline{G}^+ + G^+$$

and the protonation constant

$$K'_1 = \frac{K_1}{\bar{K}_{1\gamma}} = \frac{(HL)a_{G^+}}{(L)a_H(G^+)} = \frac{(HL)}{(L)(H^+)}$$

For the second and further steps of protonation we obtain

$$K'_2 = \frac{(H_2L)}{(HL)(H^+)},$$
$$\vdots \qquad \vdots$$
$$K'_j = \frac{(H_jL)}{(H_{j-1}L)(H^+)}, \quad (j = 1, 2 \ldots N)$$

According to the principles of electroneutrality:

$$\frac{\overline{G}_K^+}{\overline{H_2O}} + (H^+) - (OH^-) = N(L^{N-}) + (N-1)(HL^{(N-1)}) + \ldots (N-j)(H_jL^{(N-j)-}) + \ldots = (L)\sum_{j=0}^{N}(N-j)\beta'_j(H^+)^j \quad (30)$$

where β'_j is the overall protonation constant:

$$\beta'_j = K'_1K'_2 \ldots K'_j$$

and, from equation (19),

$$(H^+) = a_H \frac{(G^+)}{a_{G^+}}$$

According to the mass-balance equation:

$$\bar{C}_L = \frac{Q}{\overline{H_2O}} = (L) + (HL) + \ldots + (H_NL) = (L)\sum_{j=0}^{N}\beta'_j(H^+)^j \quad (31)$$

From equations (30) and (31):

$$\bar{\alpha}^* = \frac{\overline{G}_K^+}{\overline{H_2O}} + \frac{(H^+) - (OH^-)}{\bar{C}_L} = \frac{\sum_{j=0}^{N}(N-j)\beta'_j(H^+)^j}{\sum_{j=0}^{N}\beta'_j(H^+)^j} \quad (32)$$

(*b*) If the base is not negatively charged, then again using the charge and mass-balance equations, for $(N - \bar{\alpha}^*)$ we obtain:

$$(N - \bar{\alpha}^*) = \frac{\overline{G}_K^-}{Q} - \frac{(H^+) - (OH^-)}{\bar{C}_L} = \frac{\sum_{j=0}^{N} j\beta'_j(H^+)^j}{\sum_{j=0}^{N}\beta'_j(H^+)^j} \quad (33)$$

where

$$(H^+) = a_H \frac{a_{G^-}}{(G^-)}$$

After rearrangement of (32) or (33) the general equation describing the protonation equilibria can be written as:

$$\sum_{j=0}^{N}(\bar{\alpha}^* - N + j)\beta'_j(H^+)^j = 0 \quad (34)$$

This equation applies when the fully protonated base is an N-basic acid and is titrated with a strong base.

For water-soluble monomers, equation (34) reduces to the Schwarzenbach relationship, equation (1). In this case the following identities exist:

$$(H^+) = a_H$$
$$\bar{\alpha}^* = \alpha^* \quad (I = \text{const.})$$
$$\bar{K}_\gamma = 1.$$

We reported earlier the preparation of chelating resins containing various polyethylene–polyamino

and polyethylene–polyamino–polycarboxylic acid functional groups.[6] Equation (34) has been used to calculate the protonation constants of such chelating resins.[7] In the next paper[8] further applications of the general equation will be shown.

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STUDIES ON CHELATING RESINS—II

DETERMINATION OF THE PROTONATION CONSTANTS OF A CHELATING RESIN CONTAINING IMINODIACETIC ACID GROUPS

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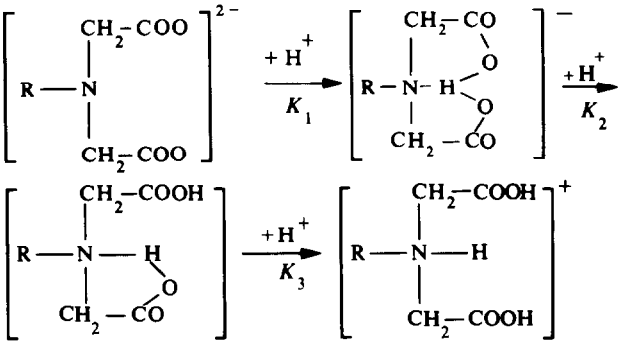
Summary—A previously derived equation for the calculation of the protonation constants of immobilized ligands is used for description of the protonation equilibria of a chelating resin containing iminodiacetic acid groups. The following values were obtained for the three steps of protonation: $\log K_1 = 9.12 \pm 0.05$; $\log K_2 = 3.10 \pm 0.07$; $\log K_3 = 1.44 \pm 0.03$.

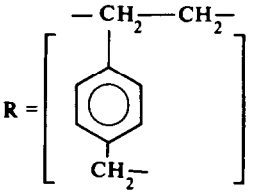
Chelating resins containing iminodiacetate groups are commercially produced in various purities by several firms under various trade names (Dowex A-1; Bio-Rad Chelex 100; Permutit S 1005). These products are widely used for the enrichment and separation of traces of heavy metals.[1,2] Few papers have been published, however, that deal with the quantitative description of the protonation equilibria.

The iminodiacetate base can be protonated in three steps according to the following scheme:



neutral, zwitterion form

where



is the resin matrix, and K_1, K_2, K_3 are the protonation constants.

Krasner and Marinsky[3] used the following equation for calculation of the second protonation constant, K_2, of the active group of Dowex A-1:

$$\log K_2 = \mathrm{pH} + \log \frac{1-\alpha}{\alpha} + \log \frac{a_M}{\bar{a}_M} + \Delta \log K \quad (1)$$

where K_2 is the thermodynamic protonation constant, pH is the equilibrium pH of the solution corresponding to a given degree of titration, α, a_M and $\bar{a}_M$ are the activities of the counter-ions in the solution and in the resin phases respectively, and $\Delta \log K$ represents a contribution originating from the difference in free energy between the negatively charged fixed base and its water-soluble analogue.

The last term was estimated according to Katchalsky's polyelectrolyte model.[4] The polyelectrolyte model can, however, be used for the description of the protonation equilibria of polyacidic bases having only one negative charge. Consequently equation (1) cannot be applied for the description of equilibria involving more than one step.

The first two protonation constants of the ligand of Dowex A-1 were calculated by Leyden and Underwood[5] by use of equation (2):

$$\log K_a = \mathrm{pH} + \log \frac{1-\alpha}{\alpha} + \log [\mathrm{Na}^+] - \log \alpha Q \quad (2)$$

Where Q is the capacity of the resin (mmole/cm^3), and [Na^+] is the concentration of the counter-ions in the solution.

The calculated values of the apparent protonation constants, K_{a1} and K_{a2} varied continuously with the degree of dissociation.

It is worthwhile emphasizing here that both the equations above and others in the literature were used mostly for the description of the protonation of bases carrying a single negative charge.

In the preceding paper[6] the following equation was derived for the description of the protonation equilibria of an N-acidic base;

$$\sum_{j=0}^{N} (\bar{\alpha}^* - N + j)\beta'_j(H^+)^j = 0 \qquad (3)$$

In equation (3) three terms need detailed explanation: (H^+), $\bar{\alpha}^*$ and β'_j. Their interpretation is given below.

The calculated proton activity in the resin phase, (H^+), can be calculated by equation (4):

$$(H^+) = [H^+]\left(\frac{(G)}{a_G}\right)^{\pm 1} \qquad (4)$$

where $[H^+]$ and a_G are the activities of the proton and the counter-ions in the solution phase, (G) is the concentration of the counter-ions in the imbibed water of the resin phase, (in mmole/g):

$$(G) = \frac{\bar{G}_K + \bar{G}_I}{\overline{H_2O}} \qquad (5)$$

$\bar{G}_K$ being the amount of chemically bound counter-ions, in mmole per g of resin, $\bar{G}_I$ is the amount of the electrolyte invading the resin phase from the solution, (mmole per g of resin), and $\overline{H_2O}$ is the water content (g) per g of resin.

The sign of the exponent in equation (4) corresponds to the sign of the charge of the counter-ion.

The real degree of titration in the resin, $\bar{\alpha}^*$, can be calculated from the amount of the chemically bound counter-ions, $\bar{G}_K$, and the capacity of the resin, Q, with allowance for the autodissociation of the water. The definition of this term depends on the type of the base to be protonated. The following three cases can be distinguished.

I. For the protonation of an anionic base (*e.g.*, a polycarboxylate anion: $\bar{L}^{k-} \xrightarrow{kH^+} \overline{LH_k}$), $\bar{\alpha}^*$ is defined as:

$$\bar{\alpha}^* = \frac{\bar{G}_K^+}{Q} + \frac{(H^+) - (OH^-)}{Q/\overline{H_2O}} \qquad (6)$$

II. For the protonation of a neutral base (*e.g.*, a polyamine: $\bar{L} \xrightarrow{lH^+} \overline{LH}^{l+}$), $\bar{\alpha}^*$ is:

$$\bar{\alpha}^* = l - \frac{\bar{G}_K^-}{Q} + \frac{(H^+) - (OH^-)}{Q/\overline{H_2O}} \qquad (7)$$

where l is the number of positive charges when the neutral base is fully protonated.

III. If the fixed ligand is an amphoteric group (*e.g.*, a polyaminopolycarboxylate: $\bar{L}^{k-} \xrightarrow{NH^+} \overline{LH}_N^{l+}$, $N = k + l$):

$$\bar{\alpha}^* = l + \frac{\bar{G}_K^+ - \bar{G}_K^-}{Q} + \frac{(H^+) - (OH^-)}{Q/\overline{H_2O}} \qquad (8)$$

In equations (6)–(8) Q always denotes the capacity of the resin in mmole/g, when the active group is in neutral form, which implies that in case I the ligand is in fully protonated form, in case II the ligand is in unprotonated form, and in case III the ligand is in zwitterion form.

Case III implies, in fact, case I and case II, so equation (8) is reduced to equation (6) in case I when $l = 0$, $N = k$, $\bar{G}_K^- = 0$ and to equation (7) in case II when $k = O$, $N = l$ and $\bar{G}_K^- = 0$.

The overall protonation constant, β'_j, at the ionic strength existing in the resin phase, is given by

$$\beta'_j = \Pi K'_j \qquad (9)$$

$$K'_j = \frac{(H_jL)}{(H_{j-1}L)(H^+)} \qquad (10)$$

From the K'-values the thermodynamic protonation constants, the K-values, can be calculated as follows:

$$K = K'\bar{K}_\gamma \qquad (11)$$

where $\bar{K}_\gamma$ is a term which contains the activity coefficients of the reaction partners in the resin phase:

$$\bar{K}_\gamma = \frac{\bar{\gamma}_{LH}}{\bar{\gamma}_L(\bar{\gamma}_G)^{\pm 1}} \qquad (12)$$

$\bar{\gamma}_{HL}$, $\bar{\gamma}_L$ and $\bar{\gamma}_G$ being the activity coefficients of the conjugated acid, $\overline{HL}$, of the base, $\bar{L}$, and of the counter-ions, G, in the resin phase. For the sign of the exponent the same applies as for equation (4).

EXPERIMENTAL

The chelating resin studied is commercially available from the firm Reanal (Budapest, Hungary) under the trade name Ligandex-I. In this resin the iminodiacetate active groups are covalently bound to the lightly cross-linked (2%) polymer framework. The sample (obtained in sodium form) was converted into the neutral zwitterion form by the procedure described earlier.[7]

The water content of the air-dry resin was determined from the weight loss on drying at 105° in an argon atmosphere. For the equilibrium study of the deprotonation and protonation of the zwitterion form of the active group, the static batch method was used. Known masses of the resin samples (~0.5 g) were weighed into 100-ml glass-stoppered flasks. The ionic strength was made 1.0 with potassium chloride.

To investigate the deprotonation, increasing amounts (0–50 ml) of 0.100M potassium hydroxide were added to the samples. The ionic strength and the 100-ml final volume

were adjusted by the addition of the calculated quantity of water and 2.0*M* potassium chloride.

For study of the protonation the procedure was identical, except that the potassium hydroxide was replaced by 1.00*M* hydrochloric acid.

The systems were left to equilibrate for 7 days at 25°. The pH of the solution phase was measured with a calibrated glass electrode. During preparation of the equilibrium systems and the pH measurement a protective argon atmosphere was used.

Determination of the water content of the resin phase

After the pH measurement the resin phase was separated from the solution by centrifuging and dried to constant weight at 105° under an argon atmosphere. The water content was calculated with reference to 1 g of the neutral dry resin.

Determination of the amount of counter-ions in the resin phase

The counter-ions are the ions of the potassium chloride used for adjustment of the ionic strength. In the first two protonation steps, when the base is anionic, the counter-ion is potassium. In the third step, when the active group is positively charged the counter-ion is chloride.

After the determination of the water content of the resin, the counter-ions originating from electrolyte invasion as well as from the chemical reaction were determined simultaneously as follows.

1. In the deprotonation experiments, the potassium ions were eluted from the resin with a known amount of 0.1*M* nitric acid and collected in a standard flask. The resin was washed free of chloride with water and the solution made up to the mark. The amount of chemically bound potassium ions was obtained by titration of the acid left in the solution, and that of the invasive potassium chloride from a Mohr chloride determination.
2. In the protonation experiments the chloride counter-ions were eluted with water into a standard flask. The amount of the chemically bound counter-ions was calculated from the results of titration of the acid, and the sum of the invasive and chemically bound chloride was obtained from the chloride content of aliquots.

The amounts (mmole) of the chemically bound and invasive counter-ions were calculated per g of dry neutral (zwitterion form) resin.

Determination of the capacity

To obtain the capacity, Q, the amount of chemically bound counter-ions was plotted against the pH of the equilibrium solution. In Fig. 1 the positive ordinate shows the amount of chemically bound potassium ions and the negative ordinate values refer to that of the chloride ions.

The active group takes up three protons in the reaction and three well-defined steps can be identified. The turnover of the potassium and chloride ions during the protonation can be estimated by drawing horizontal lines through the inflection points. In accordance with expectation the three steps in the curve are equally spaced and all give the capacity of the resin as Q = 3.0 mmole of active group per g of neutral, zwitterion form resin.

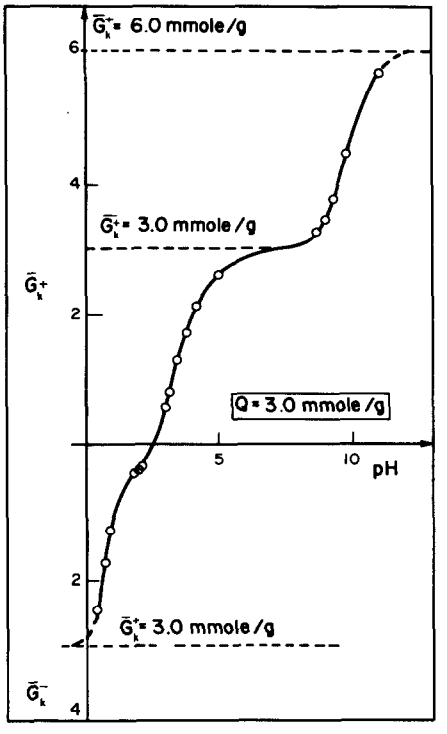


Fig. 1. The variation of the chemically bound counter-ions ($\bar{G}_K^+$ = potassium and $\bar{G}_K^-$ = chloride) as a function of the pH of the equilibrium solution. From the three horizontal inflexion lines, the capacity of the resin is Q = 3.0 mmole/g.

RESULTS AND DISCUSSION

The experimental data and some of the calculated results are given in Table 1. In the experiments 1–6 the counter-ion is chloride and in experiments 7–18 it is potassium.

Column 6 gives the molality of the counter-ions, (G), calculated from equation (5). The pH of the resin phase, $\overline{pH}$, was obtained through equation (4). The exponent is -1 in experiments 1–6 and $+1$ in experiments 7–18. Column 8 gives the real degree of titration calculated by equation (8). The last column gives the log K'-values calculated by equation (14) from each of the experiments.

In Fig. 2, the measured pH of the solution and the calculated $\overline{pH}$ of the resin are plotted against the real degree of titration, $\bar{\alpha}^*$.

When the counter-ions are positive the pH *vs.* $\bar{\alpha}^*$ curve runs above the $\overline{pH}$ *vs.* $\bar{\alpha}^*$ curve and for negative counter-ions their position is reversed. The curves intersect at the isoelectric point of the active groups, where $\bar{\alpha}^* = 1$.

As already shown, the two equations $(H^+) = [H^+]\,(G^+)/a_{G^+}$ and $(H^+) = [H^+]a_{G^-}/(G^-)$ are used for the calculation of the hydrogen-ion activity of the resin phase, for positive and negative counter-ions respectively. At the isoelectric point, of course, both equations yield the same value and give.

$$\bar{a}_{G^+}\bar{a}_{G^-} = a_{G^+}a_{G^-}$$

which is, in fact, the Donnan equation.

At the isoelectric point there is no chemically bound counter-ion in the resin phase, and the only counter-ions present are those which have migrated from the solution phase by invasion of the electrolyte (the potassium chloride). It is to be expected, there-

Table 1. The results of the protonation study of the immobilized iminodiacetate active groups

1 Expt No.	2 pH	3 $\overline{H_2O}$	4 $\bar{G}_K$	5 $\bar{G}_I$	6 (G)	7 $\overline{pH}$	8 $\bar{\alpha}^*$	9 log K'
1	0.380	1.247	2.425	0.672	2.485	0.991	0.234	1.51
2	0.660	0.879	1.703	0.576	2.592	1.289	0.447	1.38
3	0.950	0.712	1.223	0.574	2.526	1.568	0.599	1.39
4	1.860	0.638	0.376	0.614	1.550	2.266	0.876	1.42
5	1.950	0.649	0.335	0.642	1.506	2.343	0.889	1.44
6	2.010	0.654	0.293	0.664	1.463	2.391	0.903	1.42
7	2.970	1.161	0.609	1.067	1.444	2.595	1.204	3.19
8	3.120	1.398	0.830	1.212	1.460	2.740	1.277	3.16
9	3.400	1.696	1.371	1.407	1.638	2.970	1.458	3.04
10	3.700	1.934	1.745	1.520	1.689	3.257	1.582	3.11
11	4.080	2.143	2.137	1.635	1.7603	3.619	1.713	3.22
12	4.930	2.269	2.801	1.711	1.901	4.436	1.933	3.32
13	8.650	2.463	3.294	1.710	2.032	8.127	2.098	9.09
14	8.940	2.554	3.466	1.796	2.061	8.411	2.156	9.14
15	9.260	2.616	3.779	1.773	2.123	8.718	2.260	9.17
16	9.740	2.709	4.462	1.767	2.230	9.163	2.487	9.19
17	9.780	2.767	4.485	1.864	2.294	9.204	2.495	9.21
18	10.980	2.983	5.814	1.891	2.539	10.360	2.937	9.19

fore, that at this point the ionic strengths in the two equilibrium phases are equal:

$$\bar{I} = I$$

This conclusion is corroborated by Fig. 3, where the molality of the invasive electrolyte (the $\bar{G}_I/\overline{H_2O}$ ratio) is plotted against the amount of the chemically bound counter-ions (column 4 in Table 1).

With increasing amount of either chemically bound counter-ion the concentration of invasive electrolyte decreases. If the curves are extrapolated to the point where $\overline{G_K^+} = \overline{G_K^-} = 0$, then a common intercept is obtained at $(G_I) = 1.04$, which agrees with the ionic strength of the external solution. This result is also in agreement with the concept of the resin phase as a concentrated electrolyte. This concept was used at the outset of the derivation of the general equation given in Part I.[6]

Figure 4 shows the change of the water content, $\overline{H_2O}$, as a function of $\overline{pH}$ of the resin phase. From a

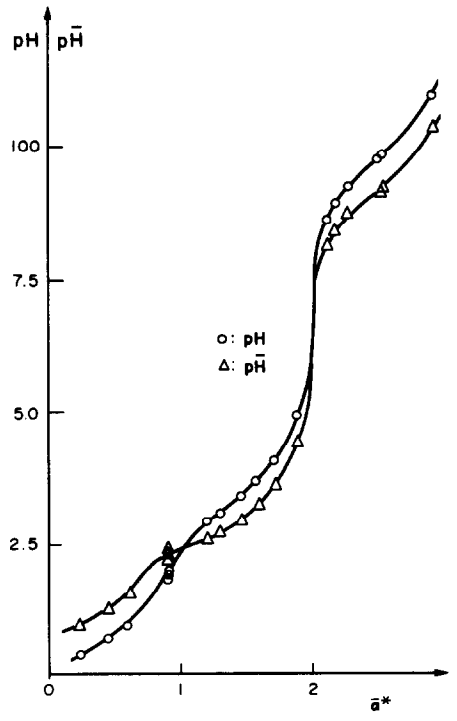


Fig. 2. Titration curves of the immobilized iminodiacetate base. ○: pH of the equilibrium solution as a function of the real degree of titration of the resin, $\bar{\alpha}^*$; △: $\overline{pH}$ of the resin phase as a function of the real degree of titration of the resin, $\bar{\alpha}^*$; [$\overline{pH} = pH \pm \log a_G/(G)$].

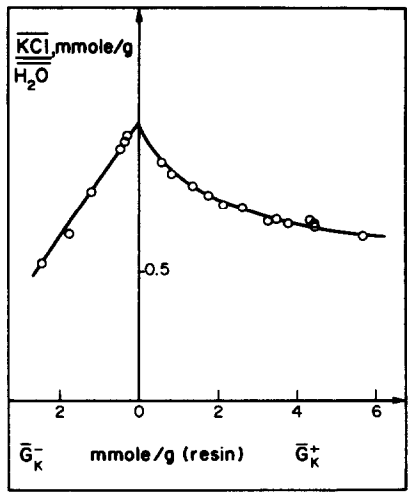


Fig. 3. The change of the invasive potassium chloride molality in the resin phase as a function of the quantity of chemically bound counter-ions; at the isoelectric point (where $\bar{G}_K^- = \bar{G}_K^+ = 0$), $\bar{I} = I$.

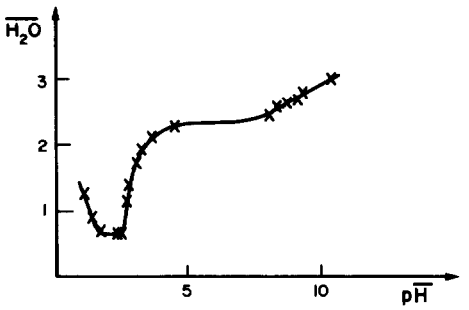


Fig. 4. Change of the water uptake of the resin, H_2O, as a function of $\overline{pH}$.

comparison of this figure with Fig. 2 it appears that the water uptake occurs in the same ranges of $\overline{pH}$ as the resin binds counter-ions chemically. This phenomenon can more directly be followed if the change of the water content is plotted as a function of the amount of the chemically bound counter-ions, as in Fig. 5.

Determination of the protonation constants

For the calculation of the log K'-values, equation (3) was applied with the corresponding data of the $\overline{pH}$ *vs.* $\bar{\alpha}^*$ function.

The summation for j in equation (3) goes from 0 to 3:

$$\bar{\alpha}^* - 3 + (\bar{\alpha}^* - 2)\beta'_1(H^+) + (\bar{\alpha}^* - 1)\beta'_2(H^+)^2 + \bar{\alpha}^*\beta'_3(H^+)^3 = 0 \qquad (13)$$

and for log K' the following values were obtained:

$$\log K'_1 = 9.12 \pm 0.05$$

$$\log K'_2 = 3.10 \pm 0.07$$

$$\log K'_3 = 1.44 \pm 0.03$$

As can be seen from Figs. 1 and 2, the consecutive steps of protonation are well separated and can be considered as individual steps, for which equation (3) takes the following simpler form:

$$\log K'_j = \overline{pH} + \log \frac{4 - \bar{\alpha}^* - j}{\bar{\alpha}^* - 3 + j} \quad (j = 1,2,3) \qquad (14)$$

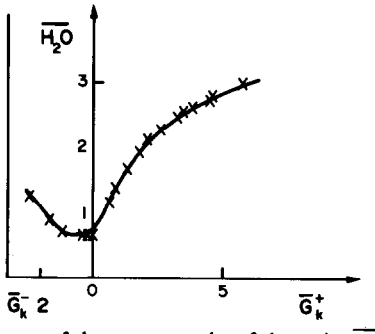


Fig. 5. Change of the water uptake of the resin, $\overline{H_2O}$, as a function of the quantity of chemically bound counter-ions.

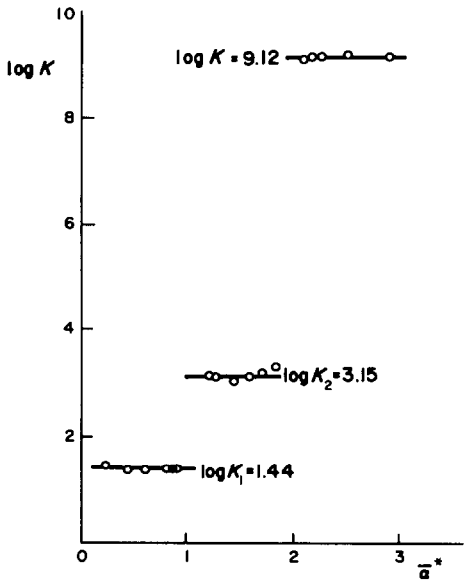


Fig. 6. Variation of the calculated log K'_j-values as a function of the real degree of titration, $\bar{\alpha}^*$.

From the corresponding $\overline{pH}$ *vs.* $\bar{\alpha}^*$ data (columns 7 and 8 in Table 1) a series of protonation constants can be calculated with equation (14) for all the three ranges of protonation (for experiments 1–6, $j = 3$; for experiments 7–12, $j = 2$; for experiments 13–18, $j = 1$). These log K'_j-values are given in column 9 of the table and are plotted as a function of $\bar{\alpha}^*$ in Fig. 6.

As can be seen from Fig. 6, the log K'_j-values are independent of the real degree of titration. The interpretation and possible extension of these results to other systems needs further consideration, however.

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THE TOTAL EMULATION OF THE INTEL 8080 INSTRUCTION SET ON A MAINFRAME COMPUTER

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Summary—A software system, ASSIM-8080, has been developed to permit the writing and debugging of Intel 8080 assembly-language programs with the aid of mainframe computers. ASSIM-8080 will assemble, with error checking and error diagnostics, an assembly-language program. If no errors are found in the source code, ASSIM-8080 will then simulate the execution of the assembly program. ASSIM-8080 will recognize a number of special instruction codes designed to simplify programming and debugging.

Microcomputers are becoming commonplace in the laboratory; they undertake automated control of equipment, data adjustment, report preparation and sometimes lengthy data-processing. Buchanan and Buchanan[1] have recently described a flexible and versatile microcomputer system as an aid in the development of microcomputer-controlled analytical instrumentation. Control programs for this type of instrumentation may be written directly in machine language, in assembly language, or in a high-level language. Machine-language programming is tedious, error-prone and difficult to debug. Programming with a high-level language, such as FORTRAN or PL/M, speeds software development but may lead to control programs of excessive length. Assembly-language programming, whilst it may be time-consuming, can lead to the most efficiently coded program for the specific task in hand.

A program written in assembly language still requires to be converted into machine language and debugged before being loaded permanently into the microcomputer's read-only-memory. The software system reported here is designed to permit the development and testing of assembly-language programs for the Intel 8080 microprocessor. The system, known as ASSIM-8080, is written in FORTRAN and will run on any mainframe computer. The assembly-language source program is input as data to ASSIM-8080. The output from ASSIM-8080 includes the source-code listing and descriptive error messages generated by programming faults detected in the source code. If the source code is error-free, machine-language code is generated and this is then used by ASSIM-8080 to simulate the performance of an Intel 8080 microprocessor, including data I/O. ASSIM-8080 will, if requested, display the contents of all registers after an instruction has been executed. A range of additional commands is available to simplify the task of debugging the source code. Besides providing a useful software tool for writing control programs for existing hardware, ASSIM-8080 is also suitable for use as a vehicle for teaching assembly-language programming of Intel microcomputers.

SYSTEM DESCRIPTION

ASSIM-8080 consists of four main parts. A short main program is used to define the hardware limitations of the target microcomputer. These include the size of random access memory (RAM), the size of read-only-memory (ROM), internal clock frequency and maximum run time for the simulation step. The sizes of a number of internal working tables are also established in the main program.

Once these parameters have been set, control passes to the major subroutine SIMUL8. This routine is responsible for internal initialization, the printing of diagnostic information and the control of the assembly and simulation steps, undertaken by the major routines ASEMBL and XEQTER. ASSIM-8080 has been written so that it may be used with minicomputers having a limited memory, if some minor changes are made. In this situation ASSIM-8080 would be reconfigured into two programs. The first would assemble the source code and the second would simulate the source code by using the machine language generated by the first program.

The features and capabilities of ASSIM-8080 will be discussed in the order in which ASSIM-8080 processes the source code. Details concerning the use of ASSIM-8080 are discussed in detail in the User's Manual. It is assumed that the reader is familiar with the function and meaning of the 78 instruction mnemonics used by the Intel 8080 microprocessor. The complete definition of these mnemonics may be found in an Intel publication.[2] An introductory text that explains each mnemonic with admirable clarity has been written by Leventhal.[3]

ASSEMBLY OF SOURCE CODE

Assembly of the source code is achieved by the subroutine ASEMBL. This routine examines each line

of source code to ensure that none of the Intel instruction-code rules have been violated. If an error is detected, an error message is issued and the next source line is examined. Some errors will cause compilation to cease and ASSIM-8080 will terminate at that point.

The operation of ASEMBL can be broken down into six steps. A flow chart of the process is shown in Fig. 1.

Step 1. Each line of source code is read in as a continuous string of 80 alphanumeric characters. Subroutine INPUT subdivides this string into four parts; label (characters 1–10), mnemonic (characters 11–15), operand (characters 16–25), and comment (characters 26–80).

Step 2. Once the label, mnemonic, operand and comment have been identified, each is checked for contextural legality. The mnemonic is first examined to determine whether it is an assembler or simulator directive, or a conventional instruction code. Assembler and simulator directives will be discussed in a later section. Assembler directives are examined by subroutine PSEUDO. Assembler directives and conventional instruction codes are processed by subroutine MNEMON. Macro definitions are catalogued within ASEMBL for subsequent expansion.

Step 3. The mnemonic field is checked by subroutine MNEMON. Besides the 78 Intel instruction codes, 12 simulator directives are also examined for legality by MNEMON. Simulator directives are recognized by ASSIM-8080 from their having a dollar sign ($) as the first character. The mnemonics are: $ASC; $BPN; $BPY; $DPN; $DYP; $HPN; $HPY; $MEM; $PRF; $RAM; $ROM; $STM.

Step 4. If no error has been found by subroutine MNEMON the operand field is checked by subroutine OPCHCK. The information required to be present in the operand depends upon the particular mnemonic. One of five types of data is required: (*a*) a register, (*b*) a register pair, (*c*) immediate data, (*d*) a 16-bit address, (*e*) no data. The information for (*a*)–(*d*) can be expressed as: (*i*) hexadecimal data; (*ii*) decimal data; (*iii*) octal data; (*iv*) binary data; (*v*) ASCII characters; (*vi*) a label that has been previously assigned a numeric value; (*vii*) a label to be interpreted as an address. It is the objective of OPCHCK to ensure that the operand and mnemonic are compatible. An error message will be issued if an incompatibility is found.

Step 5. Subroutine LABCHK is used to ensure that a label, if present, is used correctly. If the legal label is found it is added to the label table. The label table is used in the final step of the assembly process.

Step 6. At this point in the assembly step the line of source code is printed out together with a line number and the starting location of the hexadecimal object code associated with that line of source code. ASSIM-8080 then commences to process the next line of source code, starting at Step 1.

When the end of the source code is found, any error messages accumulated during the compilation of the source code are printed. The label, equate, and precision tables are printed out. These tables associate labels with memory locations, variable names with numeric values, and give the number of bytes for each variable, respectively. If requested by use of the appropriate assembler directive, the label and/or mnemonic cross-reference tables are also printed out. If no errors have been found, the assembled hexadecimal object code, with associated line numbers, is printed.

ASSIM-8080 is a one-and-a-half-pass assembler. This is achieved as follows. If a jump command is encountered before the destination label has been

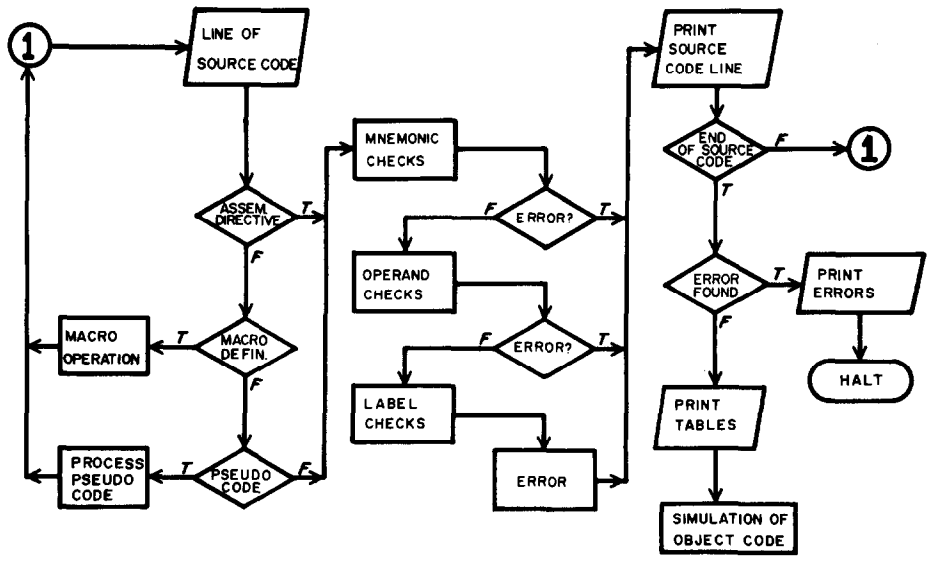

Fig. 1. Flow chart for subroutine ASEMBL, illustrating the basic steps involved in source-code error-checking.

assigned to a memory location, then that line of source code cannot be assembled immediately. A similar situation will occur for calls to subroutines. In this event a condensed version of the label, taken from the operand field, is placed in the object code. The label table contains, at the end of the assembly step, a list of the condensed label names found and their associated memory locations. The completed object code is then examined, byte by byte, for the presence of a special marker signifying that the condensed label was used rather than the absolute memory location. If the marker is found the label table is scanned for the presence of the condensed label. The associated memory location replaces the label name in the object code. This final clean-up of the object code takes less than 0.05% of the total time required.

ASSEMBLER AND SIMULATOR DIRECTIVES

Assembler and simulator directives are recognized by ASSIM-8080 but not by the Intel 8080 microprocessor. These codes have been added to the instruction set to provide the programmer with debugging aids. Assembler directives do not produce any object code. Simulator directives produce, in most instances, a two-character object code, the first character being "$".

Assembler directives

There are 17 assembler directives that are recognized by ASSIM-8080. They will be discussed as related groups.

1. *The group EQU, DB, DW, DS, ORG.* The purpose of this group of assembler directives is to equate variable names with numeric values, EQU; to place one byte, DB, or two bytes, DW, into RAM; to reserve a number of bytes of RAM and label that space with a variable name, DS; to set the start of the program in ROM or to reset the program counter, ORG. The first four codes are interrelated, and detailed explanations of their function and usage are presented in the ASSIM-8080 User's Manual. ORG must appear at least once in the source code to establish the program origin. It may also be used more than once, for example, to subdivide the first 64 ROM locations into 8-byte segments in preparation for the use of the Intel RST instruction.[4]

2. *The group FRLB, FRMN, LNLB, LNMN.* This group of codes provides cross-referencing capabilities. FRLB and FRMN give a list of the number of times each label (FRLB) has appeared in the label and operand fields, and the number of times each mnemonic (FRMN) has been used in the source code. LNLB and LNMN give the line number where a label of mnemonic, respectively, has occurred in the source code. These two assembler directives will provide this information only for a specified list of labels or mnemonics that are part of the source code. FRLB and FRMN give frequencies for all labels and mnemonics.

3. *The group MAC, ENDM.* The directive MAC, in the mnemonic field, signifies that a short section of code, known as a macro, is to be defined. The name of the macro will appear in the label field of the MAC source line. Macros are usually defined at the beginning of the source code. Subsequently, when the name of the macro appears in the mnemonic field, ASSIM-8080 places, after that line, the previously defined macro. Error checks, as outlined in steps 1–6, are then performed as each line of the macro is expanded. The principal advantage of a macro is that it permits new instruction codes to be established by the programmer; these are then used according to the rules of their definition. However, the use of a macro rather than a subroutine must be carefully weighed, since a macro generates a number of lines of code each time it is expanded whereas a subroutine reuses the same section of code each time it is called. These points are discussed further in reference 2 (introduction to Chapter 5).

The code ENDM signifies the end of the macro definition.

4. *The group LIST, NLST, PNCH, END, COMP, EXEC.* These six codes are used to control various routine operations of ASSIM-8080. LIST and NLST control the listing, or not, of all or part of the source code. END is used to signify the physical end of the source code. PNCH will cause the object code to be punched onto computer cards or, if the mainframe computer has the capability, onto paper tape. COMP and EXEC are complementary. The former will cause only the assembly of the source code to be completed. The latter causes ASSIM-8080 to simulate the 8080 microprocessor, given preassembled object code. This particular feature is useful when checking code received from software vendors, or using ASSIM-8080 as two distinct programs.

Simulator directives

There are twelve simulator directives, which will be discussed in related groups. Each directive is converted into a two-character object byte that is recognized, during the simulation step, by ASSIM-8080. They are used, in the source code, as mnemonics.

1. *The group $BPN, $BPY, $DPN, $DPY, $HPN, $HPY.* These six codes turn on, or off, the printing of the contents of each of the Intel 8080 registers, program-status word and program counter. The general form of these directives is $*x*P*y*; where *x* is either B (register contents in base 2), D (register contents in base 10), or H (register contents in base 16), and *y* is either Y (start print-out) or N (stop print-out). It is therefore possible to monitor the effect of one or more source code statements on the registers, program-status word and program counter. The default for ASSIM-8080 is that no print-out is produced during the simulation step. Once a $*x*PY has been found, the register contents are printed after each execution step until $*x*PN is encountered.

2. *The group* $MEM, $RAM, $ROM, $STM. These four directives will display various portions of memory if data exist in the memory locations. The general form is $xyM where xy is ME for all memories, RA for RAM only, RO for ROM only, and ST for the stack section of RAM. These directives produce one memory dump for each occurrence. A starting memory location, for the dump, may be specified within the operand field for each of the directives. However, if the operand field is left blank, all filled locations for the particular memory will be printed out. Together with the ability to examine selectively the contents of each microprocessor register, these four directives permit the complete monitoring of each Intel instruction step for all or part of the simulation of the source code.

3. *The directive* $ASC. When ASCII character strings are to be read into, or printed from, RAM, the characters are stored as their hexadecimal equivalents. The $ASC directive permits, during debugging of source code, the direct use of ASCII characters. $ASC invokes a conversion, within ASSIM-8080, from the character into its hexadecimal equivalent. This directive is most useful when debugging code that employs the Intel IN and/or OUT codes. When the object code is loaded into the ROM of the target machine, conversions between ASCHII and the hexadecimal equivalent are performed by the I/O device.

4. *The directive* $PRF. This directive provides a histogram of the percentage of execution time devoted to each step in the source code. This option is particularly useful when the program has to be recoded in order to reduce execution time. The histogram will indicate those areas of code for which more efficient writing could result in a significant decrease of execution time.

SIMULATION OF SOURCE CODE

Provided that there are no syntax errors or violations of the Intel instruction-code rules, the object code generated by ASEMBL is passed to subroutine XEQTER, where the operation of the 8080 microprocessor is simulated. The object code is in hexadecimal format and may include the special object-code bytes generated by one or more of the twelve simulator directives. Subroutine XEQTER examines the first character of the current object-code byte and if this character is "$" then control passes to a special section designed to process the particular simulator directive. Otherwise the object byte is assumed to be a conventional instruction code.

XEQTER is composed of a number of short sections, each related to one specific mnemonic code. The instruction byte is interpreted in the following manner. The byte, in hexadecimal, is converted into its octal equivalent, OCT. The particular value of OCT is then used to direct control to the required part of XEQTER. Figure 2 shows the first step in this process. The 8080 instruction set can be subdivided

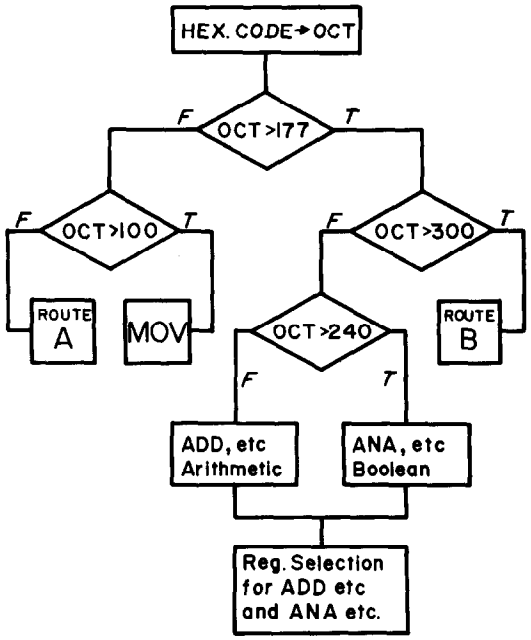

Fig. 2. Flow chart illustrating the basic algorithm for subroutine XEQTER.

into six groups. For example, if the octal equivalent of the object-code byte is between 100_8 and 177_8, then the instruction to be simulated is one of the 64 MOV instructions. Therefore, a value of OCT = 145_8 would direct control to the section of XEQTER designed to simulate MOV. It then remains to determine which two registers are involved in the particular MOV

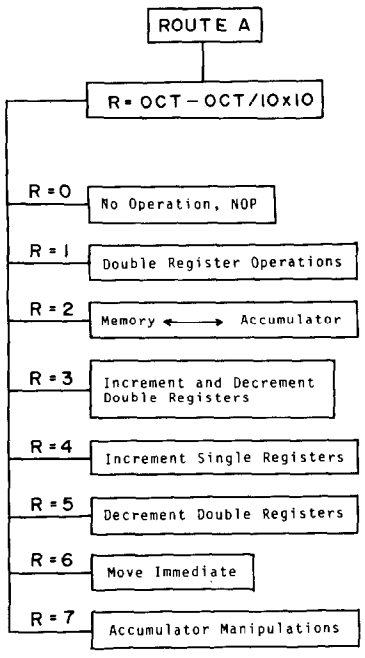

Fig. 3A. Flow chart illustrating the details of Route A (see Fig. 2).

instruction. In this particular example, since $1XX_8$ means MOV, then the second and third digits indicate which registers are involved. For OCT = 145_8 the mnemonic would have been MOV H, L. Figures 2 and 3 indicate the general method by which the value of OCT points to the simulation of the particular object-code byte.

Once the simulation of a particular instruction code has been completed, the contents of the registers are printed if $BPY, $DPY or $HPY has been set. The total number of machine cycles is updated and is used to limit the execution time of the simulation. This feature prevents the consumption of inordinate amounts of main frame computer time in the event that an endless loop is present in the source code.

OUTPUT DESCRIPTION

After this brief description of the various options of ASSM-8080 that are available to the user, it is instructive to examine a short program that has been processed by ASSIM-8080. Appendices I and II show a typical program and the output generated by ASSIM-8080. It can be seen, from Appendix I, that provided the label, mnemonic and operand are within the column constraints described earlier, there is no left-justify requirement. The source code contains a macro called WRTE. Macro construction and usage follows the normally accepted procedures.[2,3]

The output from ASSM-8080 is shown in Appendix II. Given below the header are the current constraints imposed by the target microcomputer and the maximum sizes of various key arrays within ASSM-8080.

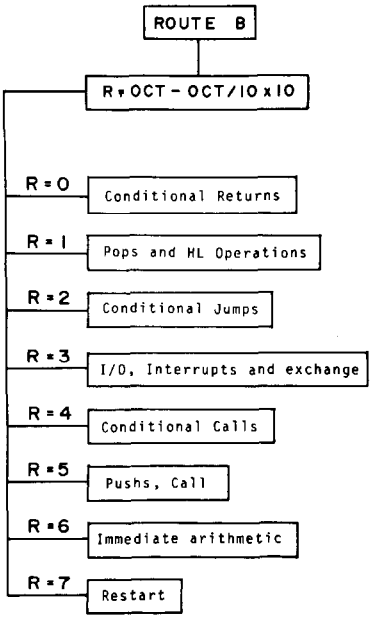

Fig. 3B. Flow chart illustrating the details of Route B (see Fig. 2).

Changes to these variables are made in the main program of ASSM-8080. Only the total available memory of the microcomputer and that portion which is ROM are specified in the main program. The actual allocations of total memory to ROM, RAM and the stack are left to the user. This is achieved through the appropriate use of the EQU assembler directive. In this example RAM starts at memory location 501_{10}, ROM starts at 16_{10} and the stack occupies the last 100 bytes of RAM. Note that the first three EQUate statements use the reserved identifiers RAMST, ROMST and STACK, respectively, to effect these memory allocations.

There follow five EQUate statements that define which particular I/O port will be used; the number of characters in the string PHRSE; and values for the multiplier and multiplicand that will be used in a simple 8-bit unsigned multiplication routine. Thirty-one bytes are reserved for the character string PHRSE, using the DS code. The character string is then defined, using the DB code. The two # signs are used by ASSM-8080 to terminate the line of output to the I/O simulator. When the character string is converted into ASCII hexadecimal codes the # signs are placed by 0D and 0A, the ASCII codes for "carriage return" and "line feed". (See RAM locations 530 and 531.) The origin for the source code is then established, using ORG. Irrespective of any other assembler directives that may be used, the first three and the last must be RAMST, ROMST, STACK EQUates and ORG, respectively. More than one origin may be established, a feature useful when dealing with restarts (RST) involved with interrupt-processing.[4]

The first line of executable code sets the stack pointer at the top of RAM, *i.e.*, location 2000_{10}. The next four lines of code indicate that the cumulative frequencies of all mnemonics (FRMN) and labels (FRLB) are to be noted; the current contents of RAM ($RAM) are to be displayed; and that a simulation time profile ($PRF) for each executable statement is needed. A listing of the macro is then provided, for reference purposes, followed, in this instance, by the actual macro calling statement. The argument list for the definition and the call need only be of the same word size. Changes in variable names are permitted.

Lines 4–11, inclusive, are the macro expansion that is assembled and simulated by ASSIM-8080. This particular example causes a character string of 31 characters to be displayed at a terminal. The macro name appears between the statement number and the ROM location counter as a reminder that this section of code is the result of a macro expansion. The assembler directive $ASC, at line 6, permits the display of the actual ASCII character during the simulation. Omission of $ASC results in the hexadecimal equivalents of each ASCII character being displayed in place of the string. It should be noted that the label PRT, in the macro definition, is renamed as PRT11 during expansion. Should WRTE be expanded a

second time the label would be renamed PRT12, thereby avoiding a repetion of the label in the same source code.

Comment statements are preceded, in the source code, by a semicolon, which is replaced by COMMENT if the complete line is a comment. However, if the comment is appended to an executable source statement the text is moved to beyond the end of the operand field.

Lines 12–27 are a straightforward 8-bit multiplication routine. In this particular example the multiplier uses register E (set to 50_{10}), the multiplicand uses register C (set to 120_{10}) and register B is the bit counter. The result is held in the double register D, E and ultimately stored in RAM location 601_{10} and 602_{10}.

The simulator directives $HPY, $DPY and $BPY, which permit the user to examine the contents of all registers in hexadecimal, decimal and binary formats, are found at lines 28, 29 and 30. Since 601D appears in the operand field of $RAM (line 33) this RAM dump starts at memory location 601_{10}.

Any errors detected during the assembly step are printed immediately following the source listing. Forty-nine messages are available that cover violations of the Intel instruction-set rules as well as improper use of assembler or simulator directives. The contents of the equate, label and precision tables are then printed, followed by a line-number-annotated object code listing. It should be noted that the simulator directives have been converted into the '$x' code. For example line 2 of the source code is $RAM which is converted into $80000. Once the source code has been completely debugged, all simulator directives must be removed and a clean object code generated before it is loaded into the target machine. The frequency table that follows the object code listing displays the number of times each mnemonic and label has appeared in the source code. A more detailed analysis of these occurrences is provided by the pseudo code LNMN and LNLB.

The simulation of the source code is now started and the contents of RAM, at line 2, are printed. Memory locations 501_{10}–531_{10} contain the hexadecimal equivalents of the ASCII string PHRSE. The string that will be printed at the terminal is displayed during the simulation, directly beneath the RAM dump. In this example the string would be output through port $2E_{16}$.

The current status of the 8080's CPU registers is then printed out in hexadecimal, decimal and binary format. The result of the multiplication, $66_{16} \times 32_{16}$, is found in RAM memory locations 601_{10} and 602_{10} and is seen to be $13EC_{16}$ ($= 5100_{10}$), as expected.

The termination of the simulation step is indicated, as shown, and followed by the execution time, in seconds, together with the number of statements executed during simulation. The execution time is calculated by assuming that the CPU is devoted entirely to the execution of the source code and therefore represents the minimum time needed for execution, at the selected clock frequency.

Since the directive $FRN was used at line 3 an execution time profile is given for the section from line 4 to the program end. As expected, most of the time is spent in writing data to the terminal.

A number of error conditions may arise during the simulation step. Notable among these are attempts to load "data" byte(s) into ROM, or access a memory location outside the available memory area. In these situations an error message is generated indicating the source-code line number where the error condition arose. Normally, although not always, simulation is terminated immediately if an error is detected.

The assembler and simulator directives used by ASSIM-8080, and their implementation, are described in detail in the user's manual.

ASSIM-8080 is written in ANSI-standard FORTRAN and has been developed and run on an IBM 370-158 and a Honeywell 66–60 computer. Memory requirements for arrays will depend upon the specific configuration of the target machine and user-declared sizes of tables.

Copies of ASSIM-8080 and the user's manual are available, on request, from the author.

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4. See reference 2, pp. 3–54. Also reference 3, p. 12-3.

APPENDIX I

Listing of a sample program for ASSIM-8080

```
RAMST     EQU  501D
ROMST     EQU  10H
STACK     EQU  1900D
RESLT     EQU   601D
PORT      EQU   02EH
LONG      EQU  31D
MLIER     EQU   32H
MCAND     EQU   66H
PHRSE     DS   LONG
PHRSE     DB   'EVERY GOOD BOY DESERVES FAVOR##'
          ORG  ROMST
          LXI  SP,2000D
          FRMN
          FRLB
          $RAM
          $PRF
WRTE      MAC   STRIN,LNGTH,PORT
          LXI  H,STRIN
          MVI  B,LNGTH
          $ASC
PRT:      MOV  A,M
          OUT  PORT
          INX  H
          DCR  B
          JNZ  PRT
          ENDM
          WRTE   PHRSE,LONG,PORT
;
  ;   THIS IS A MULTIPLICATION ROUTINE FOR TWO UNSIGNED
;    8-BIT NUMBERS.
;
           MVI  C,MCAND
          MVI  E,MLIER
;
;    INTIALIZE MOST SIGNIFICANT BYTE OF RESULT
;
MULT:     MVI   D,0H
          MVI  B,9H       ;BIT COUNTER.
;
;      ROTATE LEAST SIGNIFCANT BIT OF MULTIPLIER
;      TO CARRY AND SHIFT LOW ORDER BYTE OF RESULT.
;
MULT0:    MOV   A,E
           RAR
          MOV  E,A
          DCR  B
          JZ   DONE        ;EXIT IF DONE.
          MOV  A,D
          JNC  MULT1
;
;  ADD MULTIPLICAND TO HIGH-ORDER BYTE OF RESULT
;  IF BIT WAS ONE.
;
          ADD  C
;
;   CARRY=0 HERE, SHIFT HIGH-ORDER BYTE OF RESULT.
;
MULT1:    RAR
          MOV   D,A
          JMP   MULT0
DONE:     NOP
          $HPY
          $DPY
          $BPY
          XCHG
          SHLD    RESLT
          $RAM    601D
          HLT
          END
```

APPENDIX II

Listing of the Output from ASSIM-8080 for the program shown in Appendix I

```
**********************  A S S I M - 8 0 8 0  **********************
*                                                                   *
*******************  INTEL-FORTRAN CROSS ASSEMBLER  *****************
*                        VERSION (4.1 MAY 1981)                     *
*                                                                   *
*********************************************************************

  EXECUTION-TIME DIMENSION LIMITS ARE :-
  TABLES= 40; SOURCE LINES= 300; TOTAL MEMORY= 2000;
  ROM= 500; TRCMAX= 30; OCCMAX= 20; NMAC= 50
  MAXIMUM SIMULATION TIME=    10.0 MILLISECONDS
  CLOCK FREQUENCY= 2.048E 06


 * * *   S O U R C E    P R O G R A M   * * * * * * * * * *

                      RAMST     EQU   501D
                      ROMST     EQU   10H
                      STACK     EQU   1900D
                      RESLT     EQU   601D
                      PORT      EQU   02EH
                      LONG      EQU   31D
                      MLIER     EQU   32H
                      MCAND     EQU   66H
                      PHRSE     DS    LONG
                      PHRSE     DB    'EVERY GOOD BOY DESERVES FAVOR**'
                                ORG   ROMST
     1        0010H             LXI   SP,2000D
                                FRMN
                                FRLB
     2        0013H             $RAM
     3        0016H             $PRF

                      MACRO DEFINITION
                      WRTE      MAC   STRIN,LNGTH,PORT
                                LXI   H,STRIN
                                MVI   B,LNGTH
                                $ASC
                      PRT:      MOV   A,M
                                OUT   PORT
                                INX   H
                                DCR   B
                                JNZ   PRT
                                ENDM
                      END OF MACRO DEFINITION

                                WRTE  PHRSE,LONG,PORT
     4 WRTE   0017H             LXI   H,PHRSE
     5 WRTE   001AH             MVI   B,LONG
     6 WRTE   001CH             $ASC
     7 WRTE   001DH   PRT11:    MOV   A,M
     8 WRTE   001EH             OUT   PORT
     9 WRTE   0020H             INX   H
    10 WRTE   0021H             DCR   B
    11 WRTE   0022H             JNZ   PRT11
  COMMENT
  COMMENT       THIS IS A MULTIPLICATION ROUTINE FOR TWO UNSIGNED
  COMMENT        8-BIT NUMBERS.
  COMMENT
    12          0025H           MVI   C,MCAND
    13          0027H           MVI   E,MLIER
  COMMENT
  COMMENT         INTIALIZE MOST SIGNIFICANT BYTE OF RESULT
  COMMENT
    14          0029H   MULT:   MVI   D,0H
    15          002BH           MVI   B,9H      ;BIT COUNTER.
  COMMENT
  COMMENT           ROTATE LEAST SIGNIFCANT BIT OF MULTIPLIER
```

```
COMMENT         TO CARRY AND SHIFT LOW ORDER BYTE OF RESULT.
COMMENT
   16       002DH  MULT0:     MOV    A,E
   17       002EH             RAR
   18       002FH             MOV    E,A
   19       0030H             DCR    B
   20       0031H             JZ     DONE        ;EXIT IF DONE.
   21       0034H             MOV    A,D
   22       0035H             JNC    MULT1
COMMENT
COMMENT     ADD MULTIPLICAND TO HIGH-ORDER BYTE OF RESULT
COMMENT     IF BIT WAS ONE.
COMMENT
   23       0038H             ADD    C
COMMENT
COMMENT     CARRY=0 HERE, SHIFT HIGH-ORDER BYTE OF RESULT.
COMMENT
   24       0039H  MULT1:     RAR
   25       003AH             MOV    D,A
   26       003BH             JMP    MULT0
   27       003EH  DONE:      NOP
   28       003FH             $HPY
   29       0040H             $DPY
   30       0041H             $BPY
   31       0042H             XCHG
   32       0043H             SHLD   RESLT
   33       0046H             $RAM   601D
   34       0049H             HLT

 * * *   ERRORS IN SOURCE PROGRAM    * * * * * * * * * *

THERE ARE NO ERRORS FOR THIS STEP

******    EQUATE  TABLE    ******
VARIABLE                   VALUE
  NAME
LONG                       001FH
MCAND                      0066H
MLIER                      0032H
PHRSE                      01F5H
PORT                       002EH
RAMST                      01F5H
RESLT                      0259H
ROMST                      0010H
STACK                      076CH

******     LABEL TABLE     ******
LABEL                     MEMORY
NAME                     LOCATION
DONE                       003EH
MULT0                      002DH
MULT                       0029H
MULT1                      0039H
PRT11                      001DH

******   PRECISION TABLE   ******
VARIABLE                  NUMBER
  NAME                   OF BYTES
PHRSE                      001FH
RAMST                      0002H
ROMST                      0002H
STACK                      0002H

LINE NO. *     1*     2*     3*     4*     5*     6*     7*     8*
OBJ.CODE *31D007*$80000*$7    *21F501*061F  *$1    *7E    *D32E  *

LINE NO. *     9*    10*    11*    12*    13*    14*    15*    16*
OBJ.CODE *23    *05    *C21D00*0E66  *1E32  *1600  *0609  *7B    *

LINE NO. *    17*    18*    19*    20*    21*    22*    23*    24*
OBJ.CODE *1F    *5F    *05    *CA3E00*7A    *D23900*81    *1F    *
```

```
LINE NO. *    25*    26*    27*    28*    29*    30*    31*    32*
OBJ.CODE *57    *C32D00*00    **5    **C    **3    *EB    *225902*

LINE NO. *    33*    34*
OBJ.CODE **80259*76    *

 * * *   ERRORS IN OBJECT CODE    * * * * * * * * * *

THERE ARE NO ERRORS FOR THIS STEP

******  FREQUENCY TABLE  ******
LABEL AND              FREQUENCY
MNEMONIC
   ADD                      1
   DCR                      2
   DONE                     2
   HLT                      1
   INX                      1
   JMP                      1
   JNC                      1
   JNZ                      1
   JZ                       1
   LNGTH                    1
   LXI                      1
   MCAND                    1
   MLIER                    1
   MOV                      5
   MULTO                    2
   MULT                     1
   MULT1                    2
   MVI                      5
   NOP                      1
   OUT                      1
   PORT                     1
   PRT                      1
   PRT11                    1
   RAR                      2
   RESLT                    1
   SHLD                     1
   STRIN                    1
   XCHG                     1

* * * *  EXECUTION STEP COMMENCES  * * * *

***  RAM MEMORY DUMP  ***  AT LINE    2

MEM. LOCATION *   501*   502*   503*   504*   505*   506*   507*   508*
MEM. CONTENTS *    45*    56*    45*    52*    59*    20*    47*    4F*

MEM. LOCATION *   509*   510*   511*   512*   513*   514*   515*   516*
MEM. CONTENTS *    4F*    44*    20*    42*    4F*    59*    20*    44*

MEM. LOCATION *   517*   518*   519*   520*   521*   522*   523*   524*
MEM. CONTENTS *    45*    53*    45*    52*    56*    45*    53*    20*

MEM. LOCATION *   525*   526*   527*   528*   529*   530*   531*
MEM. CONTENTS *    46*    41*    56*    4F*    52*    0D*    0A*


THE FOLLOWING STRING WILL BE DISPLAYED AT THE OUTPUT PORT  2E
EVERY GOOD BOY DESERVES FAVOR

        CODE =  EB
B=  0;C=102;D=  2;E= 20;H= 19;L=236;A=236;PSW= 86;SP=07D0H;PC=0042H;NEW=0043H
B=00H;C=66H;D=02H;E=14H;H=13H;L=ECH;A=ECH;PSW=56H;SP=07D0H;PC=0042H;NEW=0043H
   B    0 0 0 0 0 0 0 0    C    0 1 1 0 0 1 1 0
   D    0 0 0 0 0 0 1 0    E    0 0 0 1 0 1 0 0
   H    0 0 0 1 0 0 1 1    L    1 1 1 0 1 1 0 0
   A    1 1 1 0 1 1 0 0 PSW    0 1 0 1 0 1 1 0
  SP    0 0 0 0 0 1 1 1 1 1 0 1 0 0 0 0
  PC    0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 1
```

```
        CODE =  22
B=  0;C=102;D=  2;E= 20;H= 19;L=236;A=236;PSW= 86;SP=07D0H;PC=0043H;NEW=0046H
B=00H;C=66H;D=02H;E=14H;H=13H;L=ECH;A=ECH;PSW=56H;SP=07D0H;PC=0043H;NEW=0046H
   B     0 0 0 0 0 0 0 0    C     0 1 1 0 0 1 1 0
   D     0 0 0 0 0 0 1 0    E     0 0 0 1 0 1 0 0
   H     0 0 0 1 0 0 1 1    L     1 1 1 0 1 1 0 0
   A     1 1 1 0 1 1 0 0 PSW     0 1 0 1 0 1 1 0
  SP     0 0 0 0 0 1 1 1 1 1 0 1 0 0 0 0
  PC     0 0 0 0 0 0 0 0 0 1 0 0 0 1 1 0


***  RAM MEMORY DUMP  ***  AT LINE   33

MEM. LOCATION *    601*    602*
MEM. CONTENTS *     EC*     13*

        CODE =  76
B=  0;C=102;D=  2;E= 20;H= 19;L=236;A=236;PSW= 86;SP=07D0H;PC=0049H;NEW=0049H
B=00H;C=66H;D=02H;E=14H;H=13H;L=ECH;A=ECH;PSW=56H;SP=07D0H;PC=0049H;NEW=0049H
   B     0 0 0 0 0 0 0 0    C     0 1 1 0 0 1 1 0
   D     0 0 0 0 0 0 1 0    E     0 0 0 1 0 1 0 0
   H     0 0 0 1 0 0 1 1    L     1 1 1 0 1 1 0 0
   A     1 1 1 0 1 1 0 0 PSW     0 1 0 1 0 1 1 0
  SP     0 0 0 0 0 1 1 1 1 1 0 1 0 0 0 0
  PC     0 0 0 0 0 0 0 0 0 1 0 0 0 1 1 0


HALT INSTRUCTION HAS BEEN FOUND. CONTROL RETURNS TO MAIN PROGRAM.

 * * * * * *  S I M U L A T I O N  T E R M I N A T E S  N O R M A L L Y


                EXECUTION TIME =    0.000863
                NUMBER OF STATEMENTS EXECUTED =      254.

 LINE  FREQ.      PERCENTAGE OF TOTAL ENCOUNTERS FOR EACH LINE
              !0%       !10%      !20%      !30%      !40%      !50%      !60%
    4       1  *
    5       1  *
    7      31  ************
    8      31  ****************
    9      31  ********
   10      31  ********
   11      31  ****************
   12       1  *
   13       1  *
   14       1  *
   15       1  *
   16       9  **
   17       9  **
   18       9  **
   19       9  **
   20       9  *****
   21       8  **
   22       8  ****
   23       3  *
   24       8  *
   25       8  **
   26       8  ****
   27       1  *
   31       1  *
   32       1  *
   34       1  *
```

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TITFIT, A COMPREHENSIVE PROGRAM FOR NUMERICAL TREATMENT OF POTENTIOMETRIC DATA BY USING ANALYTICAL DERIVATIVES AND AUTOMATICALLY OPTIMIZED SUBROUTINES WITH THE NEWTON–GAUSS–MARQUARDT ALGORITHM

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Summary—TITFIT, a computer program written in HP-enhanced BASIC is able to fit potentiometric titration curves with up to 400 points by using the Newton–Gauss–Marquardt technique supplemented by the use of analytical derivatives. The program contains a part which writes an optimized subroutine as needed by the model. The program also has additional facilities such as visual interactive adjustment of parameters and a plotting subroutine for graphical presentation of the final results. Data can be entered either manually through the keyboard or automatically through an RS-232 serial interface. The performance of the program is discussed for the well studied Ni^{2+} glycine system; the results are similar to those already published in the literature.

Potentiometric titrations with a pH electrode have been widely used in the study of the stability of metal complexes with ligands having basic properties.[1] In order to obtain reliable results the collection of a large amount of data and a powerful program for the calculation are both necessary.

Data collection has been discussed in the literature and several automatic data-acquisition systems have been described.[2,3] These generally allow accumulation of the data necessary for a complete potentiometric analysis.

Several computer programs have been proposed for the evaluation of pH-titrations.[4] The use of computers in the calculation of equilibrium constants began with Sillén's LETAGROP program,[5] which is based on the "pit-mapping" technique. Later SCOGS[6] and MINIQUAD[7] appeared and are still the programs most used. SCOGS is based on the Newton–Gauss algorithm and minimizes the sum of squares of the titration-volume residuals by using the general subroutine COGSNR to calculate the concentrations of each species. MINIQUAD employs a different approach; the minimization is done on the sum of residuals of the three mass equations for total hydrogen, total metal, and total ligand. It is based on the Newton–Gauss or Newton–Raphson algorithm, together with linear optimization of the shifts.

In recent years, however, Marquardt's method,[8] which combines the steepest-descent and Newton–Gauss algorithms in a powerful way, has been introduced in DALSFEK[9] and in MARFIT.[3] The main advantage of this procedure is that it will converge to the minimum without good estimates of the initial parameters.

SCOGS, MINIQUAD and DALSFEK are programs designed for large computers, and they generally do not allow direct interaction[10] between the user and the program, which is especially important in view of the large amount of time usually needed to find the "best" model. MARFIT, on the other hand, is a smaller program written for a computer with limited memory. It allows direct interaction with the user, but it does not have all the other facilities of the large programs, for example, a new subroutine must be written for each model to be tested.

We have now written a new program which has all the facilities mentioned above. An innovation is the use of analytical derivatives combined with the Marquardt technique for finding the minimum. In addition an optimized subroutine is generated automatically by the program itself. The working of the program TITFIT will be illustrated by using data for the Ni^{2+}/glycine system which has been suggested as a test for titration and calculation methods.[11]

EXPERIMENTAL

Chemicals of analytical grade were used without further purification. Titrisol sodium hydroxide (Merck) was diluted with doubly distilled water to give a 0.4M solution. The temperature was kept constant at 25.0 ± 0.2° and the ionic strength at $\mu = 1M$ (NaCl).

The titrations were done with the automatic titrator unit described previously,[3] after calibration of the glass electrode with two buffers of pH 4 and 7. The activity coefficient γ for H^+ and the value of pK_w were determined from

Table 1. Experimental conditions for the titrations of glycine and Ni^{2+} at 25° and $\mu = 1M$ (NaCl)

c_L, M	c_M, M	c_L/c_M	Number of points n	pH range
7.0×10^{-3}	—	—	51	<11.3
1.4×10^{-2}	1.28×10^{-2}	0.914	51	<7.3
1.4×10^{-2}	6.4×10^{-3}	0.457	39	<9.9
1.4×10^{-2}	3.2×10^{-3}	0.228	36	<11.2
3.5×10^{-3}	3.2×10^{-3}	0.914	51	<8.3
3.5×10^{-3}	1.6×10^{-3}	0.457	41	<9.9
3.5×10^{-3}	8.0×10^{-4}	0.228	38	<10.8

titrations of hydrochloric acid under the same experimental conditions as for the complexation of nickel ions with glycine.

The pK_H-values of glycine were determined by titrating $7 \times 10^{-3}M$ glycine to which an excess of acid had been added. The titrations with nickel present were run at two total concentrations with different ratios of c_L/c_M (Table 1). Titrations were stopped when the pH started to drift because of incipient formation of $Ni(OH)_2$ and/or polymeric hydroxo-complexes. The maximum pH values which were used for the calculation are also given in Table 1.

The calculations were done with the aid of a Hewlett-Packard HP9835 computer equipped with a plotter (HP7225A), a serial input/output interface (HP98036A) and a Heathkit H14 printer connected through a second serial interface.

THE PROGRAM TITFIT

TITFIT is written in HP-enhanced BASIC and is a turn-key system for a Hewlett-Packard HP9835. After transcription into standard BASIC it could be run on any desk-top computer with at least 48 kbytes of memory. The program can perform eight different tasks which are shown in the flow diagram (Fig. 1).

The experimental data can be introduced either through the keyboard (task 7) or through an RS-232 serial interface (task 5) which is adapted to accept data from our data-acquisition system.[3] If other types of automatic data-collection are used, this part of the program (Subroutine Tr) must be changed. For both input modes, a new file is first created on tape and some control operations are done.

Manual input starts with an identification, then the user is asked whether the volume increment is variable, or constant, in which case only Δml need be entered. Then in a loop the values of pH and, if necessary, those of the titration volume (ml_{exp}) are typed in. The value 0 for pH indicates the end of the input.

Transfer of data through the RS-232 interface is done by setting the interface to 8-bit characters with two stop bits, no parity and 1/64 bit-rate factor. The 8-bit values are then transformed automatically into the values pH and ml_{exp}.

The dimension statement in the program is such that a total number of 400 titration points (points per curve times number of curves) can be used. For a single curve up to 100 points can be accepted.

After the titration points have been read in, the values of the constants (*e.g.*, total concentrations, known equilibrium constants) and those of the parameters (*e.g.*, unknown equilibrium constants) are entered. Constants and parameters are distinguished by an index (0 and 1, respectively) and the equilibrium constants also must have the indices m, l and h which define the species $M_mL_lH_h$.

Sometimes it is necessary to change the pH and ml_{exp} values (task 8) and to have a print-out before the data are recorded on tape. Of course the data on tape can also be retrieved (task 1).

The program can be used to plot a species distribution (task 6). To do this a set of equilibrium constants to define the model must be given, then TITFIT creates a specific subroutine (Subroutine Ohb) for calculation of the concentrations of the species, and links it to the main program. Then a further subroutine (Subroutine Plot) is called automatically to plot the species distribution (per cent *vs.* pH) over the pH range 2–12. Each species is identified by the use of a different plotting symbol and is only plotted when its concentration is >1%.

The model defined by the set of parameters can readily be changed (task 4) so that the user can see how a new model affects the species distribution.

Experimental data and calculated titration curves can be plotted to give a graphical display (task 3). The plotting subroutine does not need any special input; only the number of the curves selected for plotting in one figure need be entered. The establishment and labelling of the pH and ml_{exp} axes are done automatically. The titration points for each curve are characterized by a different symbol and the calculated curves are plotted. At the end a caption can be written on the figure. An example is shown in Fig. 2.

The main part of the program, however, is the fitting procedure (task 2). To fit the experimental points to a theoretical model given by the chosen set of complex species a subprogram must be generated to describe the model mathematically. This is done by the subroutine Wr, which writes a series of BASIC statements which together will give the subroutine Ohb. In contrast to other computer programs such as SCOGS,[6] MINIQUAD[7] and DALSFEK[9] which use general subroutines, or MARFIT[3] for which the oper-

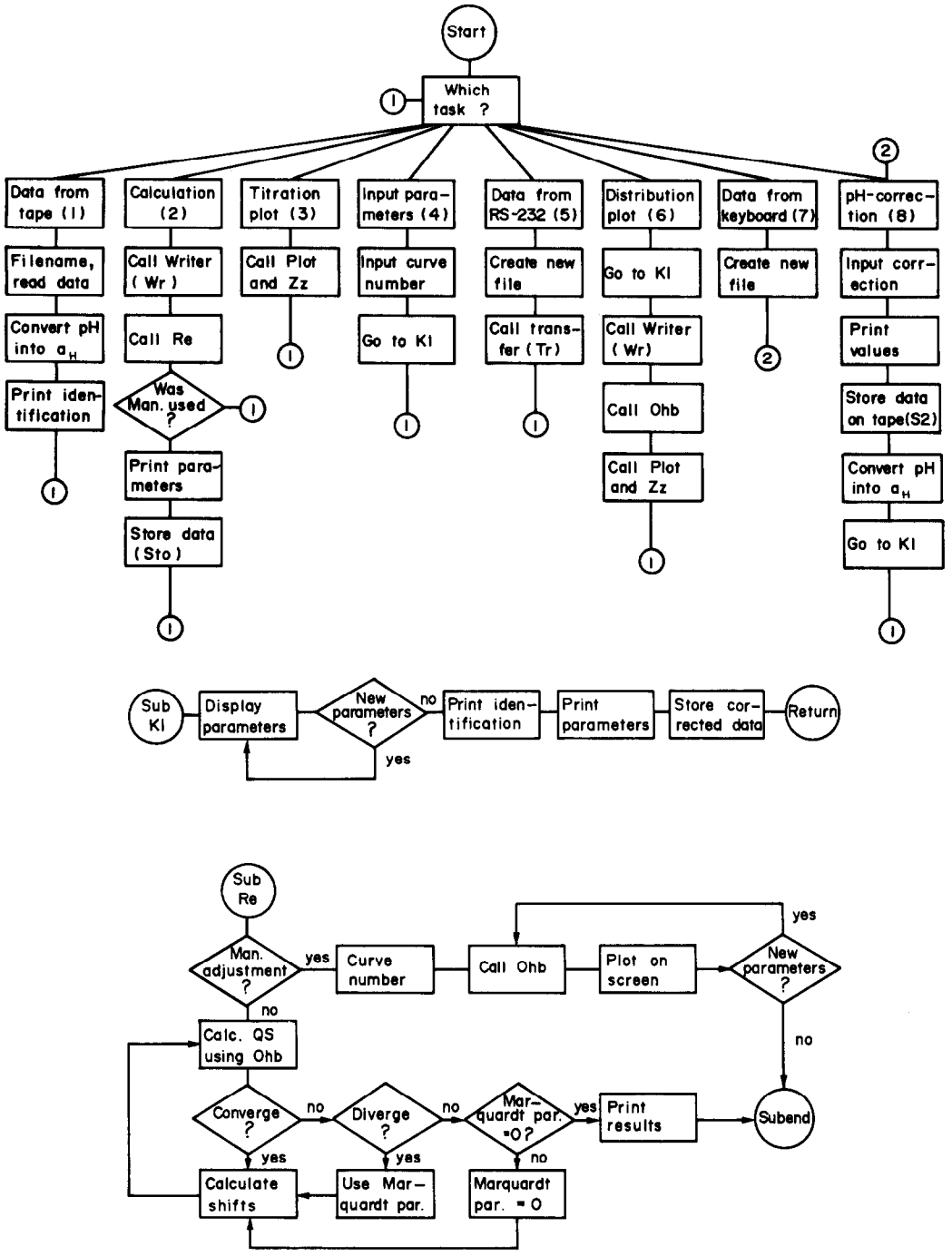

Fig. 1. Flow diagram for the program TITFIT.

ator has to write a new subroutine for each new model, subroutine Wr generates a specifically optimized subroutine for the model to be examined. The advantages are many; the user does not have to bother about the subroutine, the subroutine Ohb is much faster than a general one, and it also saves memory space, since all dimension statements are adapted to the need. All these aspects are important when a desk-top computer is used.

The subroutine Ohb first solves for the concentrations of free ligand L and free metal ion M, using a standard Newton–Raphson procedure.[14] Then the analytical derivatives $\partial \mathrm{ml}_{\mathrm{calc}}/\partial \log\ p_i$ $(i = 1, \ldots, I)$, where p_i are the I parameters to be determined, are calculated according to Nagypal *et al.*[12] In our experience use of $\partial \mathrm{ml}_{\mathrm{calc}}/\partial \log p_i$ instead of $\partial \mathrm{ml}_{\mathrm{calc}}/\partial p_i$ is more satisfactory by far since this automatically generates a Hessian[14] with diagonal elements of

roughly equal size. With the derivatives (elements of the Jacobian) the Hessian[14] (or curvature matrix[15]) is then built up.

Back in the main program the Newton–Gauss–Marquardt algorithm[8,15] is applied. The shifted parameters so obtained are used to calculate a new sum of squares QS and it is checked that convergence takes place. This is continued iteratively until the relative change in QS is less than 0.01%. In the case of divergence the Marquardt parameter is introduced. It is first set equal to the first diagonal element of the curvature matrix and is added to all diagonal elements. After each step of convergence the Marquardt parameter is reduced by a factor of 3 and in the final iteration it is set equal to zero. Once the minimum in QS is found the "best" parameters and their standard errors are printed and the values of pH, ml_{exp} and ml_{calc} are stored on tape.

TITFIT is 26 kbytes long in its original form, but will need more memory for the subroutine Ohb once this is generated and for the data (about 13 kbytes). If a computer with insufficient memory has to be used, space can be saved by deleting all the remark statements, and by linking to the main program only those subroutines which are needed in a specific task.

MATHEMATICAL BACKGROUND

For a model which consists of a set of species $M_mL_lH_h$, the equations related to the equilibrium constants β_{mlh} and to the mass balance for total metal c_M, total ligand c_L, and total hydrogen c_H are:

$$\beta_{mlh} = [M_mL_lH_h]/[M]^m[L]^l a_H^h \tag{1}$$

$$F = c_L - \sum_{mlh} l[M_mL_lH_h] \tag{2}$$

$$G = c_M - \sum_{mlh} m[M_mL_lH_h] \tag{3}$$

$$H = c_H - \left\{\sum_{mlh} h[M_mL_lH_h] + a_H/\gamma - K_w/a_H\right\} \tag{4}$$

where a_H is the measured proton activity, γ the activity coefficient for H^+ and K_w the dissociation constant of water. From these, the calculated titration volume (ml_{calc}) can be derived:

$$ml_{calc} = EQ - \frac{EQ}{n_L \cdot c_L}\left(\sum_{mlh} h.[M_mL_lH_h] + a_H/\gamma - K_w/a_H\right) + Exac \tag{5}$$

where EQ is the equivalence-point volume, Exac the volume corresponding to the excess of strong acid, and n_L the total number of protons of the ligand that can be titrated.

From equations (2) and (3) the two unknown concentrations [M] and [L], represented by M and L, can be calculated by the Newton–Raphson[14] method since $F = G = 0$. With the shifts ΔM and ΔL, calculated by using the partial derivatives $\partial F/\partial M$, $\partial F/\partial L$, $\partial G/\partial M$, and $\partial G/\partial L$ the solutions are obtained by iteration. At this point the concentrations of all species $M_mL_lH_h$ are also known and hence ml_{calc} [equation (5)] and the error square sum [equation (6)] can be calculated.

$$QS = \sum_{points} (ml_{exp} - ml_{calc})^2 \tag{6}$$

To obtain the "best" parameters the minimum of QS must be found and for the parameters p_i there are I equations of the type (7)

$$0 = \frac{\partial QS}{\partial \log p_i} = \sum_{points} \frac{\partial\, ml_{calc}}{\partial p_i} p_i \tag{7}$$

which must be solved. As parameters the unknown β_{mlh} values are taken, but also if necessary the equivalence-point volume EQ, the excess of acid Exac, the ion-product of water K_w or the activity coefficient γ. For p_i equal to EQ, Exac, K_w and γ the derivatives $\partial\, ml_{calc}/\partial p_i$ are trivial and equations (8)–(11) can be written at once.

$$\partial\, ml_{calc}/\partial \log EQ = EQ - EQ\left(\sum_{mlh} h[M_mL_lH_h] + a_H/\gamma - K_w/a_H\right)\Big/(n_L c_L) \tag{8}$$

$$\partial\, ml_{calc}/\partial \log Exac = Exac \tag{9}$$

$$\partial\, ml_{calc}/\partial \log K_w = K_w . EQ/(n_L c_L a_H) \tag{10}$$

$$\partial\, ml_{calc}/\partial \log \gamma = a_H . EQ/(n_L c_L \gamma). \tag{11}$$

However, for p_i equal to β_{mlh} the situation is a little more complicated. As discussed by Nagypal *et al.*,[12] equation (12) can be written:

$$\frac{\partial\, ml_{calc}}{\partial \log \beta_{mlh}} = -\frac{EQ}{n_L c_L}\left\{h[M_mL_lH_h] + \frac{\partial H}{\partial L}\frac{\partial L}{\partial \log \beta_{mlh}} + \frac{\partial H}{\partial M}\frac{\partial M}{\partial \log \beta_{mlh}}\right\} \tag{12}$$

$\partial H/\partial L$ and $\partial H/\partial M$ can be calculated from equation (4), whereas $\partial L/\partial \log \beta_{mlh}$ and $\partial M/\partial \log \beta_{mlh}$ are determined from the two linear equations (13) and (14):

$$0 = \frac{\partial F}{\partial \log \beta_{mlh}} = l[M_mL_lH_h] + \frac{\partial F}{\partial L}\frac{\partial L}{\partial \log \beta_{mlh}} + \frac{\partial F}{\partial M}\frac{\partial M}{\partial \log \beta_{mlh}} \tag{13}$$

$$0 = \frac{\partial G}{\partial \log \beta_{mlh}}$$

$$= m[M_m L_l H_h] + \frac{\partial G}{\partial L}\frac{\partial L}{\partial \log \beta_{mlh}} + \frac{\partial G}{\partial M}\frac{\partial M}{\partial \log \beta_{mlh}} \quad (14)$$

where $\partial F/\partial L$, $\partial F/\partial M$, $\partial G/\partial L$ and $\partial G/\partial M$ are already known from the Newton–Raphson solution for the concentrations.

Once the partial derivatives $\partial \mathrm{ml}_{\mathrm{calc}}/\partial \log p_i$ have been calculated, the Hessian matrix is directly built up and the standard Newton–Gauss–Marquardt procedure is used to compute the shifts of the parameters.

DISCUSSION

The program TITFIT was developed for use on a computer to which the operator has direct access for interaction. The program is easy to use, since it is written in blocks (subroutines and subprograms) which perform a single task and which can be assessed in any logical order. It is also possible to interrupt one task and continue with another at any moment. For the user who is not interested in writing programs or in their detailed function it can be used as a turn-key system with "black box" characteristics.

We would like to emphasize the following comparisons with the working of other programs.

(a) In contrast to other programs which minimize titration-volume differences, analytical derivatives $\partial \mathrm{ml}_{\mathrm{calc}}/\partial \log p_i$ are used for the calculation. No numerical differentiation (with all its problems of step size control and loss of significant figures) is needed in the entire program. Analytical derivatives are also used in MINIQUAD[6] but it employs a completely different approach, since it performs a least-squares fit on all three mass-balance equations (2)–(4), thus mixing independent and dependent variables.

As shown in the mathematical part, the analytical derivatives $\partial \mathrm{ml}_{\mathrm{calc}}/\partial \log p_i$ can be calculated by using the derivatives of the functions F, G and H with respect to M and L, which have been already calculated to solve for the concentrations. The advantages of the use of analytical derivatives are higher speed and better convergence.

(*b*) The program automatically writes its own optimized subroutine, including the analytical derivatives and the algorithm for building the Hessian.[14] The user need only define the complex species through their stoichiometric indices m, l and h and indicate whether a value should be refined or kept constant.

(*c*) The program has facilities which help with the choice of model and reasonable values for the parameters. Each titration curve can be displayed on the screen and the parameters can be adjusted manually. A new plot and the error square sum QS are then shown. Through visual interaction the operator can obtain a rough fit to the titration curve before the automatic refinement starts.

(*d*) At any moment the user can decide whether he wants to keep a certain value constant or whether it should be considered as a parameter and be refined. Any change requires rewriting of the subroutine, as described in (*b*).

(*e*) Like DALSFEK[9] and MARFIT[3] this program uses the Newton–Gauss–Marquardt algorithm,[8] which seems to be one of the best for a non-linear least-squares problem such as this. The Marquardt parameter is used only when divergence occurs.

(*f*) In contrast to the authors of DALSFEK[9] we think that the algorithm used here is not ideal for other types of measurements, and especially not for spectrophotometric ones. It has been shown[13] that for such data, another approach, which uses eigenvector analysis for data-reduction and elimination of the linear parameters, can greatly improve the convergence condition and decrease calculation time as well as memory requirement.

The program has been tested and used in our laboratory for more than a year with many different examples.

No difficulties have been found in fitting titration curves provided that reasonable models are chosen. In general we can fit titration curves to 0.1–0.5% of the total volume of the titrant.

RESULTS

The system Ni^{2+}/glycine was chosen for use as a model system to allow comparison of the reproducibility of techniques and calculation procedures.[11] The titration data for the free ligand were fitted with two pK_H values, taking the excess of strong acid and the equivalence-point volume as additional parameters. For 51 data points 7 sec were required for an iteration and the complete fitting was done after 3 or 4 iterations. The Ni^{2+}/glycine system was investigated by using six titration curves with a total of 256 data points. The values of the three parameters β_{110}, β_{120} and β_{130} were found after four iterations in about 10 min. This time is only slightly dependent on the number of parameters used for the fit. The best values, together with those published in the literature,[11] are given in Table 2.

Table 2. Acid dissociation constants of glycine and formation constants of the Ni^{2+}–glycine complexes at 25° and $\mu = 1M$ (NaCl)

Species m,l,h	$\log \beta_{mlh}(\sigma_{\log\beta})$†	$\log\beta_{mlh}$
0 1 1	9.653 (0.011)	9.63
0 1 2	12.083 (0.031)	12.08
1 1 0	5.638 (0.071)	5.58
1 2 0	10.391 (0.089)	10.30
1 3 0	13.922 (0.191)	13.75
pK_w	13.693 (0.02)	13.69

† Mean values and standard deviations taken from Bottari *et al.*[11]

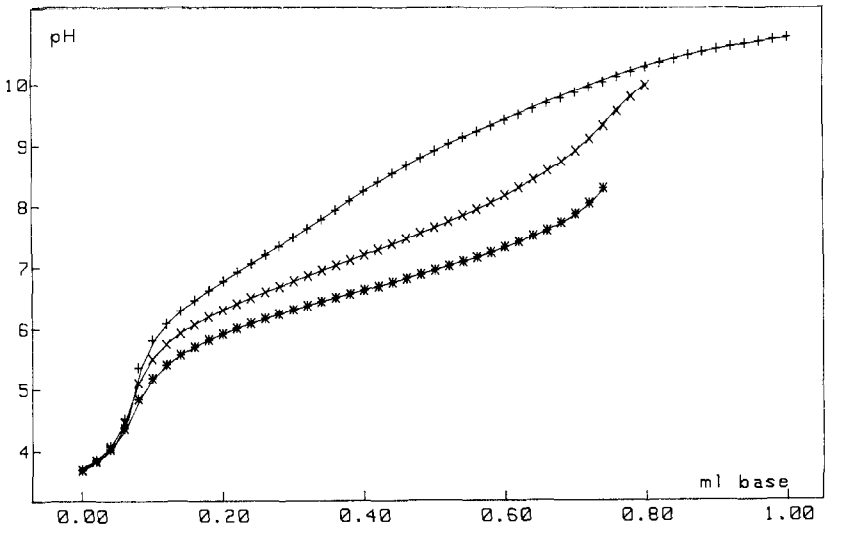

Fig. 2. Experimental and calculated titration curves for $3.5 \times 10^{-3} M$ glycine with 0.914, 0.457 and 0.228 equivalents of Cu^{2+}, plotted by the computer program TITFIT.

The values found are all well within the limits of the results obtained by the other groups and agree with the mean of the previous results within less than one standard deviation. The standard errors of our values calculated from a single set of six titration curves range from 0.002 to 0.008 and are considerably smaller than the "true" uncertainties obtained by repeating the experiments several times and by comparing results from different groups. An example of experimental and calculated curves is shown in Fig. 2.

Since one of the authors of the paper mentioned[11] proposed the presence of hydroxo-species in addition to the 1:1, 1:2 and 1:3 complexes, we also postulated the presence of Ni(gly)OH. The sum of squares decreased insignificantly from 1.766×10^{-3} to 1.765×10^{-3} and β_{11-1} was 8.6×10^{-7} with a standard error $\sigma_\beta = 3 \times 10^{-6}$, clearly indicating that the species must be rejected. We assume the hydroxo-complexes to be artefacts which are obtained only if titration points from the unstable pH region are included.

In conclusion we can say that TITFIT combines the facilities of the larger computer programs with the easy and direct access of desk-top computer systems. Direct interaction of the operator with a graphical display greatly facilitates quick testing of different models. Enhanced speed and improved numerical safety are provided by the automatic design of an optimized subprogram and the use of analytical derivatives.

Listings of TITFIT or copies on an HP200 cartridge are available from the authors upon request.

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AUTOMATED SQUARE-WAVE ANODIC-STRIPPING VOLTAMMETRY WITH A FLOW-THROUGH CELL AND MATRIX EXCHANGE

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Summary—An extremely sensitive and versatile instrument is described that performs square-wave anodic-stripping voltammetry. The instrument incorporates a flow-through cell and is capable of changing the solution matrix between the deposition and stripping steps. The working electrode is a static mercury drop electrode constructed in the authors' laboratories. The entire system is controlled by a microcomputer that allows the usual variation of the square-wave parameters, as well as setting of the initial and final scan potentials, the deposition time, the scan-rate, the instrument sensitivity and the drop size. To show the performance of the instrument, calibration graphs for Cd in the ranges 0.2–40 and 0.1–1 ng/ml are described and the reproducibility of the drop is discussed. Analysis of a NaCl sample for Cd is given as an example of application of the method.

In the last decade anodic-stripping voltammetry (ASV) has become an important electroanalytical technique, especially for trace analysis, because of its high sensitivity and specificity.[1,2] The sensitivity of ASV can be increased by increasing the deposition time: cadmium at concentrations below the ng/ml level were determined by ASV as early as 1957, but a 60-min deposition time was required.[3] The sensitivity can also be improved by increasing the rate of convection in the solution, increasing the area of the electrode or employing a more sophisticated form of voltammetry for the determination or stripping process. A number of potential–time wave-forms, such as sine-wave,[4] pulse,[5] differential pulse[6,7] and square-wave,[8] have been superimposed on a linear potential ramp for stripping purposes. Desimoni *et al.*[9] have recently shown that the specificity can be increased by coupling matrix exchange with stripping analysis.

This paper deals with an instrument that has been developed to carry out automated square-wave stripping voltammetry coupled with matrix exchange. A number of parameters which allow the analyst to achieve sensitivities in the sub-ng/ml range without the use of lengthy deposition times, *i.e.*, in less than 5 min have been investigated. By the use of a mini flow-through cell, sufficient convection is obtained during the deposition period for mechanical stirring to be unnecessary. The enhancement of sensitivity is derived from the use of square-wave voltammetry, which incorporates a square-wave combined with a staircase wave-form in the stripping stage of analysis.

EXPERIMENTAL

Apparatus

The mini flow-through cell is constructed of $\frac{1}{2}$-in. thick "Plexiglas" sheet and dowelling. It consists of two sections. The manifold control block, or first section, controls the flow of the solutions that enter the second section, the cell block, where the working, auxiliary and reference electrodes are located. The manifold control block has a $\frac{1}{16}$-in. diameter channel drilled lengthwise through its centre. This manifold has two inlets and two outlets. The first inlet is for introduction of the sample; opposite it is the sample reservoir drain, which allows draining of the sample reservoir and rinsing of the sample inlet valve. The second inlet to the manifold is used for introduction of the stripping medium into the manifold when the matrix-exchange method is used. The remaining outlet serves to connect the manifold cell block to the electrode (cell) block. The placement of these inlets and outlets is shown in Fig. 1. There are two reservoirs connected to the manifold control block. One reservoir, a 600-ml beaker with a Teflon stop-cock valve fitted on its bottom, holds the sample solution. The other reservoir, a 250-ml separatory funnel, holds the de-aerated electrolyte. Both reservoirs are connected to their respective inlets with Tygon tubing.

The cell block is also shown in Fig. 1. The cell chamber itself is a $\frac{3}{8}$-in. diameter channel $1\frac{3}{8}$ in. deep. There are two outlets and one inlet to this chamber. The inlet located at the bottom of the cell chamber is 0.020 in. in diameter, which allows the solution to flow from the manifold block at a controlled rate into the cell chamber. A $\frac{1}{16}$-in. internal

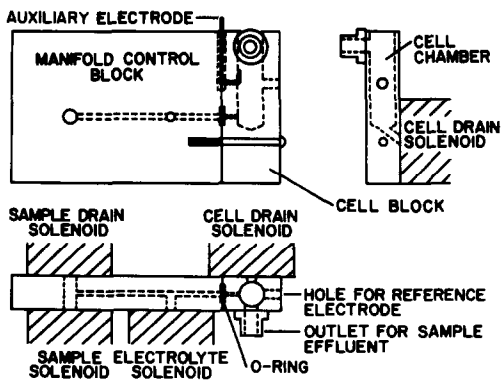

Fig. 1. Mini flow-through cell.

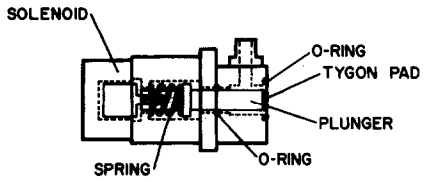

Fig. 2. Plunger assembly.

diameter O-ring is used to seal the inlet connection between the manifold and cell blocks. One outlet is located near the top of the cell chamber and is the effluent port for the solution flowing through the cell chamber during the analysis. The other outlet is at the bottom of the cell chamber and is used to drain the cell and remove used mercury from the cell chamber. Two other holes are drilled on opposite sides of the electrode block. One is for the counter-electrode (a platinum wire), and the other is for the reference electrode, a Ag/AgCl electrode employing an unfired Vycor tip as the solution/electrode interface. The two sections of the cell are held together by a bolt screwed into a hole tapped in the manifold control block.

The sample inlet, sample drain, electrolyte inlet and cell drain are controlled by plungers made from "Plexiglas" dowelling and sheet. The plungers are spring-loaded and controlled by their solenoids. When the solenoids are activated the plungers are pulled away from the corresponding valve seat on the manifold block. If the solenoids are in the non-activated state, the springs hold the plungers against the valve seats. A piece of Tygon attached to the end of the plunger makes a leak-proof seal with the seat. Figure 2 shows the plunger assembly.

The working electrode used is a static mercury drop electrode (SMDE) similar in design principle to the one described by Peterson and manufactured by E. G. & G. Princeton Applied Research Corporation.[10] In the design used in our laboratory no metal other than the platinum electrical connection comes into contact with the mercury. A "Plexiglas" plunger under the control of a solenoid regulates the flow of mercury through the glass capillary. When the solenoid is pulsed quickly and accurately, a reproducible drop is formed on the end of the glass capillary. The body of the electrode assembly is made from "Plexiglas" sheet and dowelling and is shown in Fig. 3. The drop is dislodged from the capillary by a drop-knocker of plunger–spring–solenoid design similar to that described above and shown in Fig. 4.

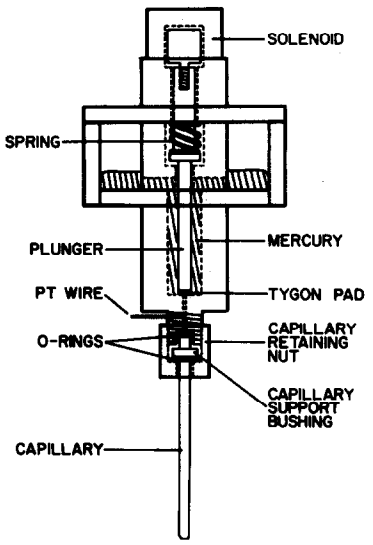

Fig. 3. Static mercury drop electrode.

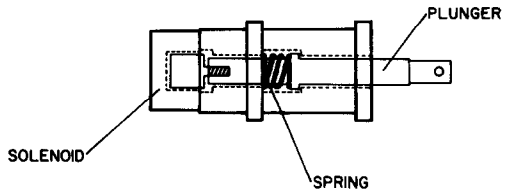

Fig. 4. Drop-knocker.

The cell potential and current signal are controlled and processed by the microcomputer-controlled square-wave polarograph designed and built by Buchanan and Sheleski.[11] The instrument applies the combined staircase voltage and square-wave signal during the stripping procedure, and monitors and processes the current flow from the cell. Table 1 lists the controllable variables applied by the square-wave polarograph.

Portions of the original software have been modified to accommodate the use of a SMDE and the stripping procedures employed with the automated flow-through cell. The main functions are retained but the use of the SMDE eliminates the need for strobe timing control. However, selection of the size of the drop formed by the SMDE is most desirable and therefore this feature was added. ASV requires the selection of deposition time and this function was also incorporated. The step duration in the staircase voltage is programmable from 1 to 65,536 msec, thus allowing the analyst to vary the scan-rate. The step height is always 5 mV. The frequency of the square-wave is selected by entering the appropriate square-wave half-period into the microcomputer. The half-period can range from 1 to 65,536 μsec, corresponding to square-wave frequencies between 8 Hz and 0.5 MHz. Square-wave amplitudes of 0, 5, 10, 25 and 50 mV are also selectable. The delay between the square-wave transition and the commencement of the current measurement is called the "transition sample delay". This parameter is adjustable from 1 μsec to the value of the square-wave half-period. The period of time over which the current signal is sampled is called the "sample aperture" and is also adjustable between 1 μsec and the square-wave half-period. The sum of these two times should not exceed the half-period of the square-wave. The deposition time can be programmed from 0 to 65,530 sec in steps of 10 sec. The initial and final potentials can cover the range ±2000 mV and are programmed in steps of 1 mV. There are four sensitivities available on the instrument. Sensitivity 1 multiplies the current produced at the working electrode by a factor of 22. Sensitivities 2, 3 and 4 provide multiplication factors of 110, 220 and 440 respectively. Four drop sizes have been made available for the SMDE. The drop size is controlled by the solenoid pulse-time when forming a drop. Drop size 1 requires the solenoid to be pulsed for 31 msec. For drop sizes 2, 3 and 4 the solenoid is pulsed for 62, 125 and 250 msec respectively. All of these parameters are constantly displayed on a cathode-ray tube (CRT) and are entered into the microcomputer from a keyboard.

Table 1. CRT display of square-wave ASV parameters

1. Square-wave amplitude	50 mV
2. Square-wave half-period	2000 μsec
3. Deposition time	180 sec
4. Transition sample delay	400 μsec
5. Sample aperture	1600 μsec
6. Initial potential	−700 mV
7. Final potential	−200 mV
8. 5-mV step time	2000 msec
9. Sensitivity	4
A. Drop size	2

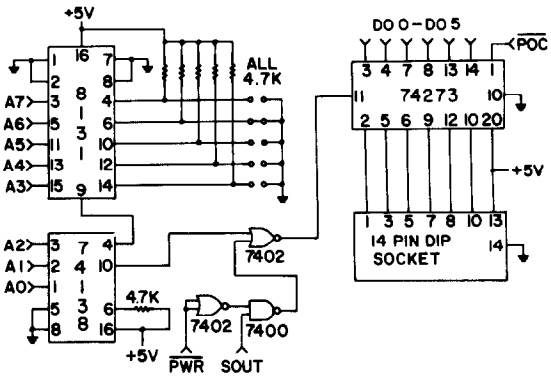

Fig. 5. Interface circuit.

Additional hardware is required for the microcomputer to manipulate the SMDE, the drop-knocker, and the solution flow through the cell during an analysis. This is accomplished through an interface board located in the microcomputer, and a manual control module connected between the interface board and the appropriate solenoids. The interface board uses the S-100 bus configuration. The circuit diagram is shown in Fig. 5. The manual control module changes the 5-V signals received from the interface board into the 24-V signals used by the solenoids. The circuit diagram of this module is shown in Fig. 6.

The sequence of events that occur during a single analysis is listed below.

1. The adjustable parameters, as shown in Table 1, are entered into the microcomputer. Comments may be entered and stored in memory.
2. The analysis is started.
3. The old drop is knocked off and a rinse drop is formed by pulsing the drop-knocker and SMDE solenoids.
4. The cell-drain solenoid plunger is activated and the cell chamber is emptied.
5. The cell-drain outlet is closed, then the electrolyte solenoid plunger is again activated and the cell is filled with electrolyte.
6. The electrolyte inlet is closed and another rinse drop is formed and knocked off.
7. An analysis drop is formed in the same manner as the rinse drop.
8. A d.c. pre-electrolysis potential of -200 mV *vs.* Ag/AgCl is applied to the cell for 40 sec to remove any oxidizable impurities from the electrode. Concurrently, the sample solenoid plunger is activated to flush the cell chamber with the sample.
9. When the pre-electrolysis period has expired, the deposition potential or initial potential is applied to the cell for the duration of the deposition time. The sample continues to flow through the cell during this period.
10. Upon completion of the deposition period the sample inlet is closed and matrix exchange is commenced by flushing the cell with the deaerated electrolyte for 40 sec. During this period the sample solution is replaced with the stripping solution. The potential applied to the cell during this period is still the d.c. deposition potential.
11. The electrolyte inlet is closed, the square-wave signal is applied to the cell and the voltage scan is started.
12. The resulting voltamperogram is recorded on a strip-chart recorder and stored digitally in the memory of the microcomputer.
13. When the final potential is reached the scan stops and the square-wave signal is terminated.
14. The instrument then waits until initialized again.

Reagents

Distilled water passed through Barnstead "Ultrapure" and "Organic Removal" cartridges was used in the preparation of all solutions. Stock solutions of cadmium and lead were prepared from reagent-grade cadmium chloride and lead carbonate. Reagent-grade ammonium chloride was used in the preparation of the stripping electrolyte. Locally purchased table salt without additives was used for preparing the sodium chloride sample solution. Oxygen was removed from the nitrogen used for deaerating the electrolyte, by bubbling the nitrogen through a chromium-(II) scrubber solution before use.

Procedure

Table 1 shows the list of operating parameters as they appear on the CRT of the instrument. The values given for each parameter are those used in all the experimentation unless otherwise noted in the text.

The pre-electrolysis potential was programmed in software to be -200 mV. The cell-chamber flush-times were also programmed in software and set at 40 sec. These parameters are not as easily changeable as those listed in Table 1, but can be varied by changing the program of the operating system.

The stripping electrolyte used for matrix exchange in all the experimentation was 0.1*M* ammonium chloride. Nitrogen was continuously bubbled through the electrolyte reservoir by means of a fritted bubbler.

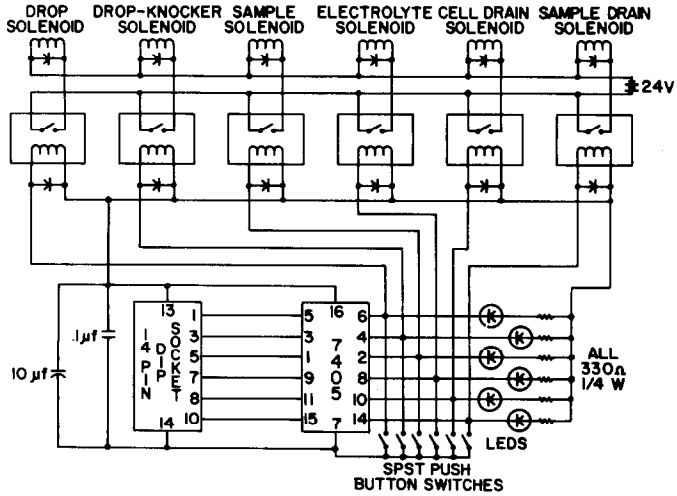

Fig. 6. Manual control module circuit.

Table 2. Data for 2-min deposition-time calibration graph for Cd

[Cd] *ng/ml*	Adjusted mean peak height, * *chart units*	Standard deviation, *chart units*	Relative std. devn., %	Sensitivity setting
0.2	6.3	0.2	3.2	4
0.4	13.3	0.7	5.3	4
0.6	22.1	0.5	2.3	4
0.8	31.3	1.8	5.8	4
1.0	39.5	0.4	1.0	4
4.1	178	3	1.7	3
6.2	262	4	1.5	2
8.2	354	1	0.3	1
10.3	434	14	3.2	1
14.4	608	6	1.0	1
20.6	894	14	1.6	1
30.9	1360	20	1.5	1
41.2	1790	30	1.7	1

* Adjusted to sensitivity setting of 4.

The flow-rate of solutions through the cell was measured and found to be 27 ± 1 ml/min. This value is fixed and controlled mainly by the diameter of the inlet to the cell.

Two calibration curves for cadmium were prepared. All of the sample solutions used had an ammonium chloride concentration of 0.1*M* in addition to the appropriate concentration of cadmium. A 2-min deposition time was employed in the preparation of the calibration curve. The concentrations of cadmium ranged from 0.2 to 40 ng/ml. The sensitivity setting of the instrument was varied according to the concentration of cadmium. A second calibration curve for cadmium was prepared, using a 3-min deposition time, cadmium concentrations of 0.1–1 ng/ml and a sensitivity setting of 4.

A sample of locally procured common table salt (sodium chloride without additives) was assayed for its cadmium content. A 42.1-g sample was dissolved in 1 litre of demineralized distilled water. The deposition conditions were the same as those for the second calibration curve described above. An additional sample containing a trace of added lead ions in the form of lead chloride was examined to confirm that the second peak observed was due to lead.

The peak height as a function of drop size was examined, with a 10-ng/ml Cd solution which was also 0.1*M* in ammonium chloride. A deposition time of 1 min and a sensitivity setting of 2 were employed for this study.

RESULTS AND DISCUSSION

The matrix-exchange method was used in the analysis of all solutions in this work. The change of cell solutions between deposition and stripping offers two advantages. The first is that the metal being determined can be stripped into a solution that is more favourable than the original sample solution for the oxidation of that particular metal. If several metals are being determined and their peaks overlap, the matrix-exchange method can often be used to resolve the peaks through changing the oxidation potentials by complex-formation. The second advantage accrues because only the stripping procedure requires a deaerated solution. Thus analysis time may be saved by employing a non-deaerated sample for the deposition step and then exchanging it for an already deaerated stripping electrolyte before starting the stripping procedure.

Table 3. Data for 3-min deposition-time calibration graph for Cd

[Cd] *ng/ml*	Mean peak weight, *chart units*	Standard deviation, *chart units*	Relative std. devn., %
0.1	4.2	0.1	2.4
0.2	9.3	0.8	8.6
0.4	19.1	1.4	7.3
0.6	28.5	1.2	4.2
0.8	38.9	1.0	2.6
1.0	49.0	1.2	2.4

Table 2 shows the data for the cadmium calibration graph obtained with a 2-min deposition time. All the cadmium peaks occurred at −535 mV *vs.* Ag/AgCl. The adjusted peak height takes into account the sensitivity employed and is the average obtained from three consecutive determinations on a single sample. A least-squares analysis of the data produced a line with a correlation coefficient of 0.9999 and a slope of 43.5 ± 0.1 chart units per concentration unit (ng/ml). The closeness of the correlation coefficient to unity

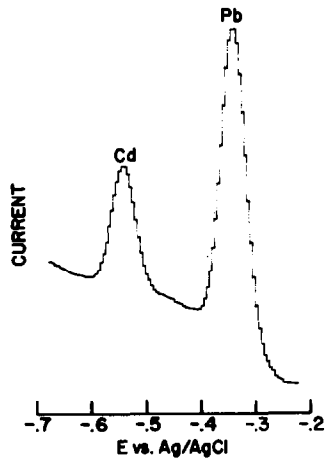

Fig. 7. Voltamperogram of a 0.72*M* NaCl solution, obtained with a 3-min deposition period.

Table 4. Drop-size comparison data

Drop size	Formation pulse time, *msec*	Mean peak height, *chart units*	Standard deviation, *chart units*	Relative std. devn., %
—	16	44	2	4.5
1	31	43.9	0.9	2.1
2	62	49.7	1.2	2.4
3	125	56.8	1.4	2.5
4	250	68	2	2.9

shows the linearity of response, and the slope indicates the sensitivity of the instrument. The mean relative standard deviation for all the data points was 2.3%.

The data for the second calibration graph are listed in Table 3. Each of the data points is a mean of 9 results taken 3 at a time on 3 separate days. The line produced by these data has a correlation coefficient of 0.9999 and a slope of 48.5 ± 0.3 chart units per concentration unit (ng/ml). The mean relative standard deviation of all the points over the 3 days was 4.6%. A comparison of the mean relative standard deviations for all the data points given in Tables 2 and 3 indicates the long-term stability of the instrument. The difference in the slopes of the two calibration graphs is indicative of the slight enhancement in sensitivity obtained by increasing the deposition time.

Figure 7 shows the voltamperogram of the table salt solution. The peak at −535 mV *vs.* Ag/AgCl is the cadmium peak and the peak at −330 mV *vs.* Ag/AgCl was identified as due to lead. The cadmium concentration in the salt solution was calculated from the second calibration graph to be 0.35 ± 0.05 ng/ml. This corresponds to 8 ± 1 ng/g in the solid sample. The magnitude of the lead content was not determined.

The cell showed no contamination problems with the cadmium and lead solutions. The cell was rinsed by filling the sample reservoir with 200 ml of distilled water and flowing it through the cell for 3 min.

Table 4 shows the results obtained in the drop-size experimentation. The peak heights are reported in chart-recorder units. Initially a drop size obtained with a 16-msec solenoid pulse was examined. The resulting peak height was the same as that obtained with a "31-msec" drop. It was concluded that this result was due to the inability of the solenoid to respond correctly to pulse times of less than 31 msec. Accordingly, the program was rewritten to make 31 msec the minimum pulse time. The increase of peak height with larger drop sizes was expected, because of the larger surface area of the drop. The reproducibility of the drop was evidenced by the mean relative standard deviation of 2.9% for the peak heights obtained for the same solution. The similarity of this value to those for the calibration graphs furthers confidence in the reproducibility of the drop sizes.

The coupling of matrix exchange with square-wave anodic-stripping voltammetry provides an analytical scheme which is capable of rapid, sensitive determinations of trace metals with minimal deposition times.

Note. Copies of the software are available from Professor Buchanan at the price of US$2.50.

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RAPID-FLOW ANALYSIS USING DIFFERENTIAL PULSE POLAROGRAPHY WITH AUTOMATIC SAMPLING

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Summary—Differential pulse polarography is used in a rapid-flow analysis system for automated determination of lead, zinc and ascorbic acid in acetate-buffered sample solutions, without the need for sample deaeration. By use of a nitrogen-segmented buffer stream at high flow-rates, high-speed sampling at up to 180 samples/hr can be obtained at a flow-rate of 22.8 ml/min through a polarographic flow-cell fitted to the dropping mercury electrode. A linear calibration range of approx. 0.1×10^{-4}–$1.0 \times 10^{-3} M$ is found for lead, zinc and ascorbic acid, with respective detection limits of 4.0, 0.8 and $0.2 \times 10^{-6} M$, limited by the high base-line current and high noise-level. Vitamin C tablets can be routinely analysed without prior separation steps, provided the sample and wash solutions are matched in electrolyte composition. A precision of better than 1% RSD is obtained at a sampling rate of 120/hr.

Although well recognized as a sensitive and selective analytical technique,[1,2] differential pulse polarography (dpp) has been little studied as a tool for automated sample analysis in continuous-flow systems. Rapid-flow analysis has been suggested[3] as a means of improving sampling rates in continuous-flow analysis with ion-selective electrodes. There have been no reports, however, on rapid sampling systems for detecting species by direct oxidation or reduction with dpp, and this study is aimed at establishing the performance limits of dpp in automated rapid-flow analysis.

Ivaska and Smyth[4] recently reviewed the status of polarography in flow-analysis systems, and found that dpp had been little used. Alexander *et al.*[5,6] first showed that dpp could be used for fast analysis in rapid-flow systems with either a mercury-pool electrode[5] or a dropping mercury electrode (DME)[6] as the indicator electrode. Sampling rates of 60–120 samples/hr were obtained with dpp operated at fixed potentials suitable for detection of certain catalytic electrode reactions.[5,6] Additionally, it has been shown[7,8] that indirect complexometric methods can be used to advantage with dpp detection in flow systems for automated determination of various proteins at routine sampling rates of 60/hr. Tóth *et al.*[9] have discussed the possible application of other modes of polarography in flow-analysis systems, including direct and indirect (or inverse) methods.

In this study we have examined the response characteristics of the DME in a rapid-flow system for dpp detection of both reduction and oxidation electrode reactions, using lead, zinc and ascorbic acid as examples of direct electrode reactions in the categories discussed by Pungor *et al.*[9] Performance limits in fast-sampling operation have been examined, and the results compared with those obtained by using d.c. polarography. The analysis of vitamin C tablets was used as an example of fast-sampling analysis where the selectivity of dpp is used to advantage. The importance is shown of matching the composition of sample and wash solutions in order to avoid high blank readings in this type of dpp analysis.

EXPERIMENTAL

Reagents and solutions

All inorganic reagents used were analytical-reagent grade. The 0.2*M* acetate buffer (pH 4.7) was prepared by mixing equal volumes of 0.2*M* sodium acetate and 0.2*M* acetic acid. A stock 0.2% solution of Triton X-100 was used.

Lead and zinc nitrates were used without purification to prepare 0.1*M* stock solutions in water, which were then diluted with 0.2*M* acetate buffer containing 0.0002% Triton X-100 to cover the range 10^{-4}–$10^{-3} M$.

Ascorbic acid solution, 0.05*M*, was prepared in boiled-out distilled water containing EDTA (0.5 g/l.) as recommended by Strohl *et al.*[10] to stabilize the solution against chemical oxidation. A standard series in the concentration range 5.0×10^{-5}–$5.0 \times 10^{-4} M$ was prepared in boiled-out distilled water containing EDTA (0.5 g/l.) and 0.0002% Triton X-100.

Tablets analysed were commercially available pharmaceutical formulations of two different types: a vitamin C tablet (Roche) containing vitamin C (500 mg) as the only vitamin component, and a coated B-group multivitamin tablet (Altorvite, Nicholas) containing vitamins C (37.5 mg) B_1 (5.0 mg), B_2 (2.5 mg), B_6 (0.5 mg), nicotinamide (25.0 mg) and calcium pantothenate (1.0 mg) in each tablet. For analysis, sample solutions were prepared in 0.2*M* acetate buffer by dissolving single tablets of each type of boiled-out distilled water and diluting to 1000 ml after addition of 0.5 mg of EDTA, 100 ml of 2.0*M* buffer solution and 1 ml of 0.2% Triton X-100 solution. The buffered solutions were then analysed directly without prior separation. The vitamin C tablet (500 mg) solution was further diluted tenfold with the same electrolyte solution to bring the concentration into the calibration range.

Polarographic instrumentation

An EG & G/Princeton Applied Research polarograph, type 174A, was used for polarographic analysis with a matched drop-timer (PAR 174/70) and a Houston Omnigraphic strip-chart recorder, type EB5117-5-S. The electrochemical cell consisted of a dropping mercury electrode (DME), a saturated calomel electrode (SCE) and an auxiliary platinum electrode. The capillary characteristics of the DME were: mercury flow-rate 2.2 mg/sec with natural drop-life of 3.8 sec at a mercury-column height of 860 mm, when measured at a fixed potential of −0.41 V with the DME immersed in 0.2*M* acetate buffer. The electrodes were used in the flow-through cell system described below, and measurements were made at room temperature.

Automated continuous-flow system

The flow-through analysis system was similar to the one previously described[6] except that an automatic sampler was added and the flow-through cell was slightly modified. Figure 1 shows the schematic diagram of the modified system and indicates the flow-rates used for automatic sample introduction into a nitrogen-segmented buffer stream.

The automatic sampler was a Chemlab type CS40 with continuously variable control of both the sample and wash times. A 6-channel peristaltic pump (Desaga, type No. 131900) was used to pump buffer and sample solutions through the system at flow-rates varied by use of a speed controller fitted to the pump. The flow-rates given in Fig. 1 were obtained with the speed-controller set at speed 8. The stream was pumped through a single mixing coil of 2 mm internal diameter, and transmission tubing of 1 mm internal diameter.

After debubbling, the sample stream flowed through a glass flow-cap fitted to the DME with the platinum auxiliary electrode sealed in the glass inlet tube, up-stream from the DME, as described previously.[6] To improve the wash-out characteristics, however, the cell was modified by reducing the internal diameter of the flow-through channel from 2.0 to 1.0 mm, and by using a DME capillary with a small outside diameter of 4.0 mm. The resulting hold-up volume of the cell was reduced from approximately 0.5 ml to 0.1 ml. The SCE was placed in the waste solution down-stream from the DME, and the waste mercury from the DME was collected in the waste solution after being flushed out of the cell by the flowing stream, thus avoiding build-up of mercury in the flow-cell.

Procedure

The buffer solution was deaerated by continuous bubbling of nitrogen through the solution vessel. In these studies, the sample, blank and wash solutions were all made 0.2*M* in acetate (with 0.0002% Triton X-100 present) in order to match the base-line currents. For the determination of ascorbic acid, the sample, blank and wash solutions all also contained 0.5 g of EDTA per litre. A steady base-line current was initially established by pumping the buffer and wash solutions through the flow-cell, the polarographic settings (unless otherwise mentioned) being as follows: the potential was fixed at the dpp peak potential of the analyte; current range 0.2 mA; drop-timer 0.5 sec; pulse amplitude 100 mV; recorder input 2 V; recorder chart-speed 1 or 2 cm/min.

Sample solutions were loaded on the sampling tray in 10-ml cups, and the sampler was then switched on with the sampling and wash times both set for normal operation at 15 sec to give an effective sampling rate of 120 samples/hr. The wash and sample solutions were pumped into the nitrogen-segmented buffer stream and were *not* deaerated before sampling. The current at the DME was recorded continuously on the strip-chart recorder for either the dpp or the d.c. mode. When required, sampling and wash times were varied from 10 to 30 sec to give corresponding sampling rates in the range 180–60 samples/hr. The sample volumes required for analysis depended on the sampling rate used and were 4.6, 3.1, 2.3 and 1.5 ml at sampling rates of 60, 90, 120 and 180 samples/hr, respectively.

RESULTS AND DISCUSSION

The continuous-flow system shown in Fig. 1 was operated at a fixed flow-rate of 22.8 ml/min through the flow-cell. The flow-rates through the sample and buffer lines were respectively 9.2 and 11.8 ml/min with a debubbling rate of 2.2 ml/min. The high flow-rate used was found to be necessary to give rapid response

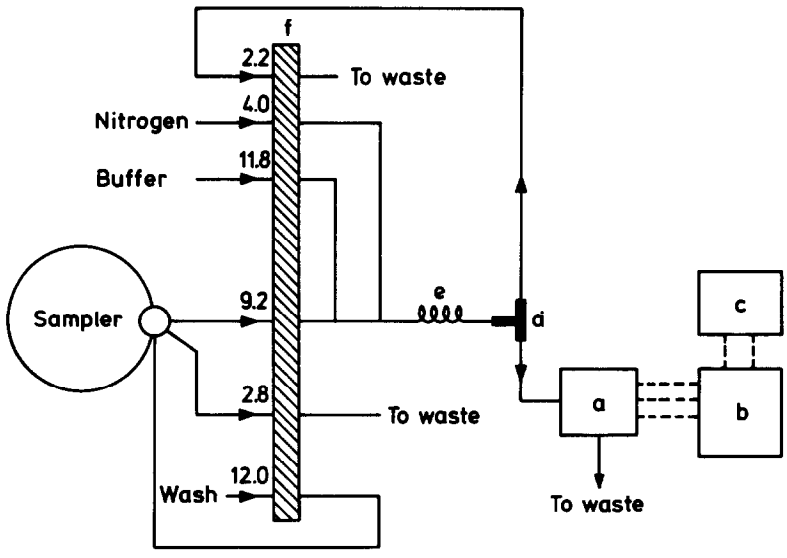

Fig. 1. Schematic diagram of flow system. (a) Flow-through electrode assembly; (b) PAR model 174A; (c) recorder; (d) debubbler; (e) mixing coil; (f) peristaltic pump. Broken lines (-----) indicate electrical connections, full-lines (——) indicate flow-path. Flow-rates (ml/min) are given above each pump line.

Table 1. Electrode characteristics in rapid-flow analysis with differential pulse polarographic detection

Analyte*	E_p, V	Baseline noise, nA	Sensitivity, mA/M	Linear range,† M	Detection limit, M
Pb^{2+}	−0.41	20	13.6	$0.05 - 1.0 \times 10^{-3}$	4×10^{-6}
Zn^{2+}	−1.00	10	22.8	$0.01 - 1.0 \times 10^{-3}$	8×10^{-7}
Vitamin C	+0.05	1.0	9.6	$0.01 - 0.5 \times 10^{-3}$	2×10^{-7}

*Dissolved in 0.2*M* acetate buffer.
† Approximate linear working concentration range.

in the dpp operational mode, as previously found by Alexander and Shah.[6] The rapid response at this high flow-rate is due to the increased mass transport from the solution to the electrode surface, when the thickness of the diffusion layer is decreased. With this flow-rate fixed, we optimized the operational parameters in this system for the automated determination of lead, zinc and ascorbic acid at fixed voltage, covering maximum sampling rates, effect of oxygen, detection limits and noise levels for both dpp and d.c. operation, effect of sample matrix composition on the baseline, and an application to tablet analysis.

Choice of fixed operating potentials

Solutions of each analyte were pumped continuously through the flow-cell while voltage scans were recorded for $1 \times 10^{-3}M$ solutions of lead, zinc and ascorbic acid, in turn, to establish optimum potentials for operation in the dpp mode. The dpp peak potentials (E_p) measured are shown in Table 1. These potentials, which agree with the $E_{\frac{1}{2}}$ values reported for static d.c. measurements,[11] were then used for automatic-sampling determinations of the analytes at fixed potential. Selective determination of a single analyte should be possible without interference from other reducible or oxidizable species unless there is serious overlap of the peaks. This is an advantage over d.c. operation where species reacting at more positive potentials than the analyte may seriously interfere with current measurements at a voltage on the plateau of a d.c. cathodic wave. In the case of oxidation reactions, the interference effects are reversed and species reacting at more negative potentials than the analyte will interfere with the current measurement on the d.c. anodic wave.

Effect of sampling rate

Results for the analysis of some lead solutions are shown in Fig. 2 for a sampling rate of 120 samples/hr. At the high flow-rate of 22.8 ml/min through the flow-cell, routine operation of the automatic sampling system gave sample peak-heights reaching at least 92% of the steady-state current values. Table 2 summarizes results obtained for lead at 60, 120 and 180 samples/hr, showing the sample volume used at each sampling rate, and the precision and carry-over observed. The results indicated that sampling at rates up to 180/hr was possible with <1% carry-over between consecutive high and low samples. Precision of ±2% RSD was observed for sampling at 180/hr, *i.e.*, a 10-sec sampling time and a 10-sec wash, with sample volumes as low as 1.5 ml.

Effect of oxygen

The recording shown in Fig. 2 was obtained without deaeration of the sample or wash solutions. We found that sample peaks could be detected by deaerating the buffer and using nitrogen-segmentation in

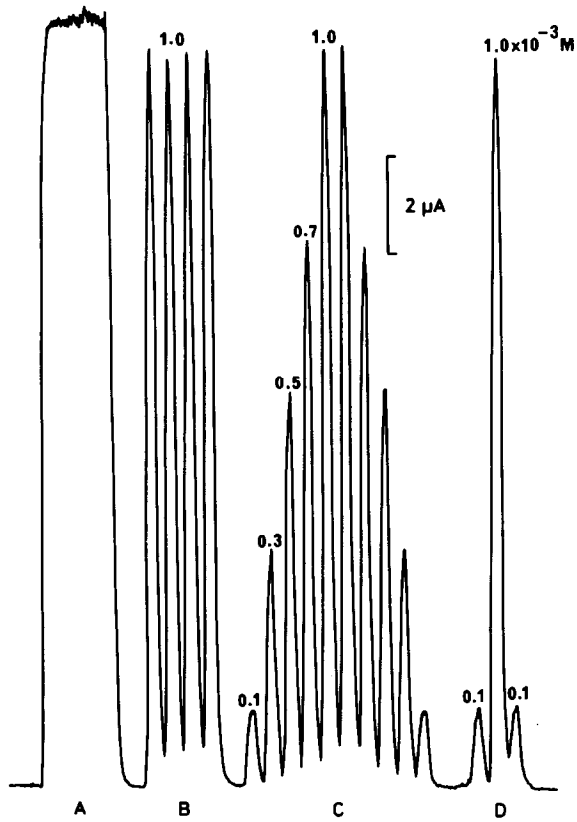

Fig. 2. Continuous recording of lead peaks at a fixed potential of −0.41 V and a rate of 120 samples/hr with 1:1 sample-to-wash ratio, using the flow-rates given in Fig. 1. (A) Steady-state reading for $1.0 \times 10^{-3}M$ lead. (B) Replicates for $1.0 \times 10^{-3}M$ lead. (C) Calibration for the concentration range $0.1–1.0 \times 10^{-3}M$. (D) Carry-over between sequential samples of 0.1, 1.0 and $0.1 \times 10^{-3}M$ lead.

Table 2. Characteristics of rapid continuous-flow determination of lead* by pulse-polarography

Sampling rate, *samples/hr*	Sample volume, *ml*	Signal, *% of steady state*	RSD,† %	Carry-over, %
60	4.6	99	0.5	nil
90	3.1	94	1.0	0.2
120	2.3	92	1.0	0.5
180	1.5	82	2.0	1.0

* Flow-rate 22.8 ml/min through the cell.
† Relative standard deviation for 5 replicates of lead solution.

the flow-system. The small amount of oxygen introduced in each sample segment was apparently not large enough to interfere with the sample peaks observed at the E_p values tested.

Base-line noise and detection limits

Table 1 shows the detection limits for lead, zinc and vitamin C, taken as twice the standard deviation of the base-line current at the operating potential. The base-line noise was found to depend on: (1) deaeration of the buffer, (2) the operating potential, (3) the flow-rate, and (4) the buffer concentration. The base-line noise for dpp operation was found to increase over the broad voltage range from approx. −0.3 to −0.9 V where oxygen is reduced at the DME. Both the sensitivity and detection limits therefore depend on base-line conditions and, since the noise levels are high in this flow-system, detection limits are not as low as those reported[12] for static dpp determination. Despite these limitations, however, many useful applications are possible.

We also operated the detector in the d.c. mode at a fixed potential (i.e., amperometric operation) on the plateau of the wave, and compared the results with the dpp data shown in Table 3 for the determination of lead. The results indicate the problems with d.c. detection at the DME, showing the high noise level on the base-line and the much lower sensitivity of the d.c. mode. The dpp mode was approximately 50 times more sensitive than the d.c. mode with the same electrode drop time of 0.5 sec and the same flow-rate through the flow-cell.

Table 3. Comparison of the detector sensitivity in the d.c. and dpp operating modes

Operating mode	Sample* concentration, *M*	Sample* current, *μA*	Operating potential, *V*
dpp	1.0×10^{-4}	1.3	−0.41
dpp noise†	nil	0.02	−0.41
d.c.	1.0×10^{-2}	2.7	−0.70
d.c. noise†	nil	0.15	−0.70

* For lead samples determined at 60/hr in 0.2*M* acetate buffer.
† Base-line noise level for 0.2*M* acetate solution.

Tablet analysis

Single tablets were selected at random from batches of vitamin C tablets, and of B-group vitamin tablets also containing vitamin C. The vitamin C content of the tablets was determined in replicate by the dpp method, and the results are summarized in Table 4. The precision of replicate analysis of each tablet was in the range 0.3–0.9% RSD, and the mean vitamin C values were within 4% of the nominal content.

Table 4. Replicate determination of vitamin C in tablets by rapid-flow analysis with dpp

	Vitamin C content, *mg**			
	Tablet A	Tablet B	Tablet C	Tablet D
	37.8	38.9	38.4	510
	38.0	39.1	38.7	505
	37.5	39.0	38.1	515
	38.2	38.7	38.8	520
	37.6	38.8	38.5	510
Average ± SD	37.8 ± 0.2	38.9 ± 0.1	38.5 ± 0.2	512 ± 4
Nominal content	37.5	37.5	37.5	500

* Tablets A, B, C: Coated B-group vitamin tablets with vitamin C (Altorvite). Tablet D: Vitamin C (Roche).
† Calculated according to British Pharmacopoeia.[13]

The major source of error in this method was non-matching of the wash and sample blank solutions, which caused high blank values. We found it was necessary to use an acetate concentration of 0.2*M* for both wash and sample solutions in order to eliminate the blank value. This type of matching would be required for all analytical applications in this flow system operating at a fixed potential, because of the large effects which variations in electrolyte concentration have on the base-line current.

CONCLUSIONS

The system reported here performs analyses as fast as any other automated single-element method. The major advantages of dpp fixed potential are the selectivity obtainable by choice of the appropriate E_p value, and the sensitivity, in the ppm range, which is at least one order of magnitude better than that offered by the d.c. amperometric mode. Large batches of samples can be rapidly analysed for a single component with this system simply by choice of the required E_p value and appropriate blank-matching, provided that peak overlap with other sample components is insignificant as a source of interference.

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HYDROXIDE COMPLEXES OF LANTHANIDES—IV*

YTTERBIUM(III) IN PERCHLORATE MEDIUM

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Summary—From the precipitation borderline in the pM′-pC_H diagram, determined experimentally under CO_2-free conditions, the stability constants of the mononuclear species of ytterbium hydroxide have been established. The values found are log $*\beta_1 = -7.7$, log $*\beta_2 = -15.5$, log $*\beta_3 = -23.2$, log $*\beta_4 = -37.5$, log $*\beta_5 = -51.9$, log $*\beta_6 = -66.2$ and log $*K_{s0} = 18.0$. The data refer to fresh precipitates, prepared at room temperature (21.5 ± 0.2°) in sodium perchlorate medium with an ionic strength of 1. The formation of polynuclear hydroxide complexes has been considered and rejected as unlikely to occur.

The determination of the hydrolysis constants from solubility measurements has been described previously for cerium(III), samarium(III) and gadolinium(III).[1–3] In this paper our results for ytterbium(III) will be discussed. Its hydrolysis constant $*\beta_1$ and $*K_{s0}$ determined previously (Table 1)[4–11] show a fair diversity, mostly caused by differences in experimental conditions. As it is necessary to exclude carbon dioxide during the manipulations we did our experiments with Yb(III) in a large nitrogen-filled glove-box, a procedure previously applied to the other rare earths. Under these conditions reproducible results have been obtained with fresh precipitates made in a sodium perchlorate medium ($I = 1.0$). From these results, besides values for $*\beta_1$ and $*K_{s0}$, values for $*\beta_2$, $*\beta_3$, ..., $*\beta_6$ hitherto absent in the literature have been deduced. The previous papers in this series[1–3] should be consulted for more details about the theory, the possible influence of carbon dioxide and the compromise between reproducibility and non-equilibrium precipitation, for which standardization of the manipulations is necessary. The precipitation procedure has been modified slightly for practical reasons and is given below. Thereby the pC_H range has been extended into the strongly alkaline region.

EXPERIMENTAL

The ytterbium stock solutions were prepared by dissolving the metal (99.99%) in 1*M* perchloric acid. The metal was cut into small discs by spark erosion under gasoline; the discs were polished mechanically to remove carbon deposits, cleaned with acetone and dried under nitrogen. Initially the dissolution is accompanied by violent evolution of hydrogen gas, but soon the reaction slows down so that heating and stirring are eventually necessary to complete the dissolution. Ytterbium oxide is still more difficult to dissolve, even in powdered form. It was only soluble in 3*M* perchloric acid with heating. The final stock solution was made 0.1*M* in ytterbium and 1*M* in perchloric acid. Before the experiments were started, the solutions were flushed in the glove-box with nitrogen for 30 min to expel carbon dioxide.

Procedure

The stock solution of ytterbium was diluted tenfold with 1*M* perchloric acid, except for the range pYb′ < 2. Carbonate-free 50% sodium hydroxide solution was added dropwise to 200 ml of this solution until a pC_H of 1 was reached. Then the pC_H was adjusted to about 4 with (carbonate-free) 5% sodium hydroxide solution. Because of the heat of neutralization the solution had to be cooled with ice (p$C_H \simeq 4$ and 1*M* sodium perchlorate) until a temperature of about 23° was attained.

The 200 ml of ytterbium solution was divided into several portions. Each portion was adjusted to a different pC_H value from the others with 0.5% and 0.05% sodium hydroxide solution (in 1*M* sodium perchlorate). Then the series of solutions was set aside for half an hour. Next the solutions were centrifuged for about 10 min, the supernatants were removed and their pC_H values were measured accurately with an electrode system calibrated as described in the gadolinium paper.[3] Because of the alkali error and the occurrence of liquid junction potentials a glass–calomel electrode system lacks accuracy when pC_H exceeds 12.5, so in this region, the pC_H was determined by potentiometric titration. The temperature when the pC_H values were measured was 21.5 ± 0.2°.

After the pC_H determination, the solutions were acidified with perchloric acid to p$C_H \simeq 1$ and removed from the glove-box. Finally, the ytterbium was titrated photometrically with EDTA at pC_H 5.8 with Xylenol Orange as indicator ($\lambda = 575$ nm).

The precipitation procedure differed from previous ones[1–3] in that all the solutions prepared from the original 200-ml portion (p$C_H \simeq 4$) were treated separately. The pre-

* Part III: *Talanta*, 1980, **27**, 1047.

Table 1. Literature values for the constants

Ref.	log $*\beta_1$	log $*\beta_4$	log $*K_{so}$	Medium	Method
4	−6.7			sulphate, 0.05*M*, 25° equilibration 4 hr	pH determination
4	−8.4			sulphate, 0.001*M*, 25° equilibration 4 hr	pH determination
5	−4.3			(H,Li)ClO_4, I = 0.1, 25°	solvent extraction radiochemical detection
6	−8.01			NaCl + $NaClO_4$, I = 0.05, 25°	potentiometric titration
7			15.4	$NaClO_4$, I = 3, 25° aged ppte	
8	−8.08			$NaClO_4$, I = 0.3, 25°	potentiometric titration
9	−7.7			I = 0	empirical relation
9	−8.05			I = 0.05	empirical relation
9	−9.3			I = 3	empirical relation
10	−8.4	< −14	18.5	non-constant I, 25°	solubility measurements
11			17.2	25°, I = 0	oscillographic activity-product measurements
11			16.0	25°, I = 0.01, Cl^-	

cipitation procedure was repeated several times—with individual 200-ml initial portions—until the whole pC_H region 6–14.5 had been covered.

RESULTS AND CONCLUSIONS

The experimental results are shown in Fig. 1. No experiments were done at pYb′ < 1 as it was impossible to apply adequate corrections for the ionic strengths, which exceeded 1.

Above pC_H 13, the pC_H was calculated from titration results. For that purpose the molar concentration product for the autoprotolysis of water was estimated as pQ_W = 13.92 [I = 1 ($NaClO_4$), 21.5°]. This value was deduced from the literature[12–15] by applying the temperature correction $dpQ_W/dT = 0.033$[15] to the value pQ_W = 13.80 for a sodium perchlorate medium (I = 1, 25°). We also did some experiments to establish pQ_W by the titration method of Molina *et al.*[12] Contrary to their findings ($pQ_W^{2.5}$ = 13.60) we confirmed the selected value $pQ_W = 13.92 \pm 0.10$. There is a complication in using this value at high pC_H.

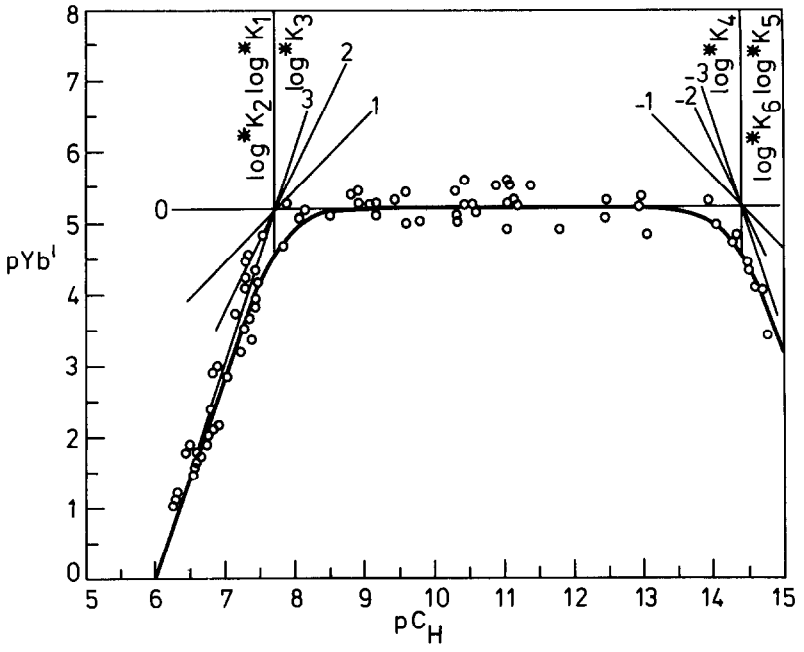

Fig. 1. The solid curve [the borderline of precipitation of $Yb(OH)_3$] was constructed with the values given in Table 3. The numbers −3, −2, −1, 0, 1, 2 and 3 near the straight lines correspond to the slopes of the straight-line segments [equations (1a)–(1g) in Table 2] approximating the exact envelope curve. The circles denote the experimental results. The vertical lines separate the regions where Yb^{3+}, $Yb(OH)_3$ and $Yb(OH)_6^{3-}$ respectively are the major species present.

Between pC_H 13 and 14, the sodium perchlorate in the medium is replaced gradually by sodium hydroxide. This slightly changes pQ_W in an unknown way.[14,15] Above pC_H 14 the ionic strength increases sharply with pC_H. This influences pQ_W to the extent of roughly 0.10 per unit of I, as we guess by comparison with other media.[14,15] It is rather speculative to compensate for this effect, and it was not done here, so conclusions from measurements above pC_H 14 should be considered with some reserve.

The borderline of the precipitation region of Yb consists of three, nearly straight parts connected by sharp bends. Regression analysis of the steep part up to pYb′ = 4.5 shows that the slope can be estimated as 2.82 ± 0.16. From theory, we know that the slope is equal to the electrical charge of the ion with the general composition $Yb_p(OH)_q^{(3p-q)+}$ that is the major ion present in the solution and in equilibrium with the precipitate. If a mixture of ions with differing charges were present in equal amounts, the precipitation borderline would show a strongly negative curvature. From the position of the experimental points (Fig. 1), it is clear that the steep part is nearly straight. Thus the solution must contain either one ion with an electrical charge of 3+ or a mixture of ions, all with the same charge, 3+. If all possible combinations with $(3p - q) = 3$ are considered, we get a series of $(p;q)$ of which the first are (1;0), (2;3), (3;6).

The occurrence, stability and structure of polynuclear hydroxides have been investigated extensively by physical chemists. A survey of the structures that have been identified reliably and confirmed experimentally has been given by Baes and Mesmer.[16] The variety is by no means as large as many investigators once thought. In accordance with this, structures such as $Yb_2(OH)_3$ and $Yb_3(OH)_6$ are rejected as unlikely to exist in major amounts. Yb^{3+} can be regarded as the main ion in solution at pC_H below 7.5.

This conclusion leads to the following set of equations for the straight-line segments which together compose the borderline of precipitation (Table 2).

If equation (1a) from Table 2 is assigned to the steep part of the borderline, the best fit to the experimental points is obtained with

$$pYb' = 3pC_H - (17.95 \pm 0.06) \quad (2)$$

Hence

$$\log {}^*K_{S0} = (17.95 \pm 0.06) \quad (3)$$

Equation (1d) can be assigned to the horizontal part of the borderline. The best fit corresponds to

$$pYb' = 5.24 \pm 0.04 \quad (4)$$

From equations (1d), (3) and (4), it follows that

$$\log {}^*\beta_3 = -23.19 \quad (5)$$

At high pC_H values ytterbium behaves amphoterically. The best fit to the experimental points in this region is obtained by assuming $Yb(OH)_6^{3-}$ to be the ion in solution. This leads to

$$pYb' = -3pC_H + (48.29 \pm 0.06) \quad (6)$$

from which follows

$$\log {}^*\beta_6 = -(66.24 \pm 0.06) \quad (7)$$

The values of ${}^*\beta_3$ and ${}^*\beta_6$ can be substituted in the general equation of the envelope curve.[3] If the terms for which no data are available are omitted, we get the equation

$$[Yb']_{max} = 10^{-3pC_H+17.95} + 10^{-5.24} + 10^{+3pC_H-48.29} \quad (8)$$

With this equation a good fit to the experimental points is obtained. From this fact the following conclusions can be drawn.

(*a*) The other straight-line segments corresponding to equations (1b), (1c), (1e) and (1f) contribute to the envelope curve either not at all or at most marginally.

(*b*) Marginal contribution of the lines (1b) and (1c) corresponds to the situation that the line segments (1a)–(1d) all intersect in one point (7.73; 5.24). As the intersecting points determine the p^*K_i values ($pC_H = p^*K_i$), this leads to $p^*K_1 = p^*K_2 = p^*K_3 = 7.73$ ($= -\frac{1}{3}\log {}^*\beta_3$). Similarly the segments corresponding to (1d)–(1g) intersect in the point (14.35; 5.24). Hence $p^*K_4 = p^*K_5 = p^*K_6 = 14.35$.

(*c*) The foregoing positions of the two groups of line segments are limiting positions. If (1b) and (1c), with slopes 2 and 1 respectively, are situated more to the right, the envelope curve is pushed away from the experimental points near the bend and this conflicts with (*a*). If they are positioned more to the left, the multiple intersection splits up into single points lying on both sides of pC_H 7.73. In any case this leads to $p^*K_1 > 7.73$. Consequently a reverse in the logical sequence of $p^*K_1 \leq p^*K_2 \leq p^*K_3$ occurs and this is fairly unlikely. Hence, the limiting position with all

Table 2. Equations for straight line segments of the precipitation borderline

Equation number	p	q	Slope $(3p - q)$	Equation
(1a)	1	0	3	$pYb' = 3pC_H - \log {}^*K_{S0}$
(1b)	1	1	2	$pYb' = 2pC_H - (\log {}^*K_{S0} + \log {}^*\beta_1)$
(1c)	1	2	1	$pYb' = pC_H - (\log {}^*K_{S0} + \log {}^*\beta_2)$
(1d)	1	3	0	$pYb' = -\log {}^*K_{S3} = -(\log {}^*K_{S0} + \log {}^*\beta_3)$
(1e)	1	4	−1	$pYb' = -pC_H - (\log {}^*K_{S0} + \log {}^*\beta_4)$
(1f)	1	5	−2	$pYb' = -2pC_H - (\log {}^*K_{S0} + \log {}^*\beta_5)$
(1g)	1	6	−3	$pYb' = -3pC_H - (\log {}^*K_{S0} + \log {}^*\beta_6)$

Table 3. Values for the constants at 21.5°C

$\log {}^*\beta_1 = -7.7$	$\log {}^*\beta_4 = -37.5$	
$\log {}^*\beta_2 = -15.5$	$\log {}^*\beta_5 = -51.9$	$\log {}^*K_{s0} = 18.0$
$\log {}^*\beta_3 = -23.2$	$\log {}^*\beta_6 = -66.2$	

segments going through one point is the most likely. Similar arguments hold for the second group of lines in the alkaline region.

When the values of $\log {}^*\beta_i$ ($i = 1, \ldots . 6$) and $\log {}^*K_{s0}$ are substituted in the general equation for the envelope curve, equation (8) is extended by four terms to become

$$[Yb']_{max} = 10^{-3pC_H+17.95} + 10^{-2pC_H+10.22} + 10^{-pC_H+2.49} + 10^{-5.24} + 10^{pC_H-19.59} + 10^{2pC_H-33.94} + 10^{3pC_H-48.29} \quad (9)$$

The added terms contribute marginally to the shape of the envelope curve. The bold curve in Fig. 1 has been constructed with this extended equation. The constants derived (Table 3) can be regarded as useful for analytical practice.

Ivanov-Emin and co-workers[17] studied the solubility of ytterbium hydroxide in strongly alkaline solution (0.01–18M). They found that the solubility reaches a maximum at an alkali concentration of 14.1M and sharply decreases at higher concentration where the solid phase sodium hexahydroxoytterbate precipitates. This also confirms the existence of $Yb(OH)_6^{3-}$ as an entity in equilibrium with solid ytterbium trihydroxide. From their results it can be concluded that p^*K_4, p^*K_5 and $p^*K_6 = 14.1 \pm 0.2$. Although their values refer to equilibrated solutions, the agreement with our results is striking.

If we assign the uncertainty in the position of the experimental points completely to pYb′, a standard deviation of 0.45 is found for the individual pYb′ values. This holds for both the steep parts and the horizontal part.

As colloidal turbidity frequently accompanied precipitate formation in the pC_H region 6–7, the influence of this phenomenon on pYb′ was studied. The colloidal turbidity disappeared after 24 hr of aging. The pC_H hardly changed (<0.05) on aging; comparison of the titration results before and after aging showed that pYb′ varied by about 0.20 in these cases. As these changes are smaller than the standard deviation for pYb′, the influence of colloid formation on the results can be neglected.

DISCUSSION

Concerning the differences between our results and those listed in Table 1, the same comment can be made as before.[1–3] Our "fresh precipitate" measurements turned out to be fairly reproducible and rather insensitive to aging for 24 hr. Concerning the errors in the constants, only those in $\log {}^*K_{s0}$, $\log {}^*\beta_3$ and $\log {}^*\beta_6$ can be deduced from the measurements (0.06, 0.07 and 0.1 respectively). As the other constants are found partly by analogy, it is a bit speculative to discuss their errors. We estimate that their uncertainty is of the same order.

Our method is not able to distinguish between species (such as Yb^{3+}, $Yb_2(OH)_3^{3+}$) that have the same electrical charge. We said before that there are good reasons to assume that Yb^{3+} is the main component in acidic solution, but this does not, however, exclude the existence of minor amounts of polycomplexes. Other experimental techniques may clarify the situation; the existence of polycomplexes has never been indicated in the literature. Also, because for stereochemical and statistical reasons[16] the formation of polycomplexes is fairly unlikely, we assume that they are not formed under the experimental conditions in our experiment.

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SHORT COMMUNICATIONS

SIMPLE AND INEXPENSIVE MICROCOMPUTER-CONTROLLABLE STEPPING MOTOR AND DRIVE CIRCUIT

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Summary—The construction of a simple stepper motor and drive circuit made from commercial components is described. The use of the stepper motor for a monochromator wavelength drive is discussed. The rate and direction of scan can be controlled by either a hardwired timer circuit or by a microcomputer.

Stepping motors are important components of many types of analytical instrumentation where precise mechanical positioning is required.[1] Common applications include the wavelength drive for monochromators and positioning of components (*e.g.*, a burner in flame spectrometry) mounted on translational stages. Commercial stepping-motor drive and control circuitry is available to modify existing instrumentation but is usually relatively expensive. This paper is concerned with the construction of a stepper-motor and drive circuit from commercial components at cost of less than $100 for parts.

The particular application for which the stepping-motor unit was designed is the monochromator drive for a Schoeffel (Westwood, NJ) GM100 monochromator, which is used for the excitation and emission monochromators of a molecular fluorescence spectrofluorometer.[2] The monochromator is normally provided with a synchronous-motor wavelength-drive set at one prechosen scan-rate, and a stepping-motor drive is not available from the company. A stepping-motor drive was needed to allow different scan-rates to be chosen with the scan-rate controlled either by a separate clock circuit or by a microcomputer.

It was also desired to be able to synchronize the monochromator scan-rate to the recorder-drive scan-rate so that the monochromator and recorder drive could be started simultaneously and so that one inch on the recorder chart paper corresponded to a prechosen number of nanometers. The Heath (Benton Harbor, MI) SR255B recorder employed uses a stepper-motor drive and has provision for using an external pulse train. If the external pulse train is set to 50 Hz, the switch-selectable recorder scan-rates (in./min) can be used directly.

EXPERIMENTAL

Figure 1 presents the schematic of a simple stepping-motor and drive unit based on the North American Philips Controls Corp. (Cheshire, CT) SAA1027 driver IC and K82701-P2 bidirectional stepper motor ($15 and $28, respectively).[3,4] The driver IC is designed to drive directly 4-phase stepper motors which require less than 350 mA per phase. The bidirectional stepping motor has a step angle of 7°30′ (48 steps per revolution) and a torque of about 6 oz. in. (~430 g.cm) at 100 steps/sec. The maximum step-rate is about 300 steps/sec.

Normally the set is tied high and the direction and trigger inputs control the stepper motor. A logic 1 (5-V) signal applied to the direction input is translated into a +12-V signal by IC1 (buffer) and fed into IC3 (driver) which provides for a counterclockwise rotation of the stepper. Likewise, a logic 0 (0-V) signal to the directional input will result in clockwise rotation. Upon application of a positive TTL edge (0 → 5 V, 50 nsec minimum width) at the trigger input, IC2 (one-shot) delivers a 0.23-msec logic 0 pulse to IC1, which translates the TTL pulse into a +12 → 0 V pulse for IC3 and causes the motor to step one step. IC2 is set near the minimum pulse width that IC3 will recognize. Components R1, C1, and C2 provide added noise immunity for IC3, while R2 provides bias current for the four internal NPN drivers in IC3. Diodes D1–D4 are for elimination of transients caused by switching of the motor windings. Finally, IC4, IC5, C3, D5, and T1 provide regulated +5 and +12 V power for the circuit and may be eliminated if external power supplies are available.

Figure 2 is the schematic for the controller constructed to run the stepper motor when connected to the monochromator. Master clock IC1 is adjusted with R1 and a frequency meter to provide a 400-Hz pulse train. IC2 divides this signal by 8 to provide a 50-Hz signal suitable for the external drive of the chart-recorder employed, which synchronizes the chart-recorder and stepper-drive pulses. IC3 and IC4 divide the 400-Hz master clock signal by 25 to produce a 16-Hz pulse train for IC5 and IC6. IC5 and IC6 provide a dip-switch programable divide-by-*N* network,[5] the output of which is buffered by T2. Division

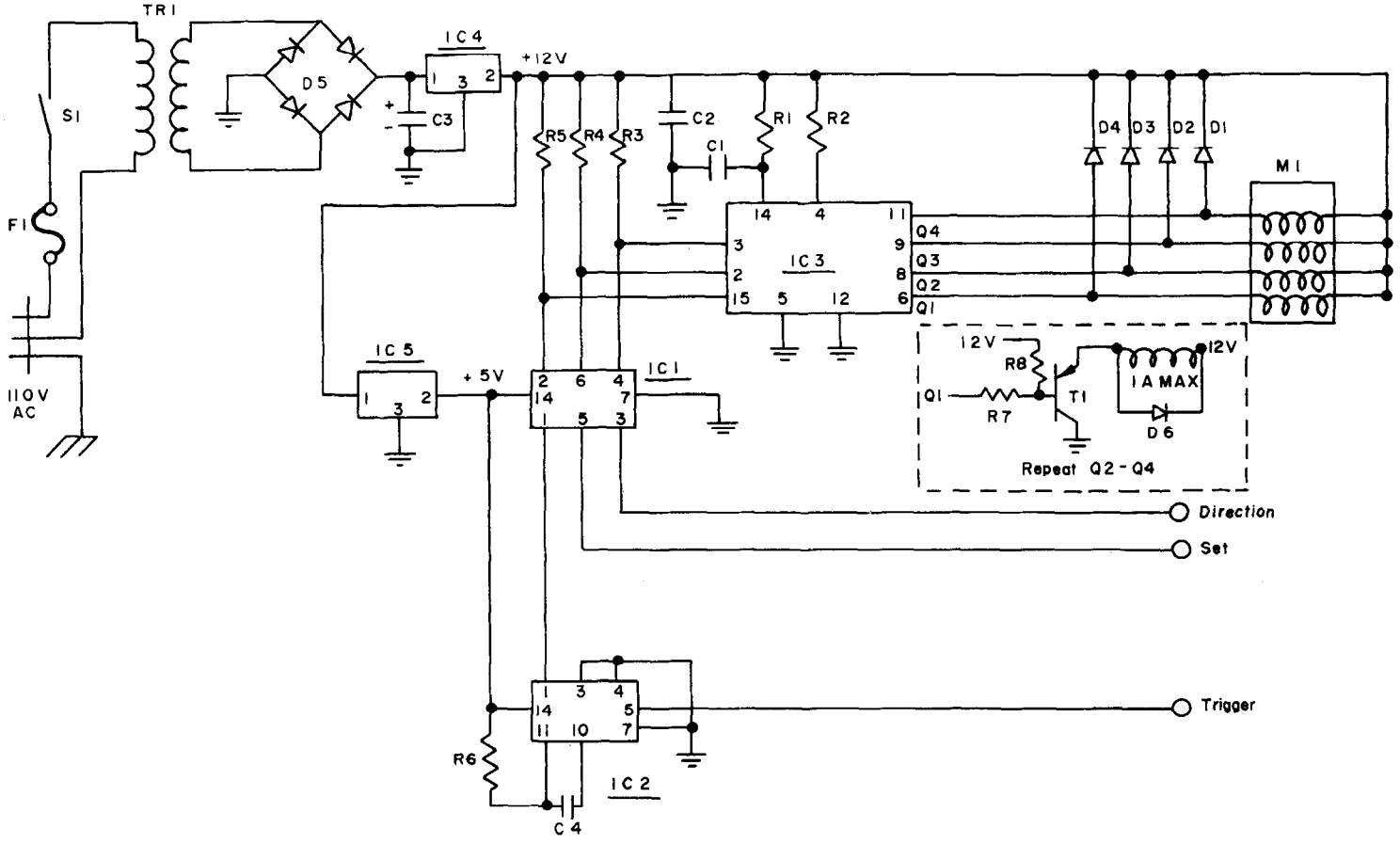

Fig. 1. Stepping motor drive circuit. IC1, 7407 buffer; IC2, 74121 one-shot; IC3, SAA 1027 driver; IC4, LM340T-12; IC5, LM340T-5; R1, 100 Ω; R2, 150 Ω, 1 W; R3, R4, R5, 3.3 kΩ; R6, 33 kΩ; R7, 330 Ω; R8, 10 kΩ; C1, C2, 0.1 μF cer; C3, 4700 μF elec; C4, 0.01 μF cer; D1, D2, D3, D4, D6 1N4119; D5, RS276-1171; T1, 2N3468; TR1, 12.6 V; S1, SPST; F1, 0.25 A; M1, K82701-P2.

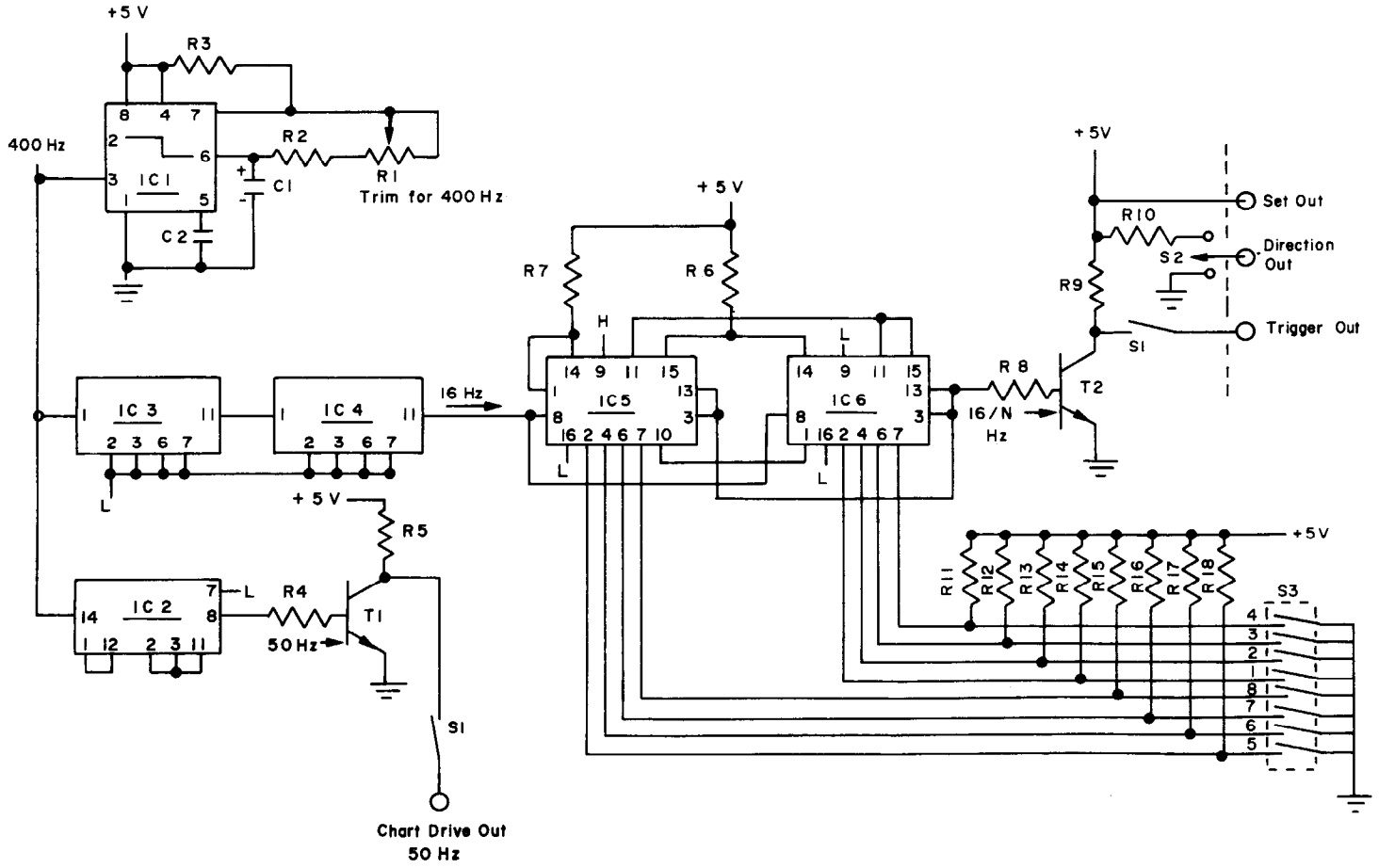

Fig. 2. Stepping-motor control circuit. IC1, 555 timer; IC2, IC3, IC4, 7490 decade counter; IC5, IC6, DM8520 modulo-*N*-divider; R1, 2 kΩ 10 turn; R2, 18 kΩ; R3, R6, R7, R9, R10, 1 kΩ; R4, R8, 10 kΩ; R5, 820 Ω; R11–R18, 1.2 kΩ; C1, 0.1 μF tant; C2, 0.01 μF cer; S1, DPST; S2, SPDT; S3, 8-pole dip switch.

by factors ranging from 2 to 255 is determined by the positions of the 8 switches in S3, allowing for output pulse trains of from 8 to 0.0627 Hz. Switch S1 allows the stepping motor and chart drive to be started simultaneously, while switch S2 allows setting of the direction of rotation of the stepping motor. The circuit is powered by a regulated +5-V, 300-mA power supply.

The shaft of the Philips stepping motor was connected to the wavelength-drive shaft of the GM100 monochromator with 1:1 gears. The worm-screw wavelength shaft of the monochromator is set up for 50 nm/revolution. With the Philips stepping motor and the control circuitry of Fig. 2, the scan-rate can be varied over the 4–500 nm/min range, with each step corresponding to 1.04 nm.

The stepping-motor drive circuit and monochromator can also be controlled directly by a KIM 1 single-board microcomputer (MOS Technology, Norristown, PA) to produce an intelligent monochromator-drive system. Only two I/O lines are needed for the direction and trigger inputs. With minimal software, the computer can duplicate the functions of the circuit in Fig. 2 except that the scan-rates and direction are keyboard-controlled. With more extensive software, the computer can be programmed to control the wavelength range scanned, to implement repetitive scanning, and to slew between wavlengths. Once the initial wavelength setting of the monochromator is keyed in, suitable software will keep track of the wavelength at any time. It is implicitly assumed that the stepper motor responds correctly to all pulses from the computer, and does not respond to any other stimulus.

Other 4-phase stepping motors (*e.g.*, with different torques or stepping angles) from Philips or other manufacturers can be used with the control circuitry described. Stepper motors with larger current requirements may be used if the four external transistors are added as shown in the inset of Fig. 1 (dashed lines) and if a larger 12-V power supply is employed.

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SELECTIVE EXTRACTIVE SEPARATION OF ZINC, CADMIUM AND LEAD FROM IODIDE MEDIA WITH MESITYL OXIDE

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Summary—The extractive separation of zinc, cadmium and lead with mesityl oxide has been investigated as a function of iodide concentration and acidity. The recovery of these metals from the organic phase by back-extraction and the behaviour of other elements under the optimal conditions established for each metal ion have been examined. The method developed has been applied to the separation of these metals from rock samples before determination by spectrophotometry.

Group extraction is generally preferred for the concentration and separation of trace elements from complex matrices before determination by atomic-absorption spectrophotometry. Such an approach, however, is unsuitable when the methods employed for the determination are poorly selective. In such cases, for speed, economy and simplicity, it would be of great advantage to be able to use successive separations from a single sample with the minimum of manipulation. This paper presents such a method for the separation of zinc, cadmium and lead and its evaluation for separation of these elements from silicate rock samples.

Extraction from iodide medium was selected because little work has been done on its use for separation of these metals, the main emphasis having been on selective extraction of cadmium from zinc with solvents such as cyclohexanone or TBP,[1] aliphatic alcohols,[2] Amberlite LA-2 in xylene[3] and tribenzylamine in chloroform.[4] Zinc and cadmium can be simultaneously extracted from 1.0*M* hydriodic acid into xylene containing Aliquat 336-S-I,[5] and the zinc selectively stripped with 1*M* sodium sulphite. Methyl isopropyl ketone has been recommended for the extraction of lead from 1*M* potassium iodide.[6]

The proposed method is based on extraction of cadmium and lead into mesityl oxide from a mixture of zinc, cadmium and lead in 0.05*M* potassium iodide, followed by extraction of the zinc into mesityl oxide after the iodide concentration has been raised to >3.0*M*. The lead can be stripped quantitatively with saturated potassium iodide solution. The parameters governing the extraction and stripping equilibria have been investigated.

EXPERIMENTAL

Reagents

Standard zinc, cadmium and lead solutions (each 100 ppm), potassium iodide solutions (saturated and 0.5*M*) and 1*M* ammonia/ammonium chloride buffer (pH 10.0). Mesityl oxide for extraction.

General procedure

Transfer a portion of sample solution into a 60-ml separatory funnel. Add enough sulphuric acid and potassium iodide to make their concentrations 1*N* and 0.05*M* respectively and shake the solution for 1 min with 10 ml of mesityl oxide. Allow the phases to separate and preserve the organic phase (**I**) for the recovery of cadmium and lead. Transfer the aqueous phase containing zinc into another separatory funnel and add sufficient potassium iodide to make its concentration >3.0*M*. Extract with 10 ml of mesityl oxide and retain the organic phase (**II**) for the recovery of zinc.

Shake the organic phase (**I**) with two 5-ml portions of saturated potassium iodide solution for 5 min each to strip the lead, leaving cadmium in the organic phase (**III**). Strip zinc and cadmium from organic phases (**II**) and (**III**) respectively by shaking each with 5 ml of ammonia buffer for 5 min and washing with 5 ml of water.

Determination

Quantitatively collect the stripping (and wash) solutions in 25-ml standard flasks and proceed as follows. If the concentrations are high, use an appropriate fraction.

Zinc.[7] Add, with mixing, 2.5 ml each of 10*N* orthophosphoric acid and 5% potassium thiocyanate solution and 5 ml of 0.005% Rhodamine 6G solution, followed by 1 ml of 1% gelatin solution. Dilute to the mark with distilled water and measure the absorbance against a reagent blank in 40-mm cells at 575 nm.

Cadmium.[8] Add 2.5 ml each of citrate buffer (pH 4.0), 10% potassium iodide solution and 0.05% pyronine G solution followed by 1 ml of 1% gelatin. Dilute to the mark and measure the absorbance against a reagent blank at 575 nm in 10-mm cells.

Lead.[9] Add 2.5 ml each of ammonia buffer (pH 10.0) and $2 \times 10^{-3}M$ 4-(2-pyridylazo)resorcinol and make up to the mark. Measure the absorbance against a reagent blank in 10-mm cells at 530 nm.

Establish the concentration by reference to appropriate calibration graphs prepared from the respective standard metal solutions for 0–5 μg of zinc, 0–15 μg of cadmium and 0–125 μg of lead.

RESULTS AND DISCUSSION

Preliminary studies were made with 100 μg of zinc, cadmium or lead in 10 ml of aqueous phase and

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extraction into 10 ml of organic phase by shaking for 5 min. The degree of extraction was determined by establishing the unextracted metal content of the aqueous phase by atomic-absorption spectrophotometry.

The extraction of zinc, cadmium and lead as a function of potassium iodide concentration is shown in Fig. 1. For quantitative extraction of cadmium and lead, the iodide concentration should be greater than 0.01 and 0.04*M* respectively. Higher iodide concentrations do not affect the extraction of cadmium, but above 1.5*M* decrease the extraction of lead, which becomes negligible at iodide concentrations $>7.0M$. The extraction of zinc is insignificant at iodide concentrations up to 0.08*M* and becomes quantitative only when the iodide is $\geqslant 3.0M$.

Figure 2 shows the effect of acidity, varied by adding sulphuric acid or sodium hydroxide solution. Though the extraction of lead is quantitative at pH $\leqslant 5.0$, acidities greater than 0.2 and 0.5*M* respectively are needed for the extraction of zinc and cadmium.

Extraction from 0.2–3.0*M* hydrochloric acid instead of sulphuric did not affect the extraction of lead but reduced the extraction of zinc and cadmium to some extent, giving 98% extraction for zinc and 84% for cadmium from 0.4–0.5 and 0.1–0.3*M* hydrochloric acid respectively.

1,2-Dichloroethane, n-butanol, cyclohexanol, isobutyl methyl ketone (IBMK), cyclohexanone, ethyl acetate and diethyl ether were tested as extraction solvents (5 min shaking) but only IBMK and cyclohexanone proved satisfactory, and then only for cadmium. Cyclohexanone gave 96% extraction of lead and IBMK 85% for zinc. Diethyl ether and 1,2-dichloroethane were found to give very poor extraction (<1%).

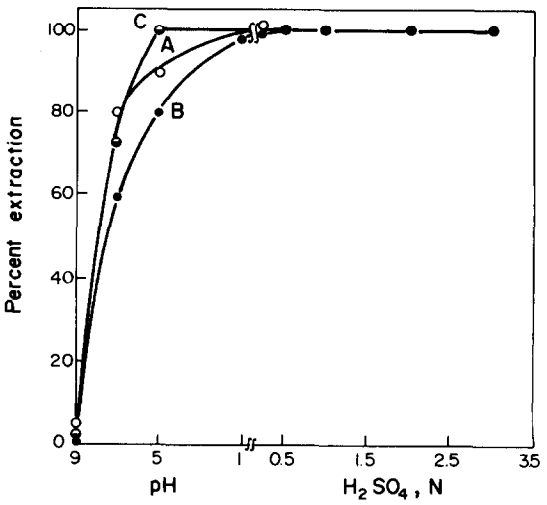

Fig. 2. Effect of H_2SO_4 or pH on extraction behaviour of A, zinc (4*M* KI); B, cadmium (0.05*M* KI); C, lead (0.05*M* KI).

Studies with mesityl oxide showed that a shaking time of 30 sec was sufficient for the quantitative extraction of zinc, cadmium and lead and that an aqueous to organic phase volume ratio of up to 10:1 was without influence on the extraction behaviour of these elements.

Ammonia buffer (pH 10.0) was found to strip both cadmium and lead quantitatively in a single step, but though water was found to strip lead quantitatively, it would also strip 65% of the cadmium. Stripping with saturated potassium iodide solution was therefore used since high concentrations of iodide prevent the extraction of lead into mesityl oxide (Fig. 1). Zinc was found to be stripped quantitatively with ammonia

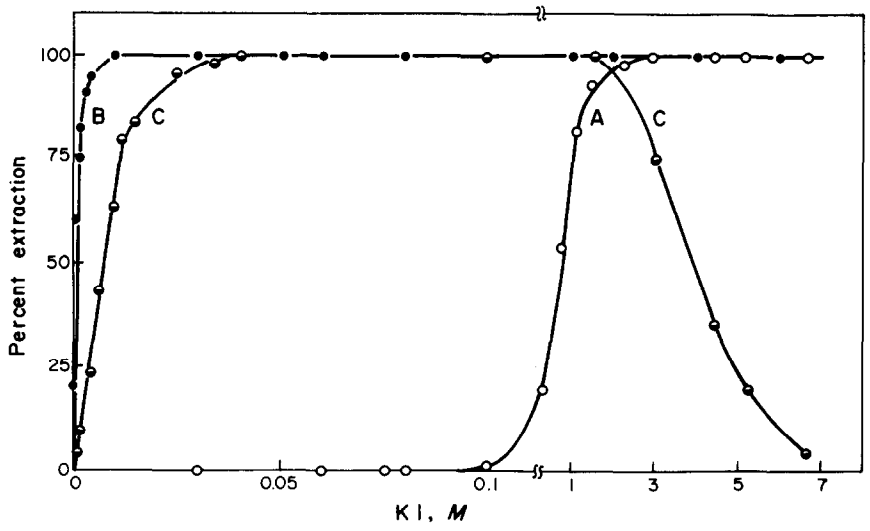

Fig. 1. Effect of potassium iodide on extraction behaviour of zinc, cadmium and lead with mesityl oxide (1*N* H_2SO_4; A, zinc; B, cadmium; C, lead).

Table 1. Results obtained by spectrophotometry for synthetic mixtures after separation by proposed method

No.	Composition (μg)	Amount found, μg		
		Zn	Cd	Pb
1	Zn(100) + Cd(100) + Pb(100)	99.6	100	101.2
2	Zn(50) + Cd(500) + Pb(20)	50.0	490	20.5
3	Zn(500) + Cd(5) + Pb(10)	496	5.2	9.6
4	Zn(5) + Cd(5) + Pb(500)	5.0	5.0	503
5*	Fe(1000) + Mn(1000) + Cu(500) + Zn(20) + Cd(20) + Pb(20)	20.8	19.6	20.5
6	Ag(500) + Co(500) + Ni(500) + Zn(10) + Cd(5) + Pb(10)	9.8	5.0	9.6
7*	Ni(500) + As(500) + Cu(500) + Zn(10) + Cd(10) + Pb(20)	10.0	10.2	20.6
8	Ca(5000) + Mg(5000) + Al(5000) + Zn(5) + Cd(5) + Pb(20)	5.0	5.2	20.0

* In the presence of 5 ml of 20% ascorbic acid solution.

buffer, but water gave incomplete stripping (85%) in a single step.

The results for extraction and stripping of each of these metals in the range 5–2000 μg, either separately or in mixtures in varying ratios with a total amount of 5 mg, showed excellent recovery for cadmium and lead over the range studied and for zinc up to 500 μg. Zinc in amounts greater than 500 μg was found to be subject to co-extraction with cadmium and lead to the extent of 2–3% from 0.05*M* potassium iodide medium, but a single wash with 10 ml of 0.05*M* potassium in 1*N* sulphuric acid would remove the co-extracted zinc without affecting the recovery of cadmium and lead.

Nature of the extracted species

The plots of log *D* *vs.* log $[I^-]$ for zinc, cadmium and lead were straight lines with slopes of 2.10, 2.08 and 2.01 respectively indicating that all three metals were extracted as MI_2 species. When the iodide concentration was greater than 1.5*M*, the plot for lead gave a slope of −4.3 indicating that formation of PbI_4^{2-} was responsible for inhibiting the extraction. However, the presence of a large excess of iodide did not affect the extraction of cadmium, although CdI_4^{2-} is known to exist. Quantitative extraction of cadmium from 1.5*M* iodide required at least 2 min of shaking, in contrast to the 30 sec required for extraction from 0.05*M* iodide, suggesting that higher iodide complexes with cadmium are formed but merely reduce the rate of extraction.

Attempts to establish the degree of solvation of the extracted species were unsuccessful, so presumably no specific solvate is formed.

Interferences

Hg(II), Bi, Sb(III), Cu(II), Tl(I), Ag, As(III), Fe(III), Co(II), Ni and Mn(II) were tested at the 100-μg level for interference. To avoid liberation of iodine, the studies on Cu(II) and Fe(III) were made after addition of ascorbic acid.

The extraction of As(III), Co(II), Fe(III), Ni and Mn(II) was found to be negligible from 0.05 and 4*M* iodide. Hg(II), Bi, Ag were quantitatively extracted and Tl(I) and Sb(III) 88% extracted from 0.05*M* iodide into mesityl oxide, and saturated potassium iodide solution gave 75% stripping of Sb(III) and complete stripping of Hg(II), Bi, Ag and Tl(I). Sb(III) could be completely stripped with ammonia buffer.

Table 2. Analysis of silicate rocks*

Sample	Metal added, μg/g			Metal found, μg/g Spectrophotometry			AAS		
	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb
1. Basalt	—	—	—	84	7.0	438	84	7.1	440
	40	5	200	126	12.4	632	125	12.4	639
	80	10	500	163	17	940	165	17.0	945
2. Dolerite	—	—	—	107	9.8	432	105	10.0	430
	50	10	200	156	20	621	155	20.6	628
	50	20	500	156	28.8	930	155	29.2	938
3. Jasperoid	—	—	—	6.8	14.0	364	6.8	14.4	360
	4	10	100	10.6	25.0	460	10.3	25.0	462
	10	20	300	16.6	35.0	652	16.8	35.6	658

Copper could be completely extracted from 4.0*M* iodide and stripped with ammonia buffer, like zinc.

The influence of 5 mg each of Al, Ca, Mg, Fe(III), Na and K, which are usually present in rock samples, on the recovery of 20 μg each of zinc, cadmium and lead was examined. The results indicated no loss in the recovery of these metals.

Application

The method developed was applied in conjunction with spectrophotometry for the determination of zinc, cadmium and lead in synthetic mixtures, by the procedures described. Zinc was determined with Rhodamine 6G[7] and cadmium with pyronine G.[8] As the colour reaction of lead with PAR[9] was unaffected by the presence of excess of iodide, this method was used for lead. The results are shown in Table 1.

The zinc, cadmium and lead contents of three rock samples—basalt, dolerite and jasperoid—were then determined by spectrophotometry after separation by the method developed and compared with those obtained by atomic-absorption spectrophotometry.[10] The samples were opened out by repeatedly heating 0.5 g of finely ground material with sulphuric acid and hydrofluoric acid.[10] The residue was dissolved in the minimum volume of dilute hydrochloric acid needed and made up to 50 ml with water. Aliquots were treated with 2 ml of 10% ascorbic acid solution before analysis. The results, and those obtained for samples spiked with the metal ions under study, are shown in Table 2. Clearly, the proposed method works satisfactorily.

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EVALUATION OF SULPHURIC ACID TREATMENT AS THE CLEAN-UP STEP FOR THE ESTIMATION OF BHC AND DDT IN FATTY AND NON-FATTY FOODS

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Summary—The potential of sulphuric acid treatment for clean-up of 10 food composites comprising 36 food items of north Indian total diet has been evaluated for the estimation of BHC and DDT residues by gas chromatography with electron-capture detection. In all cases the clean-up was satisfactory without resort to column chromatography. The chromatograms obtained were free from interference by the co-extractives. The method is simple, rapid and economical.

Adsorption column chromatography is invariably used in standard procedures for final clean-up in multiresidue analysis of organochlorine insecticides by gas chromatography with electron-capture detection (GC–EC).[1–3] Additional clean-up by passage through a Celite–sulphuric acid column[4,5] or by shaking with concentrated sulphuric acid[6] has also been suggested for eliminating interferences in the estimation of acid-stable insecticides and PCBs in fatty substrates. The direct treatment of a petroleum ether solution of butter fat with concentrated sulphuric acid in a specially designed apparatus has been used in the estimation of several organochlorine residues[7,8] and adopted for estimation of certain organochlorine insecticides in vegetable oils[9] and milk fat.[10,11] The present work extends the use of sulphuric acid as clean-up agent to non-fatty foods.

EXPERIMENTAL

Reagents

Acetone, acetonitrile and n-hexane, all redistilled in all-glass apparatus; concentrated sulphuric acid; anhydrous sodium sulphate, sodium chloride. The suitability of the reagents for residue analysis was checked by running reagent blanks.

BHC (α-, β-, γ-, δ-isomers), *p, p'*-DDD, *p, p'*-DDE and *p, p'*-DDT (all >95% purity) were obtained from the U.S. Environmental Protection Agency, and used as reference standards.

Apparatus

A Packard model No. 7624 gas chromatograph equipped with a tritium-source electron-capture detector and a 1.84 m × 2 mm glass column packed with 1.5% SP-2250 + 1.95% SP-2401 on 110–120 mesh Supelcoport. Operating temperatures were: injector 210°, column 190°, detector 200°. Nitrogen was used as carrier-gas at a flow-rate of 70 ml/min.

The extraction column[7] was made locally.

Preparation of total diet composites

Representative amounts of the constituents of each food group of the north Indian diet (Table 1) were processed (washed, peeled, baked, cooked, *etc.*) according to local practices and homogenized in a blender to obtain the composites. To avoid transfer of residues from fats and oils, boiling was used instead of frying. For foods that are eaten raw as well as cooked, half the composite was raw and the rest cooked.

Extraction and partitioning

Cereals, pulses, root vegetables and other vegetables. Composite equivalent to 50 g fresh weight was extracted with 100 ml and then 50 ml of acetonitrile by blending for 3 min each time. The combined extract was transferred to a 1-litre separatory funnel, diluted with 600 ml of 5% aqueous sodium chloride solution and partitioned with two 100-ml portions of n-hexane. The combined n-hexane layers were dried over anhydrous sodium sulphate and concentrated on a flash evaporator to about 10 ml, followed by concentration to about 5 ml by a gentle stream of nitrogen.

Fruits. Composite equivalent to 50 g fresh weight was extracted with 100 ml and then 50 ml of a 2:1 v/v acetonitrile–water mixture and further processed as for cereals *etc.*

Milk and milk products, meat and eggs and sugar, condiments, preserves, etc. Composite equivalent to 50 g fresh weight was extracted with 100 ml and then 50 ml portions of a 2:1 v/v n-hexane–acetone mixture by blending for 3 min each time. The combined extract was transferred to a 1-litre separatory funnel and washed with two 300-ml portions of 5% aqueous sodium chloride solution, then dried and concentrated as for cereals, *etc.*

Oils and fats. Three 5-g portions of composite were dissolved in separate 100, 15 and 15 ml portions of n-hexane.

Water and beverages. A 1-litre portion of composite was partitioned with two 100-ml portions of n-hexane. The combined extract was dried and concentrated as for cereals, *etc.*

Sulphuric acid clean-up

For all composites except oils and fats. The concentrated n-hexane extract was taken in a 25-ml separatory funnel and sulphuric acid was added dropwise from a Pasteur pipette till the n-hexane layer became clear. The spent sul-

Table 1. Composition of various food composites

Composites	Composition*
Cereals	Wheat flour (79) + rice (16) + bread (5)
Pulses	Rajmash (25) + black gram (25) + green gram (25) + soya beans (25)
Root vegetables	Potato (69) + onion (23) + carrot (8)
Other vegetables	Tomato (60) + cauliflower (24) + coriander leaves (6) + gourd (6) + green chillies (4)
Fruits	Mango (66) + Sapota (34)
Oils and fats	Hydrogenated vegetable oil (68) + butter fat (32)
Milk and milk products	Milk (83) + curd (16) + cheese (1)
Meat and eggs	Chicken (45) + eggs (55)
Sugar, condiments, preserves, *etc.*	Sugar (58) + salt (22) + fruit cake (4) + fennel (4) + tomato ketchup (4) + turmeric (3) + coriander (3) + chillies (2)
Water and beverages	Tap water (66) + tea infusion (26) + soft drinks (6) + coffee infusion (2)

* Numbers in brackets represent per cent of various food items (raw weight basis) in a composite. These values were based on a dietary survey.

phuric acid was discarded and the upper layer washed with 10-ml portions of distilled water till neutral.

Only 15 ml of sulphuric acid were required for complete clean-up, except for the milk and milk products, meat and eggs, and sugar, condiments, preserves, *etc.*, which required 30–40 ml.

Oils and fats composite. The Veirov and Aharonson technique[8] was used. The 100 ml of n-hexane containing 5 g of sample was taken in a glass column fitted with a stopcock, and 100 ml of sulphuric acid were added dropwise. The used sulphuric acid was occasionally discarded. The second 5-g sample (in 15 ml of n-hexane) was added to the column and again 100 ml of sulphuric acid were added dropwise. This step was repeated with the third 5-g sample. After discard of the lower phase, the n-hexane solution was washed with distilled water till acid-free, dried over anhydrous sodium sulphate and concentrated to suitable volume by flash evaporation and a gentle stream of nitrogen.

Recovery studies

All the composites except water and beverages were fortified, before extraction, with 0.016, 0.064, 0.016, 0.032, 0.02, 0.04 and 0.04 μg of α-, β-, γ-, δ-BHC, *p, p'*-DDE, *p, p'*-DDD and *p, p'*-DDT, respectively, per g. For the water and beverages composite, the fortification levels were 0.8, 3.2, 0.8, 1.6, 1, 2 and 2 μg/l. The compounds were added as a standard mixture.

Analysis

All the samples were analysed in duplicate by GC–EC, with peak-height measurement. Because of the possible presence of PCBs (which have similar retention times to some of the test compounds) in the food samples, the peaks were identified by means of the alteration in peak pattern after alkaline treatment.[12] The limit of determination was taken as the amount giving 10% of full-scale deflection, and for aldrin under the operating conditions was 0.08 μg/l. for the water and beverages composite and 0.016 μg/g for all other composites when the amounts injected for GC corresponded to 500 and 25 mg of sample respectively.

RESULTS AND DISCUSSION

Recoveries from the composites fortified with BHC isomers and *p, p'*-DDT and its derivatives ranged from 80 to 107% for all groups except milk and milk products, for which the recoveries were 74–109% (Table 2). The values for the unfortified composites are given in Table 3. The greater spread of recoveries for the milk and milk products composite may be attributed to the low level of fortification relative to the high residue values in the unfortified sample (Table 3).

Table 2. Recoveries (and range, duplicate results) of BHC and DDT residues from fortified samples of the food composites

Composites	α-BHC	β-BHC	γ-BHC	δ-BHC	*p, p'*-DDE	*p, p'*-DDD	*p, p'*-DDT
Cereals	93 ± 3	93 ± 2	92	92 ± 2	99 ± 3	94 ± 2	96 ± 2
Pulses	97 ± 2	98 ± 1	81	97 ± 2	99 ± 1	93 ± 2	96 ± 2
Root vegetables	99 ± 1	100	93 ± 2	99	89 ± 2	87 ± 1	96 ± 2
Other vegetables	93	92 ± 3	90 ± 2	92 ± 4	90 ± 4	85 ± 2	86 ± 3
Fruits	94 ± 3	99 ± 1	91 ± 2	103 ± 1	106 ± 1	107 ± 1	94 ± 3
Oils and fats	86 ± 2	88 ± 4	97 ± 7	100 ± 7	93 ± 2	101	102 ± 1
Milk and milk products	74	109 ± 5	94 ± 1	107 ± 5	79 ± 2	75 ± 2	76
Meat and eggs	91 ± 5	94 ± 4	84 ± 1	81 ± 2	95 ± 3	83 ± 3	83 ± 2
Sugar, condiments, preserves, *etc.*	84 ± 3	98	81	94 ± 2	80 ± 1	93 ± 4	99 ± 1
Water and beverages	90 ± 1	95	93	93 ± 2	85 ± 1	100 ± 1	100

Table 3. BHC and DDT residues found in unfortified samples of the food composites

Composites	α-BHC	β-BHC	γ-BHC	δ-BHC	p,p'-DDE	p,p'-DDD	p,p'-DDT
Cereals, *ng/g*	2	6	2	4	ND	ND	ND
Pulses, *ng/g*	ND	ND	6	ND	ND	ND	ND
Root vegetables, *ng/g*	ND	ND	5	ND	ND	ND	ND
Other vegetables, *ng/g*	67	ND	40	ND	171	ND	ND
Fruits, *ng/g*	26	ND	19	ND	ND	ND	33
Oils and fats, *μg/g*	0.033	0.029	0.020	0.016	0.113	0.36	0.203
Milk and milk products, *ng/g*	84	91	49	45	24	40	41
Meat and eggs, *ng/g*	26	74	17	6	29	8	47
Sugar, condiments, preserves, *etc.*, *ng/g*	36	18	18	8	11	ND	ND
Water and beverages, *μg/l.*	0.101	ND	0.143	0.140	0.60	0.35	1.00

ND = not detectable (below limit of determination).

The clean-up method resulted in gas chromatograms free from any interference by co-extracted material. The "other vegetables" group still gave a light green colour after the sulphuric acid treatment, but there were no extraneous peaks causing interference in the GC determination.

A number of other compounds, such as aldrin, α- and γ-chlordane, heptachlor and its epoxide, hexachlorobenzene, *o,p'*-TDE and Aroclor 1254 have been reported to be stable to treatment with sulphuric acid and can be determined by this method. However, dieldrin and endrin are degraded and thus cannot be determined by this method.[7] Similarly, endosulphan A, B and sulphate have been reported to be partially degraded by this treatment.[13] Aldrin was tested at the 0.05 μg/g level in the solid composites and its recovery found to be 83–96%.

Since the sulphuric acid treatment is simple, rapid and economical, it holds promise for final clean-up, instead of with adsorbents (Florisil, alumina, silica, *etc.*), for acid-stable chlorinated hydrocarbon residues in a wide variety of fatty and non-fatty substrates. Supplementary treatment with alcoholic potassium hydroxide can be used for confirmation of the identity of insecticide residues and the presence of PCBs.[12]

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QUANTITATIVE STUDY OF THE REACTION BETWEEN QUINONES AND IRON(II) IN PHOSPHORIC ACID MEDIUM

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Summary—A simple method is described for the determination of some quinones with ammonium iron(II) sulphate, by titration potentiometrically or with cacotheline, Methylene Blue, Methylene Green, thionine, Toluidine Blue, Azure A, Azure C or Azure-2-eosin as redox indicators. The reverse titrations can be used for estimation of iron(II). Alternatively, excess of iron (II) can be added and the iron(III) produced determined colorimetrically with thiocyanate.

Quinones can be estimated in acid solution by titration with chromium(II), tin(II), titanium(III) or vanadium(II), the quinones being reduced to the corresponding hydroquinones.[1] Iron(II) is a mild reducing agent in common acids, the conditional electrode potentials of the iron(II)–iron(III) couple being 0.71 V in 0.5*M* hydrochloric acid and 0.68 V in 1*M* sulphuric acid.[2] Headridge and Wilson[3] titrated quinones with iron(II) potentiometrically at pH 3.0 in the presence of hydrofluoric acid and ammonium fluoride. Rao and Sagi[4] reported that the formal potential of the iron(II)–iron(III) couple changed from 0.429 to 0.400 V as the phosphoric acid concentration was varied from 8.92 to 11.58*M*. In view of the hazard to the operator and damage to the glassware associated with use of hydrofluoric acid, and the inconvenience of using buffer solutions in which dissolved oxygen also reacts with the titrant and hence interferes with the determinations, it was thought worthwhile to investigate the reaction between quinones and iron(II) in phosphoric acid media. In this paper the estimation of four quinones with iron(II) by potentiometric or visual titration or by colorimetry is presented. The reverse determination, of iron(II) with quinones, is also possible, with either potentiometric or visual detection of the end-point.

EXPERIMENTAL

Reagents

Ammonium iron(II) sulphate solution, 0.01*M*, was prepared in 0.5*M* sulphuric acid and standardized with potassium dichromate.

Solutions (0.01*N*) of 2,6-dimethyl-1,4-benzoquinone, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and tetrachloro-1,2-benzoquinone were prepared in methanol, and of methyl-1,4-benzoquinone in water.

The indicator solutions were 0.1% except for cacotheline, which was 0.2%.[5]

Merck *pro analysi* syrupy phosphoric acid was used. Other reagents were analytical grade.

Apparatus

A bright platinum rod indicator electrode, a saturated calomel reference electrode, and a saturated potassium chloride salt bridge having sintered-glass ends were used for the potentiometric titrations, which were done with an atmosphere of carbon dioxide to exclude aerial oxygen.

Procedures

An aliquot of quinone solution and enough phosphoric acid to give a final concentration of 8*M* (for tetrachloro-1,2-benzoquinone, methyl-1,4-benzoquinone or 2,6-dimethyl-1,4-benzoquinone) or 10*M* for all four quinones were transferred to the titration vessel and diluted to 40 ml with water. Carbon dioxide was passed through the solution for 10 min. The quinone was then titrated with iron(II) solution. Stable potentials were obtained within 45 sec.

Iron(II) was similarly titrated potentiometrically with quinone solution, under a carbon dioxide atmosphere.

Alternatively, with a 10*M* phosphoric acid medium, 0.3 ml of cacotheline solution or 0.05 ml of Methylene Blue, Methylene Green, thionine, Toluidene Blue, Azure A, Azure C or Azure-2-eosin solution, can be added as indicator, the colour change being from yellow to pink for cacotheline, and from blue to colourless for the others. The indicator correction was 0.03 ml of 0.01*N* iron(II). The reverse titration is again possible.

For the colorimetric determination 2.0 ml of 20% potassium thiocyanate solution and 2.0 ml of 0.0075*M* iron(II) (and for methyl-1,4-benzoquinone, 5 ml of concentrated hydrochloric acid) are added to the quinone sample in a 50-ml standard flask, and the solution is diluted to the mark and mixed. The colour is measured at 520 nm or with a green filter (transmission at 500–560 nm).

RESULTS AND DISCUSSION

No potential break was observed in the titration of any of the quinones in phosphoric acid less concentrated than 4*M*. Quantitative results were obtained at acid concentrations between 6 and 12*M* although 2,3-dichloro-5,6-dicyano-1,4-benzoquinone required at least 8*M* phosphoric acid for a sharp break in potential at the equivalence point. Stable potentials were obtained throughout the titrations at all acid

Table 1. Conditional redox potentials (mV) of quinone-hydroquinone couples in different phosphoric acid media; temperature 28°; overall sulphric acid concentration 0.02*M*

Phosphoric acid concentration, *M*	Tetrachloro-1,2-benzoquinone	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone	Methyl-1,4-benzoquinone	2,6-Dimethyl-1,4-benzoquinone
1.0	522	286	398	314
2.0	530	293	410	316
4.0	549	320	437	318
6.0	566	339	452	322
8.0	578	373	472	326
10.0	592	408	505	338
12.0	598	434	517	348

concentrations. In the estimation of iron(II), stable potentials were obtained at once in phosphoric acid solutions down to 4*M*, except near the end-point, where stabilization took at least 5 min. Quantitative results and sharp potential breaks were obtained in acid concentrations from 9*M* to 12*M*.

The indicator colour transitions were all sluggish with phosphoric acid concentrations below 8*M*, but sharp in 9–12*M* phosphoric acid, for both the direct and reverse titrations.

One mole of each quinone consumed two moles of ammonium iron(II) sulphate, as would be expected.

The conditional redox potentials of the quinone/hydroquinone couples in media of varying phosphoric acid concentration were also determined. For each determination 3.0 ml of quinone solution, sufficient acid for reaction to be quantitative, and enough iron(II) solution to reduce half the quinone were taken and diluted to give the desired final acid concentration. The potential was then measured with the electrode system described. The results are presented in Table 1.

The limits of determination, and average standard deviations (given in brackets) for estimation of the four quinones are given in Table 2, and for the reverse titrations in Table 3.

The advantage of the method over the Headridge and Wilson method[3] is that the chemicals used are not hazardous, and no buffers are used. Interference by aerial and dissolved oxygen is also reduced. Titration of tetrahydroxy-1,4-benzoquinone and 9,10-phenanthraquinone was also tried but failed because the

Table 2. Determination of quinones

Quinone	Range of determination, *mg**		
	Potentiometric titration	Visual titration	Colorimetric method
Tetrachloro-1,2-benzoquinone	1.22–24.4 (0.03)	1.22–24.4 (0.04)	1.20–4.0
2,3-Dichloro-5,6-dicyano-1,4-benzoquinone	1.13–20.3 (0.02)	1.13–22.6 (0.05)	0.50–2.5
Methyl-1,4-benzoquinone	0.68–15.0 (0.04)	0.68–13.6 (0.03)	0.30–1.5
2,6-Dimethyl-1,4-benzoquinone	0.61–9.2 (0.03)	0.61–12.2 (0.03)	—

* Standard deviation in brackets

Table 3. Determination of iron(II)

Quinone	Range of determination, *mg**	
	Potentiometric titration	Visual titration
Tetrachloro-1,2-benzoquinone	4.39–43.9 (0.07)	4.39–43.9 (0.08)
2,3-Dichloro-5,6-dicyano-1,4-benzoquinone	4.39–39.5 (0.07)	4.39–43.9 (0.09)
Methyl-1,4-benzoquinone	4.39–48.3 (0.08)	4.39–39.5 (0.07)
2,6-Dimethyl-1,4-benzoquinone	4.39–39.5 (0.07)	4.39–48.3 (0.07)

* Standard deviation in brackets.

redox potentials of these quinones[3] are below that of the iron(II) system.[4]

Arsenic(III), antimony(III), manganese(II), zinc, magnesium, barium, citrate, tartrate, oxalate, acetate, chloride and sulphate do no interfere in any amount. Nitrate and nitrite interfere at all concentrations.

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INSTRUMENTS IN ANALYSIS—CRITICAL REVIEWS

DIFFUSE-REFLECTANCE SPECTROSCOPY IN THE INFRARED

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Summary—The theory, and the problems encountered in the development of diffuse-reflectance spectroscopy in the infrared region as an analytical technique, are reviewed. The introduction of Fourier-transform infrared spectrometers has eliminated the difficulty of detecting small scattered intensities, and diffuse-reflectance measurement is now a routine method. The first commercial instruments are now available.

The demands nowadays made on analytical chemistry frequently necessitate the use of modern physical instruments. The first step in solving some analytical problems is often the recording of an infrared spectrum of the substances to be studied. Most molecular compounds exhibit vibrations with frequencies comparable with those of electromagnetic waves in the infrared region. The central region of the infrared, usually defined by wavenumbers from 4000 to 400 cm^{-1} (*i.e.*, wavelengths from 2.5 to 25 μm) is called the "fingerprint region" because many functional groups of molecules are associated with characteristic absorptions in this region.

The measurement of infrared radiation encountered many problems in earlier years. The greatest of these was the detection of radiation with the very inefficient thermal detectors then available. No commercial instruments or constructional elements were then available and the problems of radiation filtering, collection and detection were dealt with individually.[1,2] Simple measurements had the status of physical experiments and were only carried out for special purposes. This situation changed in the 1930s, when continuous recording replaced point-by-point measurement. This possibility and the demands of the fast-growing chemical industry for rapid and more exact methods of analysis promoted the development of commercial instruments. The fundamental study of the interaction of electromagnetic radiation with chemical compounds left the experimental stage and when in the 1940s the first ratio-recording infrared spectrometers were built, the technique passed from the hands of experimental physicists into those of the analytical chemist and became a routine analytical method.

Later, demands for the improvement of this type of applied spectroscopy encouraged more and more new developments. The original thermocouples and bolometers were replaced by more sensitive pneumatic and semiconductor detectors and the prism monochromators were superseded by diffraction grating types. Recently the well-established alternative technique of interferometry has been applied.

At the present time infrared spectroscopy is a favoured method, because it can be applied to nearly all substances, in all states of aggregation and at any temperature.

This wide field of application is made possible by the advanced stage of instrumental development and the sophistication of measuring techniques and sample preparation. The most widely used technique is based on the measurement of transmittance of a sample and is applicable to all transparent media. Disperse media such as powders can often be made transparent by immersion in infrared-transmitting materials (salts or oils). Provided that the refractive index of the immersion medium is closer to that of the powder than to that of the surrounding air, radiation scattering is reduced. Regular reflection and attenuated total reflection (ATR) techniques are useful supporting methods for studies of bulk samples, surface and interface phenomena and thin layers. However, routine analysis now presents an increasing number of problems associated with disperse materials such as powders or fibres, where the abovementioned techniques are not applicable. There are, for example, adsorbents and catalysts which cannot be immersed in a matrix if their adsorption behaviour is to be characterized. The surface of the powder must not be disturbed by sample preparation and in any case a faster method of preparation is to be preferred. Sometimes the scattering of radiation is not greatly reduced by immersion techniques. In such cases the measurement of diffuse scattering would provide an alternative method, if the intensity of the diffusely reflected radiation could be detected quantitatively.

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The term "diffuse reflection" relates only to an ideally diffusing surface and there are several effects occurring when electromagnetic radiation interacts with the surface of the medium. The radiation falling onto the surface is partially reflected (diffuse and specular reflection) and is partially transmitted. The radiation penetrating the medium may be absorbed or scattered and will leave the medium in a forward or backward direction. The radiation coming from the surface in the opposite direction to that of the incident beam will thus consist of reflected and re-emitted components and the term "remission" was coined to describe it. This term is seldom used outside the German literature and so the term "diffuse reflection" is used here to describe the abovementioned phenomena.

The diffuse-reflection technique has been widely applied in the ultraviolet, visible, and near infrared spectral regions for some time. The commercial instruments and spectrometer accessories available have been described in reviews by several authors.[3–5]

The application of this technique in the infrared region is made difficult by the problem of detection of scattered infrared radiation. The thermal detectors are less sensitive than the photoelectric cells and photomultipliers used for ultraviolet and visible radiation, and are more subject to interference from ambient thermal radiation. This is often of the same order of intensity as that of the monochromatic beam which has been attenuated by scattering. This difficulty must be overcome by collecting the diffusely reflected radiation with minimal loss and directing it onto the detector, which should have a small sensitive area to minimize thermal noise. This can be achieved in two ways. The radiation source of the spectrometer is removed and replaced by the diffusely and polychromatically illuminated sample, and part of the radiation diffusely reflected into a small solid angle is analysed by the spectrometer. Alternatively, the detector may be removed and replaced by the sample. The diffusely reflected radiation from the directionally and monochromatically illuminated sample is then collected by an integrating optical element and directed onto the detector. The earlier work embodying these principles has been reviewed by Kortüm[3] and by Dunn *et al.*[6] The present article describes developments during the 12–15 years since these reviews appeared.

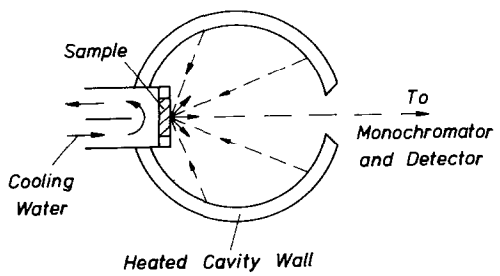

Fig. 1. "Hohlraum" type diffuse reflection cell (heated cavity).

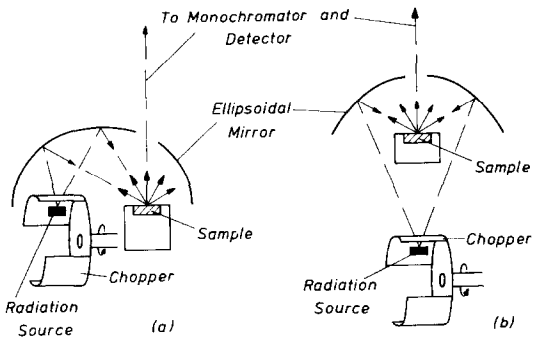

Fig. 2. Ellipsoidal mirror configurations for diffuse polychromatic sample illumination; (a) off-axis, (b) on-axis.

The first of these was realized by Gier *et al.*[7] for measurements in the mid-infrared region. The sample is placed in a heated cavity which simulates a black body (Fig. 1). Thermal radiation falls onto the sample from all directions and is diffusely reflected. The quantity of reflected radiation passing through the exit hole of the cavity may be analysed with any conventional spectrometer. This configuration has some disadvantages; samples with low thermal conductivity will be heated and changes in chemical constitution may be induced. Furthermore, thermal emission from the heated sample overlaps the reflected radiation and it is impossible to separate the two components. The spectrometer measures only a small proportion of the diffusely reflected radiation, set by the hole in the cavity and the geometry of the other optical elements, so a poor signal-to-noise ratio is obtained and long measuring times are necessary. Although some authors have reported successful applications of this method with laboratory-made cavities, and designs for commercial instruments have been developed (*e.g.*, Beckman Instruments), the method has never come into common use.

Some of the difficulties associated with this "hohlraum" type reflectometer can be overcome by illuminating the sample with an infrared radiation source and focusing with an ellipsoidal mirror in the off-axis or on-axis configuration (Fig. 2).[8,9] The radiation source is mounted at one focus of the ellipsoid and the sample is located at the second focal point where the radiation is collected by the focusing properties of the ellipsoidal mirror. The heating of the samples is reduced to 50% by chopping the incident radiation. A phase-sensitive detection system registers only the chopped radiation diffusely reflected by the sample and is insensitive to the uniform background radiation.

The second experimental arrangement, in which the sample is illuminated with monochromatic radiation, avoids all these heating problems. The same two configurations of ellipsoidal mirrors may be used, but the sample and the detector are placed at the conjugate foci (Fig. 3) as described by Kortüm and Delfs[10] and by Blevin and Brown.[11] This principle allows the diffusely reflected radiation to be measured with minimal loss. Perpendicular illumination of the sample

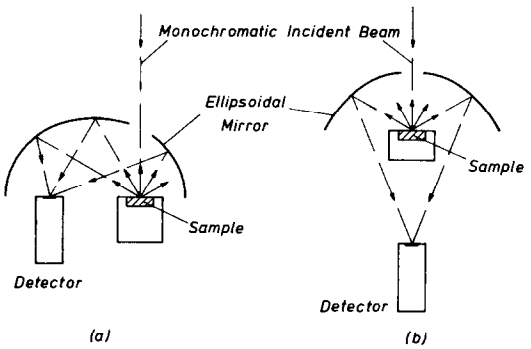

Fig. 3. Integration of the diffusely reflected monochromatic radiation over semi-infinite space with the aid of an ellipsoidal mirror, (a) off-axis, (b) on-axis.

surface serves for the separation of diffusely and specularly reflected radiation, because the latter leaves the ellipsoidal mirror through the entrance hole. The incident beam must be focused onto a small sample area, because the focusing properties of the ellipsoidal mirrors are only valid at the focal points and because the detectors have only small sensitive areas.

Brandenberg[12] and Heinisch *et al.*[13] studied these optical properties in detail for the case of the off-axis mirror and showed that there is only a small image enlargement at the second focus, caused by the rays coming from the immediate surroundings of the first focus. This configuration imposes no restrictions on the dimensions of the sample holder (*e.g.*, evacuated or temperature-controlled cells may be used), because the focal length may be chosen to suit the measurement problem before the ellipsoid is fabricated. A disadvantage of this configuration is the large solid angle of radiation incident on the detector, which will cause some loss by reflection at the surface of the detector window. This loss is lower in the on-axis configuration, but the image enlargement associated with it is very high. In the instrument described by Dunn *et al.*[14] the ratio of the illuminated areas at both focal points was 1:25. A second disadvantage is the shadow caused by the sample holder in the incident radiation flux. If it is necessary to use large

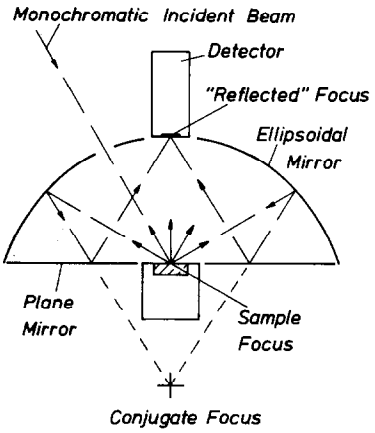

Fig. 4. On-axis ellipsoidal mirror configuration for sample holders of any dimension.

sample cells, a modification proposed by Masterson[15] can be made. A plane mirror at the sample focus and perpendicular to the main axis of an almost spherical ellipsoid ($a:b = 1.06$) reflects the rays at the detector focus through a hole in the ellipsoidal mirror on the main axis (Fig. 4).

An improvement to the off-axis type ellipsoidal mirror was made by Maulhardt *et al.*[16] who modified the ellipsoid so as to realize a true double-beam instrument, as evidenced from dispersive transmission measurements.

A disadvantage of all configurations with monochromatic sample-illumination can appear if high-temperature sample cells are used. This can cause a radiation overload of the detector, especially of semiconductor detectors. However, of the two concepts of polychromatic or monochromatic sample illumination, the second seems to be preferable, because the need for higher efficiency of radiation detection is the most outstanding problem.

It is now about 70 years since the first infrared diffuse-reflectance measurements were carried out by Royds[1] and Coblentz.[2] They used half-spheroidal mirrors instead of off-axis ellipsoids, which it was impossible to produce at that time. They placed their samples and detectors at points opposite to the centre of the sphere and as near as possible side by side to minimize the spherical aberration.

It is not surprising therefore that the detection problems in the infrared have hitherto confined successful applications of diffuse-reflection spectrometry to the ultraviolet and visible regions, for which practical integrating spheres have been designed and developed.[17] It was only after the end of World War II, when automatic recording infrared spectrometers came into common use, that the ideas of Royds and Coblentz were investigated and new concepts developed. Although today the difficulties of producing complicated ellipsoidal and paraboloidal mirrors have been overcome by using computer-controlled machinery, nearly all the early work in the infrared field was done with equipment built in the laboratory for the solution of specific problems. The development of new infrared spectrometers based on the interferometric principle and their commercial availability has radically changed the situation. The higher radiation throughput of the interferometer overcomes the detection problem. Low[18] has already measured diffuse-reflectance spectra without collection of diffusely reflected radiation. Willey[19] developed and offered a commercial spectrometer with an integrating sphere coated with diffusely reflecting gold, but its slow-scanning interferometer design was unsuccessful and the diffuse-reflectance unit was also ineffective. Fuller and Griffiths[20] and Maulhardt and Kunath[21] proposed two successful designs, combining an interferometer with an on-axis or an off-axis ellipsoidal mirror. Typical performance figures for the off-axis mirror configuration are 10% radiation efficiency (relative to the transmission intensity without the sample) and

less than 1% stray light. Only a few minutes are needed for installation of this portable instrument. Digilab Inc. (Cambridge, Mass., U.S.A.) offers the first commercially available diffuse-reflection unit for its FTS® spectrometer series, which was developed by Krishnan *et al.*[22] It is simply constructed, provides only a partial collection of diffusely reflected radiation and does not discriminate against the measurement of specularly reflected radiation. Like the development of conventional infrared spectrometers the production of these accessories has been encouraged by the requirements of analytical chemistry.

The different phenomena such as transmission, absorption, scattering and specular reflection, which affect diffuse reflection, make it difficult to obtain a quantitative estimate of optical constants, particularly the absorption coefficient, which is of paramount interest to the analytical spectroscopist. Numerous attempts have been made to derive mathematical expressions for the dependence of the diffuse reflectance on the molar absorption coefficient and sample concentration and to compare reflectance spectra with transmittance spectra (where the Beer–Lambert law applies). The theories developed fall into two classes. The basis of the first is a consideration of the interaction of the electromagnetic waves with single particles or layers of particles, the sample being considered as a "discontinuous" medium. The second group describes the scattering in terms of a "continuous" medium disregarding the effects of particles or layers of particles. Both concepts originated at about the beginning of this century. In 1899 Rayleigh[23] gave a mathematical expression for the scattering of small single particles and in 1908 Mie[24] extended it to particles of any size. Schuster[25] solved the differential equations for a continuously scattering atmosphere, in 1905. All these early treatments were carried out to provide a general description of radiative transfer through turbid media. The most commonly used theory for spectroscopic purposes is based on the continuum model of Schuster, which was rediscovered by Kubelka and Munk[26] in 1931, and in 1948 Kubelka[27] derived the formulae in the form used to the present day. The simplicity of this theory, requiring only two constants (one scattering and one absorption coefficient), promoted its widespread use. The disadvantage is that there is no exact comparison between the absorption coefficient derived from diffuse-reflectance measurements and the true absorption coefficient of the bulk material, because of the simplifications used in this theory. The "discontinuum" theories can provide more exact relationships between the optical constants of the bulk materials and those derived by reflectance measurements on powders. The phenomena of "reflection" and "refraction" of the incident radiation at the particle surface and the phenomenon of "absorption" within the particle may be considered in the form of separate processes provided that the particle size is large with respect to the wavelength of the incident radiation. This is true for normally encountered powders and wavelengths in the ultraviolet and visible regions, but not in the infrared region. Because of the necessary approximations relating to particle-size distribution and particle geometry, discrepancies appear between measured and calculated infrared reflectance values. The "discontinuum" theories are more complicated to handle because of the larger number of parameters which they take into account. The reader is referred to Hecht's comprehensive survey of the different types of theory.[28]

The theory developed by Schuster, Kubelka and Munk is the one most commonly used for studies in the infrared as well as in the ultraviolet and visible regions up to the present day, although it must be considered as only a semi-empirical method for the quantitative interpretation of diffuse-reflectance spectra. Kortüm has carefully examined the applicability of this theory to measuring techniques in all spectral regions and has pointed out its limitations. Numerous practical applications and much experience in the interpretation of reflectance spectra are summarized by Kortüm in his monograph.[3]

A problem which has not been solved up to the present time is the provision of reflectance standards which have high diffuse reflectance and no spectral absorption bands in the infrared. Such standards are necessary because diffuse-reflectance measurements carried out with the instruments described above provide no absolute reflectance data and must be related to measurements on a specimen of known reflectance. Diffusing materials such as magnesium oxide or barium sulphate are suitable "white standards" for the visible region but are not useful for the infrared. Some authors have proposed powdered sulphur as a standard but its application is restricted to the near infrared. Powders of the alkali-metal halides in the bulk form are familiar as infrared transmitting windows, and rough glass plates coated with gold or aluminium by evaporation can be used for most purposes. The alkali-metal halides have the disadvantage of being hygroscopic, while the diffusely reflecting metal coatings do not have the same radiation distribution at all solid angles, as would be the case for ideal scattering standards. This problem of reflectance standards in the infrared may be avoided by the use of alternative spectroscopic techniques, in which a reference sample assumes the function of a reflectance standard. Such a technique may have applications to a wide range of analytical problems. These include adsorbed molecules on powdered materials or fibres, material composites, mixed polymers or minerals and the quantitative analysis of molecular compounds in solids at low concentrations. Diffuse-reflectance spectrometry may be expected to become of ever-increasing importance in the non-destructive trace analysis of molecules in granular materials.

Some idea of these future possibilities for the application of the diffuse-reflectance technique in the infrared region may be gained by considering the state of development of the analogous but much

simpler technique of diffuse reflectance in the near infrared region. However, in the application of the diffuse reflectance technique in the mid infrared region there remain two severe problems. The infrared radiation reflected from the sample falls upon detectors, which have lower sensitivities and smaller sensitive areas than the photoelectric detectors used in the ultra violet, visible and near infrared regions of the spectrum and this requires increased sophistication in the spectrometer optics. In addition, there exists at the present time, no comprehensive theory, relating the absorption of the substance to its concentration.

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DEVELOPMENT OF A HIGH-RESOLUTION INDUCTIVELY-COUPLED ARGON PLASMA APPARATUS FOR DERIVATIVE SPECTROMETRY AND ITS APPLICATION TO THE DETERMINATION OF HAFNIUM IN HIGH-PURITY ZIRCONIUM OXIDE

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Summary—A high-resolution apparatus for inductively-coupled plasma emission spectrometry (ICPES) has been developed, based on an echelle spectrometer modified for wavelength modulation with a quartz refractor plate. The selectivity of the technique is thus improved, and small amounts of hafnium in high-purity zirconium oxide can be determined directly without prior separation or preconcentration. A straight-line calibration curve passing through the origin is obtained without any correction for the interference from zirconium which exists in large excess. The detection limit for hafnium is 0.06 μg/ml, and the relative standard deviation (10 replicates) for hafnium at the 1.2 μg/ml level is about 3%.

Inductively-coupled plasma emission spectrometry (ICPES) is an excellent technique for trace determination of a number of elements. Among the requirements of ICPES, selectivity is among the most important. The emission line of the determinand often suffers spectral interference from neighbouring emission lines in the analysis of high-purity materials for trace impurities and the resolution of conventional spectrometers may be inadequate for this use of ICPES. Two approaches have been proposed for improving the resolution of the spectrometer, and hence the selectivity in ICPES. One is the use of a high-resolution echelle grating instead of a conventional grating[1,2] and the other is wavelength modulation.[3] Zander *et al.*[4] succeeded in obtaining high resolution in atomic-absorption spectrometry by using a commercially available echelle monochromator, modified for wavelength modulation, so we decided to use this approach for ICPES, using a new high-resolution spectrometer recently devised by Kihachiro Murakami of Yanaco Co., Ltd., taking our requirement into consideration, combined with a commercial ICP excitation source and a data-acquisition system. In this spectrometer, an echelle grating is mounted in a computer-controlled monochromator system and wavelength modulation is introduced. With this apparatus a well-defined second derivative spectrum can be obtained and the selectivity greatly improved. The usefulness of the method has been confirmed by its application to the direct determination of a small amount of hafnium in high-purity zirconium oxide without the need[5] for correction for the interference of zirconium.

EXPERIMENTAL

Apparatus

The individual components of the equipment are listed in Table 1. A new design of spectrometer with a modified Czerny–Turner configuration is used (commercially available from Yanaco Co., Ltd., Japan, model UOE-1). It incorporates an echelle grating (79 lines/mm) and an oscillating quartz refractor plate in the light-path behind the entrance slit (to produce wavelength modulation). The optical and electronic systems of the apparatus are shown in Figs. 1 and 2, respectively. The focal length is 0.8 m and the wavelength range 190–800 nm. The reciprocal linear dispersion and resolving power are shown in Table 2, and are about one order of magnitude better than those (8 Å/mm and 0.15 Å, respectively[6]) for a conventional Czerny-Turner spectrometer having a grating with 1200 lines/mm and a focal length of 1 m.

The wavelength modulation is done sinusoidally by a refractor plate modulator. The modulation interval can be selected in the range 0–1.0 Å by computer control. When the wavelength (λ) is modulated (by rapid scanning back and forth over a small spectral interval by the oscillating refractor plate) a signal proportional to the change in spectral intensity (I) over the interval is generated. The resulting a.c. signal amplitude is proportional to the first derivative ($dI/d\lambda$) of the intensity with respect to wavelength. If a lock-in amplifier is used to detect the a.c. component of the signal, the second harmonic (*i.e.*, twice the frequency of modulation) is proportional to the second derivative, $d^2I/d\lambda^2$. With this apparatus, the second derivative spectrum can be recorded on a chart recorder, and the original emission spectrum can also be displayed directly on a cathode-ray tube. Hence any spectral interference from neighbouring lines can be directed by monitoring the spectral profile. The wavelength can be selected automatically with a precision of 0.01 Å by computer control, with the mercury 2536.52-Å emission line as standard. Further details of the spectrometer systems will be reported elsewhere by Murakami *et al.*

Determination of hafnium

On the basis of the experimental results described below, operating conditions for the determination of hafnium were decided, as shown in Table 3.

Reagents. Stock solutions containing hafnium (1000 mg/l.) and zirconium (5000 mg/l.) were prepared from 99.9% hafnium oxide or "hafnium-free" zirconium oxide (Nippon Mining Co., Ltd.), hafnium content 6.6 μg/g, in the same

Table 1. Experimental components employed

Item	Description
R.F. generator	Model HFP-1500 D, Plasma-Therm Inc. (Kresson, N.J.)
Matching network	Model MN-1500 E, Plasma-Therm Inc.
Plasma torch	Model PT-1500, Plasma-Therm Inc.
Plasma, coolant and aerosol carrier gases	Argon
Sample introduction system	Pneumatic nebulization using a concentric glass nebulizer
Spectrometer	Wavelength-modulated echelle spectrometer with a modified Czerny–Turner mounting. Adjustable slit-width: 25, 50, 100, 200, 500 μm. Adjustable slit-height: 200, 300, 500, 1000, 2000 μm
Data acquisition system	Model UM-1 controller, Yanaco Ltd. (Japan) and Model RC-125 chart recorder, Japan Spectroscopic Co., Ltd.

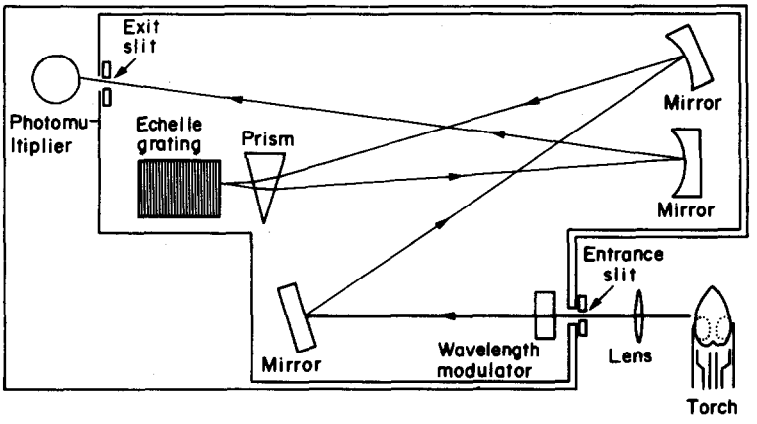

Fig. 1. Block diagram of the optical system of the spectrometer.

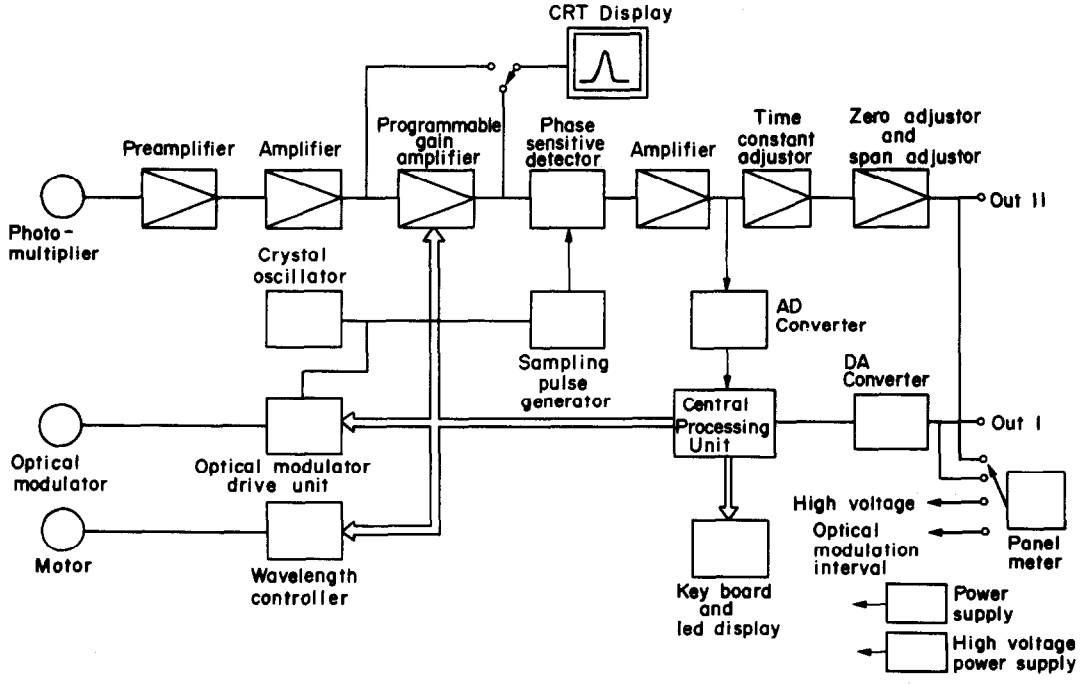

Fig. 2. Block diagram of the electronic system of the apparatus.

Table 2. Reciprocal linear dispersion and resolving power of the spectrometer used

Wavelength, *nm*	Reciprocal linear dispersion, *Å/mm*	Resolving power (25-μm slit-width), *Å*
200	0.31	0.0078
300	0.46	0.012
400	0.62	0.016
500	0.78	0.020
600	0.93	0.023
700	1.0	0.25
800	1.2	0.030

way as the sample solutions (see below). Working solutions were prepared by dilution of the stock solutions with sulphuric acid (1 + 19).

Preparation of sample solutions. Place 0.5 g or less of sample in a 50-ml beaker, and add 2.5 g of ammonium sulphate and 7 ml of concentrated sulphuric acid. Cover the beaker with a watch-glass, heat gently at first, then raise the temperature until sulphuric trioxide fumes appear, and keep at this temperature until the solution is quite clear. After cooling, dilute the solution to 100 ml (or other suitable volume) with sulphuric acid (1 + 19).

RESULTS AND DISCUSSION

Selection of spectral line for hafnium

As shown in Table 4, there are several emission lines suitable for determination of hafnium. The Hf II 2641.41-Å line is the most favourable, because it is fairly strong and is free from spectral interference by zirconium. This is well shown in Fig. 3, which shows the second derivative spectra of hafnium and zirconium near the 2641.41 Å Hf line.

Influence of modulation interval

The effect of the modulation interval on the second derivative intensity and the detection limit for hafnium was examined. The results are shown in Fig. 4, from which it is clear that enlarging the modulation interval increases the second derivative intensity and lowers the detection limit. However, if the modulation interval exceeds 0.25 Å zirconium begins to interfere because the resolution is decreased. Consequently, for determination of hafnium in the presence of a large amount of zirconium, a modulation interval of 0.25 Å is chosen as the best compromise.

Table 3. Operating conditions for the determination of hafnium

ICP equipment		
forward R.F. power		1.4 kW
reflected power		<5 W
argon flow-rate	coolant gas	10.5 l./min
	plasma gas	1.0 l./min
	aerosol carrier gas	0.43 l./min
Spectral observation		
entrance and exit	slit-width	200 μm
	slit-height	1000 μm
integration time		15 sec
observation height above work coil		20 mm
modulation interval		0.25 Å
analysis line		2641.41 Å (Hf II)

Table 4. Emission lines of hafnium

Wavelength, Å	Detection limit*, *ng/ml*	Relative second derivative intensity	Interference from Zr	
2773.36	15	strong	medium	(2774.16 Å)†
2738.76	16	medium	medium	(2739.77 Å)†
2641.41	18	strong	none	(2640.15 Å)†
2322.47	18	weak		
2638.71	18	strong	remarkable	(2639.09 Å)†
2820.22	18	strong	remarkable	(2819.56 Å)†
2512.65	20	weak		
2513.03	20	weak		
2571.69	20	weak		
1963.82	21	weak		
2393.83	21	weak		
2351.22	23	weak		
2464.19	23	weak		

*By R. K. Winge *et al.*[7]
†Nearest line of Zr (from M.I.T. tables).

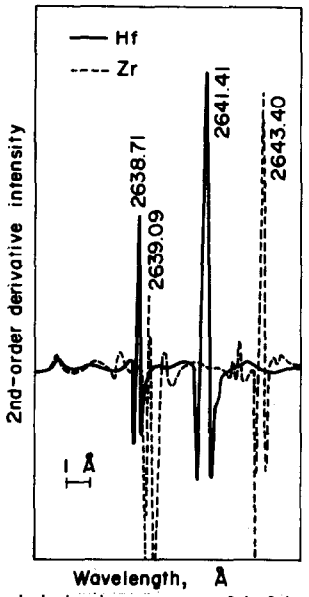

Fig. 3. Second derivative spectra of hafnium and zirconium in the vicinity of the 2641.41 Å Hf line. Hf: 20 mg/l., Zr: 3000 mg/l., slit-width: 200 μm, slit-height: 1000 μm, modulation interval: 0.25 Å.

Influence of slit width and height

With slit-widths up to 200 μm and slit-heights up to 1000 μm, the spectral resolution is good, there is no optical interference from the emission lines of zirconium, and the second derivative intensity for hafnium increases with increase in width and height of the slit, but at settings above these values the spectral resolution deteriorates and optical interference from zirconium is no longer negligible.

Influence of zirconium concentration

It can be seen from Fig. 3 that no spectral interference from emission lines of zirconium in the vicinity of the 2641.41-Å Hf line is observed even in the presence of large amounts of zirconium, because of the high resolution of the spectrometer, but high concentrations of zirconium (3 g/l.) can affect the behaviour of hafnium in the plasma, presumably by changing the population of excited hafnium atoms or ions. However, Fig. 5 shows that the zirconium concentration does not affect the second-derivative signal for hafnium if the observation height is set at 20 mm above the work coil.

Influence of acid concentration

In ICPES the acid concentration of the solution introduced into the plasma torch can affect the nebulization efficiency, so the effect of various mineral acids was examined. The results are shown in Fig. 6. The acids tested all decrease the hafnium signal; sulphuric acid has the largest effect. In spite of this, sulphuric acid is used to prepare the sample solution because the dissolution of zirconium oxide with this acid and ammonium sulphate is one of the easiest and most rapid procedures for the purpose. However, the

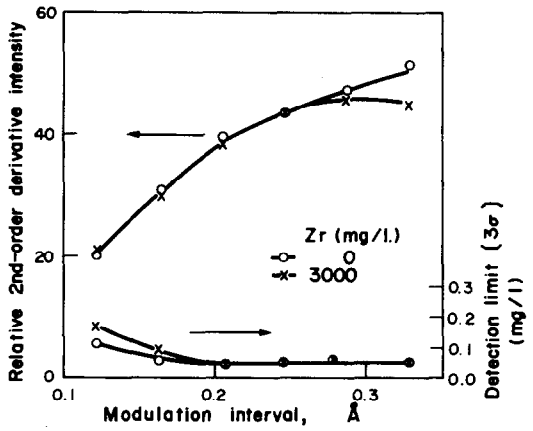

Fig. 4. Influences of modulation interval on second derivative intensity and detection limit of hafnium. Hf: 0.4 mg/l., H_2SO_4: 4*N*, $(NH_4)_2SO_4$: 2.5%.

sulphuric acid concentration of the sample solutions should match that of the standard solutions used for preparation of the calibration graph.

Influence of ammonium sulphate

Ammonium sulphate concentrations up to 4% do not affect the hafnium signal, but if the concentration exceeds 5%, solid ammonium sulphate tended to deposit on the inner wall of the quartz aerosol-injected tube.

Calibration graph, detection limit and precision

Plotting the second-derivative signal against hafnium concentration gave a straight line passing through the origin, whether zirconium was present or absent, showing that zirconium, even in large excess, did not interfere. The detection limit for hafnium, defined as the concentration giving an average net derivative signal equal to three times the standard deviation of the background noise, was found to be 0.06 μg/ml. The relative standard deviation (10 replicates) for a 1.2-μg/ml hafnium solution was ~3%.

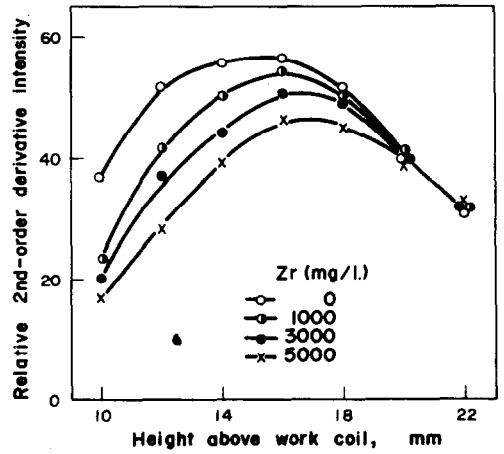

Fig. 5. Influence of observation height above work coil on second derivative intensity of hafnium. Hf: 1.2 mg/l., H_2SO_4: 4*N*, $(NH_4)_2SO_4$: 2.5%.

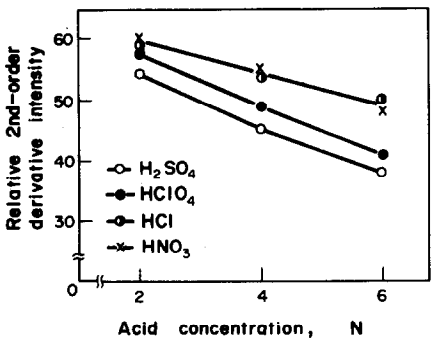

Fig. 6. Influence of mineral acid concentration on second derivative intensity of hafnium. Hf: 1.2 mg/l.

These results suggest that with this apparatus the direct determination of a small amount of hafnium in high-purity zirconium oxides, which is very difficult and almost impossible with the conventional apparatus, is feasible without prior separation, preconcentration or correction, with satisfactory precision. Further, these results indicate that such high-resolution apparatus considerably improves the selectivity in ICPES in general, and provides a very useful and powerful tool for trace element analysis, especially in a complex matrix.

Determination of hafnium in zirconium oxides

In order to confirm the high resolution and usefulness of the apparatus, hafnium present as an impurity in commercial high-purity zirconium oxides obtained from three different makers was determined with it. The results, which are summarized in Table 5 and compared with those obtained by an extraction–spectrophotometric method[8] show that the performance of the apparatus has been confirmed. In addition, it was found that the amounts of hafnium contained in these so-called "high-purity" zirconium oxides are of the order expected for zirconium oxide not subjected to purification.

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Table 5. Determination of hafnium in high-purity zirconium oxides

Sample	Sample taken, *mg*	Final volume, *ml*	Dilution ratio	Hf found, *μg/ml*	Hf content, %		Hf content by other method[8], %
W	103	100	1/10	1.84	1.78		1.8
			1/10	1.82	1.76	Average	
			1/5	3.64	1.76	1.75	
			1/5	3.50	1.69		
	504	100	1/25	3.56	1.77		
			1/25	3.48	1.72	Average	
			1/12.5	6.77	1.69	1.74	
			1/12.5	7.12	1.77		
M*	103	100	1/10	1.94	1.94		2.1
			1/10	2.11	2.06	Average	
			1/5	4.13	2.02	1.99	
			1/5	4.00	1.95		
	502	100	1/25	3.92	1.95		
			1/25	4.01	1.99	Average	
			1/12.5	8.07	2.01	1.99	
			1/12.5	7.96	1.99		
R†	104	100	1/10	1.49	1.44		1.4
			1/10	1.42	1.39	Average	
			1/5	2.99	1.45	1.44	
			1/5	3.10	1.49		
	626	100	1/25	3.61	1.44		
			1/25	3.64	1.45	Average	
			1/12.5	7.38	1.47	1.47	
			1/12.5	7.65	1.53		

* Sold as 99.9% ZrO_2.
† Sold as 99.99% ZrO_2.

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THE USE OF GLASS ELECTRODES FOR THE DETERMINATION OF FORMATION CONSTANTS—I

A DEFINITIVE METHOD FOR CALIBRATION

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Summary—The computer program, MAGEC (Multiple Analysis of titration data for Glass Electrode Calibration), described can optimize simultaneously any or all of the titration parameters pertinent to the calibration of glass electrodes. In particular, the protonation constants of a ligand and the glass-electrode parameters may be determined simultaneously from a given single set of titration data. A method is described for obtaining definitive values of these parameters, involving cyclical treatment of titration data by means of MAGEC and another program such as MINIQUAD. The use of MAGEC for evaluating the performance of a glass electrode or as an analytical tool for determining, with high precision, the concentrations of acid and base solutions, is also described.

Despite three decades of extensive effort directed towards obtaining unequivocal formation constants for ligand–metal systems by glass-electrode potentiometry, there remain worrying uncertainties concerning the accurate calibration of the electrodes. Originally, glass electrodes were calibrated against buffers of specific pH but, although this approach remains valid in work where only a relative scale of acidity is required, it is unsatisfactory nowadays in metal–ligand equilibrium studies. Indeed, owing to the difficulties involved in relating pH values to hydrogen-ion activities, IUPAC has introduced an operational definition of pH.[1]

Even if a specific mathematical relationship between the *defined* pH and the hydrogen-ion activity of a buffer is assumed, difficulties remain in inferring the hydrogen-ion concentration values of a test solution, such as would be required in the determination of the protonation constants of a ligand and the formation constants of ligand–metal complexes. First, the hydrogen-ion activity coefficients are not the same in the buffer and in the test solutions, because of their different ionic compositions. Attempts to overcome this difficulty by applying various types of theoretical or empirical correction[2–9] introduce some inherent inaccuracy.[10,11] Moreover, differences in liquid-junction potential obtained with the buffer and with the test solution impose further uncertainty.[10,11]

These problems with the use of buffers in the calibration of glass electrodes are exacerbated when high concentrations of background electrolyte are used for controlling the ionic strength of the test solutions. Thus, researchers who employ potentiometric methods to measure formation constants have long used solutions of known hydrogen-ion concentration instead.[12] This is often done by titrating strong acid solutions with strong alkali and plotting the emf at each point against the corresponding values of $\log[H^+]$, where $[H^+]$ denotes the free hydrogen-ion concentration. The procedure aims to obtain a linear calibration curve for the electrode system.

There are several reasons why an ideal response is not observed in practice, however. Chiefly, it is found that unless there is a sufficient excess of acid or alkali to ensure that the solution is concentration-buffered, small errors become significant. It has, for example, been shown that the presence of glass itself causes a large deviation from linearity,[13] a fact that can probably be attributed to adsorption of hydrogen ions onto the glass surface or to dissolution of minute amounts of the glass. Another important factor is the imperfect behaviour of glass electrodes in alkaline solution: many of the types of glass used for electrode manufacture become increasingly sensitive to metal ions, especially sodium ions, above $-\log[H^+] = 11.0$.[14] The effect of hydrogen-ion concentrations on liquid-junction potentials also tends to cause some deviation at high concentrations of hydrogen ion.

Accordingly, there is a restricted range of free hydrogen-ion concentration over which strong-acid *vs.* strong-base titration data are suitable for calibration purposes. In the authors' experience, the most nearly linear response occurs in the $-\log[H^+]$ ranges 2.3–2.9 and 10.8–11.3. Data collected inside these

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limits with most research equipment can yield a level of precision that is adequate for the determination of formation constants for biological systems. What is less satisfactory, however, is that the critical measurements with most systems lie outside the calibration range. Thus the hydrogen-ion concentrations must be procured by extrapolation. This makes them sensitive to small errors in the analytical concentrations of the acid. Also, it is well known that the standard potential of the glass membrane varies from day to day (due to asymmetry effects) and that it is difficult to reproduce liquid-junction potentials with adequate precision. These factors can be significant even from one experiment to another.[11,14–17] *Thus internal calibrations of the electrode, performed in the test solution itself, are highly desirable.*

In principle, there are two main ways of achieving this goal. The first applies to solutions in which any strong acid added is completely dissociated and gives a correspondingly increased free hydrogen-ion concentration. It is then possible to calibrate by a series of constant additions. Alternatively, in titrations of weak acids or bases, free hydrogen-ion concentrations can be calculated at various points in the titration from the protonation constants, provided these are accurately known. Very precise calibrations can be made in this way but they are of limited use. Indeed, the object of many titrations is to measure the protonation constants. In these cases a number of parameters, including glass-electrode properties and one or more equilibrium constants, must be determined simultaneously. Straightforward solutions are very rarely available so general *optimization* techniques must be employed.

This paper describes an approach for the optimization of some or all of the parameters required for calibrating glass-electrode systems and shows how protonation constants of a ligand can, if desired, be determined simultaneously. The relevant algorithms are incorporated in a Fortran program named MAGEC (Multiple Analysis of titration data for Glass Electrode Calibration), the coding for which is available to interested readers on request.*

THEORY

Consider an electrochemical cell in which a test solution surrounding a glass electrode is in electrical contact with a reference electrode through a salt bridge. It can be represented as follows:

first reference half-cell (in glass electrode)	test solution	second reference half-cell (external)
g		l

The boundaries, g and l, respectively, indicate the glass membrane and the liquid junction at the interface between the salt bridge and the test solution. There are four contributions to the measurable potential difference between the two reference electrodes.[14–17] Two arise from the reference electrodes themselves. They will have opposite signs and will usually be of comparable magnitudes. Most importantly, their contribution will be independent of the composition of the test solution and so may be represented as a fixed combined potential, E_r. On the other hand, the potential differences generated across the boundaries of g and l will depend on the activities of all the chemical species on either side of them. If these boundary potentials are represented by E_g and E_l, the measured emf of the cell is given by the equation.

$$E_{cell} = E_r + E_l + E_g \qquad (1)$$

In the case of the liquid junction, considerable changes in the composition of the test solution are required to alter E_l significantly, so, for the time being, this will be considered as a constant. Glass electrodes, in general, are found experimentally to exhibit a Nernstian response over a wide range of concentration.[16]

Equation (1) can be rewritten as

$$E_{cell} = E_r + E_l + E_g^0 + \frac{RT}{F} \ln\{H^+\} \qquad (2)$$

where E_g^0 is the standard glass-electrode potential at unit activity of hydrogen ions, R is the universal gas constant, T is the absolute temperature, F is the Faraday constant and $\{H^+\}$ represents the hydrogen-ion activity. As long as the ionic strength of the test solution remains constant, the free hydrogen-ion activity, $\{H^+\}$, can be expressed in terms of concentration. Hence, equation (3) is obtained by putting $s = 2.303RT/F$ and collecting together all the constants (including the hydrogen-ion activity coefficient and factors arising from it) as E_{const}:

$$E_{cell} = E_{const} + s \log[H^+] \qquad (3)$$

In circumstances where strong-acid *vs.* strong-base titrations are to be used in the calibration of an electrode system (*i.e.*, in the approximate pH ranges 2.3–2.9 and 10.8–11.3), MAGEC uses a subroutine entitled CALIBT. CALIBT first analyses the data by the method of Gran.[18,19] Since the potentiometric data are transformed into a linear form, this analysis gives a good indication of glass-electrode performance and also yields an end-point that is independent of the slope and intercept used in equation (3). Furthermore, if extrapolation of the data from before the end-point produces a value significantly lower than that obtained from data after the end-point, it is possible that an alkaline titrant may have become contaminated with carbon dioxide from the atmosphere.[19] The end-point obtained from the Gran extrapolations

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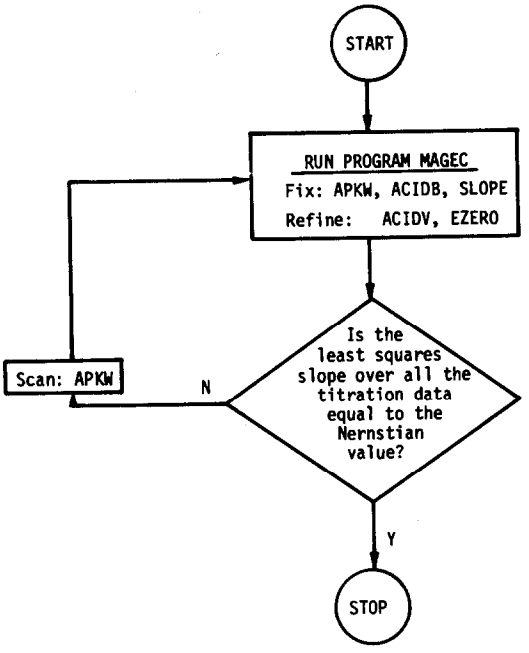


Symbols

APKW = apparent dissociation constant of water (K_w)

ACIDV = titrand acid concentration (negative for alkali)

ACIDB = titrant acid concentration (negative for alkali)

EZERO = electrode intercept (E_{const})

SLOPE = electrode slope (s)

Fig. 1.

provides an independent check of the CALIBT optimization of the strong acid concentration referred to below.

Further processing of strong-acid *vs.* strong-base titrations is divided into three stages. To begin with, the input concentration values are used to calculate free hydrogen-ion concentrations at each point, and a linear least-squares fit is performed on the data before and after the end-point and over the entire range. This first analysis is used mainly for comparison with subsequent output. Because of the effect of relatively small errors in the concentrations of titrant and titrand, the least-squares straight line does not normally possess a Nernstian slope, so the concentration of the titrand is varied slightly until the slope from the data before the end-point becomes Nernstian. It is then possible to adjust the titrant concentration in a similar manner, but on the basis of the whole range of data. Of course, to maintain the same end-point, a corresponding change in the titrand concentration also needs to be made. In this way, very close agreements between the calculated and observed values for the emf at each point can be achieved.

A factor that critically affects the refinement of the titrant concentrations in the final stage is the value used for the dissociation constant of water, K_w. This parameter is very highly correlated with the concentration of alkali in the burette, *i.e.*, a small error in its value will cause a significant deviation in the apparent slope of the electrode response, because it is used to convert the hydroxide-ion excess into free hydrogen-ion concentrations, and so it manifests itself in the optimized alkali concentration. Another way of looking at this is that either the titrant concentration or K_w (but not both) can be determined by finding the value which yields the most ideal least-squares slope. To accommodate those situations in which K_w is uncertain, CALIBT permits the user to vary the estimate systematically. The recommended procedure for using CALIBT is illustrated in Fig. 1.

As pointed out earlier, it is often necessary to determine E_{const} and the ligand protonation constants simultaneously. In such circumstances, two further equations in addition to equation (3) are applicable. These are the mass-balance equations, (4) and (5), for the ligand and for protons.

$$T_L = [L] + \sum_p \sum_r p\beta_{pr}[L]^p[H^+]^r \qquad (4)$$

$$T_H = [H^+] + \sum_p \sum_r r\beta_{pr}[L]^p[H^+]^r. \qquad (5)$$

Here, T_L and T_H are the total concentrations of ligand and protons, respectively; [L] and $[H^+]$ are the free concentrations of ligand and protons, respectively; β_{pr} is the equilibrium constant for the general reaction,

$$pL + rH^+ \rightleftharpoons L_pH_r$$

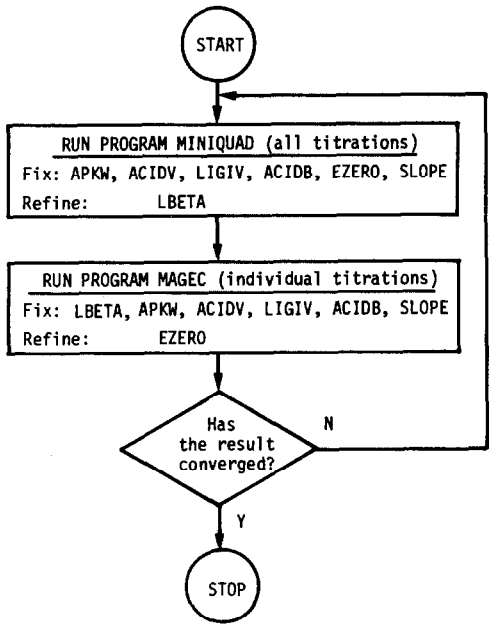
It is clear that (3), (4) and (5) constitute only two independent equations for each titration point. T_L and T_H may be expressed in terms of the initial volume of the titrant, the volume of each added increment of titrant and K_w. E is measured at each titration point. It follows that for n titration points, there are $2n$ independent equations containing $(pr + n + 2)$ unknowns, namely, the two electrode parameters E_{const} and s, pr β-values, and n free-ligand concentrations. Thus, in principle, the $2n$ equations may be solved simultaneously to yield E_{const} and the β-values from a single set of titration data, provided that the number of titration points is sufficiently large.

The values of T_L and T_H are subject to analytical error; the value of K_w is sometimes uncertain, which imposes additional uncertainty on T_H. Thus, in a sense, T_L, T_H and K_w may be regarded as additional unknowns.

In the analysis of data obtained for the titration of monoprotic ligands, the main routine in MAGEC utilizes a number of approximation formulae, for example, the Henderson–Hasselbalch equation,

$$-\log [H^+] = -\log K_a + \log \frac{[salt]}{[acid]},$$

to solve for the free hydrogen-ion concentration at each point in the titration. Analysis of the data then



Symbols

LBETA = ligand protonation constants
APKW = apparent dissociation constant of water
ACIDV = titrand acid concentration (negative for alkali)
ACIDB = titrant acid concentration (negative for alkali)
LIGIV = ligand concentration in vessel
EZERO = electrode intercept
SLOPE = electrode slope

Fig. 2.

follows a similar approach to that described above for strong acids and strong bases. On the whole, however, this is not very satisfactory because it is much more difficult to know when the solution can be considered to be reasonably well buffered.

Accordingly, the main analysis applied by MAGEC to all titrations involving ligands is one of general optimization of parameter values by using an objective function based on titration volumes. The sum of squared residuals is minimized by the simplex method introduced by Nelder and Mead.[20] Any of the parameters E_{const}, s, the β-values, T_L, T_H and K_w can be flagged for refinement, so the specific procedure is left largely in the hands of the user. Usually, the requirement is to find the value for E_{const} in each of a series of titrations with the ultimate objective of determining the protonation constants of a ligand.

THE USE OF MAGEC IN PRACTICE

In our experience, the calibration of an electrode system is best carried out in the following steps.

1. Even when the calibration is needed outside the pH ranges 2.3–2.9 or 10.8–11.3, it is recommended that an initial estimate of E_{const} be obtained by applying a CALIBT analysis to the titration data for a strong acid *vs.* a strong base. An additional advantage of this step stems from the possibility of using the CALIBT analysis for checking the reliability of an individual glass electrode. Lifetimes of glass electrodes differ considerably and it is often difficult to detect the first signs of deteriorating performance. In the MAGEC analysis of strong-acid *vs.* strong-base titration data, however, the appearance of sudden and marked increases in the titration-volume residuals signals the imminent demise of the electrode. A second useful criterion of incipient electrode unreliability is a tendency for discrepancies to develop between the end-points determined by CALIBT minimization of the Nernstian response and by Gran plot.

2. The E_{const} value obtained in step 1 should be used in conjunction with data obtained for titration of a ligand (perhaps acidified with mineral acid) with a strong base, in a suitable program for refining the values of the protonation constants for the ligand. The authors favour MINIQUAD[21] for this purpose. Individual titrations (*i.e.*, either replicate titrations or titrations differing in the total concentration of ligand) should be processed together to yield global values for each protonation constant.

3. The ligand-titration data and the protonation constants from step 2 are processed by the main routine of MAGEC to yield refined E_{const} values.

4. Steps 2 and 3 are repeated cyclically until convergence to a satisfactory degree is obtained.

5. If desired, the values of E_{const} and the protonation constants may be improved further by refining the values of T_H, T_L and K_w in a series of subsequent cycles. Care must be taken, however, to ensure that the calculation is not performed with more degrees of freedom than are warranted by the accuracy of the data. If this precaution is neglected, all optimization procedures can give rise to spurious results.

The number of parameters which can be refined with safety from data with a given experimental precision depends on the particular system being investigated. Thus, it is important to ensure that the refinement includes only those parameters for which optimization leads to a sufficient improvement in the sum-of-squares function that is to be minimized.

The flow diagram of Fig. 2 illustrates steps (2)–(4).

RESULTS

An example of the calibration of a glass electrode by strong-acid vs. *strong-base titration*

A mixture of hydrochloric acid (10.00 ml, *ca.* 50m*M* and sodium chloride (20.00 ml, 212.0m*M*) was titrated with sodium hydroxide (100.0m*M*) and sodium chloride solution. The sodium chloride was used to maintain the ionic strength (*ca.* 0.15*M*) as constant as possible. The solution was maintained at 37.0 ± 0.1°.

Table 1. Strong-acid *vs.* strong-base titration

	Before CALIBT optimization	After optimization of the acid concentration
Using only the data before the end-point (47 points)		
(*i*) electrode intercept, *mV*	362.4 ± 0.15	360.7 ± 0.04
(*ii*) electrode slope, *mV*	62.48 ± 0.07	61.53 ± 0.02
(*iii*) overall standard deviation, *mV*	1.4×10^{-1}	4.1×10^{-2}
(*iv*) number of residuals greater than 0.1 mV	26	0
Using all the buffered data (62 points)		
(*i*) electrode intercept, *mV*	360.4 ± 0.10	360.6 ± 0.05
(*ii*) electrode slope, *mV*	61.53 ± 0.02	61.50 ± 0.01
(*iii*) overall standard deviation, *mV*	5.0×10^{-1}	2.6×10^{-1}
(*iv*) number of residuals greater than 0.1 *mV*	41	15

Notes (*i*) The optimized initial acid concentration of 16.59m*M* compares with an expected value of 16.67m*M* and yields an end-point of 4.978 ml.

(*ii*) A slope of 61.50 mV corresponds to $pK_w = 13.310$. In practice, the calculation could be repeated using $pK_w = 13.305$ to obtain a better agreement with the Nernstian slope (61.54 mV).

A Gran-plot analysis for MAGEC gave end-points of 4.977 ± 0.002 and 4.97 ± 0.03 ml for the acid and alkaline pH data respectively. The CALIBT optimization analysis is summarized in Table 1.

An example of the internal calibration of a glass electrode in titration of a ligand solution

Duplicate titrations, using different electrode systems, were performed on a solution of acetic acid (20.00 ml, *ca.* 20m*M*) with 0.150*M* sodium hydroxide. A background concentration of perchlorate ions (0.150*M*) was maintained and the solution was kept at 25.0 ± 0.1°. The protonation constant and the initial concentration of the acetic acid as well as E_{const} for each electrode system were refined simultaneously by MAGEC. A Nernstian slope of 59.16 mV per log [H^+] was assumed throughout. The results are shown in Table 2.

MINIQUAD analysis of these titrations, using the electrode intercepts obtained by MAGEC and an initial value of [acetate] = 20.82 m*M*, yielded a refined pK_a value 4.523 ± 0.0004 with a sum of squared residuals of 2.3×10^{-8} and a MINIQUAD R factor of 0.0008.

Table 2. Acetic acid *vs.* strong base titrations

	Titration 1	Titration 2
pK_a	4.522	4.524
Electrode Intercept *mV*	−367.5	−379.9
[acetate], *m*M	20.83	20.81

An example of the internal calibration of a glass electrode in a student titration of ligand solution

In a fourth-year exercise, a pair of students titrated a solution of glycine (25.00 ml, 9.17m*M*) in hydrochloric acid (21.90m*M*). A background concentration of chloride ions (1.00*M*) was maintained and the solution was kept at 25.0° ± 0.1.

Table 3 indicates the progress of the MINIQUAD–MAGEC cycling refinement.

Table 3. Honours students' titration: glycine *vs.* strong base

	Initial values	Refined values after first MAGEC run (29 iterations)	Refined values after 5 MINIQUAD–MAGEC cycles
pK_w	13.69		13.63
pK_{a1}	2.4		2.318
pK_{a2}	9.6		9.583
Electrode Intercept, *mV*	400.0	382.1	381.7
[glycine], *m*M	9.171		9.176
[NaOH], *m*M	49.65		49.63
Sum of squared residuals	151.3	0.4289	0.04859

Table 4. Honours students' titration: glycine *vs.* strong base

Titre volume, *ml*	Observed emf, *mV*	Initial residuals, *ml*	Residuals after 1st MAGEC run, *ml*	Final residuals, *ml*
0.000	273.30	3.048	0.076	0.012
0.020	273.10	3.061	0.098	0.035
0.040	273.05	3.053	0.092	0.029
0.060	273.00	3.045	0.086	0.023
0.381	271.80	2.966	0.060	−0.001
0.875	269.70	2.870	0.059	0.002
1.358	267.60	2.770	0.053	0.000
1.838	265.50	2.661	0.036	−0.013
2.318	263.30	2.552	0.022	−0.022
2.792	260.90	2.459	0.030	−0.009
3.255	258.60	2.349	0.016	−0.019
3.721	256.10	2.244	0.013	−0.017
4.177	253.60	2.133	0.001	−0.024
4.632	250.90	2.027	0.000	−0.021
5.076	248.10	1.923	0.001	−0.015
5.513	245.20	1.817	0.001	−0.012
5.943	242.20	1.706	−0.003	−0.011
6.368	239.00	1.596	−0.002	−0.007
6.781	235.60	1.490	0.005	0.004
7.181	232.10	1.380	0.008	0.010
7.575	228.40	1.265	0.007	0.012
7.957	224.40	1.153	0.013	0.020
8.322	220.30	1.035	0.010	0.019
8.778	215.80	0.832	−0.075	−0.065
9.013	211.00	0.804	0.011	0.022
9.328	205.80	0.692	0.013	0.024
9.618	200.20	0.586	0.015	0.026
9.881	194.30	0.483	0.011	0.021
10.122	187.50	0.389	0.013	0.023
10.311	181.00	0.311	0.011	0.020
10.479	173.60	0.242	0.011	0.019
10.602	166.70	0.189	0.010	0.017
10.703	159.40	0.146	0.008	0.015
10.779	152.40	0.112	0.006	0.012
10.838	145.20	0.086	0.005	0.011
10.881	138.40	0.067	0.004	0.010
10.920	130.70	0.048	0.002	0.007
10.952	121.50	0.034	0.001	0.006
10.975	112.30	0.023	0.001	0.005
10.998	99.40	0.012	−0.002	0.002
11.019	77.40	0.002	−0.004	−0.000
11.039	36.50	−0.010	−0.012	−0.008
11.059	−26.00	−0.012	−0.020	−0.016
11.079	−53.40	0.002	−0.022	−0.018
11.099	−66.50	0.015	−0.024	−0.019
11.133	−80.70	0.041	−0.026	−0.020
11.173	−91.30	0.071	−0.027	−0.021
11.224	−101.30	0.115	−0.025	−0.018
11.286	−109.70	0.162	−0.026	−0.017
11.365	−117.90	0.222	−0.024	−0.013
11.458	−125.10	0.284	−0.025	−0.013
11.574	−132.40	0.358	−0.023	−0.009
11.708	−138.90	0.426	−0.027	−0.010
11.866	−146.00	0.521	−0.015	0.004
12.039	−151.40	0.565	−0.034	−0.011
12.241	−158.70	0.678	0.000	0.026
12.437	−164.10	0.732	0.004	0.033
12.664	−169.70	0.773	0.004	0.035
12.908	−175.10	0.790	−0.005	0.029
13.166	−180.70	0.800	−0.007	0.029
13.428	−186.20	0.795	−0.010	0.029
13.693	−191.60	0.773	−0.017	0.023
13.964	−197.00	0.735	−0.032	0.010
14.237	−202.50	0.688	−0.051	−0.006
14.510	−208.40	0.648	−0.061	−0.014
14.767	−214.20	0.613	−0.072	−0.021
15.022	−220.00	0.582	−0.092	−0.037

Table 4.—*continued*

Titre volume, *ml*	Observed emf, *mV*	Initial residuals, *ml*	Residuals after 1st MAGEC run, *ml*	Final residuals, *ml*
15.276	−226.10	0.571	−0.109	−0.047
15.521	−232.20	0.590	−0.123	−0.051
15.761	−238.20	0.642	−0.135	−0.050
16.003	−244.00	0.730	−0.146	−0.045
16.248	−249.40	0.849	−0.160	−0.040
16.508	−254.70	1.019	−0.169	−0.024
16.778	−259.50	1.221	−0.181	−0.007
17.068	−264.00	1.463	−0.194	0.012
17.378	−268.10	1.734	−0.214	0.029
17.713	−272.00	2.057	−0.231	0.053
18.00	−275.00	2.363	−0.240	0.081

A Nernstian slope of 59.16 mV per log $[H^+]$ was assumed throughout. The refinement, within the first run, by MAGEC of the electrode intercept to within 0.4 mV of the final value, is impressive. This is in spite of the use of approximate acid dissociation-constant values.

Table 4 shows how the magnitudes and distributions of the residuals have been improved.

DISCUSSION

The accurate experimental determination of hydrogen-ion concentrations is important in many areas of chemistry, often because the measurements provide information about other chemical species which occur in solution. Despite this, considerable differences exist in the way in which the electrode systems are calibrated.

For the determination of formation constants, many investigators still employ the pH-scale, as evidenced by the plethora of publications describing how pH measurements can be adjusted to yield values for hydrogen-ion concentration.[2–9] A standardized approach, based directly on hydrogen-ion concentrations, is clearly needed. This would facilitate both the comparison of formation-constant values and elimination of errors inherent in the use of the pH-scale. In this respect, numerical analysis of titration data, to determine parameters such as E_{const}, would appear to have many advantages.

The results presented here demonstrate the efficacy of MAGEC in the calibration of a glass-electrode system both in the strong-acid *vs.* strong-base titration range and in the hydrogen-ion concentration range where the interaction of a given ligand with protons or a metal ion is significant. A strong advantage lies in the ability of MAGEC together with MAGEC–MINIQUAD cycling to solve this problem even when the protonation constants of the ligand are not known *a priori*. Thus, E_{const} is optimized for a given titration, and any change in the liquid-junction potential is incorporated into the particular E_{const} value obtained. Small variations in the liquid-junction potential arising, for example, from poor reproducibility or concentration effects, cease to be a problem. This is a significant improvement over less general approaches such as that of Gaizer and Puskás.[22] A further marked advantage is derived from the MAGEC (CALIBT) analysis of strong-acid *vs.* strong-base data in assessing the reliability of a given glass electrode. Yet another feature of the MAGEC (CALIBT) routine lies in the analytical facility whereby the concentration of a given strong acid or strong base solution can be determined more precisely than by the Gran method.

As far as the main routine in MAGEC, as applied to ligand–proton equilibrium, is concerned, the text describes how, in principle, the parameters E_{const}, s, β-values, total concentrations of ligand and protons and K_w, can all be determined provided there are sufficient titration points. In practice, however, limitations arise, mainly from the correlation of errors between two or more of the parameters. This results from an effect in which small differences in the values of these particular parameters have similar (increasing) effects on the function to be minimized. Thus, the errors in the parameters concerned tend to compensate each other, leading to false mathematical solutions. The difficulties arising from this source may be restricted by the MAGEC–MINIQUAD cycling procedure, which provides for only one or a selected few of the parameters to be estimated by MAGEC.

While work on MAGEC has been in progress, another program with similar objectives has appeared in the literature.[23] Like MAGEC, this permits the optimization of any or all of the titration parameters. However, it does not offer the additional facilities simultaneously. Hence, the two programs partially overlap and partially complement one another.

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RADIOTRACER STUDIES ON ION-SELECTIVE ELECTRODE MEMBRANES CONTAINING METAL COMPLEXES OF A NONYLPHENOXYPOLY-(ETHYLENEOXY)ETHANOL IN POLY(VINYL CHLORIDE) MATRICES

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Summary—Radiotracer studies with ^{133}Ba, ^{45}Ca and ^{36}Cl are reported for PVC matrix membranes containing 2-nitrophenyl phenyl ether and the tetraphenylborate of a barium (or calcium) complex with a nonylphenoxypoly(ethyleneoxy)ethanol (NP), Antarox CO880. The results show that there is very limited permeation of radioactive barium and calcium ions through the membrane systems. However, the continued uptake (with time) of radioactive barium ions by the membranes (the uptake of calcium ion is less) suggests that in relation to selective electrode response the stabilization of ions by the NP ligand within the membrane is important in addition to the simple availability of membrane pathways for primary-ion transport through the membrane. Permselectivity to counter-ions is indicated by the non-permeation of radioactive chloride ions.

Nonylphenoxypoly(ethyleneoxy)ethanols (NPs) [$C_9H_{19}C_6H_4O(CH_2CH_2O)_nC_2H_4OH$] are non-ionic surfactants. They form complexes with metal cations,[1–3] and the tetraphenylborate (TPB) of the barium–NP complex, where $n = 29$ as in the commercial preparation Antarox CO880, functions as an ion-selective sensor for barium ions in liquid[4,5] and PVC matrix[1,2] membrane electrodes. The stoichiometry[2,3] of the ion-sensor is $Ba(CO880)_{0.4}TPB_2$.

Solvent extraction data (obtained by use of picrate as counter-ion) for the interaction of alkaline-earth cations with Antarox CO880 show that the affinity[2] of the cations for Antarox CO880 is in the order Ba > Sr > Ca > Mg. The electrode selectivity coefficients, $k^{pot}_{Ba,M}$, for PVC membrane electrodes containing the barium complex of Antarox CO880, with 2-nitrophenyl phenyl ether as solvent mediator, are in the order Sr > Ca > Mg.

The special properties of the barium–NP adduct have been attributed[4] to 12 ethyleneoxy units (EOU) complexing with Ba^{2+} to assume a tight helical conformation with a ring radius of about 1.3 Å in which the Ba^{2+} ion is held by a cage of 12 oxygen atoms (6 in each loop) through ion–dipole interaction.

With regard to possible pathways for ion transport in ionselective electrode membranes the use of radiotracers has yielded much useful information for calcium ion-selective PVC matrix membranes based on calcium dialkylphosphate sensors.[6–8] Similar techniques have now been extended to a study of the permeation of barium, calcium and chloride ions through PVC matrix membranes based on the barium and calcium complexes of Antarox CO880, the γ-emitting barium-133 ($t_{\frac{1}{2}}$ 7.2 years), β-emitting calcium-45 ($t_{\frac{1}{2}}$ 165 days) and β-emitting chlorine-36 ($t_{\frac{1}{2}}$ 3.1×10^5 years) radioisotopes being used for the purpose.

Non-ionic surfactants also elicit a potential response from such barium-sensor membranes,[9] and the effect of a solution of the non-ionic surfactant, Antarox CO880, on barium ion permeation through this sensor membrane system is also discussed.

EXPERIMENTAL

PVC-supported barium-sensitive membranes were prepared as previously described[1,10] from PVC (0.17 g) and a solution of the tetraphenylborate of the barium (or calcium) complex with Antarox CO880 (0.04 g) in 2-nitrophenyl phenyl ether (0.36 g). Additional membranes were based on other proportions of complex, Antarox CO880 and 2-nitrophenyl phenyl ether (see Table 1). Diffusion of radiotracers between two solutions separated by such membranes was followed by using the cell shown in Fig. 1. The cell was enclosed in an air thermostat maintained at 35°. The cell was charged with appropriate solutions (10 ml) on either side of the membrane and their activities were monitored by removing 20-μl (for barium-133) or 10-μl (for calcium-45 and chlorine-36) samples with a Lang–Levy auto-zero micropipette after the times shown in Table 1. Such samples were spotted onto filter paper (Whatman No. 1, 2.1 cm diameter) and the activity was counted with a Geiger–Müller mica end-window tube.

The activity of each membrane after conclusion of the experiment at the times shown in Table 1 was also monitored by (*i*) slicing off the membrane from its supporting PVC tubing, placing it on a planchette under the Geiger–Müller tube, and counting both sides in turn to determine C_{act}/C_{inact} (see results section) and (*ii*) dissolving the membrane in tetrahydrofuran (10 or 5 ml) and counting 20, 40 and 60 μl samples, spotted onto filter papers, in order to determine percentage uptake (see results section). All counts were taken for 300 sec and corrected for background (*ca.* 170 counts). Count-rates of *ca.* 8, 17 and 45 cps

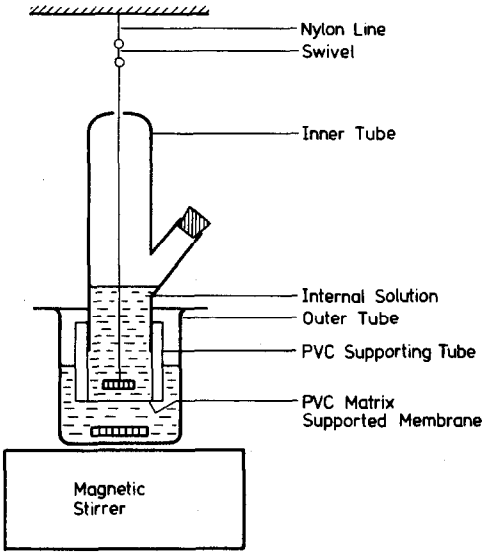

Fig. 1. Apparatus assembly for study of permeation of radiotracer ions through PVC matrix membranes by intermittent sampling of solutions.

were obtained from 10-μl samples of the barium-133, calcium-45 and chlorine-36 solutions, respectively. The half-lives of all the radiotracers were long enough for the effect of decay during the experiments to be negligible.

Barium chloride ($10^{-3}M$), calcium chloride ($10^{-3}M$) or Antarox CO880 ($10^{-2}M$) solution was placed on one side of the PVC membrane, and on the other $10^{-3}M$ barium chloride (labelled with ^{133}Ba), $10^{-3}M$ calcium chloride (labelled with ^{45}Ca) or $10^{-3}M$ barium chloride (labelled with ^{36}Cl).

The metal chloride solutions were prepared from BDH "AnalaR" grade materials. Radioactive solutions were prepared by addition of (*i*) 14 ml of $6.39 \times 10^{-5}M$ barium chloride (^{133}Ba *ca.* 0.08 mCi/ml) to 100 ml of $10^{-3}M$ barium chloride, (*ii*) 0.15 ml of $3.7 \times 10^{-2}M$ calcium chloride (^{45}Ca *ca.* 2.0 mCi/ml) to 250 ml of $10^{-3}M$ calcium chloride, (*iii*) 1.15 ml of 0.21M hydrochloric acid (^{36}Cl *ca.* 0.09 mCi/ml) to 95 ml of $10^{-3}M$ barium chloride. This ^{36}Cl-labelled solution was neutralized with barium hydroxide and the resultant solution was made up to 100 ml with distilled water. Active solutions of barium chloride (*ca.* $10^{-3}M$) were prepared by dilution of this stock solution.

RESULTS

The results presented in Table 1 refer to the permeation of barium ions (experiments 7–21, 24–30 and 33–37) or chloride ions (experiments 22, 23, 31 and 32) through PVC barium-sensitive membranes with 2-nitrophenyl phenyl ether (NPPE) solvent mediator, of barium ions through membranes containing only NPPE (experiments 4–6) and a membrane containing some free uncomplexed Antarox CO880 and NPPE (experiment 1), and of chloride ions through membranes containing Antarox CO880 and NPPE (experiments 2 and 3). The uptake of barium was also measured. The results of experiments 7–19 refer to experiments in which two barium chloride solutions were separated by a barium-sensor membrane, while those of experiments 24–32 refer to experiments in which the membrane separated barium chloride and Antarox CO880 solutions. In experiments 20 and 21 barium chloride solutions containing barium-133 were used on both sides of the membrane. Experiments 33–37 show the effect on barium-133 permeation of adding free Antarox CO880 to membranes based on barium complex plus NPPE.

Results are also presented for calcium permeation through, and uptake by, barium-sensor membranes (experiments 40 and 41), a membrane containing the calcium complex of Antarox CO880 (experiment 39) and a membrane containing only NPPE solvent mediator (experiment 38).

Since the methods involved the destruction of the membranes, fresh membranes had to be used for each experiment quoted in Table 1.

The values of C''/C' represent the ratio of counts obtained for samples taken from the radioactive solution (C') and the initially inactive solution (C''). The low ratios clearly indicate that permeation of radioactive barium, calcium and chloride ions through these membrane systems was very limited. On the other hand, the C_{act}/C_{inact} ratio represents the ratio of counts obtained from the surface of the membrane in contact with the radioactive solution (C_{act}) to counts obtained from the surface of the membrane in contact with the initially inactive counter-solution (C_{inact}) at the conclusion of each experiment.

Control membrane-counting experiments in which a number of radioactive barium chloride samples (20–100 μl) were counted (*i*) in the normal manner for PVC membranes, and (*ii*) with a barium-sensor membrane of the thickness normally used (*ca.* 0.05 cm) placed between the sample and the Geiger-Müller tube, gave values of 4.28 (s.d. 0.37), 4.17 (s.d. 0.91) and 3.89 (s.d. 0.25) for the ratio of counts obtained for three different membranes. Such values suggest that the value of C_{act}/C_{inact} for membranes in actual barium radiotracer experiments would be *ca.* 4 if all the barium radiotracer incorporated into the membrane had been concentrated at, or near, the surface of the membrane which had been in contact with the radioactive solution. The corresponding figures for the calcium-sensor membrane with radioactive calcium-45 were 34.8 (s.d. 3.7) and for barium-sensor membranes with solutions containing chlorine-36 were 2.64 (s.d. 0.18) and 2.03 (s.d. 0.21). The varying extent of the quenching of the counts from the different radioisotopes by the membranes reflects the different energies (0.384, 0.258 and 0.714 MeV for barium-133, calcium-45 and chlorine-36, respectively) and natures (β-particles for calcium-45 and chlorine-36 and γ-rays for barium-133) of the emissions from the radioisotopes.

The values of C_{act}/C_{inact} are consistent with diffusion of radioactive barium species within the barium-sensor membranes away from the radioactive solution/membrane interface with increasing contact times. No appreciable diffusion of such radioactive species occurs within membranes containing only NPPE or NPPE with Antarox CO880 (*cf.* experi-

Table 1. Radioactive count data for PVC membranes containing the tetraphenylborate of barium (or calcium) complex with Antarox CO880 and 2-nitrophenyl phenyl ether (NPPE)

Expt.	Membrane materials in 0.17 g of PVC	Time, *hr*	Active solution	Inactive solution	C''/C'	C_{act} [a]	C_{inact} [a]	$C_{act/inact}$ [a]	Uptake, %
1	CO880 0.04 g	96	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	4601	1136	4.05	0.5
2[b]	+ NPPE 0.36 g	96	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.01	1036	721	1.44	<0.1
3[b]		96	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	1163	733	1.59	<0.1
4		48	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	675	140	4.82	<0.1
5	NPPE 0.04 g	96	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	388	120	3.23	<0.1
6		44	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	558	122	4.82	<0.1
7		2	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	8503	2677	3.18	1.0
8		4	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.01	14158	5798	2.44	1.8
9		4	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	17442	8209	2.12	2.0
10		6	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	24904	8034	3.10	2.4
11		24	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.01	29166	13923	2.09	4.0
12		24	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	23661	16015	1.48	3.6
13		48	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.02	29688	21766	1.36	5.6
14		48	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.01	31660	20610	1.54	5.1
15		72	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.02	49167	27198	1.81	6.6
16		72	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.01	40868	29420	1.39	5.5
17		96	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.03	19599	16275	1.20	4.0
18		144	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.01	60344	38763	1.56	6.6
19	Barium sensor 0.04 g	503	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.03	48155	35238	1.37	NC
20	+ NPPE 0.35 g	96	$10^{-3}M$ $BaCl_2$[c]	—	[c]	19599	35419	0.82[c]	4.1
21		96	$10^{-3}M$ $BaCl_2$[c]	—	[c]	47764	41759	1.14[c]	4.9
22[b]		96	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	499	415	1.20	<0.1
23[b]		96	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	—	800	609	1.31	<0.1
24		2	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ CO880	0.01	9649	2875	3.36	1.2
25	—	4	$10^{-3}M$ $BaCl_2$	$10^{-2}M$ CO880	0.01	12654	8772	1.44	1.4
26		6	$10^{-3}M$ $BaCl_2$	$10^{-2}M$ CO880	0.02	17369	11491	1.51	2.0
27		48	$10^{-3}M$ $BaCl_2$	$10^{-2}M$ CO880	0.01	17636	14603	1.21	2.5
28		48	$10^{-3}M$ $BaCl_2$	$10^{-2}M$ CO880	0.01	16043	12674	1.27	2.0
29		96	$10^{-3}M$ $BaCl_2$	$10^{-2}M$ CO800	0.02	35533	28360	1.25	4.4
30		96	$10^{-3}M$ $BaCl_2$	$10^{-2}M$ CO880	0.02	44869	28362	1.58	4.5
31[b]		96	$10^{-3}M$ $BaCl_2$	$10^{-2}M$ CO800	—	195	133	1.47	<0.1
32[b]		96	$10^{-3}M$ $BaCl_2$	$10^{-2}M$ CO800	—	154	103	1.50	<0.1
33	Barium sensor 0.04 g	96	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.06	21789	24182	0.90	4.6
34	+ CO800 0.04 g	168	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.08	19097	23476	0.81	4.2
35	+ NPPE 0.36 g	336	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.05	30249	31110	0.97	5.4
36	Barium sensor 0.04 g + CO800 0.01 g	336	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.04	28489	21600	1.32	5.1
37	+ NPPE 0.36 g	336	$10^{-3}M$ $BaCl_2$	$10^{-3}M$ $BaCl_2$	0.03	28662	28452	1.00	5.9
38	NPPE 0.40 g	360	$10^{-3}M$ $CaCl_2$	$10^{-3}M$ $CaCl_2$	0.01	19048	NC	NC	NC
39	Calcium sensor 0.04 g + NPPE 0.36 g	360	$10^{-3}M$ $CaCl_2$	$10^{-3}M$ $CaCl_2$	0.01	91212	2918	31.2	1.6
40	Barium sensor 0.04 g	216	$10^{-3}M$ $CaCl_2$	$10^{-3}M$ $BaCl_2$	0.01	119160	15497	7.70	2.1
41	+ NPPE 0.36 g	360	$10^{-3}M$ $CaCl_2$	$10^{-3}M$ $CaCl_2$	0.01	44046	6093	7.23	0.8

[a] Direct comparison of ^{45}Ca, ^{133}Ba and ^{36}Cl values is not possible because of the different natures and concentrations of the radiotracers used.

[b] Active solutions contain chlorine-36.

[c] Active solution containing barium-133 on both sides of membrane.

NC Not counted.

ments 1, 4–6). If no concentration of radioactive ions occurs at or near one of the solution/membrane interfaces a value of *ca.* 1 for this ratio would indicate a homogeneous distribution of radioactive barium ions within the membrane.

In addition to providing evidence for diffusion of barium ions within the barium-sensor membranes, the experiments show that the uptake of barium ions by the membranes increases with time, *cf.* the increase in magnitude in C_{act} and C_{inact} in Table 1.

Further evidence for increased uptake of barium with time was obtained from the experiments involving dissolving the membranes in tetrahydrofuran and counting the total activity. The percentage uptake is the activity of the membrane at the end of the experiment, expressed as a percentage of the total initial activity in the radioactive solution. It provides a convenient measure of the extent of incorporation of radioactive ions by these membrane systems, and, by extrapolation, of the total number of ions of that type, assuming that no preferential incorporation of radioactive ions occurs.

Examination of Table 1, even when allowance is made for the limited reproducibility of the uptake

data on account of such factors as minor variations in membrane content and thickness, shows that over similar time scales

(*i*) the presence of Antarox CO880 as the counter-solution depressed the rate of barium-ion incorporation by the barium-sensor membranes;

(*ii*) there is no evidence for the uptake of significant amounts of barium radiotracer by membranes containing only the solvent mediator, NPPE;

(*iii*) the uptake of radioactive barium is considerably lower for a membrane containing only Antarox CO880 and NPPE than for membranes containing the barium-sensor material;

(*iv*) the presence of Antarox CO880 with complex and NPPE in the membranes leads to increased amounts of barium radiotracer appearing in the solution on the other side of the membranes;

(*v*) even over a much longer time scale the extent of incorporation of calcium-45 into membranes containing either the barium or calcium form of the Antarox CO880 complex is significantly lower than that for barium-133 ions;

(*vi*) the uptake of radioactive barium by membranes separating two active solutions (experiments 20 and 21) and an active and an inactive solution (experiment 17) after 96 hr of contact shows that incorporation of barium ions into the membranes occurs to essentially equal extents from the solutions on both sides of the membrane;

(*vii*) the results of the experiments with chlorine-36 provide no evidence for either the permeation of chloride co-ions through the membranes or for the uptake of chloride ions by these membranes.

DISCUSSION

The solvent mediator effects[1,8,11–15] and the chelation between ions and sites within the membrane, particularly when a strong affinity exists between the ions and such sites,[15–17] both strongly influence the selectivity characteristics of membranes based on liquid ion-exchangers. The results of this study strongly suggest that strong ion/membrane-site interactions are involved in the response of membrane systems based on the cation complexes of Antarox CO880.

Barium-133 and chloride-36 studies

The continued uptake of radioactive barium ions by barium-sensor membranes and the absence of evidence for the uptake of chloride ions (Table 1) confirm that these membranes possess the permeation selectivity characteristics of ion-exchanger membranes. The membrane takes up these radioactive ions, but there is no evidence for their large-scale permeation through the membrane into the inactive counter-solution (*cf.* experiments 7–19). The evidence for the entry of barium ions into the membrane from the solutions on both sides of the membranes (*cf.* experiments 20 and 21) implies that ions might accumulate within the membrane (or at least at the membrane side of the membrane–solution interface) rather than be involved in large-scale membrane pathways for primary ion transport through the membrane. Though the pattern of barium ion uptake could be due to a very slow exchange between barium ions and ion-exchanger sites within the membrane, the attainment of a maximum uptake of radioactive barium after *ca.* 2 days' contact with the barium chloride solutions and the failure of the radioactive barium ions to enter the counter-solution in appreciable amounts even after *ca.* 21 days' contact with the solutions (*cf.* experiment 19) strongly suggests the presence within the membrane of sites with a strong affinity for the incorporated barium ions.

Initial uptake of radioactive barium ions from solution, which occurs only with those membranes containing either the barium-sensor complex (experiments 7–21 and 24–30) or the free uncomplexed Antarox CO880 (experiment 1) is consistent with an interaction between barium ions in solution and surface sites on the membrane. Barium-sensor complex species and/or free uncomplexed Antarox CO880 species could act as such ion-exchange sites on the surface. Transport of incorporated barium ions away from the solution/membrane interface into the bulk of the membrane with increased contact time between the membranes and the radioactive solutions, as indicated by a general decrease in the C_{act}/C_{inact} ratio, can only occur if a network of the ion-exchanging sites exists within the membrane. In the absence of such a network and the need to preserve the electrical balance imposed by permselectivity, the incorporated barium ions would remain concentrated at or near the active solution/membrane interface (*cf.* C_{act}/C_{inact} and uptake values in experiment 1).

The reluctance of the radioactive ions to pass through the membrane and enter the bulk of the counter-solution, as happens with calcium in calcium dialkylphosphate membranes,[6,7] may be surprising in view of the seemingly ready availability of pathways for diffusion of barium ions within the membrane. However, the fact that the existence of a strong affinity between incorporated ions and sites within the membrane caused the entrapment of radioactive beryllium-7 species within membranes based on alkylphosphate indicates that permeation of radio-tracer through the membrane and into the solution on the other side is not obligatory.[7] In this respect, the maximum extent of incorporation of radioactive barium, *i.e.*, *ca.* 6%, is low in relation to the total number of ion-exchanger sites within the membrane ($\sim 1.3 \times 10^{18}$), which is approximately the same as the number of barium ions in the solutions ($\sim 6 \times 10^{18}$).

Elemental analysis[2,17] of the barium complex with Antarox CO880 indicates presence of *ca.* 5% free uncomplexed Antarox CO880. Such molecules present within the sensor membrane may act as intermediaries for the stabilization of incorporated barium

ions, but for actual uptake there must also be simultaneous co-ion permeation to preserve electrical balance, and significant permeation by chloride co-ions was not observed (experiments 22, 23, 31 and 32). However, free Antarox CO880 does seem to have a part to play, as can be seen by the small transfer of barium radiotracer to solutions on the other side of the membranes in experiments 33–37 where C''/C' exceeds the values of $\leqslant 0.01$ observed in most of the other experiments. The difficulty of coiling the Antarox CO880 into the helix postulated for the complex,[4] may well be related to the small transfer of barium radiotracer species.

Although the disappearance of the C–O–C stretching frequency bands at 1149 and 1060 cm^{-1}, the appearance of a new band at 1030 cm^{-1}, and the shift of the main band from 1115 cm^{-1} to 1085 cm^{-1} are all indicative of NP–barium complex formation,[2] infrared studies are not as discriminating as the radiotracer experiments in yielding clues to the mechanism of the barium ion transfer. Measurements indicating membrane resistances of *ca.* 0.2 MΩ also do not provide the necessary discrimination for any useful conclusions to be drawn.

The inability of membranes containing only the solvent mediator, NPPE, to incorporate radioactive barium ions to any significant extent (*cf.* experiments 4–6) also demonstrates a role for Antarox CO880, as does the concentration of barium ions incorporated at or near the active solution/membrane interface in a membrane containing only Antarox CO880 and NPPE solvent mediator.

It is clear from the data that the rate of uptake of barium radiotracer is faster when barium chloride is used in the inactive counter-solutions. The data best fit first-order kinetics, with linearization regression coefficients 0.9203 and 0.9338 for the experiments with barium chloride and Antarox CO880 respectively. The $t_{\frac{1}{2}}$ values were *ca.* 90 and 540 hr, respectively. The unsuitability of the data for satisfactory kinetic analysis is not surprising when it is considered that each datum point was obtained with a separate membrane and that minor variations in membrane composition and thickness could cause appreciable variations in the number of sites available within the membrane for interaction with the incorporated ions.

The lower rate of uptake of barium ions by the membrane when Antarox CO880 is used in the counter-solution may be related to participation of the solute Antarox CO880 with the membrane barium ion. In this respect, it is significant that the membrane system, when set up in a potentiometric electrode, responds to the ethoxylate.[9]

Calcium-45 studies

The overall electrode selectivity characteristics of these PVC-supported barium-sensor systems is related to stable complex formation with Antarox CO880 molecules within the membrane.[1,2,4] The selectivity of the membranes should, then, be due to the relative ability of barium and interferent ions to enter the membrane and to form stable complexes with membrane sites. The results of calcium-45 permeation studies with membranes containing the calcium complex (experiment 39) and the barium complex (experiment 40) with Antarox CO880 are consistent with such a view. These results show that the extent of incorporation of calcium ions is significantly less than that of barium ions. Moreover, they show that permeation of radioactive ions within the membranes occurs to a greater extent in a membrane containing the barium-sensor material (*cf.* the values of C_{act}/C_{inact} in experiments 39–41). The relative concentration of radiotracer near the active solution/membrane interface for the membrane containing the calcium complex could be due to either a strong affinity between surface ion-exchanger sites and the incorporated ions or a breakdown of the mechanism for the transfer of ions away from the membrane surface because of the greater affinity of barium ions. This study does not conclusively favour either of these possibilities but picrate solvent-extraction data for alkali and alkaline-earth metal cations show that barium forms a more stable complex with Antarox CO880 than calcium and lends support to the second alternative.[2]

CONCLUSION

These studies suggest that the response of membrane systems based on cation complexes with Antarox CO880 cannot be explained in terms of the ready availability of membrane pathways for primary ion transport through the membrane. They imply only that an important role should be assigned to the ability of the membranes to incorporate and stabilize ions by complexation. The barium/calcium selectivity behaviour of these membranes can also be explained on this basis.

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PREPARATION AND ANALYTICAL PROPERTIES OF A CHELATING RESIN CONTAINING PHENYLALANINE GROUPS

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Summary—A polystyrene-based macroreticular resin containing phenylalanine groups has been prepared and its analytical properties have been investigated and compared with Dowex A-1. The phenylalanine resin shows high selectivity for mercury(II) and copper(II) in the pH region 2–3. The sorption behaviour of copper has been examined in detail, with the intention of using the resin analytically. The important characteristics of the resin are fast equilibration, high selectivity and small volume change between its hydrogen form and metal forms. These enable it to be applied for the rapid concentration of trace amounts of copper in the presence of large amounts of diverse metals. It may be used for the determination of copper in sea-water and the separation of copper/cobalt and copper/nickel.

A number of chelating resins containing aminocarboxylic acid groups, (*e.g.*, ethylenediaminetetra-acetic acid,[1,2] iminodiacetic acid[3–10] sarcosine,[11] and others[12–14] have been prepared and their analytical properties investigated. Among the commercially available resins, Dowex A-1 (Chelex 100) is the most frequently employed in the concentration and/or enrichment of trace metals, including transition metals.[15–18] Although the affinity of Dowex A-1 or Chelex 100 for the metal ions is high, their selectivity is not satisfactory.[5,19,20]

It is of interest to prepare new chelating resins having specificity for a selected metal ion. The specificity or selectivity of a chelating resin is usually correlated to that of the monomeric compound corresponding to the functional group. On the basis of this idea, we have proposed use of macroreticular resins containing α-amino-acid groups which may confer greater selectivity for some metal ions than that attainable with aminopolycarboxylic acids. Schlögl and Fabitschowitz[21] have reported a glycine resin based on polystyrene, but no detailed study on its sorption of metals is available.

The present paper describes an investigation of the preparation of a macroreticular phenylalanine resin, which has essentially the same functional group as that of the glycine resin,[21] and similar metal-sorption properties. The value of the resin for the preconcentration of copper, based on its high selectivity, has been studied and compared with that of Dowex A-1.

EXPERIMENTAL

Reagents

The stock metal ion solutions were prepared by dissolving the reagent grade nitrates and chlorides in water or the matrix acids, and standardized by complexometric titration.

Preparation of phenylalanine resin

To a solution of sodium ethoxide prepared from 23.7 g of sodium and 530 ml of absolute ethanol, 246 g of diethyl acetoaminomalonate were added and the mixture was refluxed for 30 min. To chloromethylated styrene–divinylbenzene copolymer (**I**) (7.5% divinylbenzene; Cl 18.3%)[22] soaked with absolute ethanol (dry resin, 100 g), the sodium diethyl acetoaminomalonate solution prepared was added, the mixture was refluxed for 30 hr with stirring, then the product was filtered off and washed with 5 litres of water and 2 litres of methanol. The pale yellow resin (**II**) obtained was air-dried at room temperature. About 148 g (N 4.0%) of resin was obtained. This intermediate resin (140 g) was refluxed for 40 hr with 400 ml of 47% hydrobromic acid with stirring, then the resin was packed into a glass tube. The column was washed successively with 10 litres of water, 1.5 litres of 1*M* sodium hydroxide and 2 litres of 90% ethanol. The light brown resin (sodium form) obtained was air-dried at room temperature. About 130 g (N 4.5%) was obtained. The 60–100 mesh fraction of the resin was used for the metal sorption studies.

Sorption of metal ions by batch operation

Unless otherwise stated, the following method was applied. To a glass-stoppered test-tube containing 100 mg of resin, 9 ml of 1*M* acetate buffer (pH 3–7) or hydrochloric acid (pH 1.5–2.5) were added. When this mixture had been equilibrated, 1 ml of metal-ion solution was added to the

test-tube, then the mixture was shaken at room temperature for a known time. The resin was filtered off on glass-wool and the amount of metal ion remaining in the filtrate was determined complexometrically or by atomic absorption spectrophotometry (AAS).

Concentration of copper from sea-water

The surface coastal sea-water was filtered through a 0.45-μm membrane filter, and treated as follows. For determination of labile copper,[16,18] 5 litres of sample were adjusted to pH 4.0 with 1*M* acetate buffer, and for total copper, 30 ml of concentrated nitric acid were added to 2 litres of sample and this solution was boiled for 15 min, then neutralized with sodium hydroxide and adjusted to pH 4.0 with acetate buffer. The conditioned sea-water samples were passed through a resin column (phenylalanine resin 1 g, 1 × 4 cm) at a flow rate of 3 ml/min. The column was washed with 50 ml of water, and copper retained on it was then eluted with 20 ml of 2*M* nitric acid and determined by AAS.

Trace concentration of copper from the sample solutions containing large amounts of other metal ions

One litre of copper solution (10 μg/l.) containing 500 or 50 mg of various metal ions was adjusted to pH 4.0 with 1*M* acetate buffer. This solution was introduced into the resin column (1 × 4 cm) at various flow-rates with a peristaltic pump. The column was washed with 50 ml of water and then copper retained on the column was eluted with 20 ml of 2*M* nitric acid.

Separation of copper/cobalt and copper/nickel

One gram of the resin was packed into a glass tube (0.5 × 15 cm) and conditioned with 50 bed-volumes of 0.1*M* acetic acid. One ml of a solution containing the metal ions was added to the column and 50 ml of 0.1*M* acetic acid were passed through the column at a flow-rate of 1 ml/min. Copper retained on the column was eluted with 20 ml of 1*M* hydrochloric acid. Cobalt or nickel was determined in the effluent and copper in the eluate.

RESULTS AND DISCUSSION

The resin was synthesized according to Scheme 1. Spherical macroreticular styrene–divinylbenzene copolymer beads were used as starting material. After the chloromethylation, diethyl acetoaminomalonate was allowed to react with the product and then hydrolysed with hydrobromic acid in the usual way. Infrared spectra of the resins are shown in Fig. 1. In

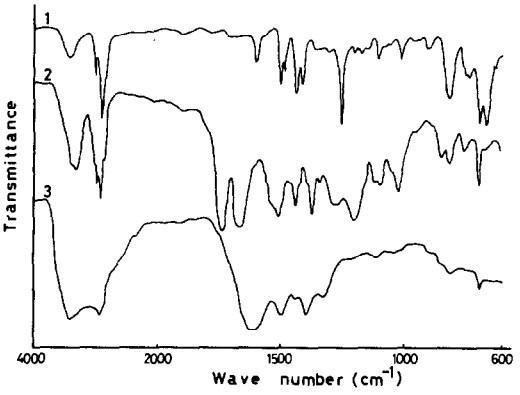
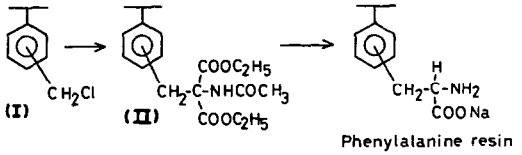


Fig. 1. Infrared spectra of resins (KBr disks). 1, Chloromethylated resin; 2, diethyl acetoaminomalonate resin; 3 phenylalanine resin.



Scheme 1

the spectrum of the intermediate resin **(II)**, the absorption band at 670 cm^{-1} (ν_{C-Cl}) found in the spectrum of the chloromethylated resin **(I)** disappeared and the characteristic absorption of the carbonyl (ester) group appeared at 1740 cm^{-1}. A characteristeric feature in the spectrum of the phenylalanine resin was the presence of the bands for the carboxylate group at 1600–1660 cm^{-1} and the amino group at 3300 cm^{-1}. Resin **(I)** also shows absorption at 3300–3400 cm^{-1} due to a small amount of residual stabilizer or water, but this is rather weak and clearly different from the absorption at 3300 cm^{-1} found for the phenylalanine resin. The functional group content in the final resin (sodium form), calculated from its nitrogen content, was 3.2 mmole/g. The spectral changes and the elemental analysis support the reac-

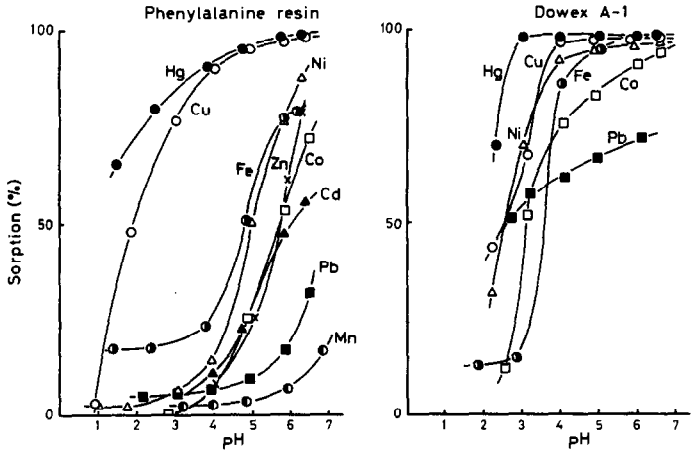


Fig. 2. Effect of pH on metal sorption with phenylalanine resin and Dowex A-1. Concentration of metal solutions 1 × 10^{-2} *M*; shaking time 24 hr.

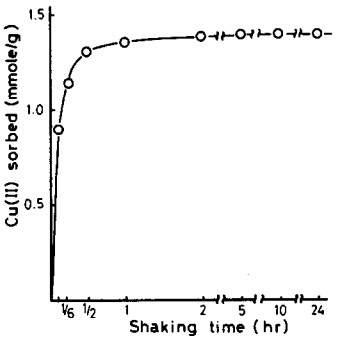


Fig. 3. Effect of shaking time on sorption of copper (II) with phenylalanine resin. Copper solution $3 \times 10^{-2}M$; pH 4.0; shaking 350 strokes/min.

tion scheme presented. The resin was found to be very stable on successive treatment with acid and alkaline solutions. The stability of the resin was checked by change of the hydrogen–sodium exchange capacity and the nitrogen content.

In a preliminary experiment, the sorption behaviour of some metal ions on phenylalanine resin and Dowex A-1 at different pH values was examined by the batch method, and the results are shown in Fig. 2. The phenylalanine resin shows high affinity for mercury(II) and copper(II). The selectivity for copper is particularly interesting. The maximum capacities for copper were 1.4 and 1.7 mmole/g at pH 4 and 7, respectively. The capacity at pH 7 suggests that the apparent molar ratio of copper to functional group in the resin is nearly 1:2, which corresponds to that of the copper–phenylalanine chelate.

The sorption kinetics of copper on the resin are shown in Fig. 3. Under the experimental conditions, sorption equilibrium is attained in 1 hr. In Table 1, the time required for 50% uptake of copper with phenylalanine resin and Dowex A-1 is shown. Its faster equilibration indicates the superiority of the phenylalanine resin. Table 2 shows the apparent volumes of various forms of both resins. The small difference in volume between the metal and hydrogen forms of the phenylalanine resin is important in column operation. The advantages of the resin over Dowex A-1 seem to be due to the difference in matrix as well as in functional group; the phenylalanine resin is a macroreticular resin but Dowex A-1 is a gel-type resin.

Table 1. The time required for 50% uptake of copper with phenylalanine resin and Dowex A-1

Resin	Time required for 50% uptake, *min*	
	pH 4.0	pH 7.0
Phenylalanine resin	2.4	4.3
Dowex A-1	10.0	13.2

Table 2. Relative resin volumes in different ionic forms

	Phenylalanine resin	Dowex A-1
Na^+	1.00	1.00
H^+	0.99	0.54
pH 4.0*	0.99	0.87
pH 6.0*	0.98	0.84
Cu^{2+} (pH 4.0)	0.95	0.64
Cu^{2+} (pH 6.0)	0.95	0.66
Co^{2+} (pH 6.0)	0.95	0.65
Ni^{2+} (pH 6.0)	0.98	0.68
Zn^{2+} (pH 6.0)	0.97	0.63

* $1M$ acetate buffer.

Sodium chloride, over a wide range of concentrations, does not affect the sorption of copper under the experimental conditions. This suggests use of this resin for trace concentration from sea-water samples. Copper was separated from sea-water by column operation. The concentrations of copper found in sea-water samples (*A–D*) collected at 4 points of Ariake Bay (Kumamoto, Japan) were as follows (in μg/l.): labile copper (*A*) 0.40, (*B*) 0.45, (*C*) 0.50, (*D*) 0.55; total copper (*A*) 0.66, (*B*) 0.71, (*C*) 0.80, (*D*) 0.96. These data agreed closely with those obtained with a Dowex A-1 column.

The separation of trace amounts of copper in the presence of large amounts of diverse metal ions with the phenylalanine resin column was examined. Figure 4 compares the results with those for a Dowex A-1 column. A 10-μg/l. copper solution containing 5×10^4 times as much cobalt(II) or nickel and 5×10^3 times as much iron(III) at pH 4 was introduced into the columns. The Dowex A-1 sorbed cobalt and nickel as well as copper and its colour changed to pink and blue, respectively, but there is no similar evidence of sorption of cobalt and nickel on the phenylalanine resin. With the Dowex A-1 column, recovery of copper decreased with increasing flow-rate,

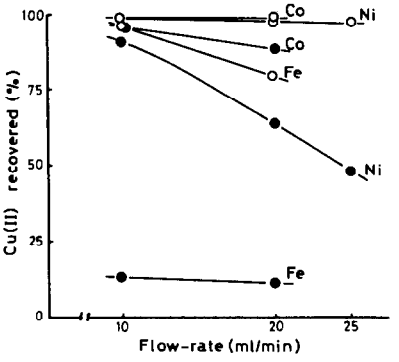


Fig. 4. Effect of flow-rate on recovery of copper in the presence of various metal ions with phenylalanine resin (—○—) and Dowex A-1 (—●—) columns. Metal concentrations, copper (10 μg/l), cobalt and nickel (500 mg/l), iron (50 mg/l).

Table 3. Separation of copper(II)/cobalt(II) and copper(II)/nickel(II) mixtures, by means of the phenylalanine resin column

Metal	Added, μg	Recovered, μg	Recovery, %
Cu	635	633	99.7
Co	5890	5798	98.4
Cu	635	635	100
Co	589	581	98.7
Cu	635	632	99.5
Ni	5870	5764	98.2
Cu	635	635	100
Ni	587	581	99.0

markedly in the presence of nickel. The presence of iron interfered strongly with the recovery of copper with Dowex A-1 column, but gave less interference on the phenylalanine resin column if the flow-rate was reduced to 10 ml/min. Thus the proposed resin is superior to Dowex A-1 for trace concentration of copper at high flow-rate.

Finally, the phenylalanine resin was applied to the separation of copper from cobalt or nickel by column operation. Cobalt and nickel are separable from copper because they are not retained on the column at pH 3 below and copper retained on the column can be eluted with 1*M* hydrochloric acid. The separation data are listed in Table 3.

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APPLICATION OF PERSONAL MICROCOMPUTERS IN THE ANALYTICAL LABORATORY—I

POTENTIOMETRIC ANALYSIS

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Summary—A novel general-purpose interface-controller unit has been designed and applied in potentiometric analysis. The unit is operated by a personal microcomputer programmed in BASIC. The interface-controller permits direct reading of specific ion-electrodes and can activate, under program control, laboratory instruments such as motor-driven burettes. The interface-controller is expandable to 16 analogue input channels, 16 binary (logic) input lines and 16 control relays but requires only one microcomputer I/O port (a total of 9 I/O lines) to handle all operations. Analogue to digital conversion is realized by counting, with the microcomputer, the output frequency of an analogue-to-frequency converter. This inexpensive method is effective in rejecting interfering signals such as power-line interference. The system has been applied in potentiometric titration analysis for determining the apparent dissociation constants of carbonic acid in sea-water and Dead Sea brines, and for ammonia determination with a gas-sensing electrode.

It is evident that the availability of cheap microprocessors and associated devices will eventually revolutionize the analytical laboratory[1] by automation of many operational, control and computation tasks which at present are performed manually. This end could be reached by interfacing existing analytical equipment with general purpose microcomputers or by designing new microprocessor-based analytical instruments. The latter approach is pursued by a number of commercial companies.

In this paper we present the alternative approach, namely the use of a general purpose microcomputer and general purpose interface operated by a high-level language. This approach has two advantages. It allows computerization of existing instrumentation and reprogramming at any stage to meet a specific need of the user, in contrast to microprocessor instrumentation which is controlled by fixed firmware. Various systems of that type were recently described by Anderson *et al.*[2]

Martin and Freiser[3] have described a microcomputer-controlled potentiometric analysis system based on a commercial microcomputer, digital pH-millivolt meter and digital burette, operated by a high-level language (CONVERS) developed at the University of Arizona. Our study differs from that of Martin and Freiser in several respects. We use a more universal high-level language (BASIC) and have developed a novel general-purpose interface which includes a high-resolution but cheap analogue to digital conversion scheme and high-impedance buffers for direct connection of pH and ion-selective electrodes. This interface can be operated in conjunction with any microcomputer which includes one 8-bit I/O port and a control line.

In this paper we describe the interface-controller and demonstrate its application in potentiometric analysis. The range of applications of this approach seems virtually unlimited.

INTERFACE DESIGN

The interface (Fig. 1) comprises 16 analogue inputs, 16 digital I/O lines and 16 uncommitted control relays. Analogue to digital (A/D) conversion is accomplished by first applying voltage to frequency (V/F) conversion and then measuring the frequency with the microcomputer. This simple and inexpensive A/D method was chosen on the basis of the intended applications, which do not call for a high rate of sampling. The method has the distinct advantage of rejecting interfering a.c. signals.

Analogue signals are fed to the V/F converter through a 16-channel multiplexer which operates under computer control. The impedance seen at the input of the analogue multiplexer is essentially the input impedance of the V/F converter, which is about 15 MΩ. Some of the channels are buffered by a very

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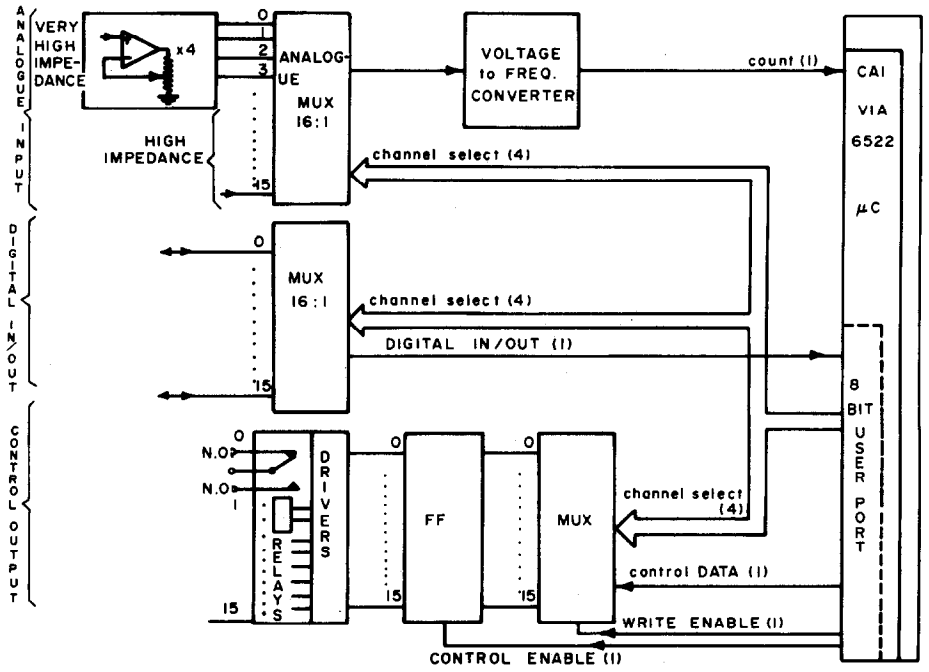

Fig. 1. Block diagram of proposed interface-controller.

high input impedance amplifier (MOSFET INPUT) to permit direct connection of pH and ion-selective electrodes. A second 16-channel multiplexer is used as a digital I/O port. Any of the lines can be a binary input or an output line. The interface-controller also includes 16 relays which are operated one at a time by the microcomputer.

Figure 1 shows a typical interconnection between the interface-controller and a microcomputer, which includes a versatile interface adaptor (VIA, type 6522). This type of interface is but one possibility, as the interface-controller is compatible with any system which has an 8-bit I/O port plus an additional binary input line for inputting the frequency signal, though it is required that any of the 8-bit lines can be programmed independently as an input or output line).

The interface-controller was built from a CMOS integrated circuit to minimize port loading and power consumption. The system used by us (Fig. 2) is, of course, just one approach of the many possible.

Figure 2a is the circuit diagram of half of the interface-controller. The complete 16 channel system is obtained by connecting two such sub-units in parallel. One half should have a break at point A of the printed circuit, and the other at point B, for proper channel decoding.

The control lines of the interface-controller are grouped as follows: PA0–PA3 (inclusive) are channel code lines. A binary combination on these lines will select one of the 16 channels of the analogue multiplexer, the digital multiplexer and one of the relay drivers. However, a relay flip-flop will be set or reset by PA4 only if line PA6 is enabled. An additional control line PA5 is used to deactivate the relays as required on turn-on or default. Digital input/out is channelled through PA7 and the output frequency of the V/F is fed to the microcomputer through line CA1.

The V/F converter (Fig. 2b) is built around a monolithic unit (RC4151 Raytheon, Inc.) and the input is buffered by a CMOS operational amplifier (CA3140, RCA). Full range is adjusted by P_1 and the offset by P_2 (Fig. 2b). In the applications considered here, the output frequency range of 0–10 kHz corresponded to an input voltage range from −500 to +500 mV. Hence, the basic sensitivity (slope) under these conditions was 10 Hz/mV.

Analogue to digital conversion

Digitization is accomplished in the system by counting the pulses of the V/F converter over a fixed period of time by a subroutine written in assembly language for maximum speed. Execution time per incoming pulse is about 100 μsec, corresponding to an upper frequency limit of 10 kHz. With a 1-sec counting window, the resolution is thus 1 part in 10^4 (*ca.* 13 bits) which is 0.1 mV. Digitization noise, estimated at ±1/2 pulse, is thus 86 db.

Besides the inherent sampling noise, the effect of integration on the signal-to-noise (S/N) ratio has to be considered. The counting technique used smooths the noise by integrating it over the counting period. Assuming a sinusoidal interfering noise of amplitude S_n, frequency ω_n and a randomly distributed phase angle ϕ_n, the total input signal S_t to the V/F is:

$$S_t = S_{in} + S_n \sin(\omega_n t + \phi_n) \quad (1)$$

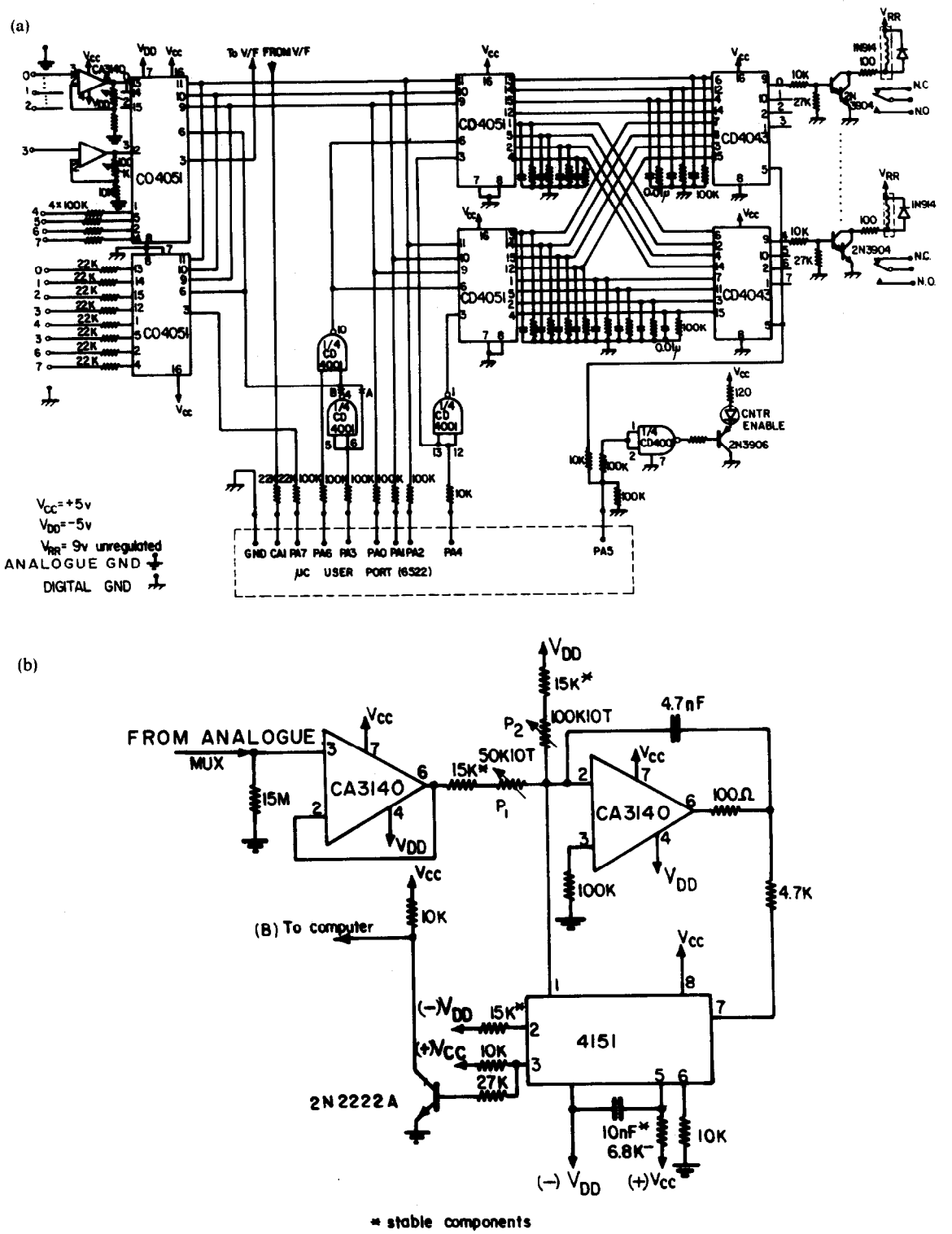

Fig. 2. Circuit diagram of interface-controller (*a*) and detailed circuit of V/F converter (*b*); 16-channel operation is obtained by using two units of type (*a*), one with a cut in the printed circuit at point A and the other with a cut at point B (shown in the 1/4 CD 4001 above PA3 in the user port).

where S_{in} is the useful input signal, which is assumed to be constant over the integration window T. Assuming linearity in the V/F conversion and neglecting sampling errors, the average total integrated (counted) signal will be

$$\bar{S}_t = \frac{1}{T}\int_0^T [S_{in} + S_n \sin(\omega_n T + \phi_n)]\,dt \qquad (2)$$

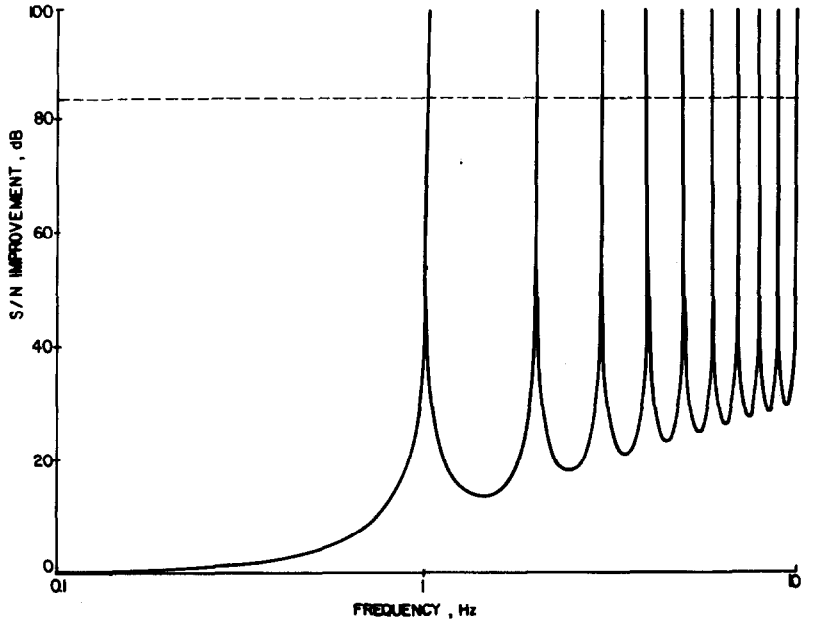

Fig. 3. S/N improvement obtained by the proposed A/D converter when the A/F frequency is counted for 1 sec. The broken line is the inherent digitization S/N (86 db) for a $1:10^4$ resolution, as used here.

$$\bar{S}_t = S_{in} + \frac{S_n}{\omega_n T}[\cos(\phi_n) - \cos(\omega_n T + \phi_n)] \quad (3)$$

The S/N ratio at the input is:

$$(S/N)_{in} = \frac{S_{in}\sqrt{2}}{S_n} \quad (4)$$

and the expected S/N ratio after integration becomes:

$$(S/N)_0 = \frac{S_{in}\omega_n T}{\sqrt{2}S_n|\sin(\omega_n T/2)|} \quad (5)$$

The improvement in S/N is thus:

$$\frac{(S/N)_0}{(S/N)_{in}} = \frac{\omega_n T}{2|\sin(\omega_n T/2)|} = \frac{\pi(T/T_n)}{|\sin(\omega_n T/2)|} \quad (6)$$

where T_n is the period of the interfering signal.

The expected improvement in S/N was calculated and plotted over the frequency range 0.1–10 Hz (Fig. 3). The broken line sets the limit of the maximum sampling error which, for a 0–10 kHz conversion range, is 86 db. The actual improvement was measured by feeding to the V/F converter a d.c. signal on which a sinusoidal signal was superimposed. It was found that the expected and actual improvement agreed very well (Fig. 4).

The effect of counting-period variation on the S/N improvement was measured by changing the counting window while corrupting the input signal with a constant interfering noise derived from the mains (50 Hz). The agreement between calculated and measured S/N improvement (Fig. 5) is good, considering the slight instability of the mains frequency and uncertainty in the counting window. The mismatch corresponds to about 0.1% variation in frequency or counting period, which is well within the tolerance of the set-up.

The ability to adjust the counting window to give optimum S/N improvement, *i.e.*, maximum rejection of an interfering noise with a given frequency, could be very useful in noisy environments. Since the mains frequency is a major interfering noise, the counting window should be adjusted for maximum rejection of this frequency. Optimum performance could be obtained by adjusting the counting window under software control to give maximum S/N improvement.

EXPERIMENTAL

Titration assembly

The titration system (Fig. 6) comprised a CBM type 2016 microcomputer (Commodore Business Machines, Inc.), the interface-controller described above, a Metrohm motor-driven burette, type E415 and a titration vessel in a thermostatic bath. The burette delivered 0.1-ml increments of titrant under program control by one relay of the interface-controller.

The sensor electrode was connected to a buffered channel of the interface controller and the reference electrode to the electrical ground of the electronics.

Electrodes

A Metrohm combination pH electrode, type EA 120, and a Broadly James NH_3 gas-sensing electrode, type 7010, were used.

Acid–base titrations

Mediterranean sea-water and Dead Sea brines were titrated with 0.5*M* hydrochloric acid. The titration was fully automatic and under microcomputer control. The titration procedure included four cycles (Fig. 7): (*a*) manual input of titration parameters; (*b*) the titration cycle; (*c*) storage of raw data; (*d*) data processing.

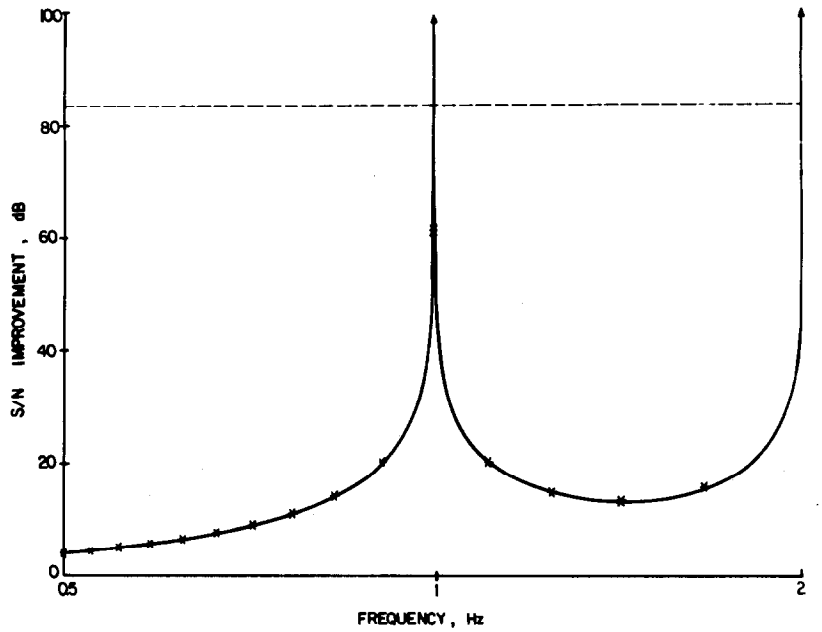

Fig. 4. Calculated (solid line) and measured (asterisks) S/N improvement in the proposed A/D converter for the frequency range 0.5–2 Hz.

Ammonia determination

The ammonia and the glass combination electrodes were connected to the interface controller through three high input impedance buffers and ground. The reference of the pH-electrode was grounded and all other lines, including the internal reference electrode of the ammonia electrode, were buffered to eliminate ground-loop problems. The pH of the solution (which had fixed ammonium chloride concentration) was adjusted manually by addition of 10*M* sodium hydroxide after addition of 2 ml of NBS standard pH-4 buffer,[4] and the outputs of the two electrodes were read by the microcomputer. These data were later used to obtain the best estimate of the constant required for calculating the total ammonia concentration from the readings of the ammonia and pH electrodes.

Computer programs

All programs, except the short subroutine for measuring the frequency of the V/F converter, were written in BASIC.

RESULTS AND DISCUSSION

Carbonate determination

The method was extensively used for studying the carbonate system in Dead Sea brines and mixtures of

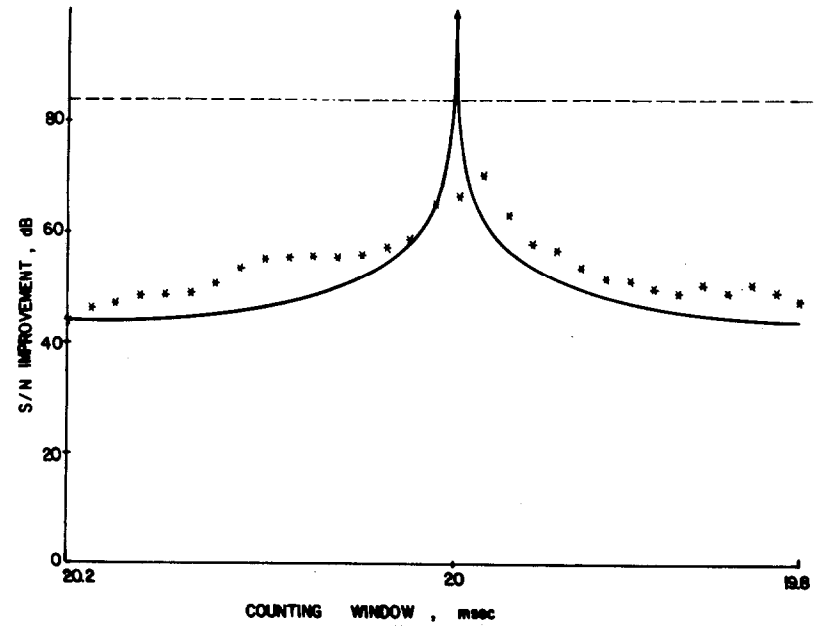

Fig. 5. S/N improvement in the proposed A/D converter as a function of counting-window width for a 50-Hz interfering signal.

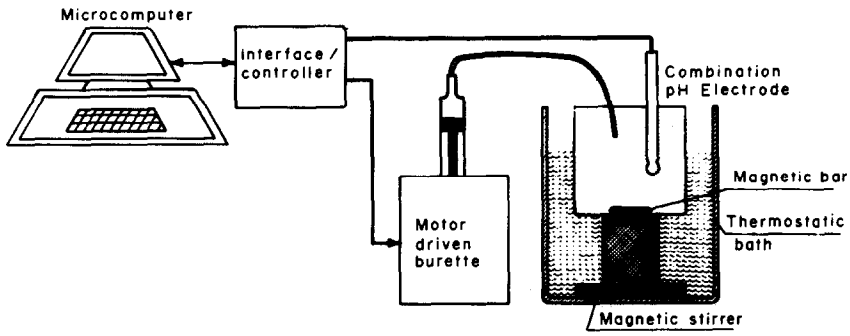

Fig. 6. Experimental system for automatic titration.

Dead Sea brines and Mediterranean sea-waters, to determine the alkalinity and first and second apparent dissociation constants of carbonic acid (K'_1 and K'_2) in the test solution. The procedure followed that described earlier by Sass and Ben-Yaakov.[5]

K'_1 and K'_2 of carbonic acid are used extensively by oceanographers[6] to describe the carbonate system in sea-water and other natural waters. The constants are defined in terms of measurable parameters:

$$K'_1 = \frac{a_{H^+}[HCO_3^-]}{[H_2CO_3]} \tag{7}$$

$$K'_2 = \frac{a_{H^+}[CO_3^{2-}]}{[H_2CO_3]} \tag{8}$$

where the brackets stand for total concentrations of the free and complexed species and a_{H^+} is the activity of the hydronium ion as determined by a glass electrode. Hence, the apparent constants are practical parameters, the use of which eliminates the need to estimate single-ion activity coefficients in complex solutions such as sea-water. However, since the constants are dependent on the ionic strength and composition of the solution, they must be determined for each case separately. They can be calculated from the pH readings during a hydrochloric acid titration. The calculation procedure[5] is basically a least-squares fit of the titration data to the model equation which describes the dependence of pH on the amount of acid added, by use of equations (1) and (2), an alkalinity mass-balance, and assumption that the total concentration of CO_2 species is constant. The alkalinity is first determined by a Gran-type titration.[5,8] The computation procedure is done by a BASIC program run after the titration is completed.

Typical titration curves obtained with the system are shown in Fig. 8. The results obtained for the sea-water ($pK'_1 = 5.95$, $pK'_2 = 9.00$ agree well with Lyman's values (5.98 and 9.06).[6]

Ammonia determination

The ammonia gas-sensing electrode responds only to NH_3 and is insensitive to the ammonium ion.[9] Since the distribution of total ammonia (ΣNH_3) between NH_3 and NH_4^+ is pH-dependent, ΣNH_3 can be directly determined by the electrode only at pH high enough for practically all the ammonia to be in the molecular form. If the ammonia and water dissociation constants

$$K_{NH_3} = \frac{[NH_4^+][OH^-]}{[NH_3]} \tag{9}$$

$$K_W = [OH^-][H^+] \tag{10}$$

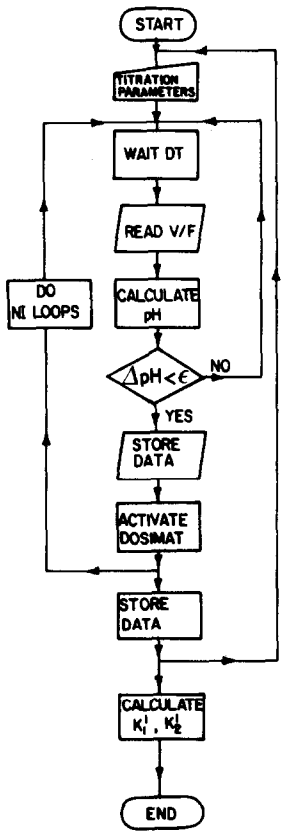

Fig. 7. Flow diagram of the titration procedure.

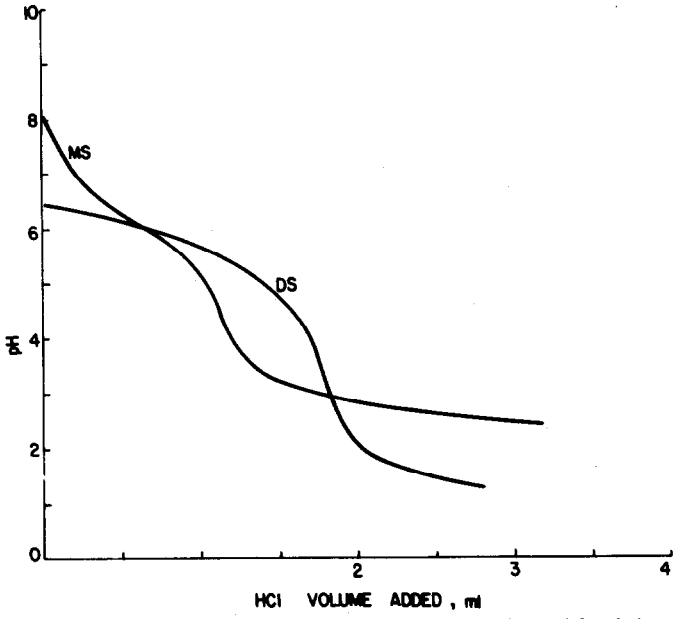

Fig. 8. Typical titration curves for Mediterranean sea-water (MS) and Dead Sea brine, obtained by the proposed system. Solutions were titrated with 0.5*M* HCl.

are known, ΣNH_3 can be calculated[10] from the readings of the ammonia and pH electrodes and the relationships

$$\sum NH_3 = [NH_3]\left\{1 + \frac{K_{NH_3}[H^+]}{K_W}\right\} \quad (11)$$

$$[NH_3] = 10^{(E^0_{NH_3} + S_{NH_3}V_{NH_3})} \quad (12)$$

$$[H^+] = 10^{(E^0_{H^+} + S_{H^+}V_{H^+})} \quad (13)$$

where E^0, S and V are the potential offset, slope and mV reading of the electrode designated by the subscript.

Direct determination of ΣNH_3 from pH and $[NH_3]$ by equation (4) is not always possible since K_{NH_3}/K_W is a function of the ionic composition of the solution and is seldom known. We have used the microcomputer system described here to calculate this constant for a given test solution by a trial and error calculation method. The procedure compares calculated ΣNH_3 values with the actual concentration for a series of guessed values for K_{NH_3}/K_W. This procedure

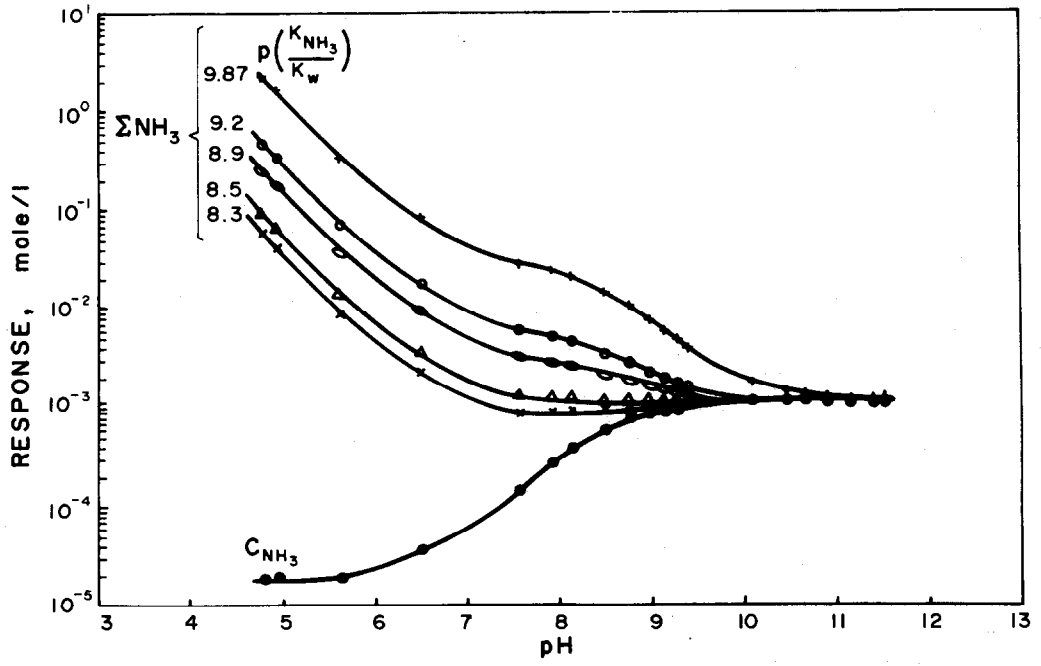

Fig. 9. Response of ammonia electrode in $10^{-3}M$ NH_4Cl at various pH values (lower curve), and calculated ΣNH_3, for various guessed values of K_{NH_3}/K_W.

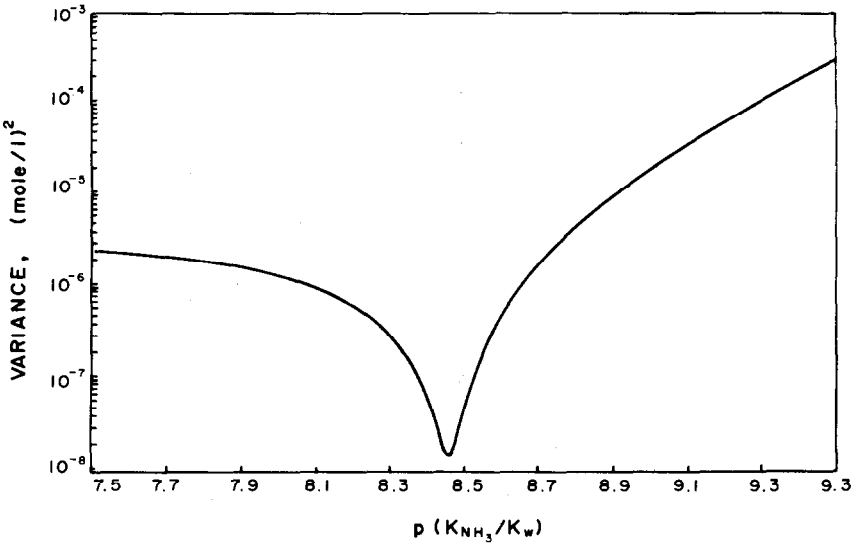

Fig. 10. Variance of the difference between calculated ΣNH_3 and known value (as measured at high pH) for various values of guessed K_{NH_3}/K_W. (For data of Fig. 9.)

is summarized graphically in Fig. 9 which depicts the original NH_3-electrode data and calculated ΣNH_3 for a number of K_{NH_3}/K_W values. The variance of the difference between the calculated values and the known concentration (as measured at high pH) was then used to find the best fit (Fig. 10). Once the constant is determined for a given solution it can be used to calculate ΣNH_3 from the reading of the electrodes at any pH provided the free ammonia concentration is not below the lower limit of linear response of the ammonia electrode. As this limit for the ammonia electrode used was about $2 \times 10^{-5}M$, ΣNH_3 at the $0.002M$ level could be determined at any pH not below 7.5 (Fig. 9).

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OBSERVATIONS ON 1-PHENYL-3-METHYL-4-TRIFLUOROACETYLPYRAZOLONE-5, A PROMISING EXTRACTING AGENT

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Summary—A simple procedure for obtaining 1-phenyl-3-methyl-4-trifluoroacetyl-pyrazolone-5 (HPMTFP), a promising metal extractant, is described. Recrystallization studies reveal that only one tautomer can be isolated, sometimes with one molecule of water of crystallization, contrary to reports that a yellow enol and a white keto tautomer can be obtained from n-hexane and aqueous ethanol respectively. The melting points and colours of some of the metal chelates of HPMTFP are tabulated and in the case of Hg(II) and Cu(II) chelates, differ from those reported by others. Solubility data for some of the metal chelates of HPMTFP are also given.

The 1-phenyl-3-methyl-4-acylpyrazolone-5 compounds, the 4-benzoyl derivative in particular, have been gaining increasing popularity for extraction of metals from acidic media,[1–15] and have been reviewed in a monograph by Zolotov and Kuzmin.[16] Recently Hasany and Qureshi employed the 4-trifluoroacetyl derivative (HPMTFP) as an extractant for some Group IB, IIB, and IIIA–VA elements[17] and obtained some encouraging results. In view of the potential of this derivative as an extractant and our general interest in this ligand and its metal complexes, we feel it necessary to clear up some confusion about some of its physical properties and those of some of its chelates. A synthesis giving very high yield of this reagent is also described.

EXPERIMENTAL

In the synthesis it was observed that the quantity and type of acid used in the acidification process affects the yield. The following procedure was found to give maximum yield.

Procedure

1-Phenyl-3-methyl-pyrazolone-5 (8.5 g) is dissolved in anhydrous pyridine (50 ml) in a 1-litre round-bottomed "Quickfit" flask. Merck pyridine (Purum grade) was found to be suitable without further purification. Next, 6.2 ml of trifluoroacetic anhydride (10 g) are added dropwise with shaking from a "Quickfit" dropping funnel, care being taken that no trifluoroacetic anhydride vapour escapes during the process. The reaction is exothermic so the flask is cooled under running water. The wine-red reaction mixture is kept at room temperature for 1 hr before being poured into 200 ml of distilled water in a 2-litre beaker. No precipitate is formed. The solution is stirred while being acidified with 3*M* hydrochloric acid (250 ml), and a pinkish-cream precipitate is obtained. This is collected on a sintered-glass funnel, washed with distilled water until the washings are colourless, and then dried in air. Yield 91%; m.p. 139–140°. The melting points of the products obtained by recrystallization from various solvents are given in Table 1.

Tautomeric forms

Jensen[1] reported that recrystallization from ethanol–water of the crude product obtained by his procedure gave 78% yield of pure product, m.p. keto tautomer 144° and enol form 132°. Hasany and Qureshi[17] reported that by Jensen's method[1] they obtained a pure product which was yellowish and had m.p. 132°. They also reported that HPMTFP exhibits keto–enol tautomerism and can be obtained in two forms, the enol being yellowish and the keto form colourless, but did not state the melting points of these tautomers. Furthermore, they depicted structure (I) for one keto form (Fig. 1) and structures (III) and (IV) for two enol forms which they claim are in "resonance". However, detailed studies carried out in our laboratories gave the results in Table 1, which do not agree with theirs. Scrutiny of the results, particularly the infrared and NMR spectroscopic data, reveals that only one tautomer is obtained throughout these studies, even though it sometimes has some molecules of water of crystallization. The result is very interesting because ordinarily for such 4-acylpyrazolones-5 as the 4-benzoyl derivative, the tautomer isolated from n-hexane (yellow form) is different from the tautomer isolated from aqueous ethanol (white form), as revealed by detailed infrared and NMR spectroscopic studies.[18] In the case of HPMTFP the tautomer isolated from carbon tetrachloride is anhydrous and assigned structure 1(IV) (Fig. 1) on the basis of conclusions arrived at during similar investigations carried out on the 4-benzoyl derivative.[18] The tautomer isolated from H_2O/MeOH (2:1 v/v) and dried over anhydrous calcium chloride in a desiccator is identical with that obtained from carbon tetrachloride, as revealed by detailed infrared and proton NMR examination.

From spectroscopic evidence both the crude product and the product recovered from n-hexane are identical and are the same tautomeric species as that obtained from both carbon tetrachloride and aqueous methanol, except that in the first of these two there is a molecule of water crystallization. The only yellow form of HPMTFP was prepared accidentally as follows.

HPMTFP [8.11 g, (0.03 mole)] was mixed with sodium triscarbonatocobaltate(III) trihydrate, $Na_3[Co(CO_3)_3].3H_2O$, (3.62 g, 0.01 mole) in 95% ethanol (120 ml), refluxed for 2 hr, cooled, and poured into excess of 3*M* hydrochloric acid. A deep yellow powder was obtained on filtration and air drying, m.p. 139–140°. Spectroscopic study

Table 1. Some physical properties, including infrared and proton NMR spectroscopic data, of HPMTFP recrystallized from various solvents

Solvent	Colour of HPMTFP	Melting point, °C	Pertinent IR frequencies, cm^{-1} (KBr)				Proton NMR chemical shifts, δ ppm (solvent, acetone-d_6)				Inference (tautomer)
			$\nu_{C=O}$	$\nu_{O-H\ldots O}$	$\nu_{OH\ of\ H_2O}$	$\beta_{OH\ of\ H_2O}$	Methyl protons	Labile proton of enol	Phenyl multiplet	H_2O protons	
(Crude unrecrystallized)	Pinkish cream	139–140	1680 (vs)	2680 (br, s)	3400 (vs, br)	1635 (vs)	2.40 (d)	5.60	7.30–7.90	5.60	enol. H_2O
H_2O/MeOH (2:1)	Bone white	138–139	1680 (vs)	2600 (br, vs)	—	—	2.40 (d)	8.60	7.30–7.90	—	enol
Aqueous ethanol	Light pink	139–140	1680 (vs)	2680 (br, s)	3400 (vs, br)	1635 (vs)	2.40 (d)	5.60	7.30–7.90	5.60	enol. H_2O
CCl_4	Creamy white	138–139	1680 (vs)	2600 (br, vs)	—	—	2.40 (d)	9.00	7.30–7.90	—	enol
n-Hexane	Creamy white	140–141	1680 (vs)	2640 (br, vs)	3400 (vs, br)	1635 (vs)	2.40 (d)	5.60	7.30–7.90	5.60	enol. H_2O
Acetone	Bone white	143–144	1680 (vs)	2680 (br, s)	3450 (br, s)	1635 (vs)	2.40 (d)	5.60	7.30–7.90	5.60	enol. H_2O

s, Strong; vs, very strong; br, broad; d, doublet, ν, stretching; β, bending or deformation.

revealed this to be the same tautomeric species as the other products, with one molecule of water of crystallization.

DISCUSSION

It is obvious from these studies that only one stable tautomeric form has been isolated, and the following explanation is offered. HPMTFP may exist in four tautomeric forms as depicted below (Fig. 1).

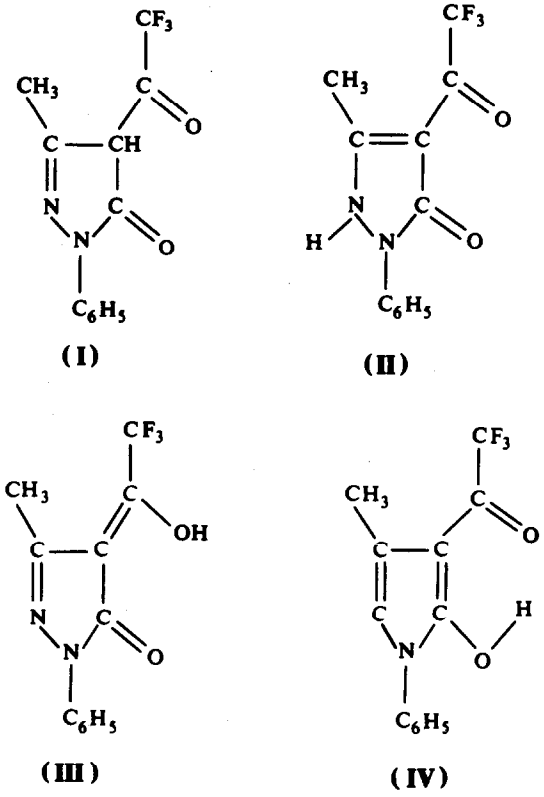
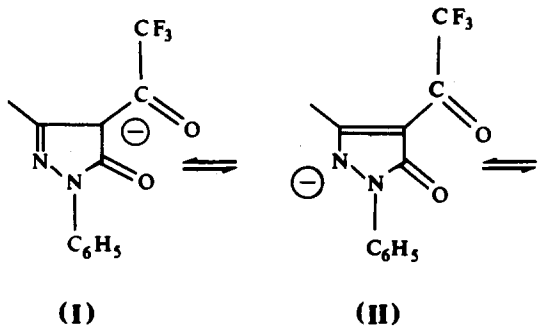
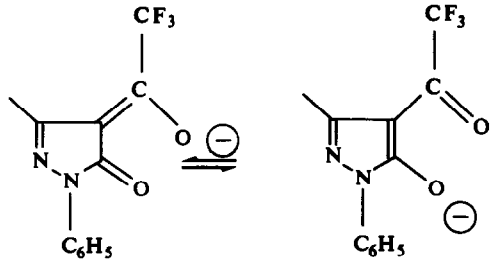


Fig. 1.

An insight into the relative stability of these forms may be gained by examining the different forms of the anion, (PMTFP)-, since the labile proton that gives rise to the tautomerism may migrate from one negative centre to another. The various resonance forms of the anion $(PMTFP)^-$ are shown below.





(III) (IV)

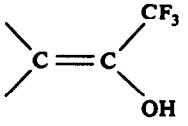
Fig. 2. Resonance forms of the anion (PMTFP).

Structure (I) (Fig. 2)

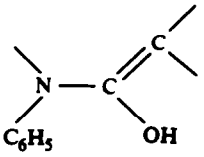
The probability of the negative charge being where located as in (I) in Fig. 2 is extremely small since several powerful electron-withdrawing groups, (C=O, –C=N– and $-CF_3$) are withdrawing negative charge from the carbon atom carrying the negative charge. Therefore this tautomer is in the least likely of the four.

Structures (II), (III), (IV) (Fig. 2)

Under suitable conditions the anions represented by (II), (III) and (IV) in Fig. 2 may plausibly be regarded as existing, and therefore the corresponding tautomers represented by (II), (III) and (IV) in Fig. 1 may be isolable. The ease of isolation of (IV) (Fig. 1) relative to that for (III) may be due to the fact that $-CF_3$ is electron-withdrawing whereas $-C_6H_5$ is electron-releasing. Therefore the hydroxyl proton in the moiety,



will be more labile than the hydroxyl proton in the moiety,



Consequently, the species represented by structure (IV) in Fig. 1 will be more stable and easier to isolate than the species (III).

Hasany and Qureshi[17] reported that they precipitated the chelates of Cu(II) and Hg(II), which were yellow to dark yellow, from buffer solutions ranging from pH 1 to 10. We have isolated and characterized some metal(II) and metal(III) chelates of HPMTFP[19] and some of the relevant data are listed in Tables 2 and 3. The metal(II) chelates were generally prepared by reacting the metal(II) acetate with HPMTFP in 95% ethanol, whereas for metal(III) chelates specific methods[19] were found desirable. The metal chelates were characterized by elemental analyses, conducti-

Table 2. Solubility data (g/l.) of HPMTFP and some of its metal chelates

Solvent	HPMTFP	$Mn(TFP)_2$	$Cu(TFP)_2$	$Be(TFP)_2$	$Mg(TFP)_2$	$Co(TFP)_2$	$Hg(TFP)_2$	$Zn(TFP)_2$	$Ni(TFP)_2$	$Pb(TFP)_2$	$Al(TFP)_3$	$Fe(TFP)_3$	$Co(TFP)_3$	$Rh(TFP)_3$	$In(TFP)_3$
Water	0.02	0.08	0.12	0.06	0.14	0.02	0.04	0.03	0.15	0.12	0.08	0.16	0.13	0.17	0.23
Methanol	90.9	39.9	0.43	0.16	24.3	4.33	0.44	15.38	16.13	10.87	1.56	3.40	7.94	142.9	250.0
Acetone	125.0	12.84	9.12	0.02	125.0	9.60	0.03	13.51	11.31	12.75	2.52	7.69	8.00	90.9	233.0
Chloroform	110.2	0.40	0.07	15.87	0.20	0.12	0.05	0.50	0.40	5.52	111.1	31.3	0.15	83.3	166.7
Carbon tetrachloride	81.3	0.28	0.05	13.90	0.15	0.08	0.03	0.20	0.30	4.82	91.1	28.1	0.07	76.4	157.0

TFP = PMTFP anion.

Table 3. Physical and analytical data for HPMTFP and some of its metal complexes

Compound and recrystallization solvent	Colour	Melting or decomp. temp., °C	Calc., %			Found, %		
			C	H	N	C	H	N
$C_{12}H_9O_2N_2F_3$ (HPMTFP) (aqueous methanol)	Bone white	138–139	53.34	3.36	10.37	53.3	3.3	10.3
$C_{12}H_9O_2N_2F_3 \cdot H_2O$ (aqueous ethanol)	Light pink	139–140	50.00	3.85	9.72	50.1	3.8	9.6
$C_{12}H_9O_2N_2F_3$ (CCl_4)	Creamy white	138–139	53.34	3.36	10.37	53.2	3.3	10.3
$C_{12}H_9O_2N_2F_3 \cdot H_2O$ (n-hexane)	Creamy white	140–141	50.00	3.85	9.72	50.0	3.8	9.7
$C_{12}H_9O_2N_2F_3 \cdot H_2O$ (Acetone)	Bone white	143–144	50.00	3.85	9.72	50.1	3.8	9.7
$Be(PMTFP)_2$	Bone white	261 dec.	52.67	2.95	10.23	52.6	3.0	10.2
$Mn(PMTFP)_2 \cdot 2H_2O$	Cream	278 dec.	45.80	3.20	8.90	45.9	3.2	8.9
$Co(PMTFP)_2 \cdot 2H_2O$	Light pink	218 dec.	45.51	3.18	8.85	45.5	3.2	8.9
$Ni(PMTFP)_2 \cdot 2H_2O$	Light green	239 dec.	45.53	3.18	8.85	45.6	3.2	8.8
$Cu(PMTFP)_2$	Olive green	281–282	47.89	2.68	9.31	47.9	2.7	9.3
$Zn(PMTFP)_2 \cdot H_2O$	White	271 dec.	46.36	2.92	9.01	46.3	3.0	9.1
$Hg(PMTFP)_2 \cdot 2H_2O$	White	224 dec.	37.19	2.60	7.23	37.2	2.6	7.3
$Pb(PMTFP)_2$	Yellow	279–280	38.66	2.16	7.51	38.6	2.2	7.5
$Mg(PMTFP)_2 \cdot 2H_2O$	White	262–263	48.14	3.37	9.36	48.2	3.4	9.3
$Al(PMTFP)_3$	Cream	198–199	51.62	2.89	10.03	51.6	2.8	9.8
$Fe(PMTFP)_3$	Wine red	184–185	49.90	2.79	9.70	49.8	2.8	9.6
$Co(PMTFP)_3$	Yellowish brown	209 dec.	49.72	2.78	9.66	49.7	2.8	9.6
$Rh(PMTFP)_3$	Orange	118 dec.	47.33	2.65	9.20	47.2	2.6	9.1
$In(PMTFP)_3$	Bone white	84–85	46.72	2.61	9.08	46.7	2.6	9.0
$Zr(PMTFP)_4$	Yellow	272–273	49.35	2.76	9.60	49.2	2.7	9.6

vity measurements, and infrared and proton NMR spectroscopy. According to our results (Table 3), the solids isolated by Hasany and Qureshi[17] were not the Hg(II) and Cu(II) chelates of HPMTFP. Our Hg(II) chelate of HPMTFP is white and not dark yellow, while the Cu(II) chelate is yellowish green and not yellow.

The selection of solvent plays an important role in establishing the optimum conditions for extraction. The solubility of the chelate is particularly important. Table 2 gives the solubility characteristics of HPMTFP and some of its metal chelates in various solvents.

Finally, it is interesting to observe that the starting material for the synthesis of HPMTFP, 1-phenyl-3-methyl-pyrazolone-5 can be regenerated from HPMTFP by boiling with a solution of sodium hydroxide in equimolar proportions, concentrating the resulting reaction mixture, and allowing the solid 1-phenyl-3-methyl-pyrazolone-5 to crystallize out.

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DETERMINATION OF Ag, Cd, Pb, Zn AND Pd IN SEA-WATER BY THERMAL-IONIZATION ISOTOPE-DILUTION MASS SPECTROMETRY

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Summary—The concentrations of Ag, Cd, Pb, Zn and Pd in sea-water have been determined by thermal-ionization isotope-dilution mass spectrometry. The concentrations found, in ng/kg, were 0.6 ± 0.4 Ag, 1.9 ± 0.4 Cd, 18 ± 8 Pb, 30 ± 8 Zn and <4 Pd. These levels are lower than some reported previously. Determination of Pd in sea-water is reported for the first time.

Although metals have been determined in sea-water for many years without apparent difficulty there is now strong evidence to suggest that much of the published data may be highly inaccurate. In the early 1970s Patterson and co-workers recognized that there were difficulties in measuring environmental lead levels. In a series of careful studies[1–5] they demonstrated that the available data were in error by orders of magnitude because of either analytical difficulties or contamination during sampling, storage or analysis. Studies of trace metal levels in sea-water by Boyle *et al.*,[6] Bruland *et al.*[7] and Bruland[8] indicate that similar difficulties may exist for cadmium and zinc. Our own studies of cadmium in river[9,10] and sea-water[11,12] also yielded lower levels than were reported elsewhere. We originally attributed this discrepancy to a particularly clean Western Australian environment, but the reason could equally well have been analytical difficulties in much of the published work.

In this paper we report the results of measurements of Ag, Cd, Pb, Zn and Pd in coastal sea-water in the Indian Ocean off Western Australia. Particular care was taken to avoid contamination during sampling, storage and analysis, and thermal-ionization isotope-dilution mass spectrometry was used for all analyses.

EXPERIMENTAL

Sampling

Water samples were collected at a location 3.3 km due west of Cape Vlaming, Rottnest Island, outside the 50-m contour. The location is approximately 20 km west of Fremantle in the Indian Ocean. The samples were collected during August and December 1980 from an aluminium work boat with a low-contamination pump constructed for the purpose. Water was transferred from a depth of 30 m to the pump through 1.2 cm bore conventional polyethylene (CPE) tubing held vertical with a stainless-steel weight. The pump operated on the principle of a simple force pump, the pumping action being achieved with an expandable Teflon bellows and two Teflon valves (inlet and outlet). All pump surfaces with which the sample came into contact were made of Teflon (PTFE). The water samples were stored in 2.5-l. high-density polyethylene (LPE) bottles, which were double sealed in LPE bags.

Reagents

Most of the sample processing and reagent preparation was done in laboratories supplied with coarsely filtered air, although laminar-flow clean-air hoods were employed for critical stages of distillation.

Quartz-distilled water (QDW). Tap water was first demineralized, then distilled in a Pyrex still. After sub-boiling distillation in a quartz still the water was stored in acid-leached LPE containers. Analysis of the water yielded 0.08 ± 0.08 ng/kg for Ag, 0.6 ± 0.4 ng/kg for Cd, 1 ± 1 ng/kg for Pb and 28 ± 10 ng/kg for Zn. Pd was not determined. Because of the possibility of contamination from handling and storage of the water these levels represent upper limits for these metals in the QDW.

Ammonia solution. A 2–3*M* solution was prepared from the analytical grade reagent with a two-bottle Teflon still.

Acetic acid. The analytical-grade reagent was distilled in a two-bottle Teflon still.

Ammonium acetate solution. Prepared by mixing appropriate quantities of acetic acid and ammonia solution to yield a solution of pH 5.0–5.5.

Hydrochloric and nitric acids. Prepared by distillation of the analytical-grade reagents, first in a quartz sub-boiling still, then in a two-bottle Teflon still. Analysis of the hydrochloric acid yielded 0.18 ± 0.06 ng/kg for Cd, 3.7 ± 0.5 ng/kg for Pb and 10 ± 3 ng/kg for Zn. Ag and Pd levels were not determined.

Ion-exchange resins. Bio-Rad 100–200 mesh AG50-X8 cation-exchange and AG1-X8 anion-exchange resins were

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stored in 4*M* and 2*M* hydrochloric acid respectively, but were cleaned and conditioned immediately before use. Bio-Rad Chelex 100, 100–200 mesh, was cleaned with dilute nitric and hydrochloric acids and stored in dilute nitric acid.

Cleaning of apparatus

The Teflon pump components were leached in dilute hydrochloric acid for 3 weeks. The CPE sampling tube was leached in dilute hydrochloric acid at 60° for the same period. The LPE sampling bottles were cleaned by successive leachings with dilute nitric and hydrochloric acids at 60° for 6 weeks. This cleaning procedure proved to be too vigorous for the LPE and one bottle became brittle and cracked during sampling; Teflon should be superior in this respect. Teflon (PTFE or FEP) laboratory ware was used, except for the ion-exchange columns (made of Vycor glass) and Pyrex conical flasks used for determination of palladium.

Isotope-dilution mass-spectrometry (IDMS)

Details of this technique have been given by Webster.[13] An accurately known amount of highly enriched stable isotope (the tracer) of the element to be determined is allowed to equilibrate with the sample. The element is then separated in a pure form and its isotopic composition measured in a mass spectrometer. From a knowledge of the isotopic composition of the tracer added and of the natural element in the sample solution, the amount of natural element present can be determined. Quantitative separation of the element is not necessary. In this study a tracer solution containing the enriched stable isotopes ^{107}Ag, ^{111}Cd, ^{204}Pb, ^{67}Zn and ^{102}Pd was prepared from materials supplied by Oak Ridge National Laboratory. The concentration of each element in this solution was determined by calibration against a gravimetrically prepared solution of the metal (Johnson and Matthey "Spec-Pure"), by the IDMS method. Before leaving the laboratory for sampling, each LPE sampling bottle was spiked with an accurately weighed amount of tracer solution. Sufficient hydrochloric acid was added to give a 0.1% solution (1 g of concentrated hydrochloric acid per kg) when the sea-water was added, to minimize loss of metals to the container surfaces. Sample bottles were protected from environmental contamination by double sealing in LPE bags. On return to the laboratory the bottles were heated to 60° under infrared lamps for at least 3 days to ensure desorption of any metal from the walls, and equilibration with the tracer.

The palladium concentrations reported were not determined by the procedures above. However, the same sampling bottles, acidification and post-sampling heating procedures were employed. Acidified 200-g samples were transferred to 250-ml Pyrex conical flasks, then tracer was added and allowed to equilibrate for 16 hr.

Chemical separations

Lead and zinc were extracted with chelating resin (Chelex 100) by procedures similar to those described by Kingston *et al.*[14]

Each 2.5-l. sample was adjusted to pH 7 with ammonia solution and then buffered to pH 5–5.5 with ammonium acetate. This enabled the samples to be transferred directly to the columns from the storage containers, and minimized the possibility of contamination. The resin was washed with ammonium acetate solution to remove calcium, magnesium and manganese, then lead and zinc were eluted with 2*M* nitric acid. The solution was evaporated to dryness and the residue treated by evaporation with *aqua regia*. This yielded a sample sufficiently pure for mass spectrometric analysis. The analytical blank was determined by processing the same quantities of reagents as used for the samples.

Silver, cadmium and palladium were extracted with anion-exchange resin (AG1-X8). This gave lower analytical blanks for these elements than could be achieved with the Chelex 100. Since the distribution coefficients for these elements on AG1 are extremely high at low hydrochloric acid concentrations, the sample solutions could be transferred directly to the columns. Following the addition of the sample the resin was washed with three 10-column-volume lots of 0.1*M* hydrochloric acid. The cadmium and silver were eluted with 6 column-volumes of 1*M* nitric acid and the eluates evaporated to dryness. Then 10 column-volumes of QDW were added to the column, and finally palladium was eluted with 5 column-volumes of 2*M* ammonia. The palladium eluate was evaporated to dryness and ammonium salts were removed by evaporating twice with a few drops of *aqua regia*. Before the palladium samples could be satisfactorily analysed they required further purification. This was achieved by passing the sample through cation-exchange resin (AG50-X8) in 0.1*M* hydrochloric acid. The silver and cadmium samples required no further processing.

Thermal-ionization mass spectrometry

The isotopic analyses were carried out in a 30.5-cm radius, 90° deflection mass spectrometer fitted with a thermal-ionization source. The ion currents were amplified with an electron multiplier and vibrating reed electrometer and the output was digitized and fed on-line to a minicomputer. The samples were loaded together with phosphoric acid and silica gel onto zone-refined rhenium ribbon single-filament assemblies. The elements were determined in three groups: (Ag, Cd), Pd and (Pb, Zn). The (Pb, Zn) group was transferred to the filament in dilute hydrochloric acid while dilute nitric acid was used for the others. The elements yielded measurable ion currents at different temperatures, so one element could be determined without loss of another. The order of appearance of the metal ions with increasing temperature was Ag, Pd, Cd, Pb, Zn. With ion currents of $>10^{-14}$ A, isotopic ratios could be measured with a precision better than 1%. The isotopic ratios measured were $^{109}Ag/^{107}Ag$, $^{112}Cd/^{111}Cd$, $^{208}Pb/^{204}Pb$, $^{66}Zn/^{67}Zn$ and $^{104}Pd/^{102}Pd$. Uncertainties introduced by the isotopic analyses made a negligible contribution to the uncertainties associated with the final element concentrations.

RESULTS AND DISCUSSION

The results of analysing sea-water for Ag, Cd, Pb, Zn and Pd are given in Table 1. The paired analyses represent determinations on 1.2-kg aliquots from the same 2.5-l. sample bottle. The 2.5-l. bottle used to determine the level of metals in the QDW was subjected to the same procedures (cleaning and pre-analysis) as the bottles containing sea-water samples. The levels found in QDW therefore represent limits of contamination acquired during the storage of samples.

Owing to the vigorous cleaning procedures employed for the polyethylene bottles, one became brittle and cracked. Analysis of sea-water samples from this bottle for silver and cadmium are shown in brackets. These values were significantly higher than those obtained from the other two bottles, indicating that contamination had occurred. Consistent results were obtained from the remaining bottles, giving 0.6 ± 0.1 ng/kg for Ag and 1.9 ± 0.4 ng/kg for Cd in sea-water.

Table 1. Levels of Ag, Cd, Pd, Pb and Zn in sea-water samples

Element	Concentration*, ng/kg Silver	Cadmium	Lead	Zinc	Palladium
Individual† analyses	0.57 ± 0.01, 0.49 ± 0.01	1.9 ± 0.2, 1.8 ± 0.2	15 ± 2, 8 ± 2	35 ± 3, 25 ± 3	0.5
	(0.64 ± 0.01, 1.6 ± 0.01)	(2.6 ± 0.2, 3.3 ± 0.2)	24 ± 2, 24 ± 2	99 ± 3, —	2, 0
	0.66 ± 0.01, 0.61 ± 0.01	2.1 ± 0.2, 1.9 ± 0.2			<4
Blanks	0.008, 0.017, 0.025, 0.008	0.1, 0.3, 0.08	4.5, 1.1, 0.9, 1.2, 2.5	10, 7, 7, 6, (51)	2
Mean§	0.6 ± 0.1	1.9 ± 0.4	18 ± 8	65 ± 43	<4

* Sample sizes of 1.2 kg were analysed in all cases except for Pd, where 200-g samples were taken.

† Three bottles of sample were analysed in duplicate for both Ag and Cd. Two other bottles were analysed in duplicate for Pb and Zn. Another three bottles were analysed for Pd but only one in duplicate.

§ Values given in brackets were not included in mean; errors shown are 95% confidence limits.

The uniformly low blank determinations for silver indicate that contamination from the reagents and the chemical laboratory was not significant. Contamination during storage was small and within the error limits quoted. The contamination during sampling could not be assessed. Environmental contamination of samples proved to be a significant problem for lead and zinc. Two bottles of sea-water were analysed. The spread in concentration was observed to be significantly greater than that found for Ag and Cd. This was principally due to intermittent contamination problems which seemed to be marginally worse for zinc than for lead. One particularly large zinc blank was rejected (shown in brackets in Table 1). Also the analysis of the second aliquot from the second sample bottle failed when measurable ion beams could not be obtained in the mass spectrometer.

The limited data given in Table 1 for zinc are supported by some less precise measurements on smaller samples (200 g) taken from the same locality. These measurements yielded 54, 73, 16 and 98 ng/kg with an accompanying blank of 80 ± 20 ng/kg. Because one blank determination was high and analytical difficulties were experienced with the second bottle it is probably wisest to accept the lower values of 25 and 35 ng/kg in Table 1 as more accurately representing the true levels. A value of 30 ± 8 ng/kg is then obtained for zinc in sea-water. The QDW analysis gave 28 ng/kg for Zn. If a significant amount of this was from contamination during storage, the true level of Zn in sea-water may be considerably lower than 30 ng/kg.

The four determinations of lead in this study gave a value of 18 ± 8 ng/kg for sea-water. These data are also supported by less precise analyses of 200-g samples, which gave 30, 28, 1 and 7 ng/kg with an accompanying blank of 23 ± 20 ng/kg. Since the QDW analyses yielded 1 ng/kg of Pb the storage container appears to be a minor source of lead contamination.

The limited usefulness of most published lead levels for environmental samples, because of contamination, has been emphasized by Patterson and co-workers.[1–5] Chow[15] has reviewed determinations of lead in ocean water and concluded that many results in the literature are unreliable. A similar picture is emerging for zinc. Bruland *et al.*[7] and Bruland[8] have reported levels of zinc in sea-water 2–3 orders of magnitude lower than the 1–10 μg/kg levels generally reported. They attribute this to serious contamination problems in other studies. Our data support the lower values. Because of the ubiquitous nature of both lead and zinc it is not surprising that contamination is the factor limiting their accurate determination. Although from our work contamination with cadmium during sampling and analysis does not appear to be as serious a problem as it is for lead or zinc, the values we report are signficantly lower than those published previously. Florence and Batley[16] express doubts about the state of knowledge of cadmium speciation in natural waters, owing to measurement difficulties, citing analytical difficulties and contamination as the principal problems. The mass spectrometric technique employed in this study enabled <0.005 ng of cadmium to be measured accurately, hence the fluctuation of the analytical blank is the principal factor limiting the precision of these analyses. Contamination during the storage of samples could contribute up to 0.6 ng/kg if the storage containers were responsible for the cadmium found in the QDW. This would still place the level of cadmium sea-water between 1 and 2 ng/kg.

Only an upper limit of 4 ng/kg could be set for palladium in the sea-water. The low precision of these measurements was a consequence of the small volumes analysed.

An assumption made in these measurements is that the tracer becomes equilibrated with the sample. This is a reasonable expectation for dissolved species. Florence and Batley[16] have reviewed speciation of elements, including Pb, Cd, Zn and Ag, in natural waters, and find the principal species in sea-water are the chloro-complexes and carbonate for Pb, the chloro-complexes for Cd, the free metal ion, chloro-complexes and carbonato-complex for Zn, and chloro-complexes for Ag. Data for Pd are very limited, although the E_h–pH conditions for a Pd–Pt–H_2O–Cl system have been described by Fuchs and Rose.[17] Their E_h–pH diagram indicates that Pd will occur as either the metal ion or the

Table 2. Comparison of some published values for element concentrations in surface (< 50 m) ocean water.

Element	Concentration, *ng/kg* This work	Literature values
Ag	0.6 ± 0.1	10[19]
Cd	1.9 ± 0.4	2–22,[12] 13,[11] 10–69,[6] 0.08–47[8]
Pb	18 ± 8	25,[18] 12–14[5]
Zn	30 ± 8	7–33,[7]* 0.8–37[8]
Pd	<4	

* Concentration in ng/l.

chloro-complex in sea-water acidified with hydrochloric acid.

Since the samples were acidified to contain 1 g of concentrated hydrochloric acid per kg, there is little risk of adsorption of these elements on the walls of the LPE storage containers, but the level of adsorption on, and dissolution from, suspended matter was not measured and is uncertain.

A comparison of the data from this study with the bulk of published data reveals a significant discrepancy for all elements, for which data could be found (*i.e.*, all except palladium). Selected published data are given in Table 2. Other data tend to be significantly higher and this is assumed to be due to contamination or analytical difficulties. The levels reported for cadmium by Boyle *et al.*[6] and Bruland[8] on samples from the Pacific Ocean are in agreement with our value of 1.9 ng/kg. Earlier measurements by Morris *et al.*[11] gave an average level of 13 ng/kg for Western Australian coastal waters. However, these samples were collected from near-shore locations where the depth of water was generally less than 2 m. Even so, some determinations gave concentrations as low as 5 ng/kg. Recent determinations by Rosman *et al.*[12] on samples taken from the seaward side of Garden Island, Western Australia, gave values as low as 2 ng/kg. Because of improvements to the sampling procedures the present results are our most reliable values. The value we report for lead compares favourably with the value of 25 ng/kg reported by Patterson *et al.*[18] for ordinary surface samples off the Californian Coast and 12–14 ng/kg reported by Schaule and Patterson[5] for near-surface samples from the Pacific Ocean. The value of 30 ± 8 ng/kg arrived at for zinc in sea-water from this study falls within the range of values found by Bruland *et al.*[7] and Bruland[8] for near-surface samples from the Pacific Ocean. These levels are approximately two orders of magnitude lower than the generally accepted levels. Confirmation of these lower levels is therefore very significant, but there remains the problem of explaining why the majority of studies appear to enhance the levels so uniformly.[16]

The value of <4 ng/kg given here for palladium may be the first reported for this element in sea-water. Because of the high level of the analytical blank compared to the level found in the water samples, we prefer to quote an upper limit for this element. The value of 0.6 ng/kg obtained for silver is significantly lower than the value of 10 ng/kg reported earlier by Robertson.[19]

The IDMS method offers significantly higher accuracy than is attainable with those methods generally used to analyse water samples. It also provides extremely high sensitivity for many elements. Mandel[20] has identified IDMS as a prime example of a definitive analytical method. Although the techniques employed by Bruland and co-workers[8,21] have adequate sensitivity for cadmium and zinc, IDMS has, in addition, high accuracy. At the levels at which many metals are present in sea-water, preconcentration is usually necessary. This will not significantly alter an isotopic ratio, hence the accuracy of IDMS is maintained. A knowledge of the chemical yield is not required and any losses at this stage will not influence the result. Hence the basic accuracy of the method is directly traceable to the gravimetrically prepared solutions used for calibration. The method is therefore ideally suited for the characterization of standard reference samples of sea-water, which are clearly needed to resolve the present uncertainties.

It should be emphasized that the method being employed in this study for isotopic analysis is thermal-ionization mass spectrometry and not spark-source mass spectrometry, as mistakenly quoted by Florence and Batley[16] in their discussion of the work by Patterson and co-workers on lead. Isotope dilution coupled with thermal-ionization mass spectrometry is a technique which has been available for some elements for more than three decades, and yet it has received very little attention from analytical chemists with the notable exception of the U.S. National Bureau of Standards (Washington, D.C.).

CONCLUSIONS

The results of the study indicate that Ag, Cd, Pb and Zn are present in sea-water at levels significantly below the currently accepted levels, confirming lower values reported in recent years by some authors. The level of Ag was found to be in the sub-ng/kg range, and a value of Pd in sea-water is reported for the first time. Evidence is accumulating which suggests that the levels of some metals in sea-water are being measured inaccurately. One serious outcome of this is that the baseline levels reported for these elements are orders of magnitude too high. To assist in resolving the discrepancies accurately characterized samples of sea-water are needed.

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DETERMINATION OF Cr, Ni, Cu, Zn AND Cd IN NIOBIUM BY RADIOCHEMICAL PROTON-ACTIVATION ANALYSIS

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Summary—A rapid radiochemical proton-activation technique based on the utilization of short-lived indicator radionuclides ($t_{1/2}$ = 7–100 min) for the determination of Cr, Ni, Cu, Zn, and Cd in niobium is described. It involves the irradiation of the samples with 13-MeV protons, the post-irradiation decontamination of the sample surface, a rapid sample dissolution, a separation procedure based on anion-exchange from HF medium, and counting the eluate with a high-resolution gamma-spectrometer. In addition, for the determination of Ni, a specific separation procedure is proposed. For a 20-min irradiation with a beam intensity of 10 μA and a delay time of 20 min, the limits of detection are 4 ng/g for Cr, 0.5 ng/g for Ni, 60 ng/g for Cu, 0.5 μg/g for Zn and 0.2 μg/g for Cd. For Cr, Ni, and Cu, the results obtained by this technique are compared with data obtained by radiochemical neutron-activation analysis and atomic-absorption spectrometry.

Several powerful activation-analysis techniques have been developed in recent years for trace analytical characterization of niobium, enabling determination of about 30 elements,[1] but none of them is capable of detecting nickel in niobium of good or high-purity grade. Radiochemical neutron-activation analysis (NAA) involving irradiation with relatively high thermal neutron flux (8×10^{13} n.cm^{-2}.sec^{-1}) does not allow detection of nickel contents below 0.1 μg/g,[2] mainly because of the low isotopic abundance of the target nuclide ^{64}Ni (0.95%) in the natural element. Limits of detection of 2 μg/g have been reported for direct optical emission spectrography[3] and 0.3 μg/g for a spectrophotometric technique involving a specific separation of nickel.[4] These limits of detection are not low enough for application of the techniques to the determination of nickel in high-purity niobium.

In our previous work,[5] it was shown that proton-activation analysis by use of (p,n)-reactions and short-lived indicator radionuclides ($t_{1/2}$ = 20–40 min) is a very sensitive technique for the determination of nickel, and in addition of chromium and copper. However, the analysis cannot be performed instrumentally, because of strong activation of the matrix through the reaction ^{93}Nb(p,n)^{93m}Nb ($t_{1/2}$ = 6.95 hr). Consequently, radiochemical separation becomes necessary if this sensitive determination technique is to be used.

The purpose of this work was to develop a radiochemical proton-activation technique for the determination of Cr, Ni, Cu, Zn, and Cd in niobium, involving a rapid decomposition of the sample and a rapid removal of the radionuclides produced from the matrix. Although, in contrast to Ni, the elements Cr, Cu, Zn, and Cd can be determined very sensitively by radiochemical NAA,[2,6,7] the present technique can be of interest as a second complementary method offering high sensitivity.

EXPERIMENTAL

Chemicals and apparatus

All reagents used prior to irradiation were of "*suprapur*" grade. They were of "*pro analysi*" grade for post-irradiation procedures. The separations were carried out on Dowex 1X8 (200–400 mesh) strongly basic anion-exchange resin in the F^--form. The original concentrations of hydrofluoric acid and nitric acid were 40% and 65%, respectively.

The separation procedure was developed by using the radioisotopes ^{54}Mn, ^{64}Cu, ^{65}Zn, ^{72}Ga, and $^{115m/115}$Cd as tracers. In the tracer experiments, a single-channel analyser with a well-type 3 × 3 in. NaI(Tl) detector was used for counting. For γ-ray spectrometry, a Ge(Li) detector having an energy resolution of 1.9 keV FWHM for the 1.332-MeV γ-ray of ^{60}Co and an efficiency of 20% relative to a 3 × 3 in. NaI(Tl) detector and a peak-to-Compton ratio of 40:1 was used. The detector was connected to a Canberra 8180 multichannel analyser.

Samples and standards

Analyses were performed on the following niobium samples:

(a) Nb-P, Plansee, Reutte, Austria
(b) Nb-Es, Heraeus, Hanau, F.R.G.
(c) Nb-WCT, Teledyn Wah Chang, Albany, Oregon, U.S.A.
(d) Nb-R-1, Max-Planck-Institut für Metallforschung, Stuttgart, F.R.G.
(e) Nb-R-2, Max-Planck-Institut für Metallforschung, Stuttgart, F.R.G.

From the niobium samples, targets with a thickness of 0.6–1 mm were cut with a diamond saw. In order to remove possible surface contamination, the samples were etched for 10 sec in a 9:1 v/v mixture of 40% hydrofluoric acid and 65% nitric acid. Thick metal targets of pure Cr, Ni, Cu, Zn, and Cd were used for standardization. A monitor foil of pure niobium was placed on the side of the target exposed to the irradiation.

After the bombardment, the monitor foils were counted with a γ-ray spectrometer, and the relative radioactivity of ^{93m}Mo produced in the irradiation of samples and standards was taken as the basis for the quantitative evaluation. The results obtained were corrected for the difference in the depth of penetration of the protons[8] in the samples and standards.

Irradiation and post-irradiation etching

Irradiations were performed in the cyclotron of the Nuclear Research Centre at Karlsruhe. Niobium samples were irradiated in water-cooled target-holders with 13-MeV protons at a beam current of 10 μA for 10–30 min. Standards were irradiated with a beam current of 100 nA for 30 sec. In order to remove possible surface contamination after irradiation the samples were etched for 30 sec in a 9:1:5 v/v mixture of 40% hydrofluoric acid, 65% nitric acid and water, then for 10 sec in 10*M* hydrochloric acid, and finally washed with water.

Radiochemical separation procedure (Fig. 1)

The systematic studies on the anion-exchange characteristics of the elements in hydrofluoric acid medium by Faris[9] and in hydrofluoric acid and hydrofluoric/nitric acid medium by us[10] was the basis for the development of the necessary radiochemical separation procedure. After surface decontamination, the samples were dissolved in 3 ml of 7:3 v/v 40% hydrofluoric acid/65% nitric acid mixture heated initially to 80°. The acid mixture contained 100 μg of each of the inactive carriers of Mn, Cu, Zn, Ga, and In. If there was incomplete decomposition (large samples), further dropwise addition of nitric acid followed. After the dissolution, the unconsumed nitric acid was removed by dropwise addition of formic acid until there was no further generation of gas (0.2–0.5 ml). Then the solution was diluted with water to about 12 ml to give about 5*M* hydrofluoric acid concentration. The dissolution was carried out in a 20-ml polyethylene syringe which was connected by a polyethylene tube, through a tube pump, to the ion-exchange column. After dilution, the pump was started and the sample solution was brought to the column at about 6–7 ml/min. Elution was done with 2*M* hydrofluoric acid. The first 25 ml of eluate were collected in a polyethylene bottle for γ-spectrometric measurements. The polyethylene column was 10 cm in height and 0.6 cm in internal diameter and filled with the resin Dowex 1X8 (200–400 mesh), pretreated sequentially with 20 ml of 10*M* hydrochloric acid, 20 ml of water and 40 ml of 2*M* hydrofluoric acid.

On the basis of recent studies on the extractability of dithizonates from hydrofluoric acid media,[11] the indicator radionuclide for the determination of Ni, ^{60}Cu, was directly separated from the hydrofluoric acid solution after the ion-exchange by extraction with two 10-ml portions of 5×10^{-3}*M* dithizone in chloroform, and then the hydrofluoric acid solution was washed with 5 ml of chloroform. The organic phase (25 ml) was used for counting with the γ-spectrometer. Standards were measured in the solid stated for 1–5 min and the count-rates were corrected for the geometry of the 25 ml of solution.

RESULTS AND DISCUSSION

The method

Table 1 reports the relevant reactions and data for the activation of the matrix element, niobium. After a 20-min irradiation with 13-MeV protons at a beam

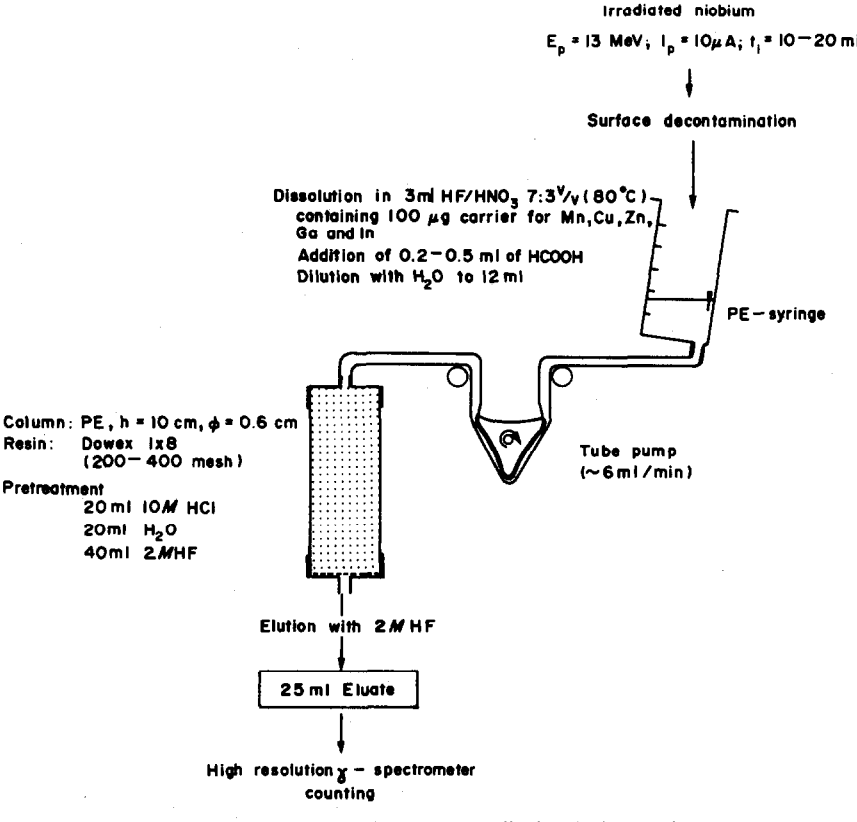

Fig. 1. Scheme of the post-irradiation radiochemical procedure.

Table 1. Reactions induced in niobium by 13-MeV protons

Reaction	Q-value, MeV	$t_{1/2}$	Major γ-rays, MeV	Intensity, %	Protein energy at σ_{max}, MeV
$^{93}Nb(p,n)^{93m}Mo$	−1.2	6.95 hr	0.2632 0.6846 1.4772	61.2 91.0 99.4	12.5
$^{93}Nb(p,pn)^{92m}Nb$	−8.8	10.16 d	0.9345	99.0	23.0
$^{93}Nb(p,\alpha n)^{89m}Zr$	−5.6	4.16 min	0.5878	89.5	—
$^{93}Nb(p,\alpha n)^{89g}Zr$	−5.6	78.4 hr	0.5110 0.9092	47.0 99.87	—

current of 10 μA, the samples give a dose-rate of 5–10 rem/hr at a distance of 10 cm, which is caused mainly by ^{93m}Mo produced from the matrix. Consequently, the samples must be processed behind appropriate lead shielding.

Data on the analytical nuclear reactions and the properties of the indicator radionuclides produced are listed in Table 2 along with primary interference reactions.[12–14]

In the determination of Cr, Ni, and Cu, the possible interferences caused by (p,pn) and (p,αn) reactions have been experimentally verified at 15 MeV,[5] and found negligible even when the content of the interfering elements is 10 times that of the element to be determined. In the determination of Zn, interference is possible from Ga and Ge, but through the reactions $^{96}Ga(p,n)^{69}Ge$ and $^{72}Ge(p,n)^{72}As$ it was proved that it could be neglected because of the extremely low content of Ga and Ge in niobium. Similarly, Sn and In can interfere in the determination of Cd, but neutron-activation analysis failed to detect these elements in the niobium samples used and the NAA detection limit is much lower[7] than that at which these elements interfere in the cadmium determination.

In trace analysis at very low levels, reliable surface decontamination can be an important factor in the accuracy. Therefore, in addition to the pre-irradiation etching and packing of the samples in clean aluminium foils for irradiation, a post-irradiation etching was performed in hydrofluoric/nitric acid mixture followed by washing in dilute hydrochloric acid. The weight loss in the post-irradiation etching is *ca.* 0.05% and is negligible.

The separation procedure described allows decontamination factors $>10^5$ to be obtained for the nuclides $^{89m,g}Zr$, ^{92m}Nb and ^{93m}Mo produced from the matrix. The chemical yields obtained for the complete post-irradiation procedure in the presence of 100 μg of carrier for each radionuclide and about 100 mg of niobium are given in Table 3. They represent the mean values and average deviations from three separate determinations.

The entire procedure, from the end of the irradiation to the start of the counting, takes on average

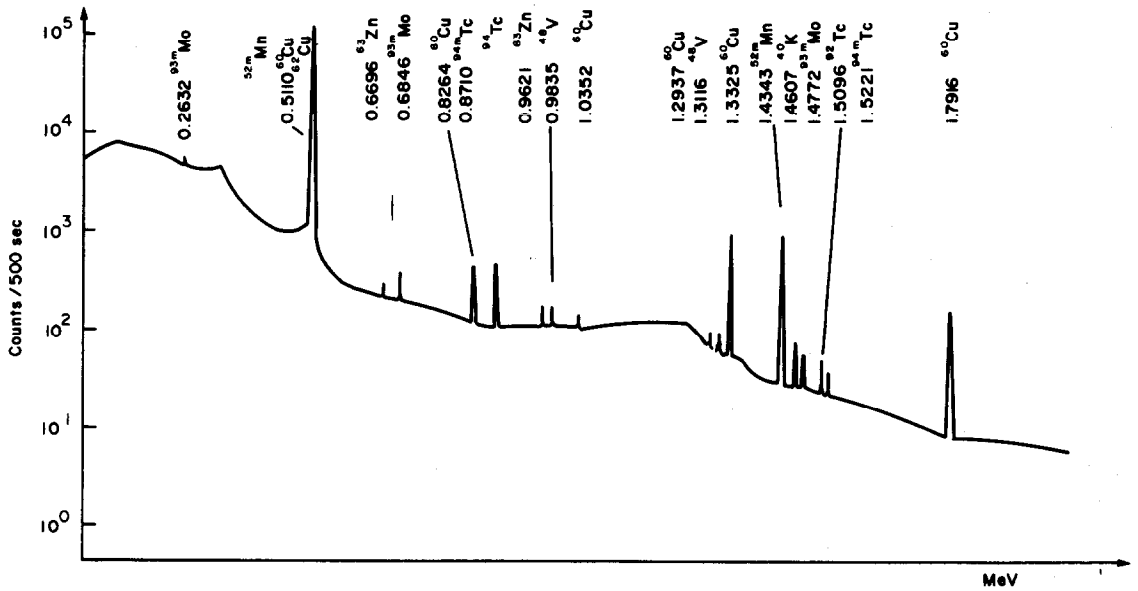

Fig. 2. γ-Ray spectrum of the eluate of a niobium sample (Nb-P) obtained by using the following experimental conditions: proton energy = 13 MeV, beam intensity = 10 μA, irradiation time = 20 min, cooling time = 18 min, counting time = 8.3 min.

Table 2. Data on production and properties of the indicator radionuclides and possible primary interference reactions

Element	Principal reaction	Isotopic abundance, %	Q-value, MeV	$t_{1/2}$, min	Major γ-ray, MeV	Intensity, %	Interfering reaction	Q-value, MeV	Isotopic abundance, %
Cr	$^{52}Cr(p,n)^{52m}Mn$	83.76	−5.5	21.3	0.5110	193.0	$^{56}Fe(p,\alpha n)^{52m}Mn$	−13.1	91.7
					1.4343	100.0			
Ni	$^{60}Ni(p,n)^{60}Cu$	26.23	−6.9	23.4	0.5110	186.0	$^{64}Zn(p,\alpha n)^{60}Cu$	−10.9	48.9
					0.8260	19.2			
					1.3325	87.3			
					1.7920	44.9			
	$^{62}Ni(p,n)^{62}Cu$	3.71	−4.7	9.76	0.5110	196.0	$^{63}Cu(p,pn)^{62}Cu$	−10.8	69.1
							$^{66}Zn(p,\alpha n)^{62}Cu$	−9.3	27.8
Cu	$^{63}Cu(p,n)^{63}Zn$	69.09	−4.1	38.4	0.5110	185.0	$^{64}Zn(p,pn)^{63}Zn$	−11.9	48.9
					0.6696	8.5			
					0.9619	6.7			
Zn	$^{68}Zn(p,n)^{68}Ga$	18.6	−3.7	68.3	0.5110	176.0	$^{69}Ga(p,pn)^{68}Ga$	−10.3	60.0
					1.0774	3.2	$^{72}Ge(p,\alpha n)^{68}Ga$	−8.7	27.5
Cd	$^{110}Cd(p,n)^{110}In$	12.4	−4.7	69.1	0.5110	121.0	$^{114}Sn(p,\alpha n)^{110}In$	−7.4	0.66
					0.6577	97.9			
	$^{111}Cd(p,n)^{111m}In$	12.8	−1.9	7.6	0.5363	87.0	$^{115}Sn(p,\alpha n)^{111m}In$	−5.1	0.35
	$^{112}Cd(p,n)^{112}In$	24.0	−3.4	14.4	0.5110	43.0	$^{113}In(p,pn)^{112}In$	−9.4	4.3
					0.6064	1.2	$^{116}Sn(p,\alpha n)^{112}In$	−6.7	14.4
					0.6182	5.3			
	$^{113}Cd(p,n)^{113m}In$	12.3	−0.5	99.48	0.3917	64.1	$^{117}Sn(p,\alpha n)^{113m}In$	−4.3	7.6
	$^{116}Cd(p,n)^{116m}In$	7.6	−1.3	54.0	0.4170	30.0	$^{115}In(n,\gamma)^{116m}In$		
					0.8188	17.0	$^{119}Sn(p,\alpha n)^{116m}In$	−3.7	8.6
					1.0971	53.0			
					1.2934	80.0			
					2.1120	16.0			

Table 3. Recovery of the indicator radionuclides with regard to the decomposition and separation in the presence of 100 μg of carrier for each element and 100 mg of Nb

Element	Yield, %
Mn	97.1 ± 0.7
Cu	99.1 ± 0.3*
	96.8 ± 1.4†
Zn	98.3 ± 0.8
Ga	97.8 ± 1.0
In	98.1 ± 1.1

* For group separation by ion-exchange.
† For specific separation including both the ion-exchange and the extraction separation steps.

Table 4. Limits of detection*

Element	Limit of detection
Cr	4 ng/g
Ni	20 ng/g
	0.5 ng/g†
Cu	60 ng/g
Zn	0.5 μg/g
Cd	0.2 μg/g

* Experimental conditions assumed; proton energy = 13 MeV; beam intensity = 10 μA; irradiation time = 20 min; cooling time = 20 min; counting time = 10 min; sample processed = Nb-R-2.
† After specific separation of Cu and using a scintillation counter; cooling time = 30 min.

about 15 min (including ~1 min transport time). The specific separation of Cu requires an additional 10 min.

In Table 4, the limits of detection are given along with the conditions under which they were obtained. The detection limit for Ni (0.5 ng/g) was obtained by counting the 1.3325-MeV γ-line of ^{60}Cu with a 3 × 3 in. NaI(Tl) detector after specific separation. This limit of detection can be improved by a factor of about 2 if the 0.511-MeV γ-line of ^{60}Cu is used for the counting. However, in counting the 0.511-MeV-line of ^{60}Cu, it must be considered that the radionuclides ^{61}Cu ($t_{1/2}$ = 3.3 hr) and ^{62}Cu ($t_{1/2}$ = 9.76 min) also contribute to formation of this peak and correct time

Table 5. Contents of Cr, Ni and Cu determined in niobium of different grades of purity

Sample	Contents determined Cr	Ni	Cu
Nb-P	9.6 ± 1.7 μg/g	53 ± 4 μg/g	0.6 ± 0.2 μg/g
Nb-ES	29 ± 5 ng/g	0.11 ± 0.03 μg/g	0.38 ± 0.04 μg/g
Nb-WCT	7 ± 2 ng/g	0.11 ± 0.04 μg/g	73 ± 21 ng/g
Nb-R-1	0.43 ± 0.08 μg/g	0.14 ± 0.02 μg/g	1.3 ± 0.3 μg/g
Nb-R-2	<4 ng/g	25 ± 7 ng/g	<60 ng/g

Table 6. Comparisons of results obtained by different determination techniques for Cr, Ni and Cu

Sample	Element	Content determined This technique	Other techniques
Nb-P	Cr	9.6 ± 1.7 μg/g	10.9 ± 0.9 μg/g[a]
	Ni	53 ± 4 μg/g	66 ± 6 μg/g[a]
Nb-ES	Cr	29 ± 5 ng/g	23 ± 6 ng/g[a]
			35 ± 12 ng/g[b]
	Cu	0.38 ± 0.04 μg/g	0.35 ± 0.12 μg/g[c]
			0.43 ± 0.08 μg/g[d]
			0.56 ± 0.14 μg/g[e]
			0.46 ± 0.03 μg/g[f]
Nb-WCT	Cu	73 ± 21 ng/g	65 ± 12 ng/g[f]
			60 ± 6 ng/g[e]

[a] Radiochemical neutron-activation analysis[6]
[b] Radiochemical proton-activation analysis using ^{52}Mn as indicator radionuclide[15]
[c] Radiochemical proton-activation analysis using ^{65}Zn as indicator radionuclide[15]
[d] Flameless atomic-absorption spectrometry[16]
[e] Radiochemical neutron-activated analysis using ^{64}Cu as indicator radionuclide[2]
[f] Radiochemical neutron-activation analysis using ^{66}Cu as indicator radionuclide[10]

normalization is possible only on the basis of the complex decay curve.

Analysis of niobium

The technique developed has been applied to the analysis of niobium samples of different grades of purity. The results are given in Table 5 as means of at least 3 determinations, and the corresponding average deviations. Zinc and cadmium could not be detected in any of the samples analysed, so only a limiting concentration can be given, for instance $\not< 0.5\ \mu g/g$ for Zn and $\not< 0.2\ \mu g/g$ for Cd in niobium Nb-R-2. As an example, the γ-ray spectrum of the separated fraction after ion-exchange of the niobium sample Nb-P is shown in Fig. 2.

For the determination of Cr, Ni and Cu, the accuracy of the method was checked by comparing the results with those obtained by other analytical techniques, *viz.* radiochemical neutron-activation analysis and atomic-absorption spectrometry. As can be seen from Table 6, which summarizes the results for niobium samples of different grades of purity, on the whole a satisfactory degree of accuracy could be achieved.

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OXIDATION OF MICRO OR TRACE AMOUNTS OF BROMIDE TO BROMATE BY PEROXODISULPHATE BEFORE THEIR IODOMETRIC DETERMINATION BY TITRATION OR SPECTROPHOTOMETRY

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Summary—The optimum conditions for the oxidation of bromide to bromate by peroxodisulphate at 120° as well as for the decomposition of the excess of oxidant have been determined. The predicted advantages of this oxidizing agent, *viz.* minimal blanks and destruction of small amounts of interfering organic matter and reducing substances, were confirmed. The bromate was determined iodometrically either by titration with thiosulphate or by spectrophotometry in absence of oxygen at 355 nm. The titrimetric finish applied to 0.8–8 μmole of bromide gave a mean yield of 100.0%, $s = 6$ nmole. The spectrophotometric finish applied to 0.05–0.25 μmole of bromide gave a mean yield of 98.9%, $s = 1.1$ nmole. Interfering amounts of iodide present in the sample and oxidized to iodate can be corrected for by making use of the pH-dependence of the reaction of iodide with bromate and iodate.

In the course of an investigation on the enrichment of bromide from natural waters by a selective ion-exchange procedure[1] a detailed study of the adopted method of analysis, oxidation of bromide to bromate followed by its iodometric determination, was found to be necessary. The oxidation of bromide to bromate as a preliminary to the iodometric determination of bromide has always been done with sodium hypochlorite.[2] Sodium hypochlorite solution, whether commercially obtained or prepared from reagent chemicals in the laboratory, invariably contains significant amounts of bromine accompanying chlorine in the chemicals used. The method is therefore marred by a considerable blank, which might be tolerated in micro analysis but constitutes a serious drawback in trace analysis. At this level, it is therefore necessary either to prepare a hypochlorite solution from specially purified reagents or to use an oxidant that does not contain chlorine. The present paper deals with peroxodisulphate used at 120° as oxidizing agent.[3] This oxidant should not only give a lower blank but also destroy smaller amounts of interfering organic matter and other reducing substances. The optimum conditions of the oxidation have been established for micro amounts of bromide. The iodine produced in the final step was titrated with thiosulphate, but in further studies on trace amounts it was determined spectrophotometrically as I_3^-.

PRINCIPLE OF THE METHOD

Bromide is oxidized to bromate by peroxodisulphate at the proper pH in a pressure vessel at 120°.

$$Br^- + 3S_2O_8^{2-} + 3H_2O = BrO_3^- + 6SO_4^{2-} + 6H^+$$

The excess of peroxodisulphate is destroyed by prolonged heating, through its oxidation of water:

$$S_2O_8^{2-} + H_2O = 2SO_4^{2-} + 2H^+ + \tfrac{1}{2}O_2$$

The bromate formed is allowed to react with an excess of iodide in acid solution:

$$BrO_3^- + 6I^- + 6H^+ = 3I_2 + Br^- + 3H_2O$$

Depending upon the amount, the iodine thus produced is determined by titration with thiosulphate or spectrophotometrically.

EXPERIMENTAL

Equipment

The reaction vessels were Jena Duran 50-ml or, in some special cases, 100-ml, bottles fitted with 14/15 glass stoppers. As the mouths of these bottles have a broad flange the stoppers could be kept in place by Quickfit JC 2/28 clamps, designed for spherical joints. The bottles were heated in a 5-litre "Flex-seal" pressure-cooker containing about 1 litre of water. The valve was dimensioned to give 1 atm overpressure, corresponding to 120°.

A 5-ml calibrated piston burette, graduated to 0.005 ml, was used for the titrations.

In the spectrophotometric finish the absorbance was measured with a Hitachi 101 spectrophotometer and a 1-cm quartz flow-through cell connected to a reaction funnel (Fig. 1) allowing the reaction between bromate and iodide to proceed in the absence of oxygen, under carbon dioxide.

Solutions

All solutions were made up from Merck analytical re-

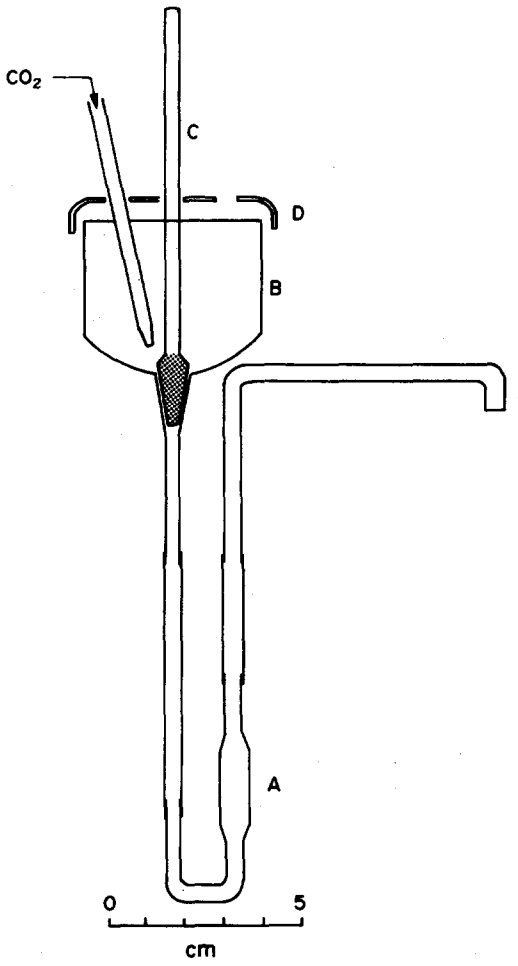

Fig. 1. Quartz cell, A, and reaction funnel, B, with stopper, C, furnished with 5/13 ground joint, and PVC cover, D. The connections are made from PVC tubing.

agents. The sodium chloride added in some cases was recrystallized from a nearly saturated solution that was acidified with hydrochloric acid and boiled after addition of hydrogen peroxide to remove traces of bromide.

Peroxo-solution": *0.100*M $K_2S_2O_8$–*0.24*M Na_2HPO_4–*0.16*M NaH_2PO_4. The decrease in peroxodisulphate concentration at room temperature due to reduction by water was determined according to Kolthoff and Carr[4] and was found to be about 1% in 16 days.

Standard bromide, bromate and iodate solutions. Prepared from potassium bromide dried at 500–600°, and potassium bromate and iodate dried at 180°.

Solutions for the titrimetric finish. These were 1*M* sodium iodide, freshly prepared, 0.2*M* sodium molybdate, 4*M* hydrochloric acid, 1.00*M* acetic acid and 1% starch solution. Standard 0.01*M* sodium thiosulphate was prepared by dilution of 0.1*M* thiosulphate before each series of experiments, and its titre was checked against a standard bromate solution in the same manner as in the actual procedure.

Solutions for the spectrophotometric finish. These must be kept free from dissolved oxygen by a continuous stream of CO_2. Enough 1.6*M* hydrochloric acid and 0.40*M* acetic acid for a series of determinations was stripped of oxygen by a stream of CO_2. The 1*M* sodium iodide used was freshly prepared for each series of determinations by dissolving the appropriate amount in water stripped of oxygen as above.

General procedure

To 10 ml of the sample solution in a 50-ml reaction bottle add 5 ml of the "peroxo solution". Stopper the bottle, apply the clamp and place it in the pressure-cooker. Heat the pot so that full pressure is attained in 7–8 min; the heating time is counted from this moment. After 20 min discontinue the heating by slowly immersing the pot in cold water until atmospheric pressure is attained. Take out the bottle and cool it in water to room temperature.

Titrimetric finish. Stir the solution in the reaction bottle for 3 min with a magnetic stirrer, under a stream of CO_2 to remove dissolved oxygen. Turn off the stream of CO_2 to avoid losses of iodine and add in order 1 ml of 1*M* sodium iodide, 3 drops of 0.2*M* sodium molybdate and 2 ml of 4*M* hydrochloric acid. Titrate with 0.01*M* thiosulphate, adding 0.5 ml of starch solution just before the end-point.

1 ml of 0.01*M* $Na_2S_2O_3$ = 10/6 μmole of Br

If iodide is present in the sample it must be corrected for by pretitration of the iodate at a higher pH. Proceed as above but substitute 2 ml of 1.00*M* acetic acid for the hydrochloric acid. Titrate the slowly forming iodine during a period of 7 min. Then add 2 ml of 4*M* hydrochloric acid and titrate the iodine produced by the bromate.

Blanks should be determined and corrected for.

Spectrophotometric finish. Transfer the solution from the reaction bottle to a 25-ml standard flask, dilute to the mark with water and mix. Fill the flow-through cell with water up to the stopper and put this in place. Pipette 10 ml of the diluted sample into the reaction funnel, place the lid on and lead a rapid stream of CO_2 through the solution for 3 min. Without turning off the CO_2 add the following oxygen-free reagent solutions in the order given: 1 ml of 1*M* sodium iodide and 2 ml of 1.6*M* hydrochloric acid. Lift the stopper, allowing the solution to replace the water in the cell, and read the absorbance 1 min after addition of the last reagent. Calibrate with a standard bromate solution (the apparent molar absorptivity for bromate is about 7.3×10^4 l.mole.cm^{-1}). Determine the blank and correct for it.

If the sample contains significant amounts of iodide a correction for this is determined with a second 10-ml portion of the diluted sample. Proceed as above but substitute 2 ml of oxygen-free 0.4*M* acetic acid for the hydrochloric acid, read the absorbance 2 min after the addition of the last reagent and apply the result as a correction.

RESULTS AND DISCUSSION

The decomposition rate of peroxodisulphate

The reaction mixture must be heated long enough to decompose the excess of peroxodisulphate, otherwise a positive error is obtained. In a similar method[3] but at pH above 12, 15 min was found to be sufficient. In the present case the final pH will be 5.8 and as the decomposition rate might be dependent on pH, some experiments were done, with various heating times; the peroxodisulphate remaining was iodometrically determined.[4] Five ml of the "peroxo solution" were diluted with 10 or 75 ml of water and heated for 5, 10, 15 and 20 min. About 10, 0.3, 0.03 and 0.00%, respectively, of the peroxidisulphate remained undecomposed, independent of the dilution. A heating time of 20 min was therefore considered as sufficient.

Influence of pH and excess of peroxodisulphate on the yield of bromate

The oxidation of bromide to bromate by peroxo-

Table 1. The effect of pH and excess of peroxodisulphate on the yield from a constant amount of bromide: each reaction mixture consisted of 5 ml of 0.01/6*M* KBr, 5 ml of a peroxodisulphate solution of varied concentration, 1 ml of 1*M* NaCl and the stated amounts of 0.4*M* buffer solutions

Buffer, *ml*		50*†	50†		100†		250*†		400†		500†	
Na_2HPO_4	NaH_2PO_4	%	%	pH	%	pH	%	pH	%	pH	%	pH
5	0	5.7	5.7	8.0	18.5	7.7	68.5	7.3	99.7	6.9	99.7	6.8
4	1	35.4	41.8	7.2	71.5	7.1	96.5	6.8	100.0	6.6	99.8	6.4
3	2	64.0	71.6	6.8	93.4	6.8	99.7	6.5	99.8	6.1	99.8	5.8
2	3	83.8	90.5	6.5	99.6	6.4	99.9	6.0	100.0	4.4	101.0	3.0
1	4	98.4	99.4	6.0	100.0	5.8	99.7	3.3	—	—	—	—
0.5	4.5	100.0	100.0	5.5	99.9	4.4	99.7	2.8	—	—	—	—
0	5	99.2	100.7	3.3	100.6	3.0	99.2	2.5	—	—	—	—

* No NaCl added.
† Peroxodisulphate added, μmole; % = yield at pH shown.

disulphate was found to be dependent on pH, necessitating the use of a buffer. On decomposition, 1 mole of peroxodisulphate forms 2 moles of hydrogen ion, which must be allowed for when the composition of the buffer is calculated. A lower limit for the pH is set by the oxidation of any chloride present to chlorine.

Experiments were performed, with the titrimetric finish, to study the influence of varying the pH and excess of peroxodisulphate on the yield of bromate from a constant amount of bromide. Sodium chloride was added in several cases in order to determine the critical pH for the appearance of chlorine. The results are given in Table 1. The sensitivity of the end-point detection was estimated to be ~0.01 ml of 0.01*M* thiosulphate; no blanks larger than that were detected. At the lowest excess (100%) of peroxodisulphate the yield is very dependent on pH but it is quantitative at about pH 5.5. As the excess is increased, the pH interval for quantitative yield broadens to about 4.4–6. At pH <4 chloride is oxidized to chlorine, noticeable by colour and smell, and a positive error is obtained, its magnitude depending on, among other things, how much of the chlorine remains after the treatment with CO_2 before the titration.

In further experiments, the pH and amount of bromide taken were varied, with a constant amount, 500 μmole, of peroxodisulphate in the presence of sodium chloride. The results, Table 2, show that a quantitative yield is obtained at molar ratio of $K_2S_2O_8$/KBr >4, over the pH interval 5.8–6.4. In practice this ratio should be kept much greater than 4, as the sample might contain other oxidant-consuming substances. At the theoretically adequate molar ratio of 3, a yield of about 80% was obtained, showing that the oxidation of bromide to bromate proceeds at a considerably higher rate than the autodecomposition of the peroxodisulphate. The oxidation could probably be performed in a reasonable time at only 100° but the removal of the excess of oxidant would then take at least 90 min.[3]

On the basis of these experiments the "peroxo solution" of the general procedure was given the composition 0.100*M* $K_2S_2O_8$–0.24*M* Na_2HPO_4–0.16*M* NaH_2PO_4. After heating, 5 ml of this solution will contain 0.20 mmole of Na_2HPO_4 and 1.80 mmole of NaH_2PO_4, giving a pH of about 5.8.

Applying this "peroxo solution" according to the general procedure to 9 portions of potassium bromide ranging from 5/6 to 50/6 μmole gave a mean yield of 100.0% with a standard deviation of 0.006 μmole. No blanks were observed.

Increasing the sample volume to 20 ml and keeping other conditions the same had no influence on the yield, but larger volumes, up to 40 ml, caused irreproducible yields varying between 97 and 100%.

The spectrophotometric finish

The iodine liberated may be determined spectro-

Table 2. Effect of pH and amount of bromide on the yield at constant peroxodisulphate concentration: in each case 5 ml of KBr solution of varied concentration, 5 ml of 0.100*M* $K_2S_2O_8$, 1 ml of 1*M* NaCl and the stated amounts of 0.4*M* buffer solutions were taken; with the larger amounts of bromide 0.05–0.1*M* thiosulphate was used for the titration

Buff, *ml*		Yield, %					pH after digestion
Na_2HPO_4	NaH_2PO_4	50/6*	250/6*	500/6*	750/6*	1000/6*	
5	0	99.7	100.0	99.9	99.8	80.2	6.8
4	1	99.8	99.9	100.0	100.1	—	6.4
3	2	99.8	99.9	100.0	100.1	78.7	5.8
2	3	101.0	100.1	100.2	—	—	3.0

* KBr added, μmole.

Table 3. Determination of the absorptivity in the spectrophotometric finish: the stated amounts of bromate, 0.20 mmole of Na_2HPO_4 and 1.80 mmole of NaH_2PO_4 were diluted to 25 ml, then 10 ml of this solution were taken for the spectrophotometric determination according to the general procedure; each result is the mean of 6 determinations

Bromate added, *μmole*	Absorbance		Absorptivity, *A*(corr)/*μmole*
	$\bar{x}$	*s*	
0.00	0.003	<0.0005	—
0.05	0.115	0.0008	2.24
0.10	0.227	0.0018	2.24
0.15	0.340	0.0008	2.247
0.20	0.456	0.0019	2.265
0.25	0.568	0.0011	2.260

Table 4. Testing the general procedure (spectrophotometric finish): the absorbances found are converted into *μmole* of Br^- by using the values in Table 3; each yield is the mean of 6 determinations; the blank, mean of 24 determinations, was 1.8 nmole, s = 0.3 nmole.

Bromide added, *μmole*	Yield, %	*s*, *nmole*
0.05	98.0	0.6
0.10	99.0	1.0
0.15	99.2	0.9
0.20	99.5	1.0
0.25	98.8	2.0

photometrically as the tri-iodide ion at 355 nm.[5] To avoid errors from aerial oxidation at low bromate concentrations, a special procedure had to be worked out. The sample solution as well as the reagent solutions must be deaerated with CO_2 and the reactions and measurements carried out under CO_2 as described under the general procedure. The molybdate used in the titrimetric procedure as a catalyst for the bromate–iodide reaction must be excluded, as it causes considerable absorption at 355 nm. According to Kolthoff and Hume,[6] however, no catalyst is required at sufficiently high acidity. As a high acidity also accelerates the oxidation of iodide by traces of oxygen left in the solution, the proper amount of hydrochloric acid to add was determined. Experiments performed on 0.2 *μmole* of bromate according to the spectrophotometric finish in the general procedure, the acidity and time of reaction being varied, showed that addition of the proposed 3.2 mmole of hydrogen chloride gave an absorbance (0.457) that was constant for 1–4 min if corrected for the absorbance of the blanks, which during that time increased from 0.003 to 0.007. Addition of 2.5 mmole of hydrogen chloride gave absorbance values that were constant only after 2 min, while addition of 4.5 mmole gave considerably higher blanks. Addition of 3.2 mmole of hydrogen chloride and a reaction time of 1 min were therefore decided on. The completeness of the reaction under these conditions was further confirmed by experiments in which iodate, which reacts at very low acidities, was substituted for bromate; nearly identical absorbances were obtained.

The absorptivity for 1 *μmole* of bromate in 25 ml of solution was calculated from the calibration data (Table 3). The average value was 2.250 and increased within the absorbance interval 0–0.6 by 0.9%. A similar series of determinations in which iodate was substituted for bromate gave an average value of 2.244. The apparent molar absorptivity calculated for BrO_3^- is $\sim 7.3 \times 10^4$ l.mole^{-1}.cm^{-1}.

The general procedure was then tested, with various amounts of bromide. The yield (Table 4) cal-

Table 5. The rate of the bromate and iodate reactions with iodide at varying pH: to 15 ml of a solution containing 50/6 *μmole* of bromate or iodate, 1 mmole of NaCl, 0.20 mmole of Na_2HPO_4 and 1.80 mmole of NaH_2PO_4 were added 1 ml of 1*M* NaI, 3 drops of 0.2*M* Na_2MoO_4 and the stated volumes of 1.00*M* CH_3COOH, and the iodine liberated was titrated with 0.01*M* thiosulphate during the time indicated

Time, *min*	Fraction of added bromate or iodate reacted, %					
	1.5* (pH 3.90)		2.0* (pH 3.78)		2.5* (pH 3.67)	
	Bromate	Iodate	Bromate	Iodate	Bromate	Iodate
3	0.0	—	0.0	96.6	0.0	98.9
4	0.0	94.4	0.0	98.5	0.0	99.6
5	0.0	96.4	0.0	99.3	0.1	99.8
6	0.0	97.6	0.0	99.7	0.2	99.9
7	0.0	98.7	0.1	100.0	0.3	99.9
8	0.1	99.1	0.2	100.0	0.4	99.9
9	0.2	99.7	0.3	100.0	0.5	—
10	0.3	99.8	0.4	—	0.6	—
11	0.3	99.8	0.5	—	—	—
12	0.4	99.9	—	—	—	—

* Volume of 1.00*M* CH_3COOH added, ml.

Table 6. Testing the general procedure, titrimetric finish, in the presence of iodide: sample 15 ml of a solution containing 1 mmole of NaCl and the stated amounts of bromide and iodide

Amount added, μmole		Yield of bromide %
KBr	KI	
50/6	50/6	99.8
		99.9
		99.9
5/6	5/6	101
50/6	5/6	99.8

culated from the calibration graph for bromate was 98.9% and the mean standard deviation 0.0011 μmole. The cause of this negative error is not known. From the standard deviation of the blank a limit of detection of 0.9 nmole and a limit of determination of 3 nmole of bromine (Br) are calculated, corresponding to 0.07 and 0.24 μg respectively.

Interferences

Substances consuming significant amounts of the oxidant will interfere by causing negative errors. On the other hand, oxidizing substances initially present or produced in the reaction may react with the iodide, causing positive errors. A separation before the oxidation is therefore sometimes necessary. In the separations generally used, bromine is accompanied by iodine; this is also the case in the ion-exchange enrichment procedure mentioned earlier. A special study of iodine interference was therefore carried out.

Any iodide present is oxidized in the same way as the bromide, to form iodate, and if the subsequent reaction with iodide is carried out at a low pH, the sum of bromate and iodate will be obtained. According to Kolthoff and Hume,[6] however, the reaction rates of bromate and iodate as a function of pH differ sufficiently to allow the iodate to be separately titrated at pH 4–5 and subsequently the bromate at a much lower pH. With the titrimetric finish, experiments were performed to determine the optimal pH and reaction time under the actual conditions used. The results, Table 5, show that the end-point of the iodate titration is attained rather slowly. A titration time of 7 min at pH 3.78 (2 mmole of acetic acid added), was adopted for further experiments, as being adequate for removal of the iodate interference. It must be emphasized, however, that this is valid only for the conditions stated. Thus, if the amounts of bromate and iodate are considerably increased, the optimum pH will approach that found by Kolthoff and Hume. Experiments with 10 times larger amounts of bromate, for instance, showed that this pH was too low; at pH 3.78 and after 7 min, 3.0% of the bromate had reacted.

The correctness of the procedure adopted was confirmed by titrations of mixtures of bromate and iodate and finally of mixtures of bromide and iodide, oxidized according to the general procedure. The results in Table 6 confirm its applicability.

With the spectrophotometric finish it is hardly possible to determine iodate and bromate separately in the same aliquot. However, the correction for iodate might be determined in a second 10-ml portion of the 25 ml of diluted sample solution. Experiments to that purpose on solutions containing iodate or bromate at the same pH as adopted for the titrimetric correction for iodate, with the absorbance read 2 min after the addition of the reagents, gave quantitative recovery of the iodate added (0.05–0.25 μmole) and no interference from the same amounts of bromate. Applying this procedure to mixtures of bromate (0.05–0.25 μmole) with 0.02 μmole of iodate gave (10 determinations) recovery of the bromate that was complete to within ±1 nmole after subtraction of the correction determined for iodate. Finally the general procedure was tested on 10-ml samples containing varying amounts of potassium bromide (0.05–0.25 μmole) and 0.02 μmole of potassium iodide. From 10 determinations an average bromide recovery of 99.1% was obtained, the individual results differing from the expected values by amounts varying within the range from −2 to +1 nmole.

As mentioned in the introduction, this investigation originated from an enrichment procedure for bromide in natural waters. As the accompanying iodide content would be low, no experiments with higher amounts of iodide were performed.

Though it was not the purpose of this investigation, it was found that the results indicate that the method may also be used for the determination of iodide.

Table 7. The destruction of some organic bromine compounds: solutions calculated to contain 50/6 μmole of Br were analysed according to the general procedure

Substance	Yield of bromine, %
p-Bromobenzoic acid	99.5, 100.0
Tetrabromo-*m*-cresolsulphonphthalein	97.7, 97.9
Tetrabromophenolsulphonphthalein	98.3
Dibromothymolsulphonphthalein	98.9, 99.5

The destruction of organic matter

It is commonly assumed that dissolved organic matter is completely destroyed by heating with peroxodisulphate in alkaline medium. Some experiments were made to test whether this assumption would also be correct at the pH of the present method, at least for disruption of the carbon–bromine bond. Aqueous solutions of some organic bromine compounds were prepared and analysed according to the general procedure with the titrimetric finish. The yield obtained, Table 7, was nearly quantitative for bromobenzoic acid (B.D.H. Organic Analytical Standard), and the yield for the indicators, not guaranteed to be of 100% purity, varied between 99.8 and 97.7%. The results indicate a complete break of the carbon–bromine bond and no interference from any residual decomposition products of the organic matter. It should, however, be observed that quantitative results for organic bromine compounds may be expected only if these are water-soluble and non-volatile under the conditions used.

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ASPECTS OF CHLORIDE INTERFERENCE IN ZINC DETERMINATION BY ATOMIC-ABSORPTION SPECTROSCOPY WITH ELECTROTHERMAL ATOMIZATION

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Summary—During the determination of zinc in a polluted stream by atomic-absorption with electrothermal atomization, chloride was found to exercise a large negative interference (40%) when a carbon-filament atomizer was used, but not when a graphite-furnace atomizer was used. The effect on the filament method was confirmed and shown to be due to the formation of zinc chloride, and further complicated by interaction of this with iron. This interference could be overcome by the use of aqueous ammonia solution or silver nitrate added as matrix modifier. The absence of interference in the graphite-furnace method is attributed to the liberation of hydrogen and removal of chloride as hydrogen chloride. It is further suggested that these observations offer a basis for the exploration of apparently contradictory reports in the literature.

Considerable discrepancy exists in literature reports on interference effects in atomic-absorption spectroscopy with electrothermal atomizers. This may in part be due to different conditions of atomization and different matrices being investigated. From the variety of reports of effects observed in similar matrices and our increased understanding of atomization processes it is clear that the design of the atomizer is of great significance. Fuller[1] has reviewed the interferences that occur when electrothermal atomizers are used and concluded that they are the major problem in the practical use of these atomizers. Of these interferences the suppression of analyte signals by chloride is perhaps the best known. While this is generally thought to be due to loss of the analyte element through vaporization of molecular halide species, this generalization may be too simple to explain the effects observed for complicated matrices. In particular, when "real" samples are dealt with, the effects of concomitant cations cannot be excluded in consideration of the effects of anions.

In a study designed to determine trace levels of metals in a stream grossly polluted by storm-water overflows and industrial effluents, conflicting results were obtained for zinc when using a carbon-filament atomizer of the type first described by West and coworkers[2,3] and a commercial graphite-tube furnace of the Massmann type.[4] The results were apparently influenced by differences in the interference of chloride in the presence and absence of iron and by the different nature of the two atomizers. While considerable mention has been made of interferences in the determination of zinc by electrothermal atomization, there is a singular lack of agreement about the extent of such interferences and no systematic study of the effects of the type of atomizer.

One of the earliest reports of the application of a carbon-filament atomizer (the Varian CRA61 with a "mini-Massmann" rod) was that of Kurz *et al.*[5] who determined zinc in aqueous solutions, serum and urine. They reported no ionic interferences, no effect of a 6000-fold concentration ratio of chloride to zinc, and good recovery of zinc added to serum and urine. In the same year Clark *et al.*[6] reported that chloride gave very severe interference in zinc determinations with a carbon furnace (the Perkin–Elmer HGA 70). Complete signal suppression at a 10000-fold concentration ratio was observed, with 40% suppression at 1000-fold ratio (chloride to zinc). Further studies of interference effects when a conventional West rod was used showed that a 2000-fold molar ratio of chloride to zinc depressed the zinc absorbance by only 10%.[7] Cruz and Van Loon[8] found major suppression of the zinc signal by 1% calcium chloride when using a carbon furnace (Perkin-Elmer HGA 2000). They reported it was not possible to volatilize the zinc selectively from high-salt matrices and thus overcome the problem. Yasuda and Kakiyama[9] found zinc chloride bands in the spectra observed when a 1-mg/ml solution of zinc in 1*M* hydrochloric acid was heated in a carbon furnace (Jarrell-Ash FLA-1).

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Losses of zinc from chloride solutions containing more than 1 μg of zinc per ml were attributed to volatilization of zinc chloride but below that zinc level no loss was observed and it was thought that the halide was then decomposed at these lower levels. Similar effects have been reported for carbon-filament atomizers.[10]

There have been a few reports of systematic studies of interferences in electrothermal atomizers, which are pertinent to a consideration of zinc. Depletion of the atom population by chloride formation in the vapour phase has been shown by time-resolved studies to be a potential interference mechanism,[11] for example in the lead/sodium chloride system. Using tracers ($^{65}ZnCl_2$) Maessen *et al.*[12] were able to show that the presence of aluminium nitrate promoted the more rapid atomization of zinc, but also that zinc was still being vaporized from a mini-furnace (Varian CRA-63) after atomization had apparently ceased. The need to consider together the several factors and interferents that may be encountered in practical situations has been stressed by many authors. Wegscheider and co-workers[13–15] have used statistical approaches in an attempt to do this. Their studies of cadmium[14] and lead[15] suggest that cation interferences are less severe than those from anions and that interaction between interferents may be used to eliminate interferences.

Specific studies of complex samples might be expected to exhibit evidence of some of these complicated interactions. Sea-water probably represents a typical such sample and indeed reports of the determination of zinc in sea-water do yield much information on interference effects. Campbell and Ottaway[16] found that magnesium chloride depressed the analytical signal and that iron or calcium up to 10000 μg/ml (added as nitrate or sulphate) was not effective for removing the interference. Zinc was lost if the ashing temperature was unduly high and this limited the usefulness of ammonium nitrate as matrix modifier. Standard-addition methods were used to circumvent the problems encountered. Sturgeon *et al.*[17] were able to use ammonium nitrate as matrix modifier to prevent the loss of zinc (in the ashing stage) by co-volatilization with the alkali metal chlorides in sea-water. A modified tube was also used to reduce the non-specific absorption and so permit direct determination of zinc in sea-water after selective volatilization of chloride-containing interferents.

One of the more promising approaches to understanding interferences in electrothermal atomizers is that which considers high-temperature thermochemistry. The use of conditions which promote the pyrohydrolysis of zinc chloride has been advocated as a way of countering severe depressive interferences.[18] One of the most conclusive studies of high-temperature equilibrium was by Frech and Cedergren[19,20] and concerned the determination of lead in steel. Theoretical calculations showed that it would be necessary to ash the sample in a sufficiently large amount of hydrogen to remove the chlorine from the graphite furnace, or volatile lead chlorides would be lost.[19] This was confirmed experimentally[20] and in addition it was shown that the necessary hydrogen could be formed in a tube furnace (Perkin-Elmer, HGA 72) but not in a mini-furnace of the modified West-rod type (Varian, CRA-63). The recent review by Sturgeon and Chakrabarti[21] is a useful source of suggested atomization mechanisms which can be considered thermochemically.

These various studies lead to the expectation that varying effects may be experienced in different atomizers, dependent on factors such as the size of the atomizer, the rate of heating, and concomitant elements. Much attention is now being devoted to the development of furnaces which allow atomization under isothermal conditions. A popular and simple approach has been to use graphite platforms, as first proposed by L'vov,[22] within conventional furnaces. This has been shown, for example, to reduce anion interferences in lead determination substantially,[23] confirming the identification of the factors influencing the interference.

This paper seeks to elucidate further information on the effect of chloride on zinc atomic-absorption signals following atomization from different designs of atomizer and in the presence of other concomitants likely to be encountered in monitoring water pollution.

EXPERIMENTAL

Apparatus

Two electrothermal atomizers were used. The first was a laboratory-constructed carbon-filament atomizer of the type previously described.[3] A graphite filament (2 mm diameter, 20 mm long, type WW5, Morganite Crucible Group, London) was supported by stainless-steel water-cooled electrodes. A small platform (3 mm long, 1.5 mm wide) was filed onto the filament to enable liquid samples (5 μl) to be injected reproducibly by a micropipette (Eppendorf) with disposable plastic tip. A perforated metal baffle was so placed, in the inert-gas shielding box beneath the rod, that only atoms up to 1 mm above the rod could be observed. Nitrogen (4 l./min) was used as sheathing gas. The filament was heated, by means of a step-down transformer with Variac control of the voltage (0–7 V), so that a temperature of 2200° could be reached within 5 sec with a current of approximately 100 A at 7 V. Typical operating conditions were: dry at 1.5 V for 30 sec; ash at 4.6 V for 10 sec; atomize at 6.5 V for 5 sec.

The graphite-furnace atomizer used was a Model H1475 with FA 236 power supply (Rank Hilger, Margate, Kent). The manufacturer's recommended water and gas pressure settings were used but the standard graphite tubes (54 mm long, 6 mm internal diameter) were modified by the addition of two extra holes (2 mm diameter) spaced either side of, and 3 mm from, the original central hole for the introduction of sample. The extra holes were placed to allow more efficient removal of smoke and particulates. Samples (5 μl) were introduced by the micropipette already mentioned. Typical operating conditions were: dry, power setting 07 (nominal 180°) for 35 sec; ash, power setting 05 (nominal 800°) for 20 sec; wait for 5 sec; atomize, power setting 06 (nominal 2380°) for 5 sec; cool for 30 sec. Nitrogen (2 l./min) was used as the sheathing gas with the flow

stopped during atomization, and the cooling water was supplied at 0.8 l./min.

All measurements were made with a Jarrell-Ash 82-000 series maximum versatility spectrometer with a 0.5-m focal-length Ebert grating monochromator. With two short focal-length convex lenses, light from a zinc hollow-cathode lamp (Activion, Halstead, Essex), or a hydrogen continuum hollow-cathode lamp (Activion) for background correction, was passed as a parallel beam through either atom cell firmly mounted on the optical rail of the instrument. Both the 213.9 and 307.6 nm zinc atomic lines were used. Readings were taken on a Honeywell Electronic Model 194 recorder, with a chart-speed of 2 mm/sec and a 10-mV span.

Reagents

All reagents were of analytical-reagent grade. Distilled demineralized water (DDW) was used throughout.

A 1000-μg/ml standard zinc solution was prepared by dissolving 0.2500 g of zinc metal in the minimum quantity of concentrated nitric acid, the solution being made up to 250 ml with DDW. Working standards were prepared each day by serial dilution in 0.1*M* nitric acid.

A 100-μg/ml standard chloride solution was prepared from standard hydrochloric acid and this was used for all the chloride additions.

A 100-μg/ml iron solution was prepared by dissolving 100 mg of the metal in the minimum quantity of concentrated nitric acid and diluting to 1 litre with DDW.

Procedures

These are included in the discussion section.

RESULTS AND DISCUSSION

Carbon filament atomizer

As would be expected from the volatility of zinc chloride (b.p. 732°), chloride (even at low levels) significantly depressed the zinc atomic-absorption signal when the carbon-filament atomizer was used (Fig. 1). The depressive effect increased with chloride concentration and then levelled off at about 40% depression, typical of the reported levels for carbon filaments. The curvature in the graph is characteristic of interference by compound formation. It is suggested that for a 0.1-mg/l. zinc solution, compound formation is complete when the molar ratio of chloride to zinc is 2:1, indicating that the volatile halide $ZnCl_2$ has been formed and that this is only partially dissociated into zinc and chloride atoms at the temperatures above the filament. As this is an open atom-cell the vapours rapidly disperse and cool before further reactions, other than condensation, take place. This can be taken as explaining the increased depressions observed when viewing at other than grazing incidence to the filament.

When iron(III) nitrate solution was added to 0.1-μg/ml zinc nitrate solution no interference was observed for the iron concentration range 1–5 μg/ml. However, in the presence of iron the chloride interference with the zinc signal was considerably modified. A whole family of graphs such as that presented in Fig. 2 was obtained. The levels in Fig. 2 (0.1-μg/ml zinc, 1-μg/ml chloride and 0–5-μg/ml iron) are typical of those studied. Decreasing the zinc or chloride concentration caused the curve to be shifted to the right or left respectively, but did not affect its general shape. The reasons for the initial further depression of the zinc signal are not clear, but presumably at low levels the iron has an antagonistic effect on whatever mechanism produces the dissociation of the zinc chloride, but this does not persist when the iron concentration is increased and the effect of the chloride interference is then substantially reduced. Apparently the competition from iron(III) for chloride complexation becomes significant and the zinc is freed to an increasing extent from the chloride. Further evidence

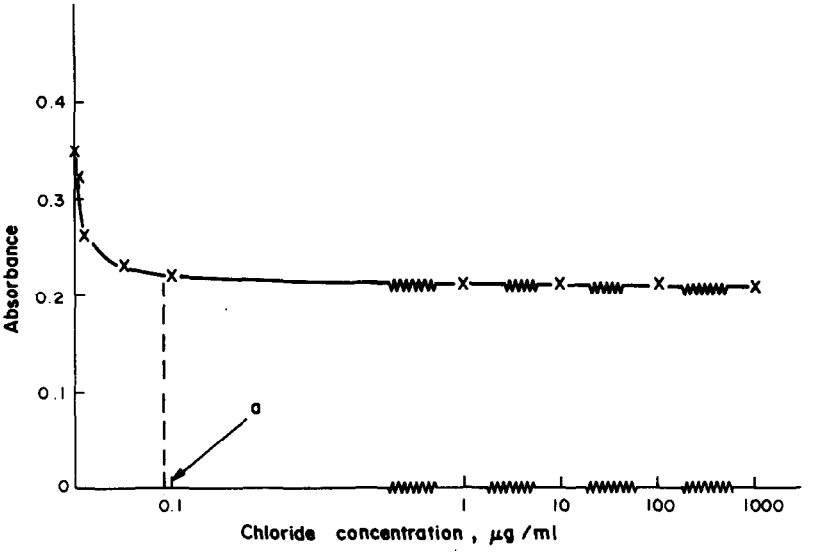

Fig. 1. Effect of chloride on the absorbance of a 0.1-μg/ml zinc solution, with a carbon-filament atomizer. The point a corresponds to a 2:1 molar ratio of chloride to zinc.

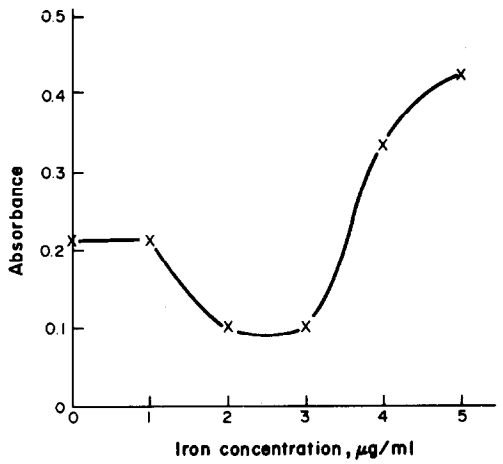

Fig. 2. Influence of iron on the interference effect from 1-μg/ml chloride on 0.1-μg/ml zinc solution, when a carbon-filament atomizer is used.

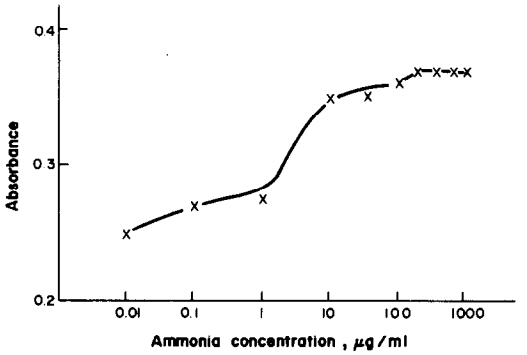

Fig. 3. Influence of ammonia solution on the interference effect from 0.1-μg/ml chloride on 0.1-μg/ml zinc solution, when a carbon-filament atomizer is used.

for this theory can be adduced from the discussion of releasing effects below. It would also appear to be consistent with recently published work[24] concerning the effect of sodium chloride on iron(III) determinations with a carbon filament. These authors added ammonia and EDTA solution to prevent premature volatilization of iron(III) chloride.

Although the effects of chloride and iron on zinc determinations with use of this atomizer can be elegantly and simply explained, this is no consolation to the practical analyst who, faced with an unknown water or steelworks effluent, will not have available sufficient evidence to correct for such complicated effects. Furthermore, standard addition, the normal resort of the practical analyst when using electrothermal atomization, is shown to be liable to error. The raising of the zinc level in the system shown in Fig. 2 would place it on a different, and not parallel, curve within the same family. Clearly it is necessary not merely to correct for this interference but to find ways of removing it.

The conventional method[25] of removing chloride interference is to add ammonium nitrate and adjust the ashing cycle so that the chloride is driven off as ammonium chloride (sublimation temperature 335°). This approach was tried by adding aqueous ammonia solution (5 μl) directly to the rod, and optimizing the ashing step so that the smoke (presumably ammonium chloride) was driven off completely without loss of zinc (10 sec at 5 V). Figure 3 shows the results of this study for 0.1 μg/ml zinc and chloride. Little effect was seen until the ammonium ion concentration was greater than the chloride concentration, and a large excess was necessary to obtain complete release. The need for a large excess might be thought to be due to the greater thermodynamic stability of the bivalent metal chloride. To test this, and to obtain further evidence about the interference mechanism, attempts were made to utilize a classical means of sequestering chloride ions, namely silver. The efficiency of silver, added as a silver nitrate solution (5 μl), to the rod can be seen from Table 1. At 20-fold w/w ratio of silver to chloride there was almost complete suppression of the interference, suggesting that the silver nitrate released the zinc from its chloride complexes. Silver can be expected to prove a useful matrix modification agent for the removal of chloride interferences in electrothermal atomization, particularly in the determination of volatile elements where there is a danger of the co-volatilization of analyte with ammonium chloride. The use of silver as matrix modifier remained effective in the presence of iron and chloride at levels typical of the stream being studied, and allowed the successful determination of zinc levels in the stream with a carbon filament atomizer.

Graphite-furnace atomizer

The interference problems encountered with the carbon-filament atomizer having been identified and overcome, an attempt was made to compare the performance of the graphite-furnace atomizer for this particular problem. The graphite furnace offered much better sensitivity than the carbon filament and great care had to be taken to avoid contamination

Table 1. Effect of adding silver ions, on the chloride interference in zinc measurements by AAS with a carbon-filament atomizer

Sample				
Zn^{2+}*, μg/ml	Cl^-, μg/ml	Ag^+, μg/ml	Fe^{3+}, μg/ml	Zinc absorbance
0.1				0.35
0.1	100			0.21
0.1	100	1000		0.30
0.1	100	2000		0.33
0.1	100	10^4		0.33
0.1		10^4		0.34
0.1	100	10^4	4	0.33
0.1	100	10^4	5	0.33

* Added as nitrate

Table 2. Effect of chloride on absorbance at 213.9 nm from 10 pg of zinc, by AAS with a graphite-furnace atomizer

Chloride concentration, μg/l.	0	1	2	10	conc. HCl
Zinc absorbance	0.135 ± 0.003	0.143 ± 0.003	0.145 ± 0.003	0.137 ± 0.005	0.138 ± 0.003

and blanks when the 213.9-nm zinc line was used. The level of zinc in the laboratory distilled and demineralized water was found to vary between 1 and 10 μg/l. and the water was further purified by passage through two 30 × 1 cm ion-exchange columns (Zerolit 225 in acid form) to bring the zinc level below 1 μg/l. Pipette tips had to be carefully washed and regularly checked to prevent high blanks. The possibility that some of the chemical effects observed in the previous study might not be reproduced at the much lower zinc levels optimal for the furnace, and that spurious blanks, *e.g.*, from contamination by airborne talcum powder, might complicate the interpretation of results, led us to also investigate results obtained by using the far less sensitive 307.6-nm zinc line. A rapid atomization step was chosen, as this enabled the zinc to be atomized within 1 sec, before the onset of non-specific absorption effects (which occurred within 2–3 sec). As detailed above, an ashing stage was also used to minimize matrix effects.

The effect of chloride concentration on the absorbance from a 2-μg/l. zinc (as nitrate) solution (5 μl) at the 213.9-nm line was investigated and the results are reported in Table 2. The results indicate no significant depression, even for concentrated hydrochloric acid medium; if they are all assumed to come from the same population, they give a mean absorbance of 0.140 with a relative standard deviation of 3.7%, compared to a mean of 0.138 with a relative standard deviation of 3.6% for replicates of a 2-μg/l. zinc nitrate solution analysed in a comparative experiment. The only problem encountered from the concentrated acid was one of increased scatter signals and limited tube-life.

As these analyte levels were significantly lower than those used on the filament, and in view of the widespread reports that the absolute quantity of interferent is an important factor,[9,26] the level of zinc was increased and, in order to accommodate these levels, the much less sensitive 307.6-nm zinc line was used. The effect of various levels of chloride on the absorbance from a 10-μg/ml zinc solution (5 μl) is shown in Table 3. The precision of these results was poorer than that of those shown in Table 2, having a relative standard deviation of 9.5% for the solution without chloride added (mean absorbance 0.145). The addition of chloride caused no significant depression and if the results are assumed to belong to the same population, the mean is 0.145 with a relative standard deviation of 13.3%.

These results for the furnace contrast strongly with those obtained for the filament. The furnace used is of the longer and larger type and the most likely explanation would appear to be that, in the furnace, sufficient hydrogen is generated during the ashing procedure to convert the chloride present into hydrogen chloride and remove it, in a manner analogous to that proposed by Frech and Cedergren.[19,20] The size of the furnace and the ashing conditions chosen presumably allow the reaction

$$ZnCl_2(g) + H_2(g) \rightarrow Zn(s) + 2HCl(g)$$

to occur, the excess of chloride also being volatilized as hydrogen chloride. This large evolution of hydrogen cannot be expected on the exposed filament or necessarily in a smaller furnace, and any hydrogen evolved in these devices would be swept away rapidly by the flow of sheathing gas.

Additionally the higher vapour phase temperature experienced in the furnace should lead to greater dissociation of zinc chloride if it is formed in the atomization stage. Confirmation of such dissociation may be inferred from the success of work on isothermal atomization, such as that by Slavin and Manning,[23] using a platform.

CONCLUSION

The results presented suggest ways in which apparently irreconcilable reports of chloride interferences in zinc determinations by atomic-absorption spectroscopy with electrothermal atomizers may be

Table 3. Effect of chloride on absorbance at 307.6 nm from 50 ng of zinc by AAS with a graphite-furnace atomizer

Chloride concentration, μg/ml	0	1	10	16	20	50	Conc. HCl
Zinc absorbance	0.145	0.146	0.141	0.146	0.146	0.147	0.150

explained. No suppression of the zinc signal was observed when using the large furnace in this work, presumably because of the removal of chloride as hydrogen chloride. The suppression of the zinc signal by chloride when the carbon-filament atomizer was used was severe even at low levels of chloride. In practical analyses it is not advisable to assume that the method of standard additions will compensate for the presence of chloride, because there is a complex interaction of other ions with a strong affinity for chloride, such as iron(III). For the analyses in question, of grossly polluted streams or steelworks effluents, a furnace atomizer is therefore to be preferred. If it is necessary to use a filament atomizer or a minifurnace the interference effects can be satisfactorily overcome by the use of ammonia solution or silver nitrate as matrix modifiers. Phenomena such as those reported here must be considered when electrothermal atomizers are being designed or selected for purchase.

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DETERMINATION OF COBALT BY PYROGALLOL CHEMILUMINESCENCE

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Summary—The chemiluminescence reaction involving pyrogallol in alkaline hydrogen peroxide solution is described. With reaction conditions selected for the determination of Co(II), the detection limit for Co(II) was at least two orders of magnitude below the detection limit of all other species tested. The results suggest that Ca, Mg, Mn, and Fe are the most likely interferents for environmental and biological samples. The log–log calibration graph for Co(II) is linear from a detection limit of 0.5 ng/ml up to 10 μg/ml.

In recent years, the analytical applications of chemiluminescence (CL) and bioluminescence systems have received considerable attention.[1–8] Some of the reasons for this interest are: simplicity of the instrumentation, low detection limits, large dynamic ranges, relatively short analysis times, and in some cases the possibility of chemical speciation. CL determination of metal ions has been restricted by the lack of selectivity. Therefore, masking of the interferent(s) or separation of the interferent(s) from the analyte is generally required before analysis of "real" samples.[9]

In our laboratory, we have detected trace amounts of cobalt by utilizing the cobalt(II) enhancement of the CL signal resulting from the reaction of alkaline hydrogen peroxide with pyrogallol (1,2,3-benzenetriol). The CL accompanying the reaction of pyrogallol (Pg) and alkaline hydrogen peroxide has long been known.[10,11] However, the analytical application of the system has not received much attention. A recent review on the development and present state of research on the oxidation and CL of pyrogallol is available.[12] The references cited in the review include a paper by Tomassi *et al.*,[13] who postulate that the enhancement in the CL signal by cobalt is due to the formation of a complex of cobalt(II) with a benzosemiquinone formed in the reaction. Zwierzchowska-Nowakowska[14] also studied this system with cobalt present and concluded that a diphenyl-cobalt phosphate complex was formed as an intermediate product.

Reported analytical applications utilizing this system are relatively few and include the determination of $5 \times 10^{-10} M$ horseradish peroxidase in distilled water.[15,16] Although the system has not been utilized for the determination of cobalt, Stieg and Nieman[17] reported a detection limit of 0.4 ng/ml for Co(II) with the gallic acid (3,4,5-trihydroxybenzoic acid) CL system. The gallic acid (GA) CL system, which is suspected to give similar results to the Pg CL system, has also been used to determine proteins,[18] tannins[19] and formaldehyde.[20] The CL spectrum of Pg under experimental conditions similar to those used in this paper was recently measured with an intensified diode-array detector system, but useful analytical information was not obtained.[21]

EXPERIMENTAL

Instrumentation

All measurements were obtained with a discrete sampling system which has been reported earlier[22] and with the modifications and instrumental conditions similar to those previously described.[23] The emission from the CL reaction was observed with no wavelength discrimination, all signals were registered as photo-anodic currents and a Spectrum model 1021 filter and amplifier was placed in line after the current-to-voltage converter and set for a 0.1-Hz cut-off frequency to reduce noise.

Reagents

All solutions were prepared with water from a Millipore (Milli-Q) reagent-grade water system. The pH of the samples and the hydrogen peroxide solution was adjusted by adding dilute nitric acid.

Stock 50m*M* Pg solution was prepared by dissolving reagent grade Pg in 0.01*M* nitric acid and diluting to the required volume with the same acid. Such solutions were stable at room temperature for at least two weeks (the duration of the test).

Stock 680m*M* hydrogen peroxide solution was prepared by diluting 30% reagent grade hydrogen peroxide and adjusting the pH to 4–4.5 as suggested by Montano and Ingle.[23] Stabilized peroxide gave the same results (*i.e.*, same analytical signal and detection limit) as unstabilized peroxide. The stock solution, at best, is stable for approximately two weeks. However, sometimes a larger than normal CL blank signal was observed, and then preparation of a new peroxide solution restored the blank CL signal to the normally observed blank level. However, even with a large blank signal the analytical signal for a given cobalt concentration remained constant.

Stock 0.5*M* sodium bicarbonate solution was prepared by dissolving the reagent-grade material in demineralized water and the pH was adjusted to 9.9 with reagent-grade potassium hydroxide solution just before use. The solution is stable for at least one month.

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The cobalt standards and the solutions for interference studies and for cleaning glassware were made as previously described.[23] Mallinckrodt reagents were used except for the humic acid (Aldrich practical grade) and formaldehyde (Baker reagent grade) standard solutions.

Procedure

The general procedure previously described for another CL system was followed.[23] Exactly 1.0 ml of blank, analyte, or interference solution, 0.5 ml of hydrogen peroxide solution, and 0.5 ml of Pg solution are added to the sample cell with Eppendorf pipettes. With the sample cell compartment lid closed and the PMT shutter open, 0.5 ml of buffer solution is injected into the cell through a septum, with a pneumatically-driven liquid dispenser. A blank CL signal is taken as the peak signal produced by use of water acidified to the same pH as the metal test solution. The CL analytical signal is taken as the difference in the CL peak height between blank and analyte determination. Between assays the reaction cell is rinsed four times with Millipore water. Typical peak shapes for blank and Co determinations are shown in Fig. 1. The CL signal lasts for over 15 min; however, the CL signal was monitored for only the first 10 sec, as shown in Fig. 1, which was enough to ensure that the peak had been registered.

RESULTS AND DISCUSSION

The optimization criteria in order of priority were as follows: detection limit, analytical signal to background ratio (S/B), dynamic range, analysis time, and use of reagent concentrations such that small changes in concentration did not significantly affect metal and blank CL signals. In most cases, the detection limit and S/B criteria indicated identical optimal conditions. If the detection limit was fairly insensitive to changes in a variable, then the S/B became the primary criterion for optimization. All optimization was carried out with Co(II) as the analyte. The detection limit was calculated as the concentration of the analyte solution producing an analytical signal equal to twice the standard deviation of the reagent blank CL signal. The relative standard deviation in the blank signal was 4–6% and was due to both irreproducibility of the blank signal and noise in the dark current and blank CL signals.

Dependence on pH

Figure 2 shows the results of the pH-dependency data obtained with a bicarbonate buffer system adjusted with potassium hydroxide. It shows a maximum in the total signal for both the reagent blank and 10-ng/ml Co(II) solutions at a pH of 10.1, and a relatively constant detection limit over the pH range 9.0–10.0. The S/B exhibits a broad maximum over the pH range 9.5–10.0 and a pH of 9.9 was chosen for all further studies. At lower pH the CL peak is broader, which makes it more difficult to distinguish, and the analysis time is longer. Sodium bicarbonate was used as the buffer because it resulted in CL signals equivalent to those obtained with sodium hydroxide solutions of the same pH and twice as great as those

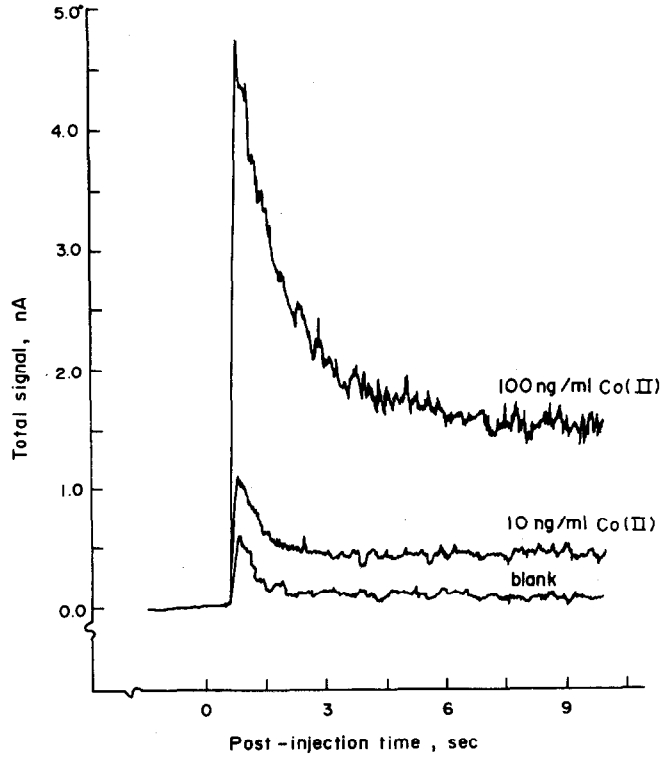

Fig. 1. Peak shapes under optimum conditions for the blank, 10, and 100 ng/ml Co(II). Cell conditions: 10m*M* Pg, 135m*M* H_2O_2, 100m*M* $NaHCO_3$ (pH 9.9), post-injection time = time elapsed after injection of the buffer.

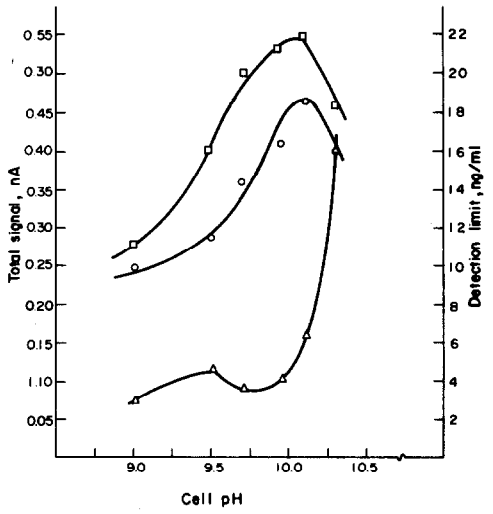

Fig. 2. Detection limit (—△—) and CL signal for 10 ng/ml Co(II) (—□—) and reagent blank (—○—) as a function of cell pH. Cell conditions: 10m*M* Pg, 156m*M* H_2O_2, 100m*M* $NaHCO_3$.

obtained with a dipotassium hydrogen phosphate buffer solution of the same pH. Since the best Co(II) detection limits were obtained if the bicarbonate concentration in the cell was 0.08*M* or higher, an initial concentration of 0.5*M* (giving 0.1*M* final concentration) was used for the rest of the studies.

Temperature

The effect of temperature in the range 18–40° was studied, and showed that no significant analytical advantage would be realized by using a temperature other than 25°, which was the temperature chosen for convenience.

Arrhenius plots for the dependence of background, 10- and 100-ng/ml Co(II) signals on temperature indicated a positive deviation from linearity. This could be due to two or more simultaneous reactions taking place or to quantum mechanical tunnelling as in electron-transfer reactions. From examination of the mechanism proposed in the literature[12,20] and our own observations of the end-products for low and high concentration of cobalt, we believe simultaneous reactions are taking place. A similar treatment of the CL signals resulting from 1- and 10-μg/ml Co(II) solutions produced linear Arrhenius plots ($r = 0.99$) and the activation energy calculated from the slope was 14 kcal/mole. Using conditions which were similar to our background reaction conditions, Slawinski[24] obtained an activation energy of 8.1 kcal/mole.

Mixing order

Montano and Ingle[23] reported that the order of mixing the reagents in the lucigenin CL system had a definite effect on the precision of the blank measurement and on the analysis time. For the Pg CL system, there are four mixing orders defined by which of the four solutions is added last. It was experimentally observed that the order of mixing the first three reagents had no significant effect on the CL signal.

The injection of the analyte or blank solution last was the only mixing order which produced an observable CL background signal prior to injection of the last solution. A 1-min delay time between mixing of the first three reagents and injection was necessary, because the CL signal derived from the mixing of Pg, peroxide and buffer decayed exponentially from a maximum which occurred in less than 1 sec to a plateau in approximately 1 min. Injection of the sample at or near the maximum gave results with relatively low precision. This mixing order was found not to be optimal, because a 50% longer analysis time was required and the precision of the measurements was dependent on the time taken to inject the analyte or blank solution.

Injection of the Pg solution last resulted in an increase in the blank signal by a factor of about four and no significant enhancement of signal with a 10-μg/ml Co(II) solution. For this reason and because the precision of the blank signal was the poorest this mixing order was abandoned.

The results obtained by injecting the peroxide or the buffer last were nearly identical. However, when peroxide was injected last the magnitude of the background or analyte signal increased with the length of time the Pg and buffer solutions were in contact before injection of the peroxide. This could be due to a higher concentration of the anionic form of Pg at the onset of the reaction or a higher concentration of an intermediate species involved in the CL reaction. In alkaline solution Pg can oxidize to various intermediate species in the absence of peroxide.[24]

The injection of the buffer last was selected as optimal because (*i*) the detection limit, S/B and analysis time were best, and (*ii*) the magnitude and precision of the CL signal were not dependent on speed of injection or delay time before the injection.

Dependence on peroxide concentration

A preliminary study indicated that the effects of the peroxide and Pg concentrations are interrelated. Therefore, approximate optimum values for both reagent concentrations were obtained by extracting S/B and detection limit information from calibration graphs for a wide range of peroxide concentrations at a number of Pg concentrations. The peroxide concentration was then reoptimized at the approximate optimum Pg concentration (10m*M*).

Figure 3 shows that the blank and analytical signals rapidly increase and the detection limit dramatically improves as the peroxide concentration is increased from 7.8 to 100m*M*, and then change more gradually at higher concentrations. A peroxide concentration of 0.14*M* was finally selected because the detection limit is still only about twice that at 1.9*M* peroxide, and the precautions which must be used when handling 9.8*M* hydrogen peroxide (1.9*M* cell

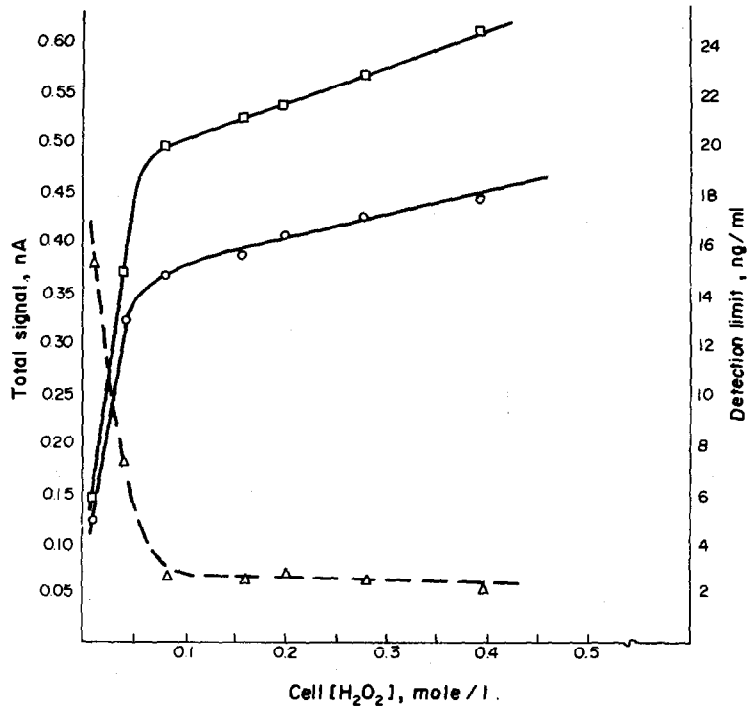

Fig. 3. Detection limit (—△—) and CL signal for 10 ng/ml Co(II) (—□—) and reagent blank (—○—) as a function of the cell H_2O_2 concentration. Cell conditions: 10m*M* Pg, 100m*M* $NaHCO_3$ (pH 9.9).

concentration) are not needed for 0.68*M* peroxide (0.14*M* cell concentration).

Dependence on Pg concentration

The effect of Pg concentration was re-examined with the selected peroxide concentration. Figure 4 shows an increase in analytical signal and decrease in detection limit as the Pg concentration increases up to 100m*M*. The increase in detection limit for Pg concentrations <5m*M* is due to a change in the peak shape when the Co(II) concentration is <30 ng/ml, resulting in a decreased S/B. There is also gradual

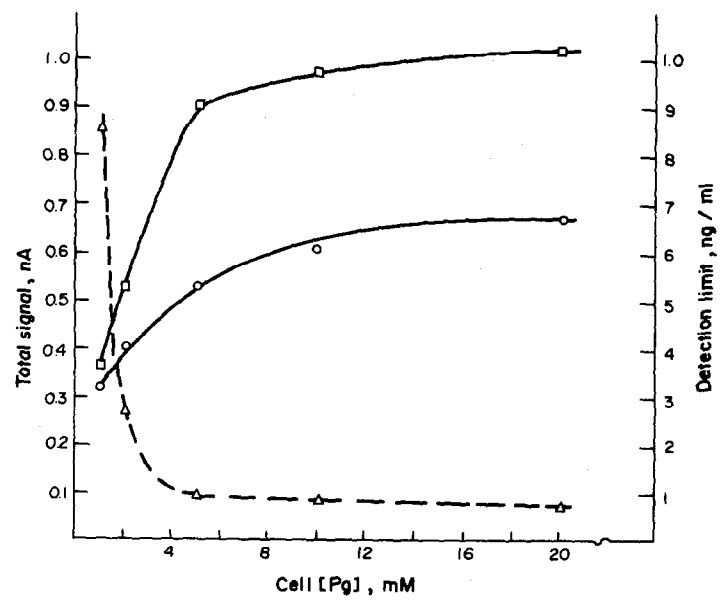

Fig. 4. Detection limit (—△—) and CL signal for 10 ng/ml Co(II) (—□—) and reagent blank (—○—) as a function of the cell Pg concentration. Cell conditions: 135m*M* H_2O_2, 100m*M* $NaHCO_3$ (pH 9.9).

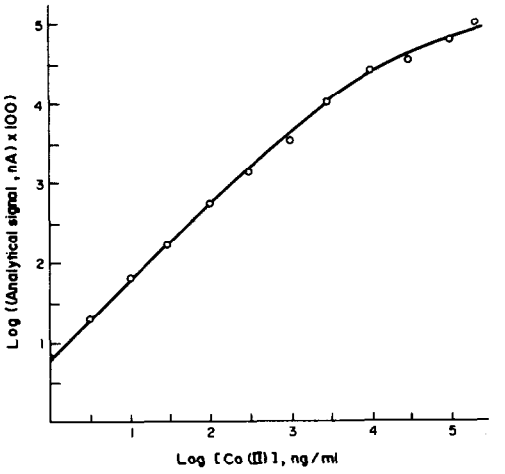

Fig. 5. Co(II) calibration curve [initial Co(II) concentrations]. Cell conditions: 10m*M* Pg, 135m*M* H_2O_2, 100m*M* $NaHCO_3$ (pH 9.9).

improvement in the detection limit as the Pg concentration is increased from 5 to 20m*M*. At Pg concentrations above 10m*M* the calibration sensitivity is lower and there is a negative deviation from linearity at lower Co(II) concentrations, which restricts the dynamic range. This deviation may have two causes: (*i*) for high cobalt concentrations a water-soluble brown polymer appears to be the principal end-product and may cause light scattering; (*ii*) examination of the Pg absorption spectrum and the CL spectrum[21] indicates that self-absorption is possible.

Calibration

Figure 5 shows a log–log calibration curve for Co(II) under the conditions determined above. The log–log plot is linear from the cobalt detection limit of 0.5 ng/ml to about 100 ng/ml, with a slope of unity, and is usable to above 10 μg/ml cobalt concentration, these concentrations being those in the sample solution. A direct plot of analytical signal *vs.* Co(II) concentration (Fig. 6a) will therefore be linear up to 100 ng/ml and is superior for this range. The slope is 0.055 nA . ml . ng^{-1}. The relative standard deviation of the signal at concentrations well above the detection limit is typically 1–2%.

Effect of dissolved oxygen

When N_2 is bubbled through the solutions (at the concentrations used for Fig. 5) to remove dissolved O_2 before initiation of the reaction, the background signal is reduced by over an order of magnitude but the net analytical signal from 10 ng/ml Co(II) does not significantly change. If O_2 is bubbled through the solutions before reaction, the blank CL signal increases by a factor of 3 and the Co(II) analytical signal increases by about 30%. If O_2 is bubbled through the solution in the absence of H_2O_2, a background CL reaction is observed with a peak at about 3 min and a peak height about 2.5 times the normal background peak. The presence of Co(II) does not affect this CL peak and no CL is observed without H_2O_2 if solutions are purged with N_2. These data indicate that dissolved O_2 is a reactant in the blank CL reaction and that the enhancement of the CL signal by Co(II) is due in part to reaction of Co(II) with H_2O_2.

Interference study

An extensive interference study was carried out and the results are summarized in Tables 1 and 2. A negative slope indicates inhibition, and a positive slope enhancement of the background CL signal by the species. The general procedure was to substitute the potentially interfering species for the cobalt solution in the standard analysis procedure, starting at the 100 μg/ml level and diluting progressively to the concentration at which the species did not interfere. The detection limit was calculated with respect to the blank reaction, as for Co(II).

All the data given represent results obtained under the optimal reaction conditions for the Co(II)-enhanced CL signal and may not be the lowest levels of the interferents that can be detected by Pg CL. For example, the detection limit for Fe(II) is listed as 2 μg/ml, but if the first three reagents are allowed to mix for 5 min before the injection of the buffer, the detection limit is lowered to approximately 34 ng/ml.

The interference effect of some species was further studied by spiking a 5-ng/ml Co(II) solution with representative interfering species from both the activator and inhibitor groups and comparing the CL signal

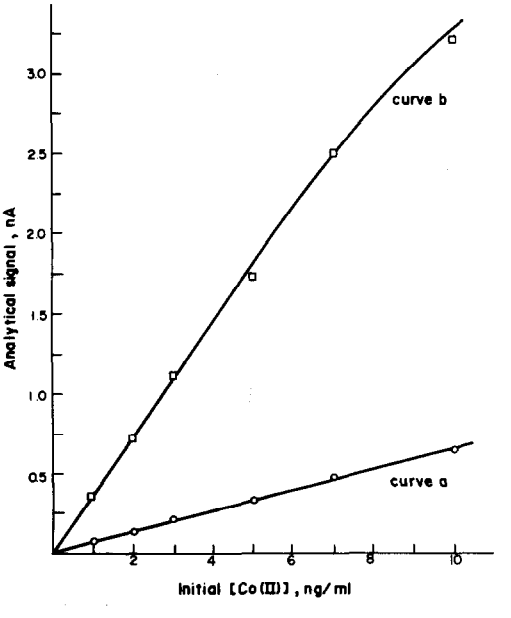

Fig. 6. Co(II) calibration curves without Ca(II) (curve a) and with 300 μg/ml Ca(II) (curve b). Cell conditions same as for Fig. 5.

Table 1. Metals and non-metals which enhance or inhibit the Pg CL reaction

Species	Slope,* $nA.ml.\mu g^{-1}$	Detection limit, $\mu g/ml$	Relative intensity†
Li(I)	—	>100	—
Be(II)	—	>100	—
Na(I)	1.7×10^{-5}	2000	0.0002
Mg(II)	3.3×10^{-3}	10	0.05
Al(III)	-1.9×10^{-3}	18	0.03
Si(IV)	4.0×10^{-4}	85	0.6
Ca(II)	5.5×10^{-3}	6	0.08
Sc(III)	-1.1×10^{-2}	3	0.2
V(V)	2.7×10^{-2}	1	0.5
Cr(III)	1.2×10^{-2}	3	0.2
Cr(VI)	5.6×10^{-2}	0.6	0.8
Mn(II)	8.6×10^{-1}	0.04	12
Mn(VII)	1.6×10^{-1}	0.07	7
Fe(II)	8.4×10^{-3}	4	0.1
Fe(III)	9.5×10^{-3}	2	0.2
Co(II)	6.5×10^{1}	0.0005	1000
Ni(II)	1.0×10^{-2}	3	0.2
Cu(II)		>100	—
Zn(II)	1.2×10^{-2}	3	0.2
Ga(III)		>100	—
Ge(IV)		>100	—
As(III)	5.5×10^{-3}	6	0.08
As(V)	2.7×10^{-3}	13	0.04
Se(IV)		>100	—
Sr(II)	1.5×10^{-2}	2	0.2
Y(III)	7.1×10^{-3}	5	0.1
Zr(IV)		>33	—
Nb(V)	-1.8×10^{-3}	19	0.03
Mo(VI)	8.5×10^{-4}	40	0.01
Ru(III)	1.6×10^{-1}	0.2	2
Rh(III)	—	>100	—
Pd(II)	—	>100	—
Ag(I)	1.6×10^{-1}	0.2	2
Cd(II)	8.4×10^{-2}	0.4	1
Sn(II)	—	>100	—
Sb(II)	7.5×10^{-3}	4	0.1
Ba(II)	2.4×10^{-3}	14	0.03
La(III)	—	>100	—
Ce(III)	1.6×10^{-2}	2	0.2
Nd(III)	1.0×10^{-3}	3	0.2
Tm(III)	—	>100	—
Hf(IV)	—	>33	—
Ta(III)	-2.0×10^{-3}	17	0.03
W(VI)	—	>100	—
Re(VII)	—	>100	—
Os(VIII)	1.7×10^{0}	0.02	25
Ir(III)	—	>100	—
Pt(IV)	9.9×10^{-2}	0.3	2
Au(III)	1.2×10^{-1}	0.3	2
Hg(II)	8.0×10^{-3}	4	0.1
Pb(II)	1.2×10^{-2}	3	0.2
Bi(II)	1.9×10^{-3}	18	0.03
Th(IV)	-2.7×10^{-2}	1	0.5
U(VI)	—	>100	—
NH_4^+	-5.7×10^{-3}	6	0.08

* Slope at the detection limit.

† Relative intensity at the detection limit compared to Co (assigned an intensity of 1000).

with that obtained from 5 ng/ml Co(II) alone. These studies showed whether the concentration of the interferent causing significant enhancement or inhibition of the Co(II) signal was the same as the detection limit for that species in the absence of Co(II). The criterion for interference was the production of a deviation from the 5-ng/ml Co(II) standard CL signal greater than twice the standard deviation of the 5-ng/ml Co(II) signal. Of the species tested, [Mg(II), Al(III), Ca(II), Mn(II), Fe(III), Zn(II), OCl^-, CH_2O, EDTA and humic acid (HA)], all gave interference at levels within a factor of two of their detection limits, except for Al(III), Fe(III), CH_2O and EDTA, which interfered at 5, 1, 0.1 and 0.05 μg/ml, respectively, levels which are below their detection limits.

Stieg and Nieman[17] noted suppression of the cobalt signal for the GA CL system in the presence of complexing agents, and could predict the degree of suppression by calculating the concentration of uncomplexed cobalt in the solution. Similar calculations for the Pg CL system with EDTA indicated that the same behaviour was observed, but the experimentally observed Co CL signal was best approximated by calculating the free cobalt concentration from the conditional stability constant K' for the 1:1 Co–EDTA complex at a pH near 4. At pH 10, the formation of the Co–EDTA complex is quantitative ($K' \sim 10^{14}$) for $10^{-7}M$ Co(II) and EDTA, but only about a 10% suppression of the CL signal was observed. Before the injection of the buffer the pH of the solution is about 3; therefore, our data indicate that the complexation of the remaining free Co is slow relative to the time it takes for the CL peak to appear (<1 sec).

The effects of HA on the Pg CL system were studied because of the role of HA as a naturally occurring organic sequestering agent[25] and since HA is known to give CL in the presence of alkaline H_2O_2.[26] Formaldehyde was tested because Slawinska and Slaw-

Table 2. Anions and complexing agents which enhance or inhibit the Pg CL reaction

Anion	Slope,* $nA.ml.\mu g^{-1}$	Detection limit, $\mu g/ml$	Relative intensity†
F^-	—	>100	—
S^{2-}	3.6×10^{-2}	0.9	0.6
SO_4^{2-}	—	>100	—
$S_2O_8^{2-}$	1.5×10^{-2}	2	0.2
Cl^-	1.2×10^{-5}	2900	0.001
OCl^-	4.0×10^{-1}	0.3	2
ClO_4^-	—	>500	—
Br^-	—	>100	—
I^-	4.6×10^{-2}	0.7	0.7
IO_4^-	1.6×10^{-2}	2	0.2
CO_3^{2-}	—	>100	—
PO_4^{3-}	7.5×10^{-4}	45	0.01
NO_3^-	—	>100	—
CH_2O	5.5×10^{-3}	3	0.1
EDTA	-2.1×10^{-1}	0.7	0.7
Humic acid	—	>100	—

* Slope at the detection limit.

† Relative intensity at the detection limit compared to Co (assigned an intensity of 1000).

inski[20] reported that formaldehyde could be detected at the $10^{-7}M$ level by the GA CL system, and Pg and GA are quite similar. The interference due to OCl^- is probably due to the CL reaction between OCl^- and H_2O_2.[27]

The apparent interference from species for which the detection limits are particularly high could be due to traces of cobalt in the chemicals used, but it would be very difficult to analyse the reagents for such low levels of cobalt.

Analysis of real samples

The interference study indicated that many species enhance or inhibit the Pg CL reaction; however, the detection limit and interference level for most species is above 1 ppm. Other than Co(II), only Cr(VI), Mn(II), Ru(III), Ag(I), Cd(II), Os(VIII), Pt(IV), and Au(III) have detection limits below 1 μg/ml.

In fresh water samples, only Ca, Mg and possibly natural complexing agents, would be potential interferences in most cases. The effect of complexing agents can be eliminated by acid digestion. In biological samples after digestion, interference can again be expected from Ca and Mg, and possibly from Fe, Zn and Mn, depending on the sample. Because the detection limit for cobalt is so much lower than for most elements, sample dilution in some cases would bring the concentration of the potential interferents to below their detection limit. Since cobalt concentrations in biological samples are typically 0.1–1 μg/ml or μg/g, the sample concentration in the final digestion solution can be as low as 0.1–1% w/w.

The applicability of the Pg CL technique was evaluated by assaying three real samples: tap water, Willamette River water, and NBS bovine liver.[28] Previous work indicated that the water samples would contain 5–15 μg/ml Ca(II), 3–5 μg/ml Mg(II) and 0.1–0.3 μg/ml Fe(III). From Tables 1 and 2, it can be seen that only calcium is a potential interferent for the determination of cobalt in the water samples. Since cobalt concentrations in natural water are usually below 10 ng/ml, and often below 1 ng/ml, dilution will not eliminate the potential calcium interference.

The calibration curve for calcium, shown in Fig. 7, indicates that the CL signal is fairly independent of the calcium concentration from 250 to 400 μg/ml. The sharp decrease in the CL signal above 400 μg/ml Ca is accompanied by the formation of a white precipitate (probably $CaCO_3$, since the buffer system will be roughly equimolar in bicarbonate and carbonate at pH ~ 10), and attendant removal of calcium from the solution. A similar effect over about the same concentration range was observed for magnesium. Addition of 1–10 ng/ml cobalt to solutions containing magnesium at concentrations in the plateau region resulted in inhibition of the Co CL signal, whereas similar treatment of calcium solutions at plateau range resulted in an enhanced Co CL signal.

The reagent blank and cobalt standards were adjusted to contain calcium at the 300-μg/ml level

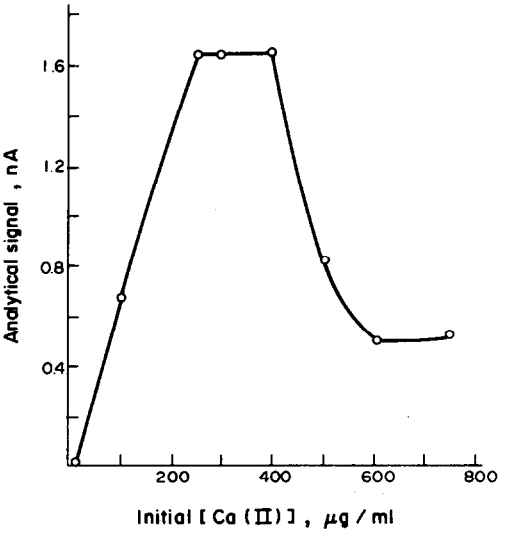

Fig. 7. Ca(II) calibration curve [initial Ca(II) concentration]. Cell conditions same as for Fig. 5.

and the cobalt calibration curve was redetermined, as shown in Fig. 6b. The curve is linear over the same region as the curve without added calcium (curve a), but the slope is significantly enhanced to 0.33 nA.ml.ng^{-1}. Hence, although the absolute standard deviation of the blank CL is greater because the mean value is increased, the detection limit remains the same owing to the increase in the calibration curve slope. However, if the samples and standards are all spiked with a 300-μg/ml calcium addition, the tolerance limit for the original calcium present is increased to 26 μg/ml, a level higher than that in most water samples. The interference levels for Mg(II), Al(III), Ca(II), Mn(II), Fe(III), OCl^-, CH_2O, EDTA and HA were tested in the presence of the 300-μg/ml calcium spike and found to be within a factor of two of the values obtained in the absence of the spike, but the interference level for Zn(II) was lowered to 1 μg/ml. Some of the HA was precipitated by the large concentration of calcium, so the supernatant solution was used for the interference study.

The results for the two water samples spiked with 300 μg/ml calcium indicated that the concentration of cobalt was below its detection limit. Standard additions of cobalt to the water samples in the 1–10 ng/ml range indicated that concentrations in this range could be determined accurately. The slope of the multiple standard addition plot was the same as the calibration slope for standard cobalt solutions.

The bovine liver sample was chosen for analysis because the matrix is well defined[28] (provisional cobalt value 0.18 μg/g). Three 0.5-g bovine liver samples were first ashed at 550° for 72 hr and then brought into solution by acid digestion with 5% v/v nitric acid. The acid-digested sample was then transferred to a 100-ml flask and the pH adjusted to 2 by the addition of 5*M* potassium hydroxide. After dilution to the mark, the sample solution was expected to

contain cobalt at the 0.9 ng/ml level, which is near the detection limit, but the Ca, Mg, Fe, and Mn concentrations were below their respective detection limits.

A cobalt concentration of 1.3 ng/ml was obtained by the multiple standard addition method with Pg CL detection. This value represents 0.26 μg/g in the original bovine liver sample, 44% greater than the expected value of 0.18 μg/g. The concentration of cobalt found from the calibration curve for the same solution was 0.8 ng/ml or 0.15 μg/g in the original sample. The iron concentration was estimated to be 415 μg/g by CL, with a 5-min wait before injection of the buffer solution. The certified iron concentration is 270 μg/g.

We attempted to confirm our results for the determination of Co in the two water samples and bovine liver sample, by neutron-activation analysis utilizing a Ge(Li) semiconductor detector after approximately 13 hr of irradiation with a flux of 3×10^{12} n.cm^{-2}.sec^{-1} from a TRIGA II reactor. Before irradiation, the water samples were preconcentrated by a factor of 20 by evaporation. The results indicated cobalt concentrations of 0.19 and 0.25 ng/ml for tap water and the Willamette River, respectively, which confirms that the concentrations were below the CL detection limit. The bovine liver sample was treated by the procedure outlined above for the Pg CL method, except that the sample solution was diluted to 5 ml so that the final concentration of cobalt in the activated sample was approximately 18 instead of 0.9 ng/ml. The results indicated a cobalt concentration of 0.28 μg/g in the original sample, which is in good agreement with the Pg CL results. A cobalt concentration of 0.25 μg/g for the bovine liver was obtained with lophine CL detection[9] and is also in good agreement with the Pg CL results.

Additional observations

The time-dependence of the Fe CL signal is only observed if Fe + H_2O_2 + Pg are all mixed together before injection of the buffer. At iron concentrations of 100 μg/ml and above, a CL signal is observed before injection of the buffer, but the CL signal is always enhanced by injection of the buffer, no matter what time delay is used.

As previously noted,[21] Mn(II) yields a unique CL response. At concentrations between 1 and 100 μg/ml, two CL peaks are observed. The first peak is observed within 0.5 sec after injection of the buffer. The second peak is observed between 30 sec and 7 min after injection of the buffer, depending on the manganese concentration. The second peak is preceded by the formation of a brown precipitate [possibly $Mn(OH)_3$ or MnO_2] and immediately before the CL emission the precipitate dissolves quickly with subsequent evolution of gas. This emission may be due to decomposition of the H_2O_2 by the $Mn(OH)_2$–MnO_2 couple.

Some of the inhibitors listed in Tables 1 and 2 are known to form complexes with Pg.[29] Thus, the inhibition may be due to an effective decrease in the free Pg concentration. However, some of the enhancers also form complexes with Pg, which suggests that more extensive research is needed to determine the nature of these complexes under the conditions of the experiments described above.

Finally, if higher concentrations of Pg and H_2O_2 are used the useful range has an upper limit of about 1 μg/ml for cobalt determination, but the detection limit could possibly be lowered by a factor of 2–4.

CONCLUSIONS

A new technique based on Pg CL has been developed for cobalt determinations in the 1–10 ng/ml range. The results indicate that the method should be useful for water samples which are not heavily polluted, and for some biological samples. For most water samples, the primary interference would be from Mg, Ca and possibly natural organic complexing agents such as humic acid. In biological samples, Fe and Mn would, in addition to Mg and Ca, be likely interferences. For a given sample, the direct applicability of the technique can be evaluated by measuring the concentration of these interfering elements by standard techniques such as flame atomic-absorption since the interference levels are above 1 μg/ml (or μg/g) except for manganese (0.04 μg/ml interference level). The calcium interference level can be raised to higher levels by spiking all standards, samples and blanks with high levels of calcium, without changing the detection limit for cobalt. Whether iron is present at a concentration high enough to cause interference can be decided by delaying the injection of the buffer for 5 min, since the signal will increase if there is interference.

As previously noted,[30] few techniques are directly applicable for determination of cobalt at concentrations below 10 ng/ml and particularly 1 ng/ml. Five different CL reagents, including Pg, provide sub-ng/ml detection limits for cobalt.[9] A rapid sample preparation procedure which removes major interfering species has recently been used for determination of cobalt by lophine CL.[9] This procedure should extend the applicability of the Pg CL to Co determinations in many types of samples.

Acknowledgements—We are indebted to Monty Smith for the neutron-activation analysis. Acknowledgement is made to the NSF (Grant No. CHE 7921293) for partial support of this research.

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THE CATION-CHELATION MECHANISM OF METAL-ION SORPTION BY POLYURETHANES

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Summary—The mechanism of sorption of ions by polyurethanes has been investigated through detailed studies of the extraction of cobalt(II) thiocyanate and the salts of several organic acids. Polyether-based polyurethanes, particularly those containing poly(ethylene oxide), were found to be distinctly superior to polyesters in the sorption of salts and performed much better than might be expected by analogy with monomeric liquid solvents. The results were judged to be inconsistent with several possible mechanisms, including adsorption, solvent extraction, weak or strong base anion-exchange, and complexation of metal anions by the polymer. A new proposal, termed the cation chelation mechanism (CCM), was advanced to account for the observations. In this view, a number of cations (including those of the alkali metals, alkaline earth metals, some transition metals, NH_4^+, RNH_3^+ and perhaps H_3O^+) may be multiply complexed (chelated) by portions of the polymer, thus facilitating the sorption of accompanying anions. As predicted by the mechanism, moderately strong and selective complexation of several cations was observed to occur with the following order of selectivity: $Li^+ < Na^+ < Cs^+ < Rb^+ < K^+ \sim NH_4^+ < Ag^+ \sim Tl^+ < Ba^{2+} < Hg^{2+} < Pb^{2+}$. Such behaviour parallels that known for many crown and non-cyclic polyethers and is therefore identified with the polyether portions of the polymer, which are thought to adopt helical conformations surrounding the complexed cations. The cation-chelation mechanism may be widely applicable to the sorption of ions of several types by polyether-based polyurethanes, particularly when large, hydrophobic anions (such as anionic metal complexes) are accompanied by an excess of chelatable cations.

Over the past decade, polyurethanes in the form of open-cell foams, microspheres or thin films, have been applied quite successfully to the extraction of a number of organic and inorganic substances from various media. Several good reviews of the literature, mainly describing analytical applications of the polyurethanes as media for separation and preconcentration, have appeared[1,2] while another including some other facets of their use is forthcoming.[3]

In many cases, other substances such as organic solvents or greases, liquid ion-exchangers, charcoal and a host of complexing agents have been added deliberately to the polyurethane before use, in order to achieve extraction. In these instances, the chief function of the polymer (usually an open-cell foam) is simply to act as a support for the added "active" materials. However, effective extraction of both organic and inorganic substances by plain polyurethane itself has also been observed and demonstrates that the polymer is not without its own sorptive capacity. Among these reports, we have been most interested in those which describe the uptake of metal ions from solution, and particularly in the mechanism whereby this might take place.

The first and perhaps most extensive such use of plain polyurethane was reported in 1970 by Bowen[4] for Hg(II), Au(III), Fe(III), Sb(V), Tl(III), Re(III), Mo(VI) and U(VI). All of these metals except the last were said to be extracted efficiently from acidic aqueous halide solutions as anionic halo-complexes (such as $FeCl_4^-$). U(VI), on the other hand, was extracted from an aqueous nitrate medium, presumably as UO_2^{2+}. Bowen found the sorption capacities to be far too large (0.5–1.5 mole/kg) to be attributable to surface adsorption phenomena, and so deduced that a true absorption into the bulk of the polymer must be occurring. In seeking to account for this, he suggested several possible mechanisms, beginning with a type of solvent extraction. In this regard, he commented that the list of substances absorbed by polyether-based polyurethane seemed to parallel closely that of compounds extractable by diethyl ether. He also suggested that some weak-base anion-exchange sites might be expected, based on the presence of nitrogen atoms in urea and urethane polymer linkages. Alternatively, it was pointed out that polyether oxygen atoms could possibly be protonated in acid, thus requiring the sorption of accompanying anionic metal complexes to maintain electrical neutrality. Which, if any, of these mechanisms was of prime importance was not established, however.

Several workers have since expanded the original work of Bowen to provide greater detail and to include other metals as well. In particular, both Schiller and Cook[5] and Sukiman[6] have reported the extraction of gold from acidic chloride solutions, while Gesser and co-workers have described the extraction of Ga(III)[7,8] from the same medium. Although no mechanistic interpretation was offered for the Au(III)/Cl^-/H^+ system, the phenomenon of Ga(III) sorption was considered to be an ether-like solvent extraction process in which $HGaCl_4$ was apparently the species extracted. Later, in a detailed study of the Fe(III)/Cl^-/H^+ system, Gesser and co-workers[7,9] also

reported the extraction of $FeCl_3$ and $HFeCl_4$. From their results, they inferred that polyether polyurethane could be regarded as a "viscous liquid" solvent of moderate dielectric constant in which some dissociation of sorbed species was evidently possible. Similar conclusions were also reached by this group on the basis of a study of U(VI) extraction from aqueous nitrate solutions.[10] Further studies by Lo *et al.* on Sn(II) and Sn(IV),[11] and on Sb(III) and Sb(V)[12] sorption from acidic chloride solutions likewise described the process as a solid "solvent" extraction.

The extraction of several metals from thiocyanate media has also been observed. Maloney *et al.*[13,14] have reported the extraction of Co(II), Fe(III), Cd(II), Zn(II) and Pb(II), while Braun and Farag[15] have provided some additional data for Co(II) and Fe(III) only. The latter authors, concurring with several others, described these processes as "etheric solvent extraction".

Moore and Chow[16] extended the use of polyurethane to the extraction of metal ions from organic solvents, and reported the removal of Ir(IV) and Pt(IV) chlorides from acetone or ethyl acetate solutions. Contrary to some earlier interpretations, these authors commented that the results clearly could not be explained solely on the basis of an ether-like solvent extraction mechanism. As previously suggested,[4,9] the possibility of specific ion-exchange sites in the polymer was therefore considered. More extensive studies on the Co(II) thiocyanate extraction system have been undertaken by Hamon[17] and similar investigations have been made by Al-Bazi[18] for the platinum group metals (Ru, Os, Rh, Ir, Pd, Pt). The results of these studies further suggest that "ether-like" solvent extraction cannot be the sole mechanism for such systems.

From our own results as well as those of others, the concept of ether-like solvent extraction as the only mechanism of sorption for metal complexes therefore seemed unlikely. Since many of the metal species absorbed were anionic in nature and extracted with very large distribution ratios, the possibility of an anion-exchange process seemed most reasonable to us. Such a mechanism has been alluded to by several authors with reference to protonation of some sites (ether oxygen atoms or linkage nitrogen atoms). Our work,[17] however, has indicated that the presence of hydrogen ions is not necessary and we therefore sought to study the mechanism in greater detail.

EXPERIMENTAL

Apparatus

Most analytical results were obtained on a Varian model 634S spectrophotometer, a Perkin-Elmer model 306 atomic-absorption spectrophotometer and an Evans Electroselenium Ltd. flame photometer. A Baird-Atomic model 530A single-channel gamma-ray spectrometer fitted with a Harshaw well-type NaI(Tl) crystal was used for radioactivity measurements.

Infrared spectra of polyurethane and sorbed materials were obtained on a Perkin-Elmer model 337 grating instrument. Solution acidities were measured with a Fisher Accumet model 520 pH meter, with glass and saturated calomel electrodes.

Polyurethanes and aqueous solutions were equilibrated in Pyrex glass distribution cells mounted within a thermostatically controlled ($25.00 \pm 0.05°$) automatic squeezing apparatus of our own manufacture. The equipment, described elsewhere,[17] was designed to produce repetitive solution-mixing and polymer-squeezing beneath the liquid surface at a rate of 25 times per minute over extended periods of time.

Reagents

Almost all materials were of reagent grade and were used as supplied by various manufacturers, without further purification. Picric acid was standardized by potentiometric titration. The *m*-cresol was of practical grade and so was purified by distillation under reduced pressure before use. ^{60}Co tracer was obtained from New England Nuclear and kept as Co(II) chloride in $10^{-3}M$ hydrochloric acid. Gamma-ray spectrometry with a semiconductor detector and multichannel analyser were used to verify its isotopic purity.

Several types of polyurethane foam were used. One (diSPo) was the product of Scientific Products Ltd., (McGraw Park, Illinois) and was of polyester type. All other polymers used were primarily of polyether composition. Two (1338 BFG and 1338 M) were produced locally from materials supplied by Goodrich and Monsanto respectively, and were used most extensively in the investigations. Five other types used on occasion (A, B and 27CGS-44-2A, -1 and -3) were the very generous gifts of Dr. C. G. Seefried of the Union Carbide Corp. Before use, all foam materials were soaked in acid (0.1*M* nitric or 1.0*M* hydrochloric) for several hours to remove possible inorganic contaminants, and then extensively rinsed with distilled water. They were next freed from non-polymeric organic materials by Soxhlet extraction with acetone for 6 hr. The residual solvent was removed by air drying in an oven at 60° or under vacuum at room temperature. No major changes in polymer properties as a result of these treatments were observed.

General procedure

Preliminary experiments established that from 6 to 12 hr of contact between polymer and solution in the squeezing apparatus was generally sufficient to establish equilibrium. Periods of 12 or 24 hr were therefore allowed for equilibration to take place between weighed pieces of polyurethane and measured volumes of solution. At the end of this time, solution samples were withdrawn for determination of the species of interest (either cation or anion). Comparison of the equilibrium concentration (C_{eq}) of the particular species with the initial concentration (C_0) before contact with the polyurethane allowed indirect calculation of the percentage extracted (E) and the distribution ratio (D) from

$$E = \frac{100(C_0 - C_{eq})}{C_0}\,\%; \quad D = \frac{VE}{(100 - E)W}$$

where V is the volume of solution (in litres) brought to equilibrium with W kg of polymer. D thus has units of l./kg.

Sorption experiments were roughly divided into two types, based on the sort of anion employed and whether the anion or the cation was to be measured.

Anionic metal complex systems

Most experiments of this type involved cobalt thiocyanate and employed ^{60}Co tracer to measure sorption.

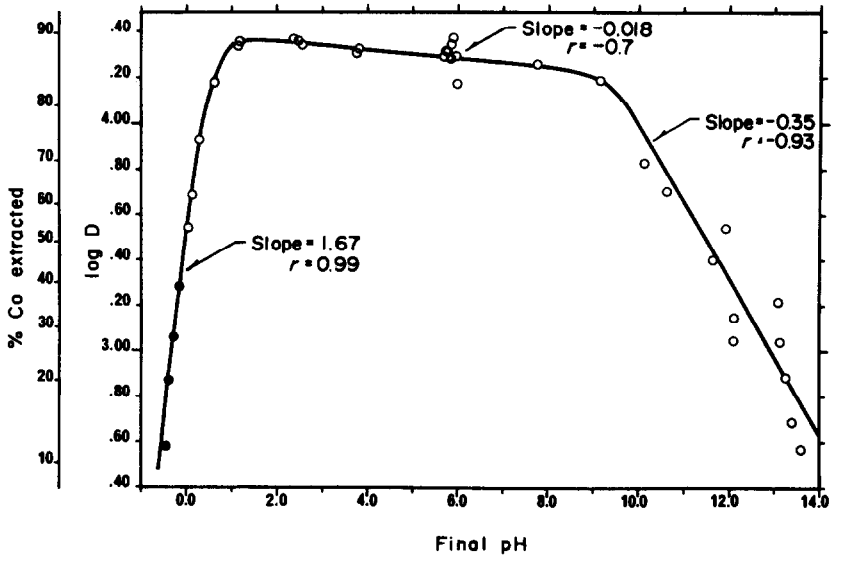

Fig. 1. Effect of pH on cobalt sorption from aqueous thiocyanate solution. Equilibrium values of pH were measured by glass electrode (open circles) or estimated by calculation (filled circles). Conditions: 0.050 g of 1338 BFG foam; 150 ml of solution $1.7 \times 10^{-6}M$ in Co(II), 0.10M in NaSCN, pH adjusted with HCl or NaOH, NaCl added to maintain 3.00M ionic strength.

In the study of dependence on acidity (Fig. 1), no buffers were used, the pH being adjusted by the addition of sodium hydroxide or hydrochloric acid of appropriate concentration, and measured before and after equilibration with 1338 BFG foam. The ionic strength and total cation concentration were adjusted to 3.0M in each case by the addition of sodium chloride in appropriate amounts.

Experiments to determine the effects of polymer type on cobalt sorption (see Table 2) were conducted similarly except that 0.1M sodium acetate/acetic acid buffer (1:1) was also added to maintain a pH near 4.7.

The influence of various cations on cobalt thiocyanate sorption was determined in two separate experiments. In the first of these (see Table 4), sorption of cobalt by 1338 M foam was measured in the presence of 2.5M concentrations of cations differing in size. Other experiments (see Table 5) were conducted with 1338 BFG foam, 0.1M acetate buffer and 0.1M concentration of different cations.

The effects of cation type on zinc thiocyanate sorption were also determined on 1338 M polyurethane foam, with 1.0M concentrations of added cations. Atomic-absorption spectrophotometry was used to determine zinc before and after equilibration.

Organic anion systems

The extraction of picrate in the presence of different cations (Fig. 2a) was investigated with $5 \times 10^{-5}M$ picrate and a 0.10M concentration of the particular cation. Samples were withdrawn after equilibration with 1338 M polyurethane foam pieces, and analysed colorimetrically for picrate. Experiments with 8-anilino-1-naphthalenesulphonate (ANS) anions instead of picrate were conducted in the same way except that 0.001M concentration of this anion and either 0.1M (Fig. 3) or 0.0225M concentrations of cation (Fig. 4) were used.

When cation extraction was measured directly (see Table 3), $10^{-4}M$ concentrations of cations with 0.025M picrate or 0.004M ANS were used, again with 1338 M foam. The initial and equilibrium cation concentrations were determined either by flame photometry (alkali metal ions) or by atomic-absorption spectrophotometry (Ag^+, Tl^+, Mg^{2+}, Ca^{2+}, Hg^{2+}, Pb^{2+}). A ^{133}Ba tracer and radiometric counting were used in the case of Ba^{2+}.

Thin film preparation and testing

Because of severe light scattering, the direct measurement of polyurethane foam spectra proved difficult. Consequently, it was necessary to prepared thin films from the foam material for observation of changes in the visible or infrared spectra, accompanying sorption of species. Thus, 0.2-ml aliquots of a solution made by dissolving 0.1 g of 1338 BFG polyurethane foam in 5 ml of *m*-cresol by refluxing at 203° were transferred to 1.5 × 3.0 cm sodium chloride plates. The *m*-cresol solvent was then removed by gentle heating under vacuum overnight. Free $-NH_2$ or $-OH$ groups in the film thus obtained were reacted for 18 hr with 0.1 g of phenyl isocyanate in 100 ml of hexane to eliminate the possibility of any complexation by such groups. The plates bearing the films were then soaked for two days in fresh hexane to remove excess of reactant, followed by evaporation of the solvent under vacuum. A film prepared in this way was soaked for 18 hr in 50 ml of saturated sodium chloride solution (to prevent dissolution of the plate) containing cobalt(II) ($8.5 \times 10^{-4}M$) and sodium thiocyanate (0.1M). Spectra of the film and sorbed species were then measured directly (Fig. 5).

RESULTS AND DISCUSSION

Possible extraction mechanisms

There are several hypotheses for the mechanism of sorption of metal ions by polyurethanes. Although we hope to identify one of these as predominant in many of the systems studied by us, others may play a role in other systems.

Surface adsorption. The concept of metal-ion adsorption at sites distributed over the polymer surface (which was assumed to be large) was one of the first to be tested and was rejected by Bowen on the basis of capacity measurements alone. Later surface-area determinations for polyurethane foam (which showed[19] a relatively small surface area of about 81 m^2/kg) as well as capacity measurements for

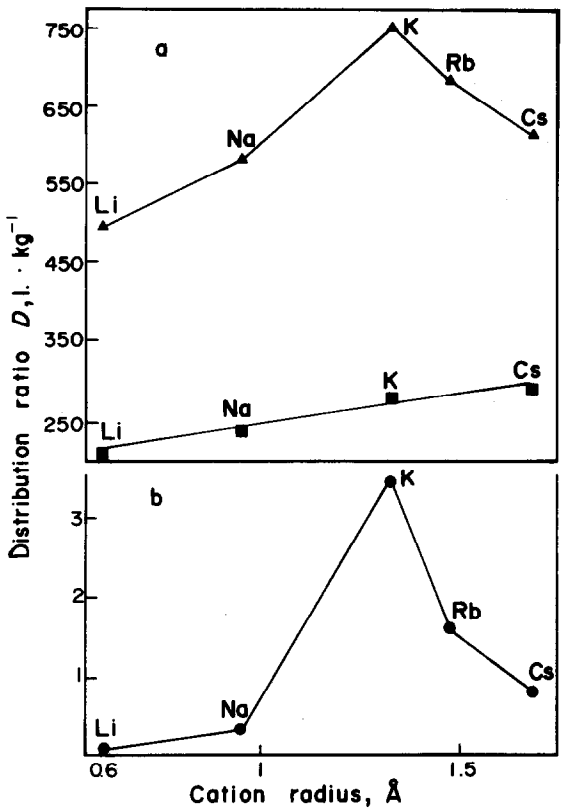

Fig. 2. (*a*) Comparison of picrate extraction from solutions containing various alkali-metal cations by polyether- and polyester-based polyurethane foams. Conditions: 0.10 g of foam; 150 ml of solution $5 \times 10^{-5}M$ in picrate and 0.10*M* in cation (added as chloride) ▲ 1338 M polyether foam, ■ diSPo polyester foam. (*b*) Extraction of $7 \times 10^{-5}M$ picrate from aqueous solution by $7 \times 10^{-5}M$ dicyclohexyl-18-crown-6 in methylene chloride; 0.10*M* cation (added as chloride); equal volumes of phases.[38]

various metal ions [including our own of 0.47 mole/kg for $Co(NCS)_4^{2-}$] have all strengthened this opinion. Furthermore, the observation that several metal species, such as $GaCl_4^-$,[8,20] $Co(NCS)_4^{2-}$,[17] $FeCl_4^-$,[20] UO_2^{2+},[20,21] are able to diffuse through intact polyurethane membranes, confirms that true absorption into the bulk of the polymer must occur.

Solvent extraction. The concept of an ether-like solvent extraction of metals was also originally suggested by Bowen[4] when he called attention to the similarity between the list of substances sorbed by polyether-based polyurethane foam and those which could be extracted by diethyl ether. Gesser *et al.*[7,9] later extended this idea to suggest that the polyether portion of the foam acted as a polymeric analogue of diethyl ether in giving "solvent extraction" of the substances tested. In this view, the long-chain portions of the polyurethane (polyether or polyester) act much as if they were the analogous liquid monomers in solvating the sorbed species, while the urethane, urea and other links joining the chains together at their ends are largely inactive in the process. This simple description is appealing and seems to have been widely accepted by most workers in the field even though it has not been rigorously tested. Such an interpretation would appear to be sufficient to account for the observed sorption of non-polar or even moderately polar compounds, but it might be expected to be inadequate when truly ionic species are involved.

Most authors have assumed that neutral complexes of the H_mMeX_n (Me = metal, X = halide or pseudohalide) or similar type are those extracted by polyurethanes. However, while this may well be the case for some elements in strongly acidic solutions, there must be doubt when sorption is observed at higher pH values. In our own studies of cobalt thiocyanate extraction, for example, we find that sorption is optimum and essentially independent of hydrogen-ion concentration over a very wide range (pH 1–9) when the solution ionic strength is maintained constant by addition of sodium chloride (see Fig. 1). If we assume that a neutral protonated species, *e.g.*, $H_2Co(NCS)_4$, is being formed and extracted by the polyurethane under these conditions, then we are forced to deduce that the conjugate anion [$Co(NCS)_4^{2-}$ in this case] is

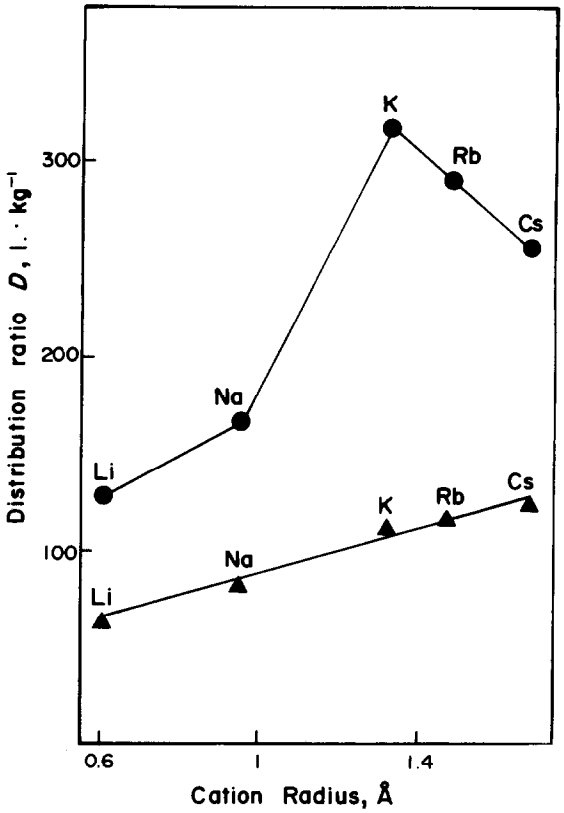

Fig. 3. Comparison of 8-anilino-1-naphthalenesulphonate (ANS^-) anion extraction from solutions containing various alkali-metal cations by polyether- and polyester-based polyurethane foams. Conditions: 0.10 g of foam; 150 ml of solution, $10^{-3}M$ in ANS^- and 0.10*M* in cation (added as chloride). ● 1338 M polyether foam, ▲ diSPo polyester foam.

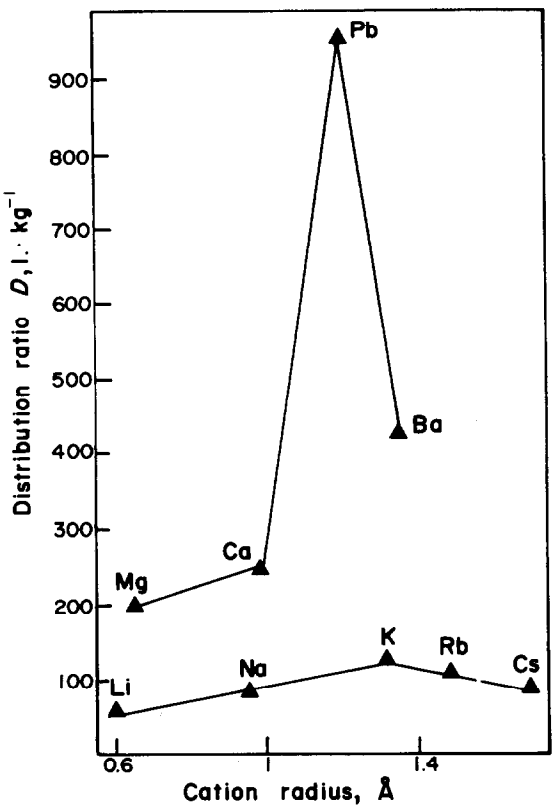

Fig. 4. Comparison of 8-anilino-1-naphthalenesulphonate (ANS^-) anion extraction from solutions containing various univalent and bivalent cations by polyether-based polyurethane foam. Conditions: 0.15 g of 1338 M foam; 90 ml of solution, 0.001*M* in ANS^- and 0.0225*M* in cation (added as chloride or nitrate).

more strongly basic than OH^-. However, basicity of this order is not at all typical of any anionic metal complexes and, moreover, we observe that extraction drops abruptly in more acidic solutions rather than increasing as might be expected. Thus, we conclude that hydrogen ions cannot be involved in the extraction of the cobalt–thiocyanate species in particular, nor of other metal complex ions, from neutral or basic solutions.

It is known that some ion-association complexes are extractable from neutral solutions, particularly when the ions involved are large and hydrophobic or only feebly hydrated. Thus, under suitable conditions, ion-association complexes of the general form $(M^{p+})_m.(MeX_n^{m-})_p$ may be formed and transferred to an organic phase (indicated by a bar) as follows:

$$mM^{p+} + pMeX_n^{m-} \rightleftharpoons \overline{(M^{p+})_m.(MeX_n^{m-})_p}$$

$$(M^{p+})_m.(MeX_n^{m-})_p \rightleftharpoons \overline{(M^{p+})_m.(MeX_n^{m-})_p}.$$

In assessing the likelihood of this type of solvent extraction phenomenon for polyurethanes, however, we must consider the following facts.

First, anion distribution ratios as high as 3×10^6 l./kg have been measured for $Co(NCS)_4^{2-}$ sorption[17] while several other anionic species, *e.g.*, $FeCl_4^-$,[9] $GaCl_4^-$,[8] $Pd(SCN)_4^{2-}$,[18] $Pt(SCN)_4^{2-}$,[18] $SnCl_4^{2-}$,[11] $SbCl_3^-$,[12] $SbCl_5^-$,[12] have all yielded values in the neighbourhood of 10^4–10^5 l./kg for aqueous solution/polyurethane systems, even when small, hydrophilic cations (H_3O^+, Na^+, NH_4^+, *etc.*) have been the only counter-ions available. Such values are much greater than the distribution ratios measured for these and similar metal complexes extracted into liquid organic solvents. The difference is especially large (several orders of magnitude) for some of the thiocyanate complexes which have been studied in our laboratories. For example, whereas *D* values in the neighbourhood of only 3 or less have been achieved for $Co(NCS)_4^{2-}$ and $Pd(SCN)_4^{2-}$ with diethyl ether,[22] values ranging from 10^5 to 10^6 l./kg have been attained with polyether-based polyurethane foam under similar conditions (see Table 1).

Moreover, Moore and Chow[16] have described the extraction of $IrCl_6^{2-}$ and $PtCl_6^{2-}$ into polyurethane foam from ethyl acetate and acetone solutions with distribution ratios ranging from 225 to 1.1×10^4 l./kg. This clearly demonstrates that if a solvent-extraction mechanism is operating polyurethane must possess some solvating characteristics which are superior not only to those of water but to those of each of these solvents as well. It is very difficult to justify treating the polymer as an analogue of diethyl

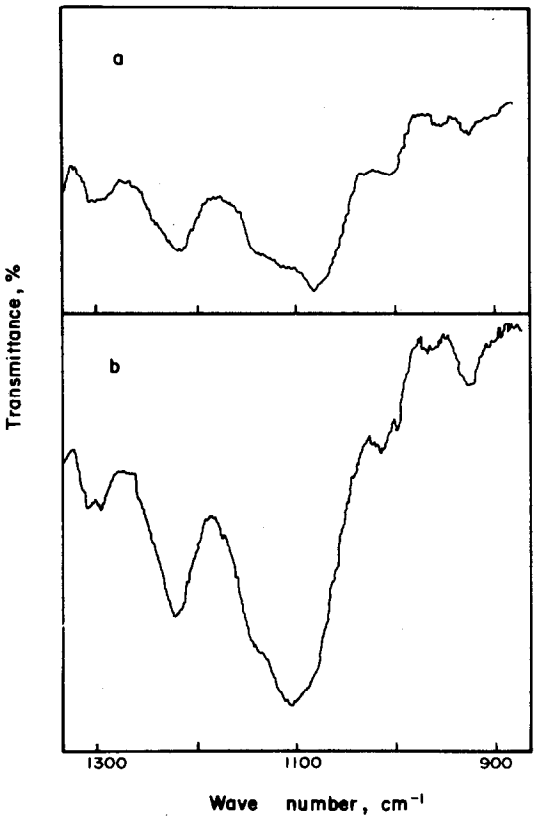

Fig. 5. Changes in the infrared spectrum of polyether-based polyurethane film on sorption of $Na_2Co(NCS)_4$ from aqueous solution (details in experimental section); (*a*) after sorption; (*b*) before sorption.

Table 1. Comparison of complex metal anion sorption by oxygen-containing organic solvents and polyether-based polyurethane foam

Metal ion	Aqueous phase	Organic phase	D^*	Reference
Sb(III)	6*M* HCl	diethyl ether	0.06	22
	6.5–8.5*M* HCl	di-isopropyl ether	0.16	22
	7*M* HCl	polyether foam	416	12
Sb(V)	6*M* HCl	diethyl ether	4.3	22
	6.5–8.5*M* HCl	di-isopropyl ether	199	22
	7*M* HCl	polyether foam	500	12
Sn(IV)	6*M* HCl	diethyl ether	0.2	22
	3*M* HCl	polyether foam	129	11
	4*M* HCl	polyether foam	141	11
Fe(III)	6*M* HCl	diethyl ether	99	22
	7.75–8*M* HCl	di-isopropyl ether	1000	22
	9*M* HCl	β,β'-dichloroethyl ether	99	22
	1*M* HCl + 6*M* LiCl	polyether foam	10^4	9
	1*M* HCl	polyether foam	10^3	9
Ga(III)	6*M* HCl	diethyl ether	32	22
	7*M* HCl	di-isopropyl ether	>1000	22
	0.85*M* HCl	polyether foam	6300	8
Co(II)	0.5*M* HCl + 1*M* NH_4SCN	diethyl ether	0.037	22
	0.5*M* HCl + 2*M* NH_4SCN	diethyl ether	0.605	22
	0.5*M* HCl + 3*M* NH_4SCN	diethyl ether	1.39	22
	0.5*M* HCl + 5*M* NH_4SCN	diethyl ether	2.98	22
	0.5*M* HCl + 7*M* NH_4SCN	diethyl ether	3.03	22
	1*M* KSCN + 1*M* buffer + 1*M* KCl	polyether foam	$>10^6$	17
	5*M* KSCN + 1*M* buffer	polyether foam	$>10^6$	17
	1*M* NaSCN + 1*M* buffer + 1*M* NaCl	polyether foam	$>10^6$	17
	2*M* NaSCN + 1*M* buffer	polyether foam	$>10^6$	17
Pd(II)	0.5*M* HCl + 1*M* NH_4SCN	diethyl ether	0.02	22
	0.5*M* HCl + 7*M* NH_4SCN	diethyl ether	0.001	22
	0.5*M* HCl + 0.15*M* KSCN	polyether foam	18200	18

* D in l./kg for polyurethane foam.

ether when neither of these complex ions exhibits any appreciable solubility in that solvent. Also, since the polarity of urethane polymers has been estimated[23] from swelling measurements to be very similar to that of acetone, a distribution ratio of around 1 l./kg for $IrCl_6^{2-}$ between acetone and polyurethane would be expected, instead of the 225 l./kg found experimentally. From these anomalously high distribution ratios, it appears that factors other than simple ether-like solvent extraction apply and that some specific interactions with the polymer must exist.

Ligand addition or exchange. Since all polyurethanes contain a large number of donor atoms (N, O) (from the urethane, urea, ether or ester links), the possibility of strong interactions arising from complex formation between any of these and the extracted species must be considered. In that case, the large distribution ratios measured, particularly for some metals, would imply the existence of very strong ligands in the polymer—a situation which seems doubtful, considering the structures typical of polyurethanes. However, if complexation did take place, it would have to do so either by addition of polyurethane ligands (L) to the extracted species [*e.g.*, $Co(NCS)_4^{2-}$]:

$$Co(NCS)_4^{2-} + 2L \rightleftharpoons Co(NCS)_4L_2^{2-}$$

or by ligand exchange for those of the polyurethane:

$$Co(NCS)_4^{2-} + nL \rightleftharpoons Co(NCS)_{4-n}L_n + n\,SCN^-$$

In the first the co-ordination number of the metal must increase. A co-ordination number of five for cobalt is rare and the resulting complex would therefore most likely be hexaco-ordinate (as shown). Such a change from tetrahedral to octahedral symmetry in cobalt complexes is accompanied by a large alteration of the absorption spectrum, resulting in a colour change from blue to pink. However, the blue-green colour and the absorption spectrum definitely show the cobalt–thiocyanate species sorbed by polyurethane (foam or film) to be tetrahedral $Co(NCS)_4^{2-}$. Similarly, the colours obtained when several other metal complexes are extracted by polyurethane foam are typical of the tetrahedral species [*e.g.*, $FeCl_4^-$, $Pd(SCN)_4^{2-}$] and it is thus apparent that appreciable complexation by ligand addition cannot be common.

In the case of ligand exchange, the symmetry of the complex would change, which should also be accompanied by some alteration of the spectrum. Since no such change is observed for the cobalt–thiocyanate system and the spectrum of the sorbed complex rapidly recovered from polyurethane into several organic solvents (acetone, methyl isobutyl ketone and tetraglyme*) is again that of $Co(NCS)_4^{2-}$, metal com-

* Tetraglyme is bis[2-(2-methoxyethoxy)ethyl] ether.

plexation by ligand exchange must also be considered unlikely.

Weak or strong base anion-exchange. The possibility of polyurethane behaving as a weak-base anion-exchanger has been mentioned by several authors. This could conceivably occur by protonation of some sites in the polymer such as nitrogen-containing urea or urethane linkages, or perhaps ether linkages in polyether-based polyurethanes. According to this view, there must be some sites in the polymer phase which become protonated in the presence of acid and at which exchange of anionic metal complexes (MeX_n^{m-}) can occur as follows:

$$H^+ + A^- + \overline{\text{site}} \rightleftharpoons \overline{(H\cdot\text{site})^+} + \overline{A^-}$$

$$m\overline{A^-} + MeX_n^{m-} \rightleftharpoons mA^- + \overline{MeX_n^{m-}}$$

where species within the polymer phase are again indicated by bars and A^- (assumed, for simplicity, to be univalent) is some counter-ion for the absorbed hydrogen ions.

In the presence of high concentrations of strong acids, this mechanism may contribute significantly to the overall sorption of anionic metal complexes. However, in the absence of appreciable amounts of strong acid, it should not play a significant role. As mentioned earlier, studies on the cobalt(II)–thiocyanate system at various solution pH values (Fig. 1) demonstrate that sorption is essentially independent of hydrogen-ion concentration over a very wide range (pH 1–9) and that extraction falls rather than increases in more acidic solutions. It seems doubtful that any sites could become protonated by contact with basic (pH 9) media. Although the possibility exists that such protonation might have initially resulted from the foam cleaning procedure (which uses up to 1*M* acid), measurements of the solution pH before and after equilibrium with the polyurethane has been reached demonstrate that insufficient hydrogen ions are either released from or absorbed by the polymer under any conditions to account for the quantity of cobalt extracted. In fact, such measurements have shown that only insignificant numbers of hydrogen ions are released from the foam to solutions of pH up to 13.5. Thus, any sites which could be protonated would have to be of extremely high basicity, but the groups typical of polyurethanes (urea, urethane, allophanate, biuret, ether and ester) are all very weak bases and could not account for the observed behaviour.

The insensitivity of Co(II) sorption with respect to hydrogen ions (Fig. 1) would be more consistent with a strong-base anion-exchange mechanism in which permanent cationic sites (*e.g.*, quaternary ammonium groups) would be involved, but no such sites are typical of any polyurethanes, especially in numbers large enough to account for the high capacities for metal-ion sorption. Hence we conclude that if anion-exchange *does* occur, it necessarily takes place at sites generated by some other mechanism.

Cation-chelation mechanism (CCM). Another possible mechanism by which anionic metal complexes may be sorbed, which does not necessarily require protonation of polyurethane sites but is, nevertheless, closely related to the weak-base anion-exchange concept, is what we will call the cation chelation mechanism (CCM). According to the view which we now propose, many cations, M^{p+}, (such as Na^+, K^+, Ag^+, NH_4^+, RNH_3^+, Pb^{2+}, Ba^{2+}, and including H_3O^+) are capable of being multiply complexed by the polymer at specific sites thus giving a solid-phase (or matrix) species which can be regarded as equivalent to an ion-exchange matrix or to a solvated cation in solid solution:

$$M^{p+} + \overline{\text{site}} \rightleftharpoons \overline{(M\cdot\text{site})^{p+}}.$$

The extraction of ion-association complexes having these cations as counter-ions will then be greatly facilitated owing to the stability of the chelate. However, which anions will accompany the cations in largest numbers will be determined by a variety of factors, including the individual hydrophobic nature and charge of the anions, and perhaps their ability to interact in other ways with the polymer. Anionic metal complexes, MeX_n^{m-}, particularly those which are co-ordinatively saturated with non-hydrophilic ligands, might be expected to be well extracted.

When little or no sorption of anions other than MeX_n^{m-} occurs (either because all other available anions are nearly unextractable in nature or because an insufficient excess of cations or other anions is present), the sorption of the ion-association complex may be regarded as taking place by a solvent extraction process in which the cation happens to be more effectively solvated than usual. The extraction mechanism could be represented by:

$$mM^{p+} + pMeX_n^{m-} + m\overline{\text{site}} \rightleftharpoons m\overline{(M\cdot\text{site})^{p+}} + p\overline{MeX_n^{m-}}$$

where the chelated cation and accompanying anion are written separately but may, in fact, be associated within the polyurethane.

On the other hand, if considerable sorption of another ion-association complex containing M^{p+} and some moderately extracted anion, A^-, occurs either before or concurrently with the sorption of MeX_n^{m-}, then the latter may be more conveniently regarded as sorbed by an anion-exchange process in which it is exchanged for A^- at the positive sites that result from the chelation of M^{p+} by the polymer. The main difference between the CCM and other ion-exchange mechanisms is that electroneutrality demands that both the cation and the anion should be more or less simultaneously extracted by the polyurethane. A matrix similar to an anion-exchanger results, at which exchange of the anionic counter-ions can occur. The extraction mechanism of MeX_n^{m-} may then be

$$M^{p+} + pA^- + \overline{\text{site}} \rightleftharpoons \overline{(M\cdot\text{site})^{p+}} + pA^-$$

$$MeX_n^{m-} + mA^- \rightleftharpoons MeX_n^{m-} + mA^-$$

Table 2. Effect of polyurethane foam type on the sorption of cobalt thiocyanate

Foam	Chief polymer type	D, l./kg
diSPo	unknown polyester	6
A	60% PEO/PPO polyether copolymer 40% styrene/acrylonitrile copolymer	7.26×10^3
B	80% PEO/PPO polyether copolymer 20% styrene/acrylonitrile copolymer	8.12×10^3
1338 BFG	unknown polyether	1.68×10^4
27CGS-44-2A	0% PEO polyether 100% PPO polyether	1.05×10^3
27CGS-44-1	8% PEO polyether 92% PPO polyether	7.26×10^3
27CGS-44-3	14% PEO polyether 86% PPO polyether	2.16×10^4

Conditions: 0.050 g of foam; 150 ml of solution, $1.7 \times 10^{-6}M$ in Co(II), 0.10M in NaSCN, 2.8M in NaCl and 0.1M in 1:1 sodium acetate/acetic acid buffer.

The cation-chelation mechanism would thus resemble both solvent extraction and ion-exchange, depending, to some extent, on solution conditions. We will now demonstrate that specific interactions do exist between some part of the polyurethanes and a number of cations.

Effects of polymer type on metal sorption

To identify which portions of the polymer might be active in chelating cations, cobalt thiocyanate sorption by several types of polyurethane foam was compared. Some of the more important results are shown in Table 2.

The most striking difference in performance is that between the polyether-based foams and the polyester-based material, there being an improvement by several orders of magnitude with the former. We infer that a polyether backbone must be an important requirement for efficient metal ion sorption. This hypothesis is further substantiated by comparison of foam types A and B, there being increased sorption ability with increasing polyether content. This is in agreement with the work of others[15,24] who have recorded significant metal extraction only by polyether polyurethane foam, particularly from solutions of low acidity. This behaviour is quite different from that of monomeric solvent analogues which generally show esters to be slightly superior to ethers as metal ion extractants, and indicates the inadequacy of a simple solvent extraction mechanism. For the cobalt(II)–thiocyanate system, for instance, D values not greater than 3 have been reported[22] for extraction by diethyl ether, and $D \sim 20$–30 with butyl acetate.[25] Moreover, although the distribution ratios for this metal species are of roughly the same magnitude for either ester or polyester extractants, the very large difference in D when ethers and polyethers are used suggests that the latter must possess some special properties.

A clue to the nature of these properties is available from the results for the 27CGS-44-2A, -1 and -3 series of polymers (Table 2) which differ only in the ratio of poly(ethylene oxide), PEO, to poly(propylene oxide), PPO. The fact that stepwise replacement of PPO by PEO produces such a marked increase in extraction of cobalt thiocyanate indicates that the latter polymer is somehow much better equipped to carry out the necessary special solvation or complexation. If, as we propose, this complexation is of cations, then the difference cannot be attributed to the electron densities on the respective oxygen atoms since the mildly electron-donating character of the methyl groups in PPO would lead us to expect higher basicity (and stronger complexation) for these ligands. The fact that the opposite behaviour results suggests that steric limitations imposed by the methyl groups must outweigh any induced electronic effects and so we surmise that specific geometry plays a very important role in the process. In seeking to clarify the mechanism, we were thus prompted to investigate further some of the complexing properties of polyethers.

Complexing properties of polyethers

In the last two decades, a number of reports of polyether–metal-ion interactions have been made, particularly for the alkali metals but also involving other cations as well (*e.g.*, complexes between certain transition metals and glymes $[CH_3O(CH_2CH_2O)_nCH_3]$). Pedersen[26,27] was the first to describe the synthesis and specific complexing abilities of a number of macrocyclic ("crown") ethers. Compounds of this type having about 5–10 oxygen atoms in the ring were shown to complex a variety of metal ions within the central cavity (crown) with the strength dependent on the ratio of ion radius to cavity size, the length of hydrocarbon chain between successive oxygen atoms, and other factors. Among the alkali-metal cations, a definite order of preference was established, as $Li^+ < Na^+ < Cs^+ < Rb^+ < K^+$, for the most extensively studied[28] crown ethers (*i.e.*, various analogues

of 18-crown-6). Such selectivity favouring members of intermediate size within a group of congeners is an important characteristic of this type of complexant in which multiple complexation (chelation) is involved.

Since then, several non-cyclic analogues of crown ethers containing similar numbers of oxygen atoms and bearing hydrophobic or complexing end-groups have also been found to complex metal ions in this fashion. The function of the end-groups in these compounds appears to be to ensure the formation of a spiral (as confirmed experimentally[29] for the complex of NaSCN with $CH_3OC_6H_5O(CH_2CH_2O)_3C_6H_5OCH_3$) which engulfs the cation completely, much like the crown ethers. Among compounds of this type, the close association of inwardly-directed polyether oxygen atoms with the cation (and not the anion) has been clearly demonstrated by X-ray crystallographic measurements[30] of Na^+ and NH_4^+ salts of $Co(NCS)_4^{2-}$ complexed by a few PEO derivatives bearing phenyl end-groups.

Later studies have revealed that even polyethers without special end-groups have cation-complexing abilities (although frequently of slightly lower effectiveness). Long-chain PEO polymers, in particular, were noted[31–33] as giving strong interactions with ions such as Na^+ and K^+,[34] and Hg(II).[35] Extension of the spiral-type configuration of oxygen atoms about a cation complexed by shorter polyethers would logically lead to a helical pattern of inwardly-directed oxygen atoms in longer chains. Pure poly(ethylene oxide) is indeed known to adopt just such a configuration in the crystalline state[36] and to retain a large part of this structure in aqueous solution.[37] On the other hand, poly(propylene oxide) does not assume a helical shape so readily[36] either in the crystalline state or in solution (evidently due to steric effects induced by the extra methyl groups). Simply on geometrical grounds, therefore, it is reasonable to assume that long-chain PEO compounds may effectively complex cations situated along the central axis of a helix whereas PPO materials would be less well disposed to behave in this manner. It also seems logical to suppose that this can occur even if the polyethers are integral parts of a larger polymer. Thus the observed structure–sorption behaviour of polyurethanes (Table 2) seems entirely consistent with the existence of cation chelation at polyether sites which are free to adopt a helical or similar configuration.

On the basis of this model of metal-ion sorption by polyurethanes, then, we may make the following general statements and predictions.

1. As with crown and other polyethers, polyurethanes will complex cations, M^{p+}, with selectivity based on size and other properties related to solvation. This selectivity will, in turn, influence the extraction of accompanying anions, because of the requirement of charge balance.

2. The nature of the anions available, A^- or MeX_n^{m-}, may likewise limit the extent to which any cation is extracted, as is the case for conventional organic solvents, ion-exchange resins and crown-ether extractants.

3. The type of polyol in the polyurethane and its freedom to assume a helical conformation within the polymer will greatly influence the degree of extraction of cations (and therefore also of anions).

To test these predictions and thus the proposed mechanism, a number of experiments were carried out.

EXPERIMENTAL TESTS OF CCM

Cation selectivity measurements

Although direct measurements of cation sorption might be preferred, the extraction of particular anions in the presence of large amounts of cations has been used most often as a measure of cation complexation. By this indirect method, Pedersen[38] has determined the selectivities of many crown ethers for cations and found the apparent order of stabilities with alkali-metal ions to be $Li^+ < Na^+ < Cs^+ < Rb^+ < K^+$ for complexes with 18-crown-6 and its derivatives. This order is presented graphically in Fig. 2b.

Christensen *et al.*,[39] using calorimetric measurements, and other workers using different physical techniques,[28] have determined directly the stability constants of various crown ether complexes with a larger number of univalent as well as bivalent cations. The trends reported by Pedersen have thus been generally confirmed and expanded with, for example, the selectivity orders for the "A" isomer of dicyclohexyl-18-crown-6 identified as $Li^+ < Cs^+ < Na^+ < Rb^+ < K^+ < Ag^+ < Tl^+$, and $Ca^{2+} < Hg^{2+} < Sr^{2+} < Ba^{2+} < Pb^{2+}$.

In our own experiments with polyurethane foam, a maximum in the extraction of picrate or 8-anilino-1-naphthalenesulphonate (ANS^-) anions was observed for K^+ in the alkali-metal ion series and for Pb^{2+} in the bivalent cation series (see Figs 2a, 3 and 4). These maxima (and to some extent the relative orders of the

Table 3. Extraction of various univalent and bivalent cations by polyether-based polyurethane foam in the presence of picrate or 8-anilino-1-naphthalenesulphonate (ANS^-) anions

Cation	*D*, *l./kg*	
	Picrate	ANS^-
Li^+, Na^+, Rb^+, Cs^+	<5	<5
K^+	<5	12 ± 2
Ag^+	80 ± 5	1050 ± 30
Tl^+	85 ± 5	208 ± 12
Mg^{2+}, Ca^{2+}	<5	—
Ba^{2+}	100 ± 7	210 ± 10
Hg^{2+}	190 ± 10	—
Pb^{2+}	400 ± 18	1290 ± 30

Conditions: 0.4 g of 1338 M foam; 100 ml of solution $10^{-4}M$ in cation and either 0.025*M* in picrate or 0.004*M* in ANS.

remaining elements) match the results of Christensen and others. The strong similarity between the trends observed for crown ether and polyether polyurethane extractions is considered to be strong evidence in favour of the CCM.

The extractability of a number of univalent and bivalent cations by crown ethers has also been determined directly by Takeda *et al.*[40,41] and found to follow the observed trends in anion extraction. We have likewise determined directly the extractability of several cations by polyurethane foam in the presence of picrate anions (see Table 3). The distribution ratios for Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+} and Ca^{2+} were all quite low [less than 5 l./kg (the limit of the analysis)] whereas several other cations had larger distribution ratios, increasing in the order $Ag^+ \sim Tl^+ < Ba^{2+} < Hg^{2+} < Pb^{2+}$. Similar trends were observed with ANS^- as the counter-anion (see Table 3) except for Ag^+ (which most likely forms a neutral complex with the anion). The higher extractability of this species also allowed us to distinguish the superior performance of K^+ amongst the alkali-metal ions. Other measurements, on the still more extractable tetraphenylborate anion, have confirmed that K^+ is extracted in preference to Cs^+ ($D = 2500$ and 1775 l./kg respectively), in contrast to the extraction of simple ion-pairs. This order of selectivity has been extended to $Na^+ < Cs^+ < K^+$ by measurements on the sorption of these alkali metal picrates from 80% aqueous methanol.

From this information we may report with some confidence the order of affinity of polyether-based polyurethane foam for various cations as $Li^+ < Na^+ < Cs^+ < Rb^+ < K^+ \sim NH_4^+ < Ag^+ < Tl^+$ and $Ca^{2+} < Ba^{2+} < Hg^{2+} < Pb^{2+}$. It is interesting to compare this with the order of stability constants reported by Christensen *et al.*[39] for the "A" isomer of dicyclohexyl-18-crown-6, given above. With a few exceptions, the series are much the same, both showing distinct maxima for K^+ and Pb^{2+}. Such similarity in behaviour to derivatives of 18-crown-6 is perhaps not too surprising since, according to Mattice,[42] the most commonly adopted conformation of poly(ethylene oxide) chains of some length contains an 18-membered spiral. The peculiar complexing abilities of such long-chain non-cyclic polyethers have been studied by Yanagida *et al.*[43,44] by solvent extraction and NMR techniques and have been compared with those of the crown ethers.

Study of the cobalt–thiocyanate polyurethane extraction system has demonstrated that sorption of the $Co(NCS)_4^{2-}$ ion is greater from solutions containing NH_4^+ or K^+ ions than from those containing either Li^+ or Na^+. Similar results were also observed in the sorption of $Zn(NCS)_4^{2-}$ (see Table 4) in agreement with data reported by Suzuki *et al.*[45] for the extraction of this ion by several non-ionic surfactants derived from poly(ethylene oxide). As shown in Table 4, other results demonstrating the influence of cations on the extraction of anions by polyurethane foam have been offered by Al-Bazi[18] for Pd(II) thiocyanate and by Lo[11] for Sn(IV) chloride. All these observations lend support to a hypothesis of cation complexation by the polyether portions of polyurethane.

Additional support for the CCM is provided by comparisons of picrate and thiocyanatocobaltate sorption in the presence of a specific series of cations differing in their hydrophobic natures. Table 5 demonstrates that anion sorption does not follow a steady trend as cation size is increased (as might be expected on the basis of simple ion-pair extraction into an organic solvent).[46] In particular, the medium-sized Me_4N^+ cation produces lower anion extraction than do either the smaller NH_4^+ or the larger Et_4N^+ and Bu_4N^+. We interpret this as indicating that although simple ion-association complex extraction may be the predominant mechanism for larger cations, the higher sorption observed for Na^+ and NH_4^+ can only be rationalized in terms of some other phenomenon. Likewise, Table 5 shows a non-monotonic trend in anion sorption for the ammonium ion and its monoalkyl derivatives. Again, the cation of medium size, $CH_3NH_3^+$, shows the least extraction of the accompanying anion. Both observations may be

Table 4. Effect of common salts on the sorption of various complex metal anions by polyether-based polyurethane foam

	D, *l./kg*			
Cation	$Co(NCS)_4^{2-}$ [a]	$Zn(NCS)_4^{2-}$ [b]	$Pd(SCN)_4^{2-}$ [c]	$SnCl_6^{2-}$ [d]
Li^+	540	550	1.77×10^3	16
Na^+	580	1.02×10^3	3.80×10^3	33
K^+	1.12×10^3	2.51×10^3	1.51×10^4	52
NH_4^+	1.90×10^3	2.57×10^3	1.33×10^4	—

[a] $1.7 \times 10^{-5}M$ Co(II), 0.05*M* SCN^-, 0.01M H^+, 2.5*M* cation; 1338*M* foam.

[b] $2 \times 10^{-4}M$ Zn(II), 0.02*M* SCN^-, 0.01*M* H^+, 1*M* cation; 1338M foam.

[c] $1.2 \times 10^{-4}M$ Pd(II), 0.006*M* SCN^-, pH 4–6, 0.1*M* cation; 1338M foam.[18]

[d] trace Sn(IV), 3*M* Cl^-, 0.12*M* H^+, 3*M* cation; A-type foam.[11]

Table 5. Effect of selected cations on the sorption of anions by polyether-based polyurethane foam

Cation[a]	D, l./kg	
	$Co(NCS)_4^{2-}$ [b]	$C_6H_2N_3O_7^-$ (picrate)[c]
Na^+	1.73×10^4	437
NH_4^+	2.92×10^4	800
$MeNH_3^+$	1.52×10^4	—
$EtNH_3^+$	1.54×10^4	—
$BuNH_3^+$	3.21×10^4	—
Me_4N^+	9.55×10^3	245
Et_4N^+		630
Bu_4N^+	$\sim 8.6 \times 10^4$	3.30×10^3

[a] 0.1M concentrations of cations added as chlorides or bromides.

[b] 0.050 g of 1338 BFG foam; 150 ml of solution, $1.7 \times 10^{-6}M$ in Co(II), 0.5M in NaSCN, 0.1M in 1:1 sodium acetate/acetic acid buffer.

[c] 0.10 g of 1338M foam; 100 ml of solution, $5 \times 10^{-5}M$ in picrate.

explained by the CCM, which predicts that the smaller Na^+ and NH_4^+ cations will be much better chelated by the polyether than are the larger bulky cations. Thus, high extractability of the anions results either when the cation is highly extractable by virtue of chelation or when it is sufficiently bulky to form ion-association complexes efficiently.

Anion selectivity measurements

We now discuss the implications, for anion extraction, of cation chelation by the polymer. The requirement of charge neutrality in both phases dictates that an equivalent number of anions must accompany any sorbed and chelated cations. However, experience with conventional solvent extraction and ion-exchange processes demonstrates that considerable selectivity for anions can exist. Qualitatively, the order of extractability of a small number of anions is given by the Hofmeister series (*i.e.*, $OH^- < F^- < Cl^- < Br^- < I^- < NCS^-$).[28] This order is related by most workers chiefly to the hydrophobicity of the ions, which is dictated by size, charge density, hydrogen-bonding ability and perhaps other factors. Thus ionic metal complexes which are large, have low charge densities and lack the ability to form strong hydrogen bonds with water (*i.e.*, those which do not contain F, N, O or acidic H atoms on the periphery of the complex) are expected to be highly extractable into organic media. Similar considerations should apply to the extraction of anions by polyurethanes and this is confirmed by our own observations which show that the extractability of various cations (K^+, Tl^+, Ag^+) is strongly dependent on the identities of the anions present. Among those anions for which measurement was possible, sorption of a given cation was found to increase in the order $NO_3^- <$ 2,4-dinitrophenolate$^-$ = 2,6-dinitrophenolate$^- < ClO_4^- <$ picrate$^- <$ ANS$^- < (C_6H_5)_4B^-$, matching both increasing size and polarizability of the anion. Also, amongst complex metal anions, it has often been observed that the extractability of thiocyanato complexes greatly exceeds that of the corresponding but smaller chloro complexes. The importance of anion size is further underlined by the very high extractabilities noted for the extremely large heteropolymolybdates of phosphate, silicate and arsenate.[47] Thus, we see that the sorption of ion-pairs by polyurethanes may be dependent on the identities of both cation and anion and that their extractability may be limited by that of either moiety. All of these characteristics are consistent with the CCM as proposed here.

Polymer sorption abilities

As already mentioned, the identity of the polyol portion of the polyurethane (polyether or polyester) is expected to be of prime importance in controlling the extraction of anions by cation chelation. We have already called attention to the increased sorption of $Co(NCS)_4^{2-}$ by polyether polyurethanes containing increasing proportions of poly(ethylene oxide), PEO, relative to poly(propylene oxide), PPO (see Table 2). Such differences are attributable to the greater tendency of PEO to form helical structures, compared to PPO, which is restricted by steric interactions between $-CH_3$ groups.[36]

Even more dramatic differences exist between polymers of polyether type and polyester type (see Table 2), the latter behaving in much the same fashion as a simple solvent. In terms of the proposed CCM, this is again partly attributable to the inherent inability of polyesters to become helically oriented about a central axis, owing to the geometry and limited flexibility of the ester bond. Also, to achieve the same physical properties, the number of carbon atoms between successive oxygen-containing groups must necessarily be much larger for polyesters than for polyethers used in foam production (because of the lower flexibility of the former). Such an increase in the number of carbon atoms leads both to a decrease in the density of oxygen atoms available and to a large increase in the size of any possible cage formed. The combination of these two effects will preclude the achievement of any significant cation chelation by polyester polymers and so only simple solvent-like properties will remain.

To support this interpretation, we have made a systematic comparison of the ability of polyether- and polyester-based foams to extract ANS$^-$ and picrate in the presence of the alkali metals. From Figs. 2 and 3 we see that polyester-based foam shows a slight but steady increase in anion extraction as the size of the accompanying cation increases from Li^+ to Cs^+. This is as expected on the basis of simple solvent-like ion-pair extraction.[46] By contrast, however, polyether foam is in all cases superior to the polyester material in extraction ability and displays a distinct maximum for the intermediate-sized K^+ cation. Such behaviour confirms that a definite and selective interaction exists between some cations and polyether-based polyureth-

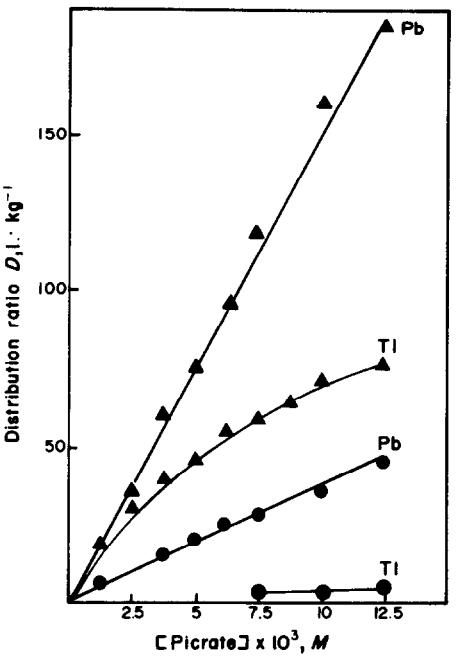

Fig. 6. Comparison of Tl^+ and Pb^{2+} extraction from aqueous picrate solutions by polyether- and polyester-based polyurethanes. Conditions: 0.4 g of foam; 100 ml of solutions $10^{-4}M$ in Tl^+ or Pb^{2+}. ● polyester foam (diSPo), ▲ polyether foam (1338 M).

ane which does not occur with the corresponding polyester polymer, as predicted by CCM.

Some further evidence is also obtained by comparing the extraction of Tl^+ and Pb^{2+} by these two types of polymer (Fig. 6). Here we see that either metal is better extracted by the polyether material over a range of picrate concentrations. This difference in behaviour is opposite to that expected on the basis of organic phase polarity since esters are more polar than ethers and we might expect this also to be true for their polymeric analogues. Such a view is supported by our own observations[47] which show that polyester foam indeed performs better than polyether foam in systems in which only simple ion-pair extraction can occur. The disparity between the expected results and those observed in Fig. 5 indicates that some specific interactions exist involving the sorbed species and polyethers, in complete agreement with the CCM.

Additional evidence in support of CCM

Further compelling evidence in support of strong polyether involvement in the extraction of ion-pairs is provided by measurement of the infrared spectrum (Fig. 5). Here we see a shift of about 30 cm^{-1} in the ether vibrational modes near 1100 cm^{-1} when Co(II) is absorbed from sodium thiocyanate solution. This change is indicative of metal–polyether interactions. However, the blue–green colour and the maximum at 615 nm and shoulder at 580 nm in the absorption spectrum both indicate that cobalt is present as the tetrahedral $Co(NCS)_4^{2-}$ ion in the polymer.[48] Moreover, the strong infrared absorption at 2055 cm^{-1} (not shown) indicates that the thiocyanate is bonded to the cobalt through the nitrogen atom.[49] Thus, it appears unlikely that cobalt atoms are involved in the interaction with the ether links. We therefore interpret this shift to be the result of sodium-ion chelation by the polymer in sorbing $(Na^+)_2Co(NCS)_4^{2-}$. Similar shifts in the infrared spectra of several other alkali and alkaline earth metal crown ether complexes have also been reported.[50]

Additional evidence for the existence of strong polyether–metal ion interactions comes from the observation that polyurethanes which are nearly saturated with cobalt(II) thiocyanate exhibit a marked increase in the polymer–glass transition temperature (*i.e.*, they fail to return to their original shape when compressed and lose much of their flexibility, to the point of becoming brittle). Such a phenomenon is not simply attributable to interchain void-filling by the sorbed species, since larger organic molecules (such as phthalates or long chain alkylamines) do not exhibit such behaviour even when present at higher concentrations. Viewed in terms of the CCM, inflexibility is easily imagined as resulting both from induced linear compression of the polyether chains in which cations are chelated along the axis, and also from electrostatic attractions between sorbed $Co(NCS)_4^{2-}$ anions and pairs of Na^+ (or, more generally, M^+) cations chelated at sites in different polymer chains (see Fig. 7). Such an arrangement of two chelated univalent cations simultaneously attracted to a single bivalent anion situated between neighbouring polymer chains constitutes a type of cross-link between them and is likely to be the largest contributing factor to polymer rigidity. Brittleness similar to this has also been observed by Moore[51] for $IrCl_6^{2-}$ and by Khan[47] for phosphomolybdate and silicomolybdate complexes, when the sorptions of univalent cations along with multivalent anions were also studied.

Although it is not our intention to reinterpret many past experiments, some other aspects of the work of Moore and Chow[16] (which records the efficient sorption by polyurethane of $IrCl_6^{2-}$ and $PtCl_6^{2-}$ from acetone and ethyl acetate solutions) are also explained by the proposed CCM. First, the existence of extraction under these conditions can be clearly seen as resulting largely from improved solvation through chelation of the cation by the polyether polymer compared to that by the organic solvent. In addition, it was previously difficult to rationalize the fact that in the capacity of polyurethane for sorption of $IrCl_6^{2-}$ from ethyl acetate is six times that from acetone. This was suggested to be the result of some unspecified interaction between the solvent and the polymer. However, physical observations of the polymer in both solvents now indicate that greater swelling (and therefore chain extension) occurs in the presence of acetone. On a molecular scale, this means reduced coiling of the polyether chains in this solvent and thus a greatly

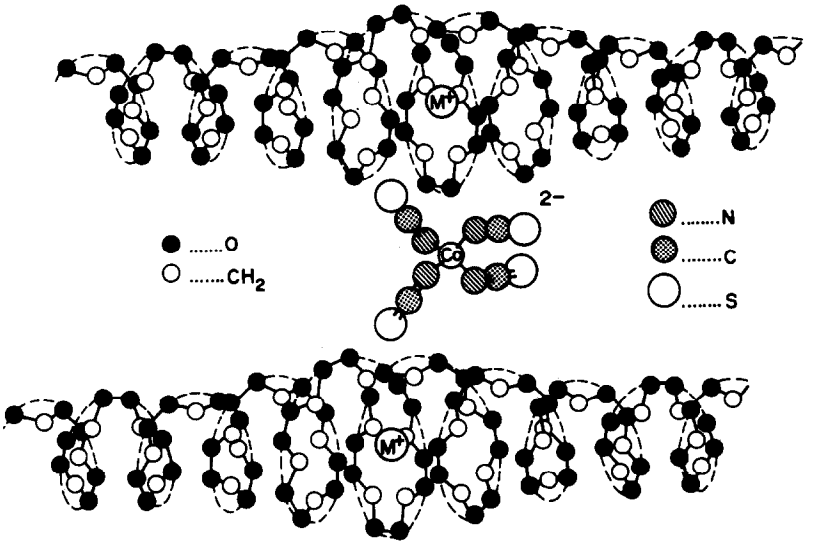

Fig. 7. Proposed helical structure (not to scale) of sorbed $M_2Co(NCS)_4$–polyether complex suggested by the cation chelation mechanism. The poly(ethylene oxide) chains have been extended beyond a length typical of polyurethane foam (~20–25 $-CH_2CH_2O-$ units) to clarify the geometry.

reduced ability to chelate cations by the proposed CCM.

Measurements of polyurethane sorption capacity for salts also appear to be consistent with the CCM hypothesis. Although a minimum of 5–10 oxygen atoms seems[27,43] to be adequate for solvating many individual cations in the case of cyclic or short-chain polyethers, the reduced freedom in an extended polymer analogue might be expected to increase this number considerably. In particular, significant disruptions would be expected to occur in any helical or other regular closed geometry of the polyether chains in the vicinity of the links (urethane, urea, *etc.*) binding them together in a polyurethane. Further losses of order in the polyether would also result from clustering of the polar linking groups into "hard" domains since bending of some polyether "soft" segments would then be necessary. Thus, considering Fig. 7, it might well be expected that in the case of many polyether chains only the central portion may be properly disposed to produce effective chelation of cations. In polyols typical of flexible polyurethane foam manufacture (molecular weight about 1000), there may be some 20–25 polyether oxygen atoms, so the undisturbed central portion might accommodate one or possibly two cations. If any further distortion of the polyether chain results from chelation of the first of these, the capacity may well be effectively limited to one cation per chain. Under these conditions, a cation capacity of roughly 1.0 mole per kg of polymer would be expected, with some variation dependent mostly on the exact polymer formulation and morphology. This is in close agreement with our own measurements[17] for $Na_2Co(NCS)_4$: 0.47 mole of Co per kg of polymer.

CONCLUSIONS

Strong interactions appear to exist between some polyurethanes and various cations. The results indicate that these occur only with polymers containing substantial polyether segments and that poly(ethylene oxide) is more effective than poly(propylene oxide) in this respect.

We note further that some selectivity of the polyether chain is demonstrated for particular cations. In the extraction of $Co(NCS)_4^{2-}$, $Zn(NCS)_4^{2-}$, picrate, ANS^- and other anions, the order of extractability follows the series $Li^+ < Na^+ < Cs^+ < Rb^+ < K^+ \sim NH_4^+ < Ag^+ \sim Tl^+ < Ba^{2+} < Pb^{2+}$. This order of cation preference is nearly identical to that of 18-crown-6 derivatives[39] and also of non-cyclic polyethers.[43] We thus believe that a helical arrangement of inwardly-directed polyether oxygen atoms forms the basis of complexation between cations and the polymer.

According to the proposed CCM, the efficient solvation of these cations then allows the facile extraction of an equivalent number of anions to take place. The most readily extracted anionic species will be those which are hydrophobic in nature, as given partly by the Hofmeister series (*i.e.*, $OH^- < F^- < Cl^- < Br^- < I^- < NCS^-$).[28] Notable among the most easily extracted species are many complex metal anions containing the more hydrophobic of these ligands, which explains the large number of metal-ion extractions observed. The high metal distribution ratios measured under conditions in which a large excess of some suitable cation is available, reflect the fact that effective solvation of the cation by the polymer is ensured by chelation and

thus the polyurethane can behave as a pseudo anion-exchanger.

Although expected to be widespread as a mechanism of ion-pair extraction by polyether-based polyurethanes, the CCM may not account for all observed extractions of metal ions or other apparently ionic species. In particular, when very large size or other steric constraint prevents complexation of the cation, simple solvent-like ion-pair extraction will be the dominant mode of sorption. Also, even though H_3O^+ may be a chelatable cation,[52] the uptake of undissociated neutral acid species, HA, rather than $H_3O^+ \cdot A^-$ ion-pairs may be very competitive for some anions in strongly acidic solutions.

Further investigation of polyurethane extraction mechanisms is continuing in our laboratory. A number of analytical and other applications are also being investigated and will be reported shortly.

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SHORT COMMUNICATIONS

DETERMINATION OF THIOSEMICARBAZONES BY REACTION WITH ω-BROMOACETOPHENONE

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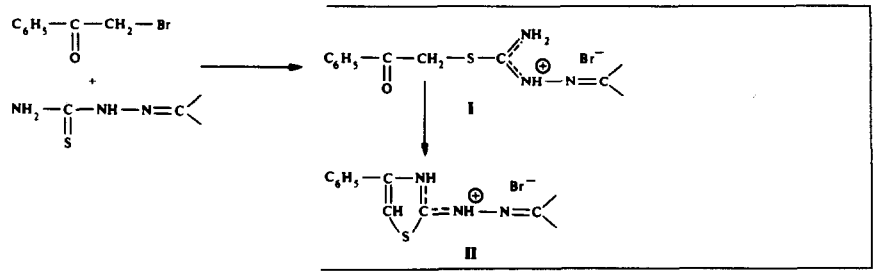
Summary—Thiosemicarbazones react quantitatively with ω-bromoacetophenone in a suitable medium; hydrobromic acid thus formed is titrated alkalimetrically with Thymol Blue as indicator.

A few titrimetric methods have been developed for determination of thiosemicarbazones: mercurimetric titration with copper 4-phenylthiosemicarbazide as indicator,[1] potentiometric titration of aromatic thiosemicarbazones,[2] and amperometric titration of thiosemicarbazones of aromatic aldehydes with mercury(II).[3] Colorimetric determinations of thiosemicarbazones of aromatic aldehydes have been based on the reaction with 2,3-dichloro-1,4-naphthoquinone,[4] and 3-chloro-1,2-naphthoquinone.[5]

Thiosemicarbazones react with ω-bromoacetophenone as follows: the cyclization of I to II is facilitated by heating.[6,7]



The reaction forms the basis of a titrimetric procedure for their determination. A thiosemicarbazone is reacted with excess of ω-bromoacetophenone in a suitable medium (the reaction products generally do not have adequate solubility in ethanol, but methanol is satisfactory for the derivatives of aliphatic carbonyls, dimethylformamide for those of aromatic aldehydes and acetonitrile for those of aromatic ketones), and the hydrobromide thus formed is titrated with sodium hydroxide, with Thymol Blue as indicator. Though tested with only a selection of thiosemicarbazones, the procedure seems to be generally applicable to this type of compound. The procedure is susceptible to interference by compounds containing an –NH–CS– function and other nucleophilic species (containing hydroxy, mercapto or amino groups) which react with ω-bromoacetophenone to produce hydrobromic acid. It is simple and accurate, and uses readily available reagents.

EXPERIMENTAL

Procedure

A weighed quantity of a thiosemicarbazone, prepared and purified according to published procedures, and ω-bromoacetophenone (about 25% excess) were dissolved in 20 ml of solvent (methanol for thiosemicarbazones of aliphatic aldehydes and ketones: dimethylformamide and acetonitrile for those of aromatic aldehydes and ketones respectively) and kept for 20 min at room temperature (20–25°). Then 5 ml of 5% sodium thiosulphate solution were added to react with the excess of ω-bromoacetophenone. The solution was allowed to stand for 5 min, and then titrated with 0.1*M* sodium hydroxide, with Thymol Blue (3 drops, 0.3% solution in methanol) as indicator, to a

Table 1. Determination of thiosemicarbazones

Parent compound*	Weight range, *mg*	Average recovery, %	Standard deviation, %
Acetone (3)	40–85	100.0	0.1
Methyl ethyl ketone (4)	45–80	99.7	0.2
Methyl n-propyl ketone (3)	45–85	99.9	0.1
Methyl isopropyl ketone (3)	35–80	100.0	0.1
Methyl n-butyl ketone (4)	35–75	99.9	0.2
Methyl isobutyl ketone (3)	35–80	99.8	0.2
Diethyl ketone (4)	45–80	99.9	0.1
Cyclohexanone (3)	40–75	100.0	0.1
Propionaldehyde (3)	40–85	99.8	0.2
Butyraldehyde (4)	40–80	100.0	0.1
Acetophenone (3)	40–60	100.1	0.1
p-Methylacetophenone (3)	40–70	99.9	0.2
Benzaldehyde (4)	40–70	99.9	0.1
p-Methylbenzaldehyde (4)	40–80	99.9	0.1
Furfuraldehyde (4)	40–80	99.9	0.1

* Figures in parentheses represent the number of determinations.

distinct blue colour when methanol and acetonitrile were used as solvents and to a green colour when dimethylformamide was used. Results are given in Table 1.

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SEPARATION AND PRECONCENTRATION OF TRACE AMOUNTS OF SEVERAL METALS IN MAGNESIUM METAL AND NITRATE, WITH ACTIVATED CARBON AS A COLLECTOR

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Summary—A method is described for the preconcentration and determination of traces of Hg, Ag, Cu, Fe, In, Mn, Pb, and Zn present as impurities in magnesium metal (1g) and nitrate (100 g). After the metal sample has been dissolved in nitric acid (or the salt in water) and the pH adjusted to 8.1–9 (except for preconcentration of Hg, when pH 3 is used), the solution is filtered through a 2-cm paper coated with 50 mg of activated carbon. The trace metals are quantitatively adsorbed on the activated carbon and separated from the matrix. The rest of the procedure has already been described. The detection limits for the analysis of 1 g of Mg and 100 g of $Mg(NO_3)_2 \cdot 6H_2O$ are 0.03–1.3 ppm and 0.00031–0.013 ppm respectively, for all the trace metals except Hg. The limit is 0.00001_4 ppm for Hg in 100 g of $Mg(NO_3)_2 \cdot 6H_2O$. The coefficient of variation is 4–33%, depending on the trace metal.

Trace metals have been preconcentrated from magnesium metal and sulphate by means of activated carbon and dithizone.[1] However, dithizone was found unnecessary for adsorption (on activated carbon) of several trace metals in sodium perchlorate,[2] only pH adjustment being required. Even for a matrix such as zinc nitrate,[3] it was not necessary to use special agents for the adsorption of traces of Hg and Fe, which could be quantitatively adsorbed on activated carbon at a favourable pH. It seemed worth examining what sort of traces can be adsorbed on activated carbon (and at what pH), without auxiliary agents. These factors may depend on the type of matrix. Hence we started this work. Preliminary experiments showed that large amounts of chloride greatly decreased the recovery of traces of mercury so we used magnesium nitrate as the sample matrix. The highest grade of nitric acid available commercially [Super Special Grade (S.S.G.), Wako Pure Chemical Co. Ltd.] was used to dissolve magnesium powder, and the impurities in the nitric acid were determined to estimate the solvent background level. A sample of magnesium nitrate was also analysed.

EXPERIMENTAL

Reagents. Magnesium powder and magnesium nitrate of guaranteed-reagent grade were used as the test samples. Activated carbon (Merck *pro analysi*) and nitric acid (S.S.G., Wako Pure Chemical Co. Ltd.) were used without further purification. Standard solutions were the same as described previously.[2,3] The water used was prepared by distilling demineralized water from dilute alkaline permanganate in a glass still.

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Preparation of sample solutions

$Mg(NO_3)_2 \cdot 6H_2O$ (100.0 g) was weighed out and dissolved in 200 ml of water; the pH was about 3. Magnesium powder (1.00 g) was weighed into a 100-ml beaker and dissolved by slow addition of 8.5 ml of concentrated nitric acid during about 1 hr. The solution was heated on a hot-plate at about 200° for a few hours to remove excess of nitric acid, and then transferred to a 300-ml beaker with water, and diluted to 200 ml; the pH was 2–3. Then, the pH of each solution was adjusted to 8.0–9 with 8–10 ml of 0.1*M* sodium hydroxide (G.R.). Contamination from the sodium hydroxide used was negligible.

Procedure for mercury preconcentration

The 200 ml of sample solution were filtered through a 2-cm filter paper coated with 50 mg of activated carbon. The carbon together with the filter paper was then heated at 500° for 5 min in an Automatic Mercury Evaporator, model ANA-K801 (Tokyo Photo-Electric Co. Ltd.), and measured with a flameless AAS, microgas analyser for mercury, model ANA-K80 (Tokyo Photo-Electric Co. Ltd.). The procedure is the same as reported previously.[2,3]

Procedure for other elements

The treatment of the activated carbon with adsorbed trace metals was the same as reported previously.[2,3] The concentration of the trace metals was determined on 100-μl subsamples with a Hitachi 518 Atomic Absorption Spectrometer.

Determination of impurities in the nitric acid

Separate 20, 40, 60, 80 and 100 ml portions of the concentrated S.S.G. nitric acid and 50 ml of water were evaporated to dryness in 100-ml beakers during 5–8 hr on a hot-plate at about 200°. Then 3 ml of 20% v/v nitric acid were added to each beaker and the amounts of metals in the solution were determined by AAS. No Ag, In or Pb was detected, but appreciable amounts of Cu, Fe, Mn and Zn were present; the results are given in Table 1. For each element, plots of the amount found against volume of nitric acid taken were linear, with intercepts. The intercepts were in good agreement with the values obtained from the 50 ml

Table 1. Contamination and impurities in high-purity nitric acid (S.S.G.)*

Nitric acid, ml	Amounts of impurities, μg			
	Cu	Fe	Mn	Zn
0 (50 ml water)	0.021†	0.216†	0.00†	0.174†
20	0.084	0.726	0.033	0.300
40	0.165	1.38	0.066	0.444
60	0.228	1.82	0.102	0.525
80	0.267	2.76	0.159	0.615
100	0.345	3.03	0.186	0.768

* Ag, In and Pb could not be detected.

† These values are thought to be the contamination blanks, not from the water itself, but from the surrounding atmosphere during the standing time of 5–8 hr and from the beaker used.

of water and were thought to correspond to the contamination from the beakers and the surrounding atmosphere. Thus, the nitric acid (8.5 ml) used to dissolve 1 g of magnesium was shown to contain 0.06, 0.48, 0.009 and 0.24 μg of Cu, Fe, Mn and Zn, respectively, and these values were subtracted from the results obtained.

RESULTS AND DISCUSSION

Dependence of the recovery on pH of sample solution

The pH of the sample solutions was adjusted by adding nitric acid or sodium hydroxide; the amounts used contained negligible amounts of the trace metals. Plots of amounts of trace metal recovered against the amount added were linear with slopes of unit for 100% recovery, and in most cases, with intercepts on the recovery axis, arising from impurities in the activated carbon and presenting analytical blanks. The recovery as a function of sample solution pH is given in Table 2. Higher pH generally gives better recovery.

Table 2. Dependence of the recovery on pH of the sample solutions*

Trace metal	Calibration range, μg	Recovery %*, pH				
		3.0	7.1	8.1	8.3	9.0
Ag	0.1–2	0	22	81	78	82
Cd	0.1–2	0	0	0	0	49
Co	0.4–4	0	0	0	0	46
Cu	0.1–2	59	96	100	92	92
Fe	4.0–40	64	74	97	100	96
In	0.2–10	45	92	97	88	98
Mn	0.1–2	0	26	72	93	89
Ni	1.0–10	0	0	0	0	20
Pb	0.2–10	0	52	91	80	62
Zn	2.0–10	0	42	74	94	40
Tl	8.0–40	0	0	0	0	0
Ca	2.0–10	0	0	0	0	0
Hg	0.001–0.008	95–100% in pH range 0.8–5.0				
	0.008–0.04					
	0.05–0.2					

* $Mg(NO_3)_2 \cdot 6H_2O$ (100 g) dissolved in 200 ml of water. Six experimental points were plotted for each calibration line; the error was, in general, ±2% in the recovery.

Table 3. Determination of trace metals in magnesium and magnesium(II) nitrate*

Sample	Trace metal	Content, μg/g	C.V. (N = 10), %	Limit of detection (*d.l.*) 3 σ (N = 24), μg/g
$Mg(NO_3)_2 \cdot 6H_2O$ (100 g)	Hg	0.00025	25	0.00001_4
	Ag	< *d.l.*	—	0.0003
	Cu	0.0027	33	0.0016
	Fe	0.078	12	0.013
	In	< *d.l.*	—	0.004
	Mn	0.038†	6	0.002
	Pb	0.0085	11	0.0033
	Zn	0.150†	4	0.0034
Mg (1.00 g)	Ag	< *d.l.*	—	0.03
	Cu	8.6	5	0.2
	Fe	9.3	7	1.3
	In	< *d.l.*	—	0.4
	Mn	3.6	33	0.2
	Pb	11	8	0.4
	Zn	70	16	0.4

* C.V. = coefficient of variation, N = number of replicates, σ = standard deviation.

† In this case, 10.00 g of $Mg(NO_3)_2 \cdot 6H_2O$ was used.

At pH >9.0 the solutions were slightly turbid from precipitation of magnesium hydroxide. The partial precipitation of the matrix ion may assist the adsorption of traces on the activated carbon. On the other hand, the recovery of Hg was quantitative even in highly acidic solutions ranging in pH from 0.8 to 5.0. In preliminary experiments it was found that Hg was not recovered at all from 200 ml of solution containing 100 g of $MgCl_2 \cdot 6H_2O$ instead of $Mg(NO_3)_2 \cdot 6H_2O$, but the recovery was 88% when only 10 g of $MgCl_2 \cdot 6H_2O$ were used. This indicates that the recovery of Hg is reduced by the presence of chloride ion, presumably by formation of chloro-complexes.

Determination of mercury

When 100 g of $Mg(NO_3)_2 \cdot 6H_2O$ were dissolved in 200 ml of water, the pH was about 3, the correct range for Hg recovery. The results are given in Table 3.

Determination of Ag, Cu, Fe, In, Mn, Pb and Zn

It is seen from Table 2 that these traces were quantitatively adsorbed on the activated carbon (within experimental error) at pH > 8.1. The results obtained for the pH range 8.1–9 are also listed in Table 3.

Preconcentration coefficient

This is defined[4] as $K = (Q_t/Q_m)/(Q_t^\circ/Q_m^\circ)$ where Q_m° and Q_m are the quantities of matrix before and after preconcentration, respectively, and Q_t° and Q_t are the quantities of the trace elements in the sample and in the concentrate (*i.e.*, activated carbon in this work). When the recovery is quantitative, $K = Q_m^\circ/Q_m$. The magnesium adsorbed on the activated carbon (*i.e.*,

Q_m) was found to be 270 μg under the conditions in Table 3 [100 g of $Mg(NO_3)_2 \cdot 6H_2O$; $Q_m^\circ = 9.5$ g]. Thus, the preconcentration coefficient is 3.5×10^4. This value is thought to be satisfactorily large. In general, the matrix itself is also adsorbed on the activated carbon and the value of Q_m is dependent on the experimental conditions such as pH of the sample solutions.

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COLORIMETRIC DETERMINATION OF *p*-AMINOPHENOL IN THE PRESENCE OF PARACETAMOL WITH 3-CYANO-*N*-METHOXYPYRIDINIUM PERCHLORATE

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Summary—Two simple and sensitive colorimetric procedures for the determination of *p*-aminophenol with 3-cyano-*N*-methoxypyridinium perchlorate are presented. One is based on reaction in methoxyethanol in presence of sodium acetate, with direct measurement at 410 nm. The reaction product obtained by this procedure has been separated and identified. The other is based on reaction in methoxyethanol in presence of chloramine-T and direct measurement at 448 nm. The method has been applied to the determination of *p*-aminophenol in pure form and as an impurity in paracetamol and paracetamol-containing tablets, with a coefficient of variation less than 2%.

Various spectrophotometric methods have been reported for the determination of *p*-aminophenol as an impurity in paracetamol, and are based on measurement of a coloured product formed through reaction with certain reagents, either before or after chromatographic separation from the paracetamol.[1–13] In many of these colour reactions[4–12] the product has not been identified. Other methods are based on non-aqueous titration with perchloric acid,[14] polarographic analysis,[15,16] HPLC with electrochemical detection,[17] and direct and indirect fluorimetric analysis.[18,19] Recently Schnekenburger *et al.*[20] used 3-cyano-*N*-methoxypyridinium perchlorate (CMPP) as a chromogenic reagent for qualitative determination of primary and secondary aromatic amines and sulphonamides in a reaction analogous with those used by Zincke,[21] König,[22] and Baumgarten.[23] All these authors have studied the hydrolysis and ring-opening of pyridinium salts by treatment with base. Salts such as 2,4-dinitrophenylpyridinium chloride,[21] *N*-cyanopyridinium bromide[22] and pyridinium sulphonic acid[23] undergo base-induced ring cleavage to form derivatives of glutaconic dialdehyde.

N-Alkoxy quaternary salts of aromatic nitrogen heterocycles are prepared from the corresponding *N*-oxides by reaction with alkylating agents.[24,25] Thus 3-cyanopyridine-*N*-oxide was synthesized according to the method of Ochiai[26] by reacting 3-cyanopyridine with hydrogen peroxide in acetic acid. The *N*-oxide was quaternized with excess of dimethyl sulphate to afford the 3-cyano-*N*-methoxypyridinium salt, which was conveniently isolated and purified as the perchlorate (CMPP).[27]

CMPP reacts with aromatic amines and sulphonamides, especially in dipolar aprotic solvents, to produce yellow solutions of 1-methoxyimino-2-cyanopentadiene derivatives. Some of these reactions require the presence of a base.[20] Accordingly, we suggest the use of CMPP as a chromogenic reagent for colorimetric determination of *p*-aminophenol. It reacts with *p*-aminophenol in presence of sodium acetate to give the intensely yellow product **I** (Scheme 1). This product, 1-methoxyimino-5-(4-hydroxyphenylamino)-2,4-pentadien-2-carbonitrile, has been synthesized and its physicochemical properties are reported. The addition of chloramine-T to a solution of **I** changes the colour from yellow to red, but the product (postulated as compound **II** in Scheme 1) is not stable enough for separation or synthesis, probably because of the quinone-imine structure.[28]

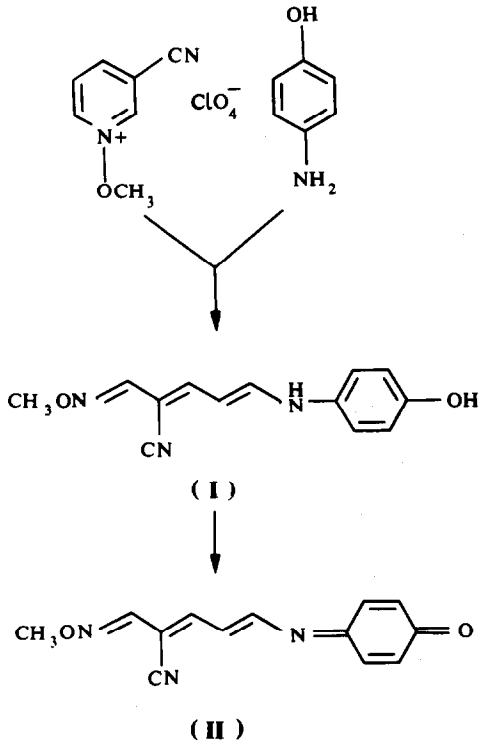
Here we use both reactions for the colorimetric determination of *p*-aminophenol either alone or in presence of paracetamol.

EXPERIMENTAL

Reagents

*3-Cyano-*N*-methoxypyridinium perchlorate (CMPP).* Dissolve 200 mg of CMPP, synthesized according to Schnekenburger and Heber[27] and recrystallized from methanol (m.p. 110°), in 100 ml of methoxyethanol. Prepare immediately before use.

Synthesis of compound I. 4-Aminophenol (1.1 mmole) and then triethylamine (5 drops) were added in one portion to a solution of 3-cyano-*N*-methoxypyridinium perchlorate (CMPP) (1 mmole) in dimethylformamide (2 ml) with vigorous stirring, and cooling in an ice-bath. The deep yellow mixture was neutralized with dilute hydrochloric acid



Scheme 1.

and then poured over crushed ice (5 g), diluted with water (5 ml), and stirred until the ice had melted. The product was filtered off, washed with distilled water, and air-dried. The crude product was purified by dissolving it in acetone (3 ml) and shaking with charcoal (500 mg). After filtration the solution was poured over crushed ice and worked up as described above (131 mg, 54% yield, m.p. 158°). TLC with chloroform–methanol (1:1) showed the product to be homogeneous; infrared (KBr) 3300, 2210, 1640 and 980 cm^{-1}; ^1H-N.M.R. (DMSO-d_6, δ[ppm]) 3.80 (s, 3H), 6.00 (t, 1H), 6.94 (m, 4H), 7.40 (d, 1H), 7.85 (t, 1H), 7.90 (s, 1H), 9.40 (s, 1H), 10.15 (d, 1H). Analysis gave C 64.1%, H 5.4%, N 17.2%; $C_{13}H_{13}N_3O_2$ (m.w. 243.26) requires C 64.19%, H 5.39%, N 17.27%.

The product was soluble in methoxyethanol, ethanol, acetone, chloroform, and dimethylformamide and sparingly soluble in water.

Chloramine T solution, 0.5% in methoxyethanol. Prepared fresh daily.

Sodium acetate solution, 0.1% in methoxyethanol.

Standard p-aminophenol solution. Prepared by dissolving 50 mg in 100 ml of methoxyethanol, and diluting a 2-ml portion to 50 ml with methoxyethanol.

Procedure A

Pipette standard *p*-aminophenol solution (0.5–2.5 ml) into 25-ml standard flasks. Add 1 ml of CMPP solution and 1 ml of sodium acetate solution. Mix and leave for 5 min. Make up to volume with methoxyethanol. Measure the absorbance in 1-cm cells against a reagent blank at 410 nm.

Procedure B

Pipette standard *p*-aminophenol solution (2–10 ml) into 25-ml standard flasks. Add 1 ml of CMPP solution and leave for 5 min. Add 1 ml of chloramine-T solution. Mix and leave for 5 min. Make up to volume with methoxyethanol. Measure the absorbance in 1-cm cells against a reagent blank at 448 nm.

Determination of p-aminophenol in paracetamol tablets

Weigh accurately 10 tablets of paracetamol and grind them in a mortar to homogeneity. Weigh a portion of the powder containing 400 ± 4 mg of paracetamol and transfer it to a 100-ml standard flask. Add 70 ml of methoxyethanol, agitate gently for 10 min, then dilute to volume with methoxyethanol. Filter through a medium-speed filter paper. Apply procedure A or B to 1 ml of the final clear solution. Calculate the amount of *p*-aminophenol from the appropriate calibration graph.

RESULTS AND DISCUSSION

Methoxyethanol, ethanol, dimethylformamide, chloroform, acetone, and dioxan were tried as solvents for the coloured products. The most suitable was methoxyethanol. The colour produced in both procedures was found to be stable in methoxyethanol for 30 min. Investigations on the effect of the concentrations of the different reagents in both procedures, with respect to maximum sensitivity, and obedience to Beer's law, led to procedures A and B above.

The yellow product (procedure A) has maximum absorption at 410 nm. No difference was found with pure synthetic I (Fig. 1). Addition of chloramine-T (procedure B) resulted in a bathochromic shift and the red colour developed showed absorption peaks at 448 and 476 nm (Fig. 1).

The colour produced in both procedures under the conditions described was found to obey Beer's law over the concentration ranges 10–60 and 40–200 μg/25 ml for procedures A and B, respectively. Five separate determinations at different concentration levels were carried out to test the reproducibility; the coefficient of variation was found to be less than 2% for both procedures. Procedure A was more sensitive (log ϵ = 4.720, 1-cm cell) than procedure B (log ϵ = 4.233 at 448 nm, 4.183 at 476 nm). Procedure B can be recommended when other amino-compounds are present that do not interfere with the measurement at 448 or 476 nm. Procedures A and B have been applied to the determination of *p*-aminophenol

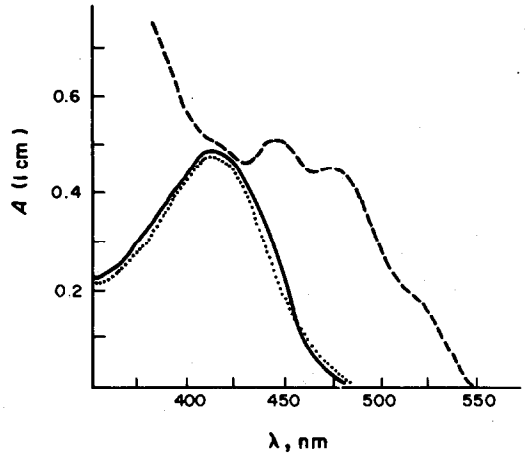


Fig. 1. Absorption spectra for the colour produced on reaction of *p*-aminophenol with 3-cyano-*N*-methoxypyridinum perchlorate (——); after addition of chloramine-T (-----); and for pure compound I (· · · · · ·).

Table 1. Determination of *p*-aminophenol alone and in presence of paracetamol, with 3-cyano-*N*-methoxypyridinium perchlorate

	Recovery, %					
	Procedure A			Procedure B		
Sample	Alone*	In paracetamol†	In paracetamol tablets†	Alone‡	In paracetamol†	In paracetamol tablets†
1	98.3	96.9	98.2	98.1	102.1	98.3
2	102.6	99.3	99.2	99.8	101.6	99.6
3	99.3	100.7	100.4	102.1	99.4	100.9
4	102.3	99.7	101.6	99.6	98.9	98.1
5	100.5	99.2	100.6	99.1	99.7	99.7
Mean	100.6	99.1	100.0	99.7	100.3	99.3
±SD	±1.9	±1.4	±1.3	±1.5	±1.4	±1.1

* Added in concentration range 10–60 μg per 25 ml.
† *p*-Aminophenol added in 1% ratio to paracetamol.
‡ Added in concentration range 40–160 μg per 25 ml.

added to pure paracetamol and to paracetamol tablets. Paracetamol does not interfere with either reaction and the method can be used to detect *p*-aminophenol even in the presence of a 10^5-fold amount of paracetamol. The tolerance limit for the pharmaceutical product is 1 in 2×10^4. The results obtained (Table 1) are acceptably precise and accurate for the levels concerned.

Several drugs and related compounds have been tested for interference in both procedures. Codeine, salicylamide, phenacetin, acetanilide, and phenol give no colour. Aniline, *o*-aminophenol and *m*-aminophenol give a yellow colour with CMPP. Addition of chloramine-T gives no further response with aniline or *m*-aminophenol, the colour remaining yellow, but with *o*-aminophenol the colour changes slowly to red. The negative response of the *m*-aminophenol product to addition of chloramine-T supports our assumption that *p*-quinone-imine derivatives are formed after the addition of chloramine-T to *p*-aminophenol. The method described is simple, selective, and accurate and can be extended to determination of primary and secondary amines and aminophenols.

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ANALYTICAL DATA

POTENTIOMETRIC STUDY OF THE ASSOCIATION CONSTANT OF ISOTHIOCYANIC ACID

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Summary—The association constant of isothiocyanic acid, HNCS, has been determined potentiometrically and found to be 0.135 ± 0.007 and 0.168 ± 0.007 at ionic strengths ($NaClO_4$) of 2.0 and 4.0*M* respectively. The indicator electrode was $Hg(SCN)_4^{2-}$/Hg. For accuracy and precision the metal phase was used in the form of a dropping electrode. Because the association constant is so low, liquid-junction potentials would cause significant errors, since a strong acid was added to the working solutions in the procedure, and an estimate of this effect was taken into account.

Thiocyanate is a ligand of analytical importance for the determination of several metals.[1] In our studies on the uranium–thiocyanate system[2] the medium was made acidic to minimize hydrolysis. Hence it was important to know the association constant for formation of isothiocyanic acid. HNCS (which is known to be a moderately strong acid) for use in calculating the free ligand concentration. Previous values for this constant are not in complete agreement,[3–6] and were not determined at the ionic strengths used in the work reported here.

In the present work an indirect potentiometric method was used to monitor the free SCN^- concentration, from the reversible response of an $Hg(SCN)_4^{2-}$/Hg electrode, this method having previously been found to be reliable.[7] Changes in junction potential, ΔE_j were also taken into account.

EXPERIMENTAL

An Orion 801-A potentiometer coupled to a Metrohm EA 876 cell and a calomel electrode ($NaCl_{sat}$) was used in the potentiometric measurements. A dropping mercury electrode, at a constant height and with drop-time of about 5 sec, was used as indicator electrode. This kind of electrode was preferred owing to its high stability in potentiometric work.[8]

The measurements were taken when the maximum surface was attained.

The potentiometric cell used was:

Ag	$AgCl_{sat}$ $NaClO_4 + NaCl = 2.00M$ (or 4.00*M*)	$NaCl_{sat}$, Hg_2Cl_2	Hg (cell II)

The standard solution of tetrathiocyanatomercury(II) used was prepared by reacting perchloric acid with the stoichiometric amounts of mercury(II) oxide and sodium thiocyanate:

$$HgO + 2H^+ + 4SCN^- = Hg(SCN)_4^{2-} + H_2O$$

A volume of 8.00 ml of the thiocyanate test solution (composition as shown above for cell I) was placed in the cell, and the initial potential E_i was measured. Then various volumes of perchloric acid (at the same ionic strength as the test solution) were added and the corresponding potentials were measured. The maximum hydrogen-ion concentration reached was always comparable with the concentration of the thiocyanate ions.

For measurements of ΔE_j the following cell was used:

Hg	$Hg(SCN)_4^{2-} = 10.0mM$ $NaSCN + NaClO_4 = 1.97M$ (or 3.97*M*)	$NaCl_{sat}$, Hg_2Cl_2	Hg (cell I)

The ΔE_j was estimated on the basis of changes in $E^{0'}_{AgCl}$ caused by addition of perchloric acid (at the same ionic strength). All measurements were made at 25.0 ± 0.1°.

RESULTS AND DISCUSSION

The potentiometric determination

A linear relationship between E and $[SCN^-]$ was found over a wide range of free ligand concentration, for a fixed mercury(II) concentration. A virtually Nernstian response was obtained, but the slope was slightly lower than expected for the existence of only $Hg(SCN)_4^{2-}$. This is, in fact, the dominant species for

the range of ligand concentrations examined, but the small concentrations of $Hg(SCN)_3^-$ and $Hg(SCN)_2$ should also be taken into account. Thus the following Nernst equation can be applied for the potential of cell I and 25.0°, constant ionic strength (2.0 or 4.0*M*) and analytical mercury(II) concentration C_{Hg}:

$$E = E_x^{0\prime} + \frac{0.05916}{2}\log C_{Hg} - \frac{0.05916\bar{n}}{2}\log[SCN^-] \quad (1)$$

where

$$\bar{n} = \frac{2\beta_2[SCN^-]^2 + 3\beta_3[SCN^-]^3 + 4\beta_4[SCN^-]^4}{\beta_2[SCN^-]^2 + \beta_3[SCN^-]^3 + \beta_4[SCN^-]^4} \quad (2)$$

$E_x^{0\prime}$ is calculated from the measured potential E at a known C_{Hg} and free thiocyanate concentration.

The initial free thiocyanate concentration $[SCN^-]$ corresponding to the initial potential E_i would be

$$[SCN^-]_i = C_{SCN^-} - \bar{n}C_{Hg} \quad (3)$$

It was obtained by an iterative calculation starting from the estimate

$$[SCN^-] = C_{SCN^-} - 4C_{Hg} \quad (4)$$

the value of $[SCN^-]$ thus obtained being used to calculate $\bar{n}$ from equation (2) with the values $\log \beta_2 = 16.07$, $\log \beta_3 = 18.95$ and $\log \beta_4 = 20.94$, taken from the literature.[9] This value of $\bar{n}$ was then used in equation (3) to refine the estimate of $[SCN^-]_i$, and so on until a constant value for $[SCN^-]_i$ was obtained. Only one iteration was usually needed, and the value of $\bar{n}$ was not significantly affected by moderate dilution of the cell solution.

When a volume v ml of perchloric acid of concentration C_H was added to the initial V ml of cell solution, the potential shifted to E_v, corresponding to the equilibrium for the new free thiocyanate concentration $[SCN^-]_{exp}$. The following equations were then introduced into the calculations.

$$[H^+] = C_H v/(v + V) \quad (5)$$

$$[SCN^-]_v = [SCN^-]_i V/(v + V) \quad (6)$$

$$E_v + \Delta E_j = E_x^{0\prime} + \frac{0.05916}{2}\log[C_{Hg^{2+}}V/(v + V)] - \frac{0.05916\bar{n}}{2}\log[SCN^-]_{exp} \quad (7)$$

$[SCN^-]_v$ in equation (6) is the free thiocyanate concentration calculated solely from the dilution factor, without regard to other effects, and thus differs from $[SCN^-]_{exp}$. Combination of equations (1) and (7) and rearrangement gives

$$[SCN^-]_{exp} = \text{antilog}\left\{\frac{2(E_i - E_v - \Delta E_j)}{0.05916\bar{n}} + \log\frac{V}{(v + V)\bar{n}} + \log[SCN^-]_i\right\}. \quad (8)$$

In the first step of the iterative calculation no ΔE_j is considered, and $[SCN^-]_{exp}$ is calculated from equation (8) and compared with the $[SCN^-]_v$ obtained from equation (6). The difference between the two values arises because of protonation of some of the thiocyanate. An estimate of [HNCS] is thus obtained from

$$[HNCS] = [SCN^-]_i V/(v + V) - [SCN^-]_{(exp)} \quad (9)$$

and of $[H^+]_{exp}$ from

$$[H^+]_{exp} = C_H v/(v + V) - [HNCS] \quad (10)$$

The calculation is then refined by inserting

$$E_j = k[H^+]_{exp} \quad (11)$$

into equation (8) to obtain a better estimate of $[SCN^-]_{exp}$ and so on. The iterative calculations involving equations (8)–(11) take a few cycles to converge to constant values for $[H^+]_{exp}$, [HNCS] and $[SCN^-]_{exp}$. These values are then used to calculate the association constant:

$$\beta = [HNCS]/[H^+]_{exp}[SCN^-]_{exp} \quad (12)$$

for the equilibrium $H^+ + SCN^- \rightleftharpoons HNCS$.

ΔE_j measurements

Perchloric acid was added to the Ag/AgCl,Cl compartment of the cell (II for which the potential is:

$$E = E_{AgCl}^{0\prime} - 0.05916\log[Cl^-]$$

$E_{AgCl}^{0\prime}$ is held constant if sodium perchlorate of the same ionic strength is added, because the equivalent conductance of ClO_4^- is close to that of Cl^- (and also of SCN^-). If perchloric acid is added, however, $E_{AgCl}^{0\prime}$ does not remain constant, and the change to a more negative potential is interpreted as a change in junction potential, ΔE_j, caused by hydrogen ions.

The ΔE_j for addition of acid to the thiocyanate solutions was assumed to be the same as that measured for a similar addition of acid to chloride solutions of the same ionic strength, because the equivalent conductance of protons is considerably higher than that of perchlorate, thiocyanate or chloride. ΔE_j has been found to be a linear function of the acidity for both ionic strengths tested. Thus ΔE_j is directly calculated from equation (11), where k is 7.9 $mV.l.mole^{-1}$ for ionic strength 2.0*M* and 11.4 $mV.l.mole^{-1}$ for ionic strength 4.0*M*.

RESULTS

Table 1 shows the final results obtained from eight series of potentiometric measurements. Slightly smaller values were obtained if $\bar{n}$ for mercury(II) was taken as 4.00, with species other than $Hg(SCN)_4^{2-}$ being ignored. However, the ΔE_j values cannot be neglected in the measurements.

A close agreement was found with the results from potentiometric titration, 0.11, at various ionic strengths,[3] but other methods, albeit at different ionic strengths, give a spread of values from 0.01–1.6.[3–6]

Table 1. Association constant of HNCS

Ionic strength, M	C_{SCN^-}, M	β_1*	Overall averages
2.0	0.24	0.135 ± 0.0050	0.135 ± 0.007
		0.138 ± 0.0042	
	0.54	0.125 ± 0.0033	
		0.141 ± 0.0012	
4.0	0.24	0.167 ± 0.0008	0.168 ± 0.007
		0.158 ± 0.0008	
	0.54	0.175 ± 0.0033	
		0.171 ± 0.0046	

* Average ± standard deviation, obtained from 6 experiments.

Since the potentiometric method is usually more precise and reliable, we believe that the present measurements are more acceptable, since the ionic strength was held constant. The high thiocyanate concentrations used eliminate any possible effects of hyperprotonation (to H_2NCS^+) or hydrolysis to OCS and HCN. It should be added that quite inconsistent results are obtained if ΔE_j is neglected.

The SCN^- ligand can co-ordinate through sulphur (soft base) or nitrogen (hard base). Since the hydrogen ion is considered to be a "hard" acid, the dominant species formed with thiocyanate should be HNCS (isothiocyanic acid) rather than HSCN.

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SYNTHESIS AND METALLOCHROMIC PROPERTIES OF SOME HYDRAZONES OF 2-, 3- AND 8-HYDRAZINOQUINOLINE

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Summary—Twenty-two new hydrazones were synthesized for evaluation as possible colorimetric reagents for trace determinations of iron, cobalt, nickel, copper and zinc. Spectrophotometric studies revealed that several show promise as sensitive reagents, with possible utility for simultaneous determinations of two or more of the metals. Conclusions regarding geometric structures of some of the hydrazones and chelates were deduced from mole-ratio studies.

Continuing our exploration of hydrazones[1–5] in search of new chromogenic reagents for trace metal determinations, we report here the synthesis of some hydrazones of 2-, 3-, and 8-hydrazinoquinoline and the results of studies of their reactions with some first-row transition metal ions.

EXPERIMENTAL

Reagents

Preparation of hydrazones (**I–XXII**). The 2-hydrazinoquinoline[6] and 8-hydrazinoquinoline[7] were prepared as described earlier. For the preparation of 3-hydrazinoquinoline, the method of Clemo and Felton[8] was followed, except that the tin(IV) salt was decomposed with concentrated sodium hydroxide solution instead of hydrogen sulphide. To prepare the hydrazones, 8 mmole of aldehyde or ketone and hydrazinoquinoline dissolved in 25 ml of absolute ethanol and treated with 1 ml of glacial acetic acid were heated under reflux for 2 hr. The cooled solution was made alkaline with ammonia solution, and the precipitate was filtered off, dried and crystallized from the solvent indicated in Table 1.

Reagent solutions (*0.01M*). A weighed amount of each compound to be tested was dissolved in the necessary volume of ethanol containing 0.05 mole of hydrochloric acid per litre.

Other solutions. Standard solutions of metal ions, buffer solutions, and ascorbic acid were prepared by procedures already described.[1]

Chelation studies

The procedures used to determine the pH ranges over which colour formation occurred, wavelengths and molar absorptivities of maximum absorbance, conformance to Beer's law, and ligand: metal-ion ratios have been already described.[1,9,10]

RESULTS AND DISCUSSION

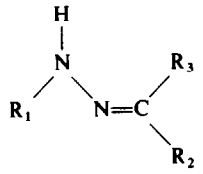
The hydrazones dissolved readily in strong acids to give intensely orange solutions and in ethanol (neutral) to yield yellow solutions. Those which formed coloured metal chelates did so over the pH range 2–11, with maximum colour formation generally at pH 3–10. Depending on the pH, the iron(II) chelates displayed two colours, with the transition in colour occurring between pH 5 and 6.

Hydrazones **II, III** and **V** formed the most intensely coloured chelates, the molar absorptivities and wavelengths of maximum absorbance of which are listed in Tables 2 and 3. It is notable that in all cases an aryl group at R_2 gave rise to greater absorptivities in the chelates than an H or CH_3 substituent. Compounds **II, III** and **V** merit consideration as sensitive chromogenic reagents for iron, cobalt, nickel and zinc. Simultaneous determinations of certain combinations of these metals also appear promising, and are based on the significant, though small, differences in the location and shape of the spectral band of these chelates.

None of the hydrazones derived from 3-hydrazinoquinoline (**VIII–XII**) proved effective as chromogens for the transition metal ions tested, other than copper(I). Examination of molecular models revealed that the 3-quinolyl nitrogen atom in these compounds is incapable of participating in chelation of metal ions. The models further showed that the 3-quinolyl group of a bound ligand can sterically hinder co-ordination of a second and especially the third ligand required for full octahedral chelation of the hexaco-ordinate metal ions Fe(II), Co(II), Ni(II) and Zn(II) by bidentate ligands. For the chelation of tetraco-ordinate copper(I), which forms tetrahedral complexes, no steric hindrance was evident from the models. Thus, for structural reasons, only copper(I) would be expected to yield coloured chelates with **VIII–XII**. The experimental results confirm this. The copper(I) chelates, however, proved to give only moderate absorption and therefore are not considered promising for analytical purposes.

It is possible to deduce which of the two possible stereoisomers was obtained in the synthesis of certain

Table 1. Synthesis and analysis of hydrazones



Compound	R_1	R_2	R_3	m.p. C	Crystn. solvent	Formula	Analysis					
							Calculated, %			Found, %		
							C	H	N	C	H	N
I	2-Quinolyl	2-Pyridyl	Methyl	148	C_2H_5OH	$C_{16}H_{14}N_4$	73.2	5.4	21.4	73.1	5.27	21.4
II	2-Quinolyl	2-Pyridyl	Phenyl	193	C_6H_6	$C_{21}H_{16}N_4$	77.8	5.0	17.3	77.6	5.00	17.1
III	2-Quinolyl	2-Pyridyl	2-Pyridyl	157	C_6H_6	$C_{20}H_{15}N_5$	73.8	4.7	21.5	73.7	4.71	21.7
IV	2-Quinolyl	Pyrazinyl	Methyl	166	CH_3OH	$C_{15}H_{13}N_5$	68.4	5.0	26.6	68.5	5.10	26.4
V	2-Quinolyl	Pyrazinyl	Phenyl	227	C_2H_5OH	$C_{20}H_{15}N_5$	73.8	4.7	21.5	73.7	4.58	21.4
VI	2-Quinolyl	3-Isoquinolyl	Hydrogen	230	Methyl cellosolve	$C_{19}H_{14}N_4$	76.5	4.7	18.8	76.6	4.84	18.6
VII	2-Quinolyl	2-Quinolyl	Methyl	175	C_2H_5OH	$C_{20}H_{16}N_4$	76.9	5.2	17.9	76.8	5.29	17.9
VIII	3-Quinolyl	2-Pyridyl	Hydrogen	218	C_2H_5OH	$C_{15}H_{14}N_4O$*	67.7	5.3	21.0	67.8	5.32	21.0
IX	3-Quinolyl	2-Pyridyl	Methyl	203	CH_3OH	$C_{16}H_{14}N_4$	73.3	5.4	21.4	72.9	5.43	21.1
X	3-Quinolyl	2-Pyridyl	Phenyl	180	CH_3OH	$C_{21}H_{16}N_4$	77.8	5.0	17.3	77.9	4.96	17.3
XI	3-Quinolyl	Pyrazinyl	Methyl	250	C_2H_5OH	$C_{15}H_{13}N_5$	68.4	5.0	26.6	68.3	5.09	26.2
XII	3-Quinolyl	3-Isoquinolyl	Hydrogen	229	C_2H_5OH	$C_{19}H_{16}N_4O$*	72.1	5.1	17.7	72.6	4.91	17.8
XIII	8-Quinolyl	2-Pyridyl	Hydrogen	118	aq. CH_3OH	$C_{15}H_{12}N_4$	72.6	4.0	22.6	72.6	4.82	22.6
XIV	8-Quinolyl	2-Pyridyl	Methyl	168	C_2H_5OH	$C_{16}H_{14}N_4$	73.3	5.4	21.4	73.5	5.30	21.4
XV	8-Quinolyl	2-Pyridyl	Phenyl	145	CH_3OH	$C_{21}H_{16}N_4$	77.8	5.0	17.3	77.8	4.96	17.3
XVI	8-Quinolyl	Pyrazinyl	Methyl	172	C_2H_5OH	$C_{15}H_{13}N_5$	68.4	5.0	26.6	68.2	4.94	26.5
XVII	8-Quinolyl	2-Pyrryl	Hydrogen	150	CH_3OH	$C_{14}H_{12}N_4$	71.2	5.1	23.7	71.0	4.95	23.7
XVIII	8-Quinolyl	Benzoyl	Phenyl	128	C_2H_5OH	$C_{21}H_{15}N_5O$*	71.4	4.3	19.8	71.5	4.15	19.8
XIX	8-Quinolyl	Phenyl	Hydrogen	118	CH_3OH	$C_{16}H_{13}N_3$	77.7	5.3	17.0	77.8	5.19	17.0
XX	8-Quinolyl	2-Quinolyl	Hydrogen	125	C_2H_5OH	$C_{19}H_{14}N_4$	76.5	4.7	18.8	77.0	4.74	18.5
XXI	8-Quinolyl	2-Quinolyl	Methyl	197	Methyl cellosolve	$C_{20}H_{16}N_4$	76.9	5.2	17.9	76.7	5.16	17.9
XXII	8-Quinolyl	3-Isoquinolyl	Hydrogen	228	aq. Methyl cellosolve	$C_{19}H_{14}N_4$	76.5	4.7	18.8	76.7	4.73	18.7

* Hydrate.

Table 2. Absorption properties of iron(II) as a function of pH

	pH 4.0			pH 7.0		
Ligand	Colour	λ, nm	ϵ, 10^3 $l.mole^{-1}.cm^{-1}$	Colour	λ, nm	ϵ, 10^3 $l.mole^{-1}.cm^{-1}$
I	Orange	454	9.3	Green	588	6.3
		541	5.7			
		585	4.2			
II	Green	465	19.6	Green	457	20.0
		594	8.5		627	6.8
III	Brown-green	465	19.1	Green	454	20.9
		598	9.5		623	9.0
IV	Brown	466[a]	19.8	Yellow-brown	654	5.1
		613	4.9			
V	Orange	471	22.2	Orange-brown	468	31.1
		654	5.5		656	6.3
VI	Brown	521[a]	7.9	Brown	561	8.1
		557[a]	6.9			
VII	[b]	[b]	[b]	[c]	[c]	[c]
VIII–XII	[c]	[c]	[c]	[c]	[c]	[c]
XIII	Brown	525[a]	7.7	Brown	515	8.6
XIV	Orange	515	8.6	Orange-brown	518	8.5
XV	Red-orange	525[a]	9.3	Orange-brown	532	9.7
XVI	Orange-brown	540	3.[d]	Brown	545	10.[d]
XVII	[c]	[c]	[c]	[c]	[c]	[c]
XVIII	Green	643	9.7	Green	645	9.5
XIV	[b]	[b]	[b]	[c]	[c]	[c]
XX	[c]	[c]	[c]	[c]	[c]	[c]
XXI	[b]	[b]	[b]	[c]	[c]	[c]
XXII	Orange	493	13.4	Orange	501	13.8

[a] Shoulder.
[b] No evidence of chelate formation.
[c] Colour of complex and free ligand are similar; near-ultraviolet band of free ligand extends into visible region and is broadended and shifted bathochromically but no new band evident on complexation.
[d] Weakly formed complex; Beer's law not followed unless a large excess of ligand is present.

Table 3. Absorption properties of cobalt, nickel, copper and zinc chelates[a]

	Cobalt(II)			Nickel(II)			Copper(I)			Zinc(II)		
Ligand	Colour[b]	λ, nm	ϵ[c]	Colour[b]	λ, nm	ϵ[c]	Colour[b]	λ, nm	ϵ[c]	Colour[b]	λ, nm	ϵ[c]
I	O	495	30.7	Y	475[d]	15.0	Go	480[d]	9.3	Y	450	36.4
II	R–O	517	33.9	Go	488	20	O	466[d]	7.2	Y	475	51.7
III	R–O	513	35.2	O	488	32.6	O	525[d]	2.3	Go	478	50
IV	O–Br	522[d]	16.4	O	502	20.6	Y	500[d]	1.2	O	488[d]	30.1
V	Br	497	26.6	R	524	31.2	O	550[d]	2.0	R–O	507	14.5
VI	O	494[d]	31.2	Y	475[d]	20[e]	Go	488[d]	3[e]	Go	475[d]	40[e]
VII	O	506	8.5	O	512	20.7	Gr-Y	625[d]	0.8	Go	507[d]	5.0
VIII	Y	[f]	[f]	Y	[f]	[f]	O	500[d]	2.2	[g]	[g]	[g]
IX	Y	[f]	[f]	Y	[f]	[f]	O	525[d]	2.2	[g]	[g]	[g]
X	[g]	[g]	[g]	[g]	[g]	[g]	Go	463[d]	4.9	[g]	[g]	[g]
XI	Y	[f]	[f]	Y	[f]	[f]	Go	556[d]	2[e]	[g]	[g]	[g]
XII	Y	[f]	[f]	Y	[f]	[f]	Go	500[d]	3.0	[g]	[g]	[g]
XIII	R-O	508	20[e]	Y	488[d]	0.6	O	476	21.3	[g]	[g]	[g]
XIV	R-O	557	17[e]	Y	[f]	[f]	O	488[d]	6[e]	[g]	[g]	[g]
XV	Br	569	20[e]	Y	[f]	[f]	O	513[d]	4.5	[g]	[g]	[g]
XVI	Br	565	4[e]	Y	[f]	[f]	O	521	9[e]	[g]	[g]	[g]
XVII	O-Br	530	9[e]	Gr-Y	527	6.6[e]	Y-Br	540	12.8	[g]	[g]	[g]
XVIII	O	550[d]	2.6	Go	538[d]	1.7	Y	[f]	[f]	Y	513[d]	0.6
XIX	[g]	[g]	[g]	g	[g]	[g]	Gr	644	3[e]	[g]	[g]	[g]
XX	[g]	[g]	[g]	Y	[f]	[f]	Y-Br	525[d]	8.2	6g	[g]	[g]
XXI	Y	[f]	[f]	Y	[f]	[f]	Y	[f]	[f]	[g]	[g]	[g]
XXII	O	507[d]	8[e]	Y	475[d]	1.2	Go	488	19.5	[g]	[g]	[g]

[a] Measured in the visible region for ethanol–water solutions buffered at pH 7 with ammonium acetate, except for zinc(II) solutions which were buffered at pH 9 with a borate buffer.
[b] Colour key: Br = brown, Go = gold, Gr = green, O = orange, R = red, and Y = yellow.
[c] Molar absorptivity, 10^3 $l.mole^{-1}.cm^{-1}$.
[d] Shoulder or side of band just before absorption of blank (free ligand) begins to become appreciable.
[e] Complex is weakly formed, requiring large excess of ligand for absorbance to follow Beer's law.
[f] Spectra of metal chelate and free ligand differ only slightly.
[g] No evidence of chelate formation.

Table 4. Ligand to metal ion ratios and relative strengths of iron(II) chelates

Ligand	pH 4		pH 7	
	L/Fe	Strength*	L/Fe	Strength*
I	2	S	2	S
II	2	S	2	S
III	2	S	2	S
IV	2	S	2	S
V	2	W	2	W
XIII	2	M	2	M
XV	2	W	2	W
XVIII	3	W	†	†

* Relative strength as indicated by curvature and low degree of formation (α) in mole-ratio plots: S = strong, very little curvature; M = moderate, some curvature; W = weak, considerable curvature.

† Too weakly formed for reliable measurement.

of the hydrazones made in this study, on the basis of the ligand to metal ion ratios found for their iron(II) chelates. As described by Bell and Rose[11] and in some of our previous communications[2–5] concerning hydrazone chromogens, terdentate co-ordination may occur with one isomer of a particular hydrazone but may not be possible with the other isomer. Accordingly, if **I, II, IV, V, XIII** or **XV** were to exist in the geometric configuration depicted in Table 1, each should be capable of terdentate co-ordination to yield an iron(II) chelate with a ligand to iron ratio of 2. However, if the other isomer had been obtained instead, with the R_2 and R_3 groups interchanged, the hydrazone would be capable of bidentate but not terdentate co-ordination, and a ligand to metal ratio of 3 would be predicted for its iron(II) chelate. Inasmuch as the iron(II) chelates of **I,II,IV, V, XII** and **XV** were each found to have a ligand to iron ratio of 2, the particular stereoisomer obtained in the synthesis of each must be the one depicted in Table 1.

Hydrazones **XIII–XXII** appear to afford only marginal promise as analytical reagents. In general, the stabilities (Table 4) and molar absorptivities (Tables 2 and 3) of their chelates were appreciably less than those of the corresponding chelates of the 2-quinolyl analogues (**I–VII**). From molecular models and the results of our mole-ratio studies we conclude that the 8-quinolyl and imino nitrogen atoms of these hydrazones can co-ordinate to a metal ion to form a 6-membered chelate ring, but that this ring is strained and therefore less stable than the 5-membered chelate ring which could form if a 2-quinolyl group were at the R_1 position, and not the 8-quinolyl group. The finding that the iron(II) chelates of **XIII** and **XV** have a ligand to metal ratio of 2 indicates that the ligands are terdentate and therefore that the 8-quinolyl nitrogen atom must be co-ordinated to iron(II) in their complexes. Ligand **XVIII**, however, was found to be bidentate, with a ligand to metal ratio of 3 for its iron(II) chelate. From this we conclude that the benzoyl group neither participates nor interferes in the chelation of iron(II) by **XVIII**.

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ANNOTATION

DRYING CONDITIONS FOR POTASSIUM DICHROMATE

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Summary—The optimum conditions for drying primary-standard or SRM grade potassium dichromate have been further examined, and it has been established that if organic impurities are present in the material the drying temperature should not exceed 200°. At higher temperatures the organic matter is decomposed oxidatively by the dichromate, with a resultant error when the dichromate is used for standardization purposes.

Potassium dichromate is a particularly useful reference material for titrimetric analysis, not only because of its oxidizing power in acidic solution, but also its functions as a primary standard in acid–base work. However, in our experience and that of Knoeck and Diehl[2] the "dried" reagent is hygroscopic.

Various temperatures (from 110° to the melting point, 398°) have been recommended for the dehydration of the dichromate, and are summarized elsewhere.[3] The presence of occluded water in the crystals of the SRM 136b from the National Bureau of Standards (NBS) was observed by Knoeck and Diehl, and they found a weight loss of 0.019% on heating the reagent at 260°.[2] Sappenfield *et al.*[4] found a weight loss of 0.018% for SRM 136c heated at the same temperature. According to the specification of NBS SRM 136c, a weight loss of 0.005% may be obtained on heating at 105° and 0.01% loss on prolonged heating. Schwab and Wichers[5] earlier found 0.027 and 0.021% of water in NBS SRM 136 by a vacuum fusion–manometric method. They also found occluded water in cavities of the crystals. Svec and Conzemius[6] measured the impurities in SRM 136b by prespark mass spectroscopy, and indicated the presence of water.

For practical use for titrimetry, the SRM is usually pulverized with an agate mortar and pestle to remove occluded water, then heated at a defined temperature, cooled in a desiccator and weighed. Yoshimori and Sakaguchi[7] therefore investigated the drying conditions for potassium dichromate by coulometric microdetermination of residual water in the "dried" reagent. They showed that the water content of the dichromate decreased appreciably with heating at above 150°, and became negligibly small with heating at above 250°. Thus heating at higher than 200° was recommended.

However, it has been reported that dichromate can be decomposed to green chromium(III) oxide by high-temperature heating.[8] On the other hand, Yoshimori *et al.*[9] purified the reagent by zone melting at 400°, and obtained a very pure product, indicating stability of the reagent up to its melting point. Thus, it seems likely that production of the green oxide might be caused by reduction with some organic impurities in the sample. This problem has now been investigated both by precise coulometric assay of the dichromate and by microdetermination of carbon[10] in the reagent.

EXPERIMENTAL

The apparatus and procedure for the coulometric assay of the dichromate were fundamentally based on the work of Taylor and Marinenko[11] and have already been presented elsewhere.[9] The microdetermination of carbon has also been described[10] and is based on the principle used by Braid *et al.*[12] The dichromate sample was heated at 200° or 300° in a stream of oxygen, then the gases produced were oxidized with copper(II) oxide at 780°. The carbon dioxide thus produced was determined by titrimetry in a non-aqueous solvent.

Two reference materials (CRM-A and -B) certified by the Industrial Inspection Institute of Japan and NBS SRM 136c were tested. The first two were prepared by different producers. All samples were pulverized with an agate mortar and pestle, heated in a fan-type oven for 3 hr at each temperature, cooled for 90 min, in a desiccator containing magnesium perchlorate, and then weighed for the assay. Values of 96484.56 C/eq for the Faraday constant and 294.1844 for the molecular weight were used for the calculation.

RESULTS AND DISCUSSION

The results are shown in Table 1.

The purities found were nearly constant for all the samples heated at between 150° and 200°, though some carbon dioxide could be obtained from the

Table 1. The results of assays and the amounts of carbon by heating potassium dichromate at various temperatures

Sample (certified value)	Heating temperature, °C	No. of detns.	Purity found, % Mean	s_R	Carbon found, ppm
CRM—A (99.99%)	150	4	99.978	0.007	—
	200	6	99.976	0.015	10
	250	7	99.965	0.010	—
	300	5	99.963	0.009	30
CRM—B (99.99%)	150	4	99.981	0.001	—
	200	9	99.980	0.005	17
	250	5	99.960	0.002	—
	300	5	99.963	0.007	30
NBS SRM 136c (99.98%)	150	2	99.961	0.002	—
	200	4	99.964	0.008	5
	300	3	99.951	0.008	10

samples heated at 200°. The standard deviations for the samples heated at 200° were larger than the deviations for those dried at 150°. This indicates heterogeneous distribution of organic impurities in the samples, especially for CRM-A.

The purities found decreased significantly when the drying temperature was increased to 250°, though the decrease was only 0.01–0.02%. Residual water in the cavities of the pulverized reagent may be removed most completely by means of the phase change of the crystals at 236° (from triclinic to monoclinic) but such removal should, contrary to our results, increase the purity of the samples. However, the amounts of carbon dioxide produced by the oxidation of any organic compounds present also increased when the heating temperature was raised from 200° to 300°. These phenomena indicate that the reduction of the dichromate by organic impurities becomes significant at above 200° and could be the main cause of the decrease in purity. Since the purities for CRM-A and -B were nearly constant when they were dried at above 250°, the effect of the organic impurities evidently terminated at this temperature, and the dichromate itself did not decompose until at least 300°.

The results for the samples dried at 150° or 200° were somewhat low compared with the certified values, though the differences were less than 0.02%. They may perhaps be due to error in the current measurements for the coulometric titration, because the uncertainty of the current-measuring devices used here was about 0.01%. The contributions from uncertainty in the molecular weight of the reagent and the weights of the samples were nearly one order less than this value. The organic impurity, calculated on the basis of a 50% carbon content, would amount to not more than 0.006% in CRM-A and -B and 0.002% in NBS SRM 136c, but could easily reduce several times as much dichromate, depending on its composition.

CONCLUSION

Potassium dichromate reference material for titrimetry should be dried at between 150° and 200° after pulverization in an agate mortar and pestle, but if the dichromate is completely pure or does not contain any organic impurities, it may be dried at 200–300° and the contamination by occluded moisture thus more thoroughly avoided.

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TALANTA REVIEW*

THE SPECIATION OF TRACE ELEMENTS IN WATERS

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Summary—Speciation (determination of the different physico-chemical species formed by an element) in a water sample is necessary for an understanding of the toxicity, bioavailability, bioaccumulation and transport of a particular element. The importance of speciation measurements and the various factors leading to changes in speciation are discussed in this review. Speciation in natural waters is a difficult task, and the analytical methods available and the results obtained are critically assessed.

Speciation of an element is the determination of the individual *physico-chemical forms* of that element which together make up its total concentration in a sample. It is now well established that speciation measurements are necessary for the study of toxicity of metals for aquatic organisms and for an understanding of trace-metal transport in rivers and estuaries. Measurement of the *total* concentration of a trace element provides no information about its bioavailability or its interaction with sediments and suspended particulates.

Perhaps the most important features which distinguish metals from other toxic pollutants are that they are not biodegradable and that, once they have entered the environment, their potential toxicity is controlled to a large extent by their physico-chemical form.[1]

Some elements are essential to life. To date, iron, iodine, copper, manganese, zinc, cobalt, molybdenum, selenium, chromium, tin, vanadium, fluorine, silicon and nickel have been established as essential trace elements, and arsenic, cadmium, lead and tungsten are suspected to be essential.[2] Since life evolved in the presence of all the natural elements of the Periodic Table, it is likely that many more trace elements will be identified as essential.

For all these trace elements there exists a fairly narrow "concentration window" between the essential and toxic levels.[2] An element which is indispensable for normal body function may be highly toxic when present at higher concentrations. Some essential elements, for example selenium and vanadium, are much more toxic than some non-essential elements such as mercury and thallium.[2]

Most people are now subjected to environmental concentrations of metals much higher than those to which their forebears were exposed.[3] The body burden of lead and cadmium in modern urban man is at least 500 times that of primitive man, and is disturbingly close to the concentrations known to produce clinical toxicity.[3,4]

Variation in the speciation of trace elements can dramatically change their bioavailability or toxicity. Cobalamin (vitamin B_{12}) is the only assimilable chemical form of cobalt, and the group of chromium amino-acid complexes, known as the glucose tolerance factor, provides most of the usable chromium.[1,2] The alkyl compounds of mercury and lead are especially dangerous because they are lipid-soluble,[1] and materials such as the lead halide aerosols emitted by automobiles can enter the lungs and be absorbed directly into the blood stream.

Change in the oxidation state of an element can have a profound effect on bioavailability and toxicity. Chromium(III) is an essential element; chromium(VI) is highly toxic. Arsenic(III) is much more toxic than arsenic(V).[5–7] Significant changes in the diet may also decrease the bioavailability of trace elements; *e.g.*, high-fibre foods and soybean proteins adsorb essential elements and can cause mineral deficiencies.[8]

Most studies of the toxicity of heavy metals for fish have shown that the free (hydrated) metal ion is the most toxic form.[7] In the case of copper, hydroxy complexes are also believed to be toxic, although to a lesser extent.[9] Strong complexes, and species associated with colloidal particles, are usually assumed to be non-toxic.

The toxicity of a particular dissolved metal species towards aquatic organisms is probably related to its ability to react with a biological membrane. Transport of oxygen, sodium, potassium and chloride are affected by metal-induced morphological changes in the gill epithelia of a fish. Penetration of the membrane by a metal ion to react with cell components depends on the direct lipid-solubility of the metal species (usually only uncharged organometallic species), or the extent and rate of reaction of the metal ion

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Table 1. Possible physico-chemical forms of metals in natural waters

Physico-chemical form	Possible examples	Approximate diameter, *nm*
Particulate	Retained by 0.45-μm filter	>450
Simple hydrated metal ions	$Cd(H_2O)_6^{2+}$	0.8
Simple inorganic complexes	$Pb(H_2O)_4Cl_2$	1
Simple organic complexes	Cu–glycinate	1–2
Stable inorganic complexes	PbS, $ZnCO_3$	1–2
Stable organic complexes	Cu–fulvate	2–4
Adsorbed on inorganic colloids	Cu^{2+}–Fe_2O_3, Pb^{2+}–MnO_2	10–500
Adsorbed on organic colloids	Cu^{2+}–humic acid	10–500
Adsorbed on mixed organic/inorganic colloids	Cu^{2+}–humic acid/Fe_2O_3	10–500

with a membrane transport protein. Metal–protein interaction, leading to carrier-mediated transport of the metal across the membrane, will, for bivalent ions, be thermodynamically favoured when the metal is in the simplest chemical form, *e.g.*, $Cu(H_2O)_4^{2+}$, $CuCl^+$ or $Cu(OH)^+$. For tervalent ions such as Fe(III), however, the most bioavailable form may be an organic complex, because hydrolysis and polymerization could render the free ion inactive.[10] In some cases, kinetics rather than thermodynamics may dictate the biologically-active chemical species. The toxic form of aluminium appears to be $Al(OH)_2^+$, which reacts with gill mucus to hinder the transport of oxygen, potassium and sodium.[11] This species was previously shown to be the kinetically-favoured species in the reaction between aluminium(III) and a hydroxyazo compound.[12] The reaction of metal ions with biological membranes is a particularly complex process, and cannot be explained by simple[13] diffusion models.

Nature has provided fish with an effective defence against ingested heavy metals, which are eliminated through the gut and liver. This detoxification process allows fish to cope with the normal levels of heavy metals present in the food chain and sediments. The evolutionary process has not, however, equipped them to tolerate free metal ion or other reactive metal species in the water pumped through their gills. Unpolluted sea-water or fresh water contains vanishingly low concentrations of these toxic metal forms, most of the dissolved metal being adsorbed on organic or inorganic colloidal particles.[7] Man-made pollution* may add metal directly in a toxic form (*e.g.*, copper leachings from a mine) or, by lowering the pH of the water (*e.g.*, by acid rain or seepage from a mine tailings dam), may convert an inactive metal form, such as polymerized aluminium hydroxide, into a reactive toxic species, *e.g.*, $Al(OH)_2^+$.

Although the total concentrations of a dissolved metal may be similar in two water systems, the chemical forms of that metal may be quite different. Some of the possible dissolved forms of a bivalent trace metal present in a natural water are shown in Table 1. Note that, by convention, "dissolved" metal is defined as all metal species which pass through a 0.45-μm filter. Most colloidal particles will therefore be included in the dissolved fraction. "Filterable" may be a better term than "dissolved", but it is still not definitive since the pore size of the filter would also have to be stated.

There is increasing evidence[7,14–16] that iron oxide and clay particles coated with humic acid strongly adsorb heavy metal ions and control their concentrations in natural waters. It may be expected then that the predominant form of *dissolved* heavy metal will be metal adsorbed on metal–humate colloidal particles.

The concentration of many heavy metals and other elements in natural waters is often below 1 μg/l., and sometimes below 0.1 μg/l. Chemical analysis at these concentration levels is a specialized science. There are severe problems with contamination from a multitude of sources and, at the same time, serious losses of metal can occur by adsorption on the walls of sample bottles or analysis vessels. A clean-room, or at least a special room with a filtered air supply is necessary for work at the ultratrace level,[7] but even then considerable experience is needed before reliable results can be obtained. The subdivision of these very low total metal concentrations into various fractions for speciation is obviously an even more difficult task, taxing both the skill of the analytical chemist and the sensitivity of the analytical method.

* The term "anthropogenic pollution" though widely used as meaning "man-made pollution" is etymologically incorrect. The correct adjective is "anthropurgic" [Ed.].

Anodic stripping voltammetry (ASV) is the technique most commonly used for speciation of heavy metals in waters. It has high sensitivity for the common toxic heavy metals, copper, lead, cadmium and zinc (which are measured in the same analysis), and the ASV-labile metal (*i.e.*, the metal measurable by ASV at the natural pH of the water or in mildly acid conditions) may approximate to the bioavailable fraction.[7,17,18] However, for elements such as mercury, selenium and arsenic, gas chromatography or atomic-absorption spectrometry (AAS) are used, and for aluminium and iron, spectrophotometric procedures may be more suitable. Any separation or analytical procedure used in speciation measurements must be designed to minimize contamination and adsorptive losses. A direct procedure (*e.g.*, ASV-labile metal measurement) is ideal, but if a preliminary separation is needed, ion-exchange is less contamination-prone than solvent extraction.

The various physico-chemical forms of a trace element present in a water sample are not necessarily in equilibrium with one another, but even if they are, any procedure applied to the sample may disturb the equilibria and hence alter the speciation, although if the equilibrium changes are slow and the separation rapid, the disturbance may be minimal. Even the initial act of filtering the sample can affect the equilibria by changing the concentrations of dissolved O_2 and CO_2, and by removing adsorbents in the form of particulate matter. The only analytical technique which may possibly measure a particular metal species without affecting the solution equilibria is potentiometry with an ion-selective electrode (ISE). Unfortunately, ISE measurements are unreliable below $1 \times 10^{-6} M$ concentrations for most elements in natural waters, which is far too insensitive. For speciation measurements during studies on acute toxicity, however, where the total metal concentration may be much higher, ISE potentiometry can be useful.

Other analytical or separation methods, such as ASV, solvent extraction, and ultrafiltration and dialysis, will cause some metal to dissociate from metal complexes or colloidal particles as a result of the electrical potential across the electrode–solution interface (in ASV)[19] or the concentration gradient across the membrane–solution interface (in ultrafiltration and dialysis).[20] Under these circumstances, it is pointless to specify [21,22] that ASV or ultrafiltration procedures should be applied only at the natural pH of the sample, since the original solution equilibria will be disturbed by the procedure, whatever the analytical pH.[23] In any case, it is important to consider the *purpose* of the speciation measurements. If, as is frequently the case, they are needed for toxicity evaluation, then the method should provide, as far as possible, a measure of the toxic metal fraction. For ASV, the best estimate of this fraction will be obtained when the test sample conditions (pH, buffer concentration, *etc.*) and ASV conditions (rotation or stirring speed, pulse frequency, *etc.*) lead to electro-deposition of the fraction of the total metal that will dissociate at, and be transported across, the relevant biomembrane.[24] Only by coincidence would the pH for this ideal condition be identical to that of the original sample.[23]

Bioassays with algae, fish or other plants or animals may appear to provide the best speciation measurement, since they give the toxicity information directly. Bioassays, however, are lengthy and expensive, provide results on one biological species only, and demonstrate only gross effects. Chemical speciation schemes are more amenable to standardization, and can yield information on the different chemical forms present in the water. This information may help to identify a source of pollution. If the relative toxicity of these different forms is known, reliable predictions about toxicity can be made.

ADSORPTION OF TRACE METALS AND REACTION WITH NATURAL ORGANIC MATTER

Most fresh waters have a pH which is in the critical range for adsorption of heavy metals onto particles; a change of as little as 1 pH unit can cause the difference between complete adsorption or desorption (Fig. 1).[25] The high fish mortality observed[26] when the pH of some lakes is lowered as a result of acid rain may be partly due to the desorption and release of copper from lake sediments (Fig. 2).[27,28]

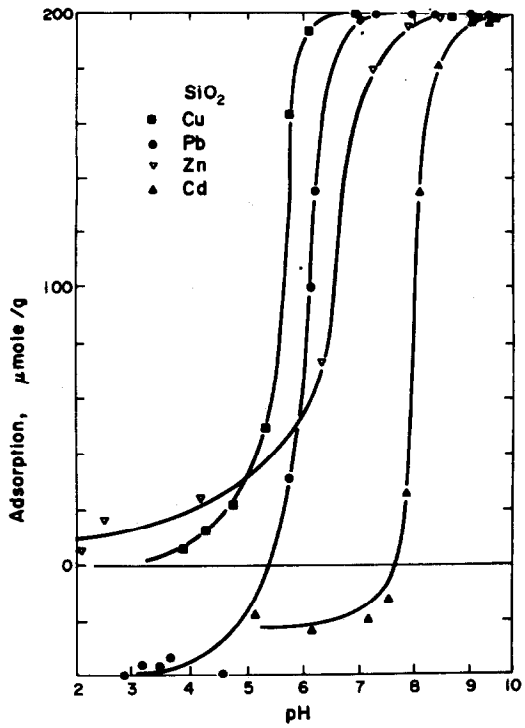

Fig. 1. Adsorption of metals on silica. The apparently negative adsorption of cadmium and lead means that adsorbed metal species are *desorbed* at these pH values, (From C. P. Huang, H. A. Elliott and R. M. Ashmead, *J. Water Pollut. Control Fed.*, 1977, **49**, 745. With permission.)

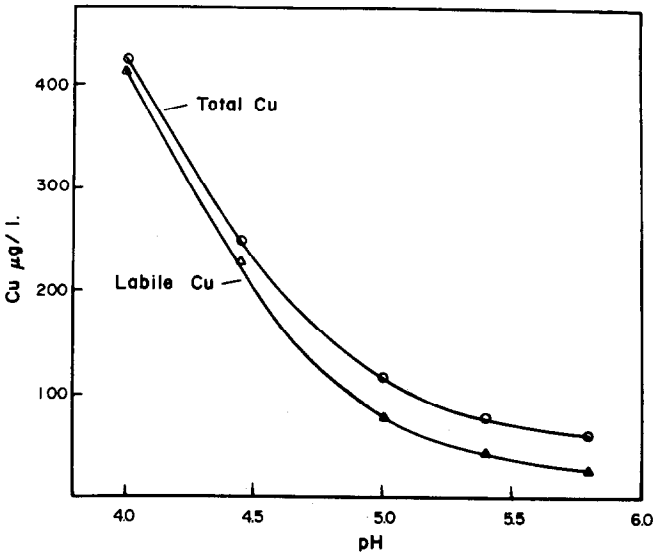

Fig. 2. Effect of pH change on the speciation of dissolved copper (measured by anodic stripping voltammetry) released from a mineralized sediment. (From P. S. Rendell, G. E. Batley and A. J. Cameron, *Environ. Sci. Tech.*, 1980, **14**, 314. With permission.)

There is little doubt that, for many heavy metals, a major fraction of the dissolved metal in fresh waters exists as species adsorbed on colloidal humic acid and colloidal particles of iron oxide coated with humic acid.[29–34] The interaction of humic material with colloidal iron oxide results in a very stable colloid, whereas uncoated iron oxide colloids are relatively unstable.[29,35] To test its ability to retain trace heavy metals, a humic acid–iron colloid with adsorbed copper and lead has been prepared, and the colloidal solution (pH 5.7) passed through a column of Chelex-100 chelating resin: no copper and only 10% of the lead was removed by the column (Batley and Florence, unpublished results). Florence[36] found that even in a low-pH river water containing little dissolved organic matter, over 50% of the copper present was associated with colloidal organic matter. In fresh waters with a high organic content, molecular complexes of heavy metals with fulvic and tannic acids may also be formed.[37–39]

In the fresh water/sea-water mixing zone of estuaries, the precipitation of high molecular-weight humic substances and hydrous oxides of iron and manganese results in the transfer of much of the dissolved heavy metals from the river water to the estuarine sediment.[40–44] The river water is thus effectively scavenged of trace heavy metals. A fraction of the most stable precipitated forms will stay in solution as colloidal particles and be transported to the oceans, along with their load of adsorbed heavy metals. The most stable colloids are likely to be iron and manganese oxides and clay particles, coated with humic material. These colloids will then carry an important part of the measured concentrations of dissolved copper, lead, cadmium and zinc in sea-water.[7,45–47] Most computer models of heavy metal speciation in sea-water have used silica as a model adsorbent.[48,49] Silica is not, however, nearly as powerful an adsorbent as a particle coated with humic acid, nor does it have the same adsorption capacity.[7,15,29]

The formation of simple, molecular organic complexes of bivalent heavy metals in sea-water is unlikely because of competition by chloride, magnesium, iron(III) and chromium(III). Even a ligand as powerful as EDTA would not complex significant amounts of copper, lead, cadmium or zinc unless present at concentrations unrealistically high for natural sea-water (Table 2).[45]

SAMPLING, FILTRATION, AND STORAGE OF WATER SAMPLES

The collection of a natural water sample, even surface water, for trace metal analysis, is fraught with dangers of contamination.[7,50–53] The risk of contamination when collecting a depth sample is at least one order of magnitude greater. Yet unless this initial step is carried out correctly, many hours of expensive chemical analysis will be wasted.

Table 2. Calculated effect of EDTA on speciation of Cu, Pd, Cd and Zn in sea-water (pH 8.1)*

	Fraction of metal as EDTA complex, %			
EDTA, *M*	Cu	Pb	Cd	Zn
2×10^{-9}	0.3	0.3	<0.1	0.2
2×10^{-8}	4.8	5.2	0.7	2.9
2×10^{-7}	35	37	6.8	24

* Fe(III) = $2 \times 10^{-9}M$, Cr(III) = $1 \times 10^{-9}M$.

The type of sampling bottle and method of cleaning must first be decided. Conventional linear-polyethylene bottles are now almost universally used for collection of surface water samples. It has been suggested, however, that Teflon is the only material which will not yield zinc to a water sample.[54] Laxen and Harrison[55] reported that soaking linear-polyethylene bottles in 1.5*M* nitric acid for 48 hr at room temperature removed all Cu, Pb, Cd and Zn leachable by synthetic and natural river water, although a simple rinse with the acid was inadequate. They found less than 0.1 μg/l. zinc contamination from a cleaned polyethylene bottle. Mart[56] and Patterson and Settle[50] recommended that polyethylene bottles be first treated with hot acid before room-temperature acid leaching, but Nürnberg *et al.*[57] claimed that this treatment could activate the surface of the polyethylene and cause losses of metal by adsorption. For fresh water samples they found it necessary to remove these activated sites by first rinsing the bottle with a solution of calcium and magnesium sulphates. Mart[56] used 1*M* hydrochloric acid for the initial hot acid cleaning, although Laxen and Harrison[55] reported that polyethylene absorbs traces of HCl, which interferes in the carbon-furnace AAS determination of some metals, and in the determination of copper by ASV.

In our laboratory we soak new polyethylene bottles in 1.5*M* reagent-grade nitric acid for a minimum of one week. The acid is emptied before the sample is collected, then the bottle is filled and emptied with the sample water at least four times before retaining a sample. Surface samples are taken over the bow of a fibreglass boat which is moving into the wind or current. Samples collected from the stern of a large power boat are invariably contaminated with copper and lead. If natural waters of similar type are being collected at intervals, the bottles are not acid-rinsed between samples, but are simply rinsed several times with the new sample. We have not observed any changes in the concentrations of Cu, Pb, Cd or Zn in sea-water or fresh waters stored at 4° over periods of weeks in bottles treated in this manner.[36,47]

The collection of depth samples is a specialized task[51] and will not be discussed here. The all-Teflon Patterson C.I.T. sampler[50] is one of the few devices available which can collect deep sea-water samples without contaminating them with metals.[58]

For speciation measurements, the water sample must not be acidifed before storage, because this would change the speciation. It should also be kept in mind, for general trace-metal analysis, that acidification of fresh water samples can cause the precipitation of humic material, which usually carries down some heavy metals. Likewise, freezing of fresh water samples is not permissible. As a water sample freezes, metal ions are continuously concentrated in the unfrozen liquid remaining in the centre of the container. Irreversible hydrolysis and polymerization of iron and other metal ions can take place at these relatively high transient concentrations, and heating with acid may be necessary to return them to solution in the thawed sample. In sea-water, the high chloride concentration may prevent hydrolysis reactions from taking place, and freezing could be an acceptable method of preservation.[47] However, storage at 4° appears to be the safest method for both fresh water and sea-water samples.[36,47]

There has been a great deal of controversy over losses of trace-metal ions to polyethylene storage bottles.[7,51] Most of the disagreement can be traced to some workers using synthetic metal solutions and others using natural waters. In general, those testing synthetic solutions found serious losses[59–62] while, with the exception of Subramanian *et al.*,[54] those using natural sea-water and fresh waters found that the losses of most metals were negligible.[36,47,55–57] This result is not surprising. Natural waters are in equilibrium with many potent adsorbents, including sediments containing iron, aluminium, manganese and silicon oxides, and humic materials. The water also contains suspended particles of these adsorbents, which have very large internal surfaces available for adsorption.[30,43] It would be most remarkable then, if a polyethylene container, which has both a relatively small surface area and weak adsorbing power, could remove any of the ionic metal (usually a minute fraction of the total metal) which is in equilibrium with the suspended particles. The colloidal particles in natural waters show little tendency to adsorb on polyethylene or even Pyrex glass.[36,63] Free metal ions, on the other hand, adsorb strongly on glass and to a lesser extent on polyethylene. Figure 3a shows the effect of pH on the adsorption of ionic Cu, Pb, Cd and Zn from distilled water onto an empty borosilicate tube containing a 1-cm thick glass-wool support. Most of the adsorption took place on the glass-wool plug. A river water sample with similar concentrations of Cu, Pb, Cd and Zn lost a negligible amount of these metal ions to the empty column. The pH at which significant metal-ion adsorption first occurs on silica can be related to the value of the first hydrolysis constant (${}^*\beta_1$); $pH = p{}^*\beta_1 - 1.5$ is a semi-empirical expression for the onset of adsorption.[7] Adsorption onto organic material can, however, occur at a much lower pH. Figure 3b shows the adsorption of metals onto a 6-cm bed of Bio-Rad SM2 resin (equivalent to Amberlite XAD-2) in the same column, but with a polyethylene frit support.

Dissolved metal in a water sample is defined by convention as metal which can pass a 0.45-μm membrane filter. The filtration step arbitrarily separates particulate metal from metal in true solution plus that associated with colloidal particles (Table 1). Open-ocean sea-water samples rarely require filtration because the concentration of particulate matter is so low, but filtration is necessary for most fresh waters. Unless filtration is done under clean-room conditions (preferably in a laminar-flow cabinet), contamination of the sample can easily occur.[7,56] The membrane

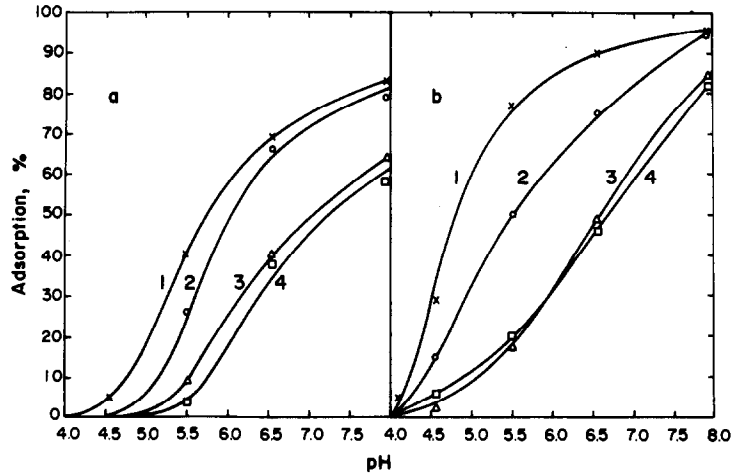

Fig. 3. Effect of pH on adsorption of (1) Cu^{2+}, (2) Pb^{2+}, (3) Zn^{2+}, (4) Cd^{2+} from distilled water; (a) empty column with 1-cm glass wool support, (b) same column, but with polyethylene frit support and filled with Bio-Rad SM2 resin.

filter must be carefully washed with acid and water before use, otherwise it can contribute both metal and organic matter to the filtrate.[64] Nuclepore membrane filters (polycarbonate) have the lowest metal content, but they block rapidly, and cellulose acetate membranes are more practical for most waters.[47,53] Polycarbonate (*e.g.*, Sartorius) filter assemblies are easier to decontaminate than glass assemblies (*e.g.*, Millipore). The filter apparatus must be cleaned with acid as carefully as the storage bottles. Pressure filtration under nitrogen is faster and less likely to result in contamination than vacuum filtration, but rupture of phytoplankton cells and contamination of the sample by their contents can occur with pressures above 0.25 atm gauge.[7]

Workers who used synthetic metal ion solutions[57,60,65] reported serious losses of several metal ions by adsorption on the membrane filter or the sintered-glass support of the Millipore filtration appartus. However, as in the case of storage containers, those using natural waters found no such losses.[35,56,57] Both river water and sea-water have been filtered through an all-glass Millipore apparatus, and the filtrate refiltered up to four times through the same filter, without any change in the Cu, Pb, Cd or Zn concentrations (Florence, unpublished results). Mart[56] found that sea-water containing as little as 15 ng of lead per kg could be filtered through a Sartorius cellulose acetate membrane without loss.

METHODS FOR THE MEASUREMENT OF TRACE-METAL SPECIATION

Trace-metal speciation in waters has been based on use of two distinctly different techniques: computer (chemical) modelling, and experimental measurement.

Computer modelling

The computer modelling approach to trace-metal speciation in waters involves the use of published stability-constant data, together with known concentrations of various ions and suspended solids in the water, to compute the equilibrium concentrations (or activities) of the various species. The rationale of chemical modelling was discussed by Jenne in the opening address to a symposium on chemical modelling held in Miami, Florida in 1978.[66] The proceedings of this symposium provide a wealth of information on the current status of the chemical modelling approach, and give details of the sophisticated computer codes now available. Gaizer[67] has also recently reviewed the computer evaluation of complex equilibria.

The main obstacle to the successful use of computer modelling of trace-metal speciation in waters is the lack of reliable thermodynamic data.[7] Just as an analysis is only as good as the sample, so a computer model is only as good as the data provided. Unfortunately, no reliable data are available for metal-ion interactions with many of the natural ligands, especially the colloidal particles present in natural waters. Indeed, even the nature and concentration of these ligands are usually unknown. In addition, there is still serious disagreement about the values for many of the simple inorganic complexes in waters.[7,66] The formation constant of the important copper species, $Cu(OH)_2$, is in dispute,[68] there is doubt about the importance of mixed-ligand complexes in sea-water,[69] and the effect of failure to correct for major ion-pair formation on the calculation of heavy-metal species concentration has been pointed out.[70] For these reasons, computer modelling results for heavy metal speciation in natural waters at present may bear little relation to reality.[7,22]

Computer modelling is a powerful technique, and will make an important contribution towards an understanding of speciation in waters once the necessary thermodynamic data are accumulated. There is an

urgent need to identify the important ligands in natural waters and to measure the relevant stability constants.

Experimental methods

The experimental determination of trace-metal speciation in natural waters is a most difficult task because of the very low concentrations of these metals. In contaminated waters, however, the analysis may be much easier because of the possibility of using ion-selective electrodes to determine the free metal-ion activity.[71] For natural waters, with presently available techniques, it is possible only to divide each metal into operational classifications or "boxes", based on the thermodynamic or kinetic behaviour of the different physico-chemical forms of the metal in the sample. Since the operations involved in measurements or separations will generally alter the original equilibria to some extent, the speciation results will be method-dependent, and it is important that full details of the analytical procedure be provided with any published speciation data.

The procedures mainly used to study trace-metal speciation in waters are anodic stripping voltammetry, ion-exchange chromatography, ultrafiltration, dialysis, and bioassay. Other analytical techniques are not as widely applicable.

Anodic stripping voltammetry. The theory and application of ASV to water analysis has been adequately reviewed.[7,57,72,73] Hanging mercury drop (HMDE) or thin mercury film (MFE) electrodes can be used. An MFE using glassy carbon as support for the mercury film provides the highest sensitivity,[57,74–76] although zinc determinations are more reproducible with an HMDE.[77] The use of a differential pulse waveform leads to lower detection limits with both types of electrodes; at a glassy carbon MFE the limit of detection for lead and cadmium is lower by a factor of about five when the differential pulse method is used instead of a simple linear voltage scan.[74] There are, however, subtle and sometimes serious problems associated with the use of differential pulse ASV for natural water analysis. Because it is a high-frequency method, the differential pulse signal is more sensitive to substances which alter the rate of the electrode reactions.[74,77] Surface-active compounds which adsorb on the mercury electrode may cause either an increase or decrease in peak height, and a shift in peak potential.[74,78–82] These effects are brought about both by natural substances such as fulvic and humic acids, and by some typical industrial pollutants, such as long-chain amines and alcohols. A more insidious phenomenon in differential pulse ASV is the production of tensammetric waves.[78] These are the result not of redox reactions, but of adsorption–desorption processes at the mercury electrode. In the past, tensammetric waves have been mistaken for metal-ion waves, and have led to the reporting of high results for Pb, Cd and Cu in sea-water. Natural organic compounds in most samples of sea-water give rise to tensammetric waves which, at pH 5, occur near the Pb and Cd waves.[83] These adsorption–desorption waves are eliminated when the sea-water is irradiated with ultraviolet light or treated with activated charcoal. They do not appear if a simple linear voltage scan is used.[83]

Another practical problem in the use of differential pulse ASV is that the test solution must have a fairly high ionic strength (0.02*M*) or consistent results are not obtained.[74] ASV measurements on river water samples without the addition of an electrolyte[21] or after buffering the solution by simply bubbling carbon dioxide through it,[22] may not be permissible.

The application of alternating current, differential pulse, and other high-frequency methods to the analysis of waters by ASV must be carefully studied for interference effects before results are reported. If it can be established that interferences are not present or can be eliminated, the higher sensitivity of the differential pulse method is a great advantage, especially for the analysis of sea-water.

Anodic stripping voltammetry is one of the very few analytical techniques that is sufficiently sensitive for direct determination of heavy metals in natural waters, and also has an intrinsic capability for speciation work. It has this ability because the amount of metal (M) deposited at the electrode during the deposition step depends, amongst other factors, on the rate of dissociation (k_{-1}) of complexed metal species (ML_n) at the electrode–solution interface;

$$ML_n \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} M + nL$$

The amount of M deposited also depends on parameters such as total metal concentration, deposition time, electrode area and temperature.[77] However, the contribution of a metal complex species to the ASV peak height (relative to that of the free metal ion) is independent of these parameters and depends only on k_{-1} and the thickness of the diffusion layer.[19] The diffusion-layer thickness decreases with increasing stirring rate, and dictates the period that the complex is "resident" at the electrode surface. The thicker the diffusion layer, the higher the contribution of a metal complex to the ASV wave height.[84–86]

These considerations have led to the widespread use in water analysis of measurement of "ASV-labile" metal, *i.e.*, the metal concentration determined by ASV at the natural pH of the water, or in mildly acid solution (acetate buffer, pH 4.7). Metal concentrations measured in this manner are usually lower than the total metal concentrations, and have been related to the metal fraction which is bioavailable.[7,17,36,87,88] Total metal can be measured by ASV after fuming the sample with nitric–perchloric acid mixture,[89] digestion with persulphate[90] or, preferably, exposure to ultraviolet irradiation.[91] It is likely that sea-water samples collected on a cruise, acidified to below pH 2, and stored for several weeks, will also yield total metal on analysis.

The ability of ASV to measure labile metal, plus its excellent sensitivity (about $10^{-10}M$[72,77]) for the four common toxic heavy metals, copper, lead, cadmium and zinc, which can be determined in the same analysis, make it a powerful tool for trace-metal speciation studies. Considerable care must be taken, however, in the intepretation of ASV measurements of labile metal since, as previously discussed, natural organic matter can have a drastic effect on the results.[23,92,93]

Ion-exchange. Ion-exchange is an attractive technique for trace-metal speciation in natural waters because separations can be carried out with little manipulation and little opportunity for contamination, a most important consideration when working with such low metal concentrations. Conventional cation- and anion-exchange resins have been used for heavy metal speciation,[7] but the iminoacetate chelating resin, Chelex-100 (Bio-Rad), has found much more application. Florence and Batley[45,94] first used this resin to separate ionic and colloidally-associated Cu, Pb, Cd and Zn in sea-water. Chelex-100 resin binds ionic metal strongly, but because it has a pore size of about 1.5 nm,[36] large molecules and colloidal particles are excluded from the resin beads and are not retained on the column. Florence[36] showed that solutions of colloidal hydrated ferric oxide and bulky organic dyes are quantitatively rejected by the resin. As reported earlier in this review (Batley and Florence, unpublished results), copper and lead adsorbed on an iron–humic acid colloid are not retained by Chelex-100. The resin therefore provides a simple, rapid, and almost contamination-free method for the separation of ionic and colloidally-associated (*i.e.*, pseudocolloid[62]) metal. This is an important function, because it is believed that these two fractions of the dissolved metal represent the main classes of toxic and non-toxic metal, respectively, in natural waters.[7]

Hart and Davies[95] used a batch equilibration technique with Chelex-100 to separate ionic metal from fresh and estuarine waters, and found this method useful for analysis of samples in the field. Figura and McDuffie[96] adopted a different approach. They used both batch and column equilibration techniques with Chelex-100 to vary the contact time of the water sample with the resin. By this method they were able to classify Cu, Pb, Cd and Zn in river water and sewage samples into the empirical groups of "very labile", "moderately labile", "slowly labile" and "inert", depending on the apparent rates of transfer to the resin. Cadmium and zinc were mainly in the very and moderately labile fractions, while significant percentages of copper and lead were classified in the slowly labile and inert fractions.

Chelex-100 resin has iminoacetate groups, which complex metals by oxygen and nitrogen bonds. This type of bonding may not, however, simulate the critical reaction between metals and biomembrane proteins, and hence may not serve as the best model for bioavailability and toxicity.[23] Passage of heavy metals across biological membranes probably occurs principally by carrier-mediated transport with metallothionein-type proteins to which the metal is complexed by sulphur (thiol) bonds.[97–101] Measurement of the metal fraction which reacts with a thiol resin may therefore be a more realistic estimate of bioavailable metal.[23] Thiol resins have been described by Phillips and Fritz[102] and Slovák *et al.*,[103] and a porous glass with bound thiol groups is available from Pierce Chemical Company, Illinois. All three thiol materials effectively remove ionic Cu, Pb, Cd and Zn from both sea-water and fresh waters (Florence and Batley, unpublished results). Results for some waters are shown in Table 3, where the effectiveness of Chelex-100 resin and the thiol porous glass are compared. It is evident that the thiol material has a somewhat lower affinity for Pb, Cd and Zn, but is much more efficient than Chelex-100 for the removal of copper. This is because of the relatively higher affinity of copper for sulphur ligands.[104] Table 4 shows that there is a much greater difference between the complexation constants of copper and the other three metals when the ligand has a sulphur donor atom, than when the ligand is iminoacetate.[105] The preference of copper for suphur ligands and their ready reduction of copper(II) to copper(I) may partly explain the very high toxicity of copper towards aquatic organisms.

Table 3. Removal of metals from sea-water and river water by Chelex-100 resin and thiol porous glass

Sample and adsorbent	Residual metal, %			
	Cu	Pb	Cd	Zn
*Sea-water,				
Chelex-100	2	<0.5	<0.5	<0.5
Thiol glass	<0.2	1.0	0.6	1.1
†River water,				
Chelex-100	75	45	18	60
Thiol glass	21	48	25	60

* Spiked with $1 \times 10^{-7}M$ Cu, Pd, Cd, Zn.
† Sewage-contaminated, unspiked.

Ultrafiltration. Ultrafiltration membranes are commercially available with apparent pore diameters from 1 to 15 nm, and can be used to separate molecular from colloidal species, or to classify colloidal particles on the basis of molecular size.[39,106] Although this appears to be a very useful technique, there are some problems. (*a*) The membranes are expensive, (*b*)

Table 4. Equilibrium constants for some metal complexes

	$\log K_1$			$-\log K_{s0}$
	EDTA	Iminodiacetic acid	Glutathione	Sulphide
Cu	18.5	10.6	14.6	49
Pb	17.7	7.5	10.6	28
Cd	16.5	5.7	10.5	28
Zn	16.5	7.1	8.3	25

The filters as supplied are contaminated with metal and soluble organic matter. Laxen and Harrison[22,107,108] reported serious adsorption of metal ions on many types of filters, but found that the Amicon PM10 filter exhibited negligible adsorption. They pretreated the filters with 0.1*M* calcium nitrate to minimize losses. Contamination or loss in ultrafiltration is especially serious, because the volume of filtrate is small, and there is a large surface-to-volume ratio. (*c*) Metal combined with large organic molecules or adsorbed on colloidal particles may be released and pass through the filter as a result of dissociation of the complex under the concentration gradient existing at the surface of the membrane filter.[20,109]

Despite these problems, ultrafiltration is certainly worthy of further study for the speciation of trace metals and trace organics,[39] particularly if correlations can be obtained between the size fractions and biological activity.

Dialysis. Beneš and co-workers have carried out much of the work on the application of dialysis to trace-metal speciation in waters.[62] Dialysis membranes commonly have pore diameters of 1–5 nm, and can effectively separate molecular and colloidal species. Like ultrafilters, dialysis membranes suffer from metal contamination, metal adsorption, and dissociation of metal complexes at the membrane surface. Dialysis, moreover, is a much slower process than ultrafiltration. Beneš[110] described an interesting semi-continuous method for monitoring dissolved ionic metal in a stream. A dialysis cell containing pure water is immersed in the stream, and ionic species diffuse into the cell, where they are removed continuously by, *e.g.*, adsorption on a suspended adsorbent. If the concentration of a dissolved species in the cell is kept very low by this method, the diffusion flow is directly proportional to the concentration of the species in the stream. This system is similar in principle to that of Hart and Davies.[111]

Bioassay. Heavy metals are toxic to a wide range of aquatic animals.[112–116] Based on the free metal-ion concentration, the order of toxicity to a marine diatom was found[116] to be

$$Hg^{2+} > Ag^{+} > Cu^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+} > Tl^{+}$$

but in the presence of different complexing agents, this order can change dramatically. Poldoski[117] found that humic acid, pyrophosphate, and aminopolycarboxylic acids reduced the uptake of cadmium by *Daphnia magna*, but that diethyldithiocarbamate increased its uptake. Rathsack and Lohs[118] found that triethanolamine increased the accumulation of copper by *Chlorella pyrenoidosa*. Ligands which increase metal accumulation and toxicity in aquatic animals probably do so by forming lipid-soluble complexes with the metals. In waters of very high organic content, the addition of low concentrations of some essential heavy metals may actually increase productivity.[119] Bivalve molluscs and other filter feeders appear to accumulate larger amounts of some metals if the metal is in particulate, rather than ionic, form.[120]

Most workers who have studied the effect of speciation on the toxicity of metals towards aquatic organisms used chemical modelling to calculate the concentrations of the different chemical species in the test tanks.[121–128] Young *et al.*[17] found that ASV-detectable copper species are the forms most toxic to shrimp zoneae, and Sunda *et al.*[129] used a cadmium ion-selective electrode to show that the toxicity of cadmium to grass shrimp is related to the free cadmium-ion activity. The modelling studies generally suggest that the most toxic forms of copper are the free cupric ion and its hydroxo complexes; carbonato complexes were found non-toxic.[122,128,130] Guy and Kean[126] suggested that the ethylenediamine and citric acid complexes of copper are also toxic to algae.

An important problem with bioassay–computer model studies of toxicity is that they cannot adequately take into consideration the effects of adsorption of the test metal on the container, the test animals and suspended particulate matter. Nor can they accurately compute the complexing of the metal by excretion products of the animal.[131]

Other methods. Ion-selective electrodes are generally too insensitive for the measurement of heavy-metal ion concentrations in natural waters. The copper ISE has been applied to sea-water,[132] but the determination is fraught with difficulties, and there are now doubts about the selectivity of the response of the electrode to cupric ion activity. The electrode may be unsuitable for use in chloride media,[133] and appears to respond to certain natural and synthetic complexing agents[134] and to hydroxo and bicarbonato complexes of copper.[135]

Gel chromatography is unsuitable for the speciation of natural waters because the relatively small sample volume and large eluent volume lead to dilution of the sample and large blank values. For sewage or polluted waters, however, gel chromatography may be an attractive method for separating metals into molecular size fractions.[136] Gas chromatography, with an atomic-absorption spectrometer as detector, is a powerful technique for the separation and determination of volatile organometallic compounds of metals such as lead, mercury and arsenic.[137]

The application of radiotracers to speciation studies is hindered by the slow equilibration of the ionic radiotracer with the non-ionic forms of the metal naturally present in the water sample. This equilibration period may be days, months or even years,[89,138,139] and underlines the danger of using standard-addition techniques in trace-metal speciation analysis.[7]

Determination of organically-bound metal

The measurement of the metal fraction which is

complexed by organic ligands, or adsorbed on organic colloidal particles, is an important requirement of any comprehensive speciation scheme. Some organometallic complexes may be directly lipid-soluble and constitute a toxicity hazard, while other organic compounds may be involved in a significant mechanism for metal transport.

Only a limited number of methods can be used for the measurement of organically-bound metal. Some rest on the premise that metals can be selectively liberated from organic complexes. The most successful techniques are ion-exchange and solvent extraction, ozone oxidation, and ultraviolet oxidation carried out at the natural pH of the sample. Oxidation in acid solution with chemicals such as persulphate and nitric or perchloric acids liberates metals from inorganic species[7,91] as well as from organic species.

Slowey *et al.*[140] used chloroform extraction to measure organically bound copper in sea-water, but chloroform is a poor choice for this analysis, because even analytical-reagent grade chloroform is badly contaminated with copper, and complete decontamination is difficult. Solvent extraction would probably not separate organic colloidal particles, which may be expected to accumulate at the liquid interface.

Ozone oxidation does not destroy all organic matter in natural waters[91] and, in any case, serious losses of lead can occur during ozone treatment, presumably as a result of the formation of lead(IV) and precipitation of PbO_2.[22]

The destruction of organic matter by ultraviolet irradiation at the natural pH of the sample has been the method most frequently used.[7,22,36,89,141,142] Florence[36] found that it could not be applied to anoxic fresh waters because oxidation of Fe(II) leads to the precipitation of hydrous ferric oxide, which carries down trace metals. Laxen and Harrison[22] and Blutstein and Smith[143] reported that brown deposits formed on the bottom of the tubes when river water samples were irradiated, and that low results for Cu, Pb, Cd and Zn were obtained after ultraviolet irradiation. This problem was not encountered by Florence,[36] but he was analysing river waters of a much lower iron content. In the speciation scheme used by Florence[36] for fresh waters, and Batley and Florence[89] and Batley and Gardner[47] for sea-water, total metal was always measured both before and after ultraviolet irradiation. No significant differences were found for any of the water samples analysed. However, when a colloidal solution of a hydrous iron(III) oxide coated with humic acid (Fe 4 mg/l.) with adsorbed Cu, Pb and Cd was irradiated, a brown precipitate and losses of the trace heavy metals to the precipitate were observed (Batley and Florence, unpublished results).

Humate-protected iron colloids may be the predominant type of colloid in many natural waters,[23,144] and humic acid appears to be essential for stability;[145] destruction of humate by ultraviolet irradiation leads to the precipitation of hydrous iron oxide. Problems[22] arising from the irradiation of fresh waters containing high concentrations of this type of colloid are a serious drawback to the general use of ultraviolet irradiation in speciation schemes, although there are apparently no difficulties with the irradiation of sea-water.

In an attempt (Florence and Batley, unpublished results) to prepare an artificial lipid membrane for measurements of solubility in lipids, Millipore prefilter cellulose discs (type AWO3) were soaked in an ethanolic solution of a mixture of cholesterol, phosphatidyl choline and phosphatidyl ethanolamine,[146] then dried. The discs were shaken with water samples to study the forms of metals which they extracted. However, serious difficulties were encountered with heavy metal impurities in the phospholipids, and adsorption of ionic metal by the membrane filter. These artificial membrane systems are too difficult to purify and too unstable for use in a routine speciation scheme. The directly lipid-soluble metal fraction is best determined by using a simple, reproducible extraction system, especially since the permeability coefficient of biomembranes is so critically dependent on the composition of the lipid bilayer, which varies from species to species.[97]

Bio-Rad Bio Beads SM2 resin (similar to Amberlite XAD-2 resin[147]) is a neutral, non-polar macroporous styrene–divinylbenzene copolymer that has a high affinity for molecules that contain both hydrophilic and hydrophobic moieties. It has a molecular-weight exclusion limit of 1.4×10^4. The material as supplied has little leachable metal impurity, is easily purified and is simple to use. A less convenient simulation model for the lipid bilayer is extraction with hexane–butanol mixtures.[146] Residual oganic solvent in the aqueous phase must be removed completely before ASV analysis, by evaporation followed by ultraviolet irradiation. Some results for the removal of organometallic complexes from water by SM2 resin and 10% tert.-butanol in hexane are shown in Table 5. Because ionic metal is strongly adsorbed by SM2 resin at pH values higher than 4.5 (Fig. 3b), the water samples were adjusted to pH 4.0 before passage through the column. This pH adjustment may, of course, alter the

Table 5. Extraction of Cu, Pb, Cd and Zn complexes from sea-water (pH 4.0) by Bio-Rad SM2 resin and hexane–10% tert.-butanol*

Extractant	Ligand	Extraction, %			
		Cu	Pb	Cd	Zn
SM2 resin	Tannic acid	22	3	0	0
	APDC†	79	89	86	92
	Fulvic acid	0	0	0	0
	Humic acid	100	45	0	0
Hexane–butanol	Tannic acid	0	0	0	0
	APDC†	89	78	77	80

* $1 \times 10^{-7} M$ metal, $2 \times 10^{-5} M$ ligand.
† Ammonium pyrrolidinedithiocarbamate.

Table 6. Average recent values for the concentration of some metals at various depths in open ocean water.

Metal	Concentration, *ng/l.*		
	Surface	0.5 km	2 km
Cu	120	100	220
Pb	14	12	2
Cd	15	75	75
Zn	10	300	600
Ni	150	270	400
Co	5	<3	<3
Fe*	750	275	500
Mn	500	—	—
Cr	100	—	—
Hg	8	8	8

* Dissolved iron.

equilibrium concentration of complexes, so the determination with SM2 resin is, like all speciation measurements, operationally defined.

Activated charcoal has been used to extract both ionic and organically-complexed copper from sea-water,[148] but the recovery is not quantitative and the activated charcoal is difficult to purify from heavy metals.

SPECIATION RESULTS FOR SELECTED ELEMENTS

The concentrations of some metals in surface sea-water, averaged from results recently reported[7] by laboratories experienced in this area of analysis, are shown in Table 6. Some of these results are in dispute, especially those for zinc, and they will be discussed in the appropriate sections below.

Copper

Two recent comprehensive reviews have discussed the speciation of copper in natural waters.[7,149] Nriagu's two-volume compilation[149] contains a wealth of information on the chemistry, determination, speciation and toxicity of copper.

Computer modelling programs[150] suggest that the predominant inorganic forms of copper in sea-water are $Cu(OH)_2$ (40%) and $CuCO_3$ (50%), and in a typical river water, $CuCO_3$ (95%). In sea-water, up to 50% of the total copper may be associated with organic matter,[7,47,68,89] and a high percentage combined with inorganic colloidal particles.[47,151] Moore and Burton,[152] however, failed to detect any significant amounts of organically-associated copper in the eastern Atlantic Ocean. Because of the strong adsorption of copper on flocculated particles of humate-coated iron oxide during estuarine mixing,[145] it seems most likely that pseudocolloids of copper, both organic and inorganic, will be present in the oceans. In fresh water streams the percentage of organically-bound copper may be very high,[7,36,130,149,153] and in both types of waters the ASV-detectable fraction of copper (similar in amount to the toxic fraction) is usually much less than 10%.[7,9,149]

Analytical chemists should be aware that acidification of fresh water samples for preservation may lead to the precipitation of humic material, which carries down with it a considerable fraction of the copper content of the water.[145]

Lead

Computer models predict[150] that the two main inorganic species of lead in sea-water are $PbCO_3$ (83%) and $PbCl_2$ (11%), while in river water the only significant form is $PbCO_3$ (91%). It is possible, however, that basic carbonates such as $Pb_2(OH)_2CO_3$ may actually be present.[7]

Chau and Lum-Shue-Chan[87] found that in 16 out of 17 Canadian lakes studied, no lead was present in the ASV-detectable form. Beneš *et al.*[154] in a study of the Litávka River, Czechoslovakia, reported that it contained no soluble acid-exchangeable forms of lead. Lead is readily adsorbed on inorganic adsorbents, and Batley and Gardner[47] found that in sea-water 40–80% of the lead was associated with inorganic colloids.

Ionic lead spikes added to sea-water and fresh waters at the natural pH equilibrate very slowly with the lead species already present. Batley and Florence[89] found that after three days at 25°, only 20–50% of the added lead had chemically exchanged with the lead in sea-water, and Beneš *et al.*[155] showed that pseudocolloids of lead exist when the pH of fresh water is greater than 4. Harrison and Laxen[156] investigated the physico-chemical forms of lead in drinking water, and concluded that filterable lead was associated with both inorganic and organic colloidal particles. In general, lead does not show the marked preference for organic over inorganic adsorbents that copper does.[7]

Batley[157] described an *in situ* electrodeposition method for depositing labile lead in sea-water onto an atomic-absorption spectrophotometric carbon furnace tube, which was used as the working electrode. The tube was then inserted into an AAS instrument and lead determined in the usual manner. This method has the advantage of long unattended deposition times and limited opportunity for contamination.

There is evidence that tetra-alkyl-lead compounds can be formed in natural aquatic sediments.[158,159] If the presence of these organolead compounds can be confirmed, it will have serious implications for man-made lead pollution of waterways, because tetra-alkyl-lead compounds are considerably more toxic than inorganic lead salts. Settle and Patterson[160] have summarized the evidence for environmental lead toxicity in humans, and pointed out the massive increase in the lead content of the human body in modern urban man. Recent results on the lead concentration in blood from tribesman in remote areas of the Himalayas[161] and New Guinea[162] showed that

the urban populations of techologically-advanced countries have blood-lead concentrations at least a factor of five higher than those of these isolated, non-industrialized tribes.

Cadmium

In sea-water, cadmium is computed to exist as the $CdCl^+$ and $CdCl_2$ complexes (92%), while in river water the dominant forms are Cd^{2+} and $CdCO_3$, depending on pH.[150] Experimental measurement of the speciation of cadmium in sea-water[47] showed that ASV-detectable forms were predominant, while in a fresh water stream, 70–90% of the cadmium was present as the free hydrated ion or other labile complexes.[36] Batley and Gardner[47] reported that 44% of cadmium in a deep, anoxic region of an estuary existed as an non-electroactive inorganic species, possibly[150] $CdHS^+$. In general, cadmium requires a significantly higher pH than copper or lead for adsorption on organic or inorganic particles.[7]

The speciation[7] and ecological cycling[163,164] of cadmium have been reviewed recently. Bruland *et al.*[165] found that the cadmium concentration in Northeast Pacific waters increased rapidly with depth, and was correlated with phosphate and nitrate concentration at all depths. They found no evidence for colloidally-associated cadmium.

Bruland *et al.*[165] suggested that microplankton and their organic decomposition products play a dominant role in the biogeochemical cycling of cadmium. Benayoun *et al.*[166] studied the flux of cadmium through the euphausiid *Meganyctiphanes norvegica* and found that the metal was most concentrated in the viscera; faecal pellets accounted for 84% of the total cadmium flux through the euphausiid.

Zinc

Computer models[7] of sea-water suggest that inorganic zinc is divided between Zn^{2+} (27%), chloro complexes (47%) and $ZnCO_3$ (17%) whereas in a typical fresh water the main species are Zn^{2+} (50%) and $ZnCO_3$ (38%).

The speciation of zinc in natural waters has recently been reviewed by Florence[46] and Florence and Batley.[7] These reviews emphasized the current confusion about the correct value for the concentration of zinc in sea-water. Most analytical chemists who determine zinc in surface sea-water find concentrations of 1–4 μg/l.; values within this range have been reported recently by workers using a wide variety of analytical techniques, including ASV, AAS, inductively-coupled plasma emission spectrometry, isotope-dilution spark-source mass spectrometry, and neutron-activation analysis.[7,46,167] Recently, however, Bruland *et al.*[151,168] and Martin *et al.*[169] reported a classical study of zinc in the Pacific Ocean, in which they found only 8.5 ng/l. at the surface, increasing to 600ng/l. at a depth of 3 km. Bruland *et al.*[168] believed all previous results to be high as a result of contamination during sampling and analysis. Table 7 is a compilation of some recent results for the determination of zinc in sea-water by chelation extraction–carbon furnace AAS, together with the total method blanks reported by the authors.[170–175] The extremely small total blank of 2 ng/l. measured by Bruland *et al.*[151] is lower by factors of 30–435 than the blanks given by the other workers.[170–175] Since the zinc blank in the reagents used in this procedure[174] is equivalent to 1–3 ng/l. in the test sample, Bruland *et al.* must have been able to carry out the complete analytical procedure with virtually zero contamination from extraneous sources such as apparatus, atmosphere and clothing. This is a remarkable achievement considering the ubiquitous nature of zinc contamination.[46]

Accepting the accuracy of the Bruland results[168] for the location sampled, an explanation is needed for the narrow grouping of zinc results (1–4 μg/l.) for surface sea-water found by many different analysts in different countries by different techniques.[7] If all values above 10 ng/l. for surface sea-water were due to random contamination during sampling and analysis, a much wider spread of results would be expected. It is very likely that coastal sea-water sampled near sewage outfalls has an elevated zinc concentration. Since thousands of tons of zinc per year can be introduced into a coastal region from this human source,[169] the effect may be detectable at a great distance from the outfall. Martin *et al.*[169] have suggested that measurement of the zinc/silicon ratio could be

Table 7. Blanks reported for chelation/extraction/atomic-absorption spectrometric determination of zinc in surface sea-water

Source of water	Extraction system*	Total zinc, *ng/l.*	Method blank, *ng/l.*	Reference
Baltic Sea	APDC–DDDC–Freon	3000	870	170
Atlantic Ocean	APDC–MIBK	3600	620	174
Indian Ocean	APDC–DDDC–Freon	600	100	171
Atlantic Ocean	DT–chloroform	373	90	172
Gulf of Aden	APDC–DDDC–Freon	10,800	70	173
Pacific Ocean	APDC–MIBK	30	60	175
Pacific Ocean	APDC–DDDC–chloroform	10	2	151

* DT = dithizone; APDC = ammonium pyrrolidinedithiocarbamate; DDDC = diethylammonium diethyldithiocarbamate; MIBK = methyl isobutyl ketone.

used to detect man-made zinc pollution. Another possible origin of high zinc values, that would be common to workers in different countries, is contamination from polyethylene sampling bottles. Both Subramanian *et al.*[54] and Landy[176] reported that polyethylene containers slowly released zinc to water samples. Landy found that the zinc concentration of ultrapure water stored in 500-ml acid-cleaned polyethylene bottles increased from 0.02 to 1.7 μg/l. over a period of days. No increase was found for copper, lead or cadmium.

The average concentration of zinc in the oceans can be estimated from the relationship between the water–rock partition coefficients[K_Y(SW)] and electronegativity functions (Q_{YO}) of the elements.[177] From the regression line calculated by Turner *et al.*[177] for the log K_Y(SW) and Q_{YO} values of 56 elements, and the crustal rock abundance of zinc (127 μg/g),[177] a concentration of 780 ng/l. is predicted for zinc in sea-water. This result is remarkably (and perhaps fortuitously!) close to the concentration of 600 ng/l. found by Bruland *et al.*[168] at depths below 1 km.

The 60-fold decrease in zinc concentration from deep to surface sea-water is probably a result of biological activity in the euphotic zone. Algae show concentration factors (based on a sea-water value of 500 ng/l.) of 10^4–10^7 for zinc,[178] and would act as a most efficient scavenger of this metal in surface water, releasing it in deeper water as they die and decompose. Anderson *et al.*[179] have, in fact, suggested that zinc may be a limiting nutrient for the growth of coastal diatoms. The surface-water zinc concentration may depend on the phytoplankton density.

Florence and Batley[45] found that approximately 50% of the zinc in surface coastal Pacific Ocean water was ASV-detectable and extractable by Chelex-100 resin, and that only 23–59% could be extracted by ammonium pyrrolidinedithiocarbamate at pH 4.5, even though ionic zinc spikes were completely extractable at this pH. When the sea-water was acidified to pH 0.7, heated, cooled, and adjusted to pH 4.5, all the zinc could be extracted. Fukai *et al.*[180] were able to extract 13–90% of zinc from Mediterranean coastal sea-water with dithizone; the extractable zinc concentration depended on the time of the year.

Florence[36] analysed three samples of soft, unpolluted river water (pH 6.0–6.1) containing 2–6 μg/l. and found that the zinc was completely absorbed by a Chelex-100 resin column. The speciation was simple, being equally divided between ASV-detectable species and inorganic species (possibly basic carbonates) which were electrochemically inactive, but decomposed by the Chelex-100 resin. Hart and Davies,[95] however, found that in the polluted Yarra River, Australia (total Zn 65 μg/l.) only 50% of the zinc was exchangeable with Chelex-100, and Figura and McDuffie[181] reported that only 34% of the total zinc (7.4 μg/l.) in a sample of Susquehanna River water was removed by the chelating resin.

Great care must be taken with analysis for zinc at the μg/l. level. Because zinc is a component of so many materials present in the laboratory, particularly paints, talcs, rubber and human skin, and its compounds are relatively soluble, contamination of samples is a greater problem than with most other heavy metals. An efficient clean-room, or at least a laminar-flow clean hood, is essential for the determination and speciation of this element in natural waters.

Iron

Computer models[150] suggest that iron(III) exists almost entirely as $Fe(OH)_4^-$ in both sea-water and fresh waters. River water contains a high concentration of iron, which exists principally as negatively-charged iron oxide–organic matter colloids.[7,16,22,62,95,145] When sea-water and river water mix in estuaries, these colloids are neutralized and flocculated, carrying down with them most of the other heavy metals.[16,145,182,183] Some low molecular-weight colloidal particles of iron oxide stabilized with humic acid escape this estuarine scavenging and enter the ocean.[7]

Ellaway *et al.*[184] found that iron was present in the Yarra River (Australia) in mainly (>90%) non-ion-exchangeable forms, and that filterable iron decreased with increasing salinity. Mill[185] was able to recover 5–53% of the total filterable iron (7–500 μg/l.) in southwestern English rivers as a non-dialysable, macromolecular form (m.w. $>10^4$). He suggested that much of the dialysable iron was present as fulvic acid complexes. Sholkovitz[145] showed that of the bivalent metal ions, copper(II) is the most strongly adsorbed on iron–humic acid colloids.

Picard and Felbeck[186] used cation-exchange measurements to show that low concentrations of humic acid strongly influence the ability of marine sediments to react with metal ions. Their study suggests that humic acid is a transporting agent for iron and trace metals in a marine environment. Sugimura,[187] using adsorption on Amberlite XAD-2 resin, likewise obtained results which indicated that 80–90% of filterable iron in sea-water is associated with organic matter.

Thermodynamic calculations[188] show that in all oxygenated natural waters, iron(III) should be the predominant oxidation state at pH values above 6.

Chromium

Because chromates are used extensively in water treatment, and chromium(VI) has a much higher toxicity than chromium(III),[189] most interest in chromium speciation has centred on the differentiation between Cr(III) and Cr(VI).

Most methods for separating the two chromium oxidation states involve ion-exchange, although co-precipitation and liquid–liquid extraction have also been used.[7,190] The ion-exchange methods assume that Cr(VI) exists as anionic species (CrO_4^{2-} or $Cr_2O_7^{2-}$) and that Cr(III) is entirely cationic. This is,

however, a questionable assumption, since Sibley and Morgan[48] calculated that in both sea-water and fresh waters the dominant ionic form of Cr(III) is $Cr(OH)_4^-$. Yamazaki *et al.*[191] found that in the neutral pH region, Cr(III) associates with humic acid and other organic substances of pond sediment to form uncharged or negatively charged compounds. Ion-exchange resins can reduce Cr(VI); Lee[192] found it necessary to treat Dowex 50-1X with alkaline peroxide and then dichromate solution before using it to separate Cr(III) from acid pickling solutions.

Batley and Matousek[193] used a novel electrodeposition technique to measure chromium speciation in natural waters. At pH 4.7 and a deposition potential of −1.8 V *vs.* SCE, both Cr(III) and (VI) are reduced to the metal and deposited onto a graphite AAS furnace tube. At the same pH, but at a deposition potential of −0.3 V *vs.* SCE, only Cr(VI) is deposited. In sea-water (total Cr 0.25 μg/l.) and saline river water, Cr(VI) was found to be the dominant form. Cranston and Murray[194] also reported that, of the 0.17 μg/l. in Columbia River water, 98% was present as Cr(VI).

Nickel and cobalt

Computer models suggest that in both sea-water and fresh water, both nickel and cobalt exist mainly as the aquo ion and carbonato complex,[150] but there have been very few experimental speciation studies of these metals in natural waters.[7] Batley and Matousek,[195] using an electro-deposition–AAS technique, found that about 70% of both nickel and cobalt in estuarine samples was ASV-detectable at pH 4.7. Beneš and Steinnes[196] showed that in some Norwegian lakes and rivers less than 40% of the cobalt was dialysable, suggesting a high concentration of colloidal or particulate species.

Sholkovitz[145] used simulated estuarine mixing experiments, and found that 40% of riverine nickel (total 0.28 μg/l.) and only 11% of riverine cobalt (total 0.25 μg/l.) was flocculated on mixing with sea-water, whereas 95% of the iron was precipitated.

Manganese

The natural-water chemistry of manganese is dominated by non-equilibrium behaviour. The main form of Mn(II) in both sea-water and fresh waters is computed to be the free hydrated ion, although manganese(IV) oxide is expected to be the stable form in sea-water and high-pH fresh waters.[7,150] The oxidation of Mn(II) in sea-water is, however, an extremely slow process, and this kinetic effect could account for the disagreement in results reported on the speciation of manganese in natural waters.[7,197] Angino *et al.*,[198] using electron spin resonance, found that Mn(II) was present in a fresh water stream (pH 8.5), and that this ion was not in redox equilibrium with Mn(IV). They suggested that $Mn(H_2O)_6^{2+}$ is the dominant species in rivers at pH up to 6.3, while above pH 8.0 some insoluble Mn(III) species are formed. Sholkovitz[145] showed that manganese behaved very differently from iron in his simulated estuary mixing experiments; only 25–45% of the manganese flocculated, compared with 95% of the iron. Knox and Turner[197] found that, in samples from the Tamar Estuary (S.W. England), polarographically-detectable manganese varied, over a 6-month period, from <10% up to 100% of the total manganese (31–252 μg/l.). They suggested that the inactive fraction consisted of colloidally-associated manganese.

Tin

The speciation of tin in natural waters is important, not only because of the large amount of tin mining carried out, but also because of the increased use of toxic organotin compounds, such as tri-n-butyltin oxide and tri-n-butyltin acetate, in antifouling paints.[199] Craig[200] discussed the likelihood of the environmental methylation of tin, possibly through preliminary complexing of tin with cobalamin. Alkyltin compounds have been detected in several lakes and rivers,[200] but they appear to be of man-made origin. There is, as yet, no direct evidence that tin compounds can be methylated under environmental conditions.

Macchi and Pettine[201] used ASV to measure the speciation of tin in natural waters, and obtained results which suggested that at pH 8 the predominant form is $SnO(OH)_3^-$, while at lower pH values it is $SnO(OH)_2$. Florence and Farrar[202] used a preliminary separation of tin as $SnBr_4$, followed by ASV, to determine tin in sea-water and marine organisms. Coastal surface sea-water was found to contain 0.3 μg/l. as dissolved tin, with less than 0.02 μg/l. associated with particulate matter.

Mercury

About 10^4 tons of mercury are released into the atmosphere each year by the burning of fossil fuels, the smelting of sulphide ores, cement manufacture, and the heating of other materials containing mercury.[203] Other industrial operations add huge amounts of mercury to the oceans and rivers.[204]

Although mercuric ion is one of the most toxic metal ions,[7,205] methylated forms of mercury are even more toxic, and can be concentrated from water or through the food chain by virtue of their high lipid-solubility. Aquatic sediments readily oxidize metallic mercury to Hg^{2+}, and certain organisms can rapidly methylate Hg^{2+} to mono- and dimethylmercury, perhaps even in the water column above the sediment.[206] For this reason, most of the research on the speciation of mercury in natural waters has been aimed at developing methods for the differentiation of organic and inorganic mercury species.

Computer modelling results predict that mercury(II) should exist entirely as chloro complexes in sea-water, and principally as hydroxo species in river water.[7] In open ocean water the mercury con-

centration is less than 10 ng/l., but may be higher in coastal waters affected by sewage discharge.[7,204] Mercury in estuaries, lakes and rivers can vary from as low as 2 ng/l. to as high as 500 ng/l. for polluted areas.[7,204] In estuaries and fresh waters a high proportion of the dissolved mercury appears to be associated with organic matter.[204] Seritti *et al.*[207] found 0.4–9 ng/l. of "reactive" mercury, and 1–30 ng/l. of "total" mercury (*i.e.*, after oxidation of organic matter) in surface sea-water.

The separation and determination of alkylmercury compounds in natural waters is usually carried out by GLC-flameless AAS,[208] although the cold-vapour AAS technique can be used alone by varying the reducing conditions to determine phenyl- and alkylmercury compounds.[209] Goulden and Anthony[209] found that in some lake waters as much as 20% of the total mercury is present as methylmercury.

Arsenic and selenium

The concentrations of arsenic and selenium in surface sea-water are 1.5 and 0.1 μg/l., respectively.[7] Both elements commonly exist in solution in two oxidation states: As(III) and (V), and Se(IV) and (VI). In sea-water it appears that the dominant oxidation states are As(V) and Se(IV), but in river waters the other two states may be important.[7] Organic forms of arsenic and selenium are also likely to be present in all natural waters.[210] Sanders and Windom[211] found that marine phytoplankton readily assimilate arsenic(V) and incorporate some of it into the cell. Most of the As(V) is reduced, methylated, and released to solution. They calculated that 15–20% of the total dissolved arsenic in sea-water is reduced by phytoplankton during blooms on the continental shelf.

The determination of arsenic speciation by selective hydride evolution and AAS has been proposed frequently,[7,212,213] although the accuracy of the method has been questioned.[210] Howard and Arbab-Zavar[213] described a method for the selective determination of inorganic As(III) and (V) and methyl- and dimethylarsenic species by the trapping of the arsine compounds and sequential volatilization into a heated quartz atomizer tube situated in the optical path of an atomic-absorption spectrometer. There was serious interference, however, from several metals.

Recently there has been a marked increase in interest in environmental selenium chemistry because of the strong negative correlation between cancer and the selenium concentration in diet.[214] Shamberger[215] has comprehensively reviewed the distribution of selenium in the environment.

Aluminium

Until recently there was very little interest in the environmental chemistry of aluminium, the metal generally being considered relatively non-toxic under all normal conditions.[216] This situation has changed, however, with the suspicion that aluminium is the agent causing dialysis encephalopathy in patients undergoing haemodialysis with domestic water supplies high in aluminium.[217,218] Also, aluminium is implicated as a metal toxic to aquatic life [possibly as the $Al(OH)_2^+$ species[12,219]] when unbuffered lakes become acidified by acid rainfall.[11,220,221]

The concentration of dissolved aluminium in open ocean water is about 0.5 μg/l., and in fresh waters can range from 2 to 100 μg/l.[7,222] Many natural organic compounds, *e.g.*, humic and fulvic acids, can release aluminium from soils. Hydes and Liss[223] used reaction with the chromogenic reagent lumogallion to study the forms of aluminium present in estuarine and fresh waters. Their results for most rivers suggested that dissolved aluminium was present as simple ionic species or adsorbed on colloidal clay particles.

Plutonium

Plutonium is present in sea-water as a result of nuclear-weapon tests. Aston[224] used a computer model for plutonium in sea-water to predict that Pu(IV) would be hydrolysed, and adsorbed by sediments and particulate matter. Plutonium(VI) should be soluble and present as the $PuO_2CO_3OH^-$ species. These predictions are in approximate agreement with the measurements of Schell *et al.*[225] and Nelson and Lovett.[226]

MEASUREMENT OF COMPLEXING CAPACITY OF NATURAL WATERS

Heavy-metal ions added to a body of natural water are eventually incorporated into the sediment.[227] Before they deposit in the sediment they are usually adsorbed or complexed by particulate or dissolved organic compounds. This "complexing" of toxic heavy-metal ions is of vital importance to the biota, because the "complexing capacity" of the body of water determines its ability to render pollutant metals inocuous. Since copper is the most toxic of the common metal ions, the complexing capacity for Cu^{2+} is normally measured. There are several methods in common use for measuring complexing capacity, and they may give quite different results. It is important therefore, when quoting complexing capacities, to state the method used and to give all essential procedural details. Even variations in the same method (*e.g.*, change in pH or electrode rotation speed for ASV) can significantly affect the complexing capacity value found.

Hart[228] and Florence and Batley[7] have recently reviewed methods for measuring the complexing capacity of natural waters. The method most frequently used is an ASV titration, where aliquots of a standard copper(II) solution are added to the test solution, and the ASV peak height for copper is measured after a suitable equilibration period.[228,229] A typical ASV titration of coastal sea-water[229] is shown in Fig. 4. Other methods used for determining copper-complexing capacity are[7,228] ion-selective electrode potentiometry, ion-exchange on resins[230] or manganese di-

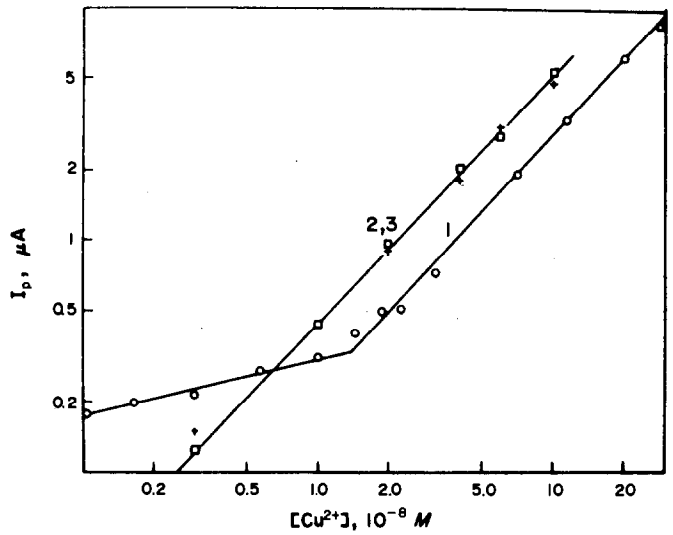

Fig. 4. ASV peak height *vs.* concentration plots for copper added to (1) sea-water, 0.015*M* acetate buffer, pH 4.8; (2) sea-water, 0.016*M* HNO_3; (3) 0.5*M* NaCl, pH 4.8. (From T. M. Florence and G. E. Batley, *J. Electroanal. Chem.*, 1977, **75**, 791. With permission.)

oxide, copper solubilization,[231] and bioassay. Copper ion-selective electrodes[232] are useful only[7,228] for detecting the end-point in the titration of a water sample with Cu^{2+}, and bioassays, while providing direct information on individual biological species,[233] are time-consuming and difficult to reproduce. Young *et al.*,[17] using larval shrimp as a test species, found good agreement between copper-complexing capacities measured by bioassay and ASV, whereas Srna *et al.*[234] reported that ASV gave values which were about half those measured by bioassay.

Some methods for determining complexing capacity also provide a simple measurement of the apparent stability constant ($^*K_1$) of the copper mixed ligand complex.[228,230,232,235,236] Values of log $^*K_1$ at pH 7 in lake or river water range[228] from 5.0 to 11.5. Sea-water and algal exudates provide stability constants within this range.[228] Hart[228] has pointed out that the commonest value of log $^*K_1$, 7.0 at pH 7, is very close to the formation constant of the copper–fulvic acid complex, and that several studies show that most of the copper-complexing ligands in fresh waters have molecular weights of 10^3–10^4.

A similar range of molecular weights has been measured for algal exudates,[131] which may dominate the complexing capacity of sea-water.[237]

The copper-complexing capacity of coastal sea-water[229] is usually in the range (1–5) × $10^{-8}M$, while rivers[228] often have values between 1 × 10^{-8} and 5 × $10^{-7}M$.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

It is inevitable that in the near future, water-quality legislation for heavy metals will include statements relating to their speciation. It may be possible to tolerate higher total concentrations of some metals as long as the labile fraction is below a certain limit. Measurement of complexing capacity may also have to be included in the water-quality criteria. In the same way, future legislation may require that the bioavailable fraction of trace elements and vitamins be measured as part of the routine analysis of foods.

Unfortunately, trace-metal speciation techniques have not yet been developed to the stage where any one method is completely acceptable for routine use. The measurement of ASV-detectable metal appears to approximate the bioavailable fraction,[17] but the term includes a wide range of techniques and procedures, and the measurement would need to be carefully defined to be internationally acceptable. The ASV technique, while almost essential for speciation analysis of saline waters, may possibly be replaced for fresh waters by equilibration with a chelating resin and measurement of the inert fraction by carbon-furnace AAS.

Potentiometric stripping analysis (PSA), developed by Jagner and co-workers,[238–240] may be more suitable than ASV for measurement of the electrochemically-active fraction of a metal. PSA is much less affected by organic matter adsorbed on the electrode than is ASV, which shows that organic matter influences the stripping, rather than the deposition step. A microprocessor-based PSA instrument has sensitivity similar to that of ASV.[240]

A trace-metal speciation scheme may be simple or complex, but it should provide the information required for the particular samples provided. If measurement of toxicity towards aquatic organisms is the purpose of the analysis, then the scheme should measure, as accurately as possible, the toxic metal fraction.[241] It should not be assumed that a chelating

resin of the Chelex-100 type is best for this purpose; thiol or other resins may be more suitable.[23] There is an urgent need for more studies of the relationship between analytical speciation schemes and direct bioassays.

A prime requirement of any speciation method is that it should require little manipulation and offer minimum opportunity for contamination. The concentrations of toxic heavy metals in natural waters are extremely low, and considerable experience is required before contamination of the sample can be consistently avoided. A laboratory must be able to demonstrate that it can routinely collect samples and measure accurately the *total* concentration of these metals in natural waters before it attempts speciation measurements. Surface sea-water from an unpolluted area is a convenient sample type to test the capability of a laboratory, because the normal concentrations of several heavy metals in sea-water are now well established (Table 6).

Computer modelling of trace-metal speciation is an attractive alternative to the difficult and tedious experimental methods. At present, however, modelling techniques are useful only for setting limits on speciation. More thermodynamic data are required on metal associations in natural waters, and the collection of these data is an urgent research need.

Humic and fulvic acids play a dominant role in trace-metal speciation in fresh waters, and probably also (through estuarine precipitation) in sea-water.[15] Humate-coated iron oxide and clay particles strongly adsorb trace heavy metals, and these "pseudocolloidal"[62,242] forms of the heavy metals are often the main dissolved species present in natural waters. More research is required to elucidate the composition, structure and behaviour of these pseudocolloids. Because of the potential toxicity of metal species which are directly lipid-soluble, improved methods for the determination of this metal fraction in natural waters should have high priority.[243–246]

Many trace-metal speciation results in the literature are undoubtedly seriously inaccurate because of contamination during collection of the sample or its subsequent analysis. This situation will continue until more laboratories establish trace-metal clean-room facilities, develop techniques for working at the ng/l. level, and train staff for this exacting area of chemical analysis.

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GLASSY-CARBON AMPEROMETRIC TRANSDUCERS AS ELECTROCHEMICAL DETECTORS IN LIQUID CHROMATOGRAPHY

THE INFLUENCE OF OXYGEN

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Summary—The electrochemical behaviour of oxygen on glassy-carbon electrodes and the suitability of this electrode material for electroreduction of organic compounds have been investigated. The observed oxygen overpotential on a glassy-carbon electrode was more negative than that on an amalgamated gold electrode, thus allowing the determination of easily reducible compounds such as polynitro-aromatics and quinones without the need for exhaustive removal of dissolved oxygen. The detection limits (3σ) were about 0.2, 0.8, and 2.5 pmole for polynitro-aromatics, mononitro–aromatics and quinones, respectively. Though the glassy-carbon material has a negative-potential limit about 250 mV more positive than that for the amalgamated gold electrode, and requires a longer equilibration time before use, it is more convenient for routine use.

The marriage of liquid chromatographic and electrochemical techniques has resulted in a great many useful applications in the last ten years, primarily to easily oxidized compounds. Progress in application to reducible substances has been slower, owing to the high background currents which can result from reduction of oxygen, hydrogen ions, and transition metal ions. Several factors must be considered when selecting the electrode material for use in such reductions, the most important being the oxygen and hydrogen overpotentials. The "operating range" of an electrode material is usually limited on the negative side by the reduction of hydrogen ions and residual oxygen, both of which can be influenced by metal deposition.

Mercury is the material of choice for most electrochemical reductions because it has a very large hydrogen overpotential, as shown in Table 1. Mercury-pool electrodes are generally not suitable as electrode materials in a thin-layer amperometric detector because they suffer from vibration and edge effects due to creeping of solution between the mercury and its container. In addition, they have poor tolerance for rapid movement of the solution. Though these effects are serious at low current densities (high sensitivity), mercury-pool electrodes have been used successfully for the determination of mercapto compounds at higher current densities.[1,2] The dropping mercury electrode (DME) detector for use in liquid chromatography was introduced in 1952 by Kemula, but its use was limited to a few academic laboratories. Recent improvements in the design of the DME detector have stimulated renewed interest and several promising applications have been reported.[3–5] The combination of liquid chromatography and electroreductive techniques has recently been reviewed.[6]

Solid electrode materials such as gold, platinum, and various forms of carbon have been used extensively to study oxidation processes:[7,8] however, their utility on the reductive side has been limited, owing to their small potential range, as summarized in Table 1. These limits should be used with caution because they were abstracted from several sources, and the experimental procedures and guidelines for their determination were not uniform.

MacCrehan,[9] and co-worker,[10] and we ourselves[11–13] initially focused attention on a mercury film on a gold substrate as electrode. As shown in Table 1, amalgamated gold and silver electrodes retain most of the desirable characteristics of liquid mercury, with only a small sacrifice in the available "potential window." The low oxygen overpotential of amalgamated electrodes has led us to investigate the electrochemical behaviour of oxygen at a glassy-carbon electrode (GCE) and to evaluate the suitability of this material for electroreduction. Several applications of glassy-carbon and mercury-film electrodes to trace determinations of reducible substances are described elsewhere.[11,13]

EXPERIMENTAL

Apparatus

A conventional liquid chromatograph (LC) with temperature control and electrochemical detection (model LC-304T from Bioanalytical Systems, Purdue Research Park, West Lafayette, Indiana) was equipped with a fixed

Table 1. Negative potential limits (V) for mercury and solid electrodes in various media

Electrode material	Electrolyte				Method*	Reference
	0.1M $HClO_4$	Acetate buffer pH 4–5	0.1M KNO_3 0.1M KCl	0.1M NaOH		
Platinum	−0.31	−0.50		−0.91		7
Platinum	−0.27			−0.96	LSV	18
Carbon	−0.32			−0.34	LSV	18
Gold	−0.37	−0.88		−1.28		7
Gold	−0.44			−1.23	LSV	18
Silver	−0.48	−0.70			LSV	19
Glassy carbon	−0.8					20
Glassy carbon		−0.8 to −1.0			LCEC	Present work
Pyrolytic graphite				−1.0	LSV	21
Carbon paste (mineral oil binder)	−0.90	−1.0	−1.1			6
Wax impregnated glassy carbon			−1.3			6
Ag/Hg on pure silver	−0.62 to −0.94	−0.8 to −1.21			LSV	19
Ag/Hg on old amalgam	−1.02	−1.27			LSV	19
Au/Hg		−1.2 to −1.3			LCEC	22
HMDE	−1.07	−1.30			LSV	19
Hg		−1.46			CV	23

*LSV—linear sweep voltammetry; CV—cyclic voltammetry; LCEC—liquid chromatography with electrochemical detection.

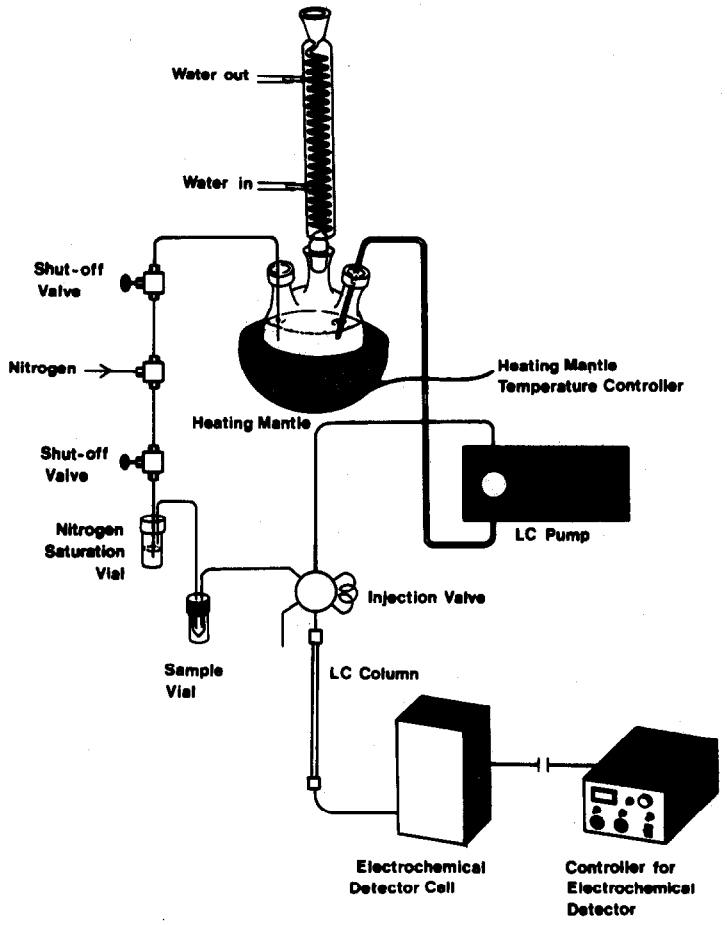

Fig. 1. Schematic diagram of the reductive electrochemical detector system for liquid chromatography.

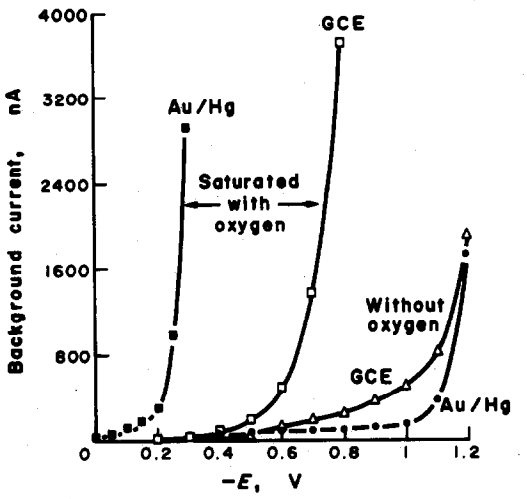

Fig. 2. Background current on a GCE (□) and Au/Hg electrode (■) without deoxygenation, and after deoxygenation with nitrogen gas on a GCE (△) and Au/Hg electrode (●) as a function of the detector potential. LC conditions: 25-cm C_8 Biophase column, 0.05*M* acetate buffer, 20% (v/v) 1-propanol, 10% (v/v) ethanol, pH 4, at 0.8 ml/min. Areas of electrodes used were 7.07 and 7.94 mm^2 for the GCE and Au/Hg electrode, respectively.

volume (20 μl) rotary sample-injection valve and an oxygen removal apparatus (Fig. 1). The LC columns were 25 cm × 4.6 mm C_{18} and C_8 5-μm Biophase.

Chemicals

Reagent grade absolute ethanol, anhydrous sodium acetate, and monochloroacetic acid were used as purchased, for the preparation of the mobile phase. Mercury was triply distilled. Picric acid (PIC), 4-nitrobenzoic acid (NBA), 3,4-dinitrobenzoic acid (DNBA), 2-chloro-3,5-dinitropyridine (CDNPY), 4-nitrophenol (NP), 4-nitroaniline (NA) and 2,4-dinitrophenol (2,4DNP) were used as purchased. 2,5-Dinitrophenol (2,5DNP) was dried under vacuum before use.

Electrode preparation and hydrodynamic voltammetry

Thin-layer mercury-film electrodes were prepared by placing triply distilled mercury on the old amalgam surface and after 2–3 min wiping the excess from the electrode surface with a piece of computer card. The potential of the electrode was initially set at −1.50 V *vs.* Ag/AgCl for "burning in" the new amalgam surface. After about 100 sec the electrode potential was lowered to a normal operating setting.

Hydrodynamic voltamperograms (HDVs) were obtained by repeated injections of standards, the potential of the thin-layer amperometric detector being changed before each injection. HDVs were plotted by using the normalized function n_{eff}, which can be derived from Faraday's law:

$$n_{eff} = \frac{Q}{FN_{inj}} = nf$$

where Q is the number of coulombs passed during the reduction process, F is the Faraday constant (9.65×10^4 coulombs/equivalent), n is the number of electrons involved in the electrochemical reaction (equivalents/mole), N_{inj} is the number of moles of analyte injected into the column and f is the fraction of injected molecules which react at the electrode surface.

RESULTS AND DISCUSSION

In our evaluation of the utility of the glassy-carbon electrode we considered hydrogen and oxygen overpotentials, reduction potentials for analytes of interest, the long term stability, and detection limits. The effect of oxygen on the magnitude of the background current is illustrated in Fig. 2. An amalgamated gold electrode exhibits detectable reduction of oxygen even at potentials around −0.1 or −0.2 V, whereas on a glassy-carbon electrode oxygen reduction occurs only at more negative potentials (about −0.5 or −0.6 V). This difference in behaviour is illustrated in Fig. 3.

Several groups have examined the electrochemical reduction of oxygen on various electrode materials.[14–16] In acidic solution, the oxygen is reduced on mercury in two 2-electron steps,

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \quad (1)$$

$$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \quad (2)$$

while on a glassy-carbon electrode only the first reduction process is observed and the second process is probably obscured by hydrogen evolution. A decrease in limiting current at high potentials was observed only for reduction of oxygen at the GCE; neither oxygen at the amalgamated gold electrode or picric acid at either electrode (under the conditions used for the oxygen experiment) gave a decrease. This rules out the possibility of the *iR* drop being the cause of the decrease in the limiting current.[17] A similar effect was observed by Taylor and Humffray for acidic media and a ring–disk glassy-carbon electrode.[14]

The chromatography of oxygen is very interesting. Oxygen is retained on reversed-phase columns, but its retention time (volume) is independent of the concentration of organic modifiers in the mobile phase, and is less sensitive to changes in column temperature than that of analytes which elute at about the same time as oxygen. Another distinguishing feature of oxygen chromatography is illustrated in Fig. 4. The oxygen peak is broad and badly skewed (the asymmetry

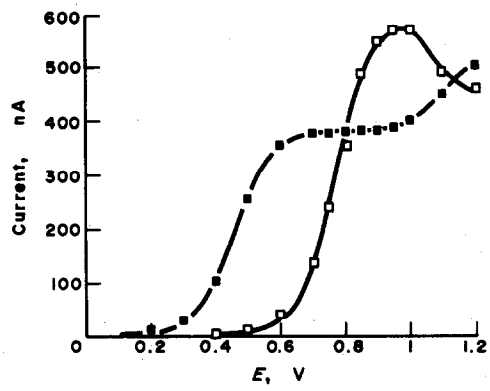

Fig. 3. Hydrodynamic voltamperograms of oxygen on a GCE (□) and Au/Hg electrode (■). LC conditions: 25-cm C_8 Biophase column, 0.1*M* acetate buffer, 20% (v/v) 1-propanol, 5% (v/v) ethanol, pH 4.8, at 1 ml/min.

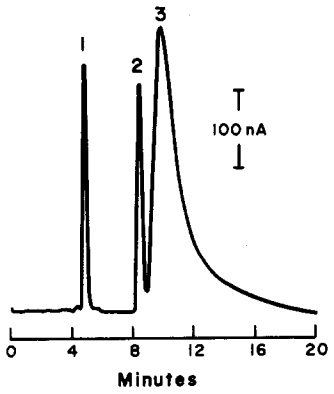

Fig. 4. A chromatogram of 170 pmole of PIC (1), 540 pmole of CDNPY (2) with injected solution saturated with oxygen (3). LC conditions: 25-cm C_8 Biophase column, 0.17*M* acetate buffer, 22% (v/v) propanol, 5% (v/v) ethanol, pH 4, at 0.8 ml/min.

factor was approximately 2.5 times larger for oxygen than for CDNPY). These factors clearly indicate that oxygen retention on the reversed-phase column is not due to the usual hydrophobic interactions, but is probably attributable to a size-exclusion mechanism. Because molecular oxygen is smaller in size than the organic analyte molecules, it is capable of penetrating smaller pores in the stationary phase.

The usefulness of the large oxygen overpotential on glassy carbon is illustrated in Fig. 5. As expected, oxygen dissolved in the injected solution does not interfere with the trace determination of easily reduced analytes (*e.g.*, picric acid, 2,4DNP), as long as the mobile phase is saturated with oxygen and the detector is operated below −0.6 V *vs.* the Ag/AgCl electrode (Fig. 5*a*, *b*). Oxygen reduction is significant at −0.6 V (manifested by high background current and increased baseline noise). Oxygen is only detected as a distinct peak when the mobile phase alone (and not the injected sample) is sparged with nitrogen (Fig. 5*c*). The lack of oxygen interference on a glassy-carbon electrode at potentials below −0.6 V simplifies the construction of the reductive electrochemical system because it eliminates the need for the oxygen-removal apparatus (Fig. 1).

Dissolved oxygen limits the available negative-potential range of the glassy-carbon detector and significantly decreases the observed detection limits for many analytes. Even though the detector response (or "sensitivity") at −0.6 V was greater than at −0.4 V (Fig. 5*a*,*b*), the detection limits under "oxygen-saturated" conditions (Fig. 5*b*) increased by a factor of 4–5 as a result of a tenfold increase in baseline noise (which is proportional to the background current). The detection limits for polynitro-aromatic compounds at −0.6 V under "oxygen-free" conditions (Fig. 5*d*) were greater by a factor of 2–3 than those at −0.4 V, and detection of less than 10 pmoles of mononitro-aromatic compounds was possible. Trace detection at potentials more positive than −0.6 V under "oxygen-saturated" conditions was not poss-

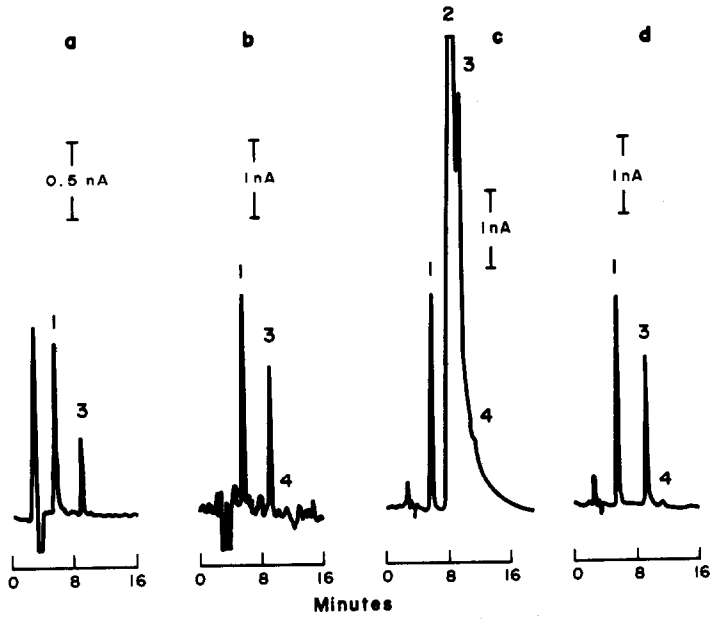

Fig. 5. The effect of dissolved oxygen (2) on the detection of 4.8 pmole of PIC (1), 9.2 pmole of 2,4DNP (3), and 28 pmole of NP (4) at GCE. (*a*) E = −0.4 V and (*b*) E = −0.6 V, both the mobile phase and injected solution being saturated with oxygen; (*c*) E = −0.6 V, the mobile phase being deoxygenated and the injected solution saturated with oxygen; (*d*) E = −0.60 V, both the mobile phase and injected solution being deoxygenated. LC conditions: 0.015*M* sodium acetate, 0.02*M* monochloroacetic acid, 0.001*M* EDTA, 17% (v/v) 1-propanol, 5% (v/v) ethanol, pH 3.5, at 1 ml/min, 25-cm column of 5-μm Biophase C_{18}.

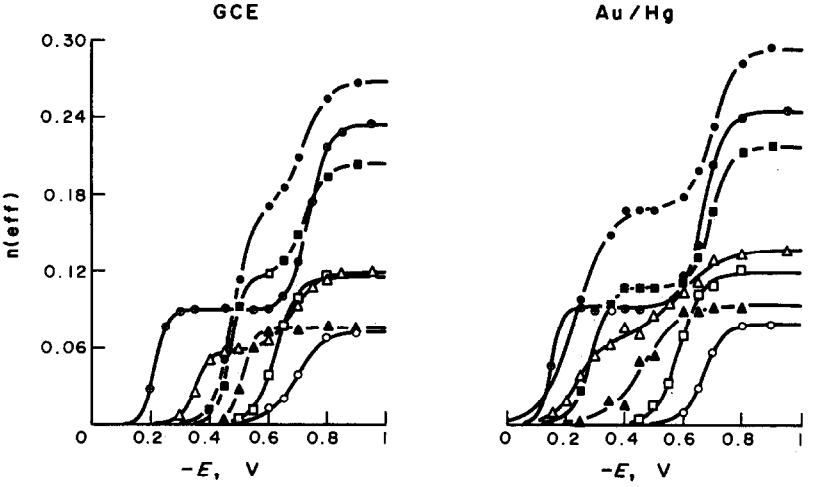

Fig. 6. Hydrodynamic voltamperograms of nitro-aromatic compounds: 160 pmole of PIC (●), 250 pmole of 2,5DNP (+), 170 pmole of 2,4DNP (■), 290 pmole of DNBA (△), 470 pmol of NBA (▲), 390 pmole of NA (□) and 650 pmole of NP (○) on a GCE and an Au/Hg electrode. LC conditions: a 25-cm Whatman Partisil PXS 5/25 ODS column; mobile phase 0.1*M* acetate, pH 4.1, 20% (v/v) 1-propanol and 5% (v/v) ethanol, and 0.9 ml/min.

ible, owing to the large background currents and associated noise.

Because of the small potential window of the GCE under "oxygen-saturated" conditions, the utility of the detector is limited to detection of analytes which contain polynitro- and nitroso-aromatic (ϕ-NO_2 and ϕ-NO), diazo, and quinone functional groups. A more negative potential limit (by about 200–400 mV) and better detection limits are achieved by deoxygenation of the mobile phase. Deoxygenation allows detection of compounds which contain a single aromatic nitro-group, aromatic *N*-oxides and thioamides.

A comparison of the redox behaviour of nitro-aromatic compounds on a glassy carbon and on an amalgamated gold electrode is illustrated in Fig. 6. The initial reduction of polynitro compounds was very sensitive to the nature of the electrode material, while the opposite was true for the reduction of mononitro compounds and secondary reduction of *meta* polynitro compounds. The detection limits on a GCE (the concentration giving a signal that is 3 times the standard deviation of the blank) for polynitro- and mononitro-aromatic compounds were about 0.15–0.25 and 0.5–1 pmole respectively. The detection limits found for quinones were higher than for nitro compounds (2.5 pmole for adriamycin) because fewer electrons are transferred in the redox reaction of quinones. These detection limits compare favourably with the detection limits obtained on an amalgamated gold electrode.[13] Lower detection limits were obtained for polynitro-aromatic compounds on the amalgamated gold electrode (by a factor of 1.5–2) if great care was used in the preparation of the amalgam surface and if the detector potential was set at the plateau of the first reduction wave.

Several groups have attempted to evaluate the reductive potential range for various electrode materials and their results are summarized in Table 1. This comparison should be used only as a qualitative guide because the criteria and the types of electrochemical techniques used in the determination of the potential ranges varied significantly among the various groups. In our experience the negative-potential limit for glassy carbon is strongly dependent on the previous history of the electrode surface and varies from one electrode to another. Often the background current at a potential between −0.8 and −1.0 V on a GCE is unstable, with an upwards drift of the order of 20–50 nA/hr, limiting the most negative useful potential to −0.8 V (for high-sensitivity measurements). On the other hand, many GCEs can be operated routinely at −1.00 V for several weeks (with only periodic surface polishing) without any significant changes in electrode sensitivity and noise (*i.e.* with invariant detection limits). A longer equilibration time of 2–3 hr is necessary if high-sensitivity measurements are to be made at glassy carbon electrodes, compared to 30–60 min for amalgamated gold electrodes. This problem can be dealt with by leaving the system on overnight at minimal mobile-phase flow (0.1–0.2 ml/min).

CONCLUSIONS

It is our experience that glassy-carbon electrodes are more reliable and easier to use than amalgamated gold electrodes for routine high-sensitivity measurements at applied potentials more positive than −0.8 V *vs.* the Ag/AgCl electrode because background current and noise levels are more reproducible. Amal-

gamated gold electrodes must be used, however, if high-sensitivity measurements (< 100 pmole) are to be made at potentials more negative than −0.8 V *vs.* the Ag/AgCl electrode. Edge effects and the spike noise common to amalgamated gold electrodes[6] are not observed with glassy-carbon electrodes. While the major drawbacks are the negative-potential limit and the longer equilibration time, glassy carbon is more convenient to use for detection of easily reduced analytes. Oxygen removal is unnecessary for trace detection of analytes on a glassy-carbon electrode at potentials less than −0.6 V *vs.* the Ag/AgCl electrode.

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DETERMINATION OF THALLIUM AT SUBTRACE LEVEL IN ROCKS AND MINERALS BY COUPLING DIFFERENTIAL PULSE ANODIC-STRIPPING VOLTAMMETRY WITH SUITABLE ENRICHMENT METHODS

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Summary—Ten international reference samples have been analysed for thallium content by differential pulse anodic-stripping voltammetry. Two separation techniques, solvent extraction and ion-exchange, were employed to preconcentrate the thallium: the results were critically compared to establish which was the better separation technique. The values found were quite satisfactory and confirmed the wide scope for application (not yet fully investigated) of voltammetry in geochemical studies.

Despite the great increase in geochemical research during the past two decades the determination of the thallium content of geological materials is rare. Consequently there are many aspects of thallium geochemistry which have not yet been settled because they are either scarcely developed or even undefined. Most of the literature data on abundance of thallium refers to minerals and rocks of magmatic suites.[1] However, the literature provides non-conclusive information about the behaviour of thallium during magmatic differentiation, mainly because of the lack of knowledge about partitioning coefficients between most major minerals and silicate melts. Little is known about the thallium content of sedimentary rocks and natural waters[1,2] or about its chemical speciation.[3] Finally, the behaviour of thallium in metamorphic and related rocks is virtually unknown.[1]

Recently, some papers[4,5] have pointed out the possibility of using thallium as a geochemical indicator in a variety of problems of geological importance such as petrogenesis, rock–sea-water interactions and the evaluation of the degree of sulphur saturation in a silicate melt.

Unfortunately, a major constraint in the improvement of geochemical information about thallium is its very low abundance in geological materials. The abundance in the earth's crust is estimated[1] at 800 ng/g but it falls to 490 ng/g in continental rocks.[6] Consequently, thallium determination at subtrace level requires high-sensitivity analytical methods combined with dissolution and separation techniques to concentrate the element from multicomponent matrices. The analytical techniques which have sufficient sensitivity are fluorimetry, neutron activation, atomic-absorption spectrometry (AAS) and differential pulse anodic-stripping voltammetry (dpasv). Fluorimetry may suffer severe limitations due to interferences, while neutron activation requires expensive facilities and a long time for irradiation and "cooling" of the samples; comparison of AAS with dpasv, taking into account the comparable sensitivities, favours the latter technique because of its lower apparatus cost and wider measurement range.

EXPERIMENTAL

Apparatus

An Amel 472 WR multipolarograph equipped with a Hewlett–Packard 7040 A X–Y recorder was used for all the stripping voltammetric analyses. A classical three-electrode system was employed: a Metrohm E 410 as working electrode, a platinum counter-electrode and an SCE as reference. The polarographic cells were made of silica glass and all glassware was conditioned with nitric acid (1 + 1) for several days before use. During the electrolysis step the solutions were stirred with a cylindrical Teflon-coated stirring bar driven by an Amel variable-speed stirring unit. All potentials reported are referred to the SCE.

Reagents

Standard Tl(I) stock solutions, 500 mg/l., were obtained by dilution of Merck "titrisol" standard solution and also by dissolving an appropriate weight of Merck R. G. $TlNO_3$ (dried at 110°), and were stored in previously conditioned polyethylene bottles. They were further diluted to give 1 and 5 ppm working solutions. All the other chemicals were Merck "Suprapur", except the Na_2EDTA, H_2SO_3, Br_2, HBr and diethyl ether, which were Merck R. G. The resin was Bio-Rad AG1 X8, chloride form, 100–200 mesh. The columns were made of borosilicate glass, bore 0.75 cm, 15 cm long, with a porous polyethylene disc as bed support. The sulphurous acid (saturated SO_2 solution) was diluted with an equal volume of water for use. It will be referred to as "1:1 H_2SO_3" in the text.

Procedures

Sample dissolution. As all the samples were silicate or silicaceous carbonate rocks, dissolution with hydrofluoric acid and perchloric acid as described earlier,[7] was chosen. The sample weight depends on the thallium content: at least 50–100 ng of Tl should be present although less can be determined. Teflon crucibles were used, because they are cheaper than platinum, and avoid introduction of platinum into the solution.[8] The advantages of the procedure are its rapidity, the oxidation of Tl(I) to Tl(III), and the lower temperature needed for evaporation. The only disadvantage may arise in the case of high-potassium samples, because of the relatively low solubility of potassium perchlorate, but this can easily be overcome by using a low sample weight and an appropriate final dilution.

Solvent extraction. The technique used is well known[9–11] and is based on the extraction of Tl(III) into diethyl ether from hydrobromic acid medium. The sample, decomposed as above, is evaporated with 5 ml of concentrated hydrobromic acid and a few drops of bromine to guarantee complete oxidation of Tl(I). The residue is dissolved with 25 ml of 1.2*M* hydrobromic acid, transferred into a separatory funnel and shaken with an equal volume of diethyl ether (presaturated with the acid). After 30 sec of standing, the organic phase is separated and the extraction repeated. The combined organic fractions are gently evaporated and the thallium recovered is reduced with excess of sulphurous acid.

Anion-exchange separation. As an alternative to solvent extraction, $TlCl_4^-$ can be isolated with a strong anion-exchange resin. This gives a fairly good selectivity because over a certain chloride concentration range only Tl(III) and a few other trace elements (noble metals) give stable anionic chloro-complexes[12] with high distribution coefficients.[13] The sample is decomposed as before, and the residue from the evaporation is taken up in water, diluted to 100 ml and made 0.5*M* in hydrochloric acid. One drop of saturated bromine water is added and the solution is passed through the exchanger column containing a 7-cm AG1 X8 (chloride form) resin bed, at a flow-rate of 1 ml/min. The column is washed successively with 20 ml of water, 50 ml of 1*M* nitric acid and 20 ml of water, each containing 1 drop of saturated bromine water. Thallium is eluted with 50 ml of 1:1 H_2SO_3, which reduces the Tl(III) to Tl(I) (which is not retained by the resin). The eluate is evaporated to dryness.

Instrumental measurement. The residue from the eluate is dissolved in 2.5 ml of supporting electrolyte (pH-4.6 1*M* acetate buffer, 0.5*M* in Na_2EDTA) and the solution is diluted to volume in a 25-ml standard flask. A 20-ml portion is transferred to a polarographic cell and deaerated with purified nitrogen for 7 min. It is then electrolysed for 2–5 min (depending on thallium concentration) at an applied potential of −700 mV *vs.* SCE, with magnetic stirring at 600 rpm. During this phase and the subsequent scanning, nitrogen is passed over the solution. All measurements are made in the dpasv mode, at the hanging mercury drop electrode (hmde) at a sweep-rate of 20 mV/sec, pulse amplitude 40 mV. The mercury drop weight used in our work was 1.66 ± 0.66 mg for an extruded drop 1 division deep; the relative standard deviation obtained, 3.8%, agreed well with previous data[14,15] for the hmde employed.

Under these conditions the E_p value found for Tl(I) was −410 mV *vs.* SCE. The standard addition method was used for the dpasv measurements, the amount added being sufficient to increase the signal by a factor of about 2.

DISCUSSION

Sample decomposition

It is an open question whether thallium is lost by volatilization in sample decomposition, when an acidic solution is evaporated. Thallium loss was first claimed to arise from fuming mixtures of hydrochloric or hydrobromic acid with perchloric or sulphuric acid.[16] Later it was reported that thallium losses of up to 40% were found in evaporation of ^{204}Tl-labelled mixtures of hydrofluoric and sulphuric or perchloric acid.[17] However, others have reported that thallium is not lost on fuming hydrofluoric/perchloric acid[18,19] and hydrofluoric/nitric acid mixtures.[19] Our experiments with spiked samples also failed to detect loss of thallium; perhaps the discrepancy between the available data can be explained in terms of the different evaporation temperatures used. We evaporated the acidic solutions at low temperature (80–90°) in 4 × 10 cm crucibles; in addition, the double evaporation was not fully completed but continued only until incipient dryness.

Calibration

Known quantities of thallium treated by the procedures described gave a linear plot of current *vs.* amount. The working range was not investigated above 2 μg of thallium because higher amounts were not the concern of this research. Even when certified chemicals are used, it is necessary to run blanks in analysis for subtrace elements. Blank determinations were therefore run on ten times the normally required volumes of reagents and gave a result equivalent to 2.1 ± 0.7 ng of Tl for a standard (0.5–1 g) sample weight: the major contributions came from the hydrofluoric, sulphurous and perchloric acids. This blank value was used to establish the limit of detection (LOD) as 4.2 ng and the limit of quantitation (LOQ) of 9.1 ng, defined as proposed by the ACS Committee on Environmental Improvement.[20] The standard Tl(I) working solutions had high stability; checked weekly over a 3-months period against freshly-prepared corresponding solutions, they never showed a relative standard deviation higher than that arising from sampling and measuring errors (less than 5% at the <500-ng level). The choice of a suitable supporting electrolyte was not problematic; it is well known that Tl(I) gives fairly well-defined peaks in different media, hydrochloric acid[21] and pH 4.6 acetate buffer[22] being the most used. With the latter supporting electrolyte and the hmde, the E_p values for Pb(II) and Tl(I) are so close that satisfactory resolution is impossible and complexation is imperative in order to shift the lead peak. Owing to the low stability constants of many Tl(I) chelates in acidic or neutral medium in comparison with those for the Pb(II) complexes (Table 1), there is ample choice of ligand. EDTA and DCTA were first utilized by Přibil *et al.*[23–25] and later by Neeb[26] and Zieglerova *et al.*[27] Jacobsen *et al.*[28] suggested the use of DTPA, and Cignini *et al.*[29] successfully used TTHA. However, in this work a 0.1*M* acetate buffer–0.05*M* Na_2EDTA mixture was chosen as supporting electrolyte, as being the most extensively studied. The earlier workers used a lower EDTA con-

Table 1. Stability constants (log K) for some Tl(I) and Pb(II) complexes[12]

	EDTA	DCTA	EGTA	NTA	DTPA
Tl(I)	6.5	5.3	4.3	4.7	5.8
Pb(II)	18.0	19.6	14.7	11.4	18.8

centration, but in rock analysis, because of the wide variation in matrix composition, a higher concentration is required. The concentration selected was shown experimentally not to lower appreciably the peak current for Tl(I). The instrumental parameters were also experimentally chosen. In particular, the plating potential chosen was the most positive that would not affect the Tl(I) deposition and the sweep-rate represented a reasonable compromise between speed of measurement and instrumental response.

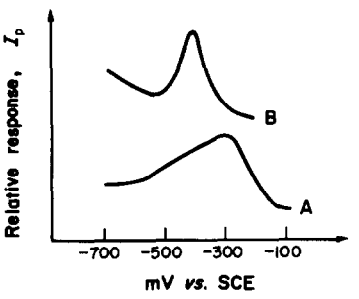

Fig. 1. (A) Signal obtained when analysing a solution containing Tl(I) recovered by solvent-extraction from a basaltic rock (Tl concentration 85 ng/g): note the matrix-dependent interference, related to the basic nature of the rock; (B) dpasv peak resulting when analysing a solution containing Tl(I) recovered by solvent-extraction from a potassium-feldspar (Tl: 50 ng/g), a typical salic mineral. The operational conditions, common to both solutions, are given in the text.

RESULTS

Solvent-extraction procedure

It was often impossible to measure the thallium recovered when the solvent-extraction procedure was used, because of an undefined Tl(I) peak. An example (a basaltic rock, Tl concentration 85 ng/g) is shown in Fig. 1A. This behaviour constantly occurred for certain samples, which all had very low thallium content. On the other hand, preliminary checks on the solvent-extraction procedure confirmed quantitative Tl recovery from synthetic solutions and from a potassium feldspar (Tl concentration 50 ng/g), with well-developed dpasv peaks (see Fig. 1B). This behaviour is due to matrix-dependent interferences. It is well known that the solvent-extraction technique used is not strictly selective, and predictions based on equilibrium constants and reagent concentrations give only a rough indication of the selectivity, because of the strong deviations introduced by the matrix. In particular Fe(III) is partly extracted even from 0.8M hydrobromic acid medium, and we have also found that micro-quantities of copper are extracted.

Table 2. Results for thallium determination (solvent-extraction procedure) in some reference samples

Reference sample	Tl, *ng/g*	$\bar{X} \pm \sigma$, *ng/g*	$100\,\sigma/\bar{X}$, %
AGV-1 Andesite	385, 354, 320	353 ± 32	9
GSP-1 Granodiorite	1498, 1580, 1605	1561 ± 54	3
SY-1 Syenite	780, 803, 896	826 ± 61	7
GH Granite	1498, 1520, 1563	1527 ± 33	2
NBS 70a K-feldspar	2720, 2931, 2496	2715 ± 217	9

Table 3. Results for thallium determination (ion-exchange procedure) in reference samples

Reference samples	Tl, *ng/g*	$\bar{X} \pm \sigma$, *ng/g*	$100\,\sigma/\bar{X}$, %
AGV-1 Andesite	295, 333, 320	316 ± 19	6
W-1 Diabase	79, 76, 96, 89	85 ± 9	11
BCR-1 Basalt	247, 292, 241	260 ± 27	11
GSP-1 Granodiorite	1433, 1410, 1527 1606	1494 ± 90	6
SY-1 Syenite	769, 799, 902	823 ± 69	8
GA Granite	708, 688, 730	708 ± 21	3
GH Granite	1322, 1351, 1405	1359 ± 42	3
NBS 70a K-feldspar	3040, 3062, 2618	2906 ± 250	9
NBS 98a Plastic clay	331, 326, 398	351 ± 40	11
DTS-1 Dunite	≤LOD		
PCC-1 Peridotite	≤LOD		

Although the fraction of iron extracted is low (<1.4%), the absolute amount is not negligible, because of the high iron content of femic minerals and ultrabasic and basic rocks. A literature survey showed that both copper and iron can interfere in thallium determination by dpasv, although for different reasons.[36–38] Further investigations showed that the solvent-extraction procedure was suitable for all salic minerals, acidic rocks (with normal thallium content) and for other minerals and rocks when the total iron (expressed as Fe_2O_3) did not exceed 4% and there was not less than 2 ppm of thallium. In other cases the ion-exchange separation technique must be used. Table 2 shows the results for thallium determination in some reference samples; comments on them are made in the following section.

Anion-exchange procedure

Table 3 shows the thallium concentrations found in the reference materials analysed by the anion-exchange procedure. The overall precision appears to be independent of the matrix composition and ranges from 3% (at the 700-ng/g concentration level) to 22% (at the 50-ng/g level). Table 4 compares our data with some values previously reported. It appears that there is a lack of systematic work on determination of thallium in the more generally used international reference materials, and that only in a few cases are the standard deviations or even ranges reported.

For these reasons, it being only rarely possible to estimate correctly the reliability of the few data available, "recommended values"[35] for thallium in reference materials have not yet been established. This situation is probably due to incomplete solution of the difficulties of thallium determination in geological samples at subtrace level. In estimating the accuracy of the new values obtained by both procedures it seemed more realistic to limit the comparison to the standard rocks for which the data show least scatter, such as W-1, BCR-1, GSP-1 and AGV-1, for which numerous reported values range from 102 to 115, 267 to 350, 1200 to 1535 and 270 to 560 ng/g, respectively. For these standards, the comparison demonstrates good accuracy. For the NBS standards, only one previous value was available for each and was not in agreement with ours. The thallium content of DTS-1 and PCC-1 may only be stated to be near or below the limit of detection for this method, so thallium determination in such materials will only be possible by other methods or by purifying the reagents to decrease the blank value.

Table 4. Literature values (ng/g) for Tl in geochemical reference samples

Standard sample	Data from this work	Wahler[30] (PP)	Sighinolfi[10] (AAS)	Böhmer[19] (F)	Laul[31] (NA)	Matthews[17] (F)	Marowsky[39] (NA)	Albuquerque[33] (S)	Heinrichs[34] (AAS)
AGV-1	316 ± 19	270	535–590	430	342 ± 50	390	1610–1650	275	330
W-1	85 ± 9	<50	105–110	350	102 ± 15		108–122	110	110
BCR-1	260 ± 67	360	280–300	480	330 ± 90	590	350	267	260
GSP-1	1494 ± 90	710	1520–1550	1780	1300 ± 200	1870	1220	1200	1280
SY-1	823 ± 69	860	950–1150					1000	740
BR	54 ± 12	<50	110						62
GA	708 ± 21	690	1100						890
GH	1359 ± 42	1380	2600						1830
NBS 70a	2906 ± 250	<50							
NBS 98a	351 ± 40	950							
DTS-1	⩽LOD	<50	11*	740	0.51 ± 0.07	130		3	6
PCC-1	⩽LOD	<50	20*	280	0.75 ± 0.1	350	0.7–0.8	5	2

NA = Neutron activation; S = spectrography; F = fluorimetry; PP = pulse polarography; AAS = atomic-absorption spectrometry.
* Data held to be unrealistic by the original author.

CONCLUSIONS

The dpasv technique can be successfully employed for determination of thallium in solid geological samples, even at subtrace level, with high sensitivity and low cost. However, the quality of the preliminary separation of thallium from the matrix is of the greatest importance and the results of this work show that

solvent extraction, though more attractive because less time-consuming, may only be used for salic minerals and acidic or slightly basic thallium-rich rocks; for femic minerals and ultra basic or basic rocks, the ion-exchange separation is recommended.

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CORRECTION OF FORMATION CONSTANTS FOR IONIC STRENGTH, FROM ONLY ONE OR TWO DATA POINTS: AN EXAMINATION OF THE USE OF THE EXTENDED DEBYE–HÜCKEL EQUATION

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Summary—The results of an extensive examination show the extended Debye–Hückel equation to be efficacious for the use of as few as one or two formation constant values, for a given metal-complex species, for calculation of the value at a lower ionic strength. Approaches to choosing values for the constants in the extended Debye–Hückel equation are described. A statistical analysis shows the chief source of error in the resulting predicted formation constants to be uncertainties in the literature formation-constant values used as data.

We are currently engaged in a project on computer simulation of metal-ion equilibria in soil solutions. Our model requires the acquisition of numerous metal–ligand formation constants valid for an ionic strength of 0.04. Though constants are reported in the literature for many of the systems concerned, only a few correspond to this ionic strength. Indeed most of the available data are given for high ionic strengths and, moreover, at only one or two levels. There are several earlier reports[1–5] on the calculation of equilibrium constants at low ionic strengths from the values at higher ionic strengths, usually with the aid of an extended form of the Debye–Hückel equation. In these, the degree of accuracy is variable and strongly dependent on the choice of values for the constants in the equation. In this paper we examine the accuracy of an extended form of the Debye–Hückel expression [equation (1)] when applied to only one or two data points:

$$-\log\gamma_i = Az_i^2[(I)^{\frac{1}{2}}/(1 + B\mathring{a}_i(I)^{\frac{1}{2}})] + cI \qquad (1)$$

where I is the ionic strength of the solution, γ_i is the activity coefficient of ion i, z_i is the charge on ion i, A and B are constants characteristic of the solvent ($A = 0.509$; $B = 0.328$ for water at 25°)[6] and $\mathring{a}_i$ is an ionic size parameter, estimates for which have been given by Kielland.[7]

We demonstrate that for systems of interest in our soil model, equation (1) has sufficient accuracy for our purpose, with use of as few as two literature values at higher ionic strengths. We also show that the equation can still be used, though with less reliability, when a formation constant at only one ionic strength is available from the literature. In that situation, either a generally recommended value of c is used, or a value based on an educated guess.

THEORY

For a given complexation reaction between a metal ion, M^{z_M}, and a ligand, L^{z_L}

$$pM^{z_M} + qL^{z_L} \rightleftharpoons (M_pL_q)^{(pz_M + qz_L)} \qquad (2)$$

the equilibrium constants, K' and K'', at ionic strengths I' and I'' are related by

$$K'' = \Gamma''K'/\Gamma'. \qquad (3)$$

In equation (3), each Γ is a quotient, expressed generally by equation (4).

$$\Gamma = (\gamma_M)^p(\gamma_L)^q/\gamma_{M_pL_q} \qquad (4)$$

where γ is the activity coefficient of the species indicated by subscripts. These activity coefficients may be calculated from equation (1) provided that values of the constants $\mathring{a}_i$ and c are available. Kielland[7] has listed $\mathring{a}_i$ values for a wide range of ions. When $\mathring{a}_i$ values are not available, we use the following formulae:

$$\begin{aligned} &\text{for small organic ions } \mathring{a}_i = z_i + 4 \\ &\text{for inorganic ions } \mathring{a}_i = 2z_i + 2 \end{aligned} \qquad (5)$$

We also make the assumption that c has the same value for each ion in any given complexation equilibrium (2). By combining this assumption with equations (1), (3) and (4), we obtain the expression

$$\log K'' = \log K' + Af(\mathring{a}_i, (I)^{1/2}) + c(I'' - I')(1 - p - q) \qquad (6)$$

where

$$\begin{aligned} f(\mathring{a}_i, (I)^{1/2}) &= (pz_M + qz_L)^2\left(\frac{(I'')^{1/2}}{1 + B\mathring{a}_{M_pL_q}(I'')^{1/2}} - \frac{(I')^{1/2}}{1 + B\mathring{a}_{M_pL_q}(I')^{1/2}}\right) \\ &\quad - pz_M^2\left(\frac{(I'')^{1/2}}{1 + B\mathring{a}_M(I'')^{1/2}} - \frac{(I')^{1/2}}{1 + B\mathring{a}_M(I')^{1/2}}\right) \\ &\quad - qz_L^2\left(\frac{(I'')^{1/2}}{1 + B\mathring{a}_L(I'')^{1/2}} - \frac{(I')^{1/2}}{1 + B\mathring{a}_L(I')^{1/2}}\right). \end{aligned}$$

Hence the overall value of c for the system concerned may be calculated from equation (6) and used in equation (1) for predicting the formation constant at the required ionic strength of 0.04.

RESULTS AND DISCUSSION

In order to examine the approach described above, several systems were considered for which formation-constant values at several ionic strengths, including infinite dilution, were available.[8,9] These values were taken in pairs and used in equation (6) to determine the system value for c. This value was subsequently used in equation (1) and the infinite-dilution (thermodynamic) formation constant was estimated. The results are presented in Tables 1 and 2 where the literature values of the respective thermodynamic constants are given for comparison. In most cases the predicted constants are sufficiently close to the literature values to be considered satisfactory for our soil model, even when the predictions are made from ionic strengths as high as 2.0 and 3.0.

For many of the systems pertinent to our soil model, the formation constants in the literature have been determined at only one ionic strength. Thus the data used to compile Tables 1 and 2 were used in a

Table 1. Values for c and log K for $I = 0.04$, and estimates of maximum errors calculated for some inorganic complexes from pairs of log K values at different ionic strengths; all literature values are taken from reference 9

Species	Predicted from c (error)	log K'	I'	log K''	I''	log K (error)
$CaNO_3^+$	0.072 (0.16)	0.06	0.5	−0.06	1.0	0.70 (0.16)
	−0.053 (0.07)	0.06	0.5	−0.02	2.0	0.64 (0.14)
	−0.071 (0.05)	0.06	0.5	0.04	3.0	0.63 (0.14)
	−0.115 (0.08)	−0.06	1.0	−0.02	2.0	0.51 (0.19)
	−0.107 (0.05)	−0.06	1.0	0.04	3.0	0.52 (0.18)
	−0.099 (0.07)	−0.02	2.0	0.04	3.0	0.54 (0.26)
						0.7 (Lit.)
$Ca(NO_3)_2$	0.033 (0.15)	−0.3	0.5	−0.5	1.0	0.72 (0.22)
	−0.042 (0.06)	−0.3	0.5	−0.4	2.0	0.61 (0.18)
	−0.035 (0.04)	−0.3	0.5	−0.4	3.0	0.62 (0.17)
	−0.080 (0.07)	−0.5	1.0	−0.4	2.0	0.39 (0.25)
	−0.052 (0.04)	−0.5	1.0	−0.4	3.0	0.47 (0.22)
	−0.024 (0.07)	−0.4	2.0	−0.4	3.0	0.72 (0.38)
						0.6 (Lit.)
$MnNO_3^+$	−0.068 (0.16)	−0.38	0.5	−0.43	1.0	0.19 (0.16)
	−0.086 (0.07)	−0.38	0.5	−0.41	2.0	0.18 (0.14)
	−0.135 (0.05)	−0.38	0.5	−0.24	3.0	0.15 (0.14)
	−0.095 (0.08)	−0.43	1.0	−0.41	2.0	0.16 (0.19)
	−0.152 (0.05)	−0.43	1.0	−0.24	3.0	0.10 (0.18)
	−0.209 (0.07)	−0.41	2.0	−0.24	3.0	−0.07 (0.26)
						0.2 (Lit.)
$Mn(NO_3)_2$	0.099 (0.15)	−0.3	0.5	−0.6	1.0	0.82 (0.22)
	0.069 (0.06)	−0.3	0.5	−0.9	2.0	0.78 (0.18)
	0.018 (0.04)	−0.3	0.5	−0.8	3.0	0.70 (0.17)
	0.054 (0.07)	−0.6	1.0	−0.9	2.0	0.69 (0.25)
	−0.002 (0.04)	−0.6	1.0	−0.8	3.0	0.52 (0.22)
	−0.058 (0.07)	−0.9	2.0	−0.8	3.0	0.02 (0.38)
						0.6 (Lit.)
$CuNO_3^+$	−0.408 (0.16)	−0.13	0.5	−0.01	1.0	0.27 (0.16)
	−0.153 (0.07)	−0.13	0.5	−0.06	2.0	0.40 (0.14)
	−0.123 (0.05)	−0.13	0.5	−0.02	3.0	0.41 (0.14)
	−0.025(0.08)	−0.01	1.0	−0.06	2.0	0.65 (0.19)
	−0.052 (0.05)	−0.01	1.0	−0.02	3.0	0.62 (0.18)
	−0.079 (0.07)	−0.06	2.0	−0.02	3.0	0.54 (0.26)
						0.5 (Lit.)
$ZnNO_3^+$	−0.148 (0.16)	−0.18	0.5	−0.19	1.0	0.35 (0.16)
	−0.133 (0.07)	−0.18	0.5	−0.14	2.0	0.36 (0.14)
	−0.147 (0.05)	−0.18	0.5	0.01	3.0	0.35 (0.14)
	−0.125 (0.08)	−0.19	1.0	−0.14	2.0	0.37 (0.19)
	−0.147 (0.05)	−0.19	1.0	0.01	3.0	0.35 (0.18)
	−0.169 (0.07)	−0.14	2.0	0.01	3.0	0.28 (0.26)
						0.4 (Lit.)
$ZnSO_4$	−0.178 (0.19)	0.93	0.5	0.89	1.0	2.10 (0.21)
	−0.084 (0.10)	0.93	0.5	0.76	2.0	2.20 (0.19)
	−0.060 (0.07)	0.93	0.5	0.70	3.0	2.22 (0.19)
	−0.036 (0.10)	0.89	1.0	0.76	2.0	2.38 (0.27)
	−0.030 (0.06)	0.89	1.0	0.70	3.0	2.40 (0.25)
	−0.024 (0.08)	0.76	2.0	0.70	3.0	2.43 (0.35)
						2.38 (Lit.)

Table 1—*continued*

Species	Predicted from c (error)	$\log K'$	I'	$\log K''$	I''	$\log K$ (error)
$FeOH^{2+}$	−0.039 (0.29)	11.17	0.1	11.01	0.5	11.64 (0.11)
	−0.177 (0.18)	11.17	0.1	11.09	1.0	11.62 (0.11)
	−0.139 (0.11)	11.17	0.1	11.14	2.0	11.63 (0.11)
	−0.125 (0.08)	11.17	0.1	11.21	3.0	11.63 (0.11)
	−0.289 (0.14)	11.01	0.5	11.09	1.0	11.51 (0.22)
	−0.166 (0.08)	11.01	0.5	11.14	2.0	11.57 (0.21)
	−0.139 (0.06)	11.01	0.5	11.21	3.0	11.59 (0.21)
	−0.105 (0.07)	11.09	1.0	11.14	2.0	11.70 (0.27)
	−0.101 (0.05)	11.09	1.0	11.21	3.0	11.70 (0.26)
	−0.098 (0.06)	11.14	2.0	11.21	3.0	11.71 (0.33)
						11.81 (Lit.)
$Fe_2(OH)_2^{4+}$	−0.242 (0.23)	24.7	0.1	24.7	0.5	25.10 (0.28)
	−0.229 (0.14)	24.7	0.1	24.9	1.0	25.10 (0.27)
	−0.200 (0.08)	24.7	0.1	25.3	2.0	25.11 (0.27)
	−0.173 (0.06)	24.7	0.1	25.6	3.0	25.12 (0.27)
	−0.219 (0.12)	24.7	0.5	24.9	1.0	25.18 (0.53)
	−0.189 (0.06)	24.7	0.5	25.3	2.0	25.18 (0.50)
	−0.162 (0.04)	24.7	0.5	25.6	3.0	25.22 (0.50)
	−0.174 (0.06)	24.9	1.0	25.3	2.0	25.27 (0.63)
	−0.148 (0.03)	24.9	1.0	25.6	3.0	25.35 (0.61)
	−0.122 (0.05)	25.3	2.0	25.6	3.0	25.58 (0.76)
						25.1 (Lit.)

second set of tests in which the value $c = -0.10$ was used for every ion in all the systems involved. This is the general value of c recommended by Davies for 1:1 electrolytes.[10] The resulting infinite-dilution formation-constant values obtained are given in Tables 3 and 4. Though the estimated constants in Tables 3 and 4 are not, generally, quite as reliable as those in Tables 1 and 2, they are often good enough for our soil model when better values cannot be obtained. Nevertheless it is evident from a comparison with the calculated c values in Tables 1 and 2 that slightly different c values would in some cases provide greater accuracy, particularly when predictions are made from higher ionic strengths. Tables 1–4 include values of estimated maximum errors in $\log K$. These were obtained by choosing, conservatively, the following values for the maximum error in $\mathring{a}_i$.

$$\text{For } \mathring{a}_i \leqslant 5, \quad \text{error} = 1.5;$$

$$\text{for } 5 < \mathring{a}_i \leqslant 10, \quad \text{error} = 2.0;$$

$$\text{for } 10 < \mathring{a}_i \leqslant 15, \quad \text{error} = 3.0.$$

When equation (6) was used to estimate c, the error in the latter was calculated from the propagation of error formula:[11]

$$s_Q^2 = \left(\frac{\partial Q}{\partial a}\right)^2 s_a^2 + \left(\frac{\partial Q}{\partial b}\right)^2 s_b^2 + \cdots \qquad (7)$$

where $Q = f(a,b\ldots)$, is a quantity which may be calculated from several observed quantities, $a,b,\ldots$, s_Q^2 is the variance of the mean of Q, s_a^2 is the variance of the mean of a, and so forth. Otherwise an error of 0.10 was assumed for c. The errors in the $\log K$ data were assumed to be 0.05 or 0.1, depending on the implied precision in the source tables. Hence, by use of the propagation of error formula, the errors in the predicted formation constants were estimated.

To date, we have calculated nearly 150 formation constants corresponding to an ionic strength of 0.04, for our soil model, by these methods. Most of these have been estimated by the method of Tables 1 and 2, that is, by using literature constants reported for two ionic strengths. Where the literature has contained a constant for only a single ionic strength, we have either put $c = -0.10$, as in Tables 3 and 4, or if c values have been available for a set of related systems, we have guessed a similar value for the system concerned. Errors in the predicted $\log K$ values were estimated as described above for Tables 1–4.

Even though the error estimates were less conservative for the literature $\log K$ values than for the other parameters, the error analysis showed these estimates to be the major contributors to the errors in the predicted constants, for 75% of the species involved, as shown in Table 5. It must be emphasized that the relative contribution of the error in c and the literature $\log K$ values to the calculated $\log K$ increases and decreases respectively, as the difference between the ionic strengths for the known and calculated $\log K$ values increases. Nevertheless these results lend some confidence to the use of equation (1) and the approach to estimating the c parameter.

Clearly the possibility of predicting an equilibrium constant for a species which might be non-existent at the required ionic strength must be borne in mind,[12] particularly when predictions are made for a very high ionic strength from the value for a very low one, or *vice versa*. The unreliability of many published constants is also a serious problem.[12] Hence predictions must be interpreted tentatively. Nevertheless the extended Debye–Hückel expression does provide a

Table 2. Values for c and log K and estimates of maximum errors calculated for some proton equilibria from pairs of log K values at different ionic strengths; all literature values taken from 8 and 9 (Sal = salicylate^{2-}, Mal = malonate^{2-})

Species	c (error)	Predicted from log K'	I'	log K''	I''	log K (error)
HPO_4^{2-}	−0.037 (0.19)	11.74	0.1	10.79	3.0	12.45 (0.13)
						12.35 (Lit.)
$H_2PO_4^-$	−2.766 (0.28)	5.72	0.1	6.57	0.5	5.87 (0.08)
	−1.235 (0.18)	5.72	0.1	6.46	1.0	6.02 (0.07)
	−0.590 (0.11)	5.72	0.1	6.36	2.0	6.09 (0.07)
	−0.372 (0.09)	5.72	0.1	6.26	3.0	6.11 (0.07)
	−0.010 (0.18)	6.57	0.5	6.46	1.0	7.25 (0.17)
	−0.009 (0.09)	6.57	0.5	6.36	2.0	7.25 (0.15)
	0.011 (0.07)	6.57	0.5	6.26	3.0	7.26 (0.15)
	−0.009 (0.09)	6.46	1.0	6.36	2.0	7.25 (0.22)
	0.016 (0.06)	6.46	1.0	6.26	3.0	7.27 (0.21)
	0.041 (0.08)	6.36	2.0	6.26	3.0	7.35 (0.30)
						7.20 (Lit.)
H_3PO_4	0.222 (0.36)	2.0	0.1	1.72	0.5	2.24 (0.11)
	0.086 (0.16)	2.0	0.1	1.70	1.0	2.21 (0.10)
	−0.011 (0.05)	2.0	0.1	1.86	3.0	2.19 (0.10)
	−0.023 (0.14)	1.72	0.5	1.70	1.0	1.99 (0.09)
	−0.049 (0.03)	1.72	0.5	1.86	3.0	1.97 (0.06)
	−0.055 (0.04)	1.70	1.0	1.86	3.0	1.93 (0.08)
						2.15 (Lit.)
$HSal^-$	0.002 (0.20)	13.4	0.1	13.15	1.0	13.77 (0.12)
	−0.021 (0.08)	13.4	0.1	13.12	3.0	13.77 (0.11)
	−0.031 (0.06)	13.15	1.0	13.12	3.0	13.74 (0.18)
						13.74 (Lit.)
H_2Sal	−0.080 (0.19)	2.81	0.1	2.78	0.5	2.98 (0.06)
	−0.057 (0.09)	2.81	0.1	2.78	1.0	2.99 (0.05)
	−0.092 (0.03)	2.81	0.1	3.16	3.0	2.98 (0.05)
	−0.038 (0.14)	2.78	0.5	2.78	1.0	3.02 (0.10)
	−0.094 (0.03)	2.78	0.5	3.16	3.0	2.97 (0.07)
	−0.108 (0.04)	2.78	1.0	3.16	3.0	2.89 (0.08)
						2.97 (Lit.)
$HMal^-$	0.081 (0.25)	5.28	0.1	5.07	0.5	5.66 (0.08)
	−0.042 (0.14)	5.28	0.1	5.07	1.0	5.65 (0.07)
	−0.089 (0.08)	5.28	0.1	5.14	2.0	5.65 (0.07)
	−0.110 (0.06)	5.28	0.1	5.26	3.0	5.64 (0.07)
	−0.140 (0.16)	5.07	0.5	5.07	1.0	5.55 (0.16)
	−0.134 (0.07)	5.07	0.5	5.14	2.0	5.55 (0.14)
	−0.141 (0.05)	5.07	0.5	5.26	3.0	5.55 (0.14)
	−0.131 (0.08)	5.07	1.0	5.14	2.0	5.56 (0.19)
	−0.141 (0.05)	5.07	1.0	5.26	3.0	5.55 (0.17)
	−0.151 (0.07)	5.14	2.0	5.26	3.0	5.52 (0.25)
						5.70 (Lit.)
H_2Mal	−0.017 (0.19)	2.65	0.1	2.57	0.5	2.84 (0.06)
	−0.046 (0.09)	2.65	0.1	2.60	1.0	2.83 (0.05)
	−0.052 (0.05)	2.65	0.1	2.68	2.0	2.83 (0.05)
	−0.059 (0.03)	2.65	0.1	2.81	3.0	2.83 (0.05)
	−0.068 (0.14)	2.57	0.5	2.60	1.0	2.78 (0.10)
	−0.061 (0.05)	2.57	0.5	2.68	2.0	2.79 (0.07)
	−0.066 (0.03)	2.57	0.5	2.81	3.0	2.79 (0.07)
	−0.057 (0.07)	2.60	1.0	2.68	2.0	2.81 (0.10)
	−0.066 (0.04)	2.60	1.0	2.81	3.0	2.79 (0.08)
	−0.074 (0.07)	2.68	2.0	2.81	3.0	2.74 (0.16)
						2.85 (Lit.)

Table 3. Values for log K and estimates of maximum errors for some inorganic complexes, assuming $c = -0.10$; all literature values are taken from reference 9

Species	Predicted from log K'	I'	log K (error)
$CaNO_3^+$	0.06	0.5	0.61 (0.14)
	−0.06	1.0	0.53 (0.20)
	−0.02	2.0	0.54 (0.29)
	0.04	3.0	0.54 (0.38)
			0.7 (Lit.)
$Ca(NO_3)_2$	−0.3	0.5	0.53 (0.20)
	−0.5	1.0	0.33 (0.29)
	−0.4	2.0	0.27 (0.47)
	−0.4	3.0	0.04 (0.66)
			0.6 (Lit.)
$MnNO_3^+$	−0.38	0.5	0.17 (0.14)
	−0.43	1.0	0.16 (0.20)
	−0.41	2.0	0.15 (0.29)
	−0.24	3.0	0.26 (0.38)
			0.2 (Lit.)
$Mn(NO_3)_2$	−0.3	0.5	0.53 (0.20)
	−0.6	1.0	0.23 (0.29)
	−0.9	2.0	−0.23 (0.47)
	−0.8	3.0	−0.36 (0.66)
			0.6 (Lit.)
$CuNO_3^+$	−0.13	0.5	0.42 (0.14)
	−0.01	1.0	0.58 (0.20)
	−0.06	2.0	0.50 (0.29)
	−0.02	3.0	0.48 (0.38)
			0.5 (Lit.)
$ZnNO_3^+$	−0.18	0.5	0.37 (0.14)
	−0.19	1.0	0.40 (0.20)
	−0.14	2.0	0.42 (0.29)
	0.01	3.0	0.49 (0.38)
			0.4 (Lit.)
$ZnSO_4$	0.93	0.5	2.18 (0.19)
	0.89	1.0	2.26 (0.27)
	0.76	2.0	2.13 (0.37)
	0.70	3.0	1.98 (0.46)
			2.38 (Lit.)
$FeOH^{2+}$	11.17	0.1	11.63 (0.11)
	11.10	0.5	11.61 (0.21)
	11.09	1.0	11.70 (0.27)
	11.14	2.0	11.71 (0.36)
	11.21	3.0	11.70 (0.45)
			11.8 (Lit.)
$Fe_2(OH)_2^{4+}$	24.7	0.1	25.14 (0.27)
	24.7	0.5	25.31 (0.52)
	24.9	1.0	25.49 (0.67)
	25.3	2.0	25.71 (0.92)
	25.6	3.0	25.77 (1.17)
			25.1 (Lit.)

Table 4. Values for log K and estimates of maximum errors for some proton equilibria, assuming $c = -0.10$; all literature values taken from references 8 and 9 (Sal = salicylate^{2-}, Mal = malonate $^{2-}$)

Species	Predicted from log K'	I'	log K (error)
HPO_4^{2-}	11.74	0.1	12.44 (0.13)
	10.79	3.0	12.26 (0.73)
			12.35 (Lit.)
$H_2PO_4^-$	5.72	0.1	6.14 (0.07)
	6.57	0.5	7.20 (0.16)
	6.46	1.0	7.16 (0.22)
	6.36	2.0	7.07 (0.33)
	6.26	3.0	6.93 (0.42)
			7.20 (Lit.)
H_3PO_4	2.0	0.1	2.17 (0.10)
	1.72	0.5	1.92 (0.08)
	1.70	1.0	1.84 (0.12)
	1.86	3.0	1.66 (0.31)
			2.15 (Lit.)
$HSal^-$	13.4	0.1	13.76 (0.11)
	13.15	1.0	13.67 (0.20)
	13.12	3.0	13.53 (0.37)
			13.74 (Lit.)
H_2Sal	2.81	0.1	2.98 (0.05)
	2.78	0.5	2.96 (0.08)
	2.78	1.0	2.90 (0.12)
	3.16	3.0	2.93 (0.31)
			2.97 (Lit.)
$HMal^-$	5.28	0.1	5.64 (0.07)
	5.07	0.5	5.57 (0.14)
	5.07	1.0	5.59 (0.20)
	5.14	2.0	5.62 (0.29)
	5.26	3.0	5.67 (0.37)
			5.70 (Lit.)
H_2Mal	2.65	0.1	2.82 (0.05)
	2.57	0.5	2.75 (0.08)
	2.60	1.0	2.72 (0.12)
	2.68	2.0	2.64 (0.22)
	2.81	3.0	2.58 (0.31)
			2.85 (Lit.)

Table 5. Percentage of calculated constants for which the given parameter was the major contributor to the error

Parameter	$\dot{a}_{ML}$	$\dot{a}_M$	$\dot{a}_L$	c	log K
%	0	4	11	10	75

satisfactory theoretical method for estimating equilibrium constants for many equilibria at ionic strengths at which they have not been measured.

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PHOTOCHEMICAL ANALYSIS STUDIES—III*

A FLUORIMETRIC AND ULTRAVIOLET SPECTROPHOTOMETRIC STUDY OF THE PHOTOLYSIS OF CHLOROQUINE ON SILICA-GEL THIN LAYERS, AND ITS ANALYTICAL APPLICATION

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Summary—The photolysis of chloroquine on silica-gel thin layers has been studied by ultraviolet spectrophotometry and fluorimetry. It has been shown that a reversible photoisomerization of chloroquine takes place and yields a fluorescent photoproduct. The effect of pH, alkali-metal halides, and the eluent system on the fluorescence intensity of chloroquine and its photoproduct was investigated. The fluorescence on silica-gel thin layers is quenched by high concentrations of sodium hydroxide; this is explained in terms of interference of sodium ions in the hydrogen-bonded adsorption of chloroquine on the silica gel. The results have been used to develop a method for determination and separation of chloroquine at the nanogram level. Linear calibration plots are obtained, covering the range between 4 and 10^4 ng.

Chloroquine is one of the antimalarial drugs most widely used in tropical regions for the prevention and treatment of malaria and other related protozoal diseases.[1] Sensitive, selective, and simple analytical techniques are needed for determining trace levels of chloroquine in human tissues, blood, urine, *etc.*, and to study its metabolism.[2]

Spot-tests have been used to detect the presence of chloroquine in urine, but their sensitivity is poor since they allow detection of only a few micrograms of the drug.[3–9] The use of ultraviolet and infrared spectrophotometric methods for determination of chloroquine suffers from spectral overlap by interfering substances in the biological sample, and from the low sensitivity.[10–13] Paper, thin-layer (TLC), and gas (GC) chromatography have mainly been applied to the identification of chloroquine and evaluation of its purity.[2] However, GC has also been used to determine chloroquine at the microgram level,[14–16] and as little as 5 ng can be detected by electron-capture detection.[17]

Fluorimetric methods have been extensively utilized for determination of chloroquine in biological media.[2,18–22] The earliest of these, because of the limitations of the instrumentation available, required photochemical conversion of the chloroquine into a more strongly fluorescent photoproduct,[18] and the procedure was very time-consuming.

Present-day spectrophotofluorimeters are sensitive enough to permit determination of the native fluorescence of chloroquine,[19–22] but require prevention of the photolysis of chloroquine. Recently, a photochemical–fluorimetric procedure was developed by Lukasiewicz and Fitzgerald.[23,24] It consisted of measuring the initial concentration of chloroquine in alkaline solution by digital integration of the decay of its native fluorescence signal or the growth of the fluorescence signal of its photochemical product. This technique is particularly sensitive, with a limit of detection of about 0.1 ng/ml (2.5 ng) of chloroquine,[23,24] but has several disadvantages, such as needing relatively expensive instrumentation and preliminary separation and purification of the chloroquine.

In a preliminary note,[25] we proposed a simple, sensitive and low-cost method for the determination and separation of chloroquine by photolysis *in situ* on a silica-gel thin layer to give a fluorescent photoproduct. Amounts of chloroquine as low as 4 ng can be measured by this method.

In the present paper, we report a detailed investigation, by ultraviolet spectrophotometry and fluorimetry, of the photolysis reaction and the effect of different parameters such as pH, alkali-metal salt concentration, and eluent system, on the analytical performance.

EXPERIMENTAL

Reagents

Aqueous solutions of chloroquine diphosphate ($C_{18}H_{26}ClN_3.2H_3PO_4$) were prepared by successive dilution of a $10^{-3}M$ stock solution.

* Part I, J. J. Aaron, V. R. Villafranca, V. R. White and J. M. Fitzgerald, *Appl. Spectrosc.*, 1976, **30**, 159. Part II, J. Fidanza and J. J. Aaron, *Analusis*, 1981, **9**, 118.

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Table 1. Chromatographic conditions for the separation of chloroquine*

Solvent	$EtOH-NH_3-H_2O$ (77:4:19) A	$MeOH-NH_3-H_2O$ (72:3:25) B	$n\text{-}BuOH-NH_3-H_2O$ (85:4:11) C	$CH_3COCH_3-H_2O-NH_3$ (70:30:2) D
R_f (×100)† chloroquine	72	30	76	42
I_F chloroquine§	14	9.5	15	9.5
I_F§‡ photoproduct	31	19	53	26

* Initial concentration of chloroquine $10^{-4}M$ (pH 7).
† For the photoproduct, $R_f = 0.0$ in all solvent systems.
‡ Value obtained after an irradiation time of 30 sec.
§ Relative fluorescence intensity.

Analytical-grade absolute ethanol, 1-butanol, acetone, concentrated ammonia solution and methanol were used for the TLC eluents. Distilled water was used for solutions. Analytical-grade sodium and potassium halides were utilized.

Instrumentation

Fluorimetric measurements were performed with a Turner model 111 filter fluorimeter (with 7-60 excitation and 2-A emission filters) equipped with a Camag model 110-710 automatic TLC scanner and a Kipp & Zonen model BD-12 recorder. The sensitivity was adjusted according to the fluorescence intensity.

For the photolysis, a 200-W Osram mercury arc HBO lamp was used in an Oriel model 6137 housing and run by an Oriel model 8500 power supply.

A Beckman model 3600 spectrophotometer was used to obtain ultraviolet absorption spectra, and a Perkin–Elmer model MPF-44B spectrophotofluorimeter for determining excitation and emission fluorescence spectra.

Photochemical–fluorimetric–TLC procedure

Twenty μl of aqueous chloroquine solution were spotted on silica-gel TLC plates with a 50-μl microsyringe. The plates were developed with various solvents (see Table 1), then dried, first with a hot air current, and then in an oven. The spots were detected by their fluorescence in the 254-nm radiation from a mercury lamp.

The dried plates were placed on the Camag TLC scanner, and irradiated for a fixed time with the image of the mercury arc focused on the chloroquine spots. The fluorescence intensity of the spots was then recorded as a function of time by automatic scanning of the plates with the Turner fluorimeter.

The entire procedure, including development of the plates, required about 2 hr, but the photochemical–fluorimetric measurement took less than 10 min. It was possible to examine successively five samples on the same TLC plate.

Procedure for study of the photolysis of chloroquine

After the samples had been put on the plates, dried, and irradiated for various periods of time, the chloroquine zones were scraped from the plates, and mixed well with 4 ml of water. The clear supernatant solutions were decanted, and studied by absorption spectrophotometry and spectrofluorimetry. Initial concentrations of chloroquine during irradiation were $1.9 \times 10^{-3}M$.

RESULTS AND DISCUSSION

Investigation of the photolysis of chloroquine

The photolysis of chloroquine on silica-gel thin layers is characterized by a rapid 3–5-fold increase in the fluorescence intensity, which reaches a maximum after an irradiation time of about 30 sec, and decreases somewhat on further irradiation, to about twice the initial chloroquine signal (Fig. 1).

The absorption spectra of the chloroquine zone at different stages of the photochemical reaction are given in Fig. 2. There are two main bands, one with maxima at 330 and 343 nm, the other with maxima at 222, 237 and 257 nm. Both bands are decreased in intensity by photolysis for 1 min (the longer wavelength band more than the other), and are then increased again on further irradiation for 3 and 6 min respectively. There is no significant shift of the peak wavelength on photolysis.

The fluorescence spectra are given in Fig. 3. The emission maximum is initially at 370 nm, but shifts slightly to 379 nm after irradiation for 1 min, a new and less intense emission band appearing simultaneously at about 470 nm. This 470-nm band remains approximately constant in position and intensity on further irradiation for up to 10 min.

The only photochemical scheme for the photolysis of chloroquine so far presented referred to *alkaline solution*;[23,24] it postulated two successive photochemical steps

$$CQ + h\nu_1 \rightarrow B \qquad (1)$$

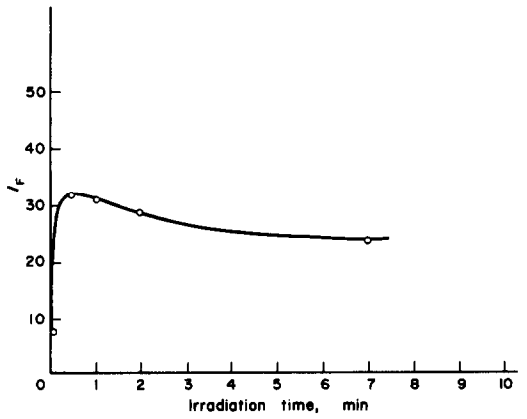

Fig. 1. Photolysis of chloroquine on silica-gel thin-layer: variation of the fluorescence signal intensity (I_F) as a function of the irradiation time. Solvent system A (Table 1). Initial chloroquine concentration 103 ppm.

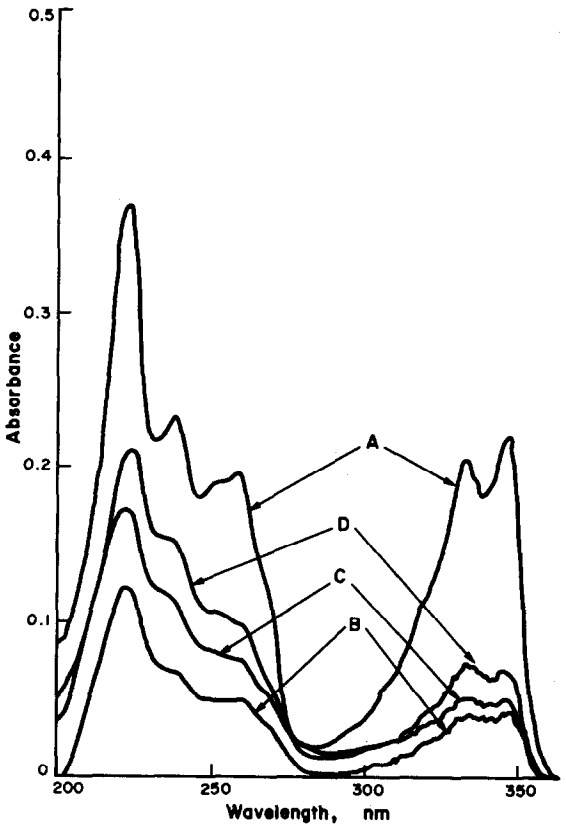

Fig. 2. Ultraviolet absorption spectra of the chloroquine zone from thin-layer chromatograms at different stages of the photochemical reaction. Chloroquine concentration 50 ppm. Photolysis time: curve A, 0; curve B, 1 min; curve C, 3 min; curve D, 6 min.

$$B + h\nu_2 \rightarrow C \qquad (2)$$

in which CQ is chloroquine, B a strongly fluorescent photoproduct and good ultraviolet absorber, and C a non-fluorescent photoproduct and poor ultraviolet absorber.

In our *solid-state photolysis* of chloroquine, the photochemical scheme seems more complicated. The rapid increase of the fluorescence intensity and the appearance of the 470-nm emission band suggest the formation of a fluorescent photoproduct B. However, the fluorescence signal decreases very slowly afterwards and then levels off, indicating that the rate of photolysis of B to C is slowed and that the reverse thermal reaction of B to chloroquine is occurring, in competition with step (2).

$$B \rightarrow CQ + \Delta H \qquad (3)$$

This interpretation is supported by the increased intensity of the ultraviolet absorption bands of chloroquine on extensive photolysis, which indicates an *increase* of its concentration, although step (1) involves a decrease.

Therefore, the primary photochemical process is probably a photoisomerization of chloroquine, a reaction which implies a limited electronic rearrangement in the molecule, and belongs to a class of aromatic heterocycle photoreactions known to be thermally reversible.[26] Step (2) implies a considerable amount of bond cleavage of the photoproduct B to give a poor ultraviolet absorber C,[23,24] the formation of which would not be evidenced in the ultraviolet spectrum because of the presence of the chloroquine yielded by step (3).

pH and salt effects on the fluorescence intensity

We have found that pH has a dramatic effect on the fluorescence intensity of chloroquine and its photoproduct; this effect is strikingly different according to whether the fluorescence measurements are made on a silica-gel thin layer or aqueous solution.

Figure 4, curve A, shows that the fluorescence intensity of chloroquine *in aqueous solution*, is practically constant for pH values between 3 and 6, but increases suddenly at around pH 8.5, and at pH 10–11 is 30 times that at pH 6. This enhancement of the fluorescence in alkaline medium is probably due to dissociation of protonated chloroquine ($pK_a = 9.94$),[2] which would give a higher fluorescence quantum yield. It also explains why earlier researchers chose a pH-10 medium for fluorimetric determination of chloroquine in aqueous solution.[2,18–22]

On the other hand, the fluorescence signal *measured on a silica-gel thin layer* is constant when the initial pH of the chloroquine solution is between 2 and 8, and decreases progressively for initial pH > 8 (Fig. 4, curve B). This behaviour can be related to quenching of the fluorescence of chloroquine adsorbed on silica gel, by the higher concentration of sodium and hydroxide ions due to the alkaline medium. It would indicate that the rate of non-radiative internal conversion of the excited singlet-state of chloroquine, and/or its rate of intersystem crossing to the triplet state, is increased relative to the rate of fluorescence emission. Recently, one of us has observed the existence of a similar quenching phenomenon of the room-temperature phosphorescence of aromatic molecules adsorbed on filter paper, in the presence of high concentrations of sodium hydroxide.[27]

There may be several causes for the quenching of the room-temperature fluorescence of chloroquine on silica gel, in the presence of sodium hydroxide. The interactions between protonated chloroquine molecules and the silica-gel matrix, which should favour the fluorescence emission, may be reduced by the dissociation of the protonated species in alkaline medium. Another, and more probable, cause may be perturbation of the silica-gel thin layer by the sodium and/or hydroxide ions, which would interfere in the adsorption of chloroquine by hydrogen-bonding to the silica-gel substrate. This would result in an increase in the probability of the non-radiative internal conversion process of the excited singlet-state chloroquine.

To decide between these interpretations, we have studied the influence of various alkali-metal halides

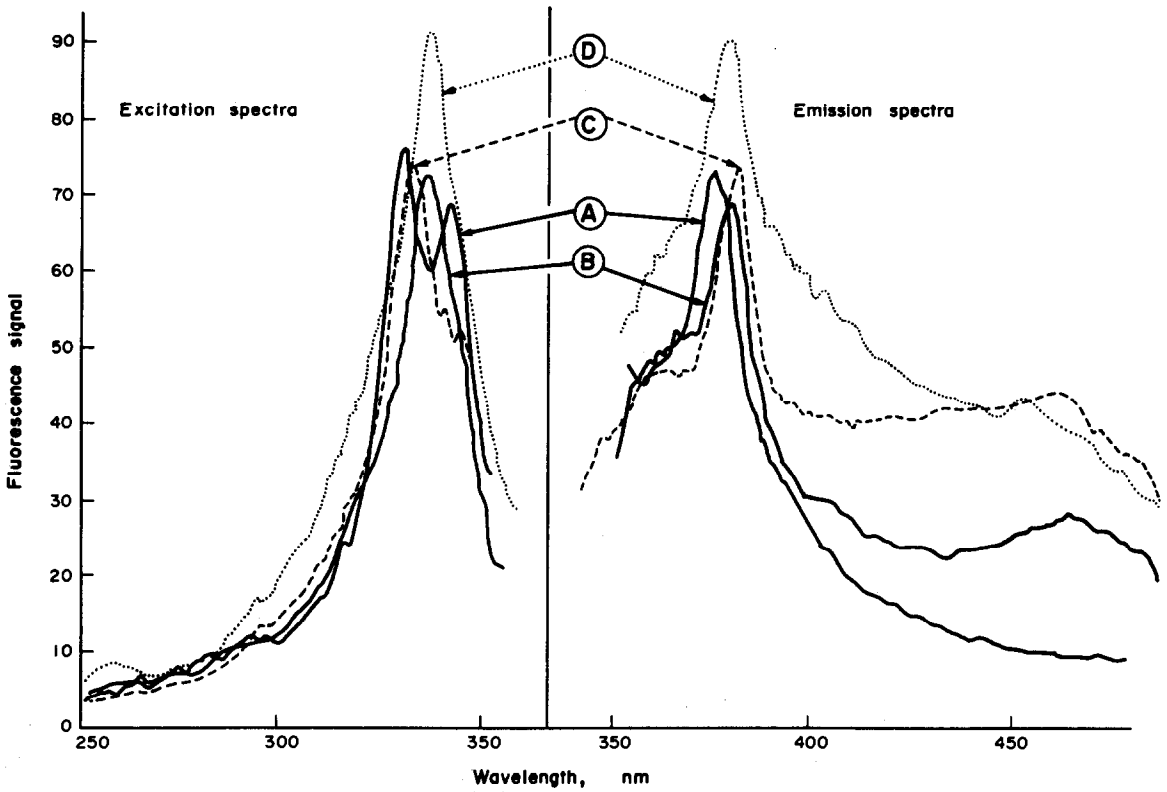

Fig. 3. Fluorescence excitation and emission spectra of the chloroquine zone from thin-layer chromatograms at different stages of the photochemical reaction. Chloroquine concentration 5 ppm. Photolysis time: curve A, 0; curve B, 1 min; curve C, 5 min; curve D, 10 min.

on the fluorescence intensity of chloroquine and its photoproduct. As can be seen in Fig. 5, the fluorescence of chloroquine and its photoproduct decreases significantly in presence of sodium fluoride, chloride or bromide at concentrations between 0.1 and 1*M*. The curves in Fig. 5 show that the quenching of the fluorescence without irradiation is practically the same for all the sodium halides tested, increasing

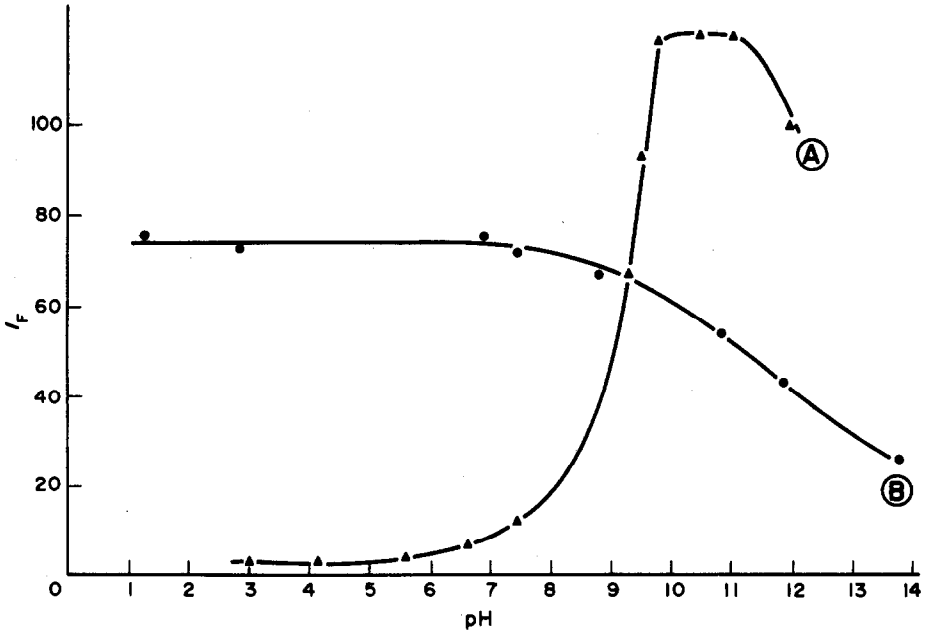

Fig. 4. Effect of pH on the fluorescence intensity (I_F) of chloroquine Curve A, fluorimetric measurement in aqueous solution (scale × 100); chloroquine concentration 10 ppm. Curve B, fluorimetric measurement on silica-gel thin-layer; chloroquine concentration 5 ppm.

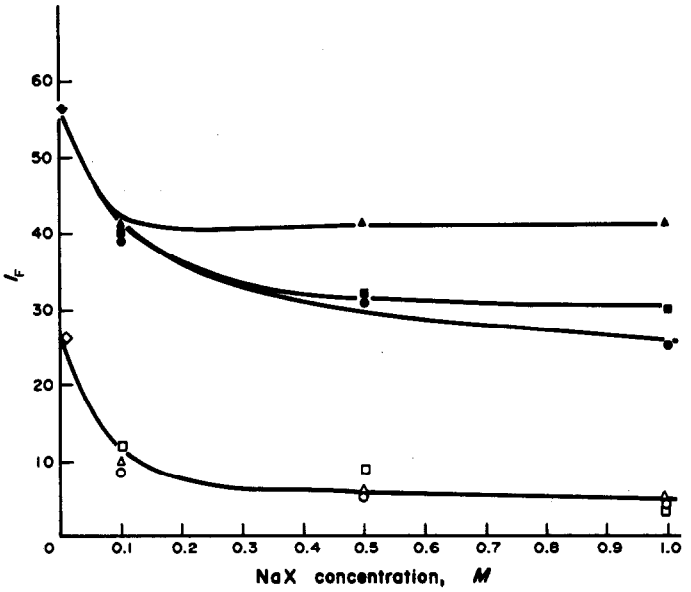

Fig. 5. Quenching effect of sodium halides on the fluorescence intensity of chloroquine and its photoproduct. ◇ ◆: water (pH-7 solution without sodium halide); ○ ●: NaF; △ ▲: NaCl; □ ■: NaBr. Open and filled symbols correspond respectively to irradiation times of 0 and 30 sec.

slightly with sodium halide concentration. The effect after 30-sec irradiation is similar, except for sodium chloride, which has less effect than the others. This suggests that the sodium ions play an important role in interfering in the hydrogen-bonding between chloroquine molecules and the silica-gel substrate. Chloride may produce a specific counter-effect.

This role of the cations is confirmed by quenching experiments performed with varying concentrations of potassium chloride and potassium bromide between 0.1 and 1.0*M*; our results show that the quenching effect due to potassium halides is about twice that of equal concentrations of the corresponding sodium halides. Therefore, the perturbation of the fluorescence by sodium halides constitutes a good model for the quenching effect of sodium hydroxide on the fluorescence intensity of chloroquine adsorbed on silica-gel thin layers.

For the maximum sensitivity of this photochemical–fluorimetric–TLC method, use of a pH-7 aqueous

Table 2. Statistical study of the photochemical–fluorimetric calibration curves for chloroquine on silica gel

Solvent system*	Irradiation time, *sec*	Linearity range, *ppm*†	Slope§	Correlation coefficient§	Detection limit,‡ *ng*
None‖	0	0.3–52	0.776	0.990	6
	30	0.2–52	0.838	0.996	4
A	0	4.6–150	0.897	0.986	93
	30	2.2–260	0.945	0.972	44
B	0	1.0–520	0.679	0.988	21
	30	0.4–520	0.730	0.996	8
C	0	1.3–520	0.576	0.989	26
	30	0.4–520	0.725	0.993	9
D	0	1.5–520	0.623	0.985	30
	30	0.7–105	0.812	0.991	14

* See Table 1 for composition.

† Defined by the chloroquine concentration limits (in ppm) for which the calibration curve is linear.

‡ Defined as the amount of compound giving a signal equal to three times the background noise, and located on the linear part of the analytical curve.

§ Calculated by least-squares treatment of the experimental data; for every calibration curve, 10–15 points were used.

‖ Calibration curve obtained without using an elution solvent.

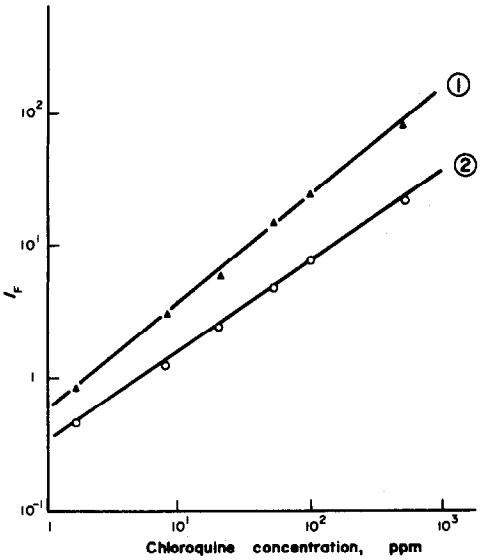

Fig. 6. Photochemical–fluorimetric calibration curves for chloroquine. Curve 1, irradiation time = 30 sec. Curve 2, irradiation time = 0 sec.

solution of chloroquine, containing no sodium halides, is optimal.

Effect of the elution solvent system

We have compared the influence of four elution solvent systems of different polarity on the conditions of TLC separation of chloroquine. We summarize our results in Table 1, which shows that the R_f of chloroquine is greater in the solvent systems A and C, which have larger proportions of ethanol and butanol. Furthermore, the fluorescence intensity of chloroquine and of its photoproduct is enhanced with these two solvent systems. However, mixture A has the drawback of a higher background fluorescence signal than that of the other mixtures. Therefore, we propose system C as the best choice.

Photochemical–fluorimetric and fluorimetric calibration curves

We present in Table 2 the statistical characteristics of the calibration curves obtained under different experimental conditions, to establish the effect of irradiation time and the solvent system on the performance of the whole method. In all cases, there is a linear log–log relation between I_F (measured for an irradiation time equal to zero and 30 sec) and the initial concentration of chloroquine (Fig. 6), over about three orders of magnitude of concentration, but the slopes are not unity, indicating non-linearity for non-logarithmic calibration plots. The photochemical–fluorimetric measurements give higher sensitivity.

The correlation coefficients are close to unity, showing that the precision is satisfactory. The precision of the photochemical–fluorimetric calibration curves is greater than that of the simple fluorimetric calibration curves, except in the case of the EtOH–NH_3–H_2O system, which gives a higher background signal, and thus decreases the accuracy.

Detection limits

We also give in Table 2 the detection limits obtained under several experimental conditions. The detection limit is defined here as the amount (or concentration) of compound giving a fluorescence signal equal to three times the fluctuations of the background fluorescence signal.

The detection limits ranged between 4 and 44 ng for the photochemical–fluorimetric measurements, and between 6 and 93 ng for the fluorimetric measurements. Therefore, the detection limit for the photochemical–fluorimetric–TLC method is lower by a factor of up to about 2 than that for the fluorimetric–TLC technique.

Comparison with other analytical methods

The detection limits for chloroquine obtained by our method are close to those given by the most sensitive analytical techniques. For instance, between 5 and 10 ng of chloroquine can be detected by GC with an electron-capture detector,[17] and 2.5 ng with a digital integration photochemical–fluorimetric procedure.[23,24] The linear range and precision of our method are comparable to those of the GC and photochemical–fluorimetric techniques.

Moreover, our TLC–photochemical–fluorimetric method has the advantage of allowing quasi-simultaneous separation of chloroquine from possible impurities and determination of amounts of chloroquine ranging between 4 and 10^4 ng, whereas the classical fluorimetric techniques[18–22] and the digital integration procedure[23,24] require preliminary purification of the chloroquine.

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THE ACCURATE DETERMINATION OF BISMUTH IN LEAD CONCENTRATES AND OTHER NON-FERROUS MATERIALS BY AAS AFTER SEPARATION AND PRECONCENTRATION OF THE BISMUTH WITH MERCAPTOACETIC ACID

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Summary—A method for determining 0.0001% and upwards of bismuth in lead, zinc or copper concentrates, metals or alloys and other smelter residues is described. Bismuth is separated from lead, iron and gangue materials with mercaptoacetic acid after reduction of the iron with hydrazine. Large quantities of tin can be removed during the dissolution. An additional separation is made for materials high in copper and/or sulphate. The separated and concentrated bismuth is determined by atomic-absorption spectrometry using the Bi line at 223.1 nm. The proposed method also allows the simultaneous separation and determination of silver.

Bismuth is a penalty impurity in lead concentrates and a quick and accurate determination in diverse materials at levels from 1 μg/g (1 ppm) upwards is important in a lead smelter. A method with high accuracy is also important in certifying primary lead standards for use in calibrating optical emission spectrometers.

Donaldson has reviewed methods for the determination of bismuth in concentrates and non-ferrous alloys and developed an iodide spectrophotometric method[1] involving separations by diethyldithiocarbamate (DDTC) and xanthate extraction, and an AAS method[2] involving DDTC extraction. These methods gave accurate results for use in the CANMET project. However, when accurate results are required in as short a time as possible, these methods take too long.

Donaldson also noted the poor sensitivity of AAS for bismuth and the numerous interferences.[1] Hence, our search for a quicker but equally accurate AAS method involved preconcentration to optimize the bismuth concentration at the measurement stage, and the elimination of interfering elements, particularly iron.

It was found that bismuth could be separated from the bulk of the lead present by precipitation with hydrogen sulphide from a 50% perchloric acid solution. In addition to bismuth, elements such as arsenic, antimony, tellurium, silver, gold, selenium and tin are also removed quantitatively. A small variable quantity of lead does precipitate, but the large bulk of the lead remains in solution.

The internal generation of hydrogen sulphide with thioacetamide was tried. It was very successful with lead alloys containing bismuth, copper, silver, arsenic, antimony *etc.*, and only a very small and constant amount of lead sulphide was precipitated with the impurity elements. However, with concentrates, the amount of lead sulphide precipitated was variable. To minimize or eliminate this variable precipitation of lead an alternative precipitant for bismuth was sought, and mercaptoacetic acid was chosen. It forms precipitates with most elements in the hydrogen sulphide group[3] in acid solution, and also reduces iron(III) to iron(II).

With solutions of the pure metals it was shown that recovery of bismuth was complete with copper as collector provided that the perchloric acid concentration did not exceed 2% v/v and the copper/bismuth ratio was at least 10. Higher acidity caused the precipitate to break up and tend to creep out of the filter paper, with a subsequent loss of bismuth.

Activated carbon was found satisfactory as the collector instead of copper, but a large amount was required: 500 mg for 5 mg of bismuth. However, the presence of copper lowered the amount of carbon required, and a combination of copper and activated carbon gave 100% recovery of bismuth from 2–15% v/v perchloric acid medium. Large amounts of copper or sulphate have an adverse effect, however, and must be separated before the mercaptoacetic acid precipitation.

EXPERIMENTAL

Reagents

Analytical-grade reagents were used throughout.

Standard bismuth solution (1 ml = 1 mg of Bi). Dissolve 0.5000 g of 99.999% pure bismuth metal and 2 g of ammonium fluoride in 50 ml of water plus 50 ml of concentrated nitric acid, and dilute to 500 ml.

Mercaptoacetic acid solution, 10% *v/v*.

Copper perchlorate solution. Dissolve 1 g of copper (Bi less than 1 ppm) in 5 ml of concentrated nitric acid and 10

ml of concentrated (72%) perchloric acid and heat to fuming. Cool. Dilute to 100 ml with water.

Activated carbon: Merck G.R. 2186.

Calibration

To each of six 150-ml beakers add 5 ml of concentrated (72%) perchloric acid. Add from a burette 0, 0.5, 1.0, 2.0, 3.0 and 4.0 ml respectively of standard bismuth solution. Heat to fuming to remove nitric acid and fluoride ion. Cool. Add 20 ml of water and 20 ml of concentrated hydrochloric acid to each and boil. Cool and dilute to volume in 100-ml standard flasks. This gives a series of bismuth standards containing, 0, 5, 10, 20, 30 and 40 mg/l.

Prepare a calibration graph of absorbance (at 223.1 nm) *vs.* bismuth content of standards; use an approximately stoichiometric air–acetylene flame. With a Varian Techtron AA6 instrument use a fuel setting of 2 and an air setting of about 6. Spray the 40-mg/l. standard and adjust the burner height so that the absorbance is about 0.6 (without scale expansion). Spray distilled water and set the zero, then adjust the gas ratio so that the zero remains constant with and without water being sprayed.

Concentrates

Weigh 2–10 g of concentrate containing 0.015–1 mg of bismuth and transfer to a 250-ml beaker. Add 5 ml of water, 5 ml of concentrated hydrochloric acid and, in proportion to sample weight, 10–20 ml of 72% perchloric acid. Heat gently on a hot-plate until the perchloric acid refluxes. Cool.

Note. Care should be taken with the dissolution. Some concentrates are difficult to dissolve and the undissolved material may react violently with the hot concentrated perchloric acid.

If the sample contains less than 50 mg of copper add 5 ml of copper perchlorate solution. Dilute to approximately 50 ml. Add a few boiling chips and 5 ml of 20% hydrazine hydrate solution. Boil. Add a further few drops of hy-

Table 1. Recovery experiments with bismuth added to various concentrates and smelter materials

Matrix	Sample wt., *g*	Nominal composition, %						Total Bi, μg	
		Pb	Zn	Cu	Fe	S	Bi	Taken	Found
Woodland Mines lead concentrate	1.000	29	12	4	17	31	0.0328	328	335
								428	435
								528	525
								628	630
								728	730
New Broken Hill Consolidated lead concentrate	2.00	74	4	1.3		16	0.0205	410	416
								510	506
								610	610
								710	714
								810	814
Zinc Corporation lead concentrates	5.00	76	3	1.0			0.0077	385	385
								485	495
								585	595
								685	675
								785	775
North Broken Hill Ltd. lead concentrates	10.00	73	5	0.8			0.0011	110	120
								210	215
								310	320
								410	425
								510	520
Zinc plant leach residue	10.00	41*	9		7		0.0023	230	230
								330	330
								430	425
								530	520
								630	625
								730	725
Electrolytic Zinc Co. of Australia leady residue	5.000	20	10		14	12	0.0131	655	663
								755	758
								855	858
								955	970
								1055	1069
								1155	1170
Copper matte	10.00	40		40	0.3	15	0.00015	15	15
								115	120
								215	210
								315	315
								415	415
								515	510

* Present as $PbSO_4$.

Table 2. Analysis of standard samples

Sample	Composition, %	Certified value and range %Bi	%Bi Found	Mean
NBS SRM 53e Bearing metal	84 Pb, 10 Sb, 6 Sn	0.052	0.0522	
			0.0526	
			0.0520	0.0520
			0.0521	
			0.0514	
NBS SRM 54d Bearing metal	88 Sn, 7 Sb, 3.6 Cu	0.044 (0.037–0.050)	0.0452	
			0.0446	
			0.0437	0.0445
			0.0445	
			0.0447	
BCS 183/3 Leaded gunmetal	84 Cu, 7 Sn, 3 Zn, 3 Pb	0.008 (0.007–0.010)	0.00726	
			0.00725	
			0.00718	0.00722
			0.00731	
			0.00710	
BCS 178/1 White metal	86.2 Sn, 6.72 Sb, 3.23 Pb	0.08 (0.073–0.10)	0.0813	
			0.0799	
			0.0818	0.0815
			0.0823	
			0.0820	
BCS 177/2	84.5 Pb, 10.1 Sb, 5.07 Sn	0.028 (0.024–0.032)	0.0260	
			0.0259	
			0.0270	
			0.0258	0.0263
			0.0268	
Canmet MP-1 Zinc, tin–copper–lead ore (bottle 1)	16.3 Zn, 2.5 Sn, 2.2 Cu, 1.9 Pb, 5.7 Fe, 0.8 As, 19.4 Si, 3.6 Al 3.4 Ca	revised figs 0.024 (0.022–0.026)	0.0214	
			0.0210	
			0.0210	0.0210
			0.0207	
			0.0211	
(bottle 2)			0.0229	
			0.0229	0.0225
			0.0222	
			0.0222	
			0.0225	
Canmet CPB-1	64.7 Pb, 4.4 Zn, 8.4 Fe, 0.014 Cd, 0.36 Sb, 0.62 Ca, 0.10 Mg, 0.039 Mn, 0.022 Sn	0.023 (0.021–0.024)	0.0219	
			0.0225	0.0222
			0.0219	
			0.0226	
			0.0221	
Canmet CZN-1	44.7 Zn, 7.4 Pb, 10.9 Ge, 30.2 S	0.0032 (0.0023–0.0040)	0.00223	
			0.00218	0.00221
			0.00219	
			0.00223	
			0.00225	
CZN-1 with carbonate separation			0.00212	
			0.00214	
			0.00216	0.00213
			0.00212	
			0.00213	
CCU-1	24.7 Cu, 3.2 Zn, 0.11 Pb, 30.8 Fe, 35.6 S	0.0026 (0.0008–0.0043)	0.00312	
			0.00313	
			0.00303	0.00310
Sample weights 5.12–5.69 g			0.00310	
			0.00311	
			0.00316	
			0.00317	
Sample weights 10.02–10.29 g			0.00320	0.00319
			0.00323	
			0.00318	

drazine hydrate solution without stirring, to check for complete reduction of iron. The precipitate should be dark green to black. The reddish brown colour of ferric hydroxide shows the need for more hydrazine hydrate to complete the iron reduction. If reduction is complete, stir to redissolve the precipitate. A pale blue solution indicates the presence of sufficient copper. Cool.

Add 5 ml of mercaptoacetic acid solution with stirring. The precipitate should be yellow. If a black precipitate is formed, either the acidity is too low or the copper content is too high (see later), and the precipitate is gelatinous and difficult to filter. Dilute to approximately 150 ml, add 0.3–0.4 g of activated carbon and stir. Filter off on a Whatman No. 2 paper and wash thoroughly with water. Discard the filtrate. Transfer the filter paper to the original beaker, add 10 ml of concentrated nitric acid, 10 ml of 72% perchloric acid, cover with a watch-glass and heat until all the carbon is destroyed. The addition of a few drops of nitric acid while the perchloric acid is refluxing will speed the removal of the carbon. When the carbon has been destroyed heat further to reduce the perchloric acid volume to 5 ml or less. Cool.

Add about 10 ml of water and 5 ml of concentrated hydrochloric acid. Boil. Cool, transfer to a 25-ml standard flask and dilute to mark with water. Mix. Determine bismuth as in the calibration procedure.

Metals and alloys

Dissolve the required mass of sample in 10–20 ml of 72% perchloric acid and 5 ml of concentrated nitric acid. Heat to fumes of perchloric acid. Cool, dilute to 50 ml with water, add copper perchlorate solution if necessary, and proceed from "Add a few boiling chips.....".

Materials high in tin

Large quantities of tin present in white metals and bearing metals, ores, *etc.* may be removed by adding hydrobromic acid during the dissolution process given for metals and alloys.

Materials high in copper and/or sulphate

Large quantities of copper (> 100 mg) present in samples of copper matte, some concentrates, ores and alloys, must be separated before the mercaptoacetic acid precipitation, and so must high sulphate contents (> 10 mg).

After dissolution and fuming is complete as in the dissolution of concentrates or metals and alloys, cool the beaker and add 50 ml of water. Add excess of ammonia and 10 g of ammonium carbonate. Dilute to 250 ml and boil. Collect the precipitate (Whatman No. 2 paper) and wash it well. Discard the filtrate. Return the filter paper to the beaker, add 5 ml of copper perchlorate solution, 20 ml of concentrated nitric acid and 20 ml of 72% perchloric acid. Cover with a watch-glass and heat to perchloric acid fumes. Cool and proceed from "Add a few boiling chips.....".

RESULTS

The method was checked by determinations on concentrates and other smelter materials with various amounts of added bismuth, and also by analysis of standard reference materials. Table 1 shows the recovery of bismuth added to various lead concentrates requiring only simple dissolution, and to smelter materials which require the carbonate separation. Table 2 shows results for standard materials.

DISCUSSION

Determination of other elements in the final analysis solutions showed complete separation from lead, zinc, iron and cadmium. Depending on the efficiency of the hydrobromic acid treatment, 50–100% of the tin and 60–80% of the antimony present are separated. Neither tin nor antimony interferes with the AAS measurements of bismuth at the levels present after this treatment.

There is no loss of bismuth in the carbonate separation; removal of copper and lead is good, and very little lead remains in the final measurement solution.

Silver is also quantitatively precipitated by mercaptoacetic acid along with bismuth and copper and thus this method may be used to determine low levels of silver in the range of materials listed in this paper. Some examples of the determination of silver are given in Table 3.

Table 3. Analysis of standard samples for silver (for composition of samples see Table 2)

Sample	Ag, *ppm* Certified value and range	Found	Mean
MP-1	revised value		
	56.3	54.6	
(bottle 1)		54.4	
	(54.2–58.5)	54.5	54.1
		53.3	
		53.6	
		57.7	
(bottle 2)		57.7	
		57.2	57.5
		57.6	
		57.4	
CPB-1	626	617	
	(620–632,	625	
		605	615
		608	
		620	
CZN-1	93	96.5	
	(90–95)	95.6	
		96.7	96.7
		97.1	
		97.4	
NBS SRM 54d	32	35.6	
	(17–41)	35.4	
		35.4	35.5
		35.5	
		35.4	

Further work on the use of this method for silver will be reported in a future paper. The results in Table 3 and recovery experiments indicate that more extensive proving of this method for silver is warranted.

The results for bismuth in the B.C.S. and NBS samples are in good agreement with the certified values. The results for the zinc–tin–copper–lead ore MP-1 are lower than the original recommended value[4] and the revised value[5,6] but in excellent agreement with that of Donaldson[1] (0.022%). Donaldson noted the possibility of bias in the recommended value.

Our value of 0.0022% for the zinc concentrate CZN-1 is lower than the recommended value but in

agreement with Donaldson's results. CZN-1 is a good "clean" concentrate and should not present any difficulties. As our figures for other more complex zinc ores, *e.g.*, MP-1, and our recovery figures for high-zinc plant samples are very satisfactory, we are confident of our result for CZN-1. In the original Canmet certification programme for CZN-1 in 1978 we obtained a mean figure of 0.00235% bismuth, by a straight AAS determination using standards accurately matched with the sample for zinc, lead and iron content.

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SPECTROPHOTOFLUORIMETRIC DETERMINATION OF TERBIUM, EUROPIUM AND SAMARIUM WITH PIVALOYLTRIFLUOROACETONE AND TRI-n-OCTYLPHOSPHINE OXIDE IN MICELLAR SOLUTION OF NONA-OXYETHYLENE DODECYL ETHER

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Summary—The fluorescence intensities of terbium, europium and samarium complexes with several β-diketone derivatives in the absence and presence of tri-n-octylphosphine oxide (TOPO) in micellar solution of nona-oxyethylene dodecyl ether (BL-9EX) were examined. The spectrophotofluorimetric determination of terbium (1.5–1500 ng/ml) has been investigated with pivaloyltrifluoroacetone (PTA) and TOPO in 0.5% micellar solution (pH 3.8–4.5) of BL-9EX. The excitation and emission wavelengths were 309 and 542 nm, respectively. Europium and samarium could be determined by a similar method.

In a previous paper, it was reported[1] that thenoyltrifluoroacetone (TTA), tri-n-octylphosphine oxide (TOPO) and their mixed complex with europium or samarium are readily soluble in aqueous solution containing polyoxyethylene iso-octylphenol (Triton X-100), and the fluorescence intensities of these complexes (which are considerably stronger than those of the corresponding mixed complexes extracted into various organic solvents) were applied for the determination of these metal ions. However, the terbium mixed complex did not give any fluorescence. Also, iron[2] and uranium[3] have been spectrophotometrically estimated with TTA and TTA–TOPO, respectively, in micellar solution of nona-oxyethylene dodecyl ether (BL-9EX).

In the present paper, the fluorescence intensities of terbium, europium and samarium complexes with several β-diketone derivatives in the absence and presence of TOPO in micellar solutions of BL-9EX are reported, and a spectrophotofluorimetric method for the determination of terbium with pivaloyltrifluoroacetone (PTA) and TOPO is described, because this mixed complex gives stronger fluorescence intensity than any other of the complexes of various β-diketone derivatives in the absence and the presence of TOPO. The determination of europium and samarium with PTA and TOPO is also described.

EXPERIMENTAL

Reagents

Standard solutions of the metal ions were prepared by dissolving the 99.9% pure oxides (for all rare earths except cerium) or the chloride (cerium and thorium) in dilute hydrochloric acid. The solutions were standardized by titration with EDTA (Xylenol Orange indicator).

Standard solutions of various β-diketone derivatives and TOPO were prepared by dissolving the reagents (Dojin Co.) in aqueous 5% w/v BL-9EX (Nikko Chemical Co.) solution. The mixed reagent solution used for the determination was $5 \times 10^{-3}M$ PTA, $5 \times 10^{-3}M$ TOPO and 5% BL-9EX.

An acetate buffer solution (pH 4.5) was prepared by adding 55 ml of 0.1*M* sodium acetate to 100 ml of 0.1*M* acetic acid.

All other chemicals used were of analytical reagent grade.

Apparatus

A Hitachi 204 spectrophotofluorimeter was used in combination with a high-pressure xenon tube and a Hitachi 200-20 automatic recording spectrophotometer.

Procedure

To a dilute hydrochloric acid solution containing 0.03–30 μg of terbium were added 2 ml of the mixed PTA–TOPO reagent solution and 2 ml of the acetate buffer solution. After adjustment to about pH 3.8–4.5 with dilute hydrochloric acid and sodium hydroxide solution, the solution was transferred to a 20-ml standard flask and made up to volume with water. The fluorescence in a 1-cm quartz cell was measured at apparent excitation and emission wavelengths of 309 and 542 nm, respectively. The procedure was also suitable for the determination of europium (0.03–30 μg) or samarium (3–30 μg) by measurement at apparent and emission wavelengths of 310 and 611 nm, respectively, for europium or 309 and 598 nm for samarium.

RESULTS AND DISCUSSION

The fluorescence intensities of the terbium, europium and samarium complexes of dipivaloylmethane

Table 1. Fluorescence intensities of terbium, europium and samarium complexes with several β-diketone derivatives in the absence and presence of TOPO

Rare earth complexes	In the absence of TOPO λ_{ex}, nm	λ_{em}, nm	Relative intensity	In the presence of TOPO λ_{ex}, nm	λ_{em}, nm	Relative intensity
Tb–DPM	—	—	—	311	545	0.36
Tb–TAA	306	542	0.03	306	542	0.73
Tb–HFA	321	545	0.01	315	542	0.78
Tb–PTA	309	544	1.00	309	542	7.29
Tb–BFA	330	542	0.03	339	543	0.04
Tb–FTA	—	—	—	330	542	0.06
Tb–TTA	—	—	—	—	—	—
Tb–H(fod)	315	542	1.26	315	543	1.47
Eu–DPM	—	—	—	—	—	—
Eu–TAA	—	—	—	311	612	0.32
Eu–HFA	—	—	—	312	613	8.54
Eu–PTA	310	611	0.24	310	611	8.01
Eu–BFA	330	612	2.04	340	613	18.01
Eu–FTA	350	611	0.06	350	612	22.00
Eu–TTA	350	612	1.00	350	612	23.60
Eu–H (fod)	316	610	7.14	316	610	6.89
Sm–DPM	—	—	—	—	—	—
Sm–TAA	—	—	—	305	601	2.08
Sm–HFA	—	—	—	320	596	2.48
Sm–PTA	305	602	3.12	309	598	3.96
Sm–BFA	340	596	0.65	340	596	5.92
Sm–FTA	357	596	0.40	357	596	7.62
Sm–TTA	355	596	1.00	355	596	6.46
Sm–H(fod)	322	596	2.44	322	596	2.44

* Tb, Eu and Sm $1 \times 10^{-5}M$, β-diketone derivatives $1 \times 10^{-4}M$, BL-9EX 0.2%, pH 5.2. The reference intensities (1.00) are those of the Tb–PTA, Eu–TTA and Sm–TTA complexes in the absence of TOPO.

(DPM), trifluoroacetylacetone (TAA), hexafluoroacetylacetone (HFA), PTA, benzoyltrifluoroacetone (BFA), furoyltrifluoroacetone (FTA), TTA and heptafluorobutanoylpivaloylmethane [H(fod)] in the absence and presence of TOPO in 0.5% micellar solutions (pH 5.2) of BL-9EX are given in Table 1. From the results, the following trends can be recognized, though the values do not always represent the intensities at the optimum pH for the complexes: (*i*) most of the fluorescences are intensified by addition of TOPO; (*ii*) the europium and samarium complexes of β-diketone derivatives having R = R′ (R and R′ represent the functional groups at both ends) such as DPM and HFA do not fluoresce; (*iii*) the complexes of terbium with FTA and TTA do not fluoresce, and the TTA complex does not fluoresce even in the presence of TOPO; (*iv*) the mixed complex of terbium with PTA and TOPO gives a stronger fluorescence intensity than any of the other mixed complexes.

Fluorescence spectra

Figures 1–3 show the apparent fluorescence spectra of aqueous 0.5% BL-9EX solutions (pH 3.4) containing the terbium, europium and samarium PTA–TOPO mixed complexes for excitation wavelengths of 309, 310 and 309 nm, respectively. The emission wavelengths of prominent maxima in the spectra are 485, 542 and 582 nm for terbium, 592 and 611 nm for europium and 560, 598 and 643 nm for samarium.

As shown in Figs. 1 and 2, no emission spectra were

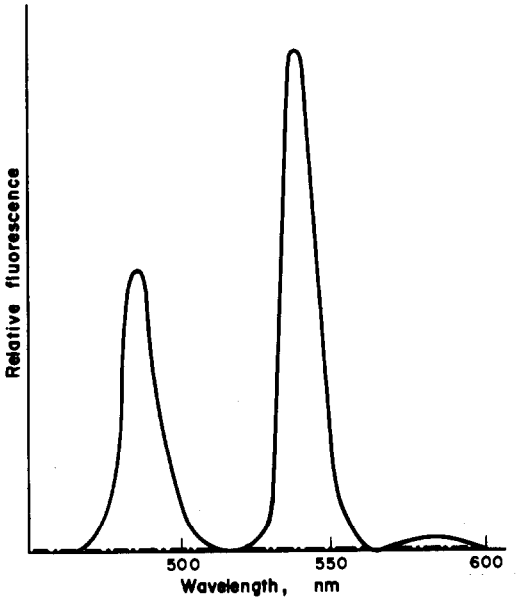

Fig. 1. Apparent fluorescence spectrum of terbium–PTA–TOPO–surfactant system. Tb^{3+} $5 \times 10^{-6}M$, PTA $5 \times 10^{-4}M$, TOPO $5 \times 10^{-4}M$, BL-9EX 0.5%, pH 3.4, fluorescence (——) and blank (— · —).

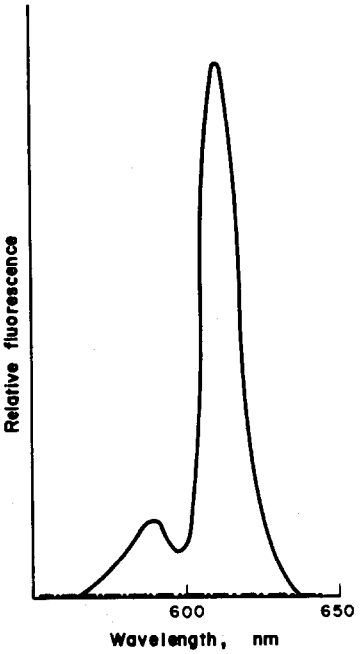

Fig. 2. Apparent fluorescence spectrum of europium–PTA–TOPO–surfactant system. Eu^{3+} $5 \times 10^{-6}M$, PTA $5 \times 10^{-4}M$, TOPO $5 \times 10^{-4}M$, BL-9EX 0.5%, pH 3.4, fluorescence (——) and blank (— — —).

found for the blank in the terbium and europium systems. On the other hand, a band was observed for the blank in the samarium system, as shown in Fig. 3. The detector sensitivity for the samarium system needs to be several hundred times that for the terbium or europium system; because the fluorescence of the samarium system is very much weaker. The band observed for the blank shifts to longer wavelength when the excitation wavelength is shifted in that direction, and this is also observed for solutions containing the surfactant only. Thus, the appearance of the band for the samarium blank system is thought to be due to

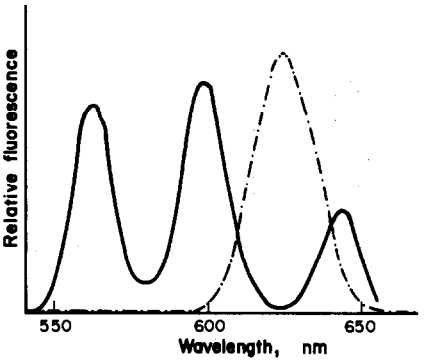

Fig. 3. Apparent fluorescence spectrum of samarium–PTA–TOPO–surfactant system. Sm^{3+} $1 \times 10^{-4}M$, PTA $5 \times 10^{-4}M$, TOPO $5 \times 10^{-4}M$, BL-9EX 0.5%, pH 3.4, fluorescence (——) and blank (— — —).

scattering of the excitation radiation by the micellar surface. However, the band gives practically no interference in the determination of samarium.

Effect of reaction variables

The variation in the fluorescence intensity at 542 nm for the terbium PTA–TOPO mixed complex was investigated as a function of pH, the intensity was constant over the pH range 3.1–4.6. The variation in intensity was also examined as a function of mole ratio of PTA to terbium (2.11×10^{-4} mmole) in the presence of a constant amount of TOPO (4.00×10^{-2} mmole) and as a function of mole ratio of TOPO to terbium in the presence of a constant amount of PTA (4.33×10^{-2} mmole). The intensities were almost constant over the mole-ratio range 100–500 for PTA:Tb and 100–1000 for TOPO:Tb. The variation in intensity was investigated as a function of the concentration of BL-9EX in the presence of excess of PTA and TOPO. Concentrations of BL-9EX in the 0.1–1.0% w/v range gave constant intensity.

Table 2. Determination of terbium by the standard-addition method

Rare earth and thorium oxides, % w/w						Tb_4O_7 found, % w/w
Synthetic samples						
Sc_2O_3	8.79	La_2O_3	6.07	CeO_2	6.78	
Pr_6O_{11}	7.36	Nd_2O_3	6.38	Sm_2O_3	6.51	
Eu_2O_3	6.37	Gd_2O_3	6.33	Tb_4O_7	0.68	0.73
Dy_2O_3	6.61	Ho_2O_3	6.42	Er_2O_3	6.70	
Tm_2O_3	6.27	Yb_2O_3	6.42	Lu_2O_3	6.25	
ThO_2	6.24					
Gd_2O_3	99.89	Tb_4O_7	0.11			0.11
Dy_2O_3	99.90	Tb_4O_7	0.10			0.10
Er_2O_3	99.91	Tb_4O_7	0.09			0.09
*Rare earth oxides (xenotime)**						
Y_2O_3	64.3	La_2O_3	0.6	CeO_2	1.4	
Pr_6O_{11}	0.2	Nd_2O_3	0.9	Sm_2O_3	1.1	
Eu_2O_3	0.1	Gd_3O_3	3.3	Tb_4O_{11}	0.9	0.75
Dy_2O_3	7.8	Ho_2O_3	1.8	Er_2O_3	6.3	
Tm_2O_3	0.9	Yb_2O_3	9.5	Lu_2O_3	0.9	

* Donated by Mr. M. Sato of the Shinetsu Chemical Co. Ltd., who also provided the XRF results.

Table 3. Comparison between the fluorescence intensities obtained by the proposed and solvent extraction methods for $1 \times 10^{-6}M$ terbium

Method	Medium	PTA, M	TOPO, M	pH	Relative fluorescence	
Proposed	Aqueous 0.5%	5×10^{-4}	5×10^{-4}	4.0	100	
Extraction	Carbon tetrachloride	5×10^{-4}	5×10^{-4}	4.0	0.08	(18.4%)*
	Carbon tetrachloride	5×10^{-3}	5×10^{-4}	4.0	0.02	(21.3%)
	Carbon tetrachloride	5×10^{-4}	5×10^{-3}	4.0	0.02	(98.2%)

* Values in parentheses show approximate degree of extraction.

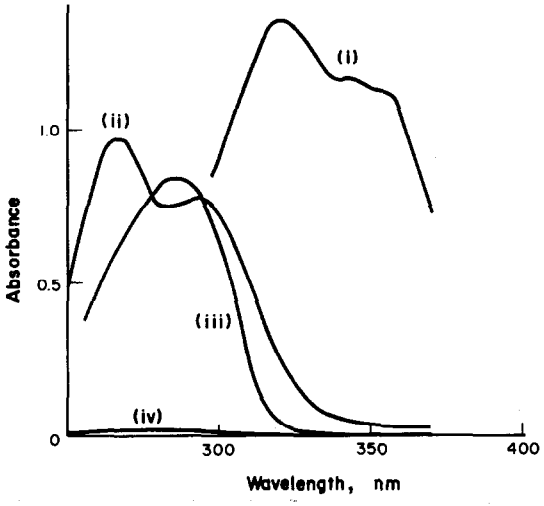

Fig. 4. Absorption spectra of TTA and PTA in aqueous BL-9EX solution and carbon tetrachloride. (*i*) $1 \times 10^{-4}M$ TTA in carbon tetrachloride (*ii*) $1 \times 10^{-4}M$ TTA in aqueous 0.5% BL-9EX solution (*iii*) $1 \times 10^{-4}M$ PTA in carbon tetrachloride (*iv*) $1 \times 10^{-4}M$ PTA in aqueous 0.5% BL-9EX solution.

Calibration graph

The fluorescence intensity was a linear function of concentration in the range 10–1500 ng/ml for terbium and europium, and 150–1500 ng/ml for samarium.

Effects of diverse metals

The effects of scandium, other rare earths and thorium on the fluorescence intensity of terbium under the optimum conditions were investigated. With 16.8-ng/ml terbium solution, 10-fold concentrations of scandium, lanthanum, gadolinium, dysprosium, lutetium and thorium did not interfere. However, larger concentrations of these metals, especially the rare earths absorbing in the ultraviolet region, interfered. Therefore the standard-addition method was used when such metals were present. It was tested for the analysis of synthetic mixtures of rare earth and thorium oxides, and for mixed rare earth oxides obtained from xenotime. The results in Table 2 shows that the method is suitable when large amounts of the interfering metals are present.

Composition of the mixed complex

The continuous variation method was used for finding the composition of the terbium mixed complex, one ligand being added in large excess, and the mole fraction of the other (with respect to terbium) being varied. The results showed that the combining ratios of Tb:PTA and Tb:TOPO were 1:3 and 1:2, respectively, the composition of the mixed complex thus being $Tb(PTA)_3(TOPO)_2$.

Comparison with the solvent extraction method

Table 3 shows the difference between the present method and the solvent extraction method. With PTA and TOPO concentrations in carbon tetrachloride equal to those of the present method (both $5 \times 10^{-4}M$), terbium is not completely extracted. Though the degree of extraction of terbium increases with increasing concentration of PTA or TOPO, the fluorescence intensity decreases, apparently as a function of the absorption intensity of PTA in the ultraviolet region. Figure 4 shows the absorption spectra of PTA and TTA in BL-9EX solution and carbon tetrachloride. For both reagents in aqueous solution the absorption bands are at shorter wavelengths than for the reagents in the organic medium. The absorption band of PTA in the aqueous solution is especially weak. There is therefore a much lower inner filter effect from the excess of reagents when the aqueous medium is used, since the excitation wavelength used for the complexes is about 310 nm.

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FLUOROMETRIC DETERMINATION OF HYDRAZINE AND AMMONIA SEPARATELY OR IN MIXTURES

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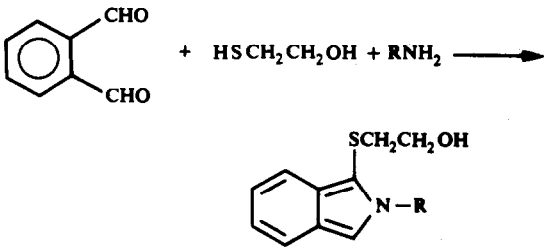
Summary—Hydrazine and ammonia are often added to boiler water to inhibit corrosion. The reagents *o*-phthalaldehyde and mercaptoethanol have been found to form derivatives with hydrazine and ammonia which can be determined by fluorimetry. Because the optimum pH values for formation of the hydrazine and ammonia derivatives were different, analysis of mixtures of the two components without prior separation was possible. Simulated wet-lay-up boiler water samples containing 5–200-μg/ml levels of hydrazine and ammonia have been analysed with an average relative error of about 10%.

Hydrazine is an important industrial chemical, used as a starting material for the synthesis of polymers, blowing agents, pesticides, and pharmaceuticals.[1] Hydrazine is also commonly added to steam-boiler water to act as a reducing agent for dissolved oxygen and metal oxides. Because its reaction products are nitrogen and water, hydrazine is the reagent of choice for inhibiting corrosion or scale build-up in boilers used by electric utility companies. Generally this protective process is carried out at alkaline pH with a mixture of hydrazine and ammonia at the ppm level, under two different circumstances. One of these is the addition of ammonia and hydrazine at the 0.1–10 μg/ml level to boiler feed-water during the time the boiler is generating steam. The other is when the boiler is idle for short periods, owing to low production requirements, plant break-downs and other events, and higher concentrations (10–200 μg/ml for ammonia and 30–300 μg/ml for hydrazine) are then generally necessary, depending on the length of down-time.[2] To minimize the possibility of any boiler degradation, both additives must be determined periodically during this period of boiler idleness. In addition, the determination of hydrazine itself is of considerable importance because it is highly toxic and a suspected carcinogen.[1]

A variety of analytical methods for the determination of either hydrazine or ammonia have been developed and reviewed.[3,4] The colorimetric procedure for hydrazine after reaction with *p*-dimethylaminobenzaldehyde[5] and the potentiometric method for ammonia[6] are probably the two most widely accepted. However, methods for the determination of small quantities of hydrazine and ammonia in mixtures are quite uncommon. An oxidation-titration method using bromide[7] for the analysis of hydrazine–ammonia mixtures is simple and inexpensive, but generally was only useful down to mg quantities. Knox and McHale[8] developed a mass spectrometric method for hydrazine, ammonia, and other gases, that required expensive or specialized equipment, which limited its use.

The use of the reaction involving *o*-phthalaldehyde (OPA) and mercaptoethanol (MCE) for forming fluorescent derivatives of primary amines has proved useful for the trace determination of amino-acids,[9] peptides[10] and proteins.[11]



The product of the reaction is an *N*-substituted 1-(2-hydroxyethyl)thio isoindole, as proposed by Simons and Johnson.[12,13]

However, this reaction has not been previously applied to the determination of simple amines such as hydrazine and ammonia. We have found this method to be useful for the fluorometric determination of both hydrazine and ammonia at the ppm level. Mixtures of hydrazine and ammonia typical of boiler wet lay-up water samples can also be analysed without the need for a separation step.

EXPERIMENTAL

Reagents

Desiccated hydrazine sulphate and dried ammonium sulphate were used to prepare aqueous 500-μg/ml hydrazine and ammonium ion stock solutions, a few drops of concentrated sulphuric acid being incorporated in the ammonium ion solution. Appropriate aliquots of these stock solutions were used to prepare the sample mixtures. Stock solutions of OPA and MCE were prepared by dissolving 0.03 g of OPA in 25 ml of methanol and by diluting 0.4 ml of MCE

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Table 1. Summary of optimum conditions for fluorescence determination of hydrazine and ammonia

Parameter	Hydrazine	Ammonia
Excitation		
Wavelength, *nm*	345	340
Slit-width, *mm*	4	1
Emission		
Wavelength, *nm*	495	465
Slit-width, *mm*	4	2
Phosphate buffer pH	3	10
Volumes of OPA, MCE, and buffer stock solutions as given in procedure		
Final pH of OPA–MCE–buffer solution	4.2	9.2
Reaction time in fluorimeter, *min*	30	8

to 25 ml with methoxyethanol. The use of methoxyethanol instead of methanol as a solvent for MCE increased the fluorescence of the ammonia and hydrazine derivatives approximately 1.3 and 1.5 times, respectively. The various 0.1*M* buffer solutions were all prepared with sodium dihydrogen phosphate and adjusted to the desired pH with either sodium hydroxide or sulphuric acid. Each buffer solution had 2% v/v of n-butanol added as a preservative to prevent mould growth. All stock solutions were stored in the dark. All of the alcohol solvents used in the stock or buffer solutions were HPLC grade (Burdick and Jackson). Demineralized distilled water was used for all solutions.

Apparatus

An Aminco SPF-125 scanning spectrofluorimeter (American Instrument Co.) was used for all fluorescence measurements.

Procedure

The OPA and MCE stock solution (1 ml of each) were mixed with 2 ml of the desired buffer solution in a glass-stoppered 14 × 150 mm test-tube. An additional 1 ml of water was added for the assay of ammonia to maintain clarity of the solution at alkaline pH. After addition of an appropriate volume of either the hydrazine or ammonium ion stock solution with a 200-μl microburette (Gilmont, precision ± 0.2 μl), the solution was transferred to a cuvette and allowed to react in the fluorimeter cell compartment for a predetermined time before measurement. A summary of the optimum conditions is presented in Table 1.

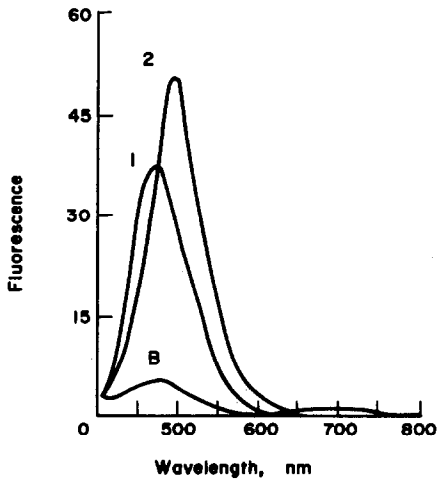

Fig. 1. Fluorescence emission spectra (uncorrected) for reactions involving OPA–MCE–ammonia (1), OPA–MCE–hydrazine (2), and the blank (OPA–MCE at pH 4, B). Excitation wavelengths, slit-widths, and other reaction conditions as in Table 1.

RESULTS AND DISCUSSION

Fluorescence of reaction products

Both the OPA–MCE–hydrazine and the OPA–MCE–ammonia reaction products had optimum excitation wavelengths at about 340 nm, consistent with those found in previous work involving primary alkyl amines.[13] The emission spectra for these reaction products as well as the blank are shown in Fig. 1. The optimum emission wavelength of 465 nm (Peak 1) for the ammonia product was also consistent with previous work.[13] The emission maximum for hydrazine occurred at slightly longer wavelength at about 495 nm (Peak 2). Only a small fluorescence signal at about 465 nm was found for the blank (Peak B). The optimum reaction times for ammonia and hydrazine were 8 and 30 min respectively. For hydrazine at about the 1-μg/ml level, the fluorescence intensity increased by approximately 8 units every 5 min until levelling off after 30 min and remained essentially constant for at least another 20 min. The hydrazine reaction took about 3 times as long if performed outside the fluorimeter cell compartment. The reaction time for ammonia was shorter, probably because of the higher pH. No fluorescence was observed for either ammonia or hydrazine when reacted with OPA in the absence of MCE. In addition, fluorescence was not observed upon reaction of hydrazine and salicylaldehyde with or without MCE present. Reaction times and fluorescence intensities observed for glycine were similar to those found for hydrazine and ammonia under the optimum conditions stated in Table 1 for each analyte. These results indicate that the fluorescent reaction product of OPA and MCE with either hydrazine or ammonia is likely to be an isoindole compound analogous to that found for other primary amines.

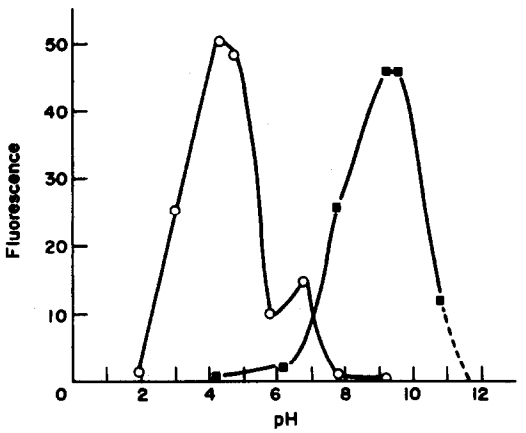

Fig. 2. Effect of pH on the fluorescence intensity of hydrazine (open circles) and ammonia (solid squares). The pH values are for the final solution, not the buffer. Other conditions as stated in Table 1.

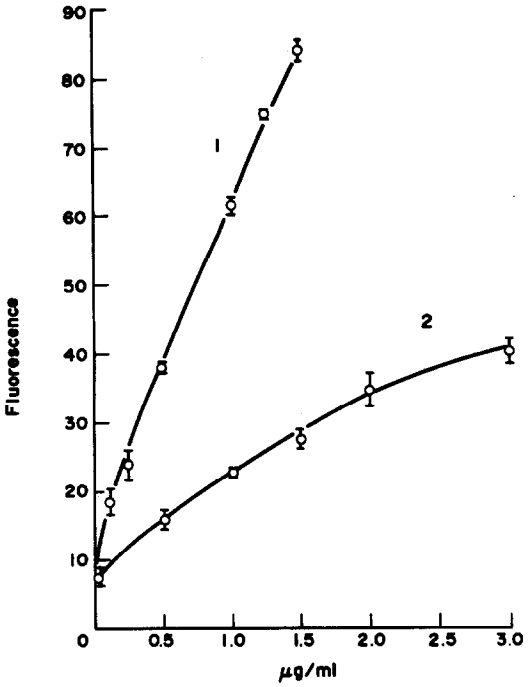

Fig. 3. Response curves for hydrazine (1) and ammonia (2). Conditions as stated in Table 1. Error bars indicate the range (n = 2 for hydrazine, n = 3 for ammonia).

Effect of pH on fluorescence

Figure 2 indicates a significant difference between the optimal pH values for the reaction of the two analytes. Maximum fluorescence for ammonia occurred when the reaction was done at pH 9–10, similarly to the reaction with amino-acids.[14] The drop in fluorescence at very high pH observed in this study was in agreement with the observation[11] that in protein analysis the background fluorescence due to ammonia could be quenched by addition of 1*M* sodium hydroxide. The optimum pH range for hydrazine was about 4–5. Essentially no fluorescence was observed for hydrazine when the reaction was done at alkaline pH. This is probably because the reaction between OPA and hydrazine at alkaline pH will produce phthalazine,[15] which is non-fluorescent. In addition, the stability of hydrazine is lower in basic solutions, owing to its greater reducing power.[1]

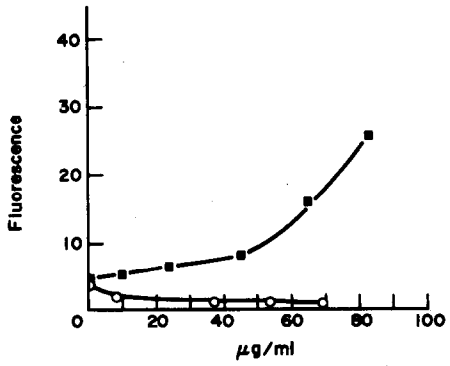

Fig. 4. Signals for of hydrazine (open circles) ammonia (solid squares), each measured under the conditions recommended for the other.

Calibration graphs

The analytical utility of the reaction was investigated by determining the response curves for hydrazine and ammonia as shown in Fig. 3. Both plots were somewhat non-linear. The fluorescence measurements were quite reproducible, as shown by an average standard deviation of ±1 fluorescence unit for both calibration curves. The detection limits for hydrazine and ammonia respectively were about 0.1 and 0.5 μg/ml. The lower sensitivity for the ammonia curve was due to the smaller slit-widths used in the measurements. The smaller slits helped to reduce the fluorescence background and minimized any interference of high concentrations of ammonia with the hydrazine determinations. Actually the molar fluorescence intensity of the ammonia derivative was about twice that of the hydrazine derivative when the same slit-width (4 mm) was used.

Interference study

The feasibility of measuring hydrazine and ammonia without serious interference was examined before mixtures were analysed. Figure 4 illustrates the results of a study in which hydrazine was measured at pH 9 under the instrumental conditions for ammonia determination and ammonia was measured at pH 4 with the instrumental settings used for hydrazine (Table 1). Because of the high pH, hydrazine produced negligible fluorescence even at high concentration. The slight quenching effect appearing at high hydrazine levels should not present a problem. Ammonia at concentrations above about 25 μg/ml could

Table 2. Analysis of hydrazine and ammonia mixtures*

Sample	Found in final solution,† μg/ml	Found in tests solution,§ μg/ml	Taken, μg/ml
1	0.49 ± 0.03	10	10
	0.97 ± 0.05	4.8	5.0
2	0.43 ± 0.05	86	100
	1.30 ± 0.20	13	10
3	0.95 ± 0.1	191	200
	0.63 ± 0.05	17	15
4	0.22 ± 0.05	1.9	2.0
	2.4 ± 0.1	61	50
5	0.20 ± 0.02	1.8	2.0
	2.70 ± 0.50	13	10
6	0.20 ± 0.0	1.8	2.0
	1.10 ± 0.10	5.5	5.0
7	0.49 ± 0.08	49	50
	1.00 ± 0.10	5.0	5.0

* Hydrazine is given first and ammonia second in each pair of results.

† For hydrazine, n = 2; for ammonia, n = 3. Precision is expressed as the range.

§ Calculated from the value found for the final solution, by use of the appropriate volume factor.

interfere in measurement of hydrazine. However, because the sample volumes used were usually less than 0.5 ml, the dilution with reagent minimized any such interference. Interference from ammonia would only be a problem with mixtures having low hydrazine concentratration and very high ammonia content. Since the hydrazine and ammonia concentrations are generally maintained at levels below 300 μg/ml in the types of sample concerned, interference effects should not be a problem with real samples.

Hydrazine–ammonia sample mixtures

Several mixtures of hydrazine and ammonia were prepared in distilled water to simulate boiler wet-lay-up water samples. No other amines are normally found in power station water.[16] Analytical results obtained for these mixtures under the conditions in Table 1 are shown in Table 2. The average relative standard deviation was about 7% and the average relative error about 10%. These values are within the 2–10% range of precision and accuracy considered characteristic of fluorescence measurements.[17] Possible sources of interference in this method would be primary amines and/or alkylhydrazines. Primary amines would interfere with the determination of ammonia, but at low levels, would not affect the hydrazine measurement. Secondary or tertiary amines do not form fluorescent derivatives with OPA and MCE.[9] Hydrazines such as monomethylhydrazine, *asym*-dimethylhydrazine and phenylhydrazine would produce fluorescence signals when mixed with OPA and MCE in slightly acidic medium, but like hydrazine gave negligible fluorescence in alkaline media. In any case, the presence of alkylhydrazines in power-station water would be unlikely. It is expected that the method could easily be adapted for the analysis of other hydrazine–primary amine binary mixtures.

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SHORT COMMUNICATIONS

DETERMINATION OF TUNGSTEN IN COMPLEX SULPHIDE ORE CONCENTRATES

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Summary—Determination of tungsten as the thiocyanato complex after separation as the α-benzoin oximate complex has been satisfactorily applied to a standard steel and a complex sulphide ore concentrate containing tungsten associated with considerable amounts of Mo, Cu, Bi, As, Pb, Zn, Fe and Sn.

Tungsten minerals that occur in sufficient abundance to be of economic significance can be divided into two groups, namely the wolframite group and the scheelite group. The three important minerals in the wolframite group are ferberite ($FeWO_4$), huebnerite ($MnWO_4$) and wolframite [(Fe, Mn)WO_4]. The tungsten minerals often occur with cassiterite, molybdenite, chalcopyrite and pyrite. This association has a high-temperature genesis.[1] The bulk sulphide concentrate used in the present study contains major amounts of sphalerite (ZnS), arsenopyrite (FeAsS) and molybdenite (MoS_2), chalcopyrite ($CuFeS_2$), galena (PbS) and quartz in intermediate amounts, and stannite (Cu_2FeSnS_4), tennantite [$(CuFe)_{12}As_4S_{13}$], bismuthinite (Bi_2S_3), pyrite (FeS_2), wolframite [(Fe, Mn)WO_4], rutile (TiO_2), cassiterite (SnO_2) and bismuth in minor amounts.

Sodium tungstate solution obtained by the decomposition of tungsten ore concentrates contains many impurities present in the original ore, and these impurities must be reduced to acceptable levels. The most frequently encountered impurities are silicon, phosphorus, arsenic and molybdenum species. Silicon species are removed by addition of aluminium and magnesium sulphates and raising the pH to 9.0–9.5. Molybdenum is precipitated as molybdenum trisulphide. Liquid ion-exchange extraction has been used to extract tungsten from sodium tungstate solution, but this method results in the co-extraction of impurities such as molybdenum, silicon, phosphorus and arsenic.

The tungsten ore concentrate used in the present study is associated with significant amounts of Mo, Cu, Bi, As, Pb, Zn, Fe, Sn and sulphide. Tungsten can be isolated from the other elements present by co-precipitation with ferric hydroxide[2] or manganese dioxide,[3] but these methods are tedious and time-consuming. Jeffery[4] isolated the element as the α-benzoin oximate complex in the course of determination of tungsten and molybdenum in silicate rocks. Cogger[5] developed a spectrophotometric method for the determination of tungsten in geological materials. A brief study has therefore been undertaken to develop a procedure for the analysis of complex tungsten ores by solvent-extraction separation of tungsten as the α-benzoin oximate complex and subsequent spectrophotometric determination, on the basis of Jeffery's and Cogger's work,[4,5] and the results obtained are reported in this paper.

EXPERIMENTAL

Dissolution of ore concentrate

Weigh a suitable amount of the ore (0.5 g) into a Teflon beaker, treat it with 10 ml of 60% perchloric acid, 10 ml of concentrated nitric acid and 10 ml of concentrated hydrofluoric acid and boil for half an hour. Evaporate to perchloric acid fumes. Add 5 ml of concentrated hydrofluoric acid, 5 ml of concentrated hydrobromic acid and 15 ml of concentrated hydrochloric acid to the residue and evaporate to perchloric acid fumes. Repeat the treatment with the three hydrohalic acids and the evaporation. Add 10 ml of concentrated hydrochloric acid and evaporate the solution to about 5 ml. Add 25 ml of concentrated hydrochloric acid, heat the contents of the beaker and then cool. Transfer the contents of the beaker into a 50-ml centrifuge tube and centrifuge at 4000 rpm for 5 min. Transfer the supernatant liquid to a 100-ml standard flask. Wash the residue with 25 ml of concentrated hydrochloric acid, centrifuge for 5 min at 4000 rpm and transfer the supernatant liquid to the 100-ml flask. Repeat the washing and transfer and finally dilute the solution in the flask to volume with 6*M* hydrochloric acid. This is solution *A*.

Transfer the residue into a zirconium crucible with water and evaporate the contents of the crucible to dryness. Fuse the residue with a minimum amount of 4:1 w/w sodium peroxide and sodium carbonate mixture. Cool, leach the solidified melt with 50 ml of water, boil for 30 min and

Table 1. Determination of tungsten in ore concentrates

Sample No.	Weight %									
	Mo	Cu	Bi	As	Pb	Zn	Fe	Sn	S	W*
1	2.84	1.17	1.67	21.4	3.56	17.3	14.5	1.08	18.0	0.47
2	3.08	1.21	0.71	25.6	0.15	18.0	16.6	0.99	19.5	0.43
3	3.15	1.29	0.71	24.6	0.11	18.4	15.1	1.04	19.6	0.42
4	3.03	1.21	1.02	24.4	0.84	17.8	15.4	0.95	19.2	0.47

* Average value of four trials. The spread of the replicates was ± 0.02.

filter (Whatman No. 42 paper). Dilute the filtrate to volume in a 100-ml standard flask with distilled water. This is solution *B*.

Separation of tungsten as α-benzoin oximate

Transfer 2 ml each of solutions *A* and *B* into a 125-ml separatory funnel and add 4 ml of water followed by 27 ml of 1.5*M* hydrochloric acid. Extract the tungsten with four 10-ml portions of 0.15% α-benzoin oxime solution in chloroform. Shake the combined extracts with 20 ml of 3*M* hydrochloric acid, transfer the organic phase into a 125-ml conical flask and evaporate it to 2–3 ml. This procedure extracts tungsten as the oximate complex along with molybdenum.[4]

Spectrophotometric determination of tungsten

Add 15 ml of concentrated hydrochloric acid and 4 ml of stannous chloride solution (15% w/v in concentrated hydrochloric acid) to the tungsten oximate solution and boil the mixture gently for 5 min. Cool the solution in an ice-bath. Transfer the solution to a 125-ml separatory funnel and use two 10-ml portions of 6*M* hydrochloric acid to rinse the flask. Add 1 ml of 0.025*M* tetraphenylarsonium chloride followed by 3 ml of 2*M* potassium thiocyanate and swirl the solution. Extract the tetraphenylarsonium tungsten(V) thiocyanato complex with three 10-ml portions of chloroform, adding 0.2 ml of tetraphenylarsonium chloride solution before the second and the third extractions. Filter the organic phase (9-cm Whatman No. 42 paper), wash the filter with chloroform and make up the filtrate and washings to volume in a 25-ml standard flask. Protect the chloroform extract from direct sunlight. Measure the absorbance of the thiocyanato complex at 405 nm against chloroform, in 1-cm cells.

Prepare the calibration curve with standard sodium tungstate solution. Beer's law holds up to 50 μg of tungsten. This procedure is a modification of the one in the literature.[5]

RESULTS AND DISCUSSION

Isolation of tungsten as the oximate complex followed by extraction with chloroform has been applied in the separation of tungsten from the U.S. National Bureau of Standards sample No. 134, which contains Mn, P, S, Si, Cr, V and Mo in addition to tungsten. The results (1.87, 1.89, 1.76, 1.72, 1.77, 1.78%, mean 1.80%, s.d. 0.07%; certified value 1.82%) show that this method for separation and determination of tungsten is acceptable. The method has also been applied to tungsten ore concentrates containing significant amounts of Mo, Cu, Bi, As, Pb, Zn, Fe and Sn as sulphides, and the results obtained are given in Table 1. The method is seen to be applicable to complex ores of this kind.

It should be noted that although some molybdenum is co-extracted with tungsten, it is tolerated in the spectrophotometric determination of tungsten.[5]

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SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNTS OF ANILINE BY DIAZOTIZATION, COUPLING WITH *N*-(1-NAPHTHYL)ETHYLENEDIAMINE AND EXTRACTION

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Summary—A spectrophotometric procedure is proposed for the determination of trace amounts of aniline by diazotization, coupling with *N*-(1-naphthyl)ethylenediamine in sodium acetate medium, extraction of the yellow dye with chloroform, and conversion into a reddish purple dye. Two methods are available for the conversion. In the first, the chloroform extract is shaken with hydrochloric acid and the dye is converted and transferred into the aqueous phase. In the other, the chloroform extract is evaporated to dryness and the residue is dissolved in glacial acetic acid. The hydrochloric acid extraction method is the more rapid. The minimum detection limits for the two methods are 0.8 and 0.6 μg/ml respectively. Both methods show satisfactory accuracy and precision.

Aniline can be determined spectrophotometrically by diazotization, followed by coupling with *N*-(1-naphthyl)ethylenediamine (**I**) in an acidic medium to produce a reddish purple dye.[1] The method has good accuracy and precision but is not readily adaptable to the determination of traces. Brodie and Axelrod[2,3] proposed a method for the determination of traces of aniline in biological materials (blood and urine) that involves diazotization, coupling with I, and extraction. In this method the aniline is extracted from up to 5 ml of sample with isoamyl alcohol–benzene mixture and is back-extracted into 5 ml of 0.1*M* hydrochloric acid. After the usual treatment with sodium nitrite and ammonium sulphamate, 1 ml of sodium acetate solution (50%) and 0.5 ml of I solution (0.2%) are added, the sample is allowed to stand for 20 min, and 0.5 ml of 6*M* sodium hydroxide is added. The dye is extracted with 0.5 ml of isoamyl alcohol–benzene mixture, 0.3 ml of the organic layer is treated with 0.05 ml of 25% solution of trichloroacetic acid in ethylene chloride, and the absorbance of the reddish purple dye is measured at 570 nm. Brodie and Axelrod did not make a comprehensive investigation of the factors affecting the method and did not report that the colour of the dye produced by the coupling in sodium acetate medium was yellow (probably because the colour is barely perceptible with minute amounts of aniline). It is the purpose of the present paper to study these factors in the determination of traces of aniline by this method, and develop an improved method applicable to the determination of minute amounts of aniline in a large volume of solution (500 ml).

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EXPERIMENTAL

Reagents

All chemicals used were reagent grade.

Standard aniline solution No. 1 (1 ml = 10.00 mg of aniline). Dissolve 1.000 g of aniline in ethanol and dilute to volume in a 100-ml standard flask with ethanol. Prepare fresh weekly.

Standard aniline solution No. 2 (1 ml = 0.50 mg of aniline). Dilute a 5-ml aliquot of standard aniline solution No. 1 to volume in a 100-ml standard flask with water. Prepare fresh every 3 days.

Standard aniline solution No. 3 (1 ml = 2.5 μg of aniline). Dilute a 5 ml aliquot of standard aniline solution No. 2 to volume in a 1-litre standard flask with water. Prepare fresh daily.

Sodium nitrite solution (1%) and sulphamic acid solution (3%). Prepare fresh every 3 weeks.

Coupling reagent solution (0.75%). Add 0.375 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride to about 45 ml of water with stirring and dilute to 50 ml. Prepare fresh every 4 days.

Preparation of calibration graphs

Hydrochloric acid method. Transfer 0.00, 1.50, 3.00, 4.00 and 5.00 ml of standard aniline solution No. 3 to 600-ml beakers and dilute to about 500 ml with water. Add 5 ml of 1*M* hydrochloric acid and 10 ml of sodium nitrite solution (1%), mix, and allow to stand for 5 min. Add 10 ml of sulphamic acid solution (3%) and allow to stand for 10 min. Add 10 g of anhydrous sodium acetate and stir to dissolve. Add 5 ml of coupling reagent solution and allow to stand for 60 min or more. Wash into a 1-litre separatory funnel, add 25 ml of chloroform, shake for about 5 sec, vent, and shake vigorously for 2 min. Transfer the chloroform layer to a dry 125-ml separatory funnel. Extract twice more with 10-ml portions of chloroform, and combine the extracts in the 125-ml separatory funnel. Add 10.0 ml of 0.2*M* hydrochloric acid (measured with a pipette) to the 125-ml separatory funnel and shake vigorously for 2 min. Drain off and discard the chloroform layer. Swirl the aqueous layer and drain off and discard about 2 ml. Drain off the remaining aqueous layer into a dry 50-ml beaker. Measure the absorbance at 555 nm against water, deduct

the blank, and plot absorbance against μg of aniline per 500 ml of solution.

Acetic acid method. Transfer 0.00, 1.00, 2.00, 3.00 and 3.50 ml of standard aniline solution No. 3 to 600-ml beakers and proceed as described in the hydrochloric acid method to the point at which the yellow dye has been extracted with chloroform, but combine the three chloroform extracts in 50-ml beakers. Place the beakers on a steam-bath and evaporate to dryness, but do not heat much further. Add 5.0 ml of glacial acid to each by pipette, swirl to dissolve the residues, and cover the beakers with watch-glasses. Let cool, then measure the absorbance at 555 nm against glacial acetic acid, deduct the blank, and plot absorbance against μg of aniline per 500 ml of solution.

Procedure

Transfer 500 ml of the sample (or a suitable smaller portion diluted to about 500 ml) to a 600-ml beaker. This volume should contain not more than 12 μg of aniline if the hydrochloric acid method is to be used, or 9 μg for the acetic acid method. Proceed as described for the calibration graphs.

RESULTS AND DISCUSSION

Formation of the yellow dye

The yellow dye was studied by diazotizing aniline in hydrochloric acid medium, treating with sulphamic acid, adding sodium acetate and coupling reagent, and measuring the absorbance at 468 nm (the wavelength of maximum absorbance). The effect of the amount of sodium acetate was investigated with 200-μg portions of aniline (4.00 ml of a tenfold dilution of aniline solution No. 2). The solutions were diluted to about 80 ml in 100-ml standard flasks and let stand for 5 min after addition of 1.0 ml of 1*M* hydrochloric acid and 1.0 ml of 1% sodium nitrite solution to each; then 2 ml of 3% sulphamic acid solution were added and the solutions were allowed to stand for 10 min. Various amounts of sodium acetate and 1.0 ml of 0.75% reagent solution were added, the solutions were diluted to the mark, and the absorbances measured at 468 nm. The results obtained (Table 1) show that the intensity of the yellow colour and the time required for its development are related to the amount of sodium acetate added. With 10 g of sodium acetate per 100 ml (which produced a pH of 6.7), the colour developed fully in 10 min. However, the use of this amount of sodium acetate seems wasteful, so the use of 2 g (which produces a pH of 5.9) and the measurement of the yellow colour after 30 min is recommended. A calibration graph prepared with 0–5 ml of the 50-μg/ml aniline solution and 2 g of sodium acetate per 100 ml was found to obey Beer's law. The method is about half as sensitive (on the basis of mg of aniline per ml) as the conventional method in which the reddish purple colour is produced by coupling in hydrochloric or sulphuric acid medium.

The yellow colour was also developed by use of a sodium hydroxide or a sodium acetate–sodium hydroxide medium, but the results were not as satisfactory as with the sodium acetate medium. The yellow dye, when present in moderately high concentrations, had a tendency to be adsorbed on glass, hence it was necessary to rinse all glassware, including the spectrophotometer cells, with 6*M* hydrochloric acid after each determination. The hydrochloric acid washings were yellow after the rinsings. The results in Table 1 on the effect of time on the absorbance would seem to indicate that the dye is adsorbed essentially as a monomolecular layer.

Extraction of the yellow dye

It was found that the yellow dye, unlike the reddish purple dye, was readily extracted into chloroform or other water-immiscible solvents and that the sensitivity of the method could be considerably increased by this means. For quantitative recovery of the very small amounts of aniline involved it was necessary for the solution to stand for 60 min before the extraction, so that the dye complex could be fully developed. The yellow colour in the aqueous solutions, however, was barely perceptible for the low concentrations of aniline used. A calibration graph for the extraction procedure was prepared by transferring 4.00, 8.00, 10.00

Table 1. Effect of sodium acetate on the development of the yellow dye (0.20 mg of aniline and dilution to 100 ml)

		Absorbance after different time intervals									
NaAc	pH	1 min	10 min	15 min	30 min	45 min	1 hr	1.5 hr	2 hr	3 hr	4 hr
0.0	2.2	reddish purple colour									
0.1*	2.6	reddish purple colour									
0.2*	4.2			0.20	0.32	0.35	0.38	0.43	0.44	0.44	0.45
0.4*	4.9			0.28	0.41	0.43	0.45	0.46	0.47	0.47	0.46
0.6*	5.1			0.34	0.45	0.46	0.47	0.47	0.47	0.47	0.47
0.8*	5.35				0.46	0.48	0.48	0.48	0.48	0.48	0.48
1.0*	5.50				0.48	0.49	0.49	0.49	0.49	0.48	0.48
1.4*	5.65				0.49	0.49	0.49	0.49	0.49	0.48	0.48
2.0*	5.85			0.45	0.49	0.49	0.49	0.48	0.48	0.48	0.48
4.0	6.2	0.28	0.46	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48
7.5	6.5	0.38	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
10.0	6.7	0.41	0.48	0.49	0.48	0.48	0.48	0.48	0.48	0.48	0.48
15.0	6.9	0.46	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48

* Added as 20% solution.

and 12.00 ml of standard aniline solution No. 3 (1 ml = 2.5 μg of aniline) to 600-ml beakers, diluting to about 500 ml, treating with 5 ml of 1*M* hydrochloric acid, 10 ml of 1% sodium nitrite solution, and 10 ml of 3% sulphamic acid solution. Then 10 g of sodium acetate and 5 ml of 0.75% reagent solution were added and the solutions were allowed to stand for 60 min. Three extractions with 15-ml portions of chloroform were performed and the combined extracts were evaporated to a volume of about 5 ml. The solutions were then diluted with chloroform to volume in 10-ml standard flasks, and the absorbances measured at 442 nm (the wavelength of maximum absorbance). Beer's law was obeyed.

Conversion of the yellow dye into the reddish purple dye

Two methods were investigated for converting the yellow dye into the more sensitive and distinctive reddish purple product. The first involved shaking the chloroform extract with 10.0 ml of dilute hydrochloric acid to make the conversion and transfer the dye to the aqueous layer. The absorbance of the aqueous layer was then measured at 555 nm (the wavelength of maximum absorbance). The acid concentration was optimized by applying the procedure to 4.00-ml portions of standard aniline solution No. 3, with use of various hydrochloric acid concentrations. The absorbances obtained were: 0.005*M* HCl, 0.40; 0.02*M*, 0.47; 0.05*M*, 0.50; 0.1*M*, 0.51; 0.2*M*, 0.51; 0.5*M*, 0.51; 1.0*M*, 0.51. A concentration of 0.20*M* was selected. The optimum acidity for this conversion and transfer is much the same as that for developing the reddish purple dye directly in the aqueous solution (0.10–1.0*M* as reported earlier[1]), but the reddish purple colour develops immediately in the conversion method and requires 75 min in the direct method. The colour is stable for several hours.

The second method involved evaporation of the chloroform extract to dryness on a steam-bath and dissolution of the residue in 5.0 ml of glacial acetic acid. As with the hydrochloric acid extraction method, the absorbance was measured at 555 nm (the wavelength of maximum absorbance). The heating on the steam-bath must not be prolonged much past the point of dryness being reached, otherwise somewhat lower results may be obtained, probably because of slight decomposition or oxidation. It is not thought that the yellow dye is volatile, since on evaporation of chloroform extracts just to dryness, and standing the beakers uncovered for 1 week, complete recovery was still obtained.

In the acetic acid method the development of the colour is also immediate. The colour is stable for 4 hr and then slightly increases in intensity, owing to appreciable volatilization of the acetic acid.

Some experiments were conducted on conversion of the yellow dye into the reddish purple form by evaporation of the chloroform extract to dryness, dissolution of the residue in a measured amount of chloroform and addition of a measured amount of glacial acetic acid. For complete conversion it was necessary that the ratio of acetic acid to chloroform be at least 5:1. The results obtained by using this method were not as satisfactory as those with acetic acid alone.

Table 2. Recovery of aniline by the hydrochloric acid extraction and acetic acid methods

Aniline added, μg/500 ml	Aniline found, μg/500 ml: HCl extraction method	Aniline found, μg/500 ml: Acetic acid method
2.5	2.5	2.4
	2.3	2.3
	2.5	2.6
	Mean 2.43	Mean 2.43
5.0	5.2	5.0
	5.0	4.9
	5.2	5.2
	Mean 5.13	Mean 5.03
7.5	7.5	7.4
	7.5	7.4
	7.6	7.6
	Mean 7.53	Mean 7.47
10.0	10.0	
	10.2	
	10.0	
	Mean 10.07	

The hydrochloric acid extraction method is faster than the acetic acid method. On the basis of mg of aniline per 500 ml of solution, the hydrochloric acid extraction method is about 0.7 times as sensitive as the acetic acid method and about 2.4 times as sensitive as the chloroform extraction method involving the measurement of the yellow colour. Taking an absorbance of 0.02 as the minimum absorbance that can be determined, the minimum detection limits for the hydrochloric acid extraction method and acetic acid method are 0.4 and 0.3 μg per 500 ml, respectively. It is to be noted that the reddish purple colour obtained in glacial acetic acid has a somewhat greater intensity than the reddish purple colour obtained in dilute hydrochloric acid (on the basis of μg of aniline per ml of solution of which the absorbance is measured). To test the precision of the two methods, portions of standard aniline solution No. 3 were added to 500 ml of distilled water and the samples carried through the recommended procedures. The results (Table 2) indicate that both methods have satisfactory accuracy and precision.

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SPECTROPHOTOMETRIC DETERMINATION OF 2-AMINO-3H-PHENOXAZIN-3-ONE AS A COLOURING MATTER IN *o*-AMINOPHENOL*

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Summary—A spectrophotometric method using 0.5*M* hydrochloric acid in methanol as solvent, has been used for the determination of 2-amino-3H-phenoxazin-3-one [APZ], occurring as a coloured impurity in *o*-aminophenol [OAP]. This impurity dissolves in the acid medium, giving a stable red solution which complies with Beer's law. The method can be applied to the determination of APZ in technical samples of OAP.

o-Aminophenol (OAP) can be manufactured by catalytic reduction of *o*-nitrophenol. It is a key intermediate in the synthesis of heterocyclic systems such as benzoxazoles, phenoxamines and oxyquinolines. It is used in the dye industry for the manufacture of azo and sulphur dyes, in the photographic industry as a developer, and as an intermediate for the manufacture of the insecticide "phosalone", which is *S*-[6-chloro-2,3-dihydro-2-oxobenzoxazol-3-yl]methyl *O,O*-diethyl phosphorodithioate. *o*-Aminophenol is unstable to air and light, undergoing oxidation readily to brown or black substances (phenoxazones). We have isolated and characterized 2-amino-3H-phenoxazin-3-one (APZ) (I) as a coloured impurity in OAP samples prepared by catalytic hydrogenation of *o*-nitrophenol in methanol.[1] In the manufacture of phosalone, estimation of this impurity in OAP is important since its presence may give rise to undesirable intermediates in the subsequent steps of the process, and these may affect the quality of the phosalone.

Some pure aminophenoxazin-3-ones, including APZ, have been determined by titration with tin(II) chloride solution.[2] Some substituted phenoxazin-3-ones have been determined by coulometric titration with titanium(III), with potentiometric indication of the end-point.[3] However, both these methods are for assay of these compounds as major or sole components of the sample. No method has been reported for determination of APZ in OAP, though a polarographic method has been given for determination of APZ produced by photolysis of OAP.[4]

This communication describes a spectrophotometric method for this determination.

EXPERIMENTAL

Instrument

A Zeiss "Specord" double-beam spectrophotometer with 1-cm quartz cells was used for all absorbance measurements.

Reagents and solutions

2-Amino-3H-phenoxazin-3-one (APZ). An authentic sample was prepared by oxidation of (OAP) with *p*-benzoquinone.[5]

APZ stock solutions. (*A*) About 10 mg of APZ accurately weighed, and dissolved in and diluted to 1 litre with concentrated hydrochloric acid. (*B*) About 10 mg of APZ accurately weighed, dissolved and diluted to 100 ml with 0.5*M* hydrochloric acid in methanol.

Calibration solutions. Two sets of calibration solutions were prepared by making appropriate dilutions of the APZ stock solutions to cover the ranges 2.5–10.0 μg/ml for *A* and 2.3–12.0 μg/ml for *B*.

Procedures

Concentrated hydrochloric acid medium. An appropriate amount of OAP, depending on the APZ content as indicated below, was accurately weighed into a conical flask (with ground-glass stopper) and 20 ml of concentrated hydrochloric acid were added. The solution was stirred for 15 min on a magnetic stirrer. If dissolution was incomplete, the mixture was allowed to stand for 4 hr, during which the hydrochloride of APZ went into solution and undissolved OAP hydrochloride settled out. The supernatant liquid was removed and its absorbance at 485 nm was measured, with concentrated hydrochloric acid as reference. Samples that dissolve completely can be measured at once.

APZ content, %	0.01	0.05	0.1	0.4	0.6	0.8
OAP, *g*	1.0	0.4	0.2	0.05	0.025	0.020

Methanolic hydrochloric acid medium. Between 50 and 2000 mg of OAP, depending on the APZ content, was accurately weighed, dissolved and diluted to the mark in a 50-ml standard flask with 0.5*M* hydrochloric acid in methanol. The absorbance of the solution was then measured at 465 nm against a solvent blank.

The amounts of APZ in the samples are calculated by using the calibration equations.

RESULTS

Pure OAP dissolved in either medium does not absorb light in the visible region. The absorption spectra of OAP containing APZ, for both media, are shown

* NCL Communication No. 2654.
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Table 1. Spectrophotometric characteristics of APZ

Medium	λ_{max}, nm	Beer's law limits, μg/ml	Molar absorptivity, $l.mole^{-1}.cm^{-1}$	Calibration equation*
Conc. HCl	480–510	2.5–10.0	1.52×10^4	$A_{485} = 1.52 \times 10^4 C - 0.003$
0.5*M* HCl in MeOH	463–472	2.3–12.0	1.38×10^4	$A_{465} = 1.38 \times 10^4 C + 0.006$

* A is the absorbance at the prescribed wavelength and C is the molar concentration of APZ solution. The coefficients must be determined with the instrument used.

in Fig. 1. The spectrophotometric characteristics of APZ in both media are given in Table 1.

Determination of APZ in laboratory made mixtures

The validity of the calibration equations was tested by determining the amount of APZ in laboratory-made mixtures prepared by taking a fixed amount of OAP (0.400 g) in concentrated hydrochloric acid (about 10 ml) or 0.5*M* hydrochloric acid in methanol (about 30 ml), adding the desired volume of appropriate APZ solution and making up to 20 or 50 ml with the solvent medium used. The absorbances of the solutions were measured against the solvent blanks at 485 and 465 nm respectively as outlined in procedures A and B. The results are presented in Table 2.

Validification tests using standard addition method and technical OAP samples

These tests were performed on a fixed quantity of technical sample (as shown in Table 3) on the same lines as for laboratory-made mixtures. The results obtained are presented in Table 3.

Repeatability

Concentrated hydrochloric acid medium. In a test of repeatability, six OAP samples (each weighing 1000 ± 3 mg) containing 0.208 mg of APZ and six OAP samples (each weighing 400 ± 1 mg) containing 0.083 mg of APZ were taken. The absorbances were measured at 485 nm as described before. The standard deviation of absorbance was calculated for each set and the results are presented in Table 4.

Hydrochloric acid (0.5M) in methanol medium. The repeatability of the method was tested in a similar manner by recording the absorbances at 465 nm of two OAP samples. Their respective weights, APZ content, mean of six determinations and standard deviations are recorded in Table 4.

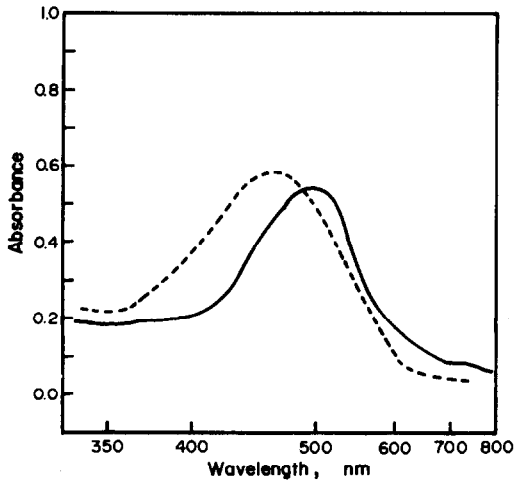

Fig. 1. Absorption spectrum of *o*-aminophenol + APZ. —— Conc. hydrochloric acid medium; ----- 0.5*M* HCl/methanol.

Table 2. Recovery of APZ added to 0.400 g of OAP

Concentrated hydrochloric acid		Hydrochloric acid in methanol	
APZ added, μg	APZ found, μg	APZ added, μg	APZ found, μg
50.0	48.8	232	230
100.0	101.2	348	343
150.0	153.8	464	461
200.0	205.0	928	916

Table 3. Validification tests on technical OAP samples (100 mg)

OAP sample	Initial amount of APZ, μg	Synthetic APZ added, μg	Total APZ, μg	APZ found, μg
Concentrated hydrochloric acid				
I	85	80	165	163
		160	245	240
		200	285	280
II	38	80	118	115
		160	198	200
		200	238	233
Hydrochloric acid (0.5M) in methanol				
I	85	174	259	254
		262	347	343
		349	434	427
		436	521	515
II	38	174	212	208
		262	300	294
		349	387	381
		436	474	469

Table 4. Repeatability of the method

Quantity of APZ, *mg*	Quantity of OAP, *mg*	Mean absorbance (6 determinations)	Standard deviation of absorbance	Relative standard deviation, %
	Concentrated HCl medium			
0.208	1000 ± 3	0.562	0.018	3.1
0.083	400 ± 1	0.192	0.008	3.9
	0.5*M* HCl/MeOH medium			
0.225	2000 ± 1	0.295	0.0033	1.1
0.443	200 ± 0.2	0.597	0.0125	2.1

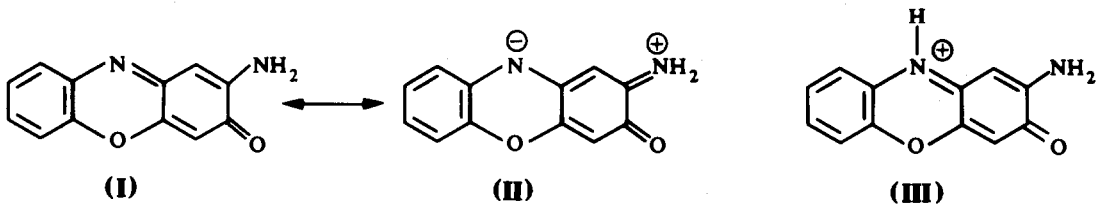

Fig. 2.

DISCUSSION

APZ can be represented by **I** and **II** (Fig. 2) as contributing canonical forms. APZ in methanol shows only one wavelength maximum in the visible region, in the range 430–434 nm. This undergoes a bathochromic shift by about 33 nm in 0.5*M* hydrochloric acid in methanol medium (λ_{max} 463–472 nm) and by about 55 nm in concentrated hydrochloric acid (λ_{max} 485–510 nm). The basicity of the amino group in APZ is so slight[6] that the first proton preferentially adds to a free electron-pair of the ring nitrogen atom (**III**). The positive charge on the ring nitrogen atom may be delocalized to give canonical forms contributing to the bathochromic shift and the colour of the protonated APZ.

The molar absorptivity of APZ in methanol is substantially higher (2.51×10^4 l.mole^{-1}.cm^{-1}) than that in concentrated hydrochloric acid (1.52×10^4 l.mole^{-1}.cm^{-1}). It was expected, therefore, that the spectrophotometric determination of APZ in methanol might serve as a quicker and more sensitive method for the determination of APZ in OAP samples. It was, however, found that under the influence of atmospheric oxygen and light, OAP undergoes rapid oxidation to APZ. A 10-g/l. solution of OAP in methanol changes its absorbance at 432 nm from the initial value of 0.20 (pale straw) to 2.38 (yellow) within 15 min. It has been reported[7] that OAP is oxidized initially to *o*-quinoneimine, which further reacts with a molecule of OAP to produce APZ. The *o*-quinoneimine is fairly stable only at temperatures below 0° in solvents such as diethyl ether, toluene, chloroform, acetone and acetonitrile, but not in methanol and ethanol.[8] However, it was found that solutions of OAP in its hydrochloride form, as in concentrated hydrochloric acid and 0.5*M* hydrochloric acid in methanol media, were quite stable with respect to APZ formation for a long period of time.

The spectrophotometric method of estimation of APZ in OAP with 0.5*M* hydrochloric acid in methanol as the medium has the distinct advantages of rapidity, better repeatability and complete dissolution even at higher concentrations of OAP. In comparison, the method using aqueous concentrated hydrochloric acid, although more sensitive, is less rapid owing to incomplete dissolution at higher concentrations of OAP, when a settling time of 4 hr has to be allowed for the undissolved OAP hydrochloride.

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A PHOTOCHEMICAL REDOX METHOD FOR THE ESTIMATION OF THALLIUM(III)

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Summary—A convenient photochemical redox method for the estimation of thallium(III) by reduction with oxalic acid followed by oxidation of thallium(I) with potassium bromate has been developed. The reduction is carried out in the presence of small concentrations of chloride and bromide as catalysts.

Oxalic acid has long been used in the photochemical reduction of metal ions, such as iron(III),[1–5] cobalt(III)[6,7] and manganese(III).[8] It is well known that thallium salts are also photosensitive.[9] Moreover, it has been observed that during the formation of oxaloto complexes of thallium(III), some of the thallium(III) is always reduced to thallium(I).[10]

The reduction of thallium(III) to thallium(I) with oxalic acid at elevated temperatures and subsequent titration of the thallium(I) with cerium(IV) sulphate has been described by Sagi and Ramana.[11] In this method the titration has to be done at 90°, a blank correction is needed, and thallium(III) is estimated by an indirect procedure involving titration of surplus oxalate after oxalate reduction of thallium(III).

Since thallium salts are photosensitive and oxalate is a photochemical reducing agent, it seemed likely that the photochemical reduction of thallium(III) with oxalic acid would be possible. Hence a critical study has been made of this reaction and a convenient photochemical method developed for estimation of thallium(III).

EXPERIMENTAL

Reagents

Thallium(III) hydroxide was prepared as reported earlier[11] and dissolved in suitable amounts of perchloric or sulphuric acid. The thallium content was estimated iodometrically[12] and verified by other methods.[11,13]

All other reagents were of analytical-reagent grade.

Procedure

The photochemical reductions were carried out by exposing the solutions in colourless glass containers to either bright sunlight or the light from a high-pressure mercury vapour daylight lamp.

RESULTS AND DISCUSSION

Effect of light on the redox reaction between thallium(III) and oxalic acid

Perchloric and sulphuric acid media were used, but not nitric acid medium, since nitric acid oxidizes organic material in the presence of light.[14] Dilute perchloric acid forms no known complexes with thallium(III) and hence the medium effects are minimal in this acid, which was therefore investigated first.

It was found that the reduction of thallium(III) with oxalic acid in perchloric acid is complete in about 3 hr at room temperature under direct light from the mercury vapour lamp. In diffused daylight in the laboratory the complete reduction of thallium(III) takes about 24 hr, but in the dark, thallium(III) is still detectable in the solution even after 15 days.

Effect of halides on the photochemical reduction

Chloride and bromide are reported to catalyse the reduction of thallium(III) with organic reagents,[15] so their effect on the photochemical reduction was examined.

A separate study showed that oxalic acid does not interfere in the bromate titration of thallium(I)[16–18] so this titration was used for monitoring the progress of the photochemical reduction.

The effect of varying amounts of chloride, bromide, oxalate and hydrogen ion is given in Tables 1–3. The total volume of solution was 75 ml, containing 0.12 mmole of thallium(III). The perchloric acid concentration was 0.5*M* and the amount of oxalic acid present 0.24 mmole except when these reagents were the variables.

The results in Table 1 indicate that (1) chloride at relatively low concentration catalyses the reaction, (2) the optimal chloride concentration is double that of the thallium(III), and (3) high chloride concentrations inhibit the reduction. Bromide has a similar but more efficient catalytic effect, and the optimal concentration is half that of the thallium(III).

Fluoride up to 0.1*M* concentration was found to have no catalytic effect, and sulphate was found to retard the reaction.

Brubaker and Andrade[19] reported that in perchloric acid medium reduction of thallium(III) with oxalic acid proceeds at a rate comparable to or faster than that of the thallium(I)–thallium(III) exchange reaction. Chloride and bromide at low concentrations

Table 1. Effect of chloride and bromide

Chloride, *mmole*	Time for complete reduction, *min*	Bromide, *mmole*	Time for complete reduction, *min*
0.0	180	0.0	180
0.008	30	0.00032	50
0.016	20	0.00064	40
0.04	18	0.0032	20
0.08	15	0.0064	11
0.16	12	0.026	5
0.25	10	0.032	3
0.40	15	0.064	2
0.80	20	0.128	3
1.60	40	0.32	20
4.00	75	0.64	45
8.00	180	1.28	180

Table 3. Effect of oxalic acid concentration

Oxalic acid, *mmole*	Time for complete reduction, *min*		
	in absence of catalyst	in presence of 0.24 mmole of chloride	in presence of 0.06 mmole of bromide
0.10	no complete reduction	no complete reduction	no complete reduction
0.15	not reduced completely even after 4 hr	12	2.5
0.20	180	10	2
0.40	120	8	1.5
0.80	90	6	1
1.60	60	5	1

reduce the exchange rate,[20] especially when their concentrations are twice and half that of the thallium(III) respectively, whereas higher concentrations considerably enhance the exchange rate. It is also reported[21] that sulphate increases the exchange rate.

Our results show that the rate of photochemical oxidation of oxalate by thallium(III) is greatest under the conditions for minimal rate of the exchange reaction. This indicates that there are two competing reactions: (1) electron transfer from thallium(I) to thallium(III), and (2) electron transfer from oxalate to thallium(III), the rate of one being at its maximum when the rate of the other is at its minimum.

Table 2 shows that the rate of reduction decreases with increase in hydrogen-ion concentration whether the chloride or bromide is present or not. Variation of the oxalic acid concentration in the presence of chloride and bromide at twice and half the concentration of thallium(III) respectively (Table 3) shows that the reduction is rapid enough at an oxalic acid concentration equal to that of the thallium(III), and that higher concentrations increase the rate comparatively slightly.

The effect of the light intensity on the reduction was studied by varying the distance between the lamp and reaction mixture, with and without halide ions present. The reduction rate decreases with decrease in the light intensity.

Table 2. Effect of hydrogen ion concentration

$[H^+]$, *M*	Time for complete reduction, *min*		
	in absence of catalyst	in presence of 0.24 mmole of chloride	in presence of 0.002 mmole of bromide
0.10	80	10	20
0.20	120	11	22
0.40	180	12	24
0.60	270	15	25
1.00	360	30	30

The light from the mercury lamp was also passed through a blue, green or red filter made out of acrylic plastic. The reaction was found to be fast under the blue light, whereas it was very slow, even in the presence of catalyst, under green or red light. The reaction was much faster when unfiltered light was used, owing to the higher intensity of the unfiltered light.

Recommended procedure for determination of thallium(III)

To 50 ml of 0.25*M* sulphuric acid in a 200-ml beaker add 0.1–0.25 mmole of thallium(III) and 0.2 mmole of oxalic acid. Stir the solution continuously, add 0.05–0.1 mmole of bromide, and expose to light from a high-pressure mercury vapour lamp for 15 min. Add 15 ml Conc. HCl to 0.1 ml of 0.1% Methyl Orange solution, heat to 60°C and titrate with 0.05*N* potassium bromate (1.392 g/l.) until the indicator is destroyed.

Application to mixtures of thallium(I) and (III)

First estimate thallium(I) directly by potassium bromate titration. Take a second sample and reduce the thallium(III) as described above. Titrate to find the total thallium and subtract the amount of thallium(I) already found. Typical results are given in Table 4.

Interferences

Cadmium, zinc, ammonium and potassium ions do not interfere. Copper(II) does not interfere if its con-

Table 4. Estimation of thallium

Thallium(I), *mmole*		Thallium(III), *mmole*	
Taken	Found	Taken	Found
0.0244	0.0244	0.2000	0.2000
0.0610	0.0608	0.1333	0.1335
0.0976	0.0976	0.0888	0.8889
0.1220	0.1222	0.0444	0.0449
0.1464	0.1459	0.0222	0.0220

centration is below 0.02*M* but at higher concentrations its colour masks the colour change at the end-point. Nitrate at below 0.03*M* concentration does not interfere, but large quantities of chloride and bromide inhibit the reduction. Iron(III) interferes.

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DETERMINATION OF SOME DITHIOCARBAMATES BY CATALYTIC THERMOMETRIC TITRATION

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Summary—The determination of dithiocarbamates by catalytic thermometric titration is described. The dithiocarbamates can be determined in the range 0.5–20 μmole with relative errors of about 5%.

Many methods for the determination of dithiocarbamates, which have been used as fungicides, vulcanization accelerators and analytical reagents, have been proposed.[1,2] Dithiocarbamates are smoothly decomposed by hot dilute mineral acids to yield carbon disulphide and the amine. The carbon disulphide generated can be collected and determined iodimetrically or colorimetrically.[3,4] Clarke *et al.*[3] proposed a method in which the CS_2 is collected in alcoholic potassium hydroxide and determined iodimetrically. The method is suited for macro determinations.

It is well known that the reaction of primary and secondary amines with carbon disulphide affords dithiocarbamic acids.[5] The dithiocarbamic acids are weak acids and can be titrated with a strong base. This principle has been utilized for the determination of amines.[6] Catalytic thermometric titrimetry is suitable for determination of small amounts of weak acids in non-aqueous solution;[7] for example, phenols, thiols, barbituric acids and the acidity of petroleum can be determined by using a hydroxide-catalysed acetone-condensation reaction as indicator.[8–11]

In this communication, a method is described in which a dithiocarbamate is decomposed by treatment with acid[4] and the carbon disulphide evolved is collected in a solution of ethylenediamine in propan-2-ol. The dithiocarbamic acid formed is titrated with potassium hydroxide solution in the presence of acetone as thermometric indicator.

EXPERIMENTAL

Apparatus

A twin-cell thermometric titrator (TOA Electric, TMT-3A) was used. It has been described in detail.[12] The titration vessels were 30-ml Dewar flasks. The apparatus used to decompose the dithiocarbamates and collect the carbon disulphide was similar to that described by Cullen.[13]

Reagents

The absorption solution was 0.5*M* ethylenediamine in propan-2-ol. Carbon disulphide stock solution (0.05*M*) was made by dissolving carbon disulphide in propan-2-ol, standardized by the xanthate method of Hofman-Bang and Szybalski[14] and then stored in a refrigerator at 0°. Dilute standard carbon disulphide solutions were prepared by diluting the freshly standardized stock solution for each measurement. Zinc dimethyldithiocarbamate, zinc and copper diethyldithiocarbamates, zinc and nickel di-n-butyldithiocarbamates, zinc dibenzyldithiocarbamate were prepared from carbon disulphide and the corresponding amine.[15] The products were recrystallized twice from acetone and dried *in vacuo* at room temperature for 40 hr, then analysed iodometrically by the method of Clarke *et al.*[3] Dithiocarbamate solutions were prepared in propan-2-ol for each measurement. Potassium hydroxide solutions (0.1 and 1.0*M*) were prepared in dry propan-2-ol and standardized with benzoic acid. All reagents were of analytical reagent grade.

Procedure

A sample of dithiocarbamate equivalent to 1–40 μmole of carbon disulphide was introduced into a dry flask. The first trap contained 20 ml of 0.1*M* sodium hydroxide and the second trap, containing 10 ml of absorption solution, was kept at 25 ± 4°. The sample was refluxed with 200 ml of 2*M* hydrochloric acid containing 2 g of $SnCl_2 2H_2O$. The carbon disulphide evolved was swept through the traps with a stream of nitrogen at a flow-rate of 20 ml/min. After 15 min the contents of the second trap were transferred, with 5 ml of acetone for rinsing, to the titration vessel. The second titration vessel was charged with an equal volume of reference solution (propan-2-ol:acetone, 2:3 v/v). One g of molecular sieve 3A pellets was introduced into each vessel. The cover with its attached thermistors, stirrers and burette tips was fitted and the stirrers were started. One burette was filled, with standard potassium hydroxide solution and the other with propan-2-ol. When the thermistor-bridge recorder showed a steady trace, the synchronous burette-motor was started to perform the titration. The recorder full-scale sensitivity was 50 mV and the rate of titrant addition was 0.05 ml/min. Blank tests were performed to determine the blanks from the reagents themselves. Calibration was done with standard benzoic acid solutions.

RESULTS AND DISCUSSION

The results obtained for the six dithiocarbamates

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Table 1. Determination of dithiocarbamates

Compound	Taken, μmole	Found, μmole	Recovery, %	C.V., % (n = 5)
Zinc dimethyldithiocarbamate	0.52	0.50	96	5
	15.6	15.6	100	2
Zinc diethyldithiocarbamate	0.58	0.56	98	4
	11.6	11.5	99	3
Copper diethyldithiocarbamate	0.55	0.53	96	5
	11.0	11.0	100	3
Zinc dibutyldithiocarbamate	0.60	0.58	97	4
	6.00	5.98	98	3
Nickel dibutyldithiocarbamate	0.55	0.54	98	4
	16.5	16.5	100	2
Zinc dibenzyldithiocarbamate	0.50	0.50	100	4
	5.03	5.00	100	3

are summarized in Table 1. In each case, the dithiocarbamate was added as a dilute solution to the digestion flask, various concentrations being used. The volume of titrant used was a linear function of amount of dithiocarbamate taken, in the range 0.5–20 μmole. Recovery of smaller amounts of dithiocarbamates ($\sim 10^{-7}$ mole) is rather low, probably because of decomposition of the dithiocarbamates in very dilute solution, as pointed out by Clarke *et al.*[3] The upper limit occurs because the reaction of ethylenediamine and carbon disulphide is not very rapid. The unreacted carbon disulphide is lost by being swept out by the nitrogen stream. Several dithiocarbamate mixtures were analysed for total dithiocarbamate by this method. Results are shown in Table 2. The recoveries of zinc dimethyldithiocarbamate and zinc diethyldithiocarbamate residues on apples are shown in Table 3.

The reactivities of primary and secondary amines such as ethylenediamine, n-propylamine, di-n-propylamine, di-isopropylamine, n-butylamine, isobutylamine, di-n-butylamine, n-heptylamine, n-octylamine, di-n-butylamine, 1,2-propyldiamine, monoethanolamine, 3-amino-1-propanol and morpholine were studied at 20–30°. Ethylenediamine reacted quantitatively with carbon disulphide within 15 min under the conditions studied. By reacting ethylenediamine with carbon disulphide in various ratios, it was found that molar ratios of amine to carbon disulphide above 30 gave quantitative results. For n-propylamine, n-butylamine and di-n-propylamine, the reaction was complete after 45 min at 30° or 60 min at 25°. For other amines, the reaction yielded low results under all conditions. Ethylenediamine solution (0.5*M*) was therefore used as absorbing solution at 25°. Titrations in an atmosphere of nitrogen were capable of greater precision than those in air. Under these conditions, 0.8–60 μmole of carbon disulphide could be determined with a coefficient of variation ($n = 5$) less than 1%. The effect of the flow-rate of nitrogen on the trapping of carbon disulphide was investigated. At nitrogen flow-rates above 50 ml/min or less than 3 ml/min, the trapping of 40 μmole of carbon disulphide was incomplete. At higher flow-rates, the vigorous bubbling causes loss, and at lower rates the dense carbon disulphide gas is incompletely transferred. A nitrogen flow-rate of 20 ml/min was chosen. The use of air instead of nitrogen as carrier-gas

Table 2. Determination of mixtures of dithiocarbamates

Mixture	Total taken, μmole	Total found, μmole	Recovery, %	C.V., % (n = 5)
Zinc dimethyldithiocarbamate and diethyldithiocarbamate	11.06	10.9	98	2
Zinc dibutyldithiocarbamate and nickel dibutyldithiocarbamate	11.55	11.5	99	2

Table 3. Recovery of dithiocarbamates from apples (100 g)

Compound	Added, μmole	Found, μmole	Recovery, %	C.V., % (n = 5)
Zinc dimethyldithiocarbamate	1.13	1.09	97	8
	2.26	2.19	98	5
Zinc diethyldithiocarbamate	1.52	1.47	97	8
	3.04	2.95	97	5

caused poor recovery because of aerial oxidation of the dithiocarbamate formed in the absorption solution, to a thiuram disulphide.

The conditions for the decomposition were investigated. The decomposition was complete in 10 min in hydrochloric acid at concentrations above 2*M* and containing 1% w/w stannous chloride. At an acid concentration of 0.5*M*, the reaction took about 30 min. The stannous chloride concentration could be varied from 0.5 to 3% without affecting the yield of carbon disulphide. A 2*M* hydrochloric acid/1% w/w stannous chloride solution and reflux time of 15 min were chosen. The formation of hydrogen sulphide during the decomposition was measured by determining the sulphide in the first trap (0.1*M* sodium hydroxide) by the Methylene Blue method.[16] A maximum of 0.2% of the available sulphur was evolved as hydrogen sulphide for all the dithiocarbamates tested. However, with manganese and zinc ethylenebisdithiocarbamates, 9–14% of the available sulphur was evolved as hydrogen sulphide, and some as carbonyl sulphide (identified by gas chromatography).

The end-point temperature change was not very sharp in the presence of water, even at concentrations as low as 0.1%. The influence of water could be avoided by adding a molecular sieve (in pellet form) to the titrand. The presence of up to 1 g of molecular sieve did not interfere mechanically or chemically with the titration. Gaseous acidic substances such as hydrogen sulphide, which are generated in the course of the acid decomposition, were completely removed by using 0.1*M* sodium hydroxide solution (20 ml) in the first trap.

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OPTICAL EMISSION SPECTROSCOPY WITH A MICROWAVE-INDUCED PLASMA IN A SEALED MICROTUBE

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Summary—A microwave system is used to induce a plasma inside a sealed microtube containing a sample or standard, and the emission from the analyte is measured. The detection limits are about 4 ng for S, Cl and Br, and 100 ng for Cd and Sn, in ~30-μl samples. Both gaseous and liquid samples can be analysed.

Most arrangements for analytical atomic spectroscopy make use of a flow system in which the residence time of the sample in the region of measurement is short. This necessitates continuous delivery of sample from a relatively large sample reservoir, as in conventional sprayed-sample systems for flame and plasma spectroscopy, or measurement of short-lifed transient signals, as in furnace atomic-absorption spectroscopy. The first approach has the disadvantage of requiring relatively large quantities of sample. The second approach requires fast response (broad bandwidth) measurement systems, with a consequent increase in the noise associated with the measurement. This report describes the results of an effort to overcome the limitations of flow systems by making use of the atomic emission from a microwave-induced plasma in an enclosed-sample system.

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EXPERIMENTAL

Apparatus

The experimental facilities are described in Fig. 1 and Table 1.

Preparation of sample tubes. Sample tubes were prepared from 1-mm bore, 3-mm outside diameter quartz tubing on a vacuum line, the major components of which are shown in Fig. 2. The tubing was cleaned by soaking in nitric/sulphuric acid cleaning solution for 24 hr and flaming with a hydrogen–oxygen torch with helium flowing through the tube. An approximately 60-cm length of the tubing was blown onto the quartz side of a graded seal on a 10/30 Pyrex joint to permit attachment to the vacuum line at V5. Different procedures were used for gaseous and liquid samples.

Gaseous samples. The cleaned quartz tube was cut into four equal lengths, one remaining attached to the 10/30 joint. The open end of the attached tube was then fused shut. The tube was connected to the vacuum line at V5, evacuated and heated to a white glow with the torch. When the tube had cooled, helium was admitted to a pressure of 25 mmHg, stopcock V1 was opened and the system was evacuated to a pressure of about 10^{-4} mmHg.

Sample, which had previously been degassed by repeated freeze–pump–thaw cycles, was admitted to the system

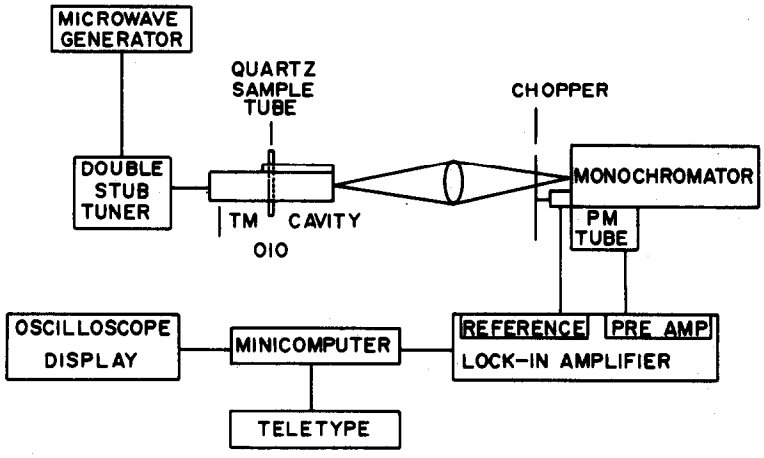

Fig. 1. Block diagram of experimental facilities.

Table 1. Experimental facilities

Microwave generator	Scintillonics HV15A, 120 W max. power
Double stub tuner	Maury Microwave, Model 1178B, 0.8–4.0 GHz
Cavity	Cylindrical TM_{010},[6] brass, locally constructed
External optics	Suprasil lens, 20-cm focal length, 1/1 image focused on entrance slit
Monochromator	Jarrell-Ash, Model 82–000, 0.5-m Ebert, f/8.6, 16 Å/mm, 1180 grooves/mm grating blazed for 3000 Å in 1st order
Detector	Hamamatsu R777
Amplifier	Princeton Applied Research, Model 126 with Model 184 photometric preamplifier, operated with 133-Hz external reference
Computer	Digital Computer Controls, Model 416, 16 K of 16-bit memory

through V4 to a pressure of about 0.2 mmHg. With V4 closed and V1 opened, the system pressure was then adjusted to the value calculated by the operator to be suitable for the intended measurement. With V1 and V2 closed, V3 was then opened to adjust the pressure of helium to the desired value (approximately 50 mmHg). A magnetically driven stirring bar in the expansion flask provided thorough mixing of the sample with the helium. Sample was admitted to the sample tube by opening V5. After a 1-min equilibration time, V5 was closed, and the sample tube was fused shut and cut from the graded seal. This provided a sample tube with a total length of about 14 cm. This tube was then sealed in the middle, cooled, and then sealed again in the middle of each of the halves to provide 4 sample tubes, each about 3 cm long, with an internal volume of about 30 μl. The amount of sample in the tube was calculated from the pressure and volumes of the vacuum system and sample tubes. Ideal gas behaviour was assumed. The volume of the vacuum system was measured by a manometric method, and that of the sample tubes was estimated from their length and bore. Additional samples were then prepared by the same procedure after sealing the remaining lengths of tubing, in turn, to the 10/30 joint.

Liquid samples. Small bulbs, each about 0.2 cm in internal diameter and 0.3 cm long, were blown every 5 cm in the 60-cm length of tube attached to the 10/30 joint. The end was sealed, the tube was attached to the vacuum system at V5, evacuated and heated to a white glow. After cooling, the tube was removed from V5, and the end was reopened.

One μl of the sample solution was placed in the lowest bulb with a micropipette. The sample was frozen in liquid nitrogen and the end of the tube was again sealed. The tube assembly was then re-attached to the vacuum system, and the sample was freeze-dried. After adjustment of the helium pressure to the desired value (about 20 mmHg), the sample tube was sealed about 1.5 cm above the bulb and cut from the tube assembly. Additional samples were prepared by filling the other bulbs in succession by the same procedure.

Measurements

The cavity was operated with the cylindrical axis vertical. Samples were mounted on the cylindrical axis at the centre of the cavity, and the radiation was viewed through an opening in the wall of the cylinder, as indicated in Fig. 1.

For gaseous samples the plasma was initiated (with a Tesla coil) at 8 W forward power, and the sample spectrum was excited at 60 W forward power. For solution residue samples the plasma was initiated at 2 W forward power, and the sample was excited at 30 W forward power. The tuning was adjusted to maintain reverse power at 2 W or less. The lower initiation power for residue samples was made possible by the lower helium pressure in the residue sample tubes. Maximum power for residue samples was

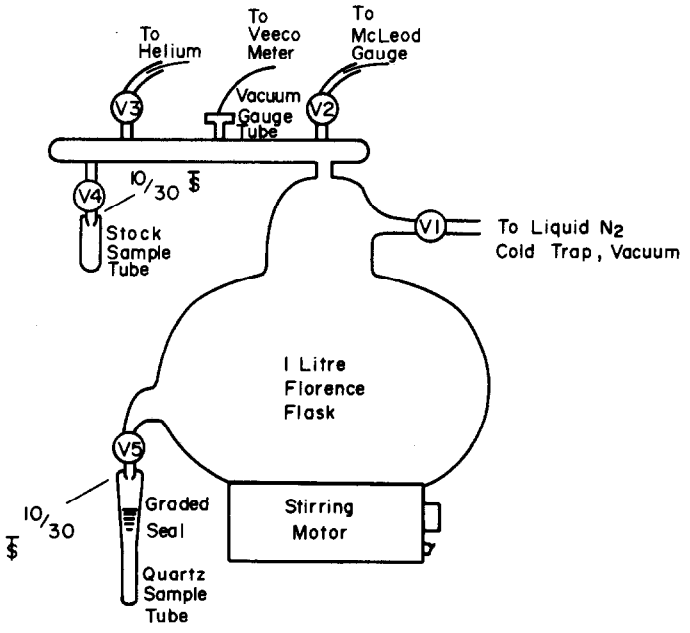

Fig. 2. Arrangement of vacuum system.

Table 2. Analysis lines and limits of detection

Analyte	Wavelength, *nm*	Detection limit, *ng*
S(II)	545.4	0.33
Cl(II)	479.5	0.54
Br(II)	470.5	0.53
Cd(I)	228.8	0.01
Sn(I)	286.3	0.18

limited to 30 W because the thinner wall produced in blowing the bulbs fused at higher power settings.

Data were acquired under computer control. Readings of the amplified photomultiplier signal were taken at 50-msec intervals, following initiation, to a maximum of 512 points. The first 29 points were taken as a background reading at the low microwave-power setting. A computer-actuated relay stepped the microwave power to the measurement value for the balance of the readings.

The data were displayed on an oscilloscope and the limits of signal integration were chosen by the operator. The integral of emission signal *vs.* time, normalized to the least sensitivie amplifier setting, and the amplifier settings used, were then printed on the teletype.

RESULTS AND DISCUSSION

Preliminary studies

Line emission from the analyte appeared quickly upon initiation of the plasma in a sample tube, increased rapidly, and then decayed rapidly. At the power level used for gaseous samples, the intensity of the emission due to analyte decayed to the level of background emission in approximately 15 sec. Decay of the signal was less rapid for lower power settings.

The reasons for this somewhat disappointing result are not known. By some process the analyte material was irreversibly removed from the plasma region. Van Montfort *et al.*[1–5] reported similar variations with time of the signal intensities in their studies, although signal decay was much less rapid with the larger sample tubes used in their studies. They were able to modify the temporal behaviour by addition of various excitation buffers. We did not examine this possibility in our studies.

The atomic emission due to helium (measured at 388.86 nm) increased with time throughout the observation period (the longest period studied was 18 min).

Calibration graphs and limits of detection

Results with the sealed-tube microwave-plasma excitation system were obtained for S, Cl and Br, introduced as gaseous samples, and for Cd and Sn, as solution residues. S, Cl and Br were introduced as CS_2, CH_2Cl_2 and C_2H_5Br, respectively. Cd and Sn were introduced as aqueous solutions of the metal chlorides.

Lines of the singly ionized element were found to be the most sensitive for S, Cl and Br. Atomic lines were most sensitive for Cd and Sn. The lines used for measurement are listed in Table 2.

The calibration graphs appeared linear up to the highest weight investigated: approximately 4 ng for S, Cl and Br, and 100 ng for Cd and Sn. Relative standard deviations for points on the analytical curves were typically 10% (3 measurements) at weights more than 10 times the detection limit. The limit of detection was taken as the amount producing a blank-corrected signal three times the standard deviation of the blank. Limits of detection are listed in Table 2. These results are preliminary in nature, and considerable improvement in limits of detection seems possible.

CONCLUSIONS

This study has demonstrated the feasibility of analytical emission spectroscopy with a microwave-induced plasma in a closed microvolume sample tube. Although the analyte signal varies with time, it lasts long enough to be measured with electronic systems with suitable time-constants.

In common with most microscale techniques, the sample handling requires considerable skill and care. Approaches for preparaing gaseous and liquid samples and standards have been developed. Liquid samples can probably be handled more conveniently by techniques such as furnace atomic-absorption spectroscopy, at least for the determination of metals, but the sealed-tube microwave-plasma technique offers promise for the determination of volatiles in air and other samples when combined with any of several trapping schemes, such as the Tenax GC approach described by Zlatkis *et al.*[6–9]

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ANALYTICAL DATA

HEAVY-ATOM ENHANCEMENT AND ANALYTICAL FIGURES OF MERIT FOR LOW-TEMPERATURE PHOSPHORIMETRY IN THE RED REGION FOR SEVERAL POLYNUCLEAR AROMATIC HYDROCARBONS

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Summary—Analytical figures of merit for the low-temperature (77 K) phosphorescence of 22 polynuclear aromatic hydrocarbons are presented. Also, heavy-atom enhancement factors have been obtained for these compounds with an n-heptane solvent with iodoethane and dimethylmercury as sources of external heavy atoms, and with a 3:1 v/v ethanol/water solvent with potassium iodide, silver nitrate, thallium acetate, and lead acetate as heavy-atom sources. Effects of these heavy atoms on the phosphorescence signals vary markedly, depending on the compound of interest.

The use of luminescence methods for the determination of polynuclear aromatic hydrocarbons (PAHs) is widespread. Room-temperature (298 K) fluorescence offers excellent sensitivity for these compounds, but because of the relatively broad-band nature of fluorescence spectra, the spectral selectivity is severely limited. One possible solution to this problem of selectivity is to cool the sample to the temperature of liquid nitrogen or helium and use Shpol'skii solvents and/or fluorescence line-narrowing methods.[1-3] Phosphorescence gives greater spectral selectivity and provides an excellent means for PAH determination. Both room-temperature (298 K) and low-temperature (77 K) phosphorescence have been used for PAH determination.[4-6]

The use of heavy atoms to enhance the phosphorescence intensity of organic compounds is common, and extensive effort has been made to explain this phenomenon.[7,8] Significant enhancement of room-temperature phosphorescence by thallium, silver, lead, iodide, and mercury has been reported.[9,10] These external heavy atoms have also been used in low-temperature phosphorescence.[11-14] LueYen-Bower and Winefordner[10] have reported a trend in the heavy-atom enhancement of room-temperature phosphorescence of several PAHs.

This paper presents the low-temperature phosphorescence figures of merit and heavy-atom enhancement factors for 22 PAHs that phosphoresce in the red region (>580 nm). These compounds are often omitted in phosphorescence data owing to instrumental limitations in the red region. A non-polar solvent, n-heptane, and a polar solvent, 3:1 v/v ethanol/water, were used, with the heavy atoms most commonly reported in the literature—silver, thallium, lead, iodide, and mercury.

EXPERIMENTAL

Instrumentation

Figure 1 shows a block diagram of the system used. A complete list of the equipment and its manufacturers is given in Table 1. A 150-W Eimac lamp, operated at 12 A, was used as the excitation source. The exciting light was focused onto the entrance slit of a JY H-10 monochromator. The radiant flux from the monochromator was then focused into the centre of a standard Aminco sample-cell compartment by an $f/4$ quartz lens. The phosphorescence was observed at 90° to the incident excitation beam and focused with an $f/4$ quartz lens into a Spex 1670 Minimate monochromator with a grating blazed at 1000 nm. Since weak phosphorescence intensities were expected for some of the PAHs, a red-sensitive 1P28 photomultiplier tube operated at −1000 V was used. This photomultiplier tube allowed phosphorescence radiation at wavelengths shorter than ~1000 nm to be detected. Phosphorescence intensity readings were taken from a laboratory-constructed nanoammeter, with a 1-sec time constant.[15] The output of the nanoammeter was fed to a strip-chart recorder for continuous monitoring of the signal and for recording excitation and emission spectra.

The sample compartment for the system had a circular opening at the top to accommodate a Dewar flask with a quartz optical window. The Dewar flask was held in place with a phenolic plastic ring slip-fitted to the compartment opening. A cylindrical cover was fitted over the Dewar and slip-fitted to the lower portion of the sample compartment. The precision-bore Spectrosil quartz sample tubes, 30 cm long, 3 mm outer diameter, 1 mm inner diameter (Thermal American Fused Quartz Co., Montville, NJ 07045) were sealed at one end and fitted into Teflon cylinders with

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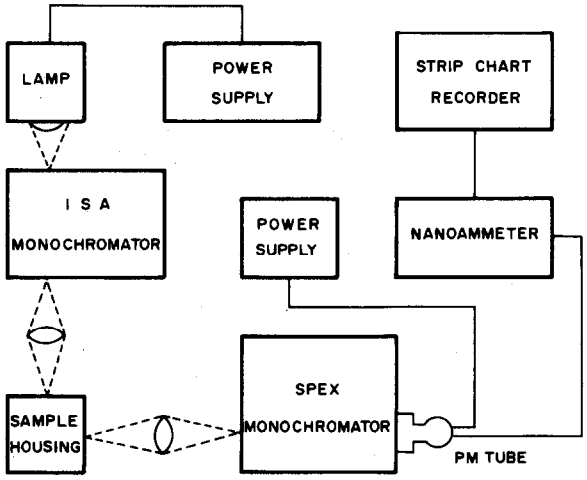

Fig. 1. Conventional red-region luminescence detection scheme.

Teflon tape. The sample tube was held rigidly in place by the Teflon cylinder and immersed in liquid nitrogen in the Dewar to attain a temperature of 77 K. To avoid frost from forming on the optical tip of the Dewar, a gentle stream of nitrogen gas was flushed into the lower section of the sample-cell compartment. A black felt cover was placed loosely over the top of the sample tube during analysis to minimize interference from room light.

Reagents

All chemicals used, and their sources, are listed in Table 2. Unless otherwise indicated, all chemicals were reagent grade or better and used as supplied. Iodoethane was purified by passage through activated alumina in a dark room and was stored over copper away from room light.

Procedure

The excitation and emission monochromators were calibrated with a low-pressure mercury lamp. Detailed phosphorescence excitation and emission spectra were obtained for each PAH in n-heptane, with a 4-nm spectral bandpass and a concentration 10–100 times the detection limit (0.1–10 μg/ml), in the linear portion of the calibration graphs. It was assumed that the peak intensity wavelengths were approximately the same as those observed when polar solvents are used. All intensity-maximum wavelengths were uncorrected for instrumental response. The sample cell was filled with about 25 μl of solution by means of a 2-ml syringe with a 12-in. long 17-gauge stainless-steel needle. The volume illuminated was about 10 μl.

Analytical calibration graphs were prepared by examination of serial dilutions of a stock 100-μg/ml solution. Detection limits were calculated from those concentrations which fell on the linear portion of the calibration graphs at the maximum excitation and emission wavelengths.

Enhancement factors are defined as ratio of the phosphorescence intensity with heavy atom present to that without heavy atom, after correction for the blank. For each measurement, a standard solution in the 0.1–1.0 μg/ml range was prepared in the particular solvent/heavy-atom combination. A 10-μg/ml solution was used for anthracene. Each phosphorescence signal was obtained at the wavelength maximum selected from the previously obtained spectra.

Table 1. Experimental equipment and manufacturers

Item	Model number	Source
Eimac xenon arc lamp 150 W	VL-150-2	Eimac, Division of Varian, San Carlos, CA 94070
Eimac illuminator power supply (operated at 12 A)	P250S-2	Eimac, Division of Varian, San Carlos, CA 94070
Monochromator *f*/3.5 holographic grating 1200 grooves/mm	H-10 UV	American ISA, Inc., Metuchen, NJ 08840
Sample housing		American Instrument Co., Silver Spring, MD 20910
Monochromator *f*/4.0 holographic grating 600 grooves/mm	1670 Minimate	Spex Industries, Inc., Box 798, Methuchen, NJ 08840
Photomultiplier tube	1P928	Hammamatsu, Waltham, MA 02154
High-voltage power supply (operated at −1000 V)	EU-42A	Heath Co., Benton Harbor, MI 49022
Nanoammeter		Laboratory constructed
Strip-chart recorder Servo/Riter Recorder II	PS01W	Texas Instrument, Inc., Santa Ana, CA 92705

Table 2. Chemicals and their sources

Compound	Company
1,12-Benzoperylene	Aldrich Chemical Co., Milwaukee, WI 53233
1,2-Benzopyrene	
3,4-Benzopyrene	
1,2:3,4-Dibenzanthracene	
9,10-Dimethylanthracene	
9,10-Diphenylanthracene	
Perylene	
Rubrene	
Lead acetate	Allied Chemical, Morristown, NJ 07960
Water	Barnstead Nanopure System, Barnstead Sybron Corp., Boston, MA 02132
Fluoranthene*	Chem Service, Inc., West Chester, PA 19386
Anthracene	Eastman Kodak, Rochester, NY 14650
1,2-Benzanthracene	
1,2:5,6-Dibenzanthracene	
7,12-Dimethyl-1,2-benzanthracene	
Naphthacene	
Pyrene	
Dimethylmercury	
Iodoethane	
Sodium iodide	Fisher Scientific Co., Fair Lawn, NJ 07410
Anthanthrene	K & K Laboratories, Inc., Plainview, NY 11803
1,2:3,4-Dibenzopyrene	
1,2:4,5-Dibenzopyrene	
3,4:8,9-Dibenzopyrene	
3,4:9,10-Dibenzopyrene	
20-Methylcholanthrene	
Silver nitrate	Mallinckrodt, St. Louis, MO 63134
n-Heptane	Matheson, Coleman & Bell, Norwood, OH 45212
5,7-Dimethyl-1,2-benzacridine	Nutritional Biochemicals, Cleveland, OH 44128
Thallium nitrate	PCR, Gainesville, FL 32601
Ethanol†	U.S. Industrial Chemicals, New York, NY 10016

* Recrystallized 3 times from ethanol.
† Technical grade (95%) distilled over KOH.

RESULTS AND DISCUSSION

Tables 3 and 4 contain the phosphorescence analytical figures of merit for PAHs in the red region, in n-heptane and ethanol/water media respectively. The limits of detection for most of the compounds are below 100 ng/ml and the average relative standard deviation is 6%. The detection limits for several of the PAHs are compared to those previously reported[14,16] for the conventional spectral region. From the wavelengths listed, some results in the early reports appear to be in error.

For most PAHs the concentration range for linear response covers four orders of magnitude. The relative standard deviations range from 5 to 10% and agree with those routinely obtained by low-temperature phosphorimetry.

The heavy-atom enhancement factors for n-heptane and 3:1 v/v ethanol/water solutions of PAH are reported in Tables 5 and 6 respectively. For alkane solutions, the sample cell was plunged into liquid nitrogen in the Dewar flask and frozen rapidly to minimize segregation of solute and solvent molecules. Aggregates exhibit much lower emission yields than isolated molecules.[17] A measurement was then taken when the signal intensity had become constant (in <30 sec). The Dewar flask was refilled after every three measurements, and the liquid nitrogen was completely replaced if flakes of ice were seen accumulating in the optical tip of the Dewar flask. For ethanol/water solutions, the sample cell was lowered slowly into the liquid nitrogen over a period of 1 min to obtain clear, frozen glasses with no cracks. If the rate of freezing was increased, severe cracks in the glass arose, lowering the phosphorescence intensity and the precision. St. John and Winefordner[18] and Hollifield and Winefordner[19] have discussed such problems

Table 3. Phosphorescence analytical figures of merit of polynuclear aromatic hydrocarbons in heptane at 77 K in the red region

Compound	Excitation maxima,* nm	Emission maxima,* nm	Limit of detection,† μg/ml	RSD,§ %
Anthanthrene‡	—	—	—	—
Anthracene	270, 350	671	3	5.4
1,2-Benzanthracene	275, 312	523, 544, 600, 652 658	0.1	9.7
1,12-Benzoperylene	366, 386	616, 634, 673, 687	0.004	4.4
1,2-Benzopyrene	331	540, 590, 638, 652	0.0005	6.7
3,4-Benzopyrene	360, 380	685, 698, 751, 756	0.2	6.3
1,2:3,4-Dibenzanthracene	337	566, 618, 678	0.05	9.3
1,2:5,6-Dibenzanthracene	345	548, 556, 587, 603 650	0.005	9.9
1,2:3,4-Dibenzopyrene	307, 362	616, 636, 684	0.04 (−)¶	7.9
1,2:4,5-Dibenzopyrene	368	614, 620, 672, 684	0.02 (0.6)	5.3
3,4:8,9-Dibenzopyrene‡	—	—	—	—
3,4:9,10-Dibenzopyrene	335	512, 554, 600	0.01 (0.5)	6.1
9,10-Dimethylanthracene‡	—	—	—	—
5,7-Dimethyl-1,2-benzacridine	360	590, 642	0.005	9.9
7,12-Dimethyl-1,2-benzanthracene	290, 365, 400	646, 702	0.05	8.6
9,10-Diphenylanthracene‡	—	—	—	—
Fluoranthene	360	545, 564, 592, 613 646	0.01	4.7
20-Methylcholanthrene	305, 359	615, 676	0.06	5.9
Naphthacene‡	—	—	—	—
Perylene‡	—	—	—	—
Pyrene	330, 350	517, 555, 592, 608 640, 656, 676, 694	0.008	2.8
Rubrene‡	—	—	—	—

* The wavelengths giving greater intensity are underlined and were determined with a 4-nm spectral bandpass. Relative intensities are uncorrected for instrumental response.

† Measurements were made at the most intense peak wavelengths, with a 40-nm spectral bandpass. The limit of detection is that concentration giving a signal 3 times the standard deviation of 16 blanks.

§ Based on 16 determinations on a 1–10 μg/ml solution at the most intense peak wavelengths.

‡ No phosphorescence observed.

¶ Reference 16.

arising from use of solvents at 77 K. The measurement was taken immediately after the sample had been completely lowered into the Dewar. Any red shifts in wavelength maxima (caused by the heavy atoms or difference in solvents) were slight. A 40-nm spectral bandpass was therefore used, centred at the wavelength maximum obtained for anlyte solutions without heavy atoms, thus compensating for any slight shift in the wavelength maximum as a result of presence of the heavy atom.

1,2:3,4-Dibenzopyrene, 1,2:4,5-dibenzopyrene, 3,4:8,9-dibenzopyrene, and 3,4:9,10-dibenzopyrene were available in only limited quantities, so were examined only in n-heptane solution. Attempts to acquire more material commercially were unsuccessful.

The relative intensities of phosphorescence peaks may change dramatically in the presence of an external heavy atom, and a relatively weak peak may become the most intense. For example, the phosphorescence of the (0, 0) peak of triphenylene or coronene is very weak in EPA as well as in crystalline matrices, but very intense in solvents containing heavy atoms.[20] Consequently, the enhancement factors reported are not necessarily the largest values obtainable for these compounds. The heavy-atom enhancement factors indicate the increase or decrease in phosphorescence observed at the wavelength maxima of analyte solutions without heavy atoms present. The concentration of the heavy atom was limited by its solubility in the solvents used. As previously reported, iodoethane,[13,14] dimethylmercury,[11] silver[11,12] and iodide[11,12] give the largest enhancement factors. Although lead and thallium(I) give large enhancement factors when used for phosphorescence work on paper,[22] they give only small enhancement effects for frozen solutions. The use of the external heavy atom is an experimentally simple means of increasing the signal levels.[16] Unfortunately, heavy atoms can also increase the background signal to the same or even a greater extent, resulting in poorer limits of detection. Therefore, the experimenter must evaluate the enhancement factor, the detection limit, the precision, and the linear response range for each analyte to determine whether the benefits justify the high cost and/or risk from the toxic effects of the heavy atoms.

Table 4. Phosphorescence analytical figures of merit of polynuclear aromatic hydrocarbons in 75/25 (v/v) ethanol/water at 77 K in the red region

Compound	Excitation wavelength,* nm	Emission wavelength,* nm	Limit of detection,† μg/ml	RSD,§ %
Anthanthrene‡	—	—	—	—
Anthracene	350	676	0.5 (0.05)¶	1.7
1,2-Benzanthracene	312	600	0.1 (0.03)	7.6
1,12-Benzoperylene	386	624	0.06 (0.09)	2.9
1,2-Benzopyrene	331	590	0.0006 (0.02)	5.7
3,4-Benzopyrene	365	685	0.03 (3.0)	5.7
1,2:3,4-Dibenzanthracene	337	618	0.05 (0.09)	4.8
1,2:5,6-Dibenzanthracene	345	603	0.002 (0.02)	7.1
9,10-Dimethylanthracene‡	—	—	—	—
5,7-Dimethyl-1,2-benzacridine	360	642	0.01	13.0
9,10-Diphenylanthracene‡	—	—	—	—
Fluoranthene	360	592	0.03	8.7
20-Methylcholanthrene	359	615	0.2 (—)	3.8
Naphthacene‡	—	—	— (0.001)	—
Perylene‡	—	—	— (—)	—
Pyrene	330	592	0.02 (0.4)	5.6
Rubrene	—	—	—	—

* Wavelengths are uncorrected for instrumental response and were determined with a 4-nm spectral bandpass.

† Measurements were made with a 40-nm spectral bandpass at the most intense peak wavelengths. The limit of detection is that concentration giving a signal 3 times the standard deviation of 16 blanks.

§ Based on 16 determinations of a 1–10 μg/ml solution at the most intense peak wavelengths.

‡ No phosphorescence observed. In the case of naphthacene, it is apparent that the value given by reference 14 is anomalous.

¶ References 14 and 16.

Note. Limited sample quantities for 1,2:3,4-dibenzopyrene, 1,2:4,5-dibenzopyrene, 3,4:8,9-dibenzopyrene, and 3,4:9,10-dibenzopyrene allowed for testing of n-heptane solutions only. These compounds are no longer available from the original suppliers.

Table 5. Heavy-atom phosphorescence enhancement factors for polynuclear aromatic hydrocarbons in n-heptane at 77 K

Compound	Excitation wavelength,* nm	Emission wavelength,* nm	Heavy-atom enhancement factor	
			1.0*M* EtI†	0.1*M* DiMeHg§
Anthanthrene‡	—	—	—	—
Anthracene	350	676	6.1	2.3
1,2-Benzanthracene	312	600	0.94	7.4
1,12-Benzoperylene	386	624	1.1	0.80
1,2-Benzopyrene	331	590	5.7	0.52
3,4-Benzopyrene	365	685	2.6	3.3
1,2:3,4-Dibenzanthracene	337	618	3.1	5.7
1,2:5,6-Dibenzanthracene	345	603	4.7	7.3
1,2:3,4-Dibenzopyrene	362	616	1.0	1.5
1,2:4,5-Dibenzopyrene	368	614	3.2	2.8
3,4:8,9-Dibenzopyrene‡	—	—	—	—
3,4:9,10-Dibenzopyrene	335	554	2.8	5.6
9,10-Dimethylanthracene‡	—	—	—	—
5,7-Dimethyl-1,2-benzacridine	360	642	6.6	1.8
7,12-Dimethyl-1,2-benzanthracene	360	646	3.2	2.7
9,10-Diphenylanthracene‡	—	—	—	—
Fluoranthene	360	592	14.0	8.0
20-Methylcholanthrene	359	615	8.8	6.6
Naphthacene‡	—	—	—	—
Perylene‡	—	—	—	—
Pyrene	330	592	9.5	7.6
Rubrene‡	—	—	—	—

* Wavelengths are uncorrected for instrumental response and were determined with a 4-nm spectral bandpass.

† 1.0*M* iodoethane.

§ 0.1*M* dimethylmercury.

‡ No phosphorescence observed, even with heavy-atom perturbation.

Table 6. Heavy-atom phosphorescence-enhancement factors for polynuclear aromatic hydrocarbons in 75/25 (v/v) ethanol/water at 77 K

Compound	λ_{ex}* nm	λ_{em}* nm	Heavy-atom enhancement factor 1.0M KI	0.1M $AgNO_3$	0.1M TlAc†	0.1M $Pb(Ac)_2$§
Anthanthrene‡	—	—	—	—	—	—
Anthracene	350	676	2.4	3.4	0.89	0.49
1,2-Benzanthracene	312	600	4.0	6.7	1.4	0.94
1,12-Benzoperylene	386	624	0.75	2.3	3.0	0.63
1,2-Benzopyrene	331	590	1.7	1.3	1.3	1.5
3,4-Benzopyrene	365	685	0.079	1.6	0.42	0.65
1,2:3,4-Dibenzanthracene	337	618	7.2	15.0	5.3	4.1
1,2:5,6-Dibenzanthracene	345	603	2.1	4.1	0.24	1.0
9,10-Dimethylanthracene‡	—	—	—	—	—	—
5,7-Dimethyl-1,2-benzacridine	360	642	2.2	11.0	1.2	1.0
9,10-Diphenylanthracene‡	—	—	—	—	—	—
Fluoranthene	360	592	3.1	4.4	1.2	1.6
20-Methylcholanthrene	359	615	2.9	69.0	3.3	2.1
Naphthacene‡	—	—	—	—	—	—
Perylene‡	—	—	—	—	—	—
Pyrene	330	592	1.1	5.7	1.1	1.1
Rubrene‡	—	—	—	—	—	—

* Wavelengths are uncorrected for instrumental response and were determined with a 4-nm spectral bandpass.

† 0.1*M* thallium acetate.

§ 0.1*M* lead acetate.

‡ No phosphorescence observed, even with heavy-atom perturbation.

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COMPORTEMENT ELECTROCHIMIQUE DES THIOCYANOSULFATES

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Résumé—Dans le cadre d'une étude systématique des propriétés sulfonantes des complexes du type SO_3B, nous nous sommes interessés aux thiocyanosulfates KSO_3SCN et NH_4SO_3SCN. Nous avons établi, par conductimétrie, que ces sels sont dissociés et que le solvant choisi, le sulfolane, solvate peu les espèces et possède un caractère aprotonique marqué. Par potentiométrie, nous avons pu déterminer la constante K de l'équilibre $MSO_3SCN \rightleftharpoons MSCN + SO_3$ en étudiant le couple électrochimique $Ag + SO_3SCN^- \rightleftharpoons AgSCN + SO_3 + e^-$. Les valeurs trouvées pour les sels d'ammonium et de potassium sont respectivement $K = 10^{-11,5 \pm 0,45}$ et $10^{-9,7 \pm 0,7}$. Les thiocyanosulfates apparaissent comme des agents de sulfonation moins faibles que les chlorosulfates. L'ion SCN^- est donc une base plus faible que Cl^- vis à vis de SO_3 dans le sulfolane.

Nous avons proposé récemment[1] un classement dans le sulfolane (TMS) de la basicité de différents solvants vis à vis de l'anhydride sulfurique, en étudiant l'équilibre:

$$TMS + SO_3 \cdot Sv \rightleftharpoons SO_3 \cdot TMS + Sv$$

Afin d'établir un classement plus complet du pouvoir sulfonant de complexes du type SO_3B dans le sulfolane, nous nous sommes intéressés aux complexants minéraux et en particulier au cas où la base B est SCN^-. Bien que la synthèse des thiocyanosulfates MSO_3SCN soit connue depuis quelques années,[2] aucune référence n'a pu être trouvée dans la bibliographie concernant le comportement physicochimique de ces composés.

La détermination de la constante K de l'équilibre

$$MSO_3SCN \rightleftharpoons MSCN + SO_3$$

nécessite la connaissance du degré de dissociation ionique, de MSO_3SCN et $MSCN$ et celle de la constante de dissociation de l'ion SO_3SCN^- en SO_3 et SCN^-. Nous avons donc tout d'abord réalisé l'étude conductimétrique des sels (M = NH_4,K) puis étudié la dissociation de l'ion SO_3SCN^- par voltampérométrie à l'électrode d'argent.

PARTIE EXPERIMENTALE

La purification du sulfolane a été décrite précédemment[3] ainsi que la préparation des thiocyanosulfates d'ammonium et de potassium.[2]

Les courbes intensité–potentiel en régime stationnaire sont tracées à 30° ± 0,1° à l'aide d'un ensemble voltampérométrique Tacussel. La vitesse de l'électrode indicatrice d'argent est de 10 t/sec. La vitesse de balayage des potentiels est de 5 mV/sec.

Les mesures potentiométriques ont été réalisées à l'aide d'un millivoltmètre Tacussel Isis 20000 suivi d'un enregistreur permettant d'apprécier la stabilité des potentiels. L'électrode de référence est constituée par la demi-pile $Ag/AgClO_4$ 0,1M.

La potentiel de demi-vague du système ferrocène–ferricinium est pris comme origine de l'échelle des potentiels.

Les conductances sont mesurées à l'aide d'un conductimètre Tacussel CD 75N. La fréquence de polarisation choisie est de 1000 Hz.

RESULTATS ET DISCUSSIONS

Etude conductimétrique

Toutes les expériences ont été réalisées avec les concentrations inférieures à $10^{-2}M$. Les résultats sont traités par la méthode préconisée par Fuoss *et al.*[4]

Les équations utilisées sont:

$$\Lambda = \Lambda_0 - S(C\gamma)^{1/2} + E'C\gamma \log 6E_1'C\gamma + LC\gamma - K_A Cf^2\gamma \quad (A)$$

si l'électrolyte est associé (constante d'association $K_A > 10$) ou

$$\Lambda = \Lambda_0 - SC^{1/2} + E'C \log 6E_1'C + (L - A\Lambda_0)C \quad (B)$$

si l'électrolyte est dissocié. Dans ces équations, les différents symboles ont leur signification habituelle, $S = \alpha\Lambda_0 + \beta$, $E = E_1'\Lambda_0 - E_2'$, et les différentes constantes α, β, E_1' et E_2' calculées à partir des équations de Fuoss *et al.*[4] ont respectivement pour valeur dans le sulfolane 0,5439, 6,996, 1,300 et 2,443. Les paramètres Λ_0 et K_A ont été déterminés par la méthode des moindres carrés.[4]

Les données expérimentales sont analysées par ordinateur IRIS 80. Leur traitement à l'aide de l'équation (A) établit que seul NH_4SCN peut être considéré comme associé ($K_A = 65$). Par contre les constantes d'association K_A des autres électrolytes étant très faibles, ces composés ont été considérés comme dissociés[4] et les résultats ont été traités à l'aide de l'équation (B). Les valeurs trouvées sont consignées dans le tableau 1.

Nous constatons que les différences entre les

Tableau 1.

	Λ_0	σ	K_A
NH_4SCN	14,45	0,04	65
KSCN	13,62	0,01	
KSO_3SCN	8,94	0,01	
NH_4SO_3SCN	9,72	0,004	

valeurs des conductivités limites des thiocyanates de potassium et d'ammonium d'une part, et celles des thiocyanosulfates correspondants d'autre part, sont voisines (respectivement $\Delta\Lambda_0 = 0,83$ et 0,78). Par ailleurs, la valeur trouvée pour Λ_0^{KSCN} est en bon accord avec celle donnée dans le même solvant par Della Monica *et al.*[5] (13,63).

Les conductivités ioniques limites $\lambda_0^{\pm}$ ont pu être déterminées à partir de celle que nous avons récemment établie[6] pour K^+ (Tableau 2). Les rayons ioniques des cations K^+ et NH_4^+ dans le sulfolane ont pu être estimés à partir de la loi de Stokes et des corrections proposées par Robinson et Stokes.[9] Les nombres de solvation (n) trouvés pour ces cations sont très petits, ce que confirme le très faible pouvoir solvatant du sulfolane (Tableau 2).

Etude électrochimique

A l'image de l'ion chlorosulfate dont nous avons étudié le comportement dans le sulfolane,[3] l'ion SO_3SCN^- doit se dissocier dans le solvant selon

$$SO_3SCN^- \rightleftharpoons SO_3 + SCN^- \qquad (I)$$

L'électrode d'argent étant indicatrice de l'ion SCN^- dans ce solvant, il fallait, pour calculer la constante $K_{SO_3SCN}^{SO_3}$ de l'équilibre (I), montrer que cette électrode était également indicatrice de l'ion SO_3SCN^-. L'étude du couple oxydo-réducteur

$$Ag + SO_3SCN^- \rightleftharpoons AgSCN + SO_3 + e^- \qquad (II)$$

a donc été entreprise.

La tracé des courbes voltampérométriques des solutions de SO_3SCN^- en présence d'électrolyte indifférent [$(C_2H_5)_4NClO_4$, $10^{-1}M$] met en évidence la présence de vagues d'oxydation reproductibles. La proximité du mur d'oxydation de l'argent rend difficile l'évaluation du courant limite de diffusion, comme le montre la Fig. 1. On peut remarquer cependant que ce courant est proportionnel à la concentration en thiocyanosulfate et que la potentiel de demi-vague en est indépendant.

Une coulométrie, faite pendant l'électrolyse d'une solution de KSO_3SCN dans le sulfolane avec une

Tableau 2.

	λ_0	r_s, Å	r_s corr., Å	n	λ_0 (Réf.)
K^+	3,96	2,01	4,01	1,7	4,05 (7)
NH_4^+	4.76	1,67	3,59	1,1	4,97 (8)
SCN^-	9,66	—	—	—	9,64 (6)
SO_3SCN^-	4,97	—	—	—	—

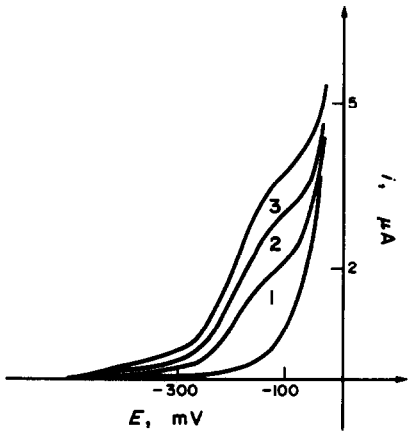

Fig. 1. Courbes voltampérométriques de solutions de thiocyanosulfate dans le sulfolane (sens cathodique–anodique). $[SO_3SCN^-]$ = (1) $7 \cdot 10^{-4}M$; (2) $9,8 \cdot 10^{-4}M$; (3) $12,7 \cdot 10^{-4}M$.

anode d'argent, montre que la réaction électrochimique fournit un électron par molécule de thiocyanosulfate.

Si l'on admet, par analogie avec le chlorodisulfate, que le thiocyanodisulfate—s'il existe—est entièrement dissocié dans le sulfolane en SO_3 et SO_3SCN^-, ces résultats permettent d'établir que la réaction d'oxydation de l'argent s'effectue bien selon (II).

Dans ces conditions, le potentiel pris par l'électrode d'argent est

$$E = E_1^0 + 0,06 \log \frac{(SO_3)}{(SO_3SCN^-)} \qquad (III)$$

avec

$$E_1^0 = E_{Ag/Ag^+}^0 + 0,06 \log \frac{K_s}{K_{SO_3SCN^-}^{SO_3}} \qquad (IV)$$

K_s étant le produit de solubilité de AgSCN.

Détermination de K_s

Les travaux de Della Monica *et al.*[10] sur différents complexes de l'argent dans le sulfolane et ceux de Parker et Alexander[11] établissent comme valeur du produit de solubilité de AgSCN dans le sulfolane à 30° respectivement $pK_s = 16,3$ et $14,5 \pm 0,3$.

Ces valeurs étant trop distinctes, nous avons redéterminé K_s dans le solvant purifié selon Pierens *et al.*,[3] par dosage potentiométrique d'une solution de KSCN par $AgClO_4$.

Le traitement mathématique des courbes potentiométriques conduit au produit de solubilité de AgSCN ($K_s = 10^{-14,8 \pm 0,2}$) et aux constantes de dissociation de $Ag(SCN)_2^-$ en AgSCN et SCN^- ($K_1 = 10^{-0,1 \pm 0,2}$) et de $Ag(SCN)_2^-$ en Ag^+ et SCN^- ($K_2 = 10^{-14,9 \pm 0,4}$).

La valeur de K_1 montre que le complexe $Ag(SCN)_2^-$ est peu stable, ce qui est en accord d'une part avec l'apparition du précipité de AgSCN avant la demi équivalence lors du dosage du thiocyanate par l'ion

argent, et d'autre part avec une inégalité des courants de diffusion correspondants aux deux vagues obtenues lors de l'étude voltampérométrique de l'oxydation de l'argent en présence de thiocyanate de potassium.

La valeur trouvée pour K_s est proche de celle proposée par Parker *et al.* bien que ceux-ci n'aient jamais signalé l'existence du complexe $Ag(SCN)_2^-$.

K_s et K_2 ont été comparées aux valeurs obtenues dans d'autres solvants.[12,13] A l'aide des coefficients de solvatation de Ag^+ déterminés par Badoz-lambling et Bardin[13] à partir de l'hypothèse de Strehlow dans différents solvants aprotoniques, nous avons pu calculer les coefficients de transfert du solvant de référence (0) au solvant (S) des ions SCN^- et $Ag(SCN)_2^-$. On peut en effet établir aisément que:

$$\log {}^{0\rightarrow S}\gamma(SCN^-) = (pK_s^S - pK_s^0) - \log {}^{0\rightarrow S}\gamma(Ag^+)$$

et

$$\log {}^{0\rightarrow S}\gamma[Ag(SCN)_2^-] = 2\log {}^{0\rightarrow S}\gamma(SCN^-) + \log {}^{0\rightarrow S}\gamma(Ag^+) + \log K_2^s - \log K_2^0.$$

Nous avons choisi, parmi les solvants dipolaires aprotoniques usuels, le nitrométhane (NM) comme solvant de référence pour ses propriétés complexantes très faibles vis à vis de Ag^+.[13]

Les valeurs de

$$\log {}^{NM\rightarrow S}\gamma$$

regroupées dans le tableau 3, sont en général voisines de zero, confirmant ainsi que ces solvants aprotoniques possèdent, comme le nitrométhane, un très faible pouvoir solvatant vis à vis des anions Cl^-, SCN^- et $Ag(SCN)_2^-$. On constate également que pour ces solvants de constantes diélectriques semblables (excepté le carbonate de propylène) les variations de solvatation diminuent quand la taille de l'anion augmente: $[Cl^- < SCN^- < Ag(SCN)_2^-]$.

$$\text{Pour } Cl^- = 0 < \log {}^{NM\rightarrow S}\gamma < 3{,}3$$

$$\text{Pour } SCN^- = -0{,}8 < \log {}^{NM\rightarrow S}\gamma < 1{,}9$$

$$\text{Pour } Ag(SCN)_2^- = -1{,}7 < \log {}^{NM\rightarrow S}\gamma < 1.$$

La différence

$$\log {}^{0\rightarrow S}\gamma(Cl) - \log {}^{0\rightarrow S}\gamma(AgCl_2^-)$$

a été choisie par Badoz-Lambling et Bardin[13] comme critère d'aprotonicité des solvants. Un calcul analogue fait à l'aide des valeurs trouvées pour les ions SCN^- et $Ag(SCN)_2^-$ confirme le classement obtenu[13] (exception faite pour le DMSO) (Tableau 3). Nous remarquons que seul le nitrométhane et le carbonate de propylène sont des solvants de caractère aprotonique plus faible que le sulfolane. Le nitrométhane ne pouvant être utilisé (les solutions concentrées de SO_3 y sont peu stables), le carbonate de propylène et le sulfolane restent donc les solvants les mieux adaptés à notre étude. Cependant, au vu de l'expérience acquise ces dernières années dans notre laboratoire, il nous a paru plus judicieux d'utiliser le sulfolane.

Constante de dissociation de l'ion SO_3SCN^- dans le sulfolane

La réaction

$$SO_3 + SCN^- \rightarrow SO_3SCN^-$$

est réalisée par addition du thiocyanate de potassium

Tableau 3.

	NM	PC	TMS	DMSO	NM_2P	DMA	DMF	HMPT
$pK_s \rightleftharpoons AgCl$	21,2(b)	20,0(c)	18,4	10,4(d)	14,5(e)	14,3(e)	14,5(e)	11,9(e)
$pK_s \rightleftharpoons AgSCN$	18,9(b)	16,4(c)	14,8	7,6(d)	11,5(e)	10,5(e)	11,5(e)	7,4(e)
pK_2 (a)	18,4(b)	16,0(c)	14,9	8,4(d)	11,9(e)	11,4(e)	11,9(e)	9,7(e)
$\log {}^{NM\rightarrow S}\gamma Ag^+$	0 (b)	−1,7(b)	−3,8(b)	−11,0(b)	−9,3(b)	−9,5(b)	−8,2(b)	−12,6(b)
$\log {}^{NM\rightarrow S}\gamma Cl^-$	0	0,5	1	0,2	2,6	2,6	1,5	3,3
$\log {}^{NM\rightarrow S}\gamma SCN^-$	0	−0,8	−0,3	−0,3	1,9	1,1	0,8	1,1
$\log {}^{NM\rightarrow S}\gamma Ag(SCN)_2^-$	0	−0,9	−0,9	−1,6	1,0	−0,3	−0,1	−1,7
$-\log \frac{(Cl)^-}{(AgCl_2^-)}$	0,3(f)	0,9(c)	1,0	1,3(f)	1,8(e)	2,3(f)	1,9(f)	3,7(f)
$-\log \frac{(SCN^-)}{Ag(SCN)_2^-}$	−0,5	−0,4	+0,1	0,8	0,4	0,9	0,4	2,3

NM = nitrométhane — PC = carbonate de propylène
TMS = sulfolane — DMSO = diméthylsulfoxide
DMF = diméthylformamide — DMA = diméthylacétamide
NM_2P = *N* méthyl 2 pyrrolidone — HMPT = hexaméthylphosphorotriamide

(a) $K_2 = \frac{(Ag^+)(SCN^-)^2}{[Ag(SCN)_2^-]}$; (b) Ref. 13; (c) Ref. 15; (d) Ref. 16; (e) Ref. 17; (f) Ref. 18.

à une solution de SO_3 dans le sulfolane en milieu $(C_2H_5)_4NClO_4$ 0,1*M* et suivie par potentiométrie à l'électrode d'argent.

L'étude conductimétrique ayant établi que KSCN et KSO_3SCN étaient dissociés, le traitement mathématique des courbes potentiométriques obtenues (Fig. 2) à partir des relations (III) et (IV) donne pour constante de dissociation de l'ion SO_3SCN^-:

$$K^{SO_3}_{SO_3SCN^-} = 10^{-11,5 \pm 0,45}$$

La valeur de cette constante, plus élevée que celle du produit de solubilité de AgSCN, montre que le thiocyanosulfate d'argent ne peut être préparé dans le sulfolane. De plus, l'écart relativement faible entre $K^{SO_3}_{SO_3SCN^-}$ et K_s^{AgSCN} ($\Delta pK = 3,3$) justifie que l'oxydation de l'argent en présence de SO_3SCN^- (Fig. 1) ait lieu à des potentiels proches de l'oxydation de l'argent seul.

Ce faible écart ne permet pas le dosage simple d'une solution de thiocyanosulfate dans le sulfolane par l'ion Ag^+, ce que nous avons pu constater expérimentalement.

Le pouvoir sulfonant d'un thiocyanosulfate est mesuré par la constante de l'équilibre:

$$MSO_3SCN \rightleftharpoons SO_3 + MSCN$$

Dans le cas où les deux sels sont dissociés (sels de potassium), la constante pour KSO_3SCN est aussi celle de l'équilibre:

$$SO_3SCN^- \rightleftharpoons SO_3 + SCN^-$$

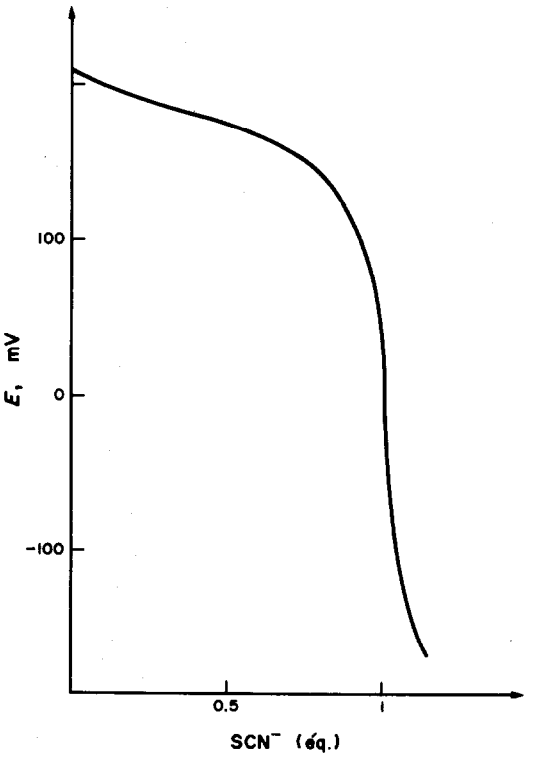

Fig. 2. Dosage potentiométrique d'une solution de SO_3 dans le sulfolane par KSCN.

soit

$$K^{SO_3}_{SO_3SCN^-} = 10^{-11,5 \pm 0,45}$$

Si M = NH_4, le thiocyanosulfate est dissocié à la différence du thiocyanate correspondant. Le pouvoir sulfonant qui peut alors s'exprimer par

$$K^{SO_3}_{NH_4^+SO_3SCN^-} = \frac{K^{SO_3}_{SO_3SCN^-}}{K^{D}_{NH_4SCN}} = 10^{-9,7 \pm 0,7}$$

augmente donc avec la stabilité du thiocyanate ($K^{D}_{NH_4SCN}$ = constante de dissociation ionique de NH_4SCN).

Notons que, dans le cas où aucun des deux sels n'est dissocié, la pouvoir sulfonant dépend du rapport des constantes de dissociation ionique $K^{D}_{MSO_3SCN}/K^{D}_{MSCN}$.

CONCLUSION

L'ion thiocyanosulfate apparaît être dans le sulfolane un agent de sulfonation faible mais plus fort que l'ion SO_3Cl^- ($K = 10^{-14,3}$) dont nous avions précédemment étudié la dissociation.[3] La différence entre le pK de dissociation de ces deux ions est en accord avec la plus faible stabilité thermique du thiocyanosulfate.[14] Elle montre que, *vis-à-vis* de l'acide SO_3, l'ion SCN^- est une base plus faible que Cl^- dans le sulfolane. Ce résultat est en accord avec les conclusions de Bréant *et al.*[19] qui constatent que SCN^- est une base plus faible que Cl^- *vis-à-vis* de l'acide H^+ dans des solvants dipolaires aprotoniques tels que le NM_2P et le DMF. Une étude plus générale du comportement de différentes bases *vis-à-vis* de l'acide de Lewis SO_3 dans le sulfolane devrait nous permettre d'établir un classement de la force d'autres agents sulfonants minéraux.

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Summary—In a systematic study of the sulphonating properties of SO_3B complexes, we have paid attention to the thiocyanosulphates KSO_3SCN and NH_4SO_3SCN. We have shown conductimetrically that these salts are dissociated and that the ionic solvation is weak in the chosen solvent—sulpholane—which is strongly aprotic in character. We have determined potentiometrically the equilibrium constant K of the reaction $MSO_3SCN \rightleftharpoons MSCN + SO_3$, by means of the electrochemical couple $Ag + SO_3SCN^- \rightleftharpoons AgSCN + SO_3 + e^-$. The values found for the ammonium and potassium salts are $K = 10^{-11.5 \pm 0.45}$ and $10^{-9.7 \pm 0.7}$ respectively. Thiocyanosulphates appear to be stronger than chlorosulphates as sulphonating agents. Therefore SCN^- is a weaker base than Cl^- in sulpholane medium.

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ANNOTATION

A COMPARATIVE STUDY OF THE APPLICATION OF THE METHOD OF LEAST-SQUARES IN THE POTENTIOMETRIC DETERMINATION OF PROTONATION CONSTANTS

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Summary—Methods of simple and multiple linear regression applied to the potentiometric determination of protonation constants of diprotic and triprotic acids are studied critically. The best way of fitting the data, according to the order of magnitude of the constants, is established. The conclusions are checked by calculating the protonation constants of succinic and citric acids.

The potentiometric determination of the protonation constants of an acid—base system may utilize graphical or analytical methods. Graphical methods[1–4] have been criticised because of their lack of accuracy, but they may assist detection of incorrect points, which is usually not possible by using only analytical methods of calculation. Analytical methods, on the other hand, are quicker and more accurate and precise. They include the unweighted least-squares procedure, which requires only a simple calculation program, and has the advantage over other analytical methods that it can be done with programmable pocket calculators.

In recent years high-speed computers have been widely used for calculation of constants.[5–8] The computer programs often include weighting factors, and are relatively sophisticated.

If the successive protonation constants of an acid differ greatly in magnitude, they can be treated separately, but when they are close, simultaneous fitting of the data is necessary. This is the case discussed here. For diprotic acids, among the various methods described, the linear least-squares procedure has been widely used, because of its precision and ease and speed of application.

For triprotic acids, the available analytical methods are much more laborious, so graphical curve-fitting methods are usually recommended.[9] Here, the least-squares method has been considered as not entirely satisfactory by some authors.[10]

In the present work the extension of the least-squares method to triprotic acids is studied with the aid of an HP 67 calculator. The conditions of its applicability to diprotic and triprotic acids are examined in parallel, and examples are shown for acids for which the successive logarithmic constants differ by unity. Systems with larger and smaller differences are also tested, with analogous results.

Potentiometric determination of protonation constants involves two well-differentiated steps: first, measurement of the experimental points to get the set of values $(\bar{n}, h)$ and secondly, an appropriate treatment of the data to obtain the constants. In this paper, hypothetical acids with known constants are assumed in order to be able to discuss not only the precision, but also the accuracy of the method. The theoretical titration curves of these acids are calculated, then "experimental" titration curves based on these are simulated by adding Gaussian errors to the volumes of reagent and the values of pH.

Finally, the conclusions are applied to the determination of the protonation constants of two real systems, and the results are compared with others in the literature.

THEORETICAL TITRATIONS

For an n-protic acid, H_nA, titrated with a strong base, the titration curve is given by:

$$v = V \frac{\left(\frac{K_w}{h} - h\right) + \frac{\Sigma(n-i)\beta_i h^i}{\Sigma\beta_i h^i} C_A}{C_v - \left(\frac{K_w}{h} - h\right)} \quad (1)$$

where h is $[H^+]$, V is the volume of acid of concentration C_A, v is the volume of strong base of concentration C_v, and β_i is the ith overall protonation constant of the system, with $\beta_0 = 1$ by definition.

Values for the protonation constants of a hypothetical acid are selected and 40 "theoretical" points along its exact titration curve $v = f(\mathrm{pH})$ are calculated, giving values to the parameters V, C_A and C_v. From this "exact" curve, 15 simulated "experimental" titration curves are obtained as follows. Each "theoretical" point is transformed into an "experimental" one by addition of a Gaussian error generated with the aid of a table of Tippett numbers,[11] which gives random deviates in units of standard deviation. To make use of this table, standard deviations of 0.01 ml in the volume of titrant and of 0.001 in pH are assumed. These values are about the lowest expected for the techniques most commonly used in the determination of stability constants. They illustrate the gradual differences, not only in precision but also in accuracy, found for the different methods of linear fitting discussed here.

For the diprotic acids the values $V = 40$ ml, $C_A = 5 \times 10^{-3}M$ and $C_v = 4 \times 10^{-2}M$ are assumed, except for the titration of the very weak acid with $\log K_1 = 12$ and $\log K_2 = 11$, for which C_v was taken as $0.1M$.

For the triprotic acids the values $V = 30$ ml, $C_A = 5 \times 10^{-3}M$ and $C_v = 5 \times 10^{-2}M$ were used, except for the titration of the acid with $\log K_1 = 12.5$, $\log K_2 = 11.5$ and $\log K_3 = 10.5$ for which C_v was $0.15M$.

TREATMENT OF THE DATA

(a) Diprotic acids

The expression

$$\bar{n} = \frac{\beta_1 h + 2\beta_2 h^2}{1 + \beta_1 h + \beta_2 h^2} \tag{2}$$

may be written in the form:

$$Y = a_0 + a_1 X \tag{3}$$

where X and Y are functions of $\bar{n}$ and h, and the parameters a_0 and a_1 are directly related to β_1 and β_2. In this way, equation (3) may lead to the three forms shown in Table 1.[9]

For each of the 40 experimental points (v, pH) a pair of values ($\bar{n}$, h) is obtained. Only points with $\bar{n}$ values given by $0.1 \leqslant \bar{n} \leqslant 0.9$ and $1.1 \leqslant \bar{n} \leqslant 1.9$ are used, because of the sensitivity of the values of X and Y to slight experimental errors in $\bar{n}$ in the other ranges. For this reason, the number of useful points, N, is reduced to about 36 in each titration.

Each of the three equations in Table 1 is fitted by the least-squares method, by using the usual equations:

$$\sum Y = a_0 N + a_1 \sum X$$
$$\sum XY = a_0 \sum X + a_1 \sum X^2 \tag{4}$$

leading to three regression lines of Y on X, from which the constants K_1 and K_2 are estimated. In the same way, three regression lines of X on Y are fitted, leading to other values for the constants.

This procedure is followed in the 15 titrations for each acid. The mean values and standard deviations of $\log K_1$ and $\log K_2$ are given in Table 2. Standard deviations (s) are given with two significant figures.

(b) Triprotic acids

In the same way the expression

$$\bar{n} = \frac{\beta_1 h + 2\beta_2 h^2 + 3\beta_3 h^3}{1 + \beta_1 h + \beta_2 h^2 + \beta_3 h^3} \tag{5}$$

may be written as the equation of a plane

$$Z = a_0 + a_1 X + a_2 Y \tag{6}$$

where X, Y and Z are functions of $\bar{n}$ and h, and the parameters a_0, a_1 and a_2 are functions of the constants β_1, β_2 and β_3 as shown in Table 3.

For the same reason as above, only the values $0.1 \leqslant \bar{n} \leqslant 0.9$, $1.1 \leqslant \bar{n} \leqslant 1.9$ and $2.1 \leqslant \bar{n} \leqslant 2.9$ are used, reducing the number of useful data, N, to about 33 in each titration.

Each of the four planes in Table 3 is fitted by the least-squares treatment by solving the system of equations

$$\sum Z = a_0 N + a_1 \sum X + a_2 \sum Y$$
$$\sum XZ = a_0 \sum X + a_1 \sum X^2 + a_2 \sum XY$$
$$\sum YZ = a_0 \sum Y + a_1 \sum XY + a_2 \sum Y^2 \tag{7}$$

to obtain four regression planes of Z on X and Y. From each plane the values of the constants K_1, K_2 and K_3 are calculated. Similarly the regression planes of Y on X and Z, and of X on Y and Z are obtained.

The mean values and standard deviations obtained from the 15 simulated titrations are shown in Table 4.

Table 1.

Straight line	Equation	Y	X
I	$\beta_1 X + \beta_2 Y = 1$	$h^2 \dfrac{(2 - \bar{n})}{\bar{n}}$	$h \dfrac{(1 - \bar{n})}{\bar{n}}$
II	$\dfrac{1}{\beta_1} X + \dfrac{\beta_2}{\beta_1} Y = 1$	$h \dfrac{(\bar{n} - 2)}{(1 - \bar{n})}$	$\dfrac{\bar{n}}{(1 - \bar{n})h}$
III	$\dfrac{1}{\beta_2} X + \dfrac{\beta_1}{\beta_2} Y = 1$	$\dfrac{\bar{n} - 1}{(2 - \bar{n})h}$	$\dfrac{\bar{n}}{(2 - \bar{n})h^2}$

DISCUSSION

As stated previously, the values for the standard deviations of the pH and volume are at the lower limit of those normally observed. This allows better observation of the gradual differences in precision and accuracy. In practice, the deviations are often a little bigger, leading to more dispersed values than the ones shown in Tables 2 and 4.

In these tables, it can be seen that for equal deviations of pH and volume, slightly better precision is

Table 2.

Theoretical value	Fitting	Straight line I log K	Straight line I s	Straight line II log K	Straight line II s	Straight line III log K	Straight line III s
log K_1 = 2.000	Y on X	1.9950	0.0050	1.9956	0.0047	2.0023	0.0072
		1.055	0.046	1.048	0.044	0.94	0.14
log K_2 = 1.000	X on Y	2.0003	0.0046	2.0002	0.0046	2.0016	0.0070
		0.988	0.049	0.992	0.050	0.96	0.12
log K_1 = 4.500	Y on X	4.4985	0.0038	4.4999	0.0015	4.5004	0.0052
		3.5011	0.0030	3.4997	0.0022	3.497	0.019
log K_2 = 3.500	X on Y	4.4987	0.0037	4.4998	0.0015	4.5003	0.0053
		3.5010	0.0030	3.4996	0.0022	3.498	0.019
log K_1 = 7.000	Y on X	7.000	0.015	7.001	0.0016	7.0011	0.0045
		6.0006	0.0054	6.0003	0.0018	5.996	0.011
log K_2 = 6.000	X on Y	7.001	0.015	7.0001	0.0016	7.0010	0.0045
		6.0005	0.0054	6.0003	0.0018	5.997	0.011
log K_1 = 10.000	Y on X	9.998	0.012	9.9998	0.0015	10.0000	0.0027
		9.0013	0.0040	9.0002	0.0019	8.9997	0.0063
log K_2 = 9.000	X on Y	9.996	0.019	9.9998	0.0015	9.9999	0.0027
		9.0013	0.0040	9.0002	0.0019	8.9999	0.0063
log K_1 = 12.000	Y on X	11.991	0.052	12.0005	0.0048	12.0015	0.0061
		11.004	0.014	11.0004	0.0032	10.9998	0.0042
log K_2 = 11.000	X on Y	11.998	0.054	12.0002	0.0048	12.0008	0.0062
		11.003	0.014	11.0003	0.0032	11.0008	0.0041

obtained in the determination of the constants of diprotic acids than for triprotic acids. It is also clear that the four methods of fitting for triprotic acids lead to much larger differences in precision and accuracy than do the three methods for diprotic acids. The different methods of fitting would lead to the same set of constants if the experimental data were not subject to errors.

From Table 2 it can be seen that, for diprotic acids with pK-values of about 3.5–11.5, the values of X and Y are interchangeable, leading to negligible differences in the values of the constants and standard deviations.

For strong acids, the regression of Y on X appears to lead to results with similar standard deviations but less accurate mean values. This is also true for triprotic acids, where the greater differences show that the regression of Z on X and Y is clearly the least accurate.

Generally, straight line II was found to give better precision and accuracy than lines I and III. Some authors have distrusted this equation,[12] but others have accepted it.[13]

For strong acids, the results from straight line I are nearer to those from straight line II than those of straight line III are. For these acids, evaluation of K_1 is more accurate and precise than that of K_2 with both lines I and II. For strong bases, the results from straight line III are close to those of the straight line II, but straight line I gives poor agreement.

For the triprotic acids (Table 4) results from plane I are not very useful, and they become worse for weaker acids, eventually resulting in interchanged

Table 3.

Plane	Equation	Z	Y	X
I	$\beta_1 X + \beta_2 Y + \beta_3 Z = 1$	$h^3 \frac{(3-\bar{n})}{\bar{n}}$	$h^2 \frac{(2-\bar{n})}{\bar{n}}$	$h \frac{(1-\bar{n})}{\bar{n}}$
II	$\frac{1}{\beta_1} X + \frac{\beta_2}{\beta_1} Y + \frac{\beta_3}{\beta_1} Z = 1$	$h^2 \frac{(\bar{n}-3)}{(1-\bar{n})}$	$h \frac{(\bar{n}-2)}{(1-\bar{n})}$	$\frac{\bar{n}}{(1-\bar{n})h}$
III	$\frac{1}{\beta_2} X + \frac{\beta_1}{\beta_2} Y + \frac{\beta_3}{\beta_2} Z = 1$	$h \frac{(\bar{n}-3)}{(2-\bar{n})}$	$\frac{(\bar{n}-3)}{(2-\bar{n})h}$	$\frac{\bar{n}}{(2-\bar{n})h^2}$
IV	$\frac{1}{\beta_3} X + \frac{\beta_1}{\beta_3} Y + \frac{\beta_2}{\beta_3} Z = 1$	$\frac{(\bar{n}-2)}{(3-\bar{n})h}$	$\frac{(\bar{n}-1)}{(3-\bar{n})h^2}$	$\frac{\bar{n}}{(3-\bar{n})h^3}$

Table 4.

Theoretical values	Fitting	Plane I log K	Plane I s	Plane II log K	Plane II s	Plane III log K	Plane III s	Plane IV log K	Plane IV s
		3.0058	0.0041	3.0079	0.0037	3.01			
	Z on X and Y	1.966	0.018	1.969	0.015	1.37	(2*)		†
$\log K_1 = 3.000$		1.190	0.086	1.208	0.080	1.69			
		3.0006	0.0034	3.0009	0.0031	3.0031	0.0089	3.01	
$\log K_2 = 2.000$	Y on X and Z	1.996	0.013	1.998	0.012	1.97	0.11	1.65	(4*)
		1.030	0.080	0.996	0.087	1.14	0.48	2.48	
$\log K_3 = 1.000$		3.0017	0.0036	3.0015	0.0031	3.0040	0.0081	3.01	
	X on Y and Z	1.991	0.014	1.995	0.012	1.968	0.098	1.20	(5*)
		1.059	0.083	1.019	0.086	1.16	0.43	2.70	
		5.01	0.10	4.9995	0.0028	4.9995	0.0062		
	Z on X and Y	4.001	0.036	4.0001	0.0031	4.006	0.013		†
$\log K_1 = 5.000$		3.0009	0.0083	3.0010	0.0037	3.022	0.022		
		4.997	0.098	4.9994	0.0028	4.9999	0.0062	5.00	
$\log K_2 = 4.000$	Y on X and Z	4.004	0.036	4.0003	0.0030	3.999	0.013	3.89	(7*)
		3.0005	0.0083	3.0009	0.0037	3.003	0.010	3.76	
$\log K_3 = 3.000$		5.10	0.15	4.9992	0.0029	4.9996	0.0062	5.00	
	X on Y and Z	3.987	0.038	3.9999	0.0031	4.000	0.013	3.91	(6*)
		3.0029	0.0087	3.0010	0.0037	3.0013	0.0098	3.63	
		7.09	0.70	7.9917	0.0082	7.9963	0.0081		
	Z on X and Y	7.35	0.33	7.016	0.015	7.018	0.023		†
$\log K_1 = 8.000$		5.9872	0.0065	5.9939	0.0079	6.011	0.015		
		7.24		7.9909	0.0082	7.9969	0.0080	8.00	
$\log K_2 = 7.000$	Y on X and Z	7.32	(6*)	7.017	0.015	7.007	0.019	6.85	(3*)
		5.99		5.9939	0.0071	5.995	0.012	6.73	
$\log K_3 = 6.000$				7.978	0.012	7.9966	0.0081	8.00	
	X on Y and Z		†	7.010	0.023	7.008	0.020	6.87	(4*)
				5.9976	0.0081	5.994	0.013	6.74	
		9.36		10.504	0.011	10.4997	0.0041	10.50	
	Z on X and Y	9.97	(5*)	9.493	0.017	9.5008	0.0051	9.53	(8*)
$\log K_1 = 10.500$		8.49		8.5032	0.0090	8.5013	0.0049	7.86	
		9.28		10.504	0.011	10.4997	0.0041	10.496	0.021
$\log K_2 = 9.500$	Y on X and Z	10.01	(5*)	9.494	0.017	9.5004	0.0052	9.497	0.083
		8.49		8.5030	0.0089	8.5007	0.0048	8.35	0.55
$\log K_3 = 8.500$				10.4954	0.0089	10.4996	0.0041	10.5004	0.0094
	X on Y and Z		†	9.487	0.021	9.5006	0.0051	9.494	0.076 (14*)
				8.5033	0.0091	8.5005	0.0048	8.39	0.45
				12.464	0.036	12.497	0.020	12.477	0.049
	Z on X and Y		†	11.541	0.045	11.5006	0.0055	11.58	0.13 (12*)
$\log K_1 = 12.500$				10.481	0.020	10.5011	0.0055	10.11	0.38
				12.457	0.042	12.498	0.020	12.498	0.053
$\log K_2 = 11.500$	Y on X and Z		†	11.549	0.052	11.4997	0.0055	11.36	0.50
				10.480	0.021	10.4990	0.0056	10.51	0.23
$\log K_3 = 10.500$				12.383	0.096	12.495	0.019	12.488	0.052
	X on Y and Z		†	11.521	0.026	11.5019	0.0053	11.520	0.086
				10.481	0.020	10.4974	0.0057	10.39	0.27

* Among the 15 titrations, only the number indicated in parenthesis lead to positive values of the constants.
† This fitting gives rise to negative values of the constants in all the 15 titrations.

Table 5.

Temp., °C	Ionic strength	log K_1	log K_2	Reference
25	0.15 ($NaClO_4$)	4.88	4.05	14
20	0.10 ($NaClO_4$)	5.28	4.00	15
30	0.1 (KCl)	5.116	3.952	16
20	0.10 ($NaClO_4$)	5.205 (s = 0.015	3.995 (s = 0.015)	This work

Table 6.

Temp., °C	Ionic strength	log K_1	log K_2	log K_3	Reference
25	0.15	5.62	4.34	2.94	17
20	0.1 ($NaClO_4$)	5.68	4.35	2.87	18
20	0.1 ($NaClO_4$)	5.68 (s = 0.02)	4.38 (s = 0.02)	2.96 (s = 0.03)	19
25	0.1 (KNO_3)	5.65	4.30	2.79	20
30	0.1 (KCl)	5.750	4.395	3.023	16
25	0.1 (KNO_3)	5.72	4.39	2.92	8
20	0.10 ($NaClO_4$)	5.660 (s = 0.015)	4.335 (s = 0.015)	2.97 (s = 0.03)	This work

values of the constants (log K_1 log K_2 *etc.*). Plane IV also does not give valid results for all acids. K_1 is always obtained with a slightly better precision than K_2 and K_3, which may become interchanged (log K_2 < log K_3).

Plane II leads to good results for all acids, but for the strongest acids, K_3 is less precise, and K_1 is the best constant. For medium-strength acids, this plane leads to acceptable values of all three constants. As the acid strength decreases, K_3 is determined with better precision (but plane III gives better precision for weak acids).

Plane III gives good results for weak and medium-strength acids (comparable to those of plane II for the latter). For strong acids, the results are not very good.

Results were also obtained for acids with greater and smaller differences between their protonation constants, and similar conclusions were reached.

CONCLUSIONS

For diprotic acids, least-squares fitting by means of straight line II is to be preferred. For strong acids, it is better to use the regression of X on Y. For other acids, both regressions should be performed, and the results with smaller dispersion, or the mean value, if both regressions have a similar precision, accepted.

For triprotic acids, the data should be fitted by means of plane II or III for strong and weak acids, respectively. For strong acids, the regression of Z on X and Y should not be used. For other acids, the three possible regressions should be calculated, and the one with the smallest dispersion, or the mean value of those with the smallest dispersion, accepted.

EXPERIMENTAL

Titrations were done at 20° with the aid of a Radiometer PHM 84, (resolution 0.001 pH unit) and a combination glass electrode, Radiometer GK2401 C, standardized against 0.05*M* potassium hydrogen phthalate (pH 4.001) and 0.025*M* potassium dihydrogen phosphate/0.025*M* disodium hydrogen phosphate (pH 6.874). The ionic strength was adjusted to 0.10*M* by addition of an appropriate amount of sodium perchlorate. All the reagents were of analytical grade.

(*a*) *Succinic acid.* Five titrations were done of 50 ml of $1.656 \times 10^{-3}M$ disodium succinate with 0.0212*M* hydrochloric acid. The mean values of the constants and standard deviations obtained by means of straight line II are shown in Table 5, where they are also compared with some literature constants obtained in similar conditions. Both regressions (X on Y and Y on X) were used because their standard deviations were of the same order of magnitude.

(*b*) *Citric acid.* Five titrations of 50 ml of $1.025 \times 10^{-3}M$ trisodium citrate were done with 0.0190*M* hydrochloric acid. The mean values of the constants and standard deviations calculated by means of plane II are shown in Table 6, where some values from the literature are also given. The three fitting possibilities were used.

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LETTER TO THE EDITOR

THE ANALYTICAL METHOD OF CALCULATING THE DERIVATIVES OF EQUILIBRIUM CONCENTRATIONS

Sir,

In connection with the letter from Bugaevsky and Nikishina[1] on the analytical method of calculating the derivatives of equilibrium concentrations we would like to state that we were not aware of the publications of Bugaevsky and co-workers when we wrote our paper. That was our fault. However, we deliberately avoided the term "new" in our paper, because the rules for the derivation of implicit function systems have been known for more than a hundred years and can be found in any textbook on mathematical analysis. The question of priority implied by Bugaevsky and Nikishina therefore seems rather academic. We think it unfortunate that the technique seems to be unknown by most of the people working on solution equilibria, although the scientists working on the fundamental aspects of equilibrium systems have been using this method for decades. The most recent advances can be found in a review by Smith.[2]

We hope that these letters - in accordance with our original intention - will draw the attention of people working in the field of solution equilibria to the possibility of calculating the derivatives analytically.

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20 January 1982

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USE OF ELECTROSTATIC PRECONCENTRATION AT HIGH SALT CONCENTRATIONS

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Summary—Apparatus is described for the electrostatic preconcentration of aerosol particles with a collector trough electrode from samples containing large amounts of matrix salts. A cooling and filtration cycle is used to precipitate and separate the matrix salts, and prevent saturation of the collector solution. The system has been applied to the preconcentration of sea-water species before analysis by flame atomic-absorption spectroscopy.

Electrostatic collection has been used successfully for the concentration of species in air[1,2] and water[3,4] samples. In the latter case the procedure requires initial conversion of the solution into an aerosol, which is then desolvated and collected either on a wire[3] or in a trough[4] electrode. In practice the trough electrode is the more convenient, as the concentrated sample is obtained as a solution and is therefore immediately available for analysis, but the collector solution rapidly becomes saturated if the original sample solution contains high concentrations of matrix salts. After this point is reached aerosol particles are still collected, but as a solid layer on the bottom of the trough rather than in solution. It is found that any analyte contained in such undissolved particles remains *in situ*, rather than being released to the solution. Hence the preconcentration process ceases to be useful at this point.

This limitation restricts the applicability of electrostatic preconcentration, at least when a liquid collector is used. Thus, for example, the preconcentration of sea-water trace species is rendered impracticable, which is a pity because the low trace element concentrations and availability of large samples make this an obvious application. The present work was carried out to determine whether the trough collector could be modified to overcome this problem and used for preconcentration of sea-water.

EXPERIMENTAL

Apparatus

The equipment has already been described.[4] The trap was modified as below.

For preconcentration of trace species in samples which contain large amounts of matrix salts, the latter must be precipitated at some point in the apparatus other than the electrostatic collector. The system used for this is shown in Fig. 1. The collection medium (water) is lifted above the collector by a peristaltic pump, then passed through a 1-ml reservoir into a filter funnel (7 cm diameter; Whatman No. 1 paper), and allowed to enter the collector trough through a drip feed. A second drip feed carries water from the other end of the collector trough back to the pump inlet. The drip feed arrangement was adopted to provide electrical isolation between the high voltage on the collec-

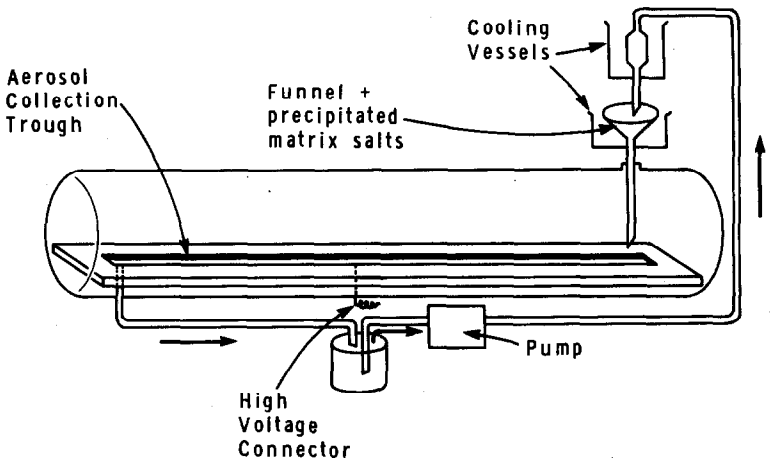

Fig. 1. The apparatus.

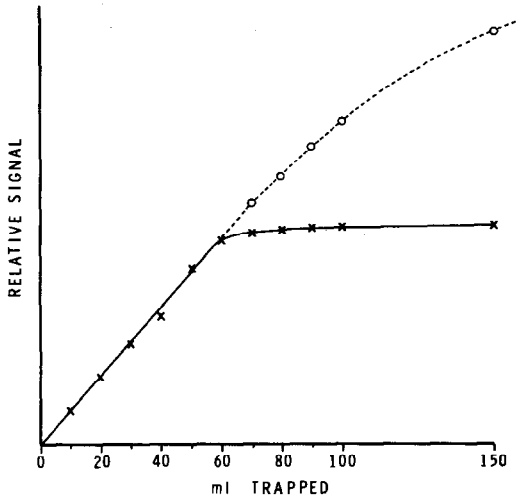

Fig. 2. Response from the collector solution *vs.* amount of sample solution which was concentrated. Open circles and crosses represent points obtained with and without use of the filtration system.

tor and the rest of the apparatus, which was grounded. The volume of solution required to fill this modified collector was 8 ml, eight times the volume used in the original trap. To minimize the amount of sample solution required to achieve a given concentration factor with this increased collector volume, it was decided to increase the efficiency of aerosol generation. This was done by preheating the air supply to the pneumatic nebulizer to 90°, which gave about 25% conversion of solution into aerosol at an uptake rate of 4.5 ml/min.

During operation, the filter funnel and reservoir are surrounded by a plastic jacket containing an ice–salt freezing mixture. During trapping, sample aerosol dissolves in the collector solution as usual, increasing its salt content. However, the cooled solution in the reservoir and funnel reaches saturation before the solution in the electrode trough. Thus the matrix salts crystallize and are retained in the funnel. After leaving the funnel the filtrate warms up sufficiently to dissolve more trapped aerosol particles. This cycle can be repeated almost indefinitely, eventually providing a solution of the concentrated trace components (together with a high loading of dissolved matrix material) for analysis, and a filter funnel in which the bulk of the matrix salts is retained as solid crystals. Any water held in these crystals is drawn from the reservoir above the funnel (Fig. 1).

This process relies on the matrix material crystallizing as the pure salt, leaving trace constituents in solution. If equilibrium is established between the salt and the solution with which it is in contact then the extent to which preconcentration is realized depends on the distribution coefficient of the material of interest between the solid and liquid phases, *i.e.*, upon its chemical potential in the two media. In practice the usual processes of occlusion, adsorption *etc.* will tend to increase the analyte concentration in the solid phase to above the equilibrium level. Therefore all these processes are expected to combine to cause reduction in the efficiency of preconcentration once collection of the matrix salts begins.

The correct rates of heating and cooling required for crystallization to proceed in the manner described were established by varying the flow-rate of the pump. A flow-rate of 2 ml/min was found to be satisfactory. The exact value does not appear to be critical.

Procedure

The procedure adopted with the present system was similar to that described previously.[4] That is, the trapping system was set up and the nebulizer allowed to aspirate a given volume of sample solution. As before, the latter process could run automatically and unattended.

To carry out an analysis the trap was initially calibrated with a known concentration of analyte in a salt solution of appropriate composition. This gave the trapping efficiency of the device (*i.e.*, the ratio of amount of analyte retained in the trap solution to the total amount entering the trap). The collector solution was analysed by the standard-addition method. Background correction was applied to all atomic-absorption readings.

It was found necessary to reduce trapping voltages to about 10–12 kV. At higher voltages the liquid in the collector tended to creep over the edges of the trough: in addition, significant liquid loss occurred in the trap because of aerosol generation. This phenomenon has been described by Mavrodineanu and Boiteux.[5] However, it was not observed in the previous work, even at trapping voltages up to 20 kV, possibly because a slightly wider collector trough was used.

RESULTS AND DISCUSSION

The effectiveness of the cooling system in removing unwanted matrix salts was tested by trapping solutions containing 1 μg of lead and 100 mg of sodium chloride per ml, with and without the cooling system in operation. The concentration of lead in the collector solution was measured as a function of the volume of sample solution aspirated. Results are given in Fig. 2, and show that the cooling system gives greater analyte preconcentration than the previous arrangement does. Salt crystals were seen to collect in the filter funnel as expected.

Measurement of trapping efficiency

Trapping efficiencies were generally determined from atomic-absorption measurements and suitably prepared calibration graphs rather than by the procedure described originally.[3,4] Figure 3(*a*) shows the calibration graph for cadmium obtained by plotting response *vs.* concentration, no trapping being used. Figure 3(*b*) shows the corresponding graph obtained

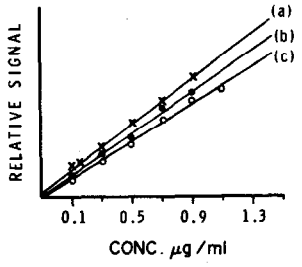

Fig. 3. (*a*) Calibration curve for Cd^{2+}, obtained by aspirating increasing concentrations into the flame without use of the trap. (*b*) Calibration curve for Cd^{2+}, obtained by preconcentrating volumes of 0–80 ml into 8 ml of collector solution. Sample solution was 0.1-μg/ml Cd^{2+} in distilled water. (*c*) Results of (*b*) repeated for 0.1-μg/ml Cd^{2+} in 3% NaCl solution.

Table 1. Trapping efficiencies for various elements in distilled water and sodium chloride media

	Trapping efficiency from calibration, %		Trapping efficiency by signal reduction, %	
Element	Distilled water	30 g/l. NaCl	Distilled water	30 g/l. NaCl
Cd	81	76	79	77
Cr	78	73	75	73
Cu	93	93	75	77
Pb	79	74	80	78
Zn	78	75	82	77

for volumes from 0 to 80 ml of 0.1 μg/ml cadmium solution with trapping into the 8 ml collector volume without the cooling system. Figure 3(*c*) is the graph for 0–80 ml of 30 g/l. sodium chloride solution containing 0.1 μg of cadmium per ml, with the cooling system in operation. It is assumed that if the device were 100% efficient all three graphs in Fig. 3 would coincide. Thus the trapping efficiency is given by the ratio of the signal on graph (*b*) or (*c*) to that from curve (*a*).

This procedure is more convenient than the original version because it simultaneously takes into account the inability of the electrostatic field to trap all the available aerosol particles, analyte loss due to retention in the filter system, and any dependence these effects may have on analyte concentration.

Table 1 gives the trapping efficiencies for various elements, measured by this procedure. They are essentially the same except for copper. They also appear to be only slightly reduced by the sodium chloride matrix. Table 1 also shows the trapping efficiencies determined separately by the original procedure, *i.e.*, by aspirating a standard solution of known, high concentration and measuring the signal reduction when the trapping voltage was applied. The difference between the two sets of values (*i.e.*, between the amount of material which is trapped and that which reappears in solution) provides a straightforward way of measuring the total amount of analyte irreversibly trapped and held by the system. It is assumed that the bulk of this unrecovered material is retained by the crystallized salts in the filter. Table 1 shows this amount was small in these experiments.

The trapping efficiencies given for copper in columns 1 and 2 of Table 1 are obviously high and inconsistent with the values in columns 3 and 4. The most likely explanation for these results is contamination of the collector solution. In fact contamination by copper is difficult to avoid with the present apparatus because of the various electrical components located around the trap.

Analysis of sea-water

After the apparatus had been tested with sodium chloride, the experiments were repeated with magnesium chloride as the matrix salt, but the results were not satisfactory. The magnesium chloride, solution in the cooling system and collector trough merely became supersaturated, with no precipitation. This caused the trap to cease functioning, because trapped aerosol particles failed to dissolve in the collector (the effect observed in the original liquid collector in the presence of high salt concentrations[4]). Therefore it was decided to proceed directly to examination of preconcentration of sea-water, to find out whether matrix salt(s) would be precipitated or whether supersaturation of the collector solution would render the process inoperative.

Sea-water was obtained from a supply pumped into the building from an adjacent inlet. Samples were acidified by addition of 1 ml of concentrated hydrochloric acid per litre immediately on collection, to give a final solution pH of about 2.0. The acid was prepared immediately before use by dissolving hydrogen chloride in demineralized distilled water.

It was found that the trap behaved towards these samples in the same way as it did to solutions of sodium chloride. The precipitated salts collected in the filter, trapped material dissolved continuously in the collector, and the concentration process could be continued almost indefinitely. Analysis of the precipitated material collected after some minutes of operation showed it to consist of a mixture of sodium chloride and magnesium chloride. It was concluded that sodium chloride probably precipitated first, and that the crystals thus formed acted as nuclei for the crystallization of other salts and thereby prevented supersaturation from occurring.

Standard-addition measurements were then made on the sea-water to test the reproducibility and trapping efficiency of the system. The behaviour of the trap was found to be indistinguishable from that already described for sodium chloride. These results gave some confidence in the ability of the system to effect a reliable concentration of the trace elements in sea-water. Accordingly, analyses were done for Cd, Cu, Cr, Pb and Zn to test the system. Each sample was divided into three immediately before analysis. The first part was preconcentrated and analysed by using the trap, followed by flame AAS, as described above for sodium chloride solutions. The other two parts were analysed directly by graphite-furnace AAS

Table 2. Comparison of concentrations found in sea-water ($\mu g/l.$)

Element	Trap/flame AAS	Anodic stripping	Graphite furnace
Cd	3.2	2.1	2.5
Cr	3.2	—	2.6
Cu	11	2.6	2.3
Pb	2.4	1.7	8.0
Zn	8.7	—	11

and by anodic-stripping voltametry for comparison. Results are given in Table 2.

Overall, the concentrations in Table 2 appear rather high, but are felt to be correct, the reason being that the source of the sea-water lay in the city and the water was piped and stored before use. The copper value obtained by use of the trap is particularly high, but as already mentioned, this is probably due to contamination from the apparatus. The other results are felt to be reasonably consistent, given the complex nature of the sample matrix,[6–8] though the high value obtained for lead by the graphite-furnace method may be noted. The reason for this apparent anomaly is under investigation.

CONCLUSIONS

The results show that it is feasible to use electrostatic preconcentration with a liquid collector for solutions which contain macroscopic quantities of matrix salts. The modified apparatus used here retains the advantages of automatic operation and an inherently low risk of contamination which characterized the original system. The cooling process used to force crystallization and separation of the salts probably occurs fast enough to cause retention of analyte by the filter system in amounts greater than those corresponding to equilibrium. Despite this, use of the cooling system reduced the trapping efficiency by only $\sim 5\%$, an encouraging sign of the potential tolerance of the system for variations in matrix composition.

Despite the success of the device with sea-water the use of a saturation and precipitation cycle to remove matrix salt(s) probably has less ability to remove matrix interferences than was apparent here. In particular, the results with magnesium chloride show that supersaturation can occur in practice. This effect is easy to recognize, however, since the trap completely ceases to operate. It also appears to be simple to cure, by addition of some nucleating species to the system. The results suggest that sodium chloride is effective for this purpose. However, it seems likely that, in addition to such obvious effects, some less apparent interferences can be expected, for instance through co-precipition, which might simply alter trapping efficiency to a greater or lesser extent. The occurrence, severity and possible remedies for such effects have not yet been investigated.

The consistency of the results found for sea-water shows that the trap is capable of functioning satisfactorily in the presence of a complicated matrix, and also demonstrates a useful and practical application of the device. It is thought likely that other types of sample containing high salt loadings could be treated with similar success, provided that the behaviour of the sample matrix in the collector system were investigated first.

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ANALYTICAL INVESTIGATION OF THE PROPERTIES AND USES OF A NEW HYDROPHOBIC MOLECULAR SIEVE*

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Summary—Distribution coefficients and capacities have been determined for many different organic compounds on a new molecular sieve called silicalite, which does not adsorb water but does adsorb small organic molecules with diameters up to 6 Å, from both liquid and gaseous streams. The characteristics of silicalite have been examined closely and new applications for it are briefly described.

Four years ago the Linde Division of Union Carbide developed a new molecular sieve[1] called silicalite, possessing a feature unique amongst all other natural and synthetic molecular sieves. Generally, molecular sieves are strongly hydrophilic and can be used to sorb water from organic solvents and from gas streams. However, silicalite is hydrophobic and can instead sorb different organic molecules from aqueous solutions and from gas streams.

Silicalite is a polymorph of silica, with a rather unusual crystal structure. It has a tetrahedral framework with a large fraction of 5-membered rings composed of silicon–oxygen tetrahedra. The material has intersecting channels defined by rings of 10 oxygen ions (Fig. 1). A mixture containing silica, hydroxide ions and alkylammonium cations, is crystallized hydrothermally in a closed system, resulting in a silica clathrate around each alkylammonium ion. This material is then calcined in air to produce the open-pore structure called silicalite. The channels are 6 Å in diameter and the total pore volume is about 33%.[2] The alkylammonium cations, which have hydroxide counterions to maintain charge balance, are driven off during the heating process. Crystalline silicalite produced in this way is in the form of particles of approximately 5–20 μm breadth. This material is referred to in this paper as unbonded silicalite. Aluminosilicate clays are used as binders to form larger particles.

Silicalite is very stable to heat: it will degrade only at temperatures higher than 1100°. It is stable in most solvents, but slowly decomposes in basic solutions. Silicalite is fairly brittle: breakage of the silicalite particles can sometimes occur during excessive shaking.

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The most important property of silicalite is that it will sorb non-polar and polar organic compounds, even some which are completely miscible with water, such as ethanol. For sorption, the diameter of the molecules must not exceed 6 Å. However, water itself is not adsorbed, because water molecules associate into clusters of 10–12 molecules with a total diameter larger than 6 Å.[2] Silicalite will sorb small molecules from liquid, vapour and gas streams.

The use of silicalite for removal of ethanol from fermented beer has been studied.[3,4] A sorption process for the production of ethanol is potentially more attractive than a distillation process because of the possible lower capital investment and energy cost. The sorbed ethanol can be removed from the silicalite column by increasing the temperature or the pressure. Another application of silicalite is the removal of chloroform from drinking water.[5]

In this work, an extensive study of the characteristics of silicalite and its applications was undertaken. Distribution coefficients and capacities for a wide variety of compounds were determined. The effects of the binder and temperature on the performance of silica-

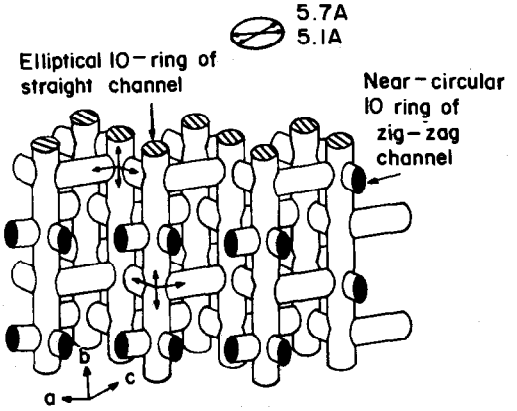

Fig. 1. Pore structure of silicalite (by courtesy of *Nature*).

lite have been investigated. The results suggest many new uses for the material.

EXPERIMENTAL

Apparatus

A Gow Mac 550 gas chromatograph with thermal conductivity detector was used for the gas-phase distribution studies. Short copper columns were used (4 mm bore, 19–20 cm length), filled with different types of silicalite. High-purity helium was used as carrier-gas. For the liquid-phase distribution studies, a Tracor 550 gas chromatograph with flame-ionization detector was used. A Tenax column (80–100 mesh) was used as the stationary phase with nitrogen as the carrier-gas.

Solutions and sorbents

All solutions for the liquid-phase distribution studies were made up in water that had been distilled and demineralized. The standard solutions were 100 ppm v/v. The chemicals were used as received, and had purities of at least 97%, with the exception of phenol (89%), 1-chlorododecane, chloroacetaldehyde (45%) and dichloroacetonitrile (90%). The gases had a purity of at least 99%, with the exception of carbonyl sulphide (97.5%).

Two types of silicalite (Linde Division, Union Carbide) were used as the sorbent: pure non-bonded silicalite (S 115) with a particle size of 5–20 μm, and bonded silicalite (LZ 115) with a particle size of up to 1 mm. Before use the silicalite was cleaned by heating in a porcelain crucible for at least 10 hr at 750° in a muffle furnace. The bonded silicalite was crushed and sieved to obtain different mesh sizes.

Measurements and calculations

Gas-phase distributions were calculated by measuring the retention time of pure compounds under isothermal conditions. The distribution data are expressed as distribution coefficients:

$$D_g = \frac{\text{(retention time} \times \text{flow-rate)} - \text{dead volume}}{\text{g of silicalite}}$$

The dead volume was determined from the retention time of 2,2,4-trimethylpentane, which is not retained by silicalite.

Liquid-phase distribution data were obtained by shaking a known amount of silicalite with 10.00 ml of standard solution for 1 hr. After the silicalite had settled, 2 μl of solution were injected into the gas chromatograph. The peak heights obtained were compared to those for corresponding standard solutions. From this ratio the distribution coefficients were calculated and expressed as

$$D_g = \frac{\text{amount retained/g of silicalite}}{\text{amount in solution/ml water}}.$$

The distribution coefficients of acids were determined by titration of 5.00 ml of equilibrated solution with standard sodium hydroxide solution, with phenolphthalein as indicator.

Capacities of silicalite for gases were measured by passing the gases through a column filled with bonded silicalite. The columns were weighed periodically until no further weight increase was detected.

The capacity for liquids was determined from breakthrough curves. Solutions (0.1% v/v) were pumped through a column (25 cm × 4 mm bore) filled with bonded silicalite. The effluent was monitored continuously with a Tracor 970A variable-wavelength ultraviolet detector. The wavelength was selected as appropriate for the compound being studied. The capacities for ethanol and chloroform were measured by fraction collection and gas chromatographic analysis. The columns used here were 31 cm long and 6 mm in bore and were filled with 4.70 g of granular (1 mm) silicalite.

RESULTS AND DISCUSSION

Two series of measurements were made to characterize silicalite, involving the determination of distribution coefficients for many compounds in both gas and liquid phases and the recording of breakthrough curves from which capacities were calculated. The results are presented in Tables 1–5.

Distribution coefficients of compounds in the gas phase

Values of the gas–solid distribution coefficients are given in Tables 1 and 2. Both gases and liquids were studied. Some compounds reached equilibrium in the gas–solid distribution very slowly, resulting in very broad peaks. For these compounds the distribution coefficient is given to only one significant figure.

The pore size is the most characteristic property of silicalite. Molecules with a diameter larger than 6 Å are sorbed very slowly or not at all. Molecules smaller than this are very strongly sorbed and give high distribution coefficients. Compare, for example, the D_g values of trimethylpentane and 2-methylpentane on non-bonded silicalite (0 and 90 respectively). However, for very small molecules, such as formaldehyde or oxygen, the distribution coefficients are lower, because while these smaller molecules fit easily into the pores of silicalite they can also easily move out again.

Within a homologous series a maximum distribution coefficient seems to exist at a certain carbon number. This effect is to be expected. Molecules should be linear to fit into the silicalite pores, so for a given molecule to be sorbed the energy needed to straighten the chain should be less than the energy of sorption by silicalite. The longer the molecule, the more energy it will take to straighten it and the lower the distribution coefficient will be.

Branching in a molecule results in a larger effective diameter of the molecule: when this diameter exceeds 6 Å the molecule will not be sorbed by silicalite. Comparison of the distribution coefficients of pentane, 2-methylpentane and 2,2,4-trimethylpentane shows this clearly (50, 90, 0 respectively).

Temperature effect

The effect of a change in temperature on the distribution coefficients of liquids is very large (see Table 1). Methanol, for example, has a D_g of 27 at 250°, 61 at 200° and 110 at 150°, methyl formate has a D_g of 41 at 250° and 88 at 200° (on non-bonded silicalite). This can be used to advantage in desorbing compounds from silicalite to clean it for future use. In general, molecules are sorbed more strongly at lower temperatures and are more easily desorbed at higher temperatures.

Table 1. Distribution coefficients of organic compounds on unbonded silicalite from the gas phase at different temperatures

Compound	D_g 250°	200°	150°	100°	50°
Alkanes					
methane	1.5	2.1	3.9	7.3	24
ethane	4.2	7.7	18	46	300
ethylene	4.2	6.4	13	29	170
propane	9.4	22	62	220	
cyclopropane	8.2	23	56	160	
pentane	51	200			
hexane	120	>400			
cyclohexane	110	>400			
cyclohexene	79	310			
heptane	310	>400			
2-methylpentane	86	380			
2,2,4-trimethylpentane	0	1.2	1.9	6.0	
benzene	0.5	2.3	4.4	11	
bromobenzene	140	>400			
Alcohols					
methanol	12	60	100		
ethanol	26	100	>200		
propanol	40	100			
isobutyl alcohol	50	400			
1-undecyl alcohol	90	>400			
Aldehydes					
acetaldehyde	30	50	170		
propanal	20	40	130		
butanal	60	140			
Acids					
acetic	26	260			
lactic	13	62			
pyruvic	5	12	27	62	
Miscellaneous					
acetone	50	200			
isophorone	5	26			
methyl formate	40	88			
ethyl acetate	100	>400			
tetrachloromethane	55	280			
chloroform	40	90	400		
water	7	16	19	74	
Gases					
O_2	2.2	2.6	2.7	3.5	8.7
N_2	1.4	1.6	2.7	3.3	9.0
N_2O	3.6	2.1	3.1	20	
CO	2.1	1.9	2.9	3.5	11
CO_2	3.3	4.9	9.2	16	77
SO_2	12	23	74	130	
air	1.4	2.0	2.7	3.4	8.6

Small gas molecules interact very little with silicalite at any temperature, with the exception of sulphur dioxide, carbon dioxide, ethane, ethylene, acetylene and propane. The low sensitivity of the thermal conductivity detector at large retention times prevented the measurement of distribution coefficients for most liquids at temperatures of 150° and below.

Effects of the binder

Since pure silicalite has a very small particle size (5–20 μm), a binder is added so that particle sizes of up to 1 mm diameter can be obtained. The binder used contains alumina, making the product more hydrophilic than the pure silicalite. The effects of this binder can be seen from the data in Table 2. The more polar compounds have a higher affinity for the bonded silicalite, which is explained by the fact that the binder is polar. Conversely, bonded silicalite sorbs non-polar compounds less than non-bonded silicalite does, perhaps because of a repulsion between the hydrophilic binder and the hydrophobic compounds.

Distribution coefficients of compounds in the liquid phase

The liquid–solid phase distribution coefficients in Table 3 show that most of the compounds tested are taken up well by silicalite. Even compounds which are

Table 2. Comparison of distribution coefficients of organic compounds on bonded and non-bonded silicalite in the gas phase at 250°

Compound	D_g, bonded	D_g, non-bonded	Compound	D_g, bonded	D_g, non-bonded
Acids			*Chloro compounds*		
acetic	25	26	hydrogen chloride	30	20
pyruvic	1.6	5	tetrachloromethane	2	55
trichloroacetic	1.6	2.0	chloroform	23	40
Alcohols			1,2-dichloroethane	60	70
ethanol	70	26	4–11 halocarbon	2.2	1.5
propanol	100	40	tetrachloroethylene	4	30
phenol	24	28	dichloromethane	20	40
Aldehydes			1-chlorododecane	25	33
formaldehyde	4.7	4.5	*Esters*		
acetaldehyde	16	30	methyl formate	20	40
acrolein	60	46	ethyl acetate	69	100
crotonaldehyde	45	61	*Ethers*		
furfural	170	290	di(2-chloroethyl) ether	143	138
propanal	50	20	diethyl ether	90	80
butanal	70	60	*Ketones*		
2-chloroacetaldehyde	>780	>290	acetone	34	50
Hydrocarbons			isophorone	16	5
methane	0.5	1.5	4-methyl-2-pentanone	100	200
ethane	1.6	4.2	*Nitriles*		
ethylene	1.6	4.2	acetonitrile	48	64
propane	3.5	9.4	acrylonitrile	82	89
cyclopropane	4.2	8.2	dichloroacetonitrile	63	85
butane	5.7	16	*Gases*		
pentane	24	51	O_2	0.7	2.2
2,2,4-trimethylpentane*	0.0	0.0	N_2	0.4	1.4
acetylene	2.0	3.9	H_2S	6	20
benzene	0.3	0.5	N_2O	1.5	3.6
hexane	53	120	CO	0.6	2.1
neopentane	0.2	39	CO_2	1.4	3.3
Bromo compound			SO_2	7	12
bromobenzene	47	140	NH_3	30	40
			H_2O	7	7

* Of all the compounds tested at this temperature, 2,2,4-trimethylpentane had the shortest retention time.

Table 3. Distribution coefficients of organic compounds between bonded silicalite and water at 25°

Compound	D_g	Compound	D_g
Acids		nonanal	130
acetic	72	decanal	240
pyruvic	29	2-chloroacetaldehyde	23.0
trichloroacetic	47	*Chloroalkane*	
Alcohols		chloroform	1230
ethanol	65	*Esters*	
propanol	250	methyl formate	2090
phenol	170	ethyl acetate	4970
Aldehydes		*Ethers*	
acetaldehyde	100	di(2-chloroethyl) ether	270
acrolein	580	*Ketones*	
crotonaldehyde	1340	acetone	270
furfural	1110	isophorone	1
propanal	1350	4-methyl-2-pentanone	2770
butanal	88	*Nitriles*	
pentanal	2800	acetonitrile	150
hexanal	940	acrylonitrile	220
heptanal	440	dichloroacetonitrile	>600
octanal	570		

Table 4. Capacity of bonded silicalite for different gases, relative to helium at 25°

Compound	mg of gas / g of silicalite	ml of gas / g of silicalite	ml of liquid gas / g of silicalite
Methane	6	8.4	0.01–0.01[a]
Ethane	53	40	0.09–0.07[a]
Propane	77	39	0.13–1.10[a]
Butane	98	38	0.16–0.12[a]
Ethylene	40	32	0.07–0.05[a]
Propylene	59	32	0.10–0.07[a]
l-Butylene	110	44	0.18–0.14[a]
Cyclopropane	99	53	0.17–0.12[a]
Isobutane	102	39	0.17–0.13[a]
Neopentane	93	29	0.16–0.12[a]
Dimethyl oxide	107	52	0.18–0.13[a]
Dichlorodifluoromethane (Freon-12)	180	33	0.12[b]
Chlorotrifluoromethane (Freon-13)	152	33	0.10[c]
Methyl sulphide	145	68	0.17[b]
Nitrogen	8	6.4	0.01[b]
Oxygen	8	5.6	0.01[b]
Carbon monoxide	13	10	0.02[b]
Carbon dioxide	70	36	0.06[b]
Sulphur dioxide	190	67	0.13[b]
Carbon disulphide	120	45	0.10[b]
Ammonia	70	92	0.09[b]
Hydrogen sulphide	80	53	0.08[d]

[a]Density = 0.6–0.8 g/ml, liquid gas.
[b]Density from CRC Handbook.
[c]Density = 1.5 g/ml, liquid gas.
[d]Density = 1.0 g/ml, liquid gas.

completely miscible in water, such as ethanol and acetic acid, are still sorbed by silicalite. Equilibrium is reached rapidly in these sorption processes, as indicated by measurements of concentrations in the aqueous solutions as a function of time. No noticeable difference was found in the degree of sorption attained after equilibration times of 1 min and 1 hr.

The existence of a maximum D_g for a homologous series is seen in the aldehyde series: pentanal is most strongly sorbed, with the highest D_g value of 2800. Both shorter and longer-chain aldehydes have smaller distribution coefficients. The effect of molecular diameter (as opposed to length) can be seen in a comparison of the distribution coefficients of acetaldehyde and 2-chloroacetaldehyde, 575 and 23 respectively. Noteworthy is the high distribution coefficient of dichloroacetonitrile, a compound which is receiving considerable attention because of its potential car-

Table 5. Capacities of bonded silicalite for different organic compounds in the liquid phase at different points on the breakthrough curve at 25°: calculated as % w/w of compound on silicalite

Compound	1% Breakthrough capacity	10% Breakthrough capacity	50% Breakthrough capacity
Acetic acid	0.02	0.3	0.9
Ethanol	12	13	15.1
Phenol	1.8		
Acrolein	2.2	2.9	
Crotonaldehyde	0.7	1.2	2.7
Propanal	6.3	8.3	
Butanal	4.2	5.5	8.1
Pentanal	3.5		
Furfural	2.4	4.2	5.8
Ethyl acetate	9	10	11
Acetone	2.0	2.9	5.2
Acetonitrile	15		
Chloroform	0.1	0.3	1.0

cinogenic danger. Possibly, silicalite could be used to isolate and concentrate this compound from natural waters.

Capacity

Measurements of the uptake of compounds from the gas phase have shown that silicalite has a very large capacity for many gases (see Table 4). The amount of gas sorbed is so much larger than the pore volume of silicalite (0.19 ml/g) that the gas must be sorbed with its molecules in contact, *i.e.*, in virtually a condensed state.[2]

It is interesting to note that the capacity is higher for the larger gas molecules: sulphur dioxide 67 ml/g, propane 39 ml/g, butane 38 ml/g, in comparison with oxygen and nitrogen, both 8 ml/g.

From the liquid phase, breakthrough capacities of up to 15% have been found (see Table 5). For phenol, propanal, pentanal and acetonitrile, breakthrough was so slow that the 50% breakthrough point could not be determined. Brief experiments were done to determine the influence of sodium chloride, humic acid and the surfactant Zonyl FSP on the capacity for ethanol. Addition of sodium chloride slightly increases the capacity for ethanol, for which 50% breakthrough was reached at a capacity of 15.1% from water and 15.8% from 1% sodium chloride solution. Addition of 0.1% v/v Zonyl FSP surfactant increased the capacity to 16.7%, while addition of 8.0 mg of humic acid per 100 ml of aqueous ethanol increased the capacity to 15.7%. The molecules of both Zonyl FSP and humic acid are too large to be sorbed by the silicalite.

Possible applications

The unique characteristics of silicalite suggest several valuable uses in analytical chemistry. The ability of silicalite to retain small organic molecules should be useful in the concentration–extraction of C_1–C_{10} aldehydes, acrylonitrile and dichloroacetonitrile from dilute aqueous samples, especially since synthetic polymer resins used to sorb organic molecules from aqueous solutions are not effective in removing those compounds that are highly miscible with water.[6,7]

The compounds retained on silicalite can be recovered by thermal desorption.[8,9] The ability of silicalite to sorb small gas molecules preferentially should also be useful. Gases could be purified by removal of their low molecular-weight impurities. Sulphur dioxide, which is very strongly retained, could possibly be removed from stack gases: the excellent temperature stability and inertness of silicalite are attractive attributes for this application.[10]

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A POROUS-JET FLOW-THROUGH ELECTRODE

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Summary—An electrochemical flow detector, based on a jet of solution directed at a thin porous carbon electrode, is described, along with its electrochemical characteristics and analytical applications. The electrode has a volume of 19 μl and surface area of 1.26 cm². The detector exhibits better sensitivity and detectability than a wall-jet detector. Dopamine and ferrocyanide were used as test systems to give detection limits at nanomolar concentration levels. Applications indicated include continuous-flow analysis (utilizing stopped-flow voltammetry) and flow-injection analysis.

With the growing interest in hydrodynamic voltammetry as a sensitive analytical tool, efforts are being made toward developing working electrodes of better properties. Flow-through solid electrodes based on various configurations have been introduced in recent years.[1] The most popular cells (which are also commercially available) are those in which the solution flows through a thin-layer channel,[2] through an open tubular electrode,[3] or onto a wall-jet electrode.[4] The wall-jet detector in particular offers the advantages of very high sensitivity, extremely small dead volume and freedom from surface adsorption.[5,6] In addition to these cells, various flow-through porous electrodes are being employed, aimed mainly at improving detector response by increasing the electrode surface area. The porous electrode material (*e.g.*, carbon, platinum or silver particles) is usually packed in cylindrical columns, which have relatively large (0.1–5 ml) dead volumes. This presents a drawback for use as a detector in monitoring chromatographic column effluents, where a low dead-volume flow-cell is required in order to maintain the separation achieved by the column.

In this article we describe the behaviour and use of a very thin porous carbon disk electrode under a jet-flow of solution. Such an electrode is a hybrid between the wall-jet electrode and a porous carbon electrode. It combines the small volume and effective mass-transport that characterize the wall-jet electrode with the large surface area of a porous electrode. The sensitivity and detectability are thus made better than those of the wall-jet electrode. The porous disk is made of reticulated vitreous carbon (RVC), a material possessing many electrochemical and hydrodynamic advantages.[7] RVC has been exploited recently as an effective working electrode in a variety of flowing systems.[8–10] The electrodes employed in these studies had volumes of about 0.1–2 ml. Although the present electrode has a volume of only 19 μl it has a substantial surface area of 1.26 cm.[2] Cell design and operation are simple. Its characteristics and advantages are elucidated in this paper.

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EXPERIMENTAL

Apparatus

A schematic diagram of the cell is shown in Fig. 1. The body consists of two 1.25-in. diameter "Plexiglas" blocks, the upper block 0.75-in. thick and the lower one 0.69-in. thick. The two blocks are held together with three stainless-steel bolts (not shown). A solution inlet (A) (0.34-mm diameter) is drilled in the upper block. An RVC disk (C) (2 × 3-S type, 0.55-cm diameter, 0.083-cm thick, 100 pores per inch, Fluorocarbon Co., Anaheim, Cal.) is placed exactly opposite the solution inlet. Electrical contact to the RVC is made through the face of a glassy carbon rod (2.5-mm diameter) which is bonded with conducting epoxy cement to a copper wire (F) that leads to the outside of the lower block. Bolting the two blocks together exerts pressure on the RVC disk and five Teflon washers (E) (each

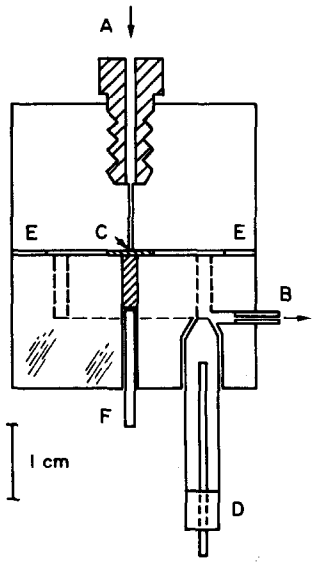

Fig. 1. Flow-through cell assembly. A, Sample solution inlet; B, Sample solution outlet; C, RVC disk; D, reference electrode; E, Teflon spacers; F, lead to working electrode.

0.005 in. thick, 1.0 cm i.d., 1.6 cm o.d.) separating the blocks. To maintain uniform flow in all directions an annular cavity (0.7 cm i.d., 0.9 cm o.d., 0.8 cm deep) is machined in the lower block (dashed line). The solution leaving the working electrode flows through the cavity, and is discarded through Tygon tubing (1/32-in. bore, 1/32-in. wall) sealed with epoxy cement into a hole drilled in the lower block (B). The Ag/AgCl reference electrode (D) was introduced into the outflow solution through an appropriate well drilled in the lower block. A rubber O-ring (not shown) forms a seal round the reference electrode. The cell construction permits easy and fast replacement of the working electrode.

The sample solution is placed in a 250-ml Nalgene beaker. Solution flows from the beaker to the cell by gravity, through 0.5-mm bore Teflon tubing and a tube-end fitting (a hydraulic head of about a foot permits flow-rates up to 3 ml/min). The flow-rates are calibrated and checked volumetrically. All our measurements are made with a Sargent-Welch Model 3001 Polarograph.

Reagents

The chemicals and reagents used have been described in detail previously.[11]

Procedure

Small air-bubbles were removed from the fresh RVC disk by pumping demineralized water up and down rapidly with a syringe located at the solution outlet. The fresh surface was activated by applying potentials of +0.9 and −0.9 V alternately, allowing 1.5 hr at each potential, over a period of 6 hr.

Daily electrode pretreatment consisted of applying potentials of +0.9 and −0.9 V alternately over a period of 10 min, allowing 2 min at each potential and ending with application of the positive potential. Following pretreatment, the desired working potential on the plateau was applied. The transient currents were allowed to decay until a steady state was reached. Following this, measurements were made on the background solution and the analyte solution. All data were corrected for background. Stopped-flow working was obtained by turning on and off a stopcock inserted between the solution reservoir and the cell. Stopped-flow voltamperograms were taken pointwise by making 100-mV changes in the applied potential and waiting for about 20 sec before applying the flow pulse.

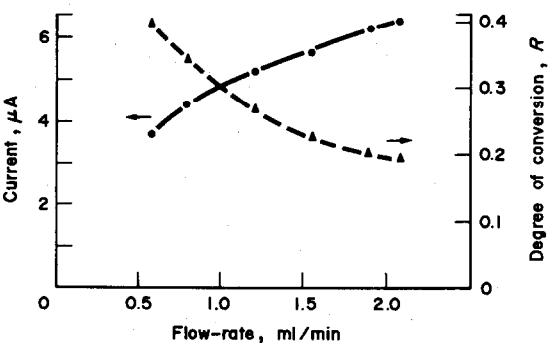

Fig. 2. Dependence of the limiting current and degree of conversion on flow-rate: 10 μ*M* $K_4Fe(CN)_6$ in 0.1*M* phosphate buffer (pH 7.4); applied potential +0.90 V.

The flow-injection measurements were performed by alternating (with a three-way stopcock) between the carrier solution (the supporting electrolyte) and the sample solution. Details are given in the following section.

RESULTS AND DISCUSSION

Mass transport

The dependence of the limiting current and of the degree of conversion (*R*) on the flow-rate is shown in Fig. 2. *R* is the fraction of electroactive species electrolysed while the solution is passing through the electrode. A log–log plot of current against flow-rate (not shown) is linear, with a slope of 0.42. *R* Values ranging from 0.32 to 0.73 for have been reported for different lengths of RVC column.[9] *R* depends on the residence time of an element of solution in the RVC electrode, so decreasing the flow-rate provides larger conversion efficiency. For the flow-rates used in this study *R* increases from 0.19 at 2.08 ml/min to 0.40 at 0.58 ml/min. At these flow-rates the calculated residence times of the solution in the electrode volume are 0.57 and 2.06 sec, respectively. The slope of the

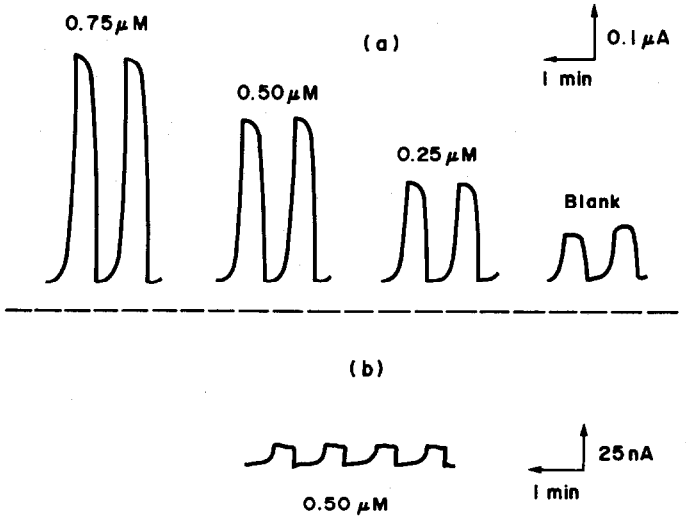

Fig. 3. Stopped-flow response at the submicromolar concentration level. (*a*) Porous-jet electrode. (*b*) Wall-jet electrode (distance between the nozzle tip and the electrode surface, 0.25 mm). Conditions: flow-rates 1.2 ml/min (on) for 20 sec, and 0 ml/min (off) for 30 sec; applied potential. +0.85 V.

log–log current *vs.* flow-rate plot for intermediate efficiency electrolysis detectors depends upon the flow regime and the degree of conversion.[12] Different electrode dimensions (and hence different degrees of conversion) will therefore result in different values of the slope.

While the mass-transport equation has not been solved exactly, for the analytical purposes for which the cell was designed the limiting current I_l can be described in the following empirical form, as was suggested for other intermediate efficiency electrolysis detectors:[12,13]

$$I_l = fC_0V^m \quad (1)$$

where f and m are experimental parameters (that may change with the degree of conversion), C_0 is the bulk concentration of the electroactive species, and V is the solution flow-rate.

Stopped-flow voltammetry

Analytical advantages may be gained when the high analytical currents at solid electrodes are combined with sensitive techniques that discriminate against the high background current. Stopped-flow modulation voltammetry has been shown to be effective in discriminating against most components of the background currents at flow-through solid electrodes.[14] Figure 3(*a*) illustrates the sensitivity and detectability obtained at the porous jet electrode by employing this modulation procedure. It is a reproduction of a chart-record for ferrocyanide at submicromolar concentration levels. The blank stopped-flow current corresponds to a concentration around 0.22 μM. The noise level is around 2 nA, corresponding to a limit of detection of about 4nM (for a signal equal to the noise). Similar values have been reported for large-volume RVC electrodes.[9,10] The stopped-flow response times are about 12 sec (on) and 20 sec (off). Rapid stopped-flow procedures, without achievement of the "off" current steady-state are under investigation. The two recorder peaks shown in Fig. 3(*a*) for 0.5μM ferrocyanide are part of a series of ten successive stopped-flow measurements, which were made for measuring the precision. The average stopped-flow current difference found was 224 nA with a range of 216–229 nA. The relative standard deviation over the complete series was 1.0%.

Figure 3(*b*) shows a stopped-flow response for 0.5μM ferrocyanide, obtained at the wall-jet electrode (using the same cell body with the RVC disk and 3 of the 5 Teflon spacers removed and the disk face of the glassy carbon contact serving as the working electrode); the other experimental conditions being the same as for Fig. 3(*a*). The wall-jet stopped-flow current (11 nA) was significantly lower than that observed for the same concentration level at the porous-jet electrode (224 nA). The noise was about 1 nA. A detection limit of about 45 nM is thus obtainable with the wall-jet electrode (for a signal equal to the noise), about 11 times that for the porous jet electrode. The improved sensitivity and detectability with the porous-jet electrode are the result of combining effective mass-transport with large surface area. The surface area of the planar wall-jet electrode is 0.049 cm^2 as compared to 1.26 cm^2 for the RVC disk (a planar disk with similar area to the RVC would require a diameter of 1.27 cm). The high linear flow-rate of the solution streaming out of the inlet nozzle is maintained inside the thin channels of the RVC. Detectors operating in the intermediate range of electrolytic efficiency (20–40%) thus provide better sensitivity than low-yield amperometric detectors, while still maintaining small dead-volumes in comparison to the electrode volumes required to achieve complete conversion.

Quantitative evaluation of the cell is based on the linear correlation between the analytical current (*i.e.*, current amplitude in the case of stopped-flow measurements) and the analyte concentration. Six concentration increments from 2.5 to 15μM ferrocyanide yielded a highly linear plot [conditions: flow-rate (on) 1.2 ml/min; pulsing time, 40 sec; applied potential, +0.9 V]. Least-squares analysis of the calibration data yielded a slope of 0.57 μA.l. μmole^{-1} (correlation coefficient 0.998). A slightly higher sensitivity (0.7 μA.l. μmole^{-1}) was reported for a larger RVC disk of 3.8 cm^2 area, indicating that the present design promotes higher efficiency of mass-transport.

Current *vs.* potential curves provide important information for the identification of compounds in flowing streams. Figure 4 shows a stopped-flow hydrodynamic voltamperogram for the oxidation of 10 μM dopamine, along with the residual current. The curve shows a well-defined wave and plateau region. The stopped-flow half-wave potential is +0.24 V, with $E_{3/4} - E_{1/4}$ equal to 135 mV. The background stopped-flow current is very low, less than 1% of the analyte stopped-flow current. The sensitivity is 2.3 μA.l. μmole^{-1}.

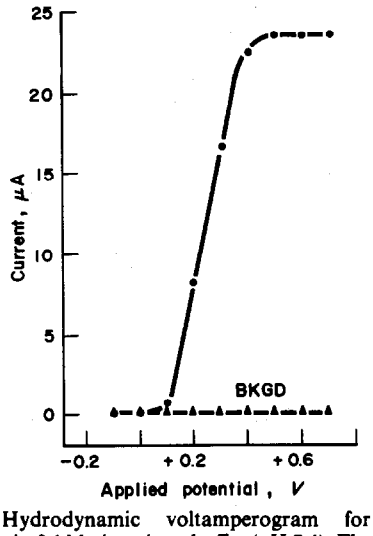

Fig. 4. Hydrodynamic voltamperogram for 10 μM dopamine in 0.1M phosphate buffer (pH 7.4). Flow-rates: 0 ml/min for 90 sec (off); 1.2 ml/min for 40 sec (on).

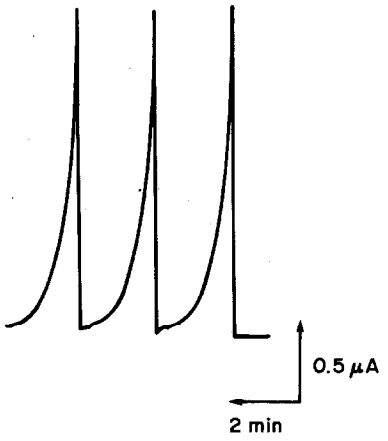

Fig. 5. Repetitive injections of 5.5 μM ferrocyanide solution. Sample flow, 15 sec; carrier (0.1 M phosphate buffer) flow, 105 sec. Flow-rate 1.3 ml/min. Sample size 0.325 ml, containing 0.75 μg of ferrocyanide. Applied potential, +0.85 V.

Flow-injection analysis

The porous-jet electrode can be used as a useful detector for flow-injection analysis. Figure 5 is a typical recording of the detection peaks for 5.5μM ferrocyanide in phosphate buffer solution (corresponding to 0.75 μg in the injection volume used). The time between sample injections was 105 sec, and the sampling rate 30 per hour. A higher sampling rate may be obtainable by use of much faster flow-rates.[10] A detection limit near 40 nM ferrocyanide (5 ng) is expected.

A detection limit of 0.7 ng of ferrocyanide was reported for flow-injection analysis at a much larger (surface area 125 cm^2) RVC electrode, utilizing higher flow-rates.[10] The three peaks shown in Fig. 5 are part of a series of eight repetitive injections for which a relative standard deviation of 3% was calculated.

Future studies will include evaluation of a porous ring–disk jet electrode, and of rapid stopped-flow scanning potential voltammetry.

Acknowledgement—The support of the Society of Analytical Chemists of Pittsburgh is gratefully acknowledged.

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TRACE METAL ANALYSIS BY ANODIC-STRIPPING VOLTAMMETRY

EFFECT OF SURFACE-ACTIVE SUBSTANCES

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Summary—The presence of cationic, anionic, and neutral surfactants and humic substances is shown to affect the peak heights of copper, lead and cadmium in synthetic sea-water analysed by differential pulse anodic-stripping voltammetry. At surfactant concentrations below 0.1 mg/l. the effect is insignificant, but at higher concentrations the peak heights usually decrease, although for copper an increase in the peak height was also observed. The peak heights do not depend to any great extent on the pH of the solution, except in alkaline solution and in the presence of humic substances. Adsorption and complex formation may account for the observed dependences.

The environmental impact of many trace metals present in natural waters depends strongly on the chemical form of the metals; increasing attention is therefore focused on analytical methods which can be used for metal speciation studies. Owing to their non-destructive nature and the ability in certain cases to discriminate between different types of species in solution, electroanalytical techniques appear to be particularly suitable for such studies. Because the concentration of trace metals in samples of environmental interest is frequently in the μg/l.–ng/l. range, the most sensitive technique, *i.e.*, anodic-stripping voltammetry (ASV) will often be the method of choice. Unfortunately, for real samples there is usually no simple relationship between the height and potential of a given stripping peak and the chemical form of the corresponding electroactive metal. For instance, the presence of surface-active substances may have a marked effect on the voltammetric response, owing to the adsorption of such compounds at the electrode surface.[1] Therefore, information about the effects of different surfactants is of importance for adequate use of ASV in metal speciation studies.

This paper describes the effects of synthetic surfactants and naturally-occurring humic substances on the ASV response of synthetic sea-water samples spiked with copper, lead and cadmium. Because the effect of a surfactant may depend on its ionic charge,[2] cationic, neutral and anionic surfactants were studied. Further, the effect of pH was studied in detail, because this is an important parameter in ASV measurements, and in addition the adsorption characteristics of surfactants may depend on pH.

EXPERIMENTAL

Apparatus

A Princeton Applied Research 174A Polarographic Analyzer and a Radiometer Servograph REC51/REA112 recorder were used. The different steps in the stripping voltammetric procedure were controlled by a home-made timer unit. A Beckman Rotating Unit, consisting of a variable speed drive (188501) and a rotating body (188551) was used with a three-edged Teflon rod for stirring.

The electrolysis cell was a Metrohm EA880-20T vessel with a thermostatic jacket, through which water at 25.0° was circulated. The working electrode was a Metrohm E410 hanging mercury drop; only capillaries with a small diameter of the mercury reservoir (2.5–2.7 mm) were used.[3] The reference electrode was either a Metrohm EA427 Ag/AgCl electrode, or a silver wire coated with silver chloride and placed inside a Luggin capillary. A platinum spiral served as counter-electrode; the wire was placed inside an open glass tube to ensure a fixed position of the electrode in the cell.

All glass equipment, particularly the electrolysis cell and the mercury drop capillary, was thoroughly cleaned before each new series of measurements. The cell and capillary were treated with dimethyldichlorosilane at regular intervals.

Reagents and solutions

The metal solutions were prepared from analytical grade salts and acidified to pH 2 with perchloric acid; solutions with a concentration below $10^{-4}M$ were prepared just before use. All acids and the sodium hydroxide used were of Suprapur quality (Merck). The water was demineralized and distilled. Solutions (1%) of the following surfactants were prepared: sodium dodecyl sulphate (M.W. 288.38), dodecylamine (M.W. 185.36), and octylphenoxypolyethoxyethanol (Triton X-100, M.W. 646.87). The dodecylamine solution was heated to 90° and shaken before use, and the dodecyl sulphate solution was heated to 30°. The "humic acid" concentrate was prepared by 20-fold concentration of a fresh-water sample from Hellerudmyra, Bærum, Norway in a rotary evaporator at 35°. The water sample, which was particularly rich in humic substances, contained[4] 23 mg of organic carbon per litre.

The synthetic sea-water was prepared from analytical-grade salts as specified in Table 1. The salts were dissolved in 25 litres of distilled water and stored in a thoroughly cleaned polyethylene container. The solution was spiked with 21.25 ml of a stock solution of copper, lead and cadmium (each $10^{-4}M$), and acidified to pH 2.3 with hydro-

Table 1. Composition of synthetic sea-water of 35‰ salinity

Salt	Concentration *M*	*g/l.*	*g/25 l.*
NaCl	4.783×10^{-1}	27.96	698.9
$MgSO_4 \cdot 7H_2O$	2.895×10^{-2}	7.136	178.4
$MgCl_2 \cdot 6H_2O$	2.561×10^{-2}	5.207	130.2
$CaCl_2 \cdot 2H_2O$	1.053×10^{-2}	1.548	38.70
KCl	9.715×10^{-3}	7.244×10^{-1}	18.11
$NaHCO_3$	2.33×10^{-3}	1.96×10^{-1}	4.89
KBr	8.51×10^{-4}	1.01×10^{-1}	2.53
H_3BO_3	4.16×10^{-4}	2.57×10^{-2}	0.643
$SrCl_2$	9.70×10^{-5}	1.54×10^{-2}	0.384
KF	7.37×10^{-5}	4.28×10^{-3}	0.107
$NaNO_3$	2.0×10^{-5}	1.7×10^{-3}	0.042
LiCl	2.6×10^{-5}	1.1×10^{-3}	0.028
$Na_2HPO_4 \cdot 2H_2O$	2.3×10^{-6}	4.1×10^{-4}	0.010
Rb_2SO_4	7.0×10^{-7}	1.9×10^{-4}	0.0047
KI	4.7×10^{-7}	7.8×10^{-5}	0.0020
$BaCl_2 \cdot 2H_2O$	2.2×10^{-7}	5.4×10^{-5}	0.0013
$AlCl_3 \cdot 6H_2O$	1.8×10^{-7}	4.3×10^{-5}	0.0011

chloric acid to prevent bacterial growth. The addition of acid resulted in a loss of hydrogen carbonate from the solution. The variation in voltammetric response with pH was studied by adding hydrochloric acid and sodium hydroxide to the sample.

Instrumental parameters

The following parameters were used for recording the differential pulse anodic-stripping voltamperograms: diameter of mercury drop 0.8 mm, deposition time 3 min, deposition potential −0.9 V *vs.* Ag/AgCl, stirring rate 20 rps, rest period 30 sec, scan-rate 5 mV/sec, modulation amplitude 50 mV, and pulse repetition time 0.5 sec. Before analysis, the solution was deaerated with nitrogen for 20 min.

RESULTS AND DISCUSSION

Effect of pH

A differential pulse anodic-stripping voltamperogram of the synthetic sea-water with metal spikes is shown in Fig. 1. As expected for chloride media, a lower and broader peak is observed for copper than for lead and cadmium.

In Figs. 2, 3 and 4, the variation in peak height with pH is shown for the three metals, in presence of the surfactants. The broken lines indicate that the measurements were poorly reproducible, or that the peak almost merged with the mercury wave. In the figures, SS refers to the pure spiked synthetic sea-water, whereas HUMIC SUB, DDS, DDA and TRITON indicate the addition of humic substances, sodium dodecyl sulphate (anionic), dodecylamine (cationic), and Triton X-100 (non-ionic), respectively. The concentration of the surfactants was 10 mg/l., corresponding to 15–54μM concentrations of the different surfactants (depending on molecular weight), while that of the humic substances was 4% (1 ml of humic acid concentrate per 25 ml), *i.e.*, organic carbon 18 mg/l. From the curves in Figs. 2, 3 and 4, it can be seen that the presence of surfactants alters the peak

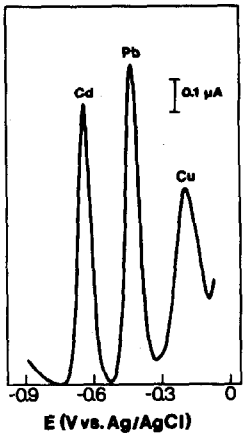

Fig. 1. Differential pulse anodic-stripping voltamperogram of synthetic sea-water spiked with copper, lead and cadmium, at pH 2.3. Total concentrations: 133nM Cu, 96nM Pb and 91nM Cd; these concentrations are the sums of the 85nM spike for each metal and the concentration in the synthetic sea-water (Cu 48nM, Pb 11nM, Cd 6nM).

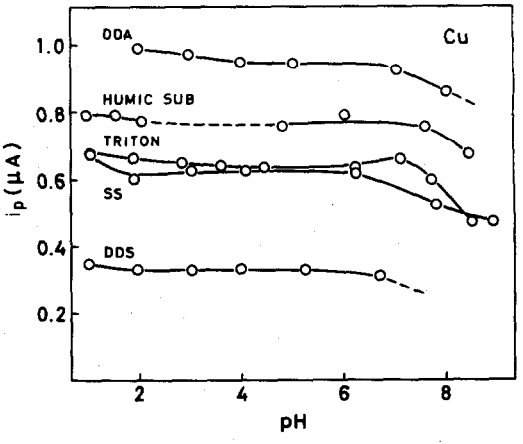

Fig. 2. Variation in the peak height of copper in synthetic sea-water as a function of pH, in absence and presence of surfactants and humic substances.

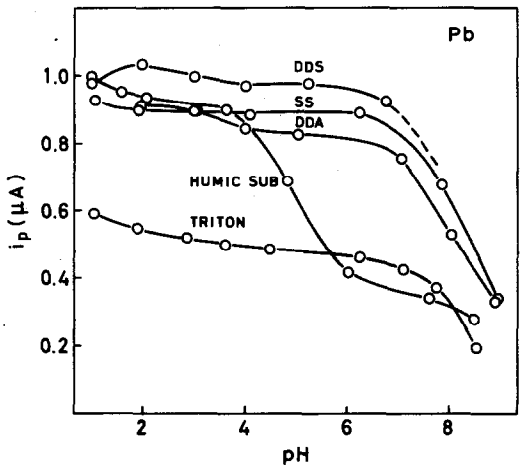

Fig. 3. Variation in the peak height of lead in synthetic sea-water as a function of pH, in absence and presence of surfactants and humic substances.

heights for the three metals. However, the peak heights do not vary to any great extent with pH in the acidic region, in contrast to results obtained by Brezonik *et al.*[1] The general pattern exhibited by the synthetic sea-water sample (SS) is also followed when the surfactants are present. For lead, and to a minor degree for copper, the peak heights decrease in alkaline solution, probably because of formation of hydroxo-complexes of these metals. The formation of carbonate complexes was disregarded, because carbonate was lost during the acidification of the synthetic sea-water. The less pronounced decrease for copper relative to lead is probably due to reduction of copper hydroxide. The decrease in peak heights was accompanied by a cathodic shift in the peak potentials, which indicates complex formation.

With humic substances present in the sample, more pronounced effects of pH were observed. For lead (Fig. 3), a decrease in peak height was seen in the pH region from 3.6 to 8.4. It was accompanied by a marked (74 mV) cathodic shift in the peak potential, indicating complex formation. For cadmium (Fig. 4), the peak height decreased as the solution was made more acidic. This decrease is not easily explained. Although a small anodic shift in the peak potential implied adsorption,[1] a well-defined stripping–polarographic curve[5] (peak height recorded as a function of deposition potential) was obtained at pH 2.3, indicating the absence of adsorption effects.

Effect of surfactant concentration

The variation in peak height with the concentration of surface-active substances (SAS) at pH 2.3 is illustrated in Figs. 5, 6 and 7 for copper, lead and cadmium, respectively. From the curves it can be seen that the change in peak heights was insignificant for concentrations of surfactant below 0.1 mg/l. Higher concentrations of dodecyl sulphate and Triton X-100 led to decreased peak heights, except for copper in presence of Triton X-100. For the highest concentrations of dodecyl sulphate, the peak heights tended to return to their original values. For dodecylamine, the peak heights remained unaffected, except for copper, where the peak increased. The absence of a peak-depression effect for dodecylamine may indicate that the electrode reactions involved negatively charged metal chloro-complexes, because these should be less affected by positively charged surfactants like dodecylamine than by anionic and non-ionic surfactants.[2]

A marked decrease in peak height was usually accompanied by a small (30–50 mV) shift in the peak potential in the anodic direction, indicating adsorption effects. For Triton X-100, the stripping–polarographic curves were ill-defined for all three metals, indicating strong adsorption. No "limiting current" plateau was observed on these curves. The same was noticed for cadmium in presence of sodium dodecyl

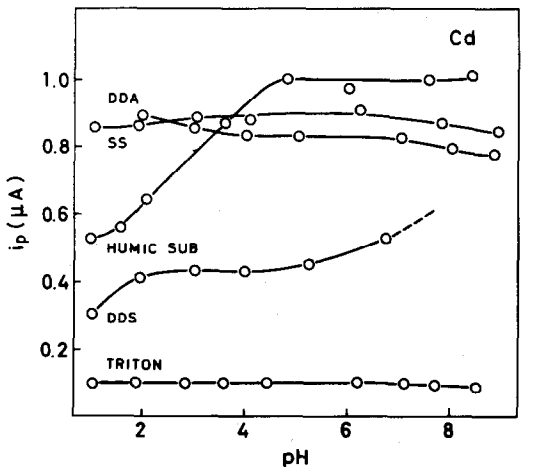

Fig. 4. Variation in the peak height of cadmium in synthetic sea-water as a function of pH, in absence and presence of surfactants and humic substances.

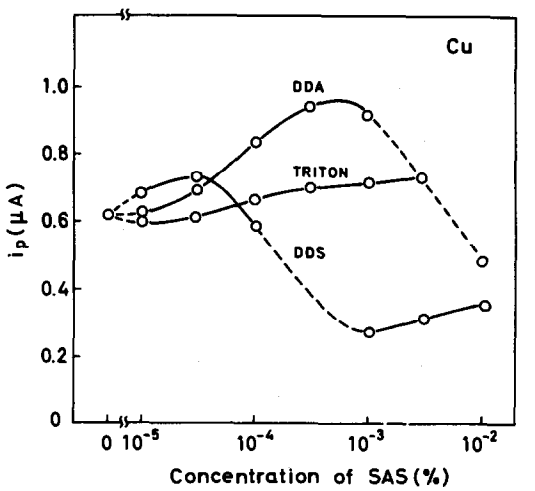

Fig. 5. Variation in the peak height of copper, as a function of surfactant concentration.

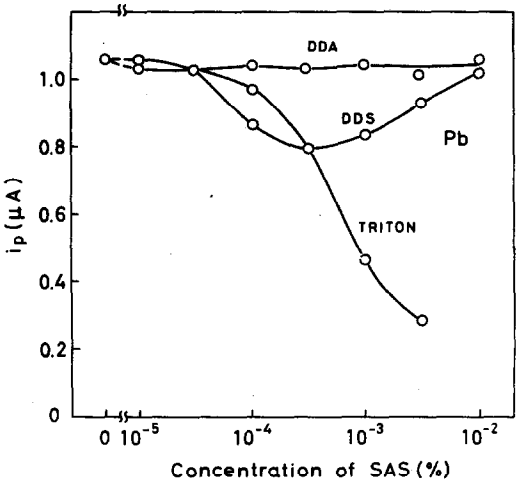

Fig. 6. Variation in the peak height of lead as a function of surfactant concentration.

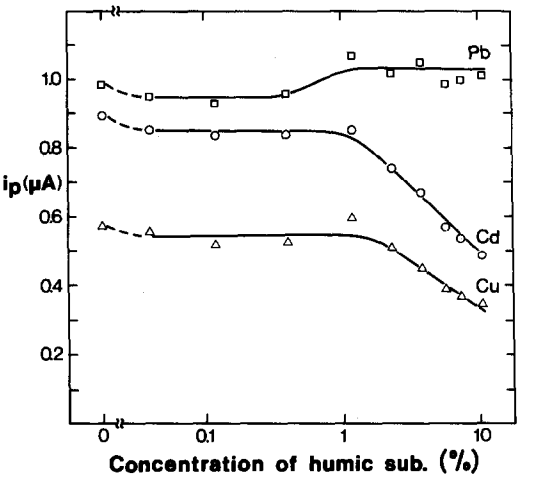

Fig. 8. Variation in the peak heights of copper, lead and cadmium as a function of the concentration of humic substances.

sulphate. The other curves resembled normal polarographic waves.

The variation in peak height with concentration of humic substances is shown in Fig. 8. The peak heights were corrected for the metal content of the humic acid concentrate. The concentration is given in terms of the "humic acid concentrate". Thus, a 1% solution contained 4.6 mg of organic carbon per litre. From Fig. 8 it can be seen that the peak heights were unaffected by low concentrations of humic substances, whereas at high concentrations the peak heights decreased for copper and cadmium, but not for lead. Only minor changes were noted for the peak potentials. The results differ from those of Ernst *et al.*,[6] who observed more pronounced changes in the height and potential of the peaks with concentration of humic acid. However, their experiments were carried out at pH 6.8, which may account for the differences.

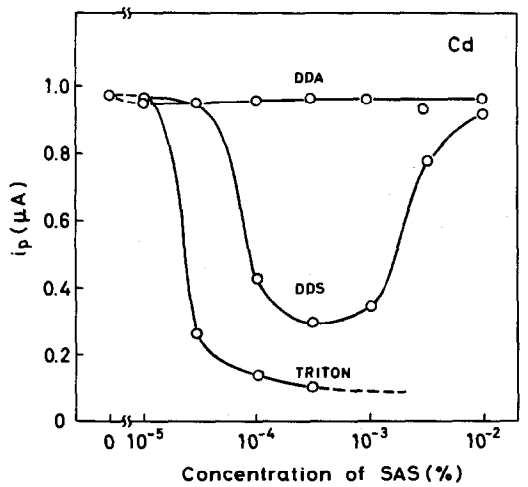

Fig. 7. Variation in the peak height of cadmium as a function of surfactant concentration.

Finally, the peak heights were recorded as a function of the metal concentration, in presence of the surfactants. Linear relationships were invariably obtained, implying that the metals can be determined quantitatively when surfactants are present, provided that the standard addition technique is used.

Conclusion

The adsorption of surfactants may lead to decreased peak heights in differential pulse anodic-stripping voltammetry. However, the effect may be reversed at high concentrations of surfactant. Further, not all surfactants exhibit a depression effect, so no general trend can be established.

The peak heights obtained in presence of a surfactant are not much affected by pH changes in the acidic region, in agreement with the general pattern observed for pure synthetic sea-water. Consequently, if a marked increase in the peak heights is observed upon acidification of a neutral water sample, this may indicate that metals are released from particles or complexes in solution, even if surfactants are present in the sample.

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ATOMIC-ABSORPTION SPECTROMETRIC DETERMINATION OF METALS AND SILICON IN TAR-SANDS FLY-ASH

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Summary—Atomic-absorption spectrometric methods are described for the determination of metals and silicon in a fly-ash which is produced during oil recovery from Alberta tar sands. The techniques of standard additions and matrix matching are compared. An experimental design for the detection, estimation and correction of interference effects arising in the determination of specific elements in the fly-ash is presented.

During the recovery of oil from the Athabasca tar sands, a boiler ash containing appreciable amounts of metals such as vanadium, nickel, titanium, iron and aluminium, as well as silicon, is collected in the boiler hoppers and cyclones of the steam-generation plants fired with petroleum coke. Thus, as well as being a petroleum resource, the tar sands are also a source of vanadium and possibly other metals, provided that efficient metal recovery processes can be developed.[1,2] Although there have been a number of recent reports on analytical methods for the chemical characterization of fly-ash emitted from coal-fired power plants,[3–5] a reliable method for the chemical characterization of the fly-ash obtained from tar sands has not yet been reported, though it is needed for metal recovery studies and for evaluation of the potential health hazards of tar-sands fly-ash. In this paper we present results which help define a reliable analytical method, based on atomic-absorption spectrometry (AAS), for such fly-ash analyses.

EXPERIMENTAL

Fly-ash sample preparation and AAS analyses

The petroleum coke fly-ash used in this investigation was provided by Great Canadian Oil Sands Ltd. (GCOS). Carbon-free fly-ash was obtained by heating the samples (as received from GCOS) at *ca.* 500° in air until all the carbonaceous fraction had been burned off. The loss in weight on ignition corresponded to 58.3% in the fly-ash.[6]

The analysis of the carbon-free fly-ash is based on an acid-digestion bomb method previously reported for the decomposition and subsequent AAS analysis of coal ash[5,7]

The carbon-free fly-ash (0.100 g) was digested with 3 ml of concentrated hydrofluoric acid and 1 ml of *aqua regia* in a 25-ml Teflon-lined Parr pressure bomb at 150° for *ca.* 45 min, then cooled to room temperature. Next, 3.8 g of boric acid were added to the solution and the whole was made up to volume in a 100-ml standard flask with demineralized water. A 10-ml aliquot was then diluted to 100 ml with dimineralized water and used for the AAS analysis, done with a double-beam Pye Unicam SP 1900 AA spectrophotometer equipped with a Pye-Unicam SP 1960 deuterium lamp background-corrector. Calibration graphs for the elements determined were obtained by use of commercially available AAS standard solutions (BDH). The accuracy of the entire analytical procedure was checked by analysis of a weighed amount of silicon standard reference material (NBS-SRM 640), an error of 0.2% being found.

Preparation of matrix-matching solution

Matrix-matching solutions containing elements present in the fly-ash were prepared from the AAS standard solutions and the reagents used in the digestion.

RESULTS AND DISCUSSION

Optimization of operating conditions

Besides the routine atomic-absorption spectrometer adjustments, the two parameters to be optimized are the fuel/oxidant flow-ratio and the burner height.[8] Metals such as vanadium which form stable oxides in the flame require a hot fuel-rich (reducing) acetylene–nitrous oxide flame. The height of the optical path in the flame is important because the temperature and composition profiles of the flame also affect the element–oxide equilibrium.

Both these parameters were optimized by continuously aspirating a suitable analyte solution into the flame and adjusting the burner height and flow-rates to maximize the signal. Figure 1 shows a typical result for silicon, titanium and aluminium. The best conditions found experimentally are summarized in Table 1 and were used in all the subsequent AAS analyses. Despite the inherent specificity of the AAS technique, under certain conditions spectral, physical and chemical interferences may arise.[8] Possible interference effects in the AAS analyses in the present study were therefore investigated.

Detection of matrix interference effects

For fairly complex samples, the method of Hansen and Hall[9] for detecting matrix interference effects should provide good results. The matrix interference effect is found by comparing the analyte absorption

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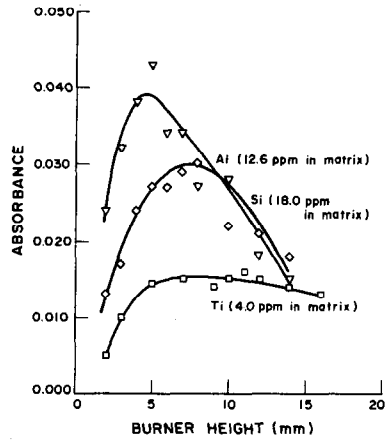

Fig. 1. Variation of absorbance of Al, Si and Ti with burner height. Other conditions as in Table 1.

signal for the series water, matrix blank, matrix + analyte, analyte + water. The results obtained are shown in Table 2.

Another method for interference detection in AAS is the graphical one proposed by Schrenk[10] in which the unknown solution is prepared at three analyte concentration levels (*e.g.*, *A*, 2*A*, 3*A*). If there is no interference, a plot of analyte signal *vs.* concentration should be linear and pass through the origin; an intercept is taken as an indication of some type of interference. The results obtained with this test are included in Table 2.

Standard-addition and matrix-matching techniques

The standard-addition technique[8,11] gave the results also provided in Table 2.

Matrix-matching solutions containing the elements listed in Table 2 were prepared so that they contained the elements (other than the analyte) at the levels indicated by the standard-addition results. This was done by using appropriate amounts of the AAS standard solutions and addition of the digestion reagents to simulate the bomb conditions used for the fly-ash dissolution. This mixture is referred to as the matrix blank. Calibration standards of given analytes in the matrix blank were prepared and when these standards were analysed by AAS, plots like those shown in Figs. 2–4 were obtained. These plots were subsequently used to determine the concentration of specific elements present in the fly-ash. The values so obtained, shown in the last column of Table 2, are the average of five replicates and have the 95% confidence interval limits shown.

The calibration graphs shown in Figs. 2–4 not only provide a value of a given analyte concentration but also provide a check for interference effects. It is readily seen from Figs. 3 and 4 that the plots for Al, Si and Ti have intercepts; thus some type of interference is expected for these elements.[10] This is in agreement with the Hansen interference-test results shown in columns 2–6 of Table 2.

In the case of iron, while the Hansen test indicates that there may be some interference, the intercept test does not. Further tests with solutions having a con-

Table 1. Optimum analytical conditions for AAS analysis of fly-ash

Element	Wavelength, *nm*	Flame	Type of flame	Oxidant/fuel flows, *l./min*	Burner height, *mm*	Lamp current, *mA*	Slit-width, *mm*
V	318.4	N_2O/C_2H_2	Reducing	4.5/3.9	7.0	15	0.10
Ni	232	air/C_2H_2	Oxidizing	4.8/0.6	7.0	12	0.10
Ti	364.3	N_2O/C_2H_2	Reducing	4.5/4.0	8.0	15	0.10
Fe	248.3	air/C_2H_2	Oxidizing	5.0/1.4	6.0	12	0.10
Al	309.4	N_2O/C_2H_2	Reducing	4.5/3.6	5.0	10	0.10
Si	251.6	N_2O/C_2H_2	Reducing	4.5/4.0	8.0	14	0.10

Table 2. Interference test results and fly-ash analysis

	Hansen interference test[9] absorbance						Carbon-free fly-ash, % *w/w*	
Element	Concentration, *mg/l.*	Demineralized water	Matrix blank	Matrix + analyte	Analyte in water	Schrenk test[10] intercept	Standard addition	Matrix matching*
V	5.0	0.000	0.000	0.013	0.012	None	3.1	2.9 ± 0.3
Ni	4.0	0.000	0.000	0.086	0.084	None	1.4	1.1 ± 0.1
Ti	5.0	0.000	0.002	0.012	0.008	+	4.0	2.4 ± 0.2
Fe	4.0	0.000	0.001	0.233	0.221	None	6.6	6.7 ± 0.8
Al	12.6	0.000	0.003	0.043	0.038	+	12.6	12.0 ± 1.1
Si	18.0	0.000	0.003	0.027	0.024	+	18.0	18.0 ± 1.0

* ±95% confidence interval.

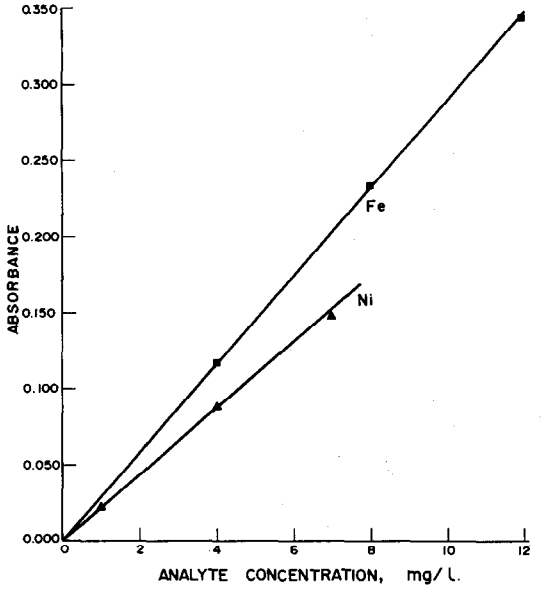

Fig. 2. Calibration curves for iron and nickel; analyte in matrix.

stant concentration of iron and varied concentrations of the other elements (over ranges close to the levels in the matrix) did not reveal any interference effects in the iron determination. No interferences were noticed for V and Ni at the concentration levels used in this work.

Comparison of the values in the last two columns in Table 2 shows that the standard-addition method gives results, in most cases, which agree reasonably well with the matrix-matching results (*i.e.*, the values

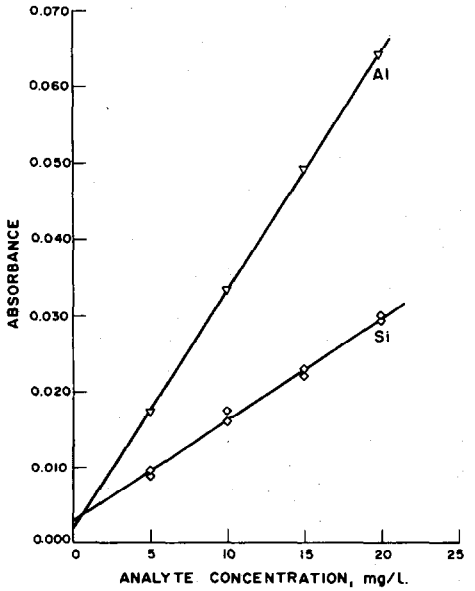

Fig. 3. Calibration curves for aluminium and silicon; analyte in matrix.

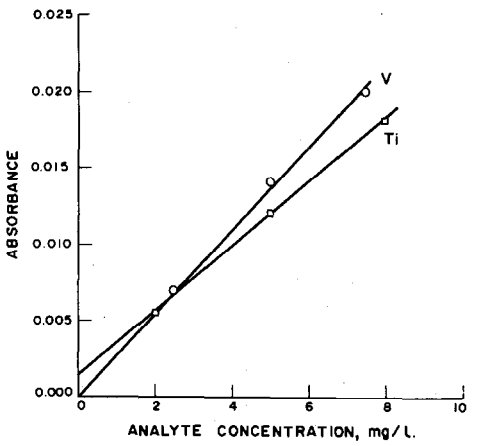

Fig. 4. Calibration curve for titanium and vanadium; analyte in matrix.

given by the matrix-matching technique are expected to provide the best estimate of the fly-ash composition[12]). The method of standard additions gives good results only in the absence of additive types of interference such as light-scattering or the effect of analyte present as impurity in the reagents.[8,11] Light-scattering interference can be overcome by using a deuterium-lamp background corrector;[12,13] this device is most effective at wavelengths shorter than 300 nm. If a calibration curve such as those shown in Figs. 2–4 passes through the origin, it is unlikely that light is scattered in the flame. It was found that the background correction for either aluminium or silicon was negligible, which suggested that the standard-addition technique should provide reasonably good values if no other source of interference is present.

When the slope of the calibration curve is enhanced (or depressed) by other matrix elements, error can arise if the calibration is done by use of pure solutions of the analyte. Such a type of error can be corrected for by the use of the standard-addition technique or by use of matrix-matched standards. Such an error appears to arise in this work for aluminium, titanium and silicon, as can be seen from Figs. 3 and 4. In order to gain a better understanding of the nature of the signal enhancement for these elements, the analyte signals from a series of synthetic solutions with varying Al, Ti and Si concentrations, with all other elements held at constant concentration (Table 3). A linear least-squares statistical analysis obtained by using a BMD 9K computer package[14] resulted in equations (1)–(3) as the ones giving the best fit to the observed data.

$$Abs_{Ti} = 0.00303\ [Ti] + 0.0000613\ [Al] \quad (1)$$

$$Abs_{Si} = 0.00191\ [Si] + 0.00119\ [Ti] \quad (2)$$

$$Abs_{Al} = 0.00469\ [Al] + 0.000267\ [Ti] \quad (3)$$

where Abs_E = absorbance of element E and [E] is the concentration of E (mg/l.). The statistical analysis

Table 3. Statistical study of interferences

Sample	Replication	Element concentration*, ppm Si	Al	Ti	Absorbance Si	Al	Ti
1	a	6	4	1	0.013, 0.014	0.0205, 0.020	0.004, 0.004
	b	6	4	1	0.0125	0.020, 0.019	0.004
2	a	6	20	1	0.013	0.096, 0.094	0.004
	b	6	20	1	0.012, 0.0135	0.096, 0.0955	0.004, 0.004
3	a	6	4	5	0.017	0.0205, 0.0195	0.015, 0.015
	b	6	4	5	0.0175, 0.0185	0.021	0.0155
4	a	6	20	5	0.018, 0.0185	0.096	0.0165
	b	6	20	5	0.017	0.0955, 0.0945	0.0165, 0.0165
5	a	22	4	1	0.043, 0.044	0.021, 0.020	0.0035, 0.004
	b	22	4	1	0.043	0.020	0.004
6	a	22	4	5	0.048	0.021	0.015
	b	22	4	5	0.048, 0.049	0.021, 0.020	0.015, 0.015
7	a	22	20	1	0.0435, 0.046	0.099, 0.097	0.004
	b	22	20	1	0.045, 0.046	0.099, 0.0975	0.004
8	a	22	20	5	0.049	0.1005, 0.0985	0.0165, 0.016
	b	22	20	5	0.050	0.0985, 0.0985	0.0165
9	a	14	12	3	0.032	0.060	0.010
	b	14	12	3	0.032, 0.033	0.060, 0.060	0.010
	c	14	12	3	0.032	0.059	0.010, 0.010
	d	14	12	3	0.031	0.059, 0.059	0.010

* Each solution also contains 2.65 ppm V, 1.20 ppm Ni, 5.50 ppm Fe, together with the bomb reagents (0.3 ml of concentrated hydrofluoric acid, 0.1 ml of *aqua regia* and 0.38 g of boric acid per 10 ml of solution).

appeared to indicate a small interelement effect between Si and Al in the silicon and aluminium determinations, but this contributed less than 5% to the observed signal and was ignored. Thus according to these equations the Ti signal is enhanced by Al, the Si signal is enhanced by Ti and the Al signal is enhanced by the presence of Ti. The first of these interferences has been previously reported[15] and so has the enhancement of the Al signal by Si (and *vice versa*).[16]

Equations (1)–(3) are of the form $y = ax + b$ when the interfering element concentration is constant. These types of curve are depicted in Figs 3 and 4; as mentioned previously, the intercept is associated with the interfering effect. Equations (1)–(3) and the concentrations of the several elements present in the fly ash, as given in Table 2 (last column) allow an estimate to be made of the errror incurred if no matrix-matching technique is used. A summary of the errors introduced by interferences in the analyte determination is given in Table 4. The errors are larger for titanium and silicon than for aluminium.

Comparison of atomic-absorption and classical analyses

In order to verify the validity of the matrix-matching technique in the atomic-absorption spectrophotometric determination of metals in fly-ash, a series of different fly-ash samples was also analysed by classical methods. The classical methods used were first checked on artificial matrices of known composition prepared from commercially available AAS standard solutions, with addition of digestion reagents to simulate the bomb-reagent conditions used for the fly-ash dissolution (see experimental section). References for the classical methods used for the fly-ash analyses are noted in Table 5 which provides a complete summary of the atomic-absorption and classical method results for four different fly-ash samples. The results of the two methods compare favourably and suggest that the atomic-absorption method can be used with a fair degree of confidence for fly-ash analyses.

Table 4. Errors caused by interferences in analyte determination

Analyte	% Error due to the presence of Al	Ti
Ti	+10	—
Si	—	+8
Al	—	+1

Table 5. Fly-ash analysis: comparison of atomic-absorption (AA)* and classical method (CM) results

Sample	Replication	Fe, % AA	Fe, % CM†	V, % AA	V, % CM§	Ti, % AA	Ti, % CM‡	Ni, % AA	Ni, % CM‖
1	a	8.3	8.4	3.5	3.4	3.8	3.8	2.7	2.5
	b	8.2	8.3	3.3	3.1	3.9	3.7	2.8	3.0
	c	8.3	8.4	3.4	3.6	3.8	3.7	2.7	2.8
2	a	6.3	6.2	3.0	2.8	3.0	2.8	1.4	1.5
	b	6.0	6.0	3.0	2.8	3.0	2.8	1.3	1.5
	c	6.3	6.2	3.0	2.8	3.0	2.8	1.3	1.5
3	a	6.2	5.9	3.1	3.0	2.7	2.5	1.3	1.5
	b	6.2	5.8	3.0	2.8	2.8	2.5	1.3	1.5
	c	6.2	5.6	3.0	2.8	2.7	2.3	1.3	1.5
4	a	6.2	6.0	3.0	2.6	2.8	2.7	1.2	1.0
	b	6.2	5.9	2.8	2.6	2.6	2.2	1.2	1.3
	c	6.2	6.2	3.0	2.8	2.8	2.6	1.2	1.3

* Matrix-matching technique employed.
† Ref. 17, p. 785.
‡ Ref. 17, p. 790.
§ Ref. 17, p. 789.
‖ Ref. 18, p. 181.

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A RAPID PROCEDURE FOR THE SIMULTANEOUS DETERMINATION OF ZIRCONIUM AND HAFNIUM IN HIGH-TEMPERATURE ALLOYS BY MEANS OF A SPECTROPHOTOMETRIC MASKING APPROACH

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Summary—Data are presented for a refined spectrophotometric procedure for the simultaneous determination of zirconium and hafnium based on the combined effects of hydrogen peroxide, sodium sulphate, and excess of zirconium ion on the hafnium and zirconium complexes with Xylenol Orange in 0.2*M* perchloric acid. Isolation procedures for the hafnium/zirconium content of complex high-temperature alloys which result in an ionic substrate compatible with the spectrophotometric masking method were devised.

Certain high-temperature alloys which are under development for jet turbine applications in aircraft for the late eighties and nineties incorporate both zirconium and hafnium. Routine procedures for the determination of zirconium and hafnium in these matrices usually involve some form of spectrographic approach (optical emission calibrated with synthetically prepared solution standards, or X-ray fluorescence with direct calculation from fundamental parameters). Chemical determinations are characteristically lengthy and tedious and, therefore, are seldom used except to establish calibration standards for spectrographic work or for referee work requiring a high degree of reliability. For this purpose, our laboratory has employed a modified version of the anion-exchange procedures of Machlan and Hague[1,2] as part of a larger separation scheme which requires at least 15 working days to complete.

The works published by Cheng[3–6] on the Xylenol Orange complexes of zirconium and hafnium pointed the way toward a spectrophotometric method which promised to be significantly faster than any devised up to that time, but quantitative data remained elusive and the complex matrices of high-temperature alloy samples presented special problems. Challis[7] expanded part of the work of Cheng into a practical simultaneous determination for nickel-base alloys which employed calibration and measurement at three precisely-adjusted acidity levels. This method requires that the zirconium and hafnium levels in the sample do not exceed 0.2% and that the matrix alloy be free from tungsten, niobium and tantalum. Kiciak and Gontarz[8] recently described the equilibria at work in the method of Challis.

However, the compositions of the powder-metallurgy alloys, AF115 and MERL 76, to list two examples, exceed the conditions for application of the Challis procedure, and in addition, the method is still too slow for our requirements. As a result, a series of studies was conducted, based on another aspect of Cheng's work—the effect of hydrogen peroxide and sodium sulphate on the Xylenol Orange complexes. It was found that with suitably adjusted conditions, aqueous mixtures of zirconium and hafnium could be accurately determined by a spectrophotometric masking technique. A separation scheme based on *p*-bromomandelic acid and ammonia separations was then applied to dissolved high-temperature alloy samples to isolate the zirconium and hafnium in a way which permitted the application of the new method.

EXPERIMENTAL

Reagents

Hafnium stock solution (1000 μg/ml). High-purity hafnium oxychloride ($HfOCl_2 \cdot 8H_2O$ 2.2943 g) was dissolved in 2*M* hydrochloric acid and diluted to 1 litre with the same acid.

Zirconium stock solution (1000 μg/ml). High-purity zirconium oxychloride ($ZrOCl_2 \cdot 8H_2O$ 3.5326 g) was dissolved in 2*M* hydrochloric acid and diluted to 1 litre with the same acid.

Zirconium working solution (100 μg/ml). An aliquot of zirconium stock solution was diluted with water (prepared fresh weekly).

Aluminium solution (5 mg/ml). High-purity aluminium rod (5.0000 g) was dissolved in 100 ml of concentrated hydrochloric acid and 25 ml of concentrated nitric acid. The cooled solution was diluted to 1 litre with water.

Xylenol Orange solution (1×10^{-3}M). Xylenol Orange (0.0761 g) was dissolved in water and diluted to 100 ml with water.

Sodium sulphate solution (1M). Anhydrous sodium sulphate (142.0 g) was dissolved in 800 ml of water with stirring and gentle heating. The cooled solution was diluted to 1 litre with water.

Perchloric acid (5M). Prepared from concentrated perchloric acid in 500-ml quantities.

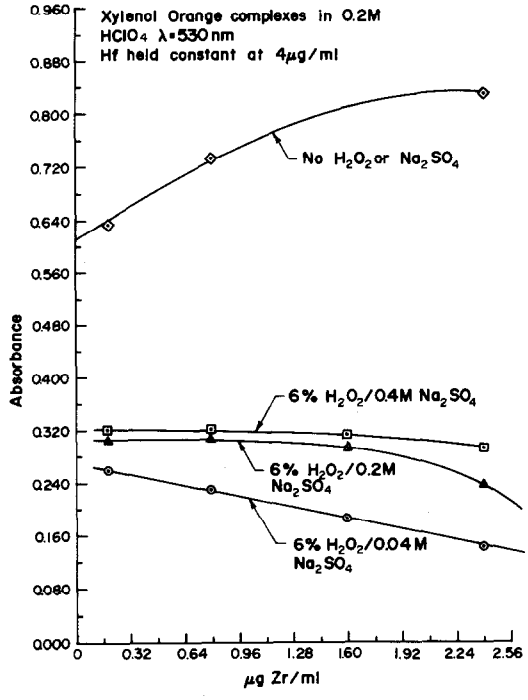

Fig. 1. Effects of hydrogen peroxide and sodium sulphate on Xylenol Orange complexes; mixtures of zirconium and hafnium.

Work with pure solutions

Cheng's studies[6] on the effect of hydrogen peroxide and sodium sulphate on the absorbance of mixtures of the zirconium and hafnium complexes with Xylenol Orange in 0.2*M* perchloric acid were repeated. In this study, the hafnium concentration was held at 4 μg/ml, while the zirconium concentration was varied between 0 and 2.4 μg/ml. Figure 1 shows the absorbance response of zirconium under four sets of conditions. In the absence of hydrogen peroxide and sodium sulphate, the apparent hafnium response increases with increasing zirconium concentration. When the same set of mixtures is examined in the presence of 6% hydrogen peroxide/0.04*M* sodium sulphate, the apparent hafnium response *decreases* with increasing zirconium concentration. Increasing the sulphate concentration produces a moderation of this effect until, with the combination of 6% hydrogen peroxide/0.04*M* sodium sulphate, the apparent hafnium response is nearly independent of zirconium concentration. Similar results were obtained by Cheng[6] who theorized that the hydrogen peroxide masks the zirconium complex completely and the hafnium complex slightly; sulphate "demasks" the hafnium complex, but only when the zirconium-complex is also present. The results in Fig. 1 give further support to this view.

Next, a series of hafnium calibration curves was prepared under the "optimized" conditions from Fig. 1 (6% hydrogen peroxide/0.4*M* sodium sulphate) with various fixed levels of zirconium. Despite the results depicted in Fig. 1, a definite effect on the hafnium calibration curve was noted for various levels of zirconium, with the greatest effect occurring between 0 and 0.8 μg of zirconium per ml. When single-element hafnium and zirconium curves were generated with use of the 6% hydrogen peroxide/0.4*M* sodium sulphate reagent (Fig. 2), the zirconium response became practically independent of zirconium concentration at around the 3–4 μg/ml level. The hafnium curve was observed to rise steeply under these conditions. On the basis of these data, it was decided to incorporate a fixed addition of zirconium (4 μg/ml) in a masking procedure for hafnium determination. The zirconium addition is intended to provide a uniform zirconium-complex background, independent of the zirconium content of the sample, against which to measure the hafnium response. This approach is analogous to the "swamping" technique used in flame photometry for overcoming interference (from anions forming thermally stable species with the analyte cation) by addition of the threshold amount of interferent to all samples and standards.

The effectiveness of this approach was verified by preparing a series of synthetic mixtures and analysing them as "unknowns." Values obtained for hafnium in the range 80–90 μg in the presence of 5–15 μg of zirconium were within 1% of the amount taken.

It remained to devise a way to extract a zirconium value from the chemistry of the Xylenol Orange complexes. It was established that in the absence of hydrogen peroxide and sodium sulphate, the complexes of hafnium and zirconium show nearly identical molar absorptivities in 0.2*M* perchloric acid, as well as a large range of linear response. The hafnium value obtained by the masking procedure, therefore, subtracted from the sum of hafnium and zirconium determined in a second run without the masking agents, was used to obtain a value for zirconium.

The method can be summarized as follows.

(1) Three calibration curves are prepared:

(*a*) Hf 0–6 μg/ml with 6% H_2O_2, 0.4*M* Na_2SO_4, 4 μg/ml Zr, 0.2*M* $HClO_4$, 8×10^{-5}*M* Xylenol Orange;

(*b*) Hf 0–6 μg/ml with 0.2*M* $HClO_4$, 8×10^{-5}*M* Xylenol Orange;

(*c*) Zr 0–2 μg/ml with 0.2*M* $HClO_4$, 8×10^{-5}*M* Xylenol Orange.

(2) The "unknowns" are analysed twice:

(*a*) once with 6% H_2O_2, 0.4*M* Na_2SO_4, 4 μg/ml Zr, 0.2*M* $HClO_4$, 8×10^{-5}*M* Xylenol Orange;

(*b*) once with 0.2*M* $HClO_4$, 8×10^{-5}*M* Xylenol Orange.

(3) The hafnium concentration of the "unknown" is derived from curve 1(*a*) and the absorbance obtained in 2(*a*).

(4) A "correction absorbance" is read from curve 1(*b*) using the hafnium concentration just obtained.

(5) The "correction absorbance" is subtracted from the absorbance obtained in 2(*b*) to derive the zirconium absorbance.

(6) The zirconium concentration of the "unknown" is derived from curve 1(*c*) and the zirconium absorbance.

Table 1 shows the results obtained for a series of synthetic mixtures of pure solutions by using this approach.

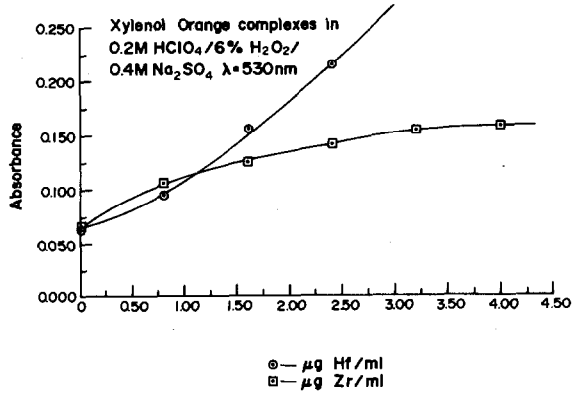

Fig. 2. Calibration curves for hafnium-only and zirconium-only, using the optimized concentrations of hydrogen peroxide and sodium sulphate.

Table 1. Results for mixtures of pure solutions (final solution volume 25 ml)

Test	Hf taken, μg	Hf found, μg	Zr taken, μg	Zr found, μg
A	60.0	59.5	10.0	10.6
B	90.0	91.0	5.0	5.8
C	100.0	96.5	15.0	15.5
D	100.0	100.0	5.0	5.4
E	120.0	120.0	5.0	5.0
F	90.0	89.7	10.0	10.6
G	110.0	108.3	10.0	10.8
H	80.0	78.1	20.0	19.5

Work with alloys

Application of the procedure to complex high-temperature alloys presents numerous difficulties: the corrosion resistance of the alloys, the tendency of certain matrix components to hydrolyse, and the very specific solution composition required by the new method. Both acid digestion and fusion/leach techniques have been developed which are effective in dissolving these alloys, but attempts to apply the masking approach directly fail owing to interference from the sample matrix. Complexing agents such as EDTA and citrate are ineffective in surmounting the interference.

Numerous separation schemes for isolation of the hafnium and zirconium from the alloy matrix were therefore investigated, including solvent extraction, mercury cathode electrolysis, and sodium hydroxide precipitation schemes. The most useful approach was found to be precipitation first with *p*-bromomandelic acid and then with ammonia.

Procedure

The method was developed specifically for iron and nickel-base alloys containing hafnium in the range 0.25–1.25% and zirconium in the range 0.05–0.30%. It is also applicable to alloys with 0.25–1.25% hafnium but no zirconium. Chromium, cobalt, molybdenum, aluminium, titanium, niobium and tungsten in the amounts normally encountered in high-temperature alloys do not interfere.

Isolation of zirconium and hafnium. Portions (0.2000 or 0.3000 g) of an appropriate matrix alloy are weighed into each of seven 125-ml Erlenmeyer flasks. (Note: if the samples to be analysed contain large amounts of hydrolysable elements such as titanium, niobium, molybdenum or tungsten, the matrix alloy is chosen to match the samples closely. Use of a master alloy containing no hafnium and zirconium is ideal; otherwise, pure iron or pure nickel can be used). The same weight of the samples is taken and 75 ml of concentrated hydrochloric acid are added to each flask. The flasks are covered with watch-glasses and allowed to stand on a warm hot-plate overnight or until the alloys have dissolved. The matrix alloy solutions are spiked with pure zirconium and hafnium solutions as follows:

Flask	μg of Hf	μg of Zr
1	—	—
2	500 or 1000	—
3	1000 or 1500	—
4	2000	—
5	—	100 or 150
6	—	300
7	—	600

The exact choice of spike-size depends on the concentrations of zirconium and hafnium expected in the samples, the sample and matrix weights taken, and the sensitivity of the given batches of reagents.

The solution volumes are reduced to about 30 ml by very gentle boiling and the flasks are cooled to approximately 40°C. Then 20 ml of concentrated perchloric acid and 20 ml of water are added to each. Next, 0.5 g of *p*-bromomandelic acid is added to each and the flasks are heated in a boiling water-bath for 20 min, then allowed to stand at room temperature overnight. The solutions are filtered through a hardened, fine-porosity filter paper (Whatman No. 542) containing a small amount of filter-paper pulp. The residues are washed thoroughly with water and the filters are transferred back to the original flasks. The organic material is destroyed by heating the filters with 50 ml of concentrated nitric acid and 10 ml of concentrated perchloric acid, over medium heat at first, then at higher temperature. Additional nitric acid is cautiously added, as necessary, *to ensure an excess at all times* (as evidenced by the evolution of dark brown fumes) until all organic matter has been destroyed. Then samples which are white or clear and evolving white fumes are heated for 1 min more, and cooled to room temperature. Next, 50 ml of water and 6 ml of aluminium solution (Al 5 mg/ml) and 30 ml of concentrated ammonia solution are added to each flask and the solutions are boiled gently for 1 min, then allowed to stand overnight. The precipitates are filtered off on glass fibre filters of medium porosity (Reeve Angel No. 934AH) folded and used like filter papers. The residues are washed five times with 1% v/v ammonia solution. The original flasks are placed under the funnels and 15.0 ml of 5*M* perchloric acid are transferred into each funnel by pipette. Next, approximately 25 ml of boiling water are added to each funnel. When the funnels have drained, 100-ml standard flasks are placed under the funnels and the solutions in the 125-ml Erlenmeyer flasks are poured through the corresponding filters in such a manner that the inner surfaces of the Erlenmeyers are wetted. The Erlenmeyer flasks are then returned to below the funnels and the solutions in the standard flasks are poured through the filters. The solutions are recycled in this way five times, then, with the 100-ml standard flasks under the funnels, the Erlenmeyer flasks and the filters are washed thoroughly with plain water. The solutions are diluted to the mark and mixed. If the solutions appear cloudy at this point they are filtered through a 0.22-μm filter (Millipore No. GSWP 04700) under suction, into a dry flask, then transferred to a dry storage flask without rinsing.

Colour development and spectrophotometry. Two sets of standard flasks are arranged—one set of 25-ml capacity and one set of 50-ml capacity. The 50-ml flasks are dried in an oven before use. Then a pipette is used to transfer a 7.0-ml portion of a sample into a 25-ml flask and a second into a 50-ml flask, for each sample in form.

To the 50-ml flasks the following reagents are added by pipette, in the sequence stated: 1500 μl of 5*M* perchloric acid; 1000 μl of zirconium working solution (Zr 100 μg/ml); 5.0 ml of 30% hydrogen peroxide; 10.0 ml of 1*M* sodium sulphate; 2.0 ml of $1 \times 10^{-3}M$ Xylenol Orange. The solutions are swirled to mix them thoroughly, *but not diluted.* After 30 min the absorbances are measured at 530 nm in 1.0-cm cells, with water as reference. To the 25-ml flasks 3.0 ml of 5*M* perchloric acid and 2.0 ml of $1 \times 10^{-3}M$ Xylenol Orange are added by pipette. The solutions are diluted to the mark and mixed thoroughly. After 30 min the absorbances are measured at 530 nm in 1.0-cm cells with water as reference.

Calculation of results. The absorbances obtained from the hafnium calibration solutions in the 50-ml standard flasks are corrected for the blank and plotted *vs.* hafnium concentration. The corrected absorbances for the "unknown" samples in the 50-ml standard flasks are translated into hafnium content by means of this plot.

The absorbances of the hafnium calibration solutions in the 25-ml standard flasks are corrected for the blank and plotted *vs.* hafnium concentration. The corresponding

Table 2. Nominal compositions (%) of high-temperature alloys studied

Element	AF115	Pyromet alloy* (CTX-2)	MERL 76	Astroloy
C	0.05	0.005 max.	0.02	0.04
B	0.02	0.01 max.	0.02	0.02
Cr	10.7	0.25 max.	12.5	15
Ni	55	38	55	55
Co	15	16	18.5	17
Mo	2.8	0.2 max.	3.2	5
Al	3.8	1	5	4
Ti	3.9	2	4.4	3.5
Nb	1.7	3	1.4	—
W	5.9	—	—	—
Fe	1 max.	40	—	0.5 max.
Hf	0.75	0.8	0.4	—
Zr	0.05	—	0.06	—

* Trademark of Carpenter Technology Corporation, registered in U.S.A.

"absorbance corrections" for the "unknown" samples are found from the hafnium contents and this curve, and then subtracted from the blank-corrected absorbances of the "unknown" samples in the 25-ml standard flasks. The difference is the absorbance for the zirconium in the "unknown" samples.

The absorbances of the zirconium calibration solutions in the 25-ml standard flasks are corrected for the blank and plotted *vs.* zirconium concentration. The "zirconium absorbances" calculated as above are then translated into zirconium content by means of the calibration plot.

RESULTS

Table 2 lists the nominal compositions of several alloy types to which the new method was applied. Table 3 lists the results obtained for hafnium and zirconium on samples of Astroloy matrix (no hafnium or zirconium) and AF115 Master Alloy (contains all the components of AF115 except hafnium and zirconium) which were spiked with known amounts of hafnium and zirconium before analysis. The hafnium level was chosen to simulate the specification level in AF115, and the zirconium was varied between 0.05 and 0.30% to check its effect on the hafnium determination (note: the zirconium range for AF115 is 0.05–0.07%). A real but slight effect was noted at 0.30% zirconium, a small positive error being produced in the hafnium results. This effect is shown to be negligible at the zirconium levels encountered in AF115 alloy.

While no primary standard materials are available which are certified for hafnium at these levels, the National Bureau of Standards SRMs 361–364 are certified for zirconium at levels which are determinable by the new method. These iron-base low-alloy standards were spiked with hafnium at the 1% level to simulate the analytical problem for which the method was designed. The results are shown in Table 4.

Table 4. Zirconium results for iron-base primary standards spiked with hafnium

Test	Sample	Certified Zr value, %	Zr found, %	Average Zr found, %
1	NBS 361 + 1% Hf	0.009	N.D.	—
2	NBS 362 + 1% Hf	0.19	0.16, 0.17	0.18
3	NBS 362 + 1% Hf		0.19, 0.19	
4	NBS 363 + 1% Hf	0.049	0.03, 0.03	0.04
5	NBS 363 + 1% Hf		0.06, 0.06	
6	NBS 364 + 1% Hf	0.068	0.06, 0.07	0.07
7	NBS 364 + 1% Hf		0.08, 0.06	

N.D.—none detected.

Table 3. Results for spiked high-temperature alloy samples

Test	Alloy matrix	Hf added, %	Hf found, %	Zr added, %	Zr found, %
1	Astroloy	0.75	0.76	0.05	0.04, 0.04
2	Astroloy	0.75	0.77	0.15	0.13, 0.12
3	Astroloy	0.75	0.77	0.30	0.25, 0.24
4	Astroloy	0.75	0.74, 0.75, 0.73, 0.74	0.05	0.04
5	Astroloy	0.75	0.79, 0.79, 0.75	0.30	0.23
6	AF115 Master Alloy	0.75	0.78, 0.72, 0.75	0.05	0.05, 0.08, 0.07
7	AF115 Master Alloy	0.75	0.82, 0.83, 0.82	0.30	0.30, 0.29, 0.29
8	AF115 Master Alloy	0.75	0.77, 0.78	0.05	0.11, 0.08

Astroloy:	grand average for Hf at all Zr levels:	0.76% Hf $2\sigma = \pm 0.04\%$.
AF115:	grand average for Hf at all Zr levels:	0.78% Hf $2\sigma = \pm 0.08\%$.
AF115:	average for Hf at 0.05% Zr added:	0.76% Hf $2\sigma = \pm 0.05\%$.

Table 5. Results from zirconium/hafnium-bearing high-temperature alloys: "given" values, except where otherwise noted, are from an ion-exchange procedure based on the work of Machlan and Hague[1,2]

Sample	Alloy type	Hf, given, %	Zr, given, %	Test	Hf found, %	Average Hf found, %	Zr found, %	Average Zr found, %
A01852	AF115	0.93	0.053	1	0.93, 0.89, 0.90	0.89	0.044, 0.067,	0.056
				2	0.87, 0.85		0.068, 0.045	
A01854	AF115	0.93	0.055	1	0.89, 0.88	0.88	0.066	0.063
				2	—		0.072, 0.051	
A01856	AF115	0.91	0.054	1	0.89, 0.94	0.91	0.038	0.048
				2	—		0.064, 0.042	
V03949B	CTX-2	0.79*	—	1	0.83, 0.88	0.84	—	—
				2	0.82, 0.82		—	
V04000T	CTX-2	0.73	—	1	0.70, 0.71	0.72	—	—
				2	0.73, 0.74		—	
V04000B	CTX-2	0.72	—	1	0.75, 0.78	0.75	—	—
				2	0.75, 0.73		—	
031580	MERL 76	0.39†	0.060†	1	0.42, 0.46, 0.44	0.43	0.049, 0.040,	0.053
				2	0.43, 0.41, 0.43		0.050, 0.063, 0.064, 0.052	

* X-Ray fluorescence result.
† Pratt and Whitney Aircraft result; sample obtained by courtesy of Pratt and Whitney Aircraft.

Table 5 shows the results from several different hafnium/zirconium-bearing high-temperature alloys, and also the results obtained by other techniques. The agreement is considered good in view of the speed of the new procedure relative to that of the modified Machlan and Hague procedure.

DISCUSSION

The new masking procedure for the simultaneous spectrophotometric determination of zirconium and hafnium has been designed for the range of these elements that occurs in some specific alloys which cannot be analysed by the method of Challis[7] (see Tables 2 and 5). Other ranges of these two elements can be determined by appropriate modification of sample weights and aliquot volumes but probably only within certain limits. Beyond these limits some modification of the chemistry would be required to achieve equivalent accuracy and precision. On the other hand, the robustness of the method is reflected in the results obtained for alloys with a difficult matrix, such as AF115.

The final form of the procedure specifies dissolution of the alloy in warm concentrated hydrochloric acid—a process which could require as long as a day, but has the advantage of minimizing the hydrolysis of elements such as titanium, niobium, molybdenum and tungsten. *p*-Bromomandelic acid is then used for selective precipitation of zirconium plus hafnium. Acid-insoluble material, if any, is retained with the precipitate and both are treated with nitric acid and fumed with perchloric acid. For the alloys investigated, this treatment proved adequate, though in other cases, where very refractory zirconium and hafnium inclusions are present in the alloy, a fusion step might be required. Next, an ammonia separation, with an aluminium carrier, removes traces of nickel, cobalt, molybdenum and tungsten and allows preparation of a sample solution with a precise perchloric acid concentration.

Trace amounts of components of the alloy matrix may survive these separations—notably niobium and titanium, which may precipitate before filtration of the *p*-bromomandelates. Any niobium dissolved in the final sample could interfere with the determination by producing a colour of its own, but its effect is minimized by the use of a precise concentration of perchloric acid.[9] Titanium interferes by forming a faint colour due to the peroxo-complex, but a suitable blank takes care of this effect. Precipitates of niobium and titanium interfere by their turbidity. When this occurs, a blank is usually not adequate for compensation and filtration of all samples is required.

The procedure described is believed to be the most rapid way to determine zirconium and hafnium in high-temperature alloys chemically (five samples have been analysed in as little as three days). The accuracy and precision have been shown to be adequate for the concentration ranges and the alloy matrices which were studied.

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DIAGRAMS FOR COMPLETE REPRESENTATION OF BINARY MONONUCLEAR COMPLEX SYSTEMS

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Summary—Solution equilibria are calculated from the system of equations expressing the mass balance and the law of mass action. The solution of these equations usually requires the use of complicated iterative procedures. It is shown in the present paper, that for mononuclear complex formation, the whole system can be represented by a single diagram exhibiting all of the important chemical information, including solubility equilibria. The data necessary for the construction of the diagrams can be calculated directly without iterative procedures.

Mononuclear stepwise complex equilibrium systems are most frequently represented by formation and distribution curves. It is well known that these functions depend only on the free ligand concentration.[1–4]

$$\bar{n} = \frac{T_L - [L]}{T_M} = \frac{\sum_0^N i\beta_i[L]^i}{\sum_0^N \beta_i[L]^i} \quad (1)$$

$$\alpha_i = \frac{[ML_i]}{T_M} = \frac{\beta_i[L]^i}{\sum_0^N \beta_i[L]^i} \quad (2)$$

where

$\bar{n}$ = average number of bound ligands (formation curve);
$\beta_i = [ML_i]/([M]\,[L]^i)$ = formation product of the ith species; $\beta_i = K_1K_2 \ldots K_i$, the product of the successive formation constants; $\beta_0 = K_0 = 1$ by definition; $K_i = [ML_i]/([ML_{i-1}][L])$;
N = maximum number of ligands;
$[L]$ = free ligand concentration;
T_L, T_M = total concentrations of the ligand and the metal ion, respectively;
α_i = partial mole fraction of the ith species (distribution curve).

The behaviour of the concentration distribution functions in this simple case is well known. The partial mole fraction of free metal ion ($i = 0$) decreases monotonically, while that of the complex containing the maximum number of ligands (ML_N, $i = N > \bar{n}$) increases monotonically with increasing free ligand concentration. Each of the distribution curves of the intermediate complexes (ML_i, $0 < i < N$) exhibits one maximum $[d(\ln\alpha_i)/d(\ln[L]) = 0]$ at the appropriate integral point of the formation curve.

The behaviour of the concentration distribution functions is much more complicated in three- and multicomponent systems. The distribution curves may then exhibit more than one extremum, as discussed by us earlier.[5,6]

CALCULATION AND USE OF EXTENDED DIAGRAMS

For mononuclear stepwise complex formation, distribution curves are very informative and useful; if the free ligand concentration is known, the distribution of the complexes is directly seen.

However, these curves are not very useful in practical situations, and particularly in analytical chemical practice, because generally only the total concentration of each component is known.

$$T_M = \sum_0^N [ML_i] = [M]\sum_0^N \beta_i[L]^i \quad (3)$$

$$T_L = [L] + [M]\sum_0^N i\beta_i[L]^i = [L] + \bar{n}T_M \quad (4)$$

If it is desired to calculate the species distribution at particular total concentrations, then first these nonlinear equations must be solved by an appropriate iteration procedure for [L] and [M]; only then may the distribution be calculated. If the concentration distribution is to be shown as a function of the total concentration, the iterative calculation must be

repeated several times. Therefore, from a practical point of view, it would be better if the distribution curves could be expressed as a function of the total concentrations.

It is shown in this paper that it is possible to relate the distribution of the complexes to the *total* concentration, by an appropriate extension of the $\bar{n},\alpha_i = f([L])$ curves.

The total concentrations of the components unambiguously determine the free ligand concentration. At the same time, at a constant ratio of the total concentrations, $q = T_L/T_M$, T_M may be expressed explicitly as a function of the free ligand concentration from equations (3) and (4):

$$T_M = \frac{[L]}{q - \bar{n}} = \frac{\sum_0^N \beta_i [L]^{i+1}}{\sum_0^N (q - i)\beta_i [L]^i} \qquad (5)$$

Values for the function $T_M = f([L])$ can be calculated directly with a simple programmable calculator, without iterative procedures. A useful diagram can then be obtained if the $\bar{n},\alpha_i = f(\log[L])$ and $\log T_M = f(\log[L])$ functions are drawn with a common

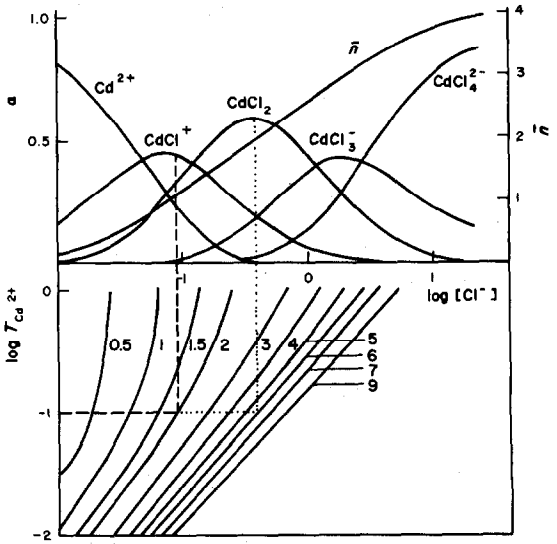

Fig. 1. Extended distribution diagram for the Cd^{2+}–Cl^- system. Upper part: formation curve and distribution of the complexes. Lower part: log $T_{Cd^{2+}}$ as a function of log[Cl^-] at different total concentration ratios. The numbers on the lines are the $T_{Cl^-}/T_{Cd^{2+}}$ values. For interpretation of the dashed and dotted lines, see text. Log $\beta_1 = 1.32$, log $\beta_2 = 2.22$, log $\beta_3 = 2.13$, log $\beta_4 = 1.68$. Medium 4.5*M* $NaClO_4$, $T = 298$ K.[11]

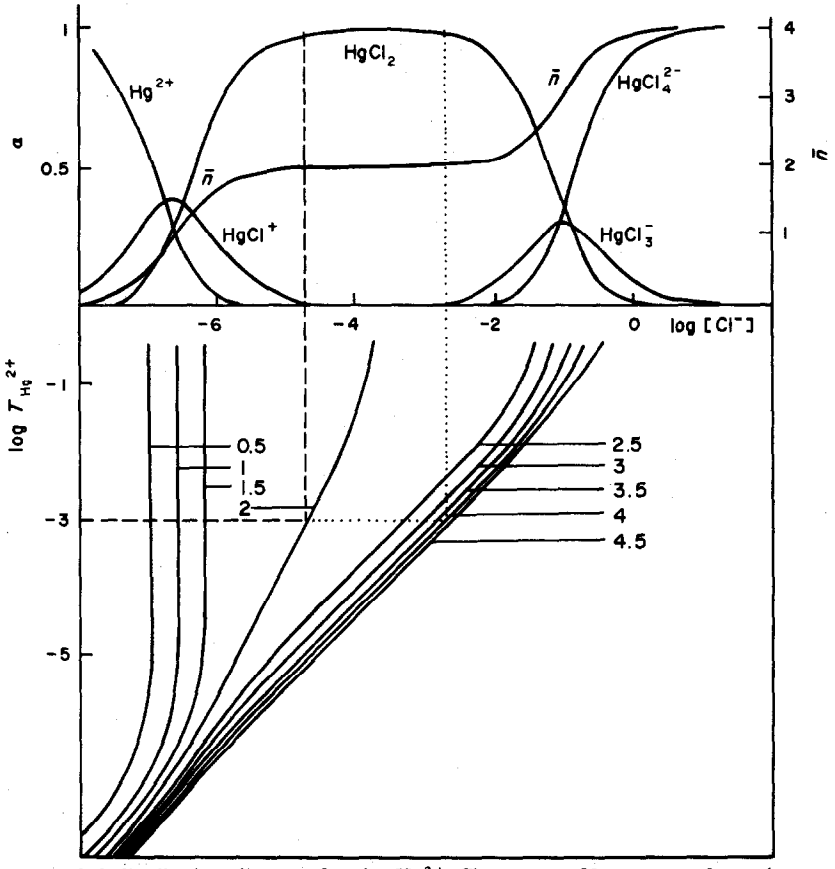

Fig. 2. Extended distribution diagram for the Hg^{2+}–Cl^- system. Upper part: formation curve and distribution of the complexes. Lower part: log $T_{Hg^{2+}}$ as a function of log[Cl^-] at different total concentration ratios. The numbers on the lines are the $T_{Cl^-}/T_{Hg^{2+}}$ values. For interpretation of the dashed and dotted lines, see text. Log $\beta_1 = 6.76$, log $\beta_2 = 13.24$, log $\beta_3 = 14.19$, log $\beta_4 = 15.24$. Medium 0.5*M* $NaClO_4$, $T = 298$ K.[12]

horizontal log[L] axis. Curves for a range of q values are drawn as illustrated in Fig. 1.

Figure 1 shows the extended distribution curves for the Cd^{2+}–Cl^- system. The upper part consists of the usual formation and distribution curves, while the lower part shows the function $\log T_{Cd^{2+}} = f(\log[Cl^-])$ at different $T_{Cl^-}/T_{Cd^{2+}}$ ratios. The thick line represents a solution of $CdCl_2$. The curves show the nature of the relationship between total and free ligand concentration. The amount of curvature towards the y-axis is indicative of the extent of complex formation. It can be directly read from the curves that, for example, in 0.1M cadmium chloride solution the dominant species are $CdCl^+$ (44%), $CdCl_2$ (31%) and free cadmium ion (23%) (see dashed line). If it is desired to prepare a solution in which the partial mole fraction of $CdCl_2$ is at its maximum, then ~0.4M potassium chloride should be added to 0.1M cadmium chloride (see dotted line).

The complexes formed in the Cd^{2+}–Cl^- system are relatively weak. Figure 2 shows the extended distribution curves for the Hg^{2+}–Cl^- system, in which strong $HgCl^+$ and $HgCl_2$ complexes are formed.

It is apparent from Fig. 2 that if either $HgCl_2$ or K_2HgCl_4 is dissolved at $10^{-3}M$ concentration in aqueous solution, $HgCl_2$ is the dominant species. It is seen moreover, that when $T_{Cl^-}/T_{Hg^{2+}} < 4$ the lines on the lower part of the figure approach asymptotically a certain log[Cl^-] value. The asymptotically vertical lines show that complex formation is practically complete; further increase in total concentrations (at constant $T_{Cl^-}/T_{Hg^{2+}}$) has no influence on the distribution of the complexes. The essential completeness of complex formation means that $\bar{n} \approx T_{Cl^-}/T_{Hg^{2+}}$ if $T_{Cl^-}/T_{Hg^{2+}} < 4$ and $\bar{n} = 4$ if $T_{Cl^-}/T_{Hg^{2+}} \geqslant 4$. Thus the log[$Cl^-$] value asymptotically reached is the one which belongs to the $\bar{n} = T_{Cl^-}/T_{Hg^{2+}}$ point of the formation curve. It has a definite value only if $\bar{n} < 4$, so asymptotically vertical lines represent the system for solutions in which $T_{Cl^-}/T_{Hg^{2+}} < 4$. If $T_{Cl^-}/T_{Hg^{2+}} > 4$, then log[Cl^-] increases monotonically as $T_{Hg^{2+}}$ increases.

These diagrams are much more informative and useful for practical purposes than the distribution curves alone. Two restrictions on their use, however, should be mentioned.

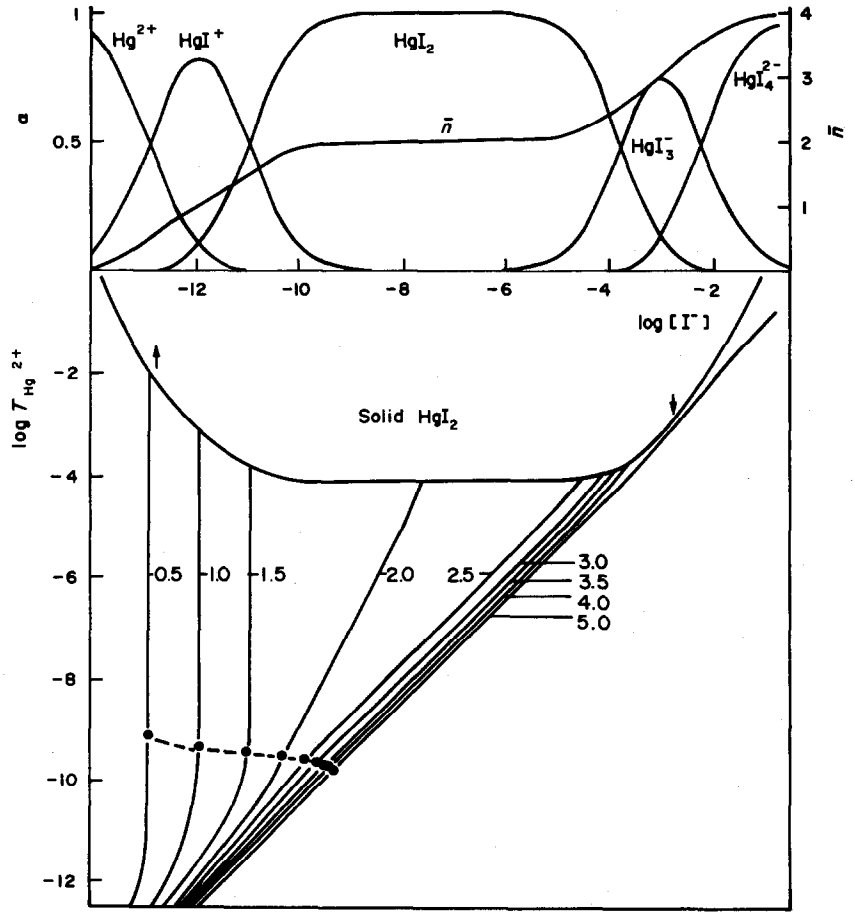

Fig. 3. Extended distribution diagram for the Hg^{2+}–I^- system. Upper part: formation curve and distribution of the complexes. Lower part: log $T_{Hg^{2+}}$ as a function of log [I^-] at different total concentration ratios, and the solubility curve. The numbers on the lines are the $T_{I^-}/T_{Hg^{2+}}$ values. Log β_1 = 12.87, log β_2 = 23.82, log β_3 = 27.60, log β_4 = 29.83, log K_s = −27.95. Medium 0.5M $NaClO_4$, T = 298 K.[13]

The β_i values used to calculate the curves are valid for a given standard state, ionic strength and temperature. The curves may be used reliably only under the same conditions. The change in total concentration along the curves causes a corresponding change in ionic strength, so the curves may be used only in a concentration range which is far less than the concentration of the background electrolyte. The requirement of keeping a constant standard state along the curves also means that the diagram can be used correctly only if dilution is done with the background electrolyte. Obviously the standard state cannot be kept constant during evaporation of the solvent, *i.e.*, if the solution is concentrated.

The other problem connected with the use of the curves is the solubility limit of the neutral complex. It is known, however, that solubility is also an unambiguous function of free ligand concentration only:[7]

$$S = \sum_{0}^{N} K_s \beta_i [\mathrm{L}]^{i-\nu} \qquad (6)$$

where

ν = the number of ligands in the neutral (sparingly soluble) complex;

$K_s = [\mathrm{M}][\mathrm{L}]^{\nu}$ = solubility product of the neutral ML_ν species.

Figure 3 shows the formation, distribution, solubility and total concentration curves for the Hg^{2+}–I^- system.

It is seen from this figure that mercuric iodide is precipitated even from $10^{-3}M$ K_2HgI_4 solution, but one equivalent of potassium iodide in excess is enough to prevent the precipitation. The individual points marked in the lower part are those that would be calculated for the solubility from the solubility product without taking into account the effect of the complex formation. The deviation of the real solubility curve from these points nicely illustrates how misleading the usual textbook-type calculation of solubility from the solubility product can be. This has already been dealt with in detail earlier.[8–10] The points marked by arrows represent the precipitation and dissolution of mercuric iodide when Hg^{2+} is added to $5 \times 10^{-3}M$ potassium iodide. At $T_{Hg^{2+}} < 10^{-3}M$, no precipitate is formed; precipitation begins at $T_{Hg^{2+}} \approx 1.2 \times 10^{-3}M$, but the precipitate dissolves if excess of mercury(II) is present ($T_{Hg^{2+}} > 10^{-2}M$).

Figure 4 shows the diagram for sparingly soluble lead iodide. It can be seen directly that lead iodide dissolves only in a very large excess of iodide. The $T_{I^-}/T_{Pb^{2+}} \approx 500$–$5000$ ratios are especially interesting, because the lines in this range may intercept the solid phase at two points. This means that if, for example, a clear solution for which $[KI] = 3M$ and $T_{Pb^{2+}} = 0.005M$ is diluted, then lead iodide first precipitates and then redissolves on further dilution.

It should be stressed that the precipitation of lead iodide on dilution differs in principle from the precipitation of hydrolysed species, which frequently occurs on dilution. In the latter case, the concentration of the reacting species (OH^-) increases on dilution, but in the Pb^{2+}–I^- system, the concentration of the reacting species (I^-) decreases. The precipitate is formed because lead iodide is formed by dissociation of PbI_4^{2-}

$$PbI_4^{2-} \rightleftharpoons PbI_2 + 2I^-$$

which is favoured by dilution.

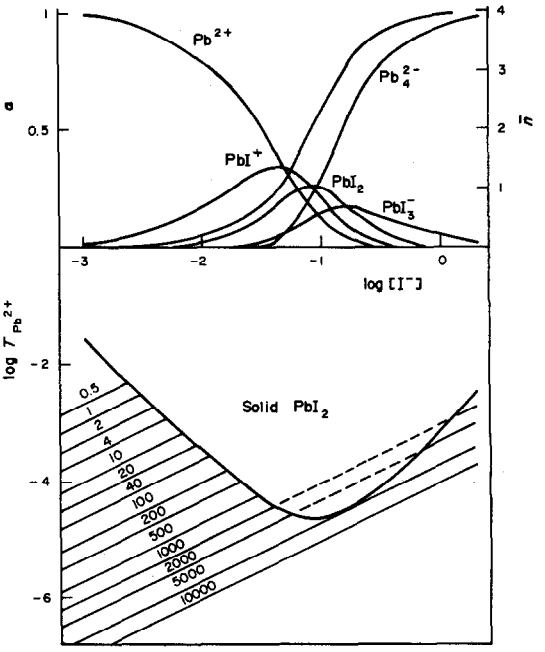

Fig. 4. Extended distribution diagram for the Pb^{2+}–I^- system. Upper part: formation curve and distribution of the complexes. Lower part: log $T_{Pb^{2+}}$ as a function of log[I^-] at different total concentration ratios. The numbers on the lines are the $T_{I^-}/T_{Pb^{2+}}$ values. log $\beta_1 = 1.30$, log $\beta_2 = 2.38$, log $\beta_3 = 3.14$, log $\beta_4 = 4.43$, log $K_s = -7.605$. Medium $2M$ $NaClO_4$, $T = 298$ K.[14]

In connection with the solubility curves some further points may be mentioned. One is that the minimum of the solubility curve is always found at $\bar{n} = \nu$ (*i.e.*, at $\bar{n} = 2$ for the cases considered).[2,3,7]

Another concerns the point at which the curve log $T_M = f(\log[L])$ is tangential to the solubility curve. This saturated solution has the interesting property that both dilution and concentration remove the saturated state. It is characterized by $T_M = S$ and $dT_M/d[L] = dS/d[L]$. Thus

$$\frac{dS}{dT_M} = \frac{dS}{d[L]} \cdot \frac{d[L]}{dT_M} = \sum_{0}^{N} K_s(i-\nu) \times \beta_i [L]^{i-\nu-1} \frac{d[L]}{dT_M} = 1 \qquad (7)$$

Taking into account that $qT_M - [L] = \bar{n}T_M$ along the curves log $T_M = f(\log[L])$, $d[L]/dT_M$ may be expressed as

$$\frac{d[L]}{dT_M} = q - \bar{n} \qquad (8)$$

Substituting this into equation (7) and multiplying both sides by $[M][L]^{\nu+1} = K_s[L] = K_s(q - \bar{n})S$, followed by simplifying gives:

$$\sum_0^N [M]i\beta_i[L]^i - \nu \sum_0^N [M]\beta_i[L]^i = S \qquad (9)$$

The sums on the left-hand side are $T_L - [L] = \bar{n}S$ and $T_M = S$. Substitution and rearrangement lead to the simple expression

$$\bar{n} = \nu + 1 \qquad (10)$$

Thus, the point of contact is at the maximum for formation of the first anionic complex $ML_{\nu+1}$. This further implicitly means that interception of the solid phase area, *i.e.*, precipitate formation by dilution, may be expected only if $N \geq \nu + 2$.

Exactly similar extended distribution diagrams can be drawn to represent acid–base systems. For the three-component proton–metal–ligand systems the extended diagrams may only be drawn in the form of two-dimensional cross-section of the full three-dimensional distribution diagram. The same applies for mixed-ligand complex formation involving two different ligands.

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ANALYTICAL APPLICATIONS OF PHOTOCHEMICAL REDUCTION OF AZUR B BY EDTA—IODIDE DETERMINATION

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Summary—A kinetic study of the photochemical reaction of Azur B and EDTA (in the absence of oxygen) has been made in connection with development of a new kinetic method for iodide. The reaction is first-order with respect to the dye, EDTA and absorbed light-intensity. The rate of photoreduction is strongly pH-dependent, and maximal at about pH 6.8. The photoreduction involves a long-lived excited state of the dye and is dramatically retarded by small amounts of iodide. A tentative mechanism is proposed, and the experimental conditions have been optimized. The variable time method appear to be the most suitable. A detection limit of 1.0 μg/ml and a coefficient of variation of about 3% can be achieved. Chloride and bromide do not interfere at levels below 100-fold mole ratio to iodide. Metal ions do not interfere if enough excess of EDTA is used. Coloured species may interfere at high concentration (filter effect).

Light-induced oxidations of aminopolycarboxylic acids by certain dyes belonging to the groups of thiazines, oxazines and phenazines have been repeatedly studied[1–4] and are the basis for several analytical determinations.[5–8] Surprisingly, EDTA shows reducing character in such photochemical processes, whereas its oxidation under normal circumstances requires the use of powerful oxidizing agents in hot and strongly acid media.

The overall light-induced reactions of EDTA with the dyes of the above-mentioned groups are irreversible and lead to the dye in its leuco form and to various oxidation products.[9–11] The mechanism of such photochemical reactions is complex and has been reported[12–15] as taking place through a series of collisions between the dye in its triplet state and specific forms of the aminopolycarboxylate anion.

This paper reports studies on the photochemical reaction of 3-dimethylamine-7-methylaminephenothiazonium chloride (Azur B) and EDTA. It has been found that the reaction is strongly inhibited by even small amounts of iodide. We have based a new method for determination of traces of iodide on this reaction.

EXPERIMENTAL

Reagents

Analytical-reagent grade chemicals and demineralized distilled water were used throughout. Aqueous Azur B solutions were prepared from the Merck product, purified by the method of Bonneau *et al.*[16] and standardized with standard sodium dithionite solution. EDTA and iodide solutions were made from the required amounts of the compounds. Buffer solutions were prepared by mixing a solution which was 0.1*M* in phosphoric, acetic and boric acids with appropriate amounts of 1*M* sodium hydroxide, and checked with a Radiometer PHM-63 pH-meter.

Apparatus

In the photolysis device the light from the lamp was passed through a small water-cooled chamber arranged so that several neutral density filters could be used to give different light intensities. Two lamps were used as sources, a low-pressure sodium lamp (Osram, 220 V, 90 W) and a halogen lamp (Sylvania, 24 V, 150 W). The emission spectra of the lamps were obtained with a Beckman DK2A spectrophotometer. The bleaching of Azur B was followed with a photometric titration unit (EEL-Unigalvo 200), a 607 filter being used. The incident intensity, I_0, was measured with a thionine–EDTA actinometer.[17]

Procedure

To the reaction cell of the photometric titration unit, add 5 ml of $10^{-4}M$ Azur B, 2 ml of 0.1*M* EDTA, 3 ml of pH-4.6 buffer and enough standard potassium iodide solution to give a final iodide concentration between 1 and 25 ppm. Dilute with demineralized distilled water to 25 ml. Deoxygenate the solution by bubbling pure nitrogen (99.99%) through it for 20 min. Switch on the halogen lamp and measure the time needed for the absorbance to be reduced to a tenth of its initial value.

Calibration graphs are constructed by plotting the iodide concentrations *vs.* t_x/t_0, where t_x is the time required for photoreduction of the sample, and t_0 the time required for an iodide-free sample. The illumination intensity chosen should give a value of about 100 sec for t_0.

Iodide in samples is determined in the same way. The reagent concentrations and light intensity must be kept constant throughout a series of measurements.

RESULTS AND DISCUSSION

When a solution containing Azur B and EDTA is illuminated at suitable pH and in the absence of oxygen, photoreduction of the dye occurs, and the blue colour disappears, but this only takes place at a fair rate when a sufficiently intense light source is used.

$$\text{Azur B}_{ox} + \text{EDTA} \rightarrow \text{Azur B}_{red} + \text{EDTA oxidation products} \quad (1)$$

A sodium lamp was used as excitation source for studying the photochemical reaction; its emission band fell within the Azur B absorption range (Fig. 1).

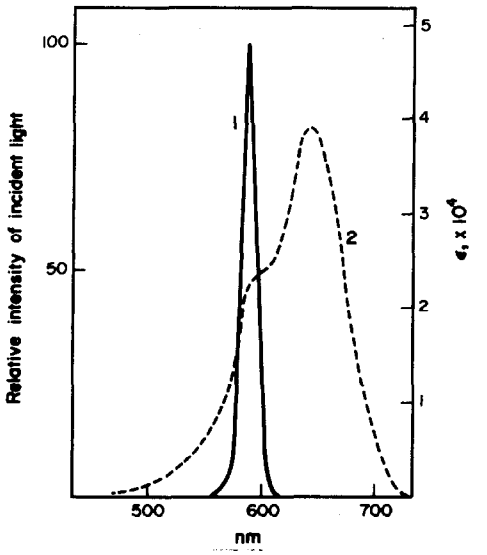

Fig. 1. Curve 1: spectral intensity of the light source. Curve 2: absorption spectrum of Azur B.

Figure 2 shows the absorption spectrum of the dye before photoreduction and the spectra obtained after reoxidation with oxygen or hydrogen peroxide. The three spectra coincide, showing that the Azur B does not undergo demethylation or irreversible breakdown during the photochemical reaction.

Stoichiometry

The stoichiometry was determined by adding excess of EDTA, at various pH values, photolysing until the dye was completely decolorized, and titrating the surplus EDTA with zinc solution or adding excess of zinc and measuring the surplus polarographically. The molar ratio found was 2:1 Azur B:EDTA.

Reagent concentrations

The initial-rate and integration techniques were used for determination of the order of reaction with respect to Azur B. The dye concentration should not exceed $3 \times 10^{-5}M$, to avoid any aggregation.[18] For the integration method the diffusion current corresponding to reduction of Azur B_{ox} on a mercury drop electrode (at *ca.* −0.5 *vs.* S.C.E.) was used. In the initial-rate method, a fixed amount of chromium(VI) was added, which reoxidized the Azur B_{red} in a fast reaction. The time needed to reduce all the chromium(VI) was dependent on the Azur B concentration (for fixed light-intensity). The results of both methods (Fig. 3 and Table 1) show a first-order dependence on Azur B concentration.

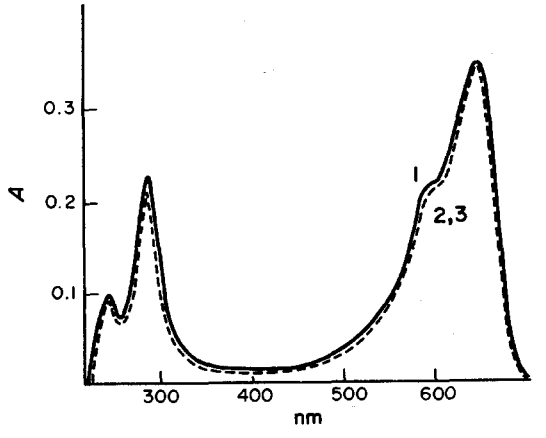

Fig. 2. Absorption spectra for $10^{-5}M$ Azur B/$10^{-2}M$ EDTA/acetate buffer (pH 5.5). Curve 1: Before the photochemical process. Curves 2 and 3: After the photochemical process and reoxidation with oxygen and hydrogen peroxide, respectively.

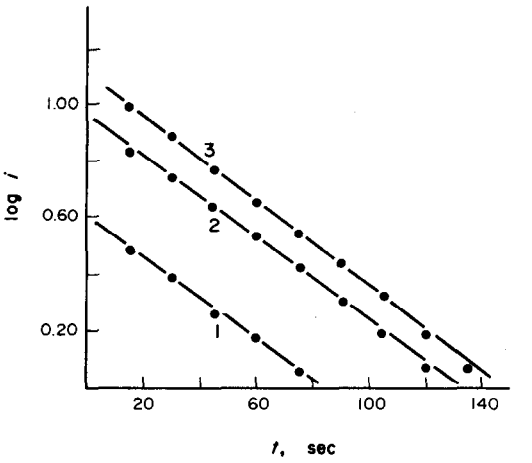

Fig. 3. Plots of logarithm of current *vs.* time. EDTA $4 \times 10^{-2}M$, acetate buffer pH 5.5; $T = 25°C$. Curve 1: Azur B $2 \times 10^{-6}M$. Curve 2: Azur B $4 \times 10^{-6}M$. Curve 3: Azur B $6 \times 10^{-6}M$.

The overall reaction order was also determined by the integration method polarographically. The results in Table 2 show an overall reaction order of 2, so the reaction is also first-order with respect to EDTA.

Effect of pH

Figure 4 shows that the reduction rate is highest at pH 6.8 and this was found to hold for all EDTA concentrations tested.

Effect of temperature

Table 3 lists the decolorization times for identical samples at different temperatures, showing that the rate increases with temperature.

Table 1. Values of the experimental pseudo first-order constant for different initial Azur B concentrations

[Azur B], μM	K, min^{-1}
9.0	3.3
8.0	3.0
7.5	3.2
7.0	3.4
6.5	3.6
6.0	3.1
5.5	3.3
5.0	3.2

[EDTA] = 0.02M; Cr(VI) = 5 μeq; acetate buffer pH 5.5; $T = 25°C$.

Table 2. Values of the experimental overall second-order constant

Photolysis time, t, *sec*	Current, *nA*	[Azur B_{red}], μM	K, $l.mole^{-1}.min^{-1}$
120	32.7	2.4	160
240	28.7	4.4	156
360	24.9	6.3	159
480	20.9	8.3	169
600	17.3	10.1	178

a = [Azur B_{ox}] = $2.0 \times 10^{-5} M$;
b = [EDTA] = $4.0 \times 10^{-4} M$;
X = [Azur B_{red}]; $K = \frac{1}{t(a-b)} \ln \frac{b(a-X)}{a(b-X)}$.

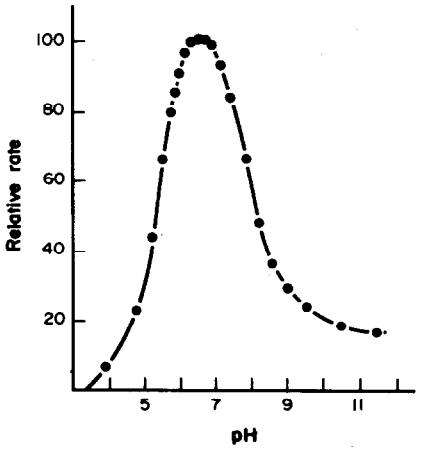

Fig. 4. Rate of photoreduction as a function of pH.

Influence of light-intensity and quantum yield

The rate of the photochemical reaction follows the general equation:

$$V = -\frac{dc}{dt} = KI_a^n \tag{2}$$

in which n denotes the reaction order with respect to the absorbed light-intensity I_a (expressed in Einstein.l^{-1}.sec^{-1}), c being the molarity of the absorbing species, and K a proportionality constant.

We have used the Fornier and Faure method[19] to determine n directly from the kinetic curves. From the Lambert-Beer law, equation (2) can be written:

$$V = -\frac{dc}{dt} = KI_0^n(1 - e^{-2.3\epsilon lc})^n \tag{3}$$

in which I_0 is the incident intensity, and ϵ the molar absorptivity of Azur B. The integrated equation is:

$$-\int_{c_0}^{c} \frac{dc}{(1 - e^{-2.3\epsilon lc})^n} = KI_0^n \int_0^t dt \tag{4}$$

From the values of ϵlc at various times, the values of the integral as a function of time can be plotted for different n values (*e.g.*, $\frac{1}{2}$, 1, $\frac{3}{2}$, 2, $\frac{5}{2}$). The straight line satisfying equation (4) gives the value of n.

Since the light-source is not monochromatic, a mean molar absorptivity has to be obtained by integrating over the waveband of the photolysis radiation used:

$$\bar{\epsilon} = \frac{\int_{\lambda_1}^{\lambda_2} \epsilon_\lambda I_\lambda^0 \, d\lambda}{\int_{\lambda_1}^{\lambda_2} I_\lambda^0 \, d\lambda} \tag{5}$$

in which ϵ_λ and I_λ^0 are the molar absorptivity and incident intensity at wavelength λ. The ϵ curve cannot be expressed analytically so a numerical integration has to be performed on the data in Fig. 1. We have found an $\bar{\epsilon}$ value of 2.20×10^4 $l.mole^{-1}.cm^{-1}$ for the range 555–600 nm.

The decolorization of Azur B by EDTA in the absence of oxygen and at different I_0 values was monitored polarographically by measurement of the dye reduction current. Equation (4) shows that the rate of the photochemical reaction is a linear function of the light-intensity:

$$K = \frac{-dc/dt}{I_a} \tag{6}$$

so K is the same as the quantum yield Φ since it represents the number of molecules transformed by each photon in a given time. Table 4 shows the quantum yield at three pH values and for different concentrations of EDTA.

Reaction mechanism

From the above, we can say that the photochemical reaction of Azur B and EDTA in the absence of oxygen takes place through a series of collisions between EDTA molecules and the dye molecules in an excited state. The strong influence of pH on the reaction rate (Fig. 4) can be explained by assuming that the only reacting species—as happens with other aminopolycarboxylic acids and dyes[1,5–8]—are the ionic forms of EDTA having at least one non-protonated nitrogen atom, and Azur B in its excited triplet state.

The photoreactive state of the dye is probably the triplet[1–4] since EDTA in low concentration does not quench the dye fluorescence although it is oxidized by

Table 3. Effect of temperature on photochemical process

Temperature, °C	20	25	30	35	40	45	50
Photo-bleaching, time, *sec*	308	200	127	98	73	56	47

[EDTA] = $1.6 \times 10^{-2} M$, [Azur B] = $4.0 \times 10^{-6} M$; acetate buffer pH 5.5.

Table 4. Values of the overall quantum yield (Azur B $10^{-5}M$)

[EDTA], M	Yield pH 7.25	pH 9.0	pH 10.0
0.040	4.16×10^{-2}	3.96×10^{-2}	3.10×10^{-2}
0.020	4.10×10^{-2}	3.71×10^{-2}	3.00×10^{-2}
0.010	4.04×10^{-2}	3.15×10^{-2}	2.95×10^{-2}

the activated dye. Further, small amounts of *p*-phenylenediamine or iodide do not quench the dye fluorescence either, but both substances dramatically retard the photoreduction of Azur B by EDTA. Obviously this is due to an energy-transfer process between the dye in the triplet state—which has a relatively long life—and the inhibitor.

According to our experimental results, we can assume the following kinetic scheme.

	Process	*Rate*
1.	Azur $B(S_0) + h\nu \longrightarrow$ Azur $B(S_1)$	K
2.	Azur $B(S_1) \longrightarrow$ Azur $B(S_0)$	$K_{ic}[S_1]$
3.	Azur $B(S_1) \longrightarrow$ Azur $B(T_1)$	$K_{isc}[S_1]$
4.	Azur $B(T_1) \longrightarrow$ Azur $B(S_0)$	$K'_{isc}[T_1]$
5.	Azur $B(T_1)$ + EDTA $\longrightarrow$ products	$K_r[T_1][EDTA]$
6.	Azur $B(T_1) + I^-(S_0) \longrightarrow$ Azur $B(S_0) + I^-(T_1)$	$K_e[T_1][I^-]$
7.	Azur $B(S_1) \longrightarrow$ Azur $B(S_0) + h\nu_f$	$K_f[S_1]$
8.	Azur $B(T_1) \longrightarrow$ Azur $B(S_0) + h\nu_p$	$K_p[T_1]$

where the quantities S_0, S_1 and T_1 are the ground and excited singlet states and the triplet state respectively, and the subscripts ic, isc, r, e, f and p mean internal conversion, intersystem crossing, reduction, excitation, fluorescence and phosphorence respectively.

Iodide determination

Taking into account the proposed kinetic scheme and applying the steady-state hypothesis for the concentration of Azur B in the triplet state, we have

$$[T_1] = \frac{K_{isc}[S_1]}{K_p + K'_{isc} + K_r[EDTA] + K_e[I^-]} \quad (7)$$

The rate of conversion of the dye into its leuco form is controlled by the rate of the chemical reaction step. This is equal to $K_r[T_1][EDTA]$ and so the expression for the quantum yield for the consumption of Azur B is:

$$\phi = \frac{K_r[T_1][EDTA]}{K} \quad (8)$$

Substituting for $[T_1]$ from equation (7) and rearranging gives:

$$\frac{1}{\phi} = \frac{K}{K_{isc}[S_1]}\left(1 + \frac{K_d}{K_r[EDTA]} + \frac{K_e[I^-]}{K_r[EDTA]}\right) \quad (9)$$

where K_d is equal to $K_p + K'_{isc}$. If the possibility of a reverse intersystem crossing ($T_1 \rightarrow S_1$, non-radiative) is considered, K_d will contain an additional constant, K''_{isc}, for this process, but this transition is regarded as unlikely.

In the absence of iodide, equation (9) reduces to:

$$\frac{1}{\phi^0} = \frac{K}{K_{isc}[S_1]}\left(1 + \frac{K_d}{K_r[EDTA]}\right) \quad (10)$$

where ϕ^0 is the quantum yield for the consumption of Azur B_{ox} in the absence of quencher. Dividing (9) by (10) we obtain:

$$\frac{\phi^0}{\phi} = 1 + \frac{K_e[I^-]}{K_d + K_r[EDTA]} \quad (11)$$

For measurements made with a fixed light intensity, with and without iodide, we have:

$$\frac{V^0}{V_x} = \frac{\phi^0}{\phi} = 1 + \frac{K_e[I^-]}{K_d + K_r[EDTA]} \quad (12)$$

V_x and V^0 being the reaction rates with and without iodide.

Operating with a large excess of reducing agent so that its concentration can be deemed constant, we have:

$$\frac{V^0}{V_x} = 1 + K[I^-] \quad (13)$$

This equation allows us to determine iodide by measuring the rate of the photochemical process in its presence and absence. The reaction conditions need to be optimized, as follows.

Figure 5 shows the inhibition of the photochemical reaction as a function of pH. The pH range 4–5 gives a high degree of inhibition, coupled with a moderate rate for the photoreduction (Fig. 4). The system needs careful buffering, however.

The decolorization time decreases with increasing EDTA concentration, but there is no further decrease for concentration ratios of EDTA to Azur B greater than 200.

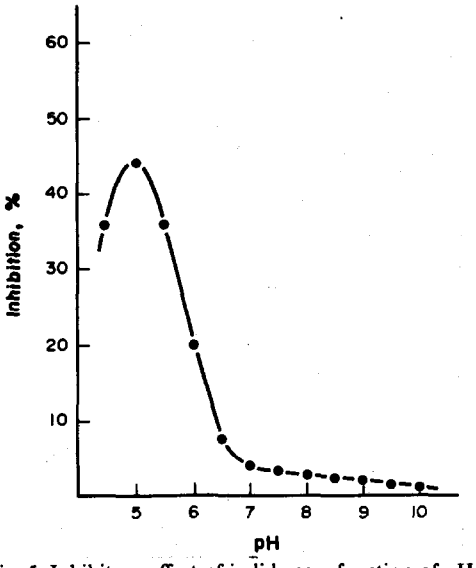

Fig. 5. Inhibitory effect of iodide as a function of pH.

Influence of iodide concentration

Equation (13) shows that iodide can be determined by means of the rate of reduction of Azur B by EDTA if the variables of the photochemical process are controlled.

We decided to use the variable time method, measuring the time required for the sample to reach a preselected absorbance (one tenth of the initial value).

To keep the process simple we used a white-light source (a halogen lamp). It gave the same results as a low-pressure sodium lamp. The method is reasonably precise under the conditions described, the maximum deviation being 3%. The limit of detection is 1.0 μg/ml. For lower levels of iodide the error increases since the decolorization time of the test sample is too close to that of the control sample. On the other hand, for iodide concentrations above 25 ppm the decolorization time is too long, and it is advisable to dilute the sample.

Interferences

Chloride and bromide do not interfere when present in up to 100-fold molar ratio to iodide. This precludes use of the method for analysing chlorides and bromides for traces of iodide.

If metal ions forming stable EDTA complexes are present, a preliminary titration with EDTA is required so that enough can be added to the test sample to fix the metal ions as chelates and leave a sufficient amount of free EDTA.

Table 5.

Sample	Iodide found, % Azur B/EDTA	Starch–iodine
I	0.024	0.022
II	0.032	0.030
III	0.034	0.035
IV	0.041	0.042

Table 6.

Salt \ Salt concentration	Iodide found, 0.02*M*	0.05*M*	0.2*M*
Na_2SO_4	4.0	4.1	3.9
K_2SO_4	4.1	4.2	3.8
KNO_3	4.0	4.3	4.1
KH_2PO_4	4.2	4.2	4.1

Coloured solutes will interfere if present in concentrations high enough to reduce the intensity of the radiation incident on the Azur B, and should be removed beforehand.

Applications

We have applied the method to determination of iodide in potassium sulphate, using 0.1–0.5 g of sample dissolved in a little doubly distilled water, and applying the procedure already given. The iodide was also determined by the starch–iodide method. The results are shown in Table 5.

The method was also applied to solutions of reagent-grade salts, containing 4.0 μg of iodide per ml. The results (average of three determinations are given in Table 6.

Finally a photographic solution containing sodium carbonate (10 g/l.), sodium sulphate (100 g/l.), potassium bromide (0.5 g/l.) and potassium iodide (0.01 g/l.) was analysed after acidification and heating to remove SO_2. The result (10.0 μg/ml) was in excellent agreement with the nominal content and also with the result obtained by the starch–iodine method (9.95 μg/ml).

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FLAME ATOMIC-ABSORPTION DETERMINATION OF BERYLLIUM AFTER EXTRACTION FROM NH_4SCN MEDIUM WITH TRIOCTYLAMINE IN MIBK

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Summary—A study has been made of the experimental conditions, errors, sensitivity, limits of detection and linear response range in the determination of beryllium by AAS in a nitrous oxide–acetylene flame, with aqueous or aqueous organic solutions or MIBK solutions containing the ion-pair $[Be(SCN)_4^{2-}][(R_3NH)_2^{2+}]$. The interference of Al, Mg, Ti, V, Fe, Ca, Mn, Na, K, SiO_3^{2-}, PO_4^{3-}, Cl^-, SO_4^{2-}, F^-, ClO_4^-, $H_2BO_3^-$ and SCN^- has been studied, and methods established for eliminating that of titanium, aluminium, fluoride and silicon.

The development of electronic and nuclear technology during the last two decades has resulted in beryllium becoming an important constituent of several materials (computer components, X-ray tube windows, *etc.*). The incidence of beryllium as an environmental pollutant ("berylliosis" or Fabroni's disease) has meant intensified study of methods for analytical control in the ng/ml range.[1]

Atomic-absorption spectrophotometry (AAS) with low-temperature flames (such as air–acetylene) is reported to give 1% absorption for a 300 μg/ml beryllium solution.[2] Use of a fuel-rich oxygen–acetylene flame gives 1% absorption with a 0.2-μg/ml ethanolic solution of beryllium.[3] A highly reducing nitrous oxide–acetylene flame gives the same response with a 0.02 μg/ml solution,[4] with a linear response range of 0.03–4 μg/ml.[5]

The effect of low concentrations of miscible organic solvents on AAS determination of beryllium in the air–acetylene flame has been reported,[6] and the effect of alcohols, ketones and glycols on AAS determination of beryllium in the nitrous oxide–acetylene flame has been also described.[7] Ramakrishna *et al.*[8] claimed improved sensitivity with use of a 10% aqueous solution of diethylene glycol diethyl ether, and studied the interference of several substances at low concentrations (up to 0.1 mg/ml). The effect of high concentrations of various metal ions (up to 10 mg/ml) has been described by Fleet *et al.*[9]

The main interfering elements suppressing the AAS signal of beryllium are aluminium and silicon. These elements form non-volatile compounds with the beryllium, which therefore cannot be effectively atomized even in the nitrous oxide–acetylene flame. Ramakrishna *et al.*[8] used addition of fluoride to eliminate the depressive effect of aluminium. Fleet *et al.*[9] recommended the addition of 8-hydroxyquinoline for this purpose, and Nakahara *et al.*[7] used diethylene glycol monobutyl ether. All these procedures give a satisfactory result up to about 4 mg of aluminium per ml.

Alternatively extraction has been used to isolate beryllium prior to its flame or flameless atomic-absorption determination either directly in the organic phase or after stripping. These methods are generally based on the extraction of acetylacetone complex into xylene,[10] benzene[11] or chloroform.[12–14] Methyl isobutyl ketone (MIBK) has also been used for extracting the acetylacetone complex, for determination by flameless AAS[15] or direct nebulization into the nitrous oxide–acetylene flame.[16,17]

The use of liquid ion-exchangers, especially ammonium quaternary salts, as extracting agents from thiocyanate or other medium is receiving increased attention for isolating beryllium. Tri-n-octylamine and tri-iso-octylamine,[18,19] 1-(3-ethylpentyl)-4-ethyloctylamine sulphate,[20,21] and tricaprylmethylammonium chloride in oxalic acid medium[22,23] have all been used to extract beryllium into n-hexane and toluene. Novoselova *et al.*[18] used MIBK as the solvent, and worked out suitable pH values for separation of binary mixtures of beryllium with Al, Mg, Zn or Mn.

The present work is intended to be both a contribution to the study of the AAS determination of beryllium in the nitrous oxide–acetylene flame, when added in mixtures of miscible organic solvents, and to the determination of interference effects of those species which frequently accompany beryllium in samples. A study was made of the extraction of beryllium from ammonium thiocyanate medium with trioctylamine–MIBK solution, and AAS determination by direct nebulization of the organic phase, with a view to eliminating interference and increasing the preconcentration and the nebulization and atomization efficiency.

EXPERIMENTAL

Apparatus

A Pye Unicam SP-9 spectrophotometer was used with a Westinghouse beryllium hollow-cathode lamp and a single-slot burner (50 mm path-length). The working wavelength was 234.86 nm.

Reagents

Stock beryllium solution (1000 μg/ml) was prepared by dissolving beryllium oxide (analytical grade) in 2*M* sodium hydroxide and immediately acidifying with 3*M* hydrochloric acid. Working solutions were obtained by suitable dilution of the stock solution just before use.

Stock solutions of interfering elements were obtained from pure chemicals, as follows: Al^{3+} from Al metal, Mg^{2+} from $Mg(NO_3)_2$, Ti^{4+} from TiO_2, V(V) from NH_4VO_3, Fe^{3+} from electrolytic Fe, Ca^{2+} from $CaCO_3$, Mn^{2+} from $Mn(NO_3)_2$, Na^+ from NaCl, K^+ from KCl, SiO_3^{2-} from Na_2SiO_3, PO_4^{3-} from Na_2HPO_4, Cl^- from KCl, SO_4^{2-} from K_2SO_4, F^- from NaF, ClO_4^- from $NaClO_4$, $H_2BO_3^-$ from H_3BO_3, SCN^- from NH_4SCN.

The remainder of the reagents used, $La(NO_3)_3$, trioctylamine, MIBK, acetone and ethanol were analytical grade.

Procedures

These are implicit in the text below.

RESULTS AND DISCUSSION

Atomic-absorption determination of beryllium in different media

A study was made of the conditions, errors, sensitivity, limit of detection and interval of linear response, for AAS analysis of aqueous, aqueous acetone, and aqueous alcohol media, and for an MIBK solution of triocytylamine, used for extracting beryllium from ammonium thiocyanate medium. Results are shown in Table 1.

From the results obtained, the optimum flame composition, slit-width and lamp current do not vary appreciably for the different media used, mainly because a strongly reducing flame was used. However, the height of observation in the flame is more critical and has to be adjusted to suit the solvent medium used. The standard deviation and the errors were larger than those generally obtained in AAS. The error of 2.6% for the water–acetone (20:80) medium was due to the increase in background noise both for the base line and the signal maximum. The sensitivity obtained in aqueous medium (0.033 μg/ml for 1% absorption) was the same as found by other authors, and improved as a function of increase in concentration of miscible organic solvent, becoming maximal for water–acetone (20:80) and for the MIBK–trioctylamine system. With this last system the sensitivity could be improved still further (to 0.001 μg/ml for 1% absorption) by using a 1:10 volume ratio of organic phase to aqueous phase, the sensitivity becoming comparable to that obtained by electrothermal atomization in a graphite furnace.

The use of organic solvents miscible with water did not improve the limit of detection, however, because of the increased background noise level. On the other

Table 1.

Medium	C_2H_2 flow-rate, *l./min*	N_2O flow-rate, *l./min*	Slit width, *mm*	Lamp current, *mA*	Observation height, *mm*	R.S.D., %	Error, %	Sensitivity, *μg/ml* for 1% absorption	Detection limit, *μg/ml*	Linear response, *μg/ml*
Water	3.6	4.5	0.11	12	30	2.1	0.6	0.033	0.019	0.7–3.0
Acetone, 20%	3.6	4.5	0.12	12.5	40	1.4	0.4	0.024	0.023	0.05–3.0
Acetone, 40%	3.6	4.5	0.14	12.5	50	2.3	0.6	0.020	0.024	0.05–3.0
Acetone, 60%	3.6	4.5	0.13	12	50	1.7	0.5	0.019	0.023	0.05–3.0
Acetone, 80%	3.6	4.5	0.14	12.5	70	6.0	2.6	0.011	0.029	0.05–3.0
Ethanol, 20%	3.6	4.5	0.12	12	30	1.1	0.1	0.025	0.017	0.05–3.0
Ethanol, 40%	3.8	4.5	0.14	11	40	1.3	0.6	0.023	0.019	0.05–3.0
Ethanol, 60%	3.9	4.5	0.12	11	30	1.6	1.0	0.022	0.023	0.05–3.0
Ethanol, 80%	3.9	4.5	0.14	11	40	1.3	0.4	0.022	0.028	0.05–3.0
MIBK–TOA	3.7	4.6	0.14	12	40	2.1	0.6	0.010	0.004	0.02–3.0

* The percentage of organic component (v/v) in the aqueous solvent is given; TOA = trioctylamine.

hand, the MIBK system gave a much better limit of detection (0.004 μg/ml) because of the physical characteristics of the MIBK. The MIBK system also gave a wider range of linear response (0.02–3.0 μg/ml), the upper limit being restricted by the read-out scale, a saturation signal being obtained for higher concentrations, even without scale expansion.

Since the optimum composition of the flame is about the same for all the media used, the increased sensitivity obtained with the MIBK system must be attributed not to higher flame temperature but to (*a*) improvement of the nebulization efficiency due to the effect of the lower density, viscosity and surface tension of the solution, and (*b*) to an increased atomization efficiency due to the fast production of ground-state beryllium from the easily combustible matrix.

Interferences

The effect of Al^{3+}, Mg^{2+}, Ti^{4+}, V(V), Ca^{2+}, Fe^{3+}, Mn^{2+}, K^+, Na^+, silicate, phosphate, chloride, sulphate, fluoride, perchlorate, boric acid and thiocyanate was studied, with a beryllium concentration of 2 μg/ml and an interferent concentration of 50–1000 μg/ml.

Of the cationic species tested, only Al^{3+} and Ti^{4+} produced a strong depression of the atomic-absorption signal of beryllium, the effect setting in at a concentration below 50 μg/ml (Table 2). None of the other cations tested interfered at levels below 200 μg/ml.

When Fleet *et al.*[9] studied the effect of a 4000-μg/ml titanium solution in hydrofluoric acid medium, they found an increase of only 1.0% in the atomic-absorption signal of beryllium. This may be because the medium was hydrofluoric acid, which eliminates the interference by formation of hexafluorotitanate, but in our case the medium is sulphuric acid and the titanium interference starts at a concentration below 50 μg/ml and is total at 300 μg/ml. Ramakrishna *et al.*[8] found that at ratios of up to 100:1 to beryllium, only palladium and silicon interfered, depressing the beryllium signal by about 29 and 8%, respectively.

The interference of aluminium and titanium can be removed by adding lanthanum, which does not affect the atomic-absorption of beryllium. Addition of increasing amounts of lanthanum to a 1000-μg/ml aluminium or titanium solution containing beryllium at the 2-μg/ml level showed that at La:Al and La:Ti ratios of 10:8 and 15:8, respectively, the absorbance was the same as for pure beryllium solution over the range 0.03–3.0 μg/ml beryllium concentration.

None of the anionic species tested gave interference, except silicate and fluoride (Table 2). Amos and Willis[4] reported no interference from 2.5*N* sulphuric acid and that a slight enhancement was produced by phosphoric acid. Fleet *et al.*[9] found a 5–7% enhancement by 10-mg/ml sulphuric acid solution, but no interference from hydrochloric, hydrofluoric, nitric or phosphoric acid. Conflicting results for interference effects are reported in several investigations in this field, including the present study. This may be due to differences in the concentration range and the efficiency of the nebulizers or burners used. We have found that there is no interference if the sample solution nebulized is 6*M* sodium hydroxide containing beryllium at the 2-μg/ml level and silicate or fluoride at 1000 μg/ml.

Although the addition of lanthanum or use of the 6*M* sodium hydroxide medium avoids the interference from aluminium and titanium or silicate and fluoride, respectively, neither increases the sensitivity, and this may be necessary for analysis of samples of very low beryllium content. For this reason we examined the preconcentration system with trioctylamine and thiocyanate.

Extraction system

The extraction of beryllium from ammonium thiocyanate medium with trioctylamine (TOA) in MIBK was used by Novoselova *et al.*[18] to separate binary mixtures of beryllium with Al, Mg, Zn, or Mn, but this system has not been used for atomic-absorption determination of beryllium.

Table 2. Interferences in flame AAS determination of beryllium (2 μg/ml)

Concentration of interfering ion, *ppm*	Be absorbance			
	Al^{3+}	Ti^{4+}	Silicate	Fluoride
0	0.384	0.292	0.392	0.391
50	0.348	0.260	0.380	0.389
100	0.292	0.168	0.332	0.384
200	0.096	0.072	0.312	0.368
300	0.040	—	0.272	0.360
400	—	—	0.244	0.356
500	—	—	0.212	0.348
600	—	—	0.124	0.347
700	—	—	0.104	0.336
800	—	—	0.076	0.328
900	—	—	0.040	0.296
1000	—	—	—	0.260

Table 3. Analysis of synthetic samples*

Be taken, μg/ml	Be found, μg/ml	Relative error, %
0.25	0.24	−4
0.32	0.30	−6
0.70	0.68	−3
1.28	1.24	−3
1.90	1.87	−2
2.50	2.53	+1

* As 0.04, B 0.24, Se 0.02, V 0.06 μg/ml in the synthetic samples.
† Average value of ten determinations.

According to our results, the optimum pH of extraction with 3% TOA in MIBK from 0.3*M* thiocyanate medium is 2.60–3.15, higher than that reported by Novoselova *et al.* With a 10:1 aqueous phase:organic phase volume ratio and shaking for at least 4 min, the recovery in a single extraction is at least 98.5%, for the beryllium range 0.2–3.0 μg/ml in the aqueous phase. When the sensitivity permitted it, the beryllium left in the aqueous phase was determined by flame AAS; otherwise, it was determined spectrophotometrically with quinalizarin. The AAS characteristics for beryllium determination in MIBK medium are shown in Table 1.

Aluminium, titanium, silicon and fluoride up to 1000 μg/ml were found not to interfere.

The American Public Health Association[24] recommends determination of beryllium in natural or sewage water by atomic-absorption in the nitrous oxide–acetylene flame with direct nebulization if the beryllium concentration is higher than 0.03 μg/ml, or by spectrophotometric measurement of the aluminium complex at 515 nm. The beryllium contents of such samples are seldom higher than 0.03 μg/ml.

Beryllium was determined in synthetic samples of the composition used by the American Public Health Association,[24] by the extraction and AAS method described. The results given in Table 3 show that the maximum relative standard deviation was 2% and relative error −6%, the average error being 3%.

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EVALUATION OF NMR SPECTROSCOPY FOR THE QUANTITATIVE CHARACTERIZATION OF UREA–FORMALDEHYDE RESINS

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Summary—A comparative evaluation has been made of both proton and ^{13}C nuclear magnetic resonance techniques in the quantitative characterization of commercial urea–formaldehyde resins. There is good agreement between data derived from ^{13}C NMR spectra and from ^{1}H high-field continuous wave, or low-field (Fourier transform) NMR spectra. Low-field continuous wave proton spectra exhibit inferior resolution and provide inaccurate quantitative data. Combination of ^{13}C and proton NMR with nitrogen analysis gives a quantitative characterization technique for these resins.

Despite the development of many new polymeric materials, interest has continued in well-established thermosetting condensation resins. Knowledge of one type of resin, urea–formaldehyde (U-F), has increased considerably with the advent of new instrumental techniques of analysis. In recent years the traditional methods of elemental analysis[1] and formaldehyde analyses[2] have been supplemented by infrared spectroscopy,[3] chromatographic methods[4–7] and NMR spectroscopy[5,8–13]

This paper evaluates methods of obtaining quantitative data on commercial U-F resins by use of both ^{1}H and ^{13}C NMR spectroscopy. The conditions required to obtain accurate and reproducible data are studied and the interpretation of such data is discussed.

EXPERIMENTAL

Aerolite UL-333, a liquid U-F resin manufactured by Ciba-Geigy Ltd., was used in this study. To obviate interference from the water present, a thin film of the sample was dried to a solid in a vacuum desiccator at 20°. All spectra were recorded at 25°.

^{1}H NMR

Continuous wave spectra were recorded, using relatively concentrated solutions (200 mg/ml), at 60 MHz (Hitachi Perkin–Elmer R24B) and 220 MHz (Hitach Perkin–Elmer R34). Pulsed (Fourier transform) spectra were obtained on more dilute solutions (40 mg/ml) at 80 MHz (Bruker WP80-WG). In each case the solvent employed was deuterated dimethylsulphoxide (DMSO-*d6*). The chemical shift was expressed in ppm relative to a tetramethylsilane (TMS) internal standard. Quantitative analysis was based on the intensity of each signal given by the integrator. In each case the integral was adjusted to the nearest 0.5%.

^{13}C NMR

These spectra were also recorded on the Bruker WP80-WG spectrometer. Gated decoupling (3-sec delay following acquisition) was employed and chromium acetylacetonate (0.02*M*) was added to the solution to obtain reliable quantitative data.[14]

Low concentrations of non-interacting paramagnetic species, such as chromium acetylacetonate, reduce thermal relaxation times (t_1) to about 0.15 sec,[15] and nuclear Overhauser enhancements (NOE) by the same factor (gated decoupling then removes the residual NOEs); 90°-pulses (duration 6.5 μsec) were employed since relaxation times were shortened sufficiently to ensure complete relaxation between pulses (~3–5 sec). The sweep width was 9000 Hz and 8000 data points were collected, so the acquisition period was 0.45 sec. The chemical shift was measured relative to TMS, and the quantitative information, based on the intensity of each band, was expressed to the nearest 0.5%.

RESULTS AND DISCUSSION

^{1}H NMR

The ^{1}H NMR spectrum of the U-F resin recorded at 60 MHz is shown in Fig. 1. The signal assignment is based on that given earlier by Chiavarini *et al.*,[10] Kambanis and Vasishth,[8] and Duclairoir and Brial.[9] The spectrum exhibits a methoxyl signal, at 2.8–3.4 ppm, and a shoulder on the –CONH– resonance, at 7.5–9.0 ppm, neither of which has previously been reported. The methoxyl signal arises because of the use of methanol-stabilized formalin in the preparation of the resin; methanol was presumably absent from the resins studied by the earlier authors.[8–10]

Resolution is improved at 220 MHz (Fig. 2). The peaks corresponding to –OH and $-NH_2$ are well resolved and the shoulder on the –CONH– signal appears as a distinct, though very broad, peak. In addition, the breadth of the $-CH_2-$ signal at 60 MHz (dotted lines in Fig. 1) is greatly reduced at 220 MHz. All these factors result in a more accurate integration profile (Table 1). At 60 MHz the width of the $-CH_2-$ band results in an underestimation of the intensity of that signal and a corresponding overestimation of the $-NH_2$, –OH and $-OCH_3$ intensities. The higher resolution at 220 MHz of the $-NH_2$ and –OH signals improves the accuracy of the intensity calculations for both peaks.

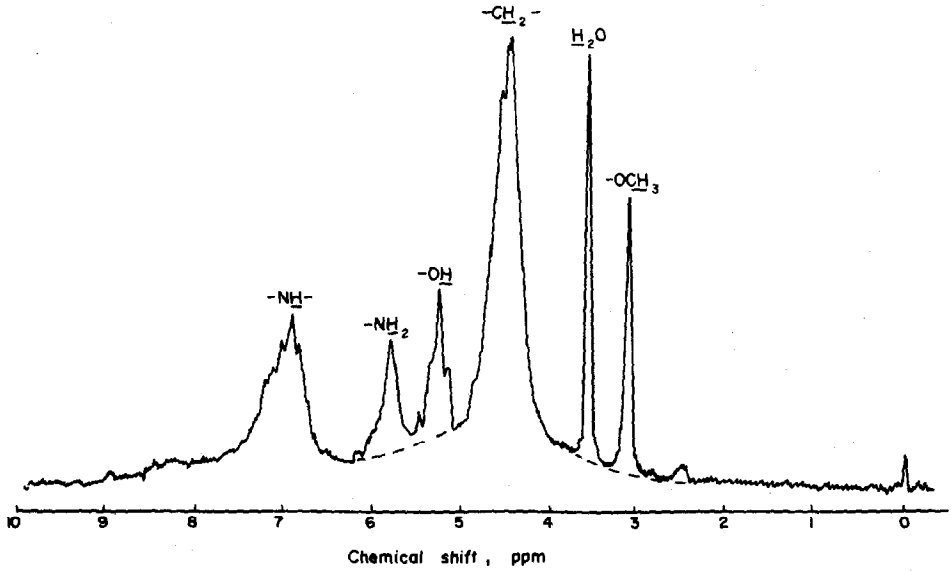

Fig. 1. 60-MHz proton NMR spectrum of a U-F resin.

In an attempt to obtain a reasonable spectrum at low field, a pulsed (FT) spectrum at 80 MHz was recorded, for a much more dilute solution of the U-F resin. The spectrum obtained (Fig. 3), although not as clearly resolved as that at 220 MHz, is superior to that obtained at 60 MHz. Integration of this spectrum (Table 1) clearly shows the improvement the Fourier transform technique makes for the low-field data, producing good agreement with results obtained at 220 MHz.

Finally, the addition of excess of deuterium oxide to the sample solution results in the replacement of all exchangeable protons, with a corresponding disappearance of the –OH, $-NH_2$ and –NH signals, includ-

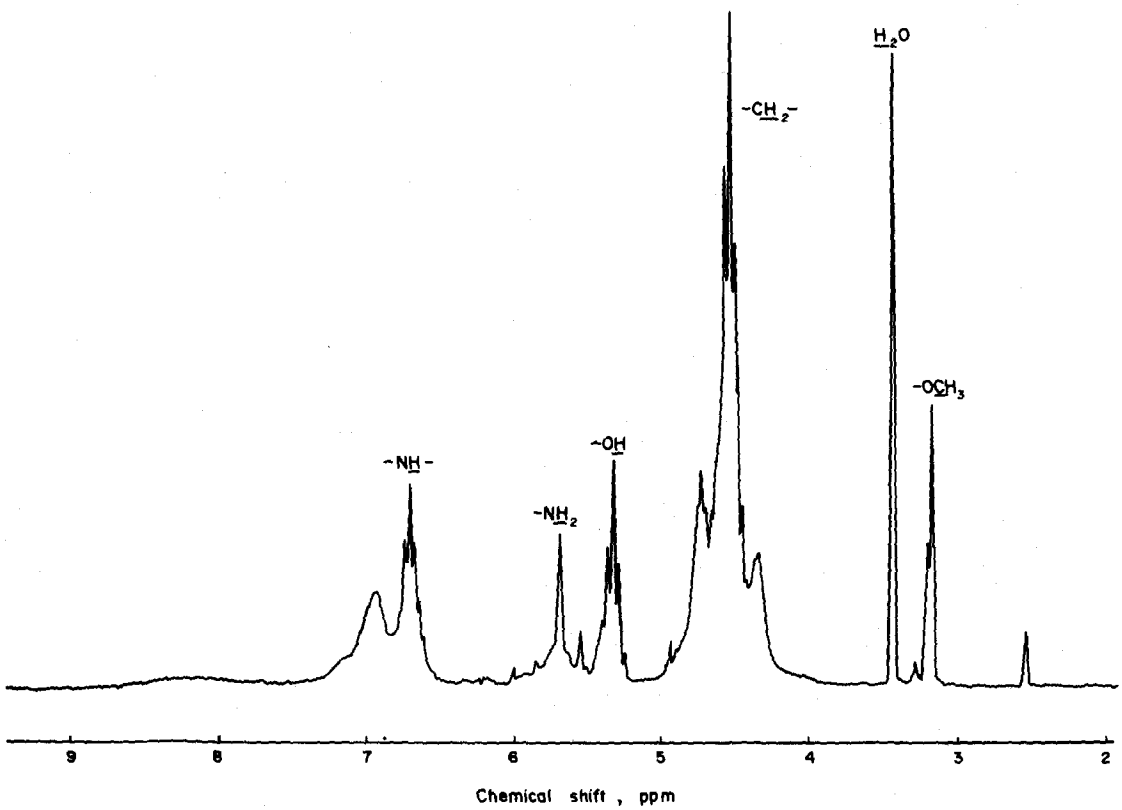

Fig. 2. 220-MHz proton NMR spectrum of a U-F resin.

Table 1. Hydrogen distribution of a U-F resin measured by ^1H NMR techniques

Functional group	Chemical shift, *ppm*	Percentage of total hydrogen		
		60 MHz	220 MHz	80 MHz (FT)
Shoulder	7.5–9.0	5.5	3.0	3.5
Monosubstituted amide (–NH–)	6.5–7.5	21.0	23.0	22.0
Non-substituted amide ($-NH_2-$)	5.5–6.5	11.0	6.5	7.0
Hydroxyl (–OH)	5.1–5.5	11.5	8.5	10.0
Methylene ($-CH_2-$)	4.0–5.1	44.0	54.0	52.5
Methoxyl ($-OCH_3$)	2.8–3.4	7.0	5.0	5.0

ing the shoulder on the latter. The shoulder would seem, therefore, to be attributable to –NH protons.

^{13}C NMR

The chemical-shift range of the methylene resonances is much larger in ^{13}C NMR than in ^1H NMR, making it possible to obtain more detailed information on the resin structure. The ^{13}C spectrum obtained with the U-F sample is shown in Fig. 4. The resonances have been assigned according to Slonim *et al.*[12] and Tomita *et al.*[13] The carbon distribution obtained from the spectrum is given in Table 2. Two points of caution must be borne in mind when assessing the intensity of the ^{13}C signals. First, care must be taken when estimating the size of the $-CONHCH_2NHCO-$ signal because of the very close proximity of the solvent resonance bands. Secondly, the presence of any uron derivatives will produce an error in the estimation of the $-N(CH_2)CH_2OCH_2-$ and $-NHCH_2OCH_3$ signals, owing to overlap of resonances. The presence of a carbonyl peak at 153.5 ppm, as observed with the resin investigated, indicates the presence of uron species.

Quantitative results

Kambanis and Vasishth, Duclairoir and Brial, and

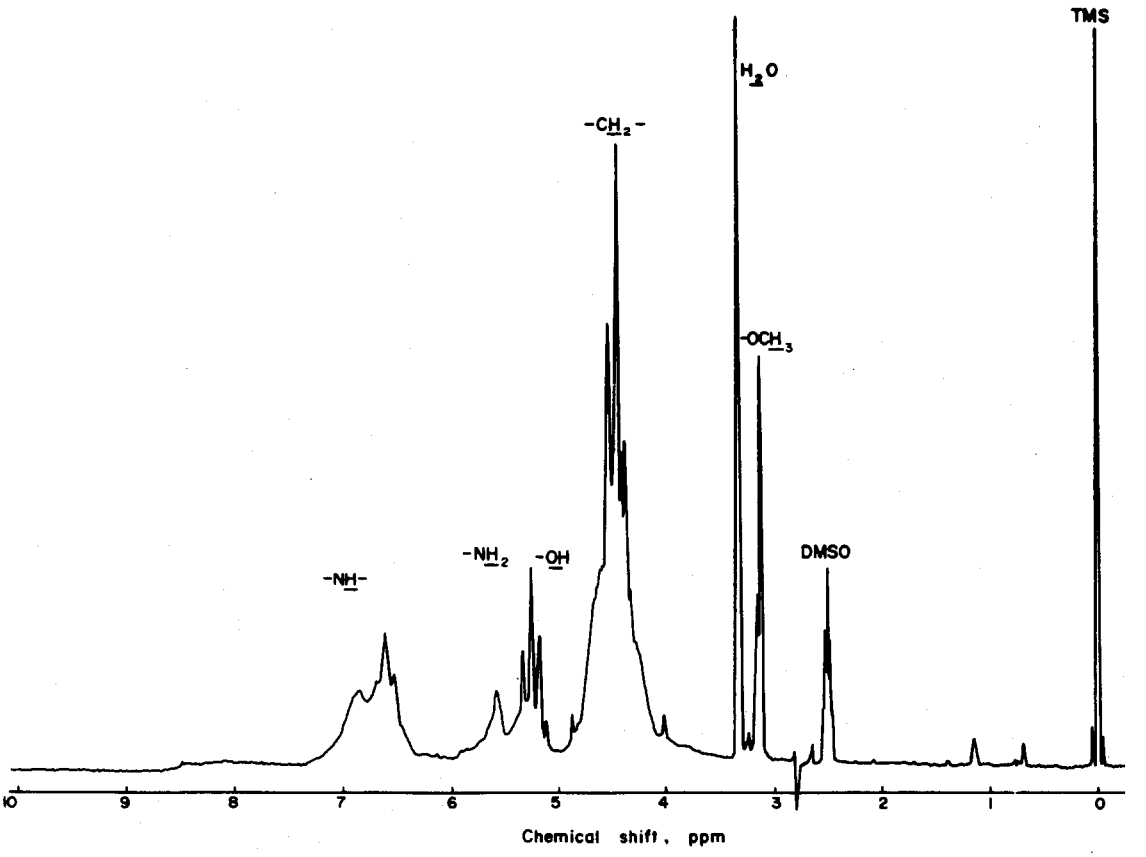

Fig. 3. 80-MHz (FT) proton NMR spectrum of a U-F resin.

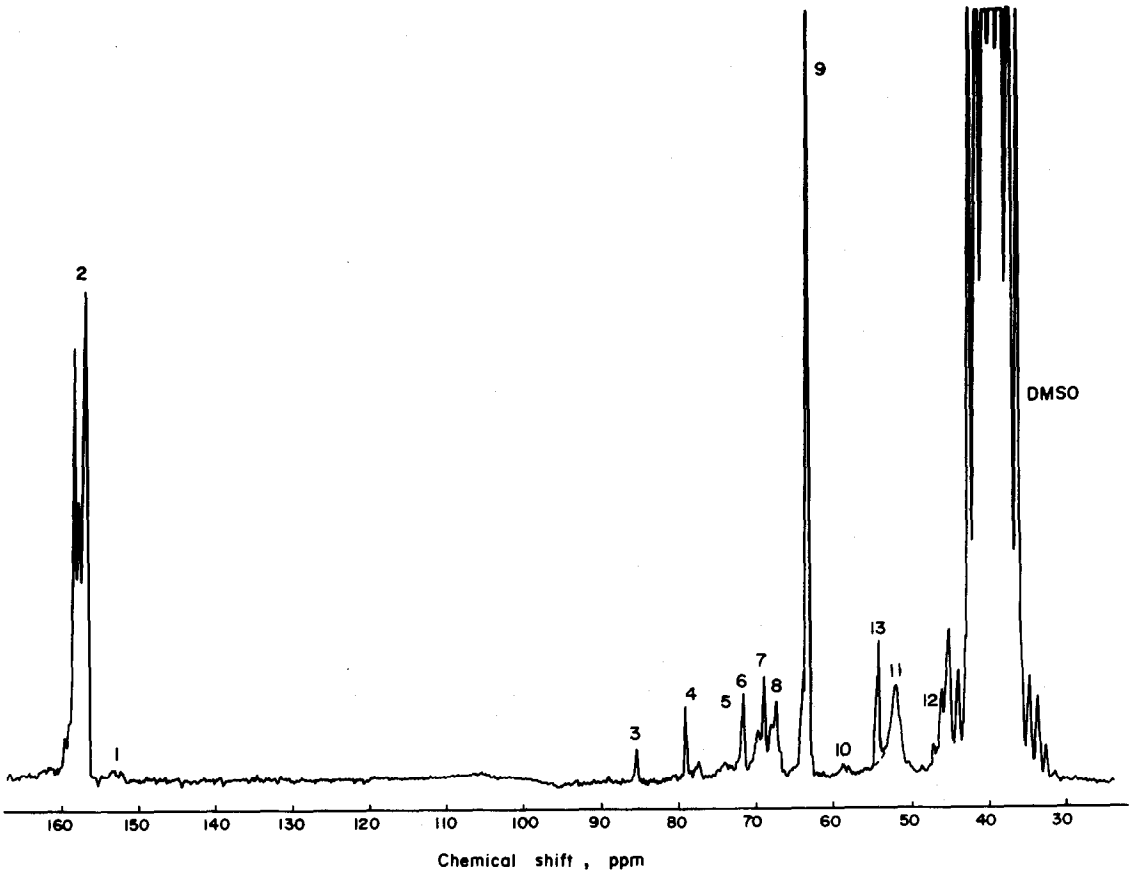

Fig. 4. ^{13}C NMR spectrum of a U-F resin (peaks identified in Table 2).

Chiavarini *et al.* all invoked some means of quantitative measurement in their early ^{1}H NMR studies. Of these, the last approach[10] was the most comprehensive, combining the information gained from the spectrum with that obtained by nitrogen and formaldehyde analyses to obtain quantitative data on a large number of functional groups present in U-F resins.

The Chiavarini formulae were therefore applied to our information obtained from the three types of proton spectra. In order to obtain the full range of data it

Table 2. Data obtained from ^{13}C NMR spectra

	Carbon type	Chemical shift, *ppm*	Percentage of total carbon	Concentration,* *mole/100 g*
	Carboxyl			
1.	Uron	153.5	1.0	0
2.	Di-substituted urea	158.6	34.5	1.0
	Tri- and tetra-substituted urea	157.5–157.7		
	Methylene†			
3.	$CH_2O\underline{CH_2}OH$	82–87	1.0	0
4.	$N(CH_2)\underline{CH_2}OCH_2$	77–82	3.5	0.1
5.	$N(CH_2)\underline{CH_2}OCH_2$	73–77	2.5	0.1
6.	$NH\underline{CH_2}OCH_3$	71.5–73	2.5	0.1
7.	$N(CH_2)\underline{CH_2}OH$	68.5–71.5	5.0	0.1
8.	$NH\underline{CH_2}OCH_2$	66.5–68.5	6.0	0.2
9.	$NH\underline{CH_2}OH$	62–66.5	17.0	0.5
10.	$N(CH_2)\underline{CH_2}N(CH_2)$	57–60	1.5	0
11.	$N(CH_2)\underline{CH_2}NH$	49–54	11.5	0.3
12.	$NH\underline{CH_2}NH$	45–49	11.0	0.3
	Methyl			
13.	$-CH_2O\underline{CH_3}$	54–55	3.0	0.1
	Error		±0.5	±0.02

* Based on total urea concentration.
† The methylene group is underlined.

Table 3. Quantitative data derived from ^1H NMR spectra by using the method of Chiavarini *et al.*[10]

Functional group	Concentration,* *mole/100 g* (±0.05) 60 MHz	220 MHz	80 MHz (FT)
Monosubstituted amide (–NH–)	2.1	1.7	1.7
Non-substituted amide ($-NH_2$)	0.4	0.2	0.2
Hydroxyl (–OH)	0.9	0.5	0.7
Methylene ($-CH_2-$)	1.7	1.7	1.7
Methoxyl ($-OCH_3$)	0.2	0.1	0.1
Disubstituted amide (–N=)	−0.5	0.1	0.1
Methylene bridges (=N–CH_2–N=)	0.7	0.7	0.7
Oxymethylene formaldehyde ($-OCH_2-$)	0.1	0.5	0.3
Acetal formaldehyde ($-OCH_2O-$)	—	0.5	0.5
Oxymethylene attached to nitrogen (=NCH_2O)	—	0.0	−0.2

* Total urea concentration = 1.02 mole/100 g, total formaldehyde concentration = 1.74 mole/100 g, oxidizable formaldehyde concentration = 1.02 mole/100 g.

was necessary to determine the total nitrogen content (Kjeldahl method), the total formaldehyde concentration (acid hydrolysis)[16] and the oxidizable formaldehyde concentration (alkaline peroxide).[17] The free formaldehyde content, estimated by the sulphite method,[18] was found to be negligible in the dried sample.

The calculated functional group concentrations are given in Table 3. They show clearly the large errors that exist in the 60-MHz data, owing to the overlap of signals. However, within the limits of accuracy of the method (approx. 0.1 mole/100 g), the information derived at 220 MHz and 80 MHz (FT) shows good agreement.

By using the total urea concentration, and assuming all the carbonyl species are derived from urea, a similar range of functional group concentrations can be derived from the ^{13}C NMR data (assuming an accuracy of 0.1 mole/100 g) (Table 2).

Good agreement is achieved between the ^1H [220 MHz or 80 MHz (FT)] and the ^{13}C data. The only instance where a serious discrepancy exists is when the Chiavarini formulae are used to calculate the concentrations of the different types of oxymethylene formaldehyde. Here, due to the nature of the calculation, any slight errors made in integrating the spectrum become greatly magnified. These groups produce well-defined signals in an area of the ^{13}C spectrum well-removed from any interfering solvent resonances, and thus should provide relatively accurate information, even when the presence of uron species is taken into account (Table 4).

CONCLUSIONS

Derivation of accurate quantitative data for U-F resins from 60-MHz ^1H NMR spectra is not possible. Such information can be obtained from high-field spectra (220 MHz) or, alternatively from low-field (Fourier transform) spectra for dilute solutions. Accurate quantitative data can also be obtained from ^{13}C NMR spectra, provided that care is taken in interpreting the intensities of the signals. Agreement between the two sets of data is good, but errors have been found when deriving oxymethylene formaldehyde data from the ^1H NMR spectrum by using previously proposed formulae. Comparison of the ^{13}C NMR results with those obtained by classical

Table 4. Comparison of quantitative data obtained by different techniques

Functional group	Concentration, *mole/100* g ^1H	^{13}C	Classical analysis
Disubstituted amide (–N=)	0.1	0.3	—
Hydroxyl (–OH)	0.7	0.6	—
Methoxyl ($-OCH_3$)	0.1	0.1	—
Total oxymethylene ($-OCH_2-$)	0.3	0.3	—
Oxymethylene attached to nitrogen (=NCH_2O)	−0.2	0.4	—
Acetal ($-OCH_2O-$)	0.5	0	—
Methylene bridges (=N–CH_2–N=)	0.7	0.6	—
Total formaldehyde	—	1.7	1.74
Oxidizable formaldehyde	—	1.1	1.01
Error	±0.05	±0.02	±0.01

methods also shows good agreement. Only when this information is combined with that derived from high-field or low-field (Fourier transform) ^{1}H NMR spectra, and the total nitrogen content, can a full characterization of a U-F resin be obtained.

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THE EFFECT OF POLYETHYLENE OXIDE MOLECULAR WEIGHT ON DETERMINATION OF ITS CONCENTRATION IN AQUEOUS SOLUTIONS

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Summary—The influence of the molecular weight of polyethylene oxide (PEO) on the results from several methods for determining its concentration in aqueous solutions has been investigated. Modified versions of the complexation reactions of PEO with molybdophosphoric acid and with tetraiodobismuthate give results that are independent of molecular-weight effects for the range *ca.* 400–10^6. The reaction with $KBiI_4$ is the less accurate. Oxidative digestion is also independent of molecular-weight, but impurities easily obscure the measurements. Other methods studied included interferometry, viscometry, and complexation with tannic acid. Interferometry and the reaction with tannic acid were only independent of molecular-weight for M > 4000. With viscometry very poor results were obtained.

Polyethylene glycols (PEG) and polyethylene oxides (PEO) find many applications[1] and are well suited for model studies on adsorption[2–6] and flocculation.[7,8] The general formula of a polyethylene oxide or a polyethylene glycol segment is —CH_2CH_2O—. The lower molecular-weight members of the series (up to about M = 2×10^4) are generally known as PEGs, the higher molecular-weight members as PEOs or polyoxyethylenes. In this paper we will use the term polyethylene oxide as a general name.

The use of PEO in model studies demands accurate and simple methods for determining the solution concentration of PEO. Several such methods exist,[3–6,9,10] especially for the lower molecular-weight series (PEGs). Little attention has been paid to the high molecular-weight PEOs[11] and to the effect of the PEO molecular weight on the determination. The latter aspect is of particular importance for polymer adsorption studies, because from a polydisperse polymer sample the higher molecular-weight species will be preferentially adsorbed.[12] As a result, the average molecular weight of the polymer remaining in solution will no longer be known. To avoid systematic errors in such a case, a method is required for determining PEO concentration that will be practically unaffected by the molecular weight of the PEO.

The objective of this study was to examine the PEO determination methods with regard to dependence on the molecular weight and to develop methods which can also be used for adsorption studies.

Existing methods

Determinations of PEO in aqueous solution can be divided into two broad classes.

(1) Methods in which a PEO solution property is measured directly, for instance interferometry,[4] viscometry,[5] or oxidative digestion.[6] In general such methods can be applied only to *pure* PEO solutions.

(2) Methods based on the ability of PEO to form precipitates with large anions in the presence of cations such as Ba^{2+}.[9,13] The following anionic reagents have been used: molybdophosphoric acid,[14,15] silicotungstic acid,[14,16] cobaltothiocyanate,[17] potassium ferrocyanide,[18] sodium tetraphenylborate (NaTPB),[10,19–21] potassium tetraiodobismuthate(III),[22] and tannic acid.[11,13] After precipitation the complex is separated from the solution and analysed. In the case of tannic acid the turbidity of the tannic acid–PEO solution is measured. Except for the reaction with tannic acid, PEOs of relatively low molecular weights have been used.

For the methods mentioned under (1), no quantitative information is available with regard to molecular weight effects. In the case of viscometry, the PEO determination is expected to be molecular-weight dependent, because the viscosity of a polymer solution is related to the molecular weight of the polymer.[25] In the case of interferometry and oxidative digestion, the nature of the segments is much more important than the length of the polymer chain. Therefore, molecular-weight effects should vanish as soon as end-group effects become negligible.

The precipitation or complexation of PEO with reagents as indicated under (2) is mostly explained by assuming that PEO forms quarternary oxonium derivatives. Two such "oxonium" groups, formed for approximately every 6 ethylene oxide units, are involved in the binding of a reagent anion.[13] For example, in the well-studied case of NaTPB in the presence of $BaCl_2$,[10,20,23] approximately 11 ethylene oxide units combine with 2 TPB ions and 1 Ba^{2+} ion. It has been suggested that this ratio is determined by the steric conformation of the PEO chain in the reagent solution.[13,23] Owing to the large number of ethylene oxide units involved in the binding mechanism, the

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reaction does not proceed with PEOs of very short chain length. For PEO chains of intermediate length, the steric conformation of the complex is still substantially dependent upon molecular weight. Consequently, most PEO determinations based on the analysis of the precipitated complex will be molecular-weight dependent when PEOs of "low" molecular weight are used. Such a dependence is clearly established for the reaction of PEO with NaTPB[10] and cobaltothiocyanate[24] and it is indicated in the case of the reaction with silicotungstic acid.[14]

In a preliminary investigation we found that the reactions with $KBiI_4$ and molybdophosphoric acid were only slightly dependent on the value of M. For the reaction of PEO of large M with tannic acid it has been reported[11] that the presence of PEOs of low molecular weight caused erroneous results.

In conclusion, we may say that little quantitative information is available on the molecular-weight dependence of the various PEO concentration determination methods. Therefore, the effect of molecular weight on the following methods was studied: interferometry, viscometry, oxidative digestion, complexation with tannic acid, precipitation with $KBiI_4$ and with molybdophosphoric acid. In the last two cases the supernatant solution was analysed instead of the precipitate. This simplified the procedure and enhanced the chance of obtaining a method not affected by the PEO molecular weight.

EXPERIMENTAL

Reagents

Polyethylene oxides. These were commercial products (Union Carbide Corporation), trademarked "Carbowax" for the lower molecular-weight members ($200 < M_n < 2 \times 10^4$) and "Polyox" for the higher molecular-weight series ($10^5 < M_w < 4 \times 10^6$). M_n is the number average molecular weight and M_w the weight average molecular weight. The "Carbowax" samples will be denoted as PEO followed by their approximate M_n, *e.g.*, PEO 600, PEO 6000; the "Polyox" types as PEO plus their approximate M_w, *viz.* PEO 100,000, PEO 600,000, *etc.* All samples were used without further purification, except for prolonged drying over phosphorus pentoxide in a vacuum desiccator.

Solutions of the PEO samples were prepared by sifting the polymer into about one-half to two-thirds of the required amount of water (at the boiling point) with mild stirring, then removing the heat source and, with continuous stirring, cooling the solution with some water at about 20°, and finally cooling the solution and transferring it to a standard flask and making up to volume with water. This procedure prevents the particles from agglomerating and facilitates the dissolution.[1]

Tannic acid solution. Made by dissolving 0.200 g of tannic acid, dried for 2 hr at 105° and 29.2 g of sodium chloride in 1 litre of water.

Bürger's reagent. Dragendorff reagent was made by dissolving 1.70 g of basic bismuth(III) nitrate ($BiO.NO_3.H_2O$) in 20 ml of glacial acetic acid and diluting to 100 ml with water, then mixing with a solution of 40 g of potassium iodide in 100 ml of water, adding 200 ml of glacial acetic acid, and making up to volume in a 1-litre standard flask. Then 100 ml of the Dragendorff reagent were combined with 50 ml of water in which 18.8 g of barium chloride dihydrate had been dissolved. The mixed reagent keeps for about two weeks in a brown bottle. It should be discarded when it turns brown.

Molybdophosphoric acid reagent. Made by dissolving 0.50 g of $H_3Mo_{10}PO_{32}.24H_2O$, 0.50 g of barium chloride dihydrate and about 1.5 ml of concentrated hydrochloric acid in 250 ml of water.

Potassium dichromate solution. Made by diluting one ampoule of Baker Chemicals "0.1*N* $K_2Cr_2O_7$" concentrate and 8 ml of concentrated sulphuric acid to 1 litre with water, to give an acidic 0.1000*N* dichromate solution.

Mohr's salt solution. Made by dissolving 39–40 g of $FeSO_4(NH_4)_2SO_4.6H_2O$ and 8 ml of concentrated sulphuric acid in 1 litre of water, and standardized with the potassium dichromate solution.

Apparatus

Interferometer (Carl Zeiss, Jena), having a double glass cell of matched 2-cm path-length, in a thermostatic water-bath.

Viscometer (Viscomatic MS, type 53000, Fica) having electronic timing (±0.01 sec) and Ubbelohde capillaries. The dilution vessel and capillaries were temperature-controlled to within 0.005° with a thermostatic water-bath.

Spectrometer (Beckman model 3600), double-beam, digital reading, equipped with a temperature-controlled dual cell holder; wavelength range 190–900 nm.

Turbidity meter (Unicam SP 1800 spectrometer), double-beam, equipped with a temperature-controlled cell-holder. The sample was placed in the secondary sample position, *i.e.*, close to the detector, in order to obtain the best accuracy.

Procedures

Interferometry and viscometry. These methods are based on the increase in refractive index or viscosity, respectively, with increasing polymer concentration. Measurements on PEO test solutions are made at constant temperature, with water as reference.

Oxidative digestion. The oxidation of PEO with dichromate is described by the equation

$$5Cr_2O_7^{2-} + 40H^+ + 3[-(C_2H_4O)-] \rightarrow 10Cr^{3+} + 6CO_2 + 26H_2O.$$

An excess of dichromate is added and the surplus back-titrated with iron(II). The amount of PEO originally present can be calculated from the mass balance as $4.4(V_C N_C - V_M N_M)$ mg, where V_C ml of dichromate (normality N_C) and V_M ml of Mohr's salt solution (normality N_M) are used. Pipette 5 ml of dichromate solution into a beaker, add b ml of PEO test solution containing about 1.5 mg of PEO, and heat for 2 hr at 90°, allowing enough evaporation to obtain a dichromate concentration high enough to oxidize the polymer completely, but without evaporation to dryness. Cool, dilute carefully, and titrate potentiometrically with the Mohr's salt solution.

Molybdophosphoric acid method. Shaffer and Critchfield[14] have described an analysis in which PEO is precipitated with phosphomolybdic acid, collected, dried and weighed. Stevenson[15] modified this procedure and measured the absorbance of a solution of the precipitate in concentrated sulphuric acid, at 520 nm. Concentrations down to 1 mg/l. could be detected. In the present study a method has been developed in which the difference in the molybdophosphoric acid concentration before and after the reaction with PEO is used as a measure of the PEO content. Pipette 2 ml of molybdophosphoric acid reagent solution into a dry centrifuge tube, add 2 ml of PEO solution (10–300 mg/l.). Shake gently, store the tube for 15 min at 20°, and centrifuge. Pipette 1 ml of the supernatant solution into a 50-ml standard flask and make up to

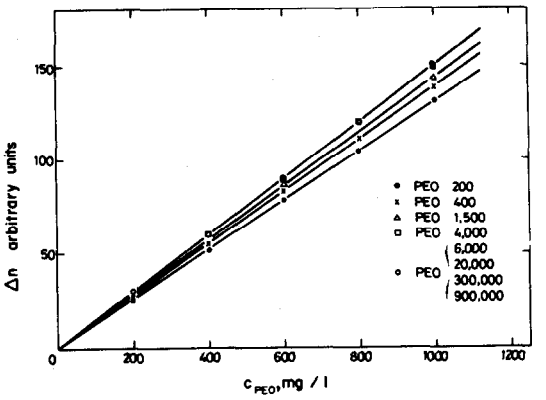

Fig. 1. Interferometry. The difference in refractive index between blank and test solution, Δn, *vs.* PEO concentration, c_{PEO}. The PEOs studied are indicated.

volume with water. Measure the absorbance at 216 nm in a 1-cm quartz cuvette against water, at 20°, directly after the cuvette has been placed in the spectrometer. The original polymer concentration, c_{PEO}, is found from the difference in absorbance between a blank and the test solution, by means of a calibration graph. The blank solution is made in the same way as the test solution but with 2 ml of water instead of the PEO solution.

Tetraiodobismuthate method. Bürger[22] precipitated PEO with $KBiI_4$ in a narrow-ended centrifuge tube and used the depth of the deposit as a measure of the PEO content. We have tried this method but could not obtain accurate results, so we have modified the procedure, determining the change in the $KBiI_4$ concentration caused by the reaction with PEO.

The effect of time, temperature and exposure to light on the absorbance of the $KBiI_4$ solution were explicit variables.

Dissolve 26.7 g of potassium iodide and 145 ml of glacial acetic acid in 1 litre of water (same concentrations as in Bürger's reagent). Store this solution in the dark. Pipette 2 ml of PEO solution (c_{PEO} 5–300 mg/l.) and 1 ml of Bürger's reagent into a dry centrifuge tube. Mix the solution carefully and store the tubes for 30 min in the dark at 20°. Centrifuge, and pipette 1 ml of the supernatant solution into a brown 50-ml standard flask containing 25 ml of the iodide/glacial acetic acid solution and make up to volume with this solution. Measure the absorbance at 337 nm in a quartz cuvette against water at 20°, directly after placing the cuvette in the spectrometer. Run a blank in the same way (with 2 ml of water instead of the sample). The PEO concentration is found from the difference in absorbance between the blank and the test solution, by means of a calibration plot.

Turbidimetry with tannic acid. Essentially the method given by Attia and Rubio[11] was followed, but the turbidity was measured with a spectrometer instead of a nephelometer. The turbidity or absorbance of a suspension depends on the number and size of the particles present. In the case of the PEO–tannic acid precipitate both the number of particles and the size are functions not only of the polymer concentration, but also of the composition of the reagent solution, the way of mixing reagent and polymer, and the temperature. To develop a standard procedure, a series of experiments has been carried out in which the concentrations of tannic acid and sodium chloride, the temperature and the measurement wavelength were varied. The molecular-weight effect was studied with the established standard procedure.

Put about 15 ml of the standard tannic acid solution into a 25-ml standard flask, add 5 ml of PEO solution (c_{PEO} 2–150 mg/l.) and make up to volume with the tannic acid solution. Mix by turning the flask upside down 10 times. Store the mixture for 1 hr in the dark at 20° and measure the absorbance of the suspension at 600 nm against water in a 1-cm cuvette.

N.B. The absorbance, A, of the suspension in a 1-cm cell is directly related to the turbidity, τ, by $A = 0.434\ \tau$, with τ in cm^{-1}.

RESULTS AND DISCUSSION

Interferometry

A typical set of curves, showing the difference in refractive index between a PEO solution and water as a function of c_{PEO} for various PEOs differing in molecular weight, is shown in Fig. 1. Apparently the difference depends on molecular weight up to at least $M_n = 1500$, and this is due to the influence of the end-groups of the PEO chain.

For $4000 \leqslant M \leqslant 9 \times 10^5$ no molecular-weight effect could be detected. The sensitivity of the method, however, is rather poor. The lower detection limit is about 100 mg/l. In the presence of salt (at constant concentration) a still lower sensitivity is observed. Hence the method is only suitable and independent of molecular weight for pure PEO solutions of sufficiently large c_{PEO} and M_{PEO}.

Viscometry

The results obtained by viscometry are shown in Fig. 2, where the relative increase in flow-time is plotted *vs.* c_{PEO} for various PEOs in aqueous or salt solutions. From Fig. 2 it follows that the determination is limited to PEOs with $M > 6000$. The sensitivity of the method is very poor except for PEO 4×10^6. The molecular-weight dependence is strong and in qualitative agreement with the Mark–Houwink–Sakurada and Flory–Fox relations[25] which give the dependence of the intrinsic viscosity on M. It is also shown that both the type and concentration of salt present in the

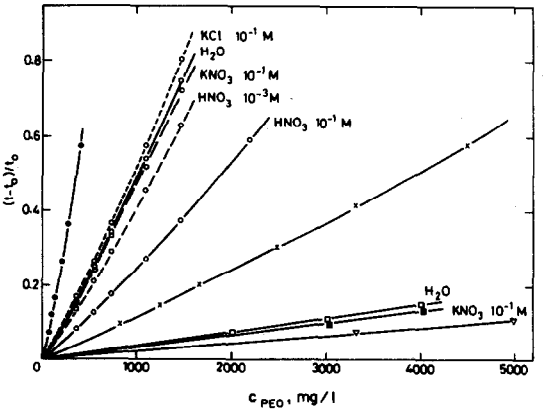

Fig. 2. Viscometry. The relative increase in flow-time, $(t - t_0)/t_0$, as a function of PEO concentration. ▽ PEO 6,000; □■ PEO 20,000; × PEO 100,000; ○ PEO 600,000; ● PEO 4,000,000.

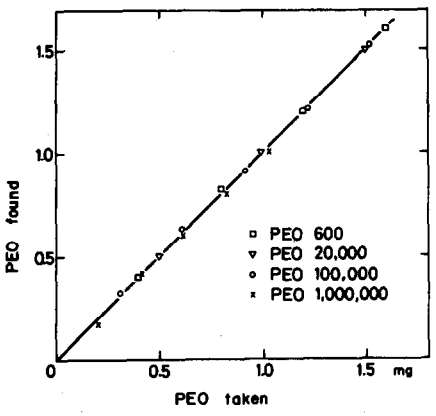

Fig. 3. Oxidative digestion with acid $K_2Cr_2O_7$.

solution can affect the determination. In conclusion, viscometry is a very poor method for determination of PEO concentration.

Oxidative digestion

Results are shown in Fig. 3. It follows that the oxidative digestion is a reasonably accurate method for PEO determination, and is independent of molecular weight. In principle, even very small PEO concentrations can be determined, but in that case a relatively large volume of solution is required and attention has to be paid to the nearly complete evaporation of the solution, or quantitative oxidation of the PEO is not attained. Moreover, other oxidizable compounds must be absent. In adsorption studies it should be remembered that any adsorbed polymer or oxidizable adsorbent still present in the supernatant solution because of incomplete sedimentation will also be determined by this method.

Molybdophosphoric acid method

The absorption spectra of the reagent solution before and after the reaction with PEO are plotted in Fig. 4; all exhibit a well-defined peak at 216 nm, indicating that only free reagent is present in the solution. Comparison of the curves for different PEOs shows that the effect of molecular weight is relatively small, except for PEO 200. The molybdophosphoric acid solution obeys Beer's law up to a concentration of 40 mg/l. The molar absorptivity is 7.5×10^4 $l.mole^{-1}.cm^{-1}$. Prolonged exposure of the reagent to ultraviolet light decreases the absorbance.

The standard PEO determination given in the experimental section is based on these findings. A maximum molybdophosphoric acid concentration of 20 mg/l. is used in order to keep the absorbance below 0.8, which enhances the accuracy of the method. To diminish the influence of ultraviolet light, water is used as a reference. The difference (ΔA_{216}) in the absorbance at 216 nm of the reagent solution before and after the reaction with PEO, is a measure of the PEO content.

In Fig. 5, ΔA_{216} is shown as a function of c_{PEO} for several PEOs differing in molecular weight. A linear relation is found, which is practically independent of molecular weight if $M \geqslant 400$. For PEOs with $M \geqslant 10^5$, the calibration curve has a slightly lower slope than for the lower molecular weights, but up to $c_{PEO} = 150$ mg/l. the differences between the different PEOs are within the experimental error. In the case of PEO 200 the reaction between polymer and reagent solution is incomplete, but it is still possible to determine the PEO concentration with the present method. It can be concluded that a rather simple and attractive new method has been developed.

The calibration curve allows us to calculate the average number of ethylene oxide units (EOUs) involved in the binding with a molybdophosphoric acid unit. An 80-mg/l. PEO solution decreases the

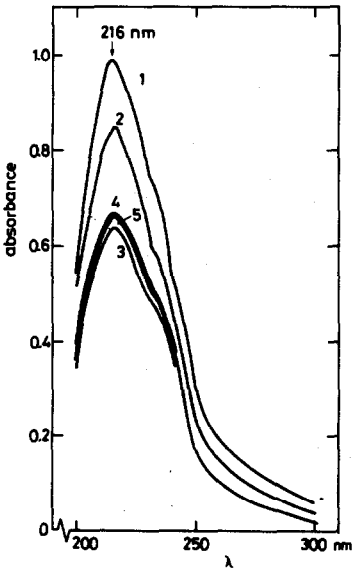

Fig. 4. Absorption spectra of the molybdophosphoric acid reagent before and after the reaction with PEO; 1, blank; 2, PEO 200; 3, PEO 400; 4, PEO 6,000; 5, PEO 600,000.

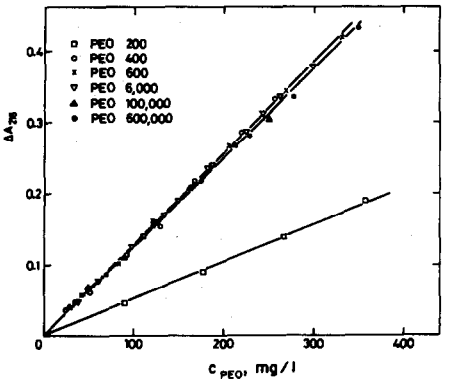

Fig. 5. ΔA_{216} (1-cm cuvette) of the molybdophosphoric acid reagent as a function of PEO concentration.

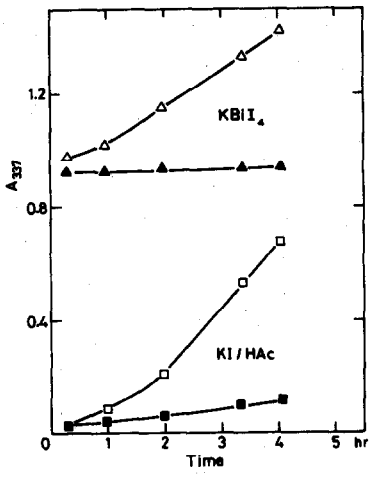

Fig. 6. Effect of time and light on the A_{337} of tetraiodobismuthate solution and a KI/acetic acid solution. Open symbols: solutions exposed to light; closed symbols: solutions kept in the dark.

absorbance by 0.1. This corresponds to a decrease in the original molybdophosphoric acid concentration of 262 mg/l. Hence, 1 molybdophosphoric acid unit reacts with about 14 EOUs or about 4 Mo atoms with 6 EOUs. Apparently, the reaction mechanism of PEO with a large unit such as molybdophosphoric acid is more complicated than that of the reaction with NaTPB.

Tetraiodobismuthate method

This method is essentially very similar to that with molybdophosphoric acid. The tetraiodobismuthate(III) anion in sulphuric acid solution exhibits absorbance maxima at 337 and 465 nm.[26] In Bürger's reagent, acetic acid is used instead of sulphuric acid. We therefore measured the absorbance of the BiI_4^- complex in the iodide/acetic acid solution. The absorption maxima are again found at 337 and 465 nm, and Beer's law is obeyed up to Bi concentrations of 10 mg/l. The molar absorptivity is 3.14×10^4 $l.mole^{-1}.cm^{-1}$.

Owing to the potassium iodide present, the reagent solution is rather sensitive to light. This can be seen from Fig. 6, where the absorbances of Bürger's reagent and a comparable iodide/acetic acid solution are shown as a function of time and exposure to light. Water was used as reference. The strong increase in absorbance in the presence of light is due to the formation of iodine. To minimize this reaction, all iodide/acetic acid solutions should be kept in the dark, brown glassware should be used, and spectrometer readings made directly after the cuvettes have been filled. Provided that these precautions are taken, the effect of temperature on the absorbance is small.

In Fig. 7 the difference in absorbance at 337 nm, ΔA_{337}, of the reagent solution before and after the reaction with PEO is shown as a function of the PEO concentration for a number of PEO samples differing in molecular weight. Except for PEO 200, all the PEO samples gave (within experimental error) the same calibration curve irrespective of the PEO molecular weight. Apparently in this case also the reaction of PEO 200 with the reagent solution is incomplete.

However, the scatter of points around the calibration curve is rather large. Probably this is connected with the presence of iodide and the fact that the precipitation reaction is reproducible only within a few per cent. Above $c_{PEO} \simeq 350$ mg/l. the slope of the calibration curve decreases strongly and the method becomes increasingly inaccurate.

All in all, the tetraiodobismuthate method gives a reasonably accurate PEO determination, the error being within about ± 10 mg/l. in the concentration range 10–350 mg/l.

From Fig. 7 it can be calculated that for low c_{PEO} 1.33 mg of PEO reacts with 1.23 mg of bismuth, indicating that about 7 EOUs are involved in the binding with one tetraiodobismuthate ion. This is somewhat larger than the quoted EOU/reagent anion ratio of 6. The discrepancy may be due to the iodide concen-

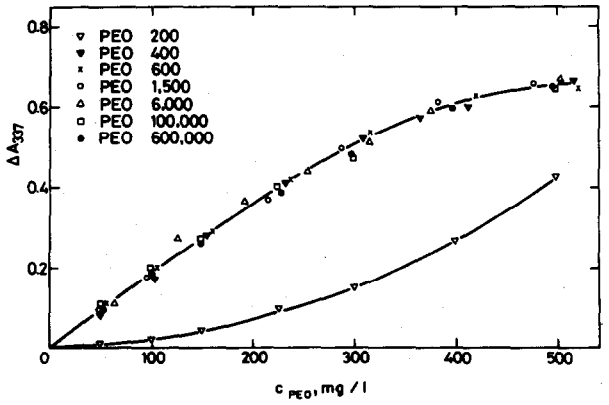

Fig. 7. ΔA_{337} for tetraiodobismuthate as a function of PEO concentration.

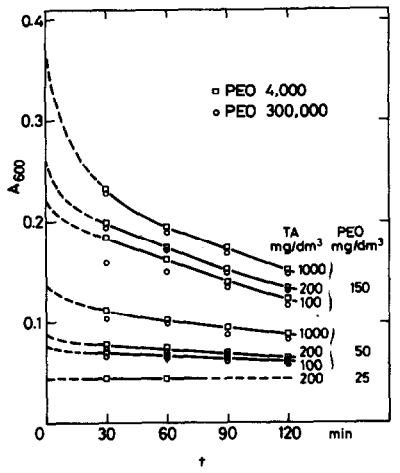

Fig. 8. The absorbance at 600 nm of tannic acid (TA)/PEO solutions as a function of time, for PEO 4,000 and PEO 300,000.

tration being limited to minimize the experimental error. In the present case, the ratio KI/Bi in the test solution is about 10^3 whereas Lisicki and Boltz[26] indicate that this ratio should be $\geq 10^4$ in order to ensure attainment of maximum absorptivity at 337 nm.

Turbidimetry with tannic acid

In the reaction of PEO with tannic acid (TA) the size and number of particles formed depend on the extent of the reaction. Hence, the turbidity of such a suspension is a function of time. This is shown in Fig. 8 where the absorbance at 600 nm, A_{600}, measured in a 1-cm cuvette, is plotted *vs.* incubation time, *t*. A_{600} is directly proportional to the turbidity (expressed in cm^{-1}).

With increasing PEO concentration an increase in the time effect is observed. Probably only the values of A_{600} extrapolated to $t = 0$ would show a linear proportionality between A_{600} and c_{PEO}. However, in practice this situation can never be attained. An increase in the tannic acid concentration also increases the time effect. At a wavelength of 400 nm instead of 600 nm, the observed time effects strongly increase.

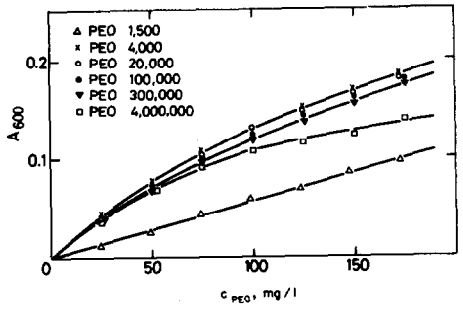

Fig. 9. The absorbance at 600 nm of tannic acid/PEO test solutions as a function of PEO concentration.

The trends mentioned are about the same for PEO 4000 and PEO 300,000, except for TA concentrations of 100 mg/l. and large PEO concentrations. In other words, the effect of molecular weight is small.

Decreasing the sodium chloride concentration from 0.5*M* to 0.1*M* lowers A_{600}. However, at low PEO and TA concentrations the time effects are similar to those shown in Fig. 8.

Temperature differences of $\pm 5°$ have only a minor effect on A_{600} and this effect is practically independent of the TA concentration.

We conclude that measurements should be made at 600 nm, a TA concentration of 200 mg/l. and an incubation time of at least 30 min. Once the time is selected, only small deviations from it are allowed, otherwise serious systematic errors occur, especially at PEO concentrations larger than 50 mg/l.

In Fig. 9 the absorbance, A_{600}, measured under standard conditions, is plotted *vs.* c_{PEO} for a series of PEO samples. As expected on the basis of the results shown in Fig. 8, the calibration plots are curved. However, for $100 < c_{PEO} < 170$ mg/l. an approximately linear relationship is found.

The reagent apparently did not react at all with PEO 600, and only very little with PEO 1500. For $M \geq 4000$ the reaction develops well, but there is still some effect of molecular weight. For very large M ($>10^6$) and large c_{PEO} (>100 mg/l.) the method becomes inaccurate. In general, the method is reasonably sensitive and accurate in the molecular weight range of 4000–10^6.

CONCLUSIONS

For the determination of PEO in aqueous solution three methods are available which are practically unaffected by the PEO molecular weight and can be used over a large PEO molecular-weight range. These three methods are oxidative digestion, precipitation with molybdophosphoric acid, and precipitation with tetraiodobismuthate. The last two methods are modifications of previously suggested procedures.

In general the precipitation reaction with molybdophosphoric acid is preferable to the other two. Oxidative digestion of PEO is very sensitive to the presence of other oxidizable compounds, and the precipitation of PEO with tetraiodobismuthate is somewhat less accurate than that with molybdophosphoric acid.

For PEOs of relatively large molecular weight, the complexation reaction with tannic acid can also be used.

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A CHELATE-FORMING RESIN BEARING MERCAPTO AND AZO GROUPS AND ITS APPLICATION TO THE RECOVERY OF MERCURY(II)

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Summary—A new chelate-forming resin bearing mercapto and azo groups was prepared from a common anion-exchange resin by treatment with azothiopyrine disulphonic acid (ATPS). ATPS resin was very stable and highly effective for the collection of mercury(II) by the batch and column methods. In the column method, the amount of mercury(II) in solution could be reduced to below 0.5 μg/l. The mercury(II) adsorbed could be eluted with thiourea solution, and the resin could be used repeatedly.

We have presented some chelate-forming resins of a new type, prepared simply by the modification of common ion-exchange resins with the proper chelating agents.[1–3] Some of them were found to be stable even in 1.0*M* sodium chloride medium and effective for the collection of heavy metal ions. The usefulness of these resins is attributed to the triple function of the chelating agents, namely ion-exchange, chelate-formation and physical adsorption on the ion-exchange resin.

A chelate-forming resin, prepared from a common anion-exchange resin by treatment with azothiopyrine disulphonic acid [disodium 4,4′-(4-diazenediyl-5-mercapto-3-methyl-1,2-diazacyclopenta-2,4-dien-1-yl)dibenzenesulphonate, abbreviated to ATPS hereafter], has been reported briefly as being effective for the adsorption of mercury(II).[2] This paper gives full details of the adsorption and recovery of mercury(II) ion with this resin (ATPS resin), together with those for copper(II) and cadmium. The synthesis and some properties of ATPS, the first example of a chelating agent bearing mercapto and azo groups, were reported previously.[4]

EXPERIMENTAL

Materials

Azothiopyrine disulphonic acid (ATPS, Fig. 1) prepared as reported previously[4] was used. The solutions of mercury(II), cadmium and copper(II) were prepared from the nitrates. Amberlite IRA-400 (8% divinylbenzene) in the chloride form, 100–200 mesh, was the anion-exchange resin used. All other reagents were of analytical-grade.

Preparation of ATPS resin

The anion-exchange resin was added to the solution of ATPS and the mixture was shaken at 30° until the supernatant liquid became colourless. The resin was filtered off, washed with water and methanol, air-dried, and stored in a refrigerator.

Determination of exchange capacity for mercury(II)

The resin, 200 mg, loaded with various amount of ATPS (0.10, 0.20, 0.40 and 0.60 mmole per g of resin) was shaken with a small excess of mercury(II). The amount of mercury(II) left in solution was determined at regular time intervals.

Determination of metal ion

Mercury(II) was determined by cold-vapour atomic-absorption spectrometry with a Shimadzu atomic-absorption/flame-emission spectrophotometer AA-630-01 equipped with a reduction/aeration apparatus. Copper and cadmium were also determined by atomic-absorption spectrometry.

Adsorption of metal ion by batch operation

Procedure A. To 1 ml of 1000 or 100-mg/l metal ion solution and 99 ml of 0.1*M* acetate buffer, 200 mg of ATPS-loaded resin (0.2 mmole/g) were added. The pH of the solution was adjusted to below 3.6 with 0.1*M* nitric acid. The mixture was shaken for about 12 hr, and the resin was then filtered off on a fritted-glass funnel. An appropriate volume of filtrate was used for determination of the metal ions.

Procedure B. To 1 ml of 1000-mg/l mercury(II), copper(II) and cadmium solution, 10 ml of 5*M* sodium chloride, 87 ml of 0.1*M* acetate buffer and 200 mg of ATPS-

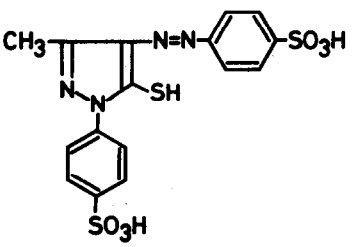

Fig. 1. ATPS.

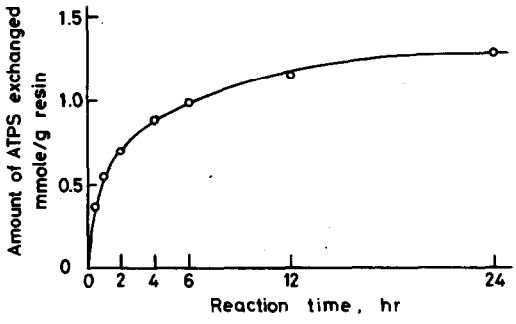

Fig. 2. Adsorption of ATPS on the anion-exchange resin.

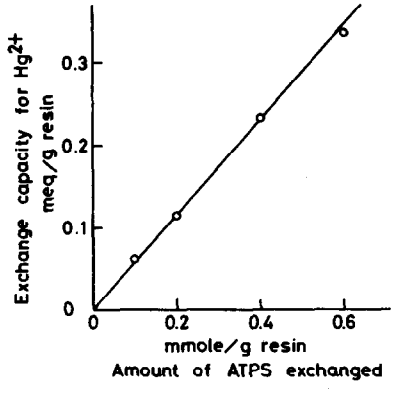

Fig. 3. Exchange capacity for mercury(II).

loaded resin (0.2 and 0.5 mmole/g) were added. Adjustment of pH, shaking of the solution and determination of the metal ions were carried out as in procedure A.

Elution of mercury(II) in column operation

In glass columns (1 cm diameter) 0.5-g amounts of ATPS resin (loading 0.2 mmole/g) which had adsorbed 1000 μg of mercury(II) were packed, to a height of 1.3 cm. The columns were eluted individually with 20 ml of 10*M* hydrochloric acid, 10*M* perchloric acid, 5, 8, 10 and 12*M* nitric acid, 10% thiourea solution, 10% thiourea solution in 0.1*M* hydrochloric acid, and 5, 7, 8 and 10% thiourea solutions in 0.1*M* perchloric acid.

Adsorption and recovery of mercury(II) ion by column operation

Columns (diameter 1.0 cm) were packed with ATPS-resin (loading, column I 0.1 mmole/g; column II 0.2 mmole/g) to a height of 10.0 cm. After the columns had been washed with 200 ml of distilled demineralized water, 50-mg/l mercury(II) solution in 0.1*M* nitric acid was passed through the column at a flow-rate of 1.5 ml/min. The resin was washed with distilled demineralized water and then the amount of mercury(II) in the eluate was determined. Mercury(II) adsorbed on the ATPS-resin in column II was eluted with 10% thiourea solution in 0.1*M* perchloric acid at a flow-rate of 1.0 ml/min, and then the column was washed with 100 ml of 0.1*M* nitric acid and 200 ml of pure water, and the amount of mercury(II) in the eluate was determined. After recovery of the mercury(II), 50-mg/l mercury(II) solution in 0.1*M* nitric acid was again passed through column II, and the amount of mercury(II) in the eluate determined.

RESULTS AND DISCUSSION

We reported earlier that the mono and disulphonic acids of azothiopyrine[4] had been obtained and that they were stable to air oxidation even in as strongly an acidic solution as 10*M* hydrochloric acid. The disulphonate was used in the present study, because of its greater solubility. As shown in Fig. 2, the exchange-capacity of the anion-exchange resin for ATPS was 1.3 mmole/g of resin. Loading with 50% of the capacity required 2 hr. The resin loaded with ATPS (0.2 mmole/g), which took 20 min to prepare, was mainly used in this study. Azothiopyrine was found to be scarcely adsorbed on the anion-exchange resin in 50% methanol. The large exchange-capacity of the resin for ATPS is attributed to an ion-exchange reaction between ATPS and the resin. The binding capacity for mercury(II) on ATPS resins (loading 0.1–0.6 mmole/g) is shown in Fig. 3. The time required for 50% uptake of mercury(II) is less than 10 min. The binding capacity for mercury(II) increases linearly with increase in the amount of ATPS loaded, and the binding ratio of mercury(II) to ATPS on the resin is about 1:2.

The adsorption behaviour of mercury(II), copper (II) and cadmium on ATPS resin as a function of pH is shown in Fig. 4 (procedure A). The adsorption of copper(II) and cadmium increases with pH. The metal ions are adsorbed more effectively at lower concentrations. Adsorption of mercury(II) is complete, the log K_d value being 6.9 in the pH range 1–7. The K_d value for a metal ion is given by the ratio (mmoles

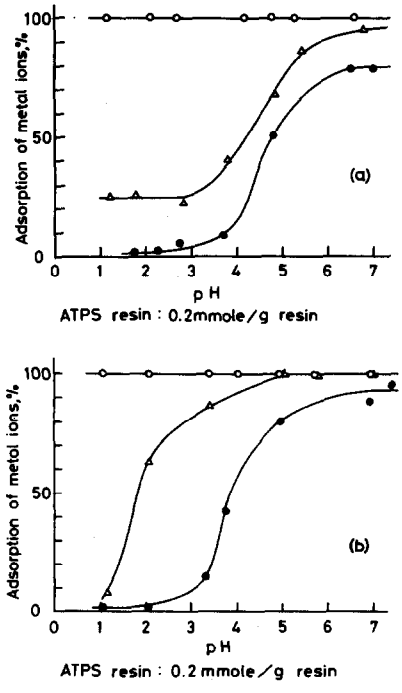

Fig. 4. Adsorption of metal ions by batch operation: ○ mercury(II); △ copper(II); ● cadmium(II). Initial concentration of metal ion: (a) 10 mg/l, (b) 1 mg/l.

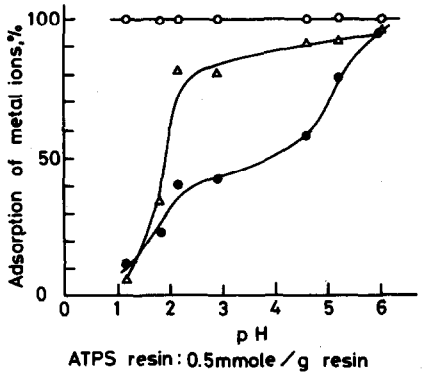

Fig. 5. Adsorption of mercury(II), copper(II) and cadmium(II) from 0.5*M* NaCl by batch operation: ○ mercury(II); △ copper(II); ● cadmium(II). Initial concentration of metal ions 10 mg/l.

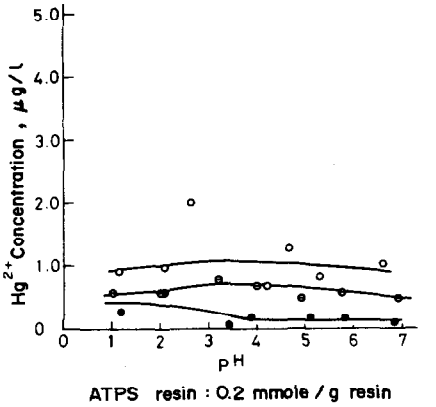

Fig. 6. Concentration of mercury(II) left in the aqueous phase. Initial concentration (mg/l) of mercury(II): ○ 10; ⊖ 1; ● 0.1.

sorbed per g of resin)/(mmoles left per ml of solution). The high K_d value for mercury(II) on ATPS resin indicates that the combination of mercapto and azo groups is extremely favourable for chelate-formation with mercury(II). In fact, the stability constant of the azothiopyrine–mercury(II) chelate[5] has been found to be as high as that of the dithizone–mercury(II) chelate.

To find whether ATPS resin would be suitable for the collection of mercury(II) from estuarine and sea-water, the influence of foreign ions was examined by use of procedure B. The resin, loaded with 0.2 mmole of ATPS per g, was found to sorb 99.6% of mercury(II) added, 71% of copper(II) and 53% of cadmium(II) at pH 6. The incomplete uptake of mercury(II) was due to insufficient loading of the resin with reagent, and the concentration of mercury(II) remaining in solution was reduced to less than 5 µg/l at pH 6 when the resin was loaded with 0.5 mmole of ATPS per g. The uptake of copper(II) and cadmium(II) was much lower than that of mercury(II) in the pH range 1–6, as shown in Fig. 5. Furthermore, these findings indicate that ATPS resin is stable even in the presence of 0.5*M* sodium chloride, and the resin can be applied effectively at pH 1 to the collection of mercury(II) from estuarine and sea-water containing copper(II) and cadmium(II). The strong fixation of ATPS on the resin is attributed to physical interaction between ATPS and the ion-exchange resin. The chelate-forming resins[3] prepared from some chelating agents which bear ion-exchange groups but are not adsorbed physically on the ion-exchange resin have been found to be unstable in the presence of chloride ion.

The amounts of mercury(II) left in the aqueous phase at various pH values were determined and the results are shown in Fig. 6. An initial mercury(II) concentration of 10 mg/l was reduced to below 2 µg/l by shaking with ATPS resin for 4 hr, indicating almost complete sorption of the mercury(II) on the resin. Initial mercury(II) concentrations of 1 or 0.1 mg/l were reduced to less than 1 µg/l.

The commercially available chelating resins for selective collection of mercury(II) cannot be regenerated and ignition is necessary for recovery of the mercury.[6] However, in the case of ATPS resin, there are two possibilities for elution of mercury(II):

$$\text{I}\quad (\text{resin–reagent–metal ion}) \xrightarrow{\text{eluent}} (\text{resin}) + \text{reagent} + \text{metal ion}$$

$$\text{II}\quad (\text{resin–reagent–metal ion}) \xrightarrow{\text{eluent}} (\text{resin–reagent}) + \text{metal ion}$$

where the species in brackets are in the solid state. As shown in Table 1, mercury(II) was completely eluted with 8*M* nitric acid by method I and the anion-exchange resin was regenerated. In method II the mercury(II) was eluted completely with a 10% solution of thiourea in 0.1*M* perchloric acid, and the ATPS resin recovered.

Table 1. Desorption of mercury(II) from ATPS resin by various eluting agents

Eluent	Recovery of mercury(II), %
6*M* HNO_3	78
8*M* HNO_3	100
10*M* HNO_3	100
12*M* HNO_3	100
10*M* $HClO_4$	18
10*M* HCl	21
5% thiourea in 0.1*M* $HClO_4$	0
7% thiourea in 0.1*M* $HClO_4$	93
8% thiourea in 0.1*M* $HClO_4$	96
10% thiourea in 0.1*M* $HClO_4$	100
10% thiourea in 0.1*M* HCl	0
10% thiourea in aqueous solution	0

Sorption conditions (batch operation): ATPS resin (0.2 mmole/g) 0.5 g; mercury(II) 1000 µg in 50 ml of 0.02*M* HNO_3; shaken for 6 hr at 30°C.

Desorption conditions (column operation): ATPS resin (1000 µg Hg sorbed) 0.5 g; column 10 × 13 mm; elution rate 1 ml/min; 20 ml of eluent.

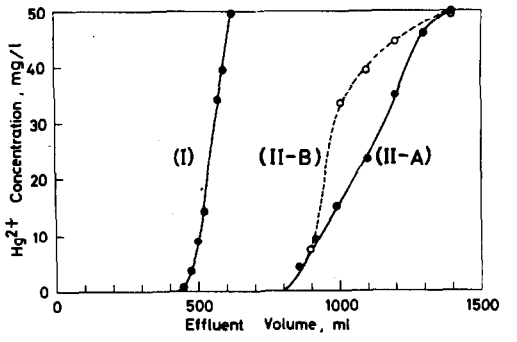

Fig. 7. Break-through curves for mercury(II). Column 1.0 × 100 mm. ATPS loading on the resin (mmole/g) (I) ●——● 0.1; (II-A) ●——● 0.2; (II-B) O---O 0.2 (resin regenerated with 10% thiourea in 0.1M $HClO_4$). Flow-rate (ml/min) (I), (II-A) 1.5; (II-B) 1.0. Concentration of mercury(II) in the sample solution: 50 mg/l in 0.1*M* nitric acid.

The removal of mercury(II) ion from 0.1*M* nitric acid medium was investigated by the column method. The break-through curve for mercury(II) is shown in Fig. 7. The concentration of mercury(II) in the effluent from column I was below 0.5 μg/l up to the break-through point. No mercury(II) was detected in the effluent from column(II), although the break-through curves varied slightly with flow-rate. At a flow-rate of 1–2 ml/min, the sorption of mercury(II) from 0.1*M* nitric acid was efficient.

In Japan, the concentration of mercury(II) in waste-water is severely controlled by law to be less than 5 μg/l. The results presented here indicate the practical applicability of ATPS resin for the final stage of waste-water treatment, after the general procedure for removal of metal ions. The volume of solution that can be treated with an ATPS resin column is doubled if the amount of ATPS loaded is doubled, in agreement with the batch operation results (Fig. 3). As shown in Fig. 7, ATPS resin regenerated with 10% thiourea solution in 0.1*M* perchloric acid can still reduce the mercury(II) concentration to below 5 μg/l although the capacity decreases slightly with repeated use.

A distinct colour change, from red to yellow, was observed on sorption of mercury(II), unlike the case for other thiol-containing chelating resins. The colour change corresponds to the spectral change of ATPS solution in chelate-formation with mercury(II), the bands at 398 and 465 nm shifting to 338 nm and 425 nm.[4]

ATPS resin was found to be stable and to hold its sorption ability for mercury(II) for at least 6 months when stored in a refrigerator. The ATPS on the resin is as stable towards air oxidation as ATPS in solution.

In conclusion, ATPS resin is regarded as a valuable sorbent for the treatment of waste-water containing mercury(II) and to have advantageous features for the preconcentration of some metal ions in trace analysis.

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EXTRACTION OF PALLADIUM THIOCYANATE WITH POLYURETHANE FOAM

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Summary—Conditions for the extraction of the thiocyanate complex of palladium by polyether-type polyurethane foam are reported. Distribution ratios of more than 10^6 with a capacity of about 0.8 mole per kg of foam were obtained. The palladium could be rapidly recovered from the foam with high efficiency by use of ammonia solution. The efficiency of palladium extraction depends on how well the cation associated with the complex fits into the polyether segment of the polyurethane foam.

The use of polyurethane foam in the separation of metals from aqueous solutions was first demonstrated by Bowen.[1] The high distribution coefficients obtained[2] and the ease of handling of the system have aroused considerable interest in using polyurethane foam for the extraction and separation of inorganic and organic species from aqueous solutions.[3–5]

The use of thiocyanate as a complexing reagent has served as a basis for the liquid-liquid extraction of a number of metals,[6] and of palladium into ethyl acetate.[7]

Recently, some attention has been devoted to the extraction of metal thiocyanate complexes onto unloaded and loaded polyurethane foam. The extraction of cobalt,[8] and of cobalt, iron, zinc and cadmium,[9] as well as the preconcentration and separation of rhodium and iridium thiocyanates[10] from aqueous solutions with polyurethane foam has been reported.

Chow and co-workers recently studied the mechanism of the extraction of alkali metals as picrates[11] and cobalt as thiocyanate[8] by polyurethane foam and concluded that the foam acts as a long acyclic polyether chain. Thus the extraction of the anion is a consequence of the complexation of a cation in the cavity of the chain, and the efficiency of extraction depends on how well the cation fits.

The purpose of the present work was to study the mechanism of extraction of palladium as a thiocyanate complex from aqueous solutions, with polyether-type polyurethane foam, and to find the optimum conditions for use of this method for preconcentration of palladium.

EXPERIMENTAL

Apparatus

A model 306 Perkin–Elmer atomic-absorption spectrometer was used for palladium determination, a Fisher Accumet model 520 for pH measurements and a Varian 634 UV-visible spectrometer for absorbance measurements.

Reagents

A stock 1000 μg/ml palladium concentration solution was prepared from palladium chloride in 0.1*M* hydrochloric acid. All other chemicals used were of analytical reagent grade. The water was doubly distilled and demineralized. Commercial polyether-type polyurethane foam sheets were obtained locally and cut into small cubes of approximately 1.0 g each. These foam cubes were soaked in 1*M* hydrochloric acid for 24 hr with occasional squeezing, washed several times with distilled water until acid-free, extracted with acetone in a Soxhlet apparatus for 12 hr and finally air-dried.

Procedure

An aliquot of the stock solution of palladium chloride was put into a 100-ml standard flask with the desired amount of thiocyanate and hydrochloric acid and made up to volume with distilled water. The acid was added after dilution of the thiocyanate solution, to minimize the decomposition of thiocyanate caused by direct contact with concentrated acid.[12]

The extraction was done by placing 95 ml of the sample solution (palladium 13.3 μg/ml) in a 250-ml glass cell containing about 0.05 g of foam. The foam was squeezed by means of a glass plunger in order to bring fresh solution into contact with the foam. The plunger was operated with a multiple automatic apparatus[8] consisting of an eccentric cam turned by a heavy-duty motor to give a 5-cm stroke at a rate of 24/min. The extraction was done at 25.0 ± 0.05°.

The percentage extraction of the metal (E) was calculated by measuring the concentration before and after extraction.

$$E = \frac{(\text{metal})_{\text{initial}} - (\text{metal})_{\text{final}}}{(\text{metal})_{\text{initial}}} \times 100$$

The distribution coefficient (D) for the extraction process was calculated from the ratio of the concentration of the metal in the foam to the concentration of the metal left in solution at equilibrium.

$$D = \frac{\%\ \text{metal on foam}}{\text{wt. of foam (g)}} \times \frac{\text{vol. of solution (ml)}}{\%\ \text{metal left in solution}}$$

RESULTS AND DISCUSSION

The rate of extraction of palladium thiocyanate was studied by varying the time of contact between the foam and 13.3-μg/ml palladium solution from 5 min

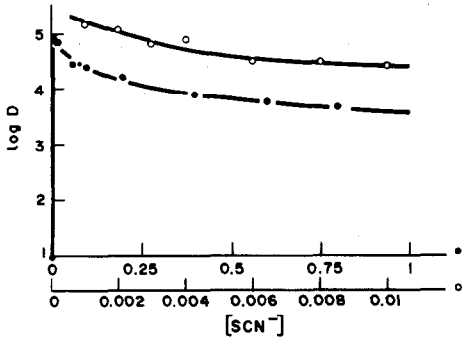

Fig. 1. Effect of thiocyanate concentration on the extraction of palladium: initial palladium concentration 13.3 μg/ml; hydrochloric acid concentration 0.8*M*; volume of solution 145 ml; weight of foam 0.05 g; time for extraction 24 hr.

to 27 hr, with thiocyanate and hydrochloric acid concentrations of 0.5 and 0.4*M*, respectively. The percentage of palladium extracted increased sharply with extraction time up to 15 min, and then more slowly up to 1 hr, after which it remained almost constant; hence a minimum of 1 hr extraction was used in further studies.

The effect of thiocyanate on the extraction of palladium is shown in Fig. 1. The results indicate a very rapid increase in extraction from 0.3% at zero thiocyanate concentration to a maximum at 0.001*M* thiocyanate, with which 98% of the palladium was extracted. Higher concentrations of thiocyanate decreased the extraction somewhat, which may be attributed to the increased influence of thiocyanic acid and 5-amino-1,2,4-dithiazole-3-thione (which forms as a result of thiocyanic acid trimerization[13]) on the extractibility of palladium thiocyanate.

The ultraviolet-region spectra of the solutions before extraction showed an absorption band at 308 nm, which is consistent with $Pd(SCN)_4^{2-}$.[14] Further, with thiocyanate concentrations above 0.1*M*, the appearance of a band at 292 nm indicated the formation of 5-amino-1,2,4-dithiazole-3-thione, the concentration of which paralleled the thiocyanate concentration.

The effect of the thiocyanate on the extraction of palladium was also studied in the presence of 2*M* potassium chloride. The results (Table 1) indicate that the extraction of palladium thiocyanate increased sharply with thiocyanate concentration up to 0.002*M*, and decreased slightly at $[SCN^-] > 0.06M$. The decrease in the extraction of palladium thiocyanate may be attributed to competitive extraction of potassium thiocyanate, which may be expected to be somewhat extractable. As in the previous experiments, the spectra of the solutions before extraction indicated the formation of $Pd(SCN)_4^{2-}$ in amounts increasing with thiocyanate concentration up to 0.06*M*, beyond which the extent of formation became constant. However, these spectra also showed that 5-amino-1,2,4-dithiazole-3-thione was not formed under these conditions, thus accounting for some of the increased palladium extraction at high thiocyanate concentrations.

Table 1. Effect of thiocyanate on palladium extraction in the presence of potassium chloride*

$[SCN^-]$, *M*	*E*, %	log *D*
0	N.D.†	N.D.†
0.002	100.0	6.95
0.004	98.4	5.09
0.006	99.9	6.95
0.008	98.3	5.05
0.012	99.9	6.16
0.02	99.2	5.38
0.06	99.7	5.76
0.1	97.1	4.80
0.5	96.1	4.69
1.0	92.0	4.34

* Initial palladium concentration 13.3 μg/ml; pH = 2.7; potassium chloride concentration 2*M*; volume of solution 95 ml; weight of foam 0.05 g; time for extraction 20 hr.
† Not determinable.

The effect of hydrochloric acid on the extraction of palladium from 0.006*M* thiocyanate medium was studied. As the hydrochloric acid concentration of the solutions was varied from 2×10^{-3} to 1.0*M*, the palladium extraction increased from 57% (log *D* = 3.59) at 0.002*M* acid to 84% (log *D* = 4.19) at 0.08*M* acid, beyond which it increased slowly to 96% (log *D* = 4.83). The spectra of these solutions before extraction showed that the amount of $Pd(SCN)_4^{2-}$ formed is not altered by the acid concentration, so the influence of the hydrochloric acid must be on the distribution of the palladium complex between foam and solution.

The effect of pH of the aqueous phase on the extraction efficiency of polyurethane foam for palladium thiocyanate in the presence and absence of 2*M* potassium chloride was studied. The pH was varied from 2.15 to 10.69 by addition of lithium hydroxide or hydrochloric acid. The results given in Fig. 2 show that in the absence of potassium chloride the extrac-

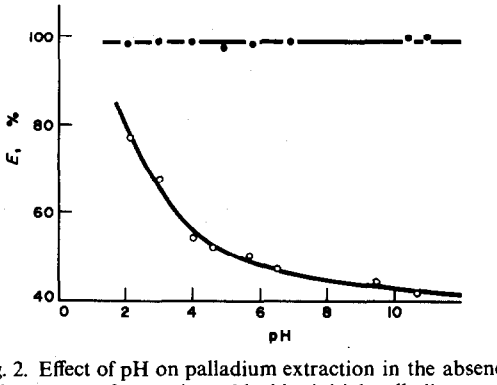

Fig. 2. Effect of pH on palladium extraction in the absence and presence of potassium chloride: initial palladium concentration 13.3 μg/ml; thiocyanate concentration 0.006*M*; volume of solution 95 ml; weight of foam 0.05 g; time for extraction 20 hr; (○) in the absence of potassium chloride; (●) in the presence of 2*M* potassium chloride.

tion of palladium decreases considerably as the pH is raised. The spectra of these solutions before extraction indicated that the $Pd(SCN)_4^{2-}$ absorption peaks were not changed in either intensity or shape by change in pH. Therefore, the decrease in extraction of palladium with increasing pH must be attributed not to the formation of palladium hydroxide but rather to change in the distribution of palladium thiocyanate between the foam and the solution.

The extraction of palladium thiocyanate from solutions containing 2*M* potassium chloride was found to be very high and independent of the pH (Fig. 2). The spectra of these solutions before extraction indicated that the concentration of $Pd(SCN)_4^{2-}$ formed is almost independent of pH and is identical to that observed in the absence of potassium chloride. Thus the presence of potassium chloride must have some effect on the distribution of palladium thiocyanate between the foam and the solution and not on formation of the complex. The presence of potassium chloride broadens the range of pH over which extraction of palladium thiocyanate is quantitative.

Under the optimum conditions for the extraction of palladium thiocyanate from solutions containing only hydrochloric acid and from those containing potassium chloride at high pH, the capacity of polyether-type polyurethane foam was studied for palladium concentrations from 10 to 300 μg/ml. The hydrochloric acid and potassium chloride concentrations were kept constant at 0.5*M*. The pH of the potassium chloride media was fixed at about 6 by addition of lithium hydroxide or hydrochloric acid. The thiocyanate concentration was varied from 6.4×10^{-3} to $5.6 \times 10^{-2}M$, according to the palladium concentration. This was done to minimize interference by potassium thiocyanate or thiocyanic acid, in order to obtain clearer information on the mechanism by which palladium thiocyanate is extracted. The spectra of the solutions indicated that the amount of $Pd(SCN)_4^{2-}$ formed was the same for both sets of experiments. The foam capacity for extraction of palladium was also about the same, about 0.8 mole/kg. Thus the polyether-type polyurethane foam is highly efficient for the preconcentration of palladium from either medium, and since the foam capacity is almost the same the extraction mechanism of the palladium thiocyanate may be the same.

The effect of ammonia on the extraction of palladium from 0.5*M* potassium chloride/0.006*M* thiocyanate medium was also studied. The pH was adjusted to about 6 with potassium hydroxide or hydrochloric acid. Ammonia solution was then added to give a total concentration of 0.01–0.1*M*, which produced a final pH of 10.54–11.14. The extraction of palladium was very low under these conditions, varying from 0 to 3%. The extraction of palladium from in 0.5*M* ammonium chloride/0.006*M* thiocyanate media was studied, the pH before extraction being varied from 4.01 to 8.27 by the addition of ammonia solution or hydrochloric acid. The extraction of palladium

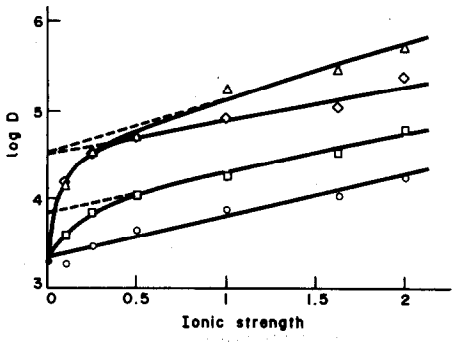

Fig. 3. Effect of ionic strength on the extraction of palladium; initial palladium concentration 13.3 μg/ml; thiocyanate concentration 0.006*M*; (●) H_2O, (○) Li^+, (□) Na^+, (◇) K^+, (△) NH_4^+; pH ~ 6, except for NH_4^+, where pH was ~4; volume of solution 95 ml; weight of foam 0.05 g; time for extraction 20 hr.

dropped from 96% at pH 4.01 to 75% at 6.25 and to zero at 7.41. The spectra before extraction for solutions containing ammonium chloride indicated that the absorbance peak at 308 nm for $Pd(SCN)_4^{2-}$ decreased with increasing pH and reached zero at pH 7.41. The same results were obtained for the 0.5*M* potassium chloride solutions, for which the absorbance at 308 nm was zero even at 0.01*M* ammonia concentration. The decrease in the extraction of palladium may thus be due to the formation of $Pd(NH_3)_4^{2+}$, which is not extractable. Therefore, the addition of ammonia solution or the adjustment of pH may be used for recovery of palladium from the foam. Qualitative studies indicate that palladium can be recovered from the foam with high efficiency.

The ionic-strength effect of various chlorides on the extraction of palladium thiocyanate by polyurethane foam was studied with lithium, sodium, potassium and ammonium chlorides. The pH of the solutions before extraction was adjusted to about 6 with lithium hydroxide or hydrochloric acid except for those containing ammonium chloride, where the pH was adjusted to around 4 to prevent the formation of $Pd(NH_3)_4^{2+}$. The results shown in Fig. 3 indicate that the effect of added salt concentration on the extraction of palladium thiocyanate was virtually independent of the nature of the salt at concentrations above about 0.5*M* (indicating a salting-out effect in this concentration range), but gave very different behaviour at lower concentrations, where the cation chelation mechanism[11] clearly predominates when potassium or ammonium salts are added. The spectra of these solutions indicated that the absorbance of the $Pd(SCN)_4^{2-}$ was the same for all the solutions before extraction, so the differences in the salt effects on the extraction are due to differences in the distribution of palladium thiocyanate between the foam and the solution rather than in formation of the complex. Furthermore, Fig. 3 shows that the extraction efficiency increases in the following order of the cations added: $Li^+ < Na^+ < K^+ \leqslant NH_4^+$. The ion-dipole interaction of ammonium ions with the oxygen sites of

Table 2. Relationship between the ionic diameter of the cation and the relative extraction of palladium*

Cation	Ionic diameter,[15] Å	$\log\left(\frac{D_{\text{(extrapolated to zero salt concentration)}}}{D_{\text{(without salt)}}}\right)$
Li^+	1.20	0
Na^+	1.90	0.5
K^+	2.66	1.2
NH_4^+ †	2.84	1.2
Rb^+	2.96	—
Cs^+	3.34	—

* Initial palladium concentration 13.3 μg/ml; thiocyanate concentration 0.006*M*; pH ~ 6.0; salt concentration 0.1–2*M*; volume of solution 95 ml; weight of foam 0.05 g; time for extraction 15 hr.

† pH ~ 4.0.

polyurethane foam may be considered as contributing to the extraction of $Pd(SCN)_4^{2-}$ being higher in the presence of ammonium chloride than of potassium chloride at concentrations above about 0.5*M*. The linear portions of the curves in Fig. 3 were extrapolated to zero concentration of the salt, and the intercept for lithium chloride was the only one which coincided with the value obtained for the extraction of $Pd(SCN)_4^{2-}$ from a 0.006*M* thiocyanate solution at pH 6 with no added salt. Table 2 shows a direct relationship between the ionic diameter of the cation and the extraction of $Pd(SCN)_4^{2-}$ relative to that with no added salt.

The effect of the different cations on the extraction of $Pd(SCN)_4^{2-}$ as shown in Fig. 4 is $Li^+ < Na^+ < K^+ \leqslant NH_4^+ > Rb^+ > Cs^+$, the ions being arranged in order of increasing diameter. This indicates that the efficiency of polyether-type polyurethane foam for the extraction of palladium thiocyanate, as a function of ionic diameter of the cation, passes through a maximum. This suggests that there are factors involved, besides the ability of the cation to form ion-pairs with $Pd(SCN)_4^{2-}$, which cause the extraction to decrease with increasing cation diameter. If it is assumed that polyether-type polyurethane foam acts as long acyclic polyethylene chains[8] and that the extraction of the anion is through a cation chelation mechanism,[11] the extraction of $Pd(SCN)_4^{2-}$ would be expected to increase with increasing ionic diameter for Li^+, Na^+, K^+ and NH_4^+ and to decrease for Rb^+ and Cs^+.

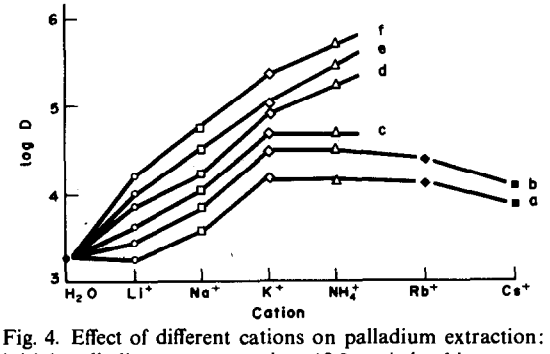

Fig. 4. Effect of different cations on palladium extraction: initial palladium concentration 13.3 μg/ml; thiocyanate concentration 0.006*M*; salt concentration: a 0.1*M*, b 0.25*M*, c 0.5*M*, d 1.0*M*, e 1.5*M*, f 2*M*; (●) H_3O^+, (○) Li^+, (□) Na^+, (◇) K^+, (△) NH_4^+, (◆) Rb, (■) Cs; pH ~ 6, except for NH_4^+ where pH was ~4; volume of solution 95 ml; weight of foam 0.05 g; time for extraction 20 hr.

Since H_3O^+ has an ionic diameter close to that of K^+ and NH_4^+, in other words, close to the cavity size of the foam, $Pd(SCN)_4^{2-}$ is highly extractable from acidic aqueous solutions. This also explains why the foam capacity for the extraction of palladium thiocyanate was almost the same whether 0.5*M* hydrochloric acid or potassium chloride was used, and also why the extraction of palladium thiocyanate decreases with increasing pH of the acid medium, whereas it is independent of pH in the presence of potassium chloride. It was also noted that the rate of extraction of $Pd(SCN)_4^{2-}$ is higher from hydrochloric acid than from potassium chloride medium. This can be explained by the fact that H_3O^+ is held in the cavity through ion–dipole interaction as well as hydrogen-bonding, whereas K^+ is held only by ion–dipole interaction.

Conclusions

Polyether-type polyurethane foam is highly efficient for the preconcentration of palladium from thiocyanate solutions containing only hydrochloric acid, and from those containing potassium chloride at high pH. The extraction is explicable in terms of the cation chelation mechanism.

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BENZYL 2-PYRIDYL KETONE 2-PYRIDYLHYDRAZONE AS REAGENT FOR THE FLUORIMETRIC DETERMINATION OF ZINC AT ng/ml LEVELS

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Summary—A sensitive fluorimetric method for the determination of zinc, based on the formation of a fluorescent chelate with benzyl 2-pyridyl ketone 2-pyridylhydrazone is described. In darkness, the fluorescence develops rapidly and remains stable for 1 hr. The detection limit is 15 ng/ml. The effect of reaction variables, and methods of removing interferences, are discussed.

Heterocyclic hydrazones with the reaction group —N=C—NH—N=C—C=N— generally interact in alkaline medium with hexaco-ordinate bivalent metal ions to form neutral 2:1 ligand:metal chelates, the secondary-amine hydrogen atom in this grouping often being important in determining the structure and behaviour of the complexes.[1,2] The determination of metal ions with such reagents is often improved by employing an extraction procedure, or an aqueous ethanol medium to keep the chelate in solution.

Benzyl 2-pyridyl ketone 2-pyridylhydrazone (BPKPH) has been reported[3] as a highly sensitive and relatively interference-free reagent for the fluorimetric determination of gallium. The fluorescence system formed by zinc(II) and this ligand is reported here. A few elements interfere but this can be prevented by appropriate conditioning of the solution. The method is suitable for determination of zinc in the presence of cadmium, gallium and indium, metals that are often produced from the same mineral source.

EXPERIMENTAL

Reagents

BPKPH, $5 \times 10^{-4}M$ solution. Prepared daily, in absolute ethanol.

Zinc solution. A 0.1*M* stock solution prepared from $ZnSO_4.7H_2O$ in 0.5*M* sulphuric acid and standardized by EDTA titration. Solutions of lower concentration were made by dilution with demineralized water.

Sodium hydroxide, 0.15M solution. Freshly prepared.

Apparatus

A Perkin–Elmer spectrofluorimeter, model MPF-43A, equipped with an Osram XBO 150-W xenon lamp, excitation and emission grating monochromators, 1 × 1-cm quartz cells, R-508 photomultiplier and a Perkin–Elmer 023 recorder. A set of fluorescence polymer samples (Perkin–Elmer) was used to adjust (daily) the spectrofluorimeter to compensate for changes in source intensity.

Procedure

Place 1 ml of 0.15*M* sodium hydroxide and 1 ml of $5 \times 10^{-4}M$ BPKPH in a 50-ml standard flask. Add an aliquot of the sample solution (pH > 3) containing 0.75–3.25 μg of zinc and dilute to volume with demineralized water. Store in the dark until measurement of the fluorescence intensity at 550 nm with excitation at 469 nm; apply a correction for a reagent blank.

RESULTS

Fluorescence spectra

Figure 1 shows the fluorescence excitation and emission spectra of the reagent and its complex with zinc at pH 11.3. Band maxima are found at 469 and 550 nm for excitation and emission, respectively. As shown, a remarkable increase in fluorescence intensity is caused by the chelation.

Effect of reaction variables

For 2% ethanol solutions, it was found that the chelate formation was complete at pH 11 and that the fluorescence intensity remained constant at pH up to at least 13 (Fig. 2). The pH can be adjusted to the desired range by addition of 1 ml of 0.15*M* sodium hydroxide. This is advantageous because potential interference of buffer ions is avoided.

The effect of solvent composition is shown in Fig. 3. The fluorescence intensity is maximal in 2% ethanol medium, but light seriously affects the time necessary for full fluorescence development. This problem is overcome by storing the solutions in darkness until they are measured, and by employing a very narrow excitation slit (3 nm for the recommended procedure). Under these conditions the fluorescence remains stable for 60 min, then slowly diminishes (Fig. 4). There is no temperature effect over the range 5–50.

The maximum fluorescence intensity is attained when the BPKPH concentration in the solution is $1 \times 10^{-5}M$, above which it decreases gradually. Hence 1 ml of $5 \times 10^{-4}M$ BPKPH is used in a final volume of 50 ml.

Using the optimum experimental conditions, it was established that the order of reagent addition is im-

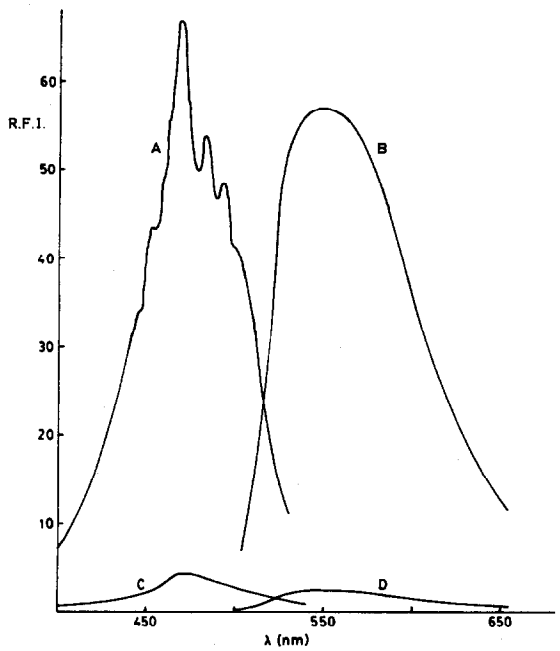


Fig. 1. Uncorrected (A, C) excitation and (B, D) emission spectra of (A, B) complex and (C, D) free ligand, at pH 11.3. [Zn] = $5 \times 10^{-6}M$, [BPKPH] = $1 \times 10^{-5}M$.

portant. The fluorescence intensity is maximal when the complex is formed in alkaline medium. Optimum results are obtained when sodium hydroxide, BPKPH, zinc and water are mixed in that order.

Calibration

The calibration graph obtained by the recommended method is linear up to 65 ng/ml, with a practical limit of detection of 15 ng/ml (defined as the zinc concentration yielding a signal-to-noise ratio of 2:1). For a series of 11 measurements on a 39-ng/ml zinc solution, a relative error of 2.4% and a relative standard deviation of 3.6% were obtained.

Interference studies

The effect of various ions on the determination of

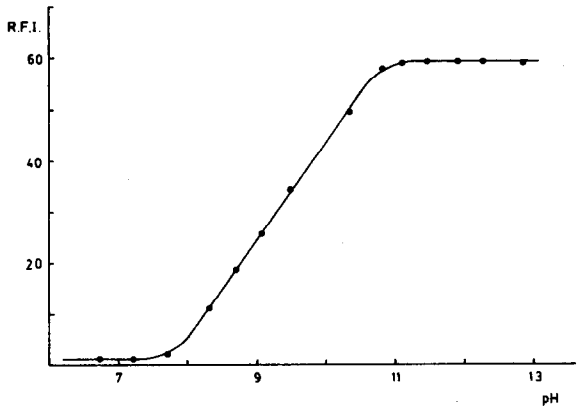


Fig. 2. Effect of pH on the formation of the zinc–BPKPH chelate. [Zn] = $1 \times 10^{-5}M$, [BPKPH] = $1 \times 10^{-5}M$.

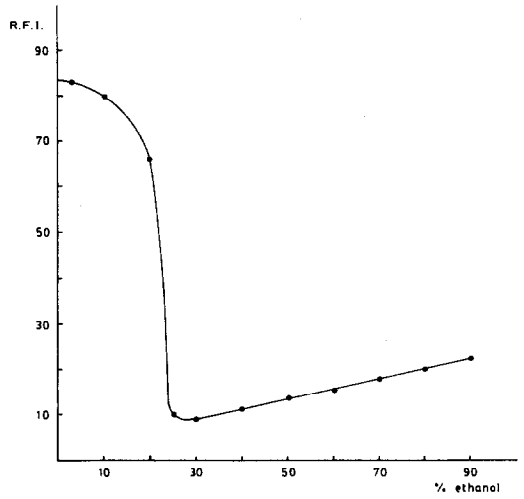


Fig. 3. Influence of solvent composition on the fluorescence intensity of the BPKPH–Zn chelate. [Zn] = $5 \times 10^{-6}M$, [BPKPH] = $1 \times 10^{-5}M$.

zinc at the 39-ng/ml level was investigated by first testing a 100-fold w/w ratio of interferent to zinc, and (if interference occurred) reducing the ratio progressively until interference ceased. Higher ratios were not tested, and the non-interference of fluoride in the aluminium bronze analysis implies that the tolerance level for fluoride is well above the 100-fold w/w level. The interference levels found were 4 ppm for Be^{2+} and F^-, 2 ppm for Tl^+, 1 ppm for PO_4^{3-}, 0.4 ppm for BrO_3^-, 0.2 ppm for Mn^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Ga^{3+}, Tl^{3+}, Cr^{3+}, Ti^{4+}, CN^- and $C_2O_4^{2-}$, 0.08 ppm for Ca^{2+}, Al^{3+}, In^{3+}, Sc^{3+}, As(V) and V(V), and 0.04 ppm of Mg^{2+}. However, Co^{2+}, Fe^{3+} and EDTA are tolerated only at the 0.02 ppm level, and Cd^{2+} (positive interference) at 0.01 ppm. Figure 5 shows the effects produced by some of these ions.

The interference of cadmium (up to 40 ng/ml) can be eliminated by masking with a fourfold molar ratio of cyanide (2 ml of 5-μg/ml potassium cyanide solution). Cobalt up to 40 ng/ml can be masked by addition of 1 ml of 0.1*M* ammonia solution, followed by 1 ml of 0.2*N* hydrogen peroxide. Iron(III) up to 200 ng/ml can be masked with 0.5 ml of 0.01*M* fluoride. The results of analysis of several synthetic samples and an aluminium bronze are presented in Table 2. The aluminium bronze was dissolved in hot nitric acid, the solution was evaporated to dryness and the residue taken up in demineralized water; this solution was made alkaline with 2*M* sodium hydroxide to precipitate interfering elements. The precipitate was filtered off and washed and the filtrate was made up to standard volume. Then an aliquot was treated with 2 ml of 0.1*M* sodium fluoride and analysed spectrofluorimetrically.

Stoichiometry

The ratio of zinc to BPKPH in the complex was established as 1:2 by both the molar-ratio and conti-

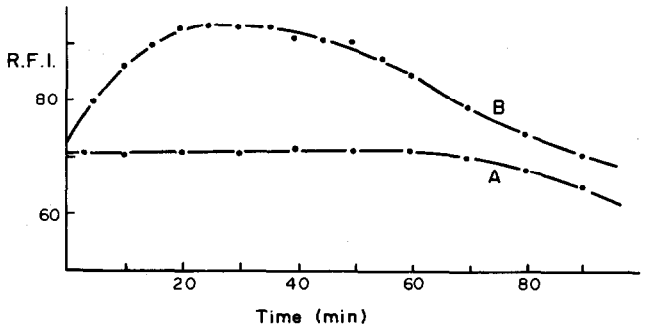


Fig. 4. Effect of light on the fluorescence intensity. A. Solution stored in darkness. B. Solution exposed to room light.

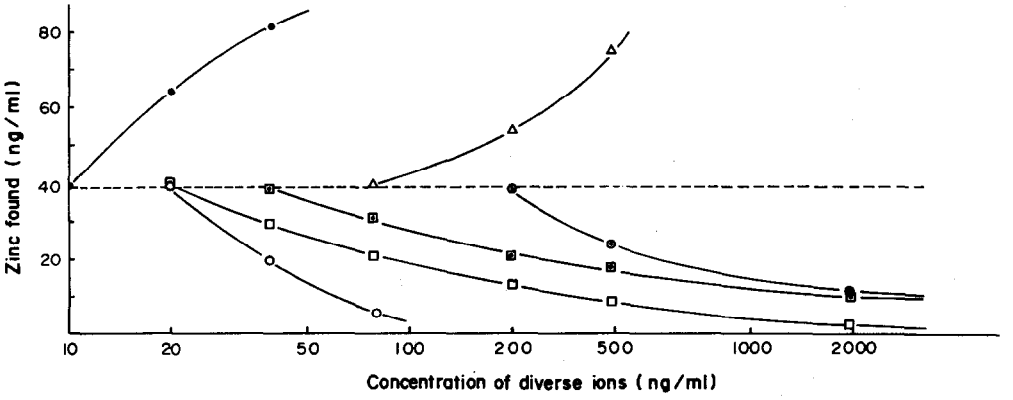


Fig. 5. Effect of diverse ions on the determination of 39 ng/ml Zn. (●) Cd; (△) In; (○) Fe(III); (□) Co, EDTA; (◎) Cr, Mn, Fe(II); (⊡) Mg.

Table 1. Spectral data of the Zn–BPKPH chelate at pH 11.3

Medium	λ_{max}, nm	λ_F, nm	$\epsilon, 10^4\ l.mole^{-1}.cm^{-1}$	R.F.I.*
2% ethanol	480	550	5.1	810
80% ethanol	455	535	5.5	190 (13†)

* The relative fluorescence intensity of 0.1 ppm of quinine sulphate is 400.
† At pH 8.0.

Table 2. Determination of zinc in different samples

Sample	Zn taken, *ng/ml*	Zn found, *ng/ml*
Zn + Fe(III) (200 ng/ml)[a]	39.0	41.4
Zn + Fe(III) (80 ng/ml)[a]	39.0	39.5
Zn + Co (II) (40 ng/ml)[b]	39.0	39.0
Zn + Cd (II) (40 ng/ml)[c]	39.0	39.5
Zn + Fe(III) (200 ng/ml)[a] + Co(II) (40 ng/ml)[b] + Cd(II) (40 ng/ml)[c]	39.0	40.2
Aluminium bronze[d]	40.0	39.0(= 0.92%)[e]
	60.0	58.2(= 0.91%)[e]

[a] In presence of 0.5 ml of $10^{-2}M$ fluoride solution.
[b] In presence of 1 ml of 0.1M ammonia solution and 1 ml of 0.2N hydrogen peroxide.
[c] In presence of 2 ml of 2.5-μg/ml potassium cyanide solution.
[d] Nominal composition: 0.94% Zn; 85.90% Cu; 2.67% Fe; 0.27% Mn; 1.16% Ni; 8.80% Al.
[e] Average of three determinations.

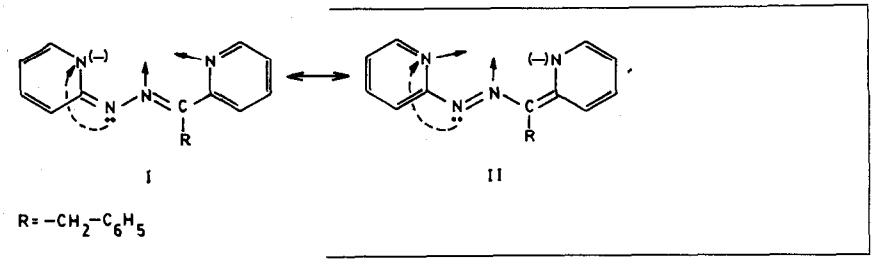
nuous-variation methods. The complex is readily extractable into solvents such as carbon tetrachloride, cyclohexane, n-hexane and benzene.

DISCUSSION

It has been stated that ferroin-type compounds with the —N=C—NH—N=C—C=N— grouping act as terdentate planar chelating agents. This is in accord with our results and some other conclusions can also be drawn.

The well-structured excitation spectrum is indicative of a planar structure for the ground state of the fluorophore, but a partial loss in planarity presumably accompanies the excitation process as indicated by the lack of resolution in the emission spectrum.[4] This occurs for both 2 and 80% ethanolic medium although there are marked differences in the spectral parameters for solutions of the complex in the two media, except for the molar absorptivity (Table 1). The similarity in the latter parameter is further evidence of planarity of the complex in both solvents, with no effect from changes in polarity or hydrogen-bonding ability of the medium. There is, however, one site in the molecule that plays an important role in the chelating behaviour of these compounds, namely the secondary-amine nitrogen atom. It is well known[5-9] that in alkaline medium these ligands act in the anionic form as one or other of the resonance structures after loss of the imine hydrogen atom, this being favoured by the chelation process.[10] On the basis of aromaticity energy arguments, however, neither of the chelates derived from structures **I** and **II** is preferred. In these circumstances,[2] the fluorescence intensity will be lower than that for chelates in which the —N=C— form is energetically favoured. That is the case for these chelates in 80% ethanol medium at pH 8.0.[11] On this basis, the abrupt increase in fluorescence intensity when the ethanol content is decreased (Fig. 3) suggests that the —N=C— form is then energetically favoured. Some evidence of this is found in the low-lying excited state of the complex in aqueous medium (Table 1). If for this type of ligand a charge-transfer process promotes the *n*-electron pair from the imine nitrogen atom towards the heterocyclic nitrogen atom upon excitation,[12] (see scheme above), then irrespective of the effect of this on the chelation process, the preferred form will be that for which the solvent most hinders displacement of the *n*-electron pair towards the site of co-ordination. In fact, chelation is more effectively achieved with structure **I** than structure **II** in water, a strong hydrogen-bonding donor solvent, which stabilizes the —N=C— form.

The results of the present investigation show that BPKPH is a suitable reagent for the fluorimetric determination of zinc. According to the structure of the ligand and the co-ordination number index of zinc ion, a neutral complex is formed. This is in agreement with the easy extraction of the chelate by low polarity solvents. Consequently, the low upper limit of the calibration graph is presumably a consequence of the insolubility of the chelate in water.



I II

R = $-CH_2-C_6H_5$

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SHORT COMMUNICATIONS

DETERMINATION OF ARSENIC IN POLLUTED WATERS BY DIFFERENTIAL PULSE ANODIC-STRIPPING VOLTAMMETRY

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(*Received* 2 *September* 1981. *Revised* 3 *November* 1981. *Accepted* 26 *November* 1981)

Summary—Experimental parameters affecting the analytical response of arsenic in differential pulse anodic-stripping voltammetry (DPASV) have been examined. DPASV offers higher sensitivity than linear-scan anodic-stripping voltammetry for similar analysis times. Both techniques have been applied to the NBS Standard Reference Water (SRM 1643) and some polluted water samples. The results on polluted waters compared favourably with those obtained by graphite-furnace atomic-absorption spectroscopy.

The direct determination of arsenic in natural waters by anodic-stripping voltammetry (ASV) generally suffers from interferences from co-existing species such as copper and iron.[1] These interferences were overcome by Davis *et al.*[2] by use of a reduction–distillation sample treatment coupled with a high-speed ASV method. This approach was adapted recently for a charge-transfer analyser in the linear-scan ASV (LSASV) mode.[3] The advantages of differential pulse ASV (DPASV) over the more conventional LSASV are well recognized,[4–6] although the slow stripping process of DPASV has hindered its wide application in routine analysis. In this study, the various experimental parameters affecting the sensitivity of arsenic determination by DPASV were examined. The DPASV results were compared with those obtained by LSASV with similar analysis times. Both techniques were applied to some natural and synthetic water samples. Some of the results were also compared with those obtained by graphite-furnace atomic-absorption spectroscopy (GFAAS).

EXPERIMENTAL

Reagents

Aqueous As(III) stock solutions, 10 and 0.1 mg/ml, were prepared from "Ultrapure" As_2O_3 (Ventron Alfa). A 1-μg/ml aqueous As(III) working standard solution was prepared fresh daily by dilution of the 0.1-mg/ml stock solution. A certified atomic-absorption arsenic standard (1 mg/ml, Fisher) was used to prepare arsenic standards of lower concentrations used in the GFAAS measurements. A 10% copper(I) chloride solution in concentrated hydrochloric acid was prepared according to Davis *et al.*[2] Nitrogen gas ("Zero Oxygen", max. 0.5 ppm O_2) was supplied by Union Carbide Canada Ltd. Distilled demineralized water was obtained from a Corning AG-11 water purification apparatus. All other reagents used were of analytical reagent grade or better.

Apparatus

All electroanalytical measurements were made with an ESA 3040 charge-transfer analyser (CTA) equipped with a Heath-Schlumberger SR-207 X–Y recorder. The functional features of the CTA have been described in detail elsewhere.[3,7] The electrochemical cell was an ESA 3600-01 with a three-electrode system: a pyrolytic tubular graphite working electrode with a gold film plated on the 2-cm^2 active inner surface; a platinum wire counter-electrode in 0.05*M* hydrazine hydrochloride solution and a silver/silver chloride reference electrode in saturated sodium chloride solution. Both the reference and counter-electrodes were isolated from the sample solution by porous Vycor plugs supplied by ESA. The porosity of the plug is unknown. Effective stirring was accomplished by a top-mounted propeller.

The distillation apparatus used to separate the As(III) from the original sample is a modified version of the one used by Davis *et al.*[2] To minimize contamination and facilitate rapid changing of apparatus between distillations, a Quickfit B-10 ground-glass joint was used instead of the original tight-fitting rubber septum to connect the nitrogen line to the distillation apparatus. To eliminate variations in the flow-rate of the nitrogen carrier-gas, regulator-equipped flowmeters (Brooks Instruments Sho-Rate 150) were used to monitor the gas flow through each set-up throughout the distillation process.

A Perkin–Elmer 603 atomic-absorption spectrophotometer equipped with a Perkin–Elmer HGA-2100 graphite furnace, a deuterium-arc background corrector, and an arsenic electrodeless discharge lamp (EDL) was used for the electrothermal atomic-absorption measurement of arsenic. The nitrogen purge-gas flow was interrupted during atomization.

Procedure

Transfer a portion of the water sample (5 ml for an arsenic level of 1–50 ng/ml) into a 25-ml test-tube placed in a thermostatically controlled metal block. For higher

Table 1. Instrumental parameters for DPASV and LSASV

Instrument parameters	DPASV	LSASV
Scan-rate, *mV/sec*	5	100
Pulse amplitude, *mV*	100	0.0
Pulse frequency, *Hz*	1	—
Deposition potential, *mV*	−50	−150
Deposition time, *sec*, stirring	150	180
resting	30	60
Final potential, *mV*	+300	+550
Total analysis time, *sec*	250	247

arsenic concentrations dilute the sample to an arsenic concentration between 20 and 50 ng/ml. Add 2 ml of a 24:24:1 mixture of nitric, perchloric and sulphuric acids to each sample. Reduce the initial sample volume to less than 1 ml at 100° to avoid losing any liquid through bumping, then continue the evaporation, to near dryness, at 200°.

Separation of the arsenic from the digested sample follows essentially a previously described reduction–distillation procedure[2,3] and is therefore not described here in detail. Using the glass reduction–distillation apparatus discussed earlier, reduce the As(V) in the digested sample with 1% copper(I) chloride solution in concentrated hydrochloric acid and simultaneously distil the $AsCl_3$ liberated, collecting it in 4 ml of distilled-demineralized water in an ASV analysis test-tube. After 16 min of distillation at a nitrogen flow-rate of 140 ml/min, add concentrated hydrochloric acid to give a total volume of 5 ml in the analysis tube. Determine the arsenic levels in the resulting solution, using the instrumental parameters listed in Tables 1 and 2. Record the stripping voltammograms on the X–Y chart recorder and also note the digital read-out of the instrument at the base and at the tip of the peak. Find the peak heights either by direct measurement on the strip chart or by calculation from the recorded digital read-outs. Obtain the concentration of total arsenic in the samples by the method of standard addition.

The atomic-absorption determination uses a direct graphite-furnace method with nickel matrix-modification. Dilute a known volume of the sample with concentrated nitric acid, 10% nickel nitrate solution and distilled-demineralized water so that the final concentrations are 1% acid, 0.05% nickel and ~20 ng/ml arsenic. Inject a 10-μl aliquot of this solution into a pyrolytically coated graphite tube with an Eppendorf pipette fitted with a precleaned[8] polypropylene tip. Initiate the sequential "dry", "char", "atomize" (100°/40 sec–900°/30 sec–2500°/7 sec) programme. Read off the peak absorbance at 193.7 nm after an integration time of 8 sec. Average five replicate measurements for each test solution. Calculate the amount of total arsenic in the sample by interpolation from the linear working curves prepared by using aqueous arsenic standards in 1% nitric acid and 0.05% nickel medium.

RESULTS AND DISCUSSION

Figures 1–5 show the variation of arsenic ASV response as a function of scan-rate, pulse frequency, pulse amplitude, deposition potential and deposition time. Since the scan-rate, pulse frequency and pulse

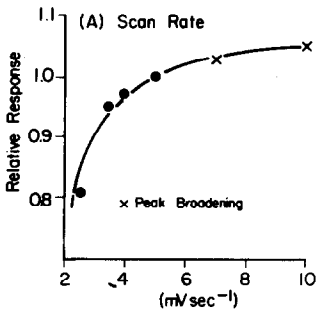

Fig. 1. Effect of scan-rate on the DPASV response to 50 ng of As(III) in 5 ml of 7*M* hydrochloric acid.

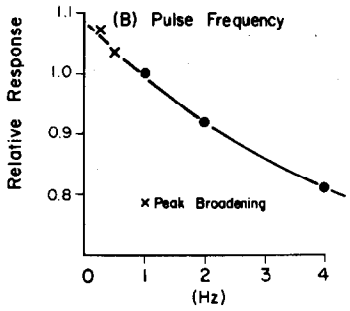

Fig. 2. Effect of pulse frequency on the DPASV response to As(III) (as in Fig. 1).

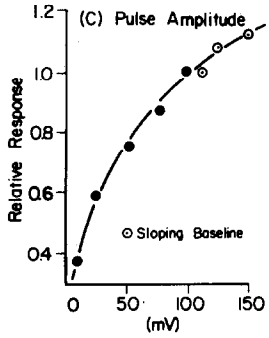

Fig. 3. Effect of pulse amplitude on the DPASV response to As(III) (as in Fig. 1).

Table 2. Charge-transfer analyser programme settings for DPASV and LSASV

Mode	Channel	Pulse amplitude, *mV*	Integration	Time per channel, *sec*
DPASV	1,2	0	No	0.06
	3,4	100	−	0.20
	5,6	0	No	0.04
	7,8	100	+	0.20
LSASV	1–8	0	No	30

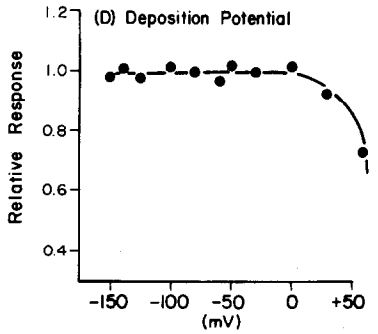

Fig. 4. Effect of deposition potential on the DPASV response to As(III) (as in Fig. 1).

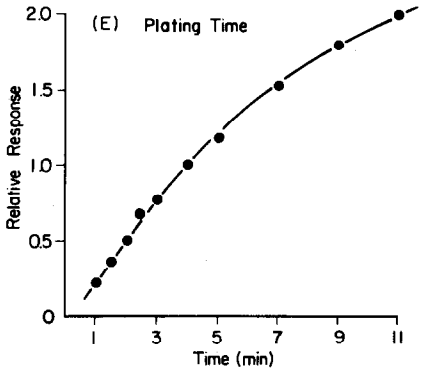

Fig. 5. Effect of plating time on the DPASV response to As(III) (as in Fig. 1).

amplitude were interactive in the ASV response, the optimum value of each parameter was also confirmed by systematically varying the other two. To obtain the highest sensitivity possible for a given analysis time, the optimum for each parameter except deposition time was chosen as the value giving the largest stripping current without peak broadening or sloping of the baseline. Higher sensitivity, if required, could be achieved by increasing the plating time within reasonable limits (Fig. 5).

Davis *et al.*[2] have made a detailed study of the experimental conditions (*e.g.*, distillation time and effect of hydrochloric acid concentration) affecting the analytical signal for arsenic. Our observations are in agreement with theirs.

Table 3 summarizes the detection limit, linear range and precision for arsenic by LSASV, DPASV and the nickel matrix-modification of GFAAS. The detection limit is defined as 3 times the standard deviation of the blank. It is apparent that DPASV gave the lowest detection limit.

Table 3. Analytical performance parameters

Parameter	DPASV	LSASV	Ni-GFAAS
Detection limit*, *ng/ml*	0.15	1.0	1.5
Linear range, *ng/ml*	0–100	0–500	0–60
Precision†, CV, %			
5 × dl	1.5	4.5	17
10 × dl	1.0	2.7	9
50 × dl	1.2	2.9	—

* Detection limit = 3 × standard deviation of blank.
† At 95% confidence interval at 5, 10 and 50 times the detection limit (dl) for arsenic, 20 replicate measurements.

Table 4. Recovery of arsenic by DPASV and LSASV (all concentrations in ng/ml)

Sample	Spiked or certified value	LSASV	DPASV
As standard in demineralized water	3.0	3.2	3.0
	10	9.9	9.6
	20	21.3	19.2
	40	36.7	40.2
NBS Standard Water 1643	77	79	78

The working calibration curves for LSASV and DPASV were obtained under the conditions listed in Tables 1 and 2. For the range 5–500 ng of arsenic, the plot of DPAS peak-heights (Y) *vs.* arsenic concentration (X, ng/ml) can be represented by the equation $Y = 3.77X + 4.2$. The correlation coefficient is 1.000. Similarly the equation for the calibration plot of the linear-scan mode can be represented by $Y = 0.83X + 4.0$ over the arsenic concentration range 2–100 ng/ml with a correlation coefficient of 0.998.

The precision, expressed as the coefficient of variation at the 95% confidence interval, was obtained at five, ten and fifty times the limit of detection for each technique. Twenty measurements were made at each concentration. Considering the levels involved, the precision is quite satisfactory.

To determine the accuracy of the ASV methods, the complete procedure was applied to several synthetic standards and the NBS Reference Material 1643 (Trace Elements in Water). The arsenic recoveries determined by DPASV and LSASV are summarized in Table 4. Very good agreement is found between the results of the two methods and the spiked or certified values.

To confirm the accuracy of the DPASV method and investigate its application to polluted waters, the arsenic contents of some environmental samples were determined by DPASV and also by GFAAS with nickel matrix-modification. The results in Table 5

Table 5. Concentration of total arsenic in some polluted water samples by electrochemical and atomic-absorption techniques (all concentrations in ng/ml)

Sample	LSASV	DPASV	GFAAS (with nickel)
ENL 1		114	115
ENL 2		94	100
ENL 3		558	550
ENL 4		433	440
ENL 5		696	645
RG	227	229	210
LG	8.06×10^3	7.71×10^3	7.50×10^3

show good correlation between the two techniques. Thus no interference was encountered in the samples analysed by DPASV when the proposed method was used in combination with the arsenic separation procedure developed by Davis *et al.*[2]

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DETERMINATION OF INDIUM BY HYDRIDE GENERATION AND ATOMIC-ABSORPTION SPECTROMETRY

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Summary—Conditions are presented for the determination of indium by atomic-absorption spectrometry following hydride generation. Indium hydride produced by addition of sodium borohydride to a solution of indium in 3*M* hydrochloric acid is flushed with argon into an electrically heated silica tube. The mass of indium giving 1% absorption is 0.3 μg.

Hydride generation coupled with atomic-absorption spectrometry is a most useful technique for the determination of elements in the middle and towards the bottom of Groups IV, V and VI of the Periodic Table, namely germanium, tin, lead, arsenic, antimony, bismuth, selenium and tellurium.[1,2] During an attempt to extend the method to neighbouring elements, it was found that indium can be determined by atomic-absorption spectroscopy after generation of indium hydride from aqueous solution. The appropriate conditions are now presented.

EXPERIMENTAL

Apparatus and reagents

The hydride was generated in a cylindrical tube of 22 mm internal diameter, sealed with a flat bottom and fitted with a detachable top. Its total capacity was 70 ml. It was constructed with a side-arm fitted with a "Suba-seal" through which sodium borohydride solution could be injected. The inlet tube for argon was centrally situated and terminated 10 mm from the bottom of the tube, *i.e.*, just above the surface of the liquid during the generation of hydride. The exit tube for argon was situated at the top of the vessel and contained a plug of glass wool to prevent any spray being carried forward. The vessel is like a Drechsel bottle with a side-arm for injection of solutions. The exit tube was connected to a silica tube (8 mm bore × 17.5 cm long) with a short length of PVC tubing. The silica tube was heated electrically and positioned within a Perkin–Elmer 300S atomic-absorption spectrometer fitted with an indium hollow-cathode lamp. The resonance line at 303.9 nm was employed.

Standard indium solution (*1 mg/ml*). Dissolve 0.1 g of indium metal in about 54 ml of hydrochloric acid (1 + 1) with warming. Dilute the solution to volume in a 100-ml standard flask to produce a solution 3*M* in hydrochloric acid.

Sodium borohydride solution, 2%. Prepared freshly each day. The solution was not stabilized with sodium hydroxide, because the acid concentration is an important factor in the hydride generation.

Preparation of the calibration graph

Into the dry generating vessel through which argon is flowing at 2 l./min, inject 20 μl of standard indium solution, onto the flat bottom. Then inject 2 ml of sodium borohydride solution. Measure the absorbance of the exit argon gas within the silica tube at 1200°. Repeat the measurements for 40, 60, 80 and 100 μl volumes of indium solution. Construct a calibration graph of peak height or peak area *vs.* mass of indium.

RESULTS AND DISCUSSION

Useful indium signals were obtained at silica-tube temperatures above 700°, with an optimum temperature range of 950–1250°. The optimum flow-rate of argon was 2 l./min. The optimum hydrochloric acid concentration of the indium solution for hydride generation was 3*M* for area measurements, but well-shaped peaks were produced within the acid concentration range 2–6*M*.

When larger volumes (0.5–5 ml) of 3*M* hydrochloric acid containing 20–100 μg of indium were injected into the generating vessel, followed by sodium borohydride, much smaller absorbances were obtained and success in obtaining useful absorbances required the injection of volumes of indium solution smaller than or equal to 100 μl.

With peak height measurements the calibration graph for 20–100 μg of indium was slightly convex but that of peak area *vs.* mass was a straight line through the origin. The mass of indium giving 1% absorption was 0.3 μg and for peak area (absorbance × time in seconds) measurements the slope of the calibration graph was 0.033 sec/μg of indium. Compared with atomic-absorption spectrometric methods involving hydride generation for germanium, tin, arsenic, antimony, bismuth, selenium and tellurium,[1,2] the method for indium has poor sensitivity. However, the characteristic mass for indium by hydride generation is not appreciably greater than that for lead.[1,2] It may be possible to improve upon the sensitivity by a detailed study of generating conditions.

Previous information on the existence of indium hydride is scanty and the compound does not appear

to have been well characterized.[3,4] Not all the indium(III) is converted into indium hydride, because, in all cases, a black precipitate (presumably of metallic indium) was also produced on addition of sodium borohydride. The signal was definitely due to atomic absorption and not molecular absorption because no absorbance signal resulted when the indium hollow-cathode lamp was replaced by a hydrogen lamp.

With the reported reciprocal sensitivity of 0.3 μg of indium for 1% absorption, the method involving hydride production has little to commend it in comparison with nebulizing an indium solution into an air–acetylene flame, for which the characteristic concentration is 0.5 μg/ml.[5] Pulse nebulization of 100 μl of indium solution into a flame should produce a greater absorbance than the hydride method with a similar volume of solution[6] but any future improvement in the hydride method will make it more competitive with methods involving nebulization into flames.

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BEHAVIOUR OF MEMBRANE GRAPHITE ELECTRODES IN POTENTIOMETRIC TITRATIONS OF REDUCING SUBSTANCES WITH BROMINE IN ACETIC ACID

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Summary—A platinum electrode, a Radelkis OP-C-7111D graphite electrode and a laboratory-prepared silicone-rubber-based membrane graphite electrode were used in potentiometric titrations of reducing substances with bromine in acetic acid. In determinations of hydroquinone, 2-methylhydroquinone, 2-chlorohydroquinone and ascorbic acid, the Radelkis electrode showed the greatest sensitivity. The advantage of the laboratory-prepared membrane graphite electrode lay in its rapid attainment of stable potential values. Titrations were improved by addition of potassium acetate to the solutions analysed. The effects of water and acetic anhydride on the results were also investigated.

According to the literature, electrodes prepared from pyrolytic graphite,[1] glassy carbon,[2] carbon fibre,[3] and by Růžička's method[4] are all suitable for end-point detection in potentiometric titrations in aqueous media. Pungor and Szepesváry have prepared silicone-rubber-based graphite electrodes of low porosity, and used them, after pretreatment with suitable oxidizing agents, as indicator electrodes in potentiometric titrations of acids and bases in water and acetone.[6,7] Since various other components can be incorporated in the membrane, making possible the preparation of electrodes having particular properties, a study has been made of the mechanism of their function in acid–base titrations.[8]

The aim of the present work was to investigate use of silicone-rubber-based membrane graphite electrodes for potentiometric titrations of reducing substances with bromine in acetic acid. The bromine reagent is used extensively as a titrant,[9–17] and conditions have been found for anodic generation of bromine in acetic acid with high current-efficiency.[18,19]

EXPERIMENTAL

Apparatus

Potentials were measured with a Radiometer PHM 26 instrument. The electrodes used were:

(1) a platinum electrode, area 6 cm^2;
(2) a Radelkis OP-C-7111D graphite electrode (C_R);
(3) a silicone-rubber-based membrane graphite electrode (C_M) made from a membrane[5] cemented to one end of a glass tube (0.5 cm bore) containing mercury for electrical contact;
(4) a mercury–mercury(I) acetate reference electrode.

Reagents and solutions

All reagents used were of analytical grade. Anhydrous acetic acid was prepared by distillation of Merck glacial acetic acid; the fraction boiling at 117.5–118° was collected. The potassium acetate was dried for 6–8 hr at 120° before use. Other reagents were used without purification. The bromine solution (0.05*M*) was prepared by dissolving 2.60 ml of Merck bromine in 1 litre of acetic acid in a dark bottle, and standardized daily by biamperometric titration[20] of a standard arsenic(III) solution.

Solutions of hydroquinone, 2-methylhydroquinone, 2-chlorohydroquinone (all 0.05*M*) and ascorbic acid (0.005*M*) in glacial acetic acid were prepared from accurately weighed amounts of the substances. Arsenic(III) chloride and antimony(III) chloride solutions in the same solvent were standardized by coulometric titration with anodically generated bromine in aqueous solution.[21,22] More dilute standard solutions were prepared by exact dilution of these solutions.

Procedure

A measured volume of the solution to be titrated was added to 15–20 ml of glacial acetic acid in a 50-ml flat-bottomed vessel and the required amount of 1*M* potassium acetate was added. The solution was stirred with a magnetic stirrer, and standard bromine solution was added from a 10-ml burette made of dark glass, the tip being immersed in the solution during the titration.

RESULTS AND DISCUSSION

All performance characteristics of the electrodes are given in Table 1 and all titration results in Table 2.

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Table 1. Total potential changes from start of titration to 10% after the end-point (E_t) and maximum (end-point) potential jumps for addition of 0.05 ml of titrant ($E_{e.p.}$) in titrations of reducing substances in 0.2*M* potassium acetate in acetic acid with bromine at a platinum electrode (Pt), a "Radelkis" graphite electrode (C_R), and a silicone-rubber-based membrane graphite electrode (C_M) (titrant additions in the vicinity of the end-points were 0.05 ml)

Sample	E_t, *mV*	$E_{e.p.}$, *mV*	Electrode	Titrant concentration, *M*
Hydroquinone	350–360	195–210	Pt	0.05
	440–460	235–245	C_R	0.05
	400–415	210–215	C_M	0.05
2-Methylhydroquinone	390–400	165–175	Pt	0.05
	500–505	280–285	C_R	0.05
	430–450	235–240	C_M	0.05
2-Chlorohydroquinone	355–370	100–190	Pt	0.05
	400–420	180–220	C_R	0.05
	340–360	120–190	C_M	0.05
Ascorbic acid	470–490	380–400	Pt	0.05
	580–620	450–490	C_R	0.05
	430–470	230–260	C_M	0.05
Arsenic(III) chloride	330–360	230–255	Pt	0.01
	250–270	100–125	C_R	0.01
	335–360	145–175	C_M	0.01
Antimony(III) chloride	290–315	110–135	Pt	0.01
	240–265	160–185	C_M	0.01

The titration of hydroquinone in acetic acid was investigated first. The absence of a pronounced potential jump at the end-point made this determination difficult, but addition of potassium acetate to give a concentration of 0.07*M* made the potential increase at the end-point large enough. A similar effect was observed in titrations of the other reductants, so all further determinations were done in 0.2*M* potassium acetate (in glacial acetic acid).

In titration of the hydroquinones, the potentials stabilized most slowly at the platinum electrode, and fastest at the membrane graphite electrode (almost immediately, except near the end-point, where stabilization took 2–3 min). Table 1 shows that the Radelkis electrode gave the best performance for these titrations.

The rather larger potential changes for the 2-methylhydroquinone system suggested that smaller amounts might be titrated successfully with more dilute titrant, but the curves obtained were too flat, and the results had low accuracy and reproducibility.

The platinum and Radelkis electrodes gave practically the same electrode potential but the membrane graphite electrode gave a much lower potential for the substituted hydroquinones (Fig. 1), and this might be due to a matrix effect or the way in which the graphite was incorporated into the membrane.[6] The hydroquinones were oxidized to the corresponding quin-

Table 2. Results of potentiometric titrations obtained by using a platinum electrode (Pt), a "Radelkis" graphite electrode (C_R), and a silicone-rubber-based membrane graphite electrode (C_M), and a mercury–mercury(I) acetate reference electrode

Sample	Taken, *mg*	Found, %*			No. of titrations
		Pt	C_R	C_M	
Hydroquinone	27.6	100.0 ± 0.1	100.5 ± 0.4	101.0 ± 0.0	6
	2.753	99.6 ± 0.0	100.0 ± 0.3	100.0 ± 0.1	7
2-Methylhydroquinone	25.3	100.2 ± 0.0	100.5 ± 0.0	100.1 ± 0.0	6
2-Chlorohydroquinone	36.2	100.4 ± 0.8	100.8 ± 0.4	100.9 ± 1.2	6
Ascorbic acid	8.80	97.8 ± 0.0	97.9 ± 0.8	97.8 ± 0.0	5
Arsenic(III) chloride	2.195	100.0 ± 0.1	99.1 ± 0.3	100.3 ± 0.1	6
Antimony(III) chloride	5.73	99.2 ± 0.1		99.3 ± 0.0	7

* Mean value ± standard deviation.

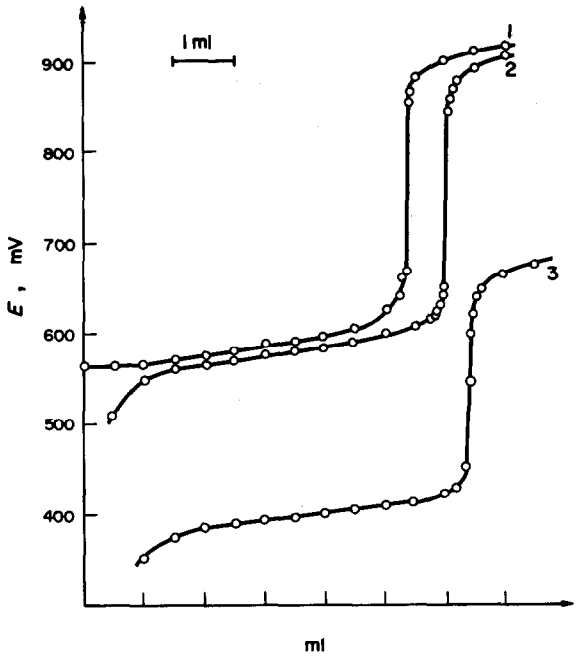

Fig. 1. Potentiometric titrations of 2-chlorohydroquinone with bromine in acetic acid, with (1) a platinum electrode, (2), a Radelkis graphite electrode and (3), a silicone-rubber-based membrane graphite electrode.

ones in a 2-electron reaction, and the titrations were quantitative (Table 2).

In the study of ascorbic acid, the solubility in acetic acid was examined first and found to be 0.005*M* maximum at room temperature. At higher temperatures, the solubility is increased, but the titration results are low by up to 9%. The response times and sensitivities of the three electrodes followed the same pattern as for the hydroquinone titrations (Table 1). The results obtained were about 2.5% low, but reproducible (Table 2).

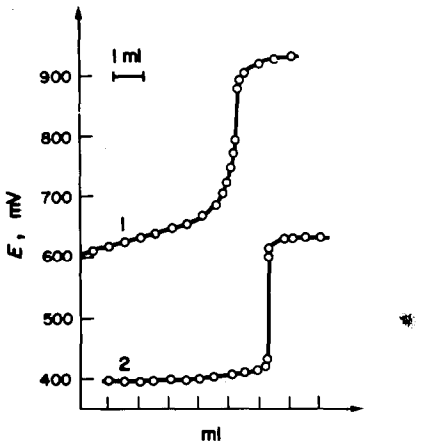

Fig. 2. Potentiometric titrations of antimony(III) with bromine in acetic acid, in the presence of (1) a platinum electrode and (2) a silicone-rubber-based graphite electrode.

The determination of arsenic(III) was also improved by addition of potassium acetate, but with 0.05*M* potassium acetate and $>10^{-3}M$ arsenic(III) a precipitate formed. Potential stabilization was very slow until the precipitate had dissolved (which was just before the end-point). The sensitivity of the electrodes is given in Table 1, and the results were accurate and reproducible (Table 2).

The addition of a small amount of iodine (reported to catalyse this reaction) does not improve the determination; the titration curves are flat, and the results low. Back-titration also gives unsatisfactory results, presumably because of the volatility of bromine, although the potential stabilizes quickly until the end-point is reached, but very slowly after it.

In titration of antimony(III), the total change of potential at the platinum electrode is equal to that obtained with arsenic(III), but the potential jump at the end-point is significantly lower (Table 1). Figure 2 shows that the membrane graphite electrode gives a better end-point than the platinum electrode.

Titration of pyrocatechol, resorcinol, acid hydrazides, mercury(I), thallium(I) and titanium(III) was also attempted but only titration of the acid hydrazides gave distinct end-points.

The presence of up to 30% water has no effect on the determination of arsenic(III) or antimony(III), but 5% of water makes the 2-methylhydroquinone results about 6% too high. The presence of water makes stabilization of the potential faster, but does not affect the total change in potential.

The presence of acetic anhydride makes it impossible to determine substances that react with it. Up to 5% of it in the solution makes the results high by up to about 3% and larger amounts result in disappearance of the potential jump at the end-point.

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ALKALIMETRIC DETERMINATION OF AMOXYCILLIN TRIHYDRATE IN NON-AQUEOUS MEDIUM

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Summary—A simple method for determination of amoxycillin by non-aqueous titration in dimethylformamide medium is described. The relative standard deviation is 0.3%.

Amoxycillin can be determined by fluorimetric assay[1] of the highly fluorescent product formed by the reaction of a blood or urine sample with formaldehyde (but it should be done without delay since the samples are not stable); colorimetry[2] based on the blue colour produced by heating with phosphomolybdic acid; spectrophotometric measurement[3] of the degradation product formed by heating with citric acid–disodium hydrogen phosphate buffer in presence of copper(II); mercurimetric titration[4] of the corresponding penicilloic acid, which is highly dependent on the speed of titration, temperature and the initial sample concentration; and by high-pressure liquid chromatography.[5]

Alkalimetric determination of amoxycillin in aqueous medium gives erratic results, though it has been reported to be successful in 80% aqueous acetone, with potentiometric indication.[6] It has now been observed that its solution in dimethylformamide can be titrated with sodium methoxide, with 2-nitroaniline as indicator; benzene–methanol (4 + 1) is unsuitable as the solvent owing to inadequate solubility of the amoxycillin trihydrate. Thymol Blue gives a diffused end-point. One mole of amoxycillin trihydrate reacts with two moles of methoxide. The procedure is precise and accurate, as is evident from the statistical analysis, and has the advantage of not requiring any sophisticated equipment. It seems to be worthy of recommendation.

Table 1. Determination of amoxycillin trihydrate*

Taken, *mg*	Found, *mg*	Apparent purity, %†
45.6	45.6	100.0
48.8	48.7	99.8
54.0	53.8	99.6
58.2	58.0	99.7
64.2	64.2	100.0
66.2	66.3	100.2
72.0	72.1	100.1
77.6	77.7	100.1
82.4	82.0	99.5
95.6	95.9	100.3

* From Amoxylin capsules, Biddle Sawyer Pvt. Ltd., Bombay; average purity found by potentiometric titration[6] 99.7%; standard deviation, 0.3%.

† Mean 99.9%, standard deviation 0.3%.

Procedure

A weighed quantity of amoxycillin trihydrate is dissolved in 25 ml of dimethylformamide, by warming. 2-Nitroaniline (2 drops, 1% solution in benzene) is introduced followed by titration with *ca.* 0.1*M* sodium methoxide[7] solution in benzene–methanol (4 + 1), standardized against benzoic acid,[8] to a distinct orange–red colour. A blank is run and deducted (typically 0.15 ml).

Applicability

The method is applicable to determination of amoxycillin trihydrate in commercial samples, mostly available in the form of capsules.[9] Typical results are shown in Table 1.

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EXTRACTION OF VANADIUM(V) CHELATES WITH *N*-BENZOHYDROXAMIC ACID (BHA) AND AMMONIUM THIOCYANATE AND ITS APPLICATION IN STEEL AND ROCK ANALYSES

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Summary—A mixed-ligand complex of vanadium(V) with *N*-benzohydroxamic acid and thiocyanate formed at various acidities can be extracted into methyl isobutyl ketone, and used for photometric determination of trace amounts of vanadium in materials such as alloy steels and rocks. The absorption maximum of the violet mixed-ligand complex is at 535 nm. The values for the simple complex are 505 nm and molar absorptivity 7.4×10^3 l.mole^{-1}.cm^{-1}.

N-Benzohydroxamic acid (BHA) reacts with vanadium(V) over a wide range of pH, and several authors[1–5] have studied the effect of different media on the reaction. The studies on the complexes in 1:1 water–ethanol and 1-hexanol are open to question as alcoholic solvents have detrimental effects on vanadium colour systems. Here we report on the methyl isobutyl ketone (MIBK) extraction of the BHA–vanadium complexes, including a mixed-ligand thiocyanate complex. These photometric methods developed for determination of traces of vanadium in alloy steels and rocks have advantages over others,[6–11] and are simple, rapid, accurate and reproducible. The composition and the extraction and stability constants of the complexes have been determined by the absorptiometric method.[12–14]

EXPERIMENTAL

Reagents

*BHA solutions (0.036*M *and 0.073*M). The reagent (m.p. 125–128°) was prepared in the usual way; 0.5% and 1.0% reagent solutions in methyl isobutyl ketone were used for the "simple" and "mixed-ligand" extractions, respectively.

Ammonium vanadate solution. Prepared by dissolving ammonium metavanadate in doubly distilled water containing a little ammonia, and standardized by complexometric titration[15] and photometrically with *N*-benzoylphenylhydroxamic acid.[16] The solution was diluted to 100 μg/ml.

*Ammonium thiocyanate solution (0.26*M). Prepared in doubly distilled water and standardized by Volhard's method.[15] A 2% solution was used and diluted as necessary.

Exactly 0.1*M* hydrochloric acid was obtained by diluting stock standard 6*M* acid. A standard buffer of pH 4.0 was prepared from 0.2*M* acetic acid and 0.2*M* sodium acetate. All other chemicals used were of general or analytical reagent grade and the solvents were purified.

Procedure A

To an aliquot of vanadium(V) solution (up to 100 μg/ml) in a 100-ml separatory funnel add 5 ml of 0.5% BHA solution and either 2 ml of the pH-4 buffer or enough 6*M* hydrochloric acid to give a final 1.5*M* concentration, and in either case dilute to 10 ml with water.

Extract the yellow-brown (pH 4) or wine-red (1.5*M* acid) vanadium complex by shaking for 5 min with 10 ml of methyl isobutyl ketone. Separate the organic layer and extract again with 5 ml of solvent. Combine the extracts, transfer to a 25-ml standard flask and make up to volume with the solvent.

Measure the absorbance against a solvent blank at 460 nm (yellow-brown complex) or 505 nm (wine-red complex).

Procedure B

To an aliquot of vanadium(V) solution (up to 100 μg/ml) in a 100-ml separatory funnel add 5 ml of 1% BHA solution and enough 6*M* hydrochloric acid to give an acidity of 1.5*M* after dilution to 10 ml. Shake with 10 ml of methyl isobutyl ketone for 2 min, then add 5 ml of 2% ammonium thiocyanate solution and shake again for 7 min more. Separate the violet extract and transfer it to a 50-ml beaker containing anhydrous sodium sulphate. Repeat the extraction with two 5-ml portions of solvent. Transfer the combined extract to a 25-ml standard flask and make up to the mark. Measure the absorbance against a solvent blank at 535 nm.

Procedure for steel analysis

Dissolve 0.5 g of steel in 20 ml of sulphuric acid (1 + 1) in a beaker on a hot-plate. Add 5 ml of concentrated nitric acid and evaporate to strong fumes of sulphur trioxide. Cool, add 50 ml of water, boil, filter with a Whatman No. 42 paper and wash the residue several times with hot dilute sulphuric acid and finally with hot water. Cool the filtrate and washings, transfer to a 250-ml standard flask, make up to the mark and mix.

To 5 ml of this solution add 0.1% potassium permanganate solution till a pink colour persists. Add this mixture to hot 15% sodium hydroxide solution in excess and boil. Filter off the precipitate and redissolve it in dilute hydrochloric acid. Reprecipitate the hydroxides as above and filter. Combine the filtrates and evaporate to low bulk. Cool, add 15 mg of sodium fluoride, and determine the vanadium content by either procedure *A* (1.5*M* acidity) or *B*. Measure the absorbance at 505 or 535 nm as appropriate.

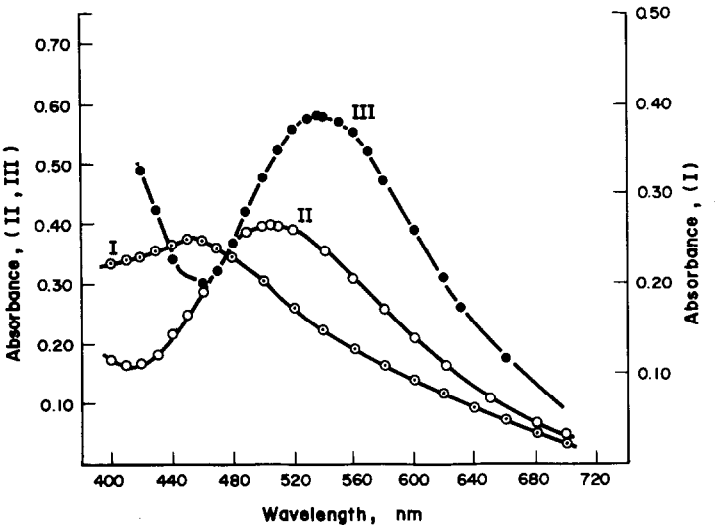

Fig. 1. The absorption spectrum of binary and ternary complex of vanadium(V) in methyl isobutyl ketone. Vanadium(V) 4 μg/ml, reagent (N-BHA) 0.0073*M* (I, II) and 0.0146*M* (III), thiocyanate 0.0866*M* (III), I: ⊙ V(V)–BHA chelate at pH 4.0; II: ○ V(V)–BHA chelate, 1.5*M* HCl; III: ● V(V)–BHA–SCN^- chelate, 1.5*M* HCl.

Procedure for rock analysis

Fuse 0.5 g of finely powdered sample (200-mesh) with 9 g of potassium bisulphate and a few drops of sulphuric acid in a silica crucible to a clear melt. Cool the mass and leach it with sulphuric acid (1 + 9). Boil and oxidize the solution with a few drops of nitric acid and then precipitate iron and titanium as hydroxides with an excess of 15% sodium hydroxide solution. Free any adsorbed vanadium from the hydroxide precipitate by double precipitation. Combine the filtrates and evaporate to about 50 ml. Transfer the solution to a 100-ml standard flask and make up to volume with water.

Take appropriate aliquots of the solution and determine the vanadium content by procedure *A* or *B*, adding 15 mg of fluoride as masking agent for any traces of iron and titanium in the solution.

RESULTS AND DISCUSSION

Absorption spectra

The yellow-brown complex forms quantitatively with vanadium(V) and reagent at pH 2.2–5.6 and has a flat absorbance maximum at 455–465 nm; it is very stable. The reagent also reacts quantitatively with vanadium(V) in 1–3*M* hydrochloric acid to form a wine-red complex [with a chloride ion attached to the sixth co-ordination position of the vanadium(V)] having an absorbance maximum at 505 nm.

The mixed-ligand complex (violet) formed in 0.75–2.1*M* hydrochloric acid (procedure *B*) has an absorbance maximum at 535 nm. A solvent reference solution can be used, as the reagent does not absorb at these wavelengths. The spectra of the complexes are shown in Fig. 1. The absorbance remains constant for at least 24 hr.

Effect of BHA

The absorbances for the yellow-brown and wine-red complexes remain constant when at least 5 ml of 0.5% reagent solution are used. When 5 ml of 2% thiocyanate solution are added, 1.5 ml of 1.0% BHA solution will be sufficient for full colour development. A large excess has no adverse effect if the order of addition is maintained. Hence, 5 ml of 1% BHA solution should be used.

Effect of thiocyanate

One ml of 2% thiocyanate solution is sufficient for complete formation of the mixed-ligand complex. A large excess has no adverse effect, so 5 ml are used.

Optical properties

The several complexes obey Beer's law in the range 1–6 ppm vanadium (in the solution measured) at 460 nm, 0.2–12.5 ppm at 505 nm, and 0.4–12 ppm at 535 nm. The optimum ranges (Ringbom plots)[17] are 1.5–6, 0.4–12.5 and 1–8 ppm, and the molar absorptivities are 0.31×10^4, 0.50×10^4 and 0.74×10^4 $l.mole^{-1}.cm^{-1}$, respectively.

Interferences

Interference studies were made for only the 1.5*M* acid systems, the tolerance limit being the amount causing a deviation of more than 0.005 in the absorbance (Table 1).

Composition, extraction and stability constants

The metal:BHA ratio in the wine-red complex was found to be 1:2 by the continuous variation, mole-ratio and slope-ratio methods. The extraction constant, K_{MR}, was found from a plot of log $A(A_{max} - A)$ *vs.* log $(HR)_0$ to be $10^{6.55}$.

The formation constants were evaluated by two photometric methods[18,19] and though the stepwise

Table 1. Determination of vanadium(V) (4 ppm in the final solution) by procedures A and B; absorbance measured at 505 and 535 nm respectively

Ions	Added as	Tolerance limits, *ppm* A	B	Ions	Added as	Tolerance limits, *ppm* A	B
Acetate	CH_3COONa	200	1200	Pd(II)	$PdCl_2$	125	100
Oxalate	$C_2O_4H_2$	300	2000	Cr(III)	$CrCl_3$	250	400
Citrate	Citric acid	1500	2000	Mn(II)	$MnCl_2$	600	600
Tartrate	Tartaric acid	2000	2000	Th(IV)	$Th(NO_3)_4$	1000	500
EDTA	Na_2EDTA	1500	2000	Co(II)	$CoCl_2$	600	400
F^-	NaF	800	1200	Ni(II)	$NiCl_2$	1000	400
NO_3^-	$NaNO_3$	8000	6000	Al(III)	$Al(NO_3)_3$	600	400
SO_4^{2-}	Na_2SO_4	8000	6000	Hg(II)	$HgCl_2$	2000	800
PO_4^{3-}	$(NH_4)_2HPO_4$	5000	6000	Cd(II)	$Cd(NO_3)_2$	2000	800
Fe(III)	$FeCl_3$	100*	N.I.(50*)	Zn(II)	$ZnCl_2$	1200	600
Ti(IV)	$TiOSO_4$	50	N.I.(100*)	Pb(II)	$Pb(NO_3)_2$	2000	600
Mo(VI)	$(NH_4)_2MoO_4$	150	100*	Cu(II)	$CuSO_4$	300	200
W(VI)	Na_2WO_4	80	100*	Nb(V)	Tartrate	40*	50*
UO_2(II)	$UO_2(NO_3)_2$	400	400	Ta(V)	Tartrate	40*	50*
Zr(IV)	$ZrOCl_2$	250	150	Ca(II)	$Ca(NO_3)_2$	600	400

* Fluoride used as masking agent.
N.I. No interference—interference removed by double precipitation.

constants were inconsistent (log $k_1 = 2.02$, log $k_2 = 4.32$ by Leden's method; log $k_1 = 2.84$, log $k_2 = 3.55$ by Yatsimirskii's method), the log β_2 values (6.34 by Leden's method, 6.39 by Yatsimirskii's method, 6.55 by an absorptiometric method, 6.52 by the Harvey and Manning method) agreed quite well.

By the same methods the metal:BHA ratio in the mixed-ligand complex was also found to be 1:2 when excess of thiocyanate was used. The metal:SCN^- ratio was similarly found to be 1:1 for the mixed-ligand complex. Thus, the composition of the mixed-ligand complex is 1:2:1.

Determination of vanadium in steel and rock

The extractive photometric methods were found excellent because of their high selectivity. They are rapid, simple and accurate when fluoride is used to mask more than a 20-fold ratio of the interfering ions [Fe(III) and Ti(IV)] to vanadium. Moreover, the mixed-ligand method has greater selectivity for the analysis of alloy steels and ilmenites. The two ilmenites were collected from Tamil Nadu and IOL, Calcutta, India and finely ground (200-mesh) for analysis directly after separation. The samples were also analysed spectroscopically and found to contain 0.1 and 0.15% vanadium, respectively.

Results of the analyses of a standard steel (BAS 64b) and the two ilmenites by procedures A and B were 1.96 and 1.97% (certified value 1.99%), 0.08 and 0.09% (certified value 0.1%) and 0.14 and 0.14% (certified value 0.15%), respectively.

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INVESTIGATION OF A NOVEL SOLID-PHASE CHEMILUMINESCENT ANALYTICAL SYSTEM, INCORPORATING PHOTOGRAPHIC DETECTION, FOR THE MEASUREMENT OF GLUCOSE

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Summary—A method has been developed for the rapid determination of substances by use of solid-phase reagents and a luminescence indicator reaction coupled with photographic detection of the light. The viability of the assay has been demonstrated for glucose estimations. The method uses small sample sizes (5–20 μl) and shows good sensitivity, *e.g.*, detection of glucose down to 28 nmole.

The major advantage offered by photographic film as a detection system in luminescence is that of simplicity, a permanent visual record being obtained without the use of complex ancillary equipment. Obviously this advantage remained relatively unappealing while photographic emulsions required conventional "wet" development, but the advent of the Polaroid "Land" system[1] and related systems of "instant" development, produced an attractive alternative. An arrangement which may make best use of such a detection system is the use of thin layers of dried or otherwise immobilized reagents, aligned so as to produce light following the application of a liquid sample and in close contact with a photographic emulsion for detection purposes.

Analytical systems employing solid-phase reagents either in the form of thin films[2] or impregnated layers[3] have been described by several groups of workers. Two different types of detection system are used to monitor such solid-phase reactions, namely reflectance spectrophotometry and conductance. As yet, the combination of solid-phase reagents with a luminescent indicator reaction and photographic detection has not been described. Luminescent indicator reactions have several advantages over conventional colorimetric or spectrophotometric reactions: they are rapid, and extremely sensitive, and they may be coupled to a wide range of analyses.[4]

The objective of this study was to explore the feasibility of a luminescent analytical system employing solid-phase reagents and photographic detection of light. Initially, a simple chemiluminescent reaction between lucigenin and hydrogen peroxide was used to compare the detection of light by photographic film and by a photomultiplier tube. An assay for glucose based on the chemiluminescent reaction of luminol with enzymatically produced hydrogen peroxide was then chosen as a model, and the reaction sequence is shown below.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Products} + H_2O_2$$

$$H_2O_2 + \text{luminol} \xrightarrow{\text{Peroxidase}} \text{Products} + \text{light}$$

EXPERIMENTAL

Reagents

Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and lucigenin (*bis*-*N*-methylacridinium nitrate) were obtained from the Aldrich Chemical Co. The monosodium salt of luminol was prepared by recrystallization from hot 1*M* sodium hydroxide, followed by recrystallization twice from water. Glucose oxidase (Type II; product no. G 6125) and peroxidase (crude; RZ approximately 0.3; product No. P8000) were obtained from the Sigma London Chemical Co. All other reagents were of analytical grade where possible.

Analytical system

Luminescent reactions were carried out in disposable plastic tubes (44 × 11 mm diameter) in a "Tufnol" mask (Fig. 1). The mask together with the tubes was placed in intimate contact with a Polaroid Land film holder No. 44-46 (Polaroid Ltd.) containing either Polaroid Type 47

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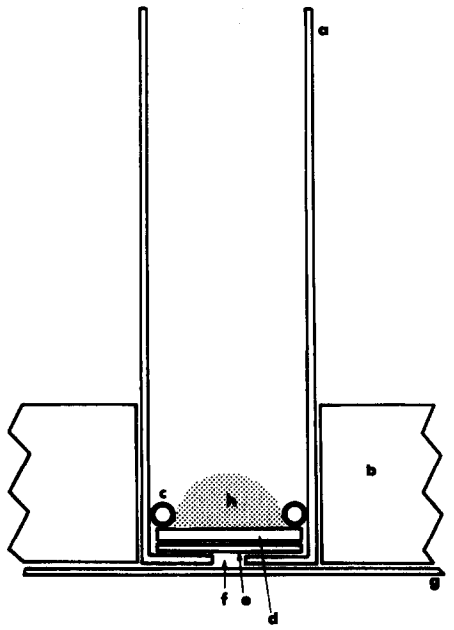

Fig. 1. Experimental arrangement used for the investigation of the solid-phase reagents (a, plastic tube; b, Tufnol mask; c, O-ring; d, enzyme-impregnated filter pad; e, luminol-impregnated Visking dialysis tubing; f, 2-mm diameter hole; g, photographic film; h, sample).

(ASA 3000) or Type 410 (ASA 10000) black and white print film. Light produced by the reactants passed downwards to the emulsion in close contact with the base of the tubes, producing, in effect, a "contact print". The mask was necessary to prevent a "halo" effect as a result of light escaping from the sides as well as the base of the tubes. No account was taken in these experiments of the relative colour sensitivities of these films.

Calibration procedure

Dilutions (1 in 1000, 2 in 1000, 3 in 1000 and 4 in 1000) of a hydrogen peroxide solution (50% v/v; 1.47*M*) were prepared and 10 μl of each of these dilute solutions were added to the disposable plastic tubes. In addition, tubes were set up with 10 μl of distilled water as blanks and all tubes were placed in the "Tufnol" mask. In a darkened room the shutter covering the film was removed and the "Tufnol" mask containing the tubes was placed in the film holder so that it covered the sensitive area. Still in the dark, a mixture of lucigenin (20 μl of a 0.2m*M* solution in water) and sodium hydroxide (1 ml of 0.1*M* solution) was injected into the tubes with a powered syringe unit.[5] The film was then exposed to the light produced by the reaction in the tubes, for 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 min.

In order to compare these results directly with those obtained by using a photomultiplier tube as a detector, identical reactions were carried out in a prototype luminometer[6] and the peak light output was recorded.

Reagent discs

Luminol-impregnated "Visking" dialysis tubing (Jennings Co., Nottingham, U.K.) was prepared by soaking lengths of tubing (type 18/32) in luminol solution (2m*M* in 1*M* potassium hydroxide) for 1 hr. Excess of luminol solution was removed by blotting with filter paper and the tubing was dried overnight at room temperature.

Enzyme discs were prepared by soaking "Millipore" filters (type AP 2502500) in glucose oxidase/peroxidase solution (glucose oxidase 22 mg/ml, *i.e.*, 400 U/ml; peroxidase 1.3 g/l., 120 U/ml) in phosphate buffer (0.1*M*, pH 7.0) for 1 hr. Finally the discs were dried overnight at room temperature.

Assay of glucose with solid-phase reagents

Disposable plastic tubes in which 2-mm diameter holes had been drilled to prevent the formation of air-locks were loaded with a disc of luminol-impregnated "Visking" tubing and a disc of enzyme-impregnated filter material. The discs were held in position by O-rings (Fig. 1).

For calibration or analysis, 5–20 μl of 5.6m*M* glucose solution in distilled water was added to tubes positioned in the "Tufnol" mask and the reaction was allowed to proceed for 5 min. Then, in a darkened room, the mask was placed in the film holder and the luminescent reaction was initiated by the addition of 200 μl of distilled water, which flushed the mixture onto the luminol-impregnated "Visking" tubing. After exposure of the film (Type 410) for 1 min, the mask was removed and the film was developed. Identical reactions were carried out in a prototype luminometer to measure the light output.

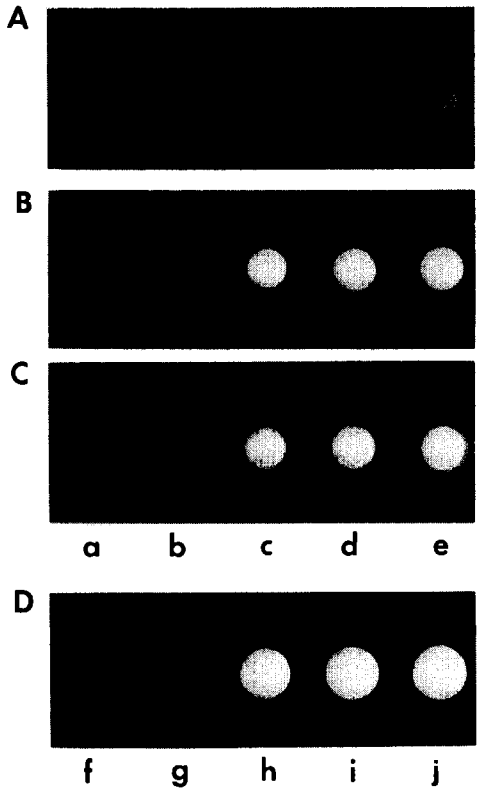

Fig. 2. Photographic film response to various chemiluminescent reactions. A, Response of Polaroid type 47 film to chemiluminescence reaction of lucigenin and different concentrations of hydrogen peroxide, exposure time 1 min; B, as for A except exposure time was 2 min; C, as for A except Polaroid type 410 film and an exposure time of 30 sec were used. Hydrogen peroxide added (μmole: a = 0, b = 14.7, c = 29.4, d = 44.1, e = 58.8. D, Response of Polaroid type 410 film to solid-phase chemiluminescent assay for glucose, exposure time 1 min. Volume of 5.6m*M* glucose added: f = 0 μl, g = 5 μl (28 nmole), h = 10 μl (56 nmole), i = 15 μl (84 nmole), j = 20 μl (112 nmole).

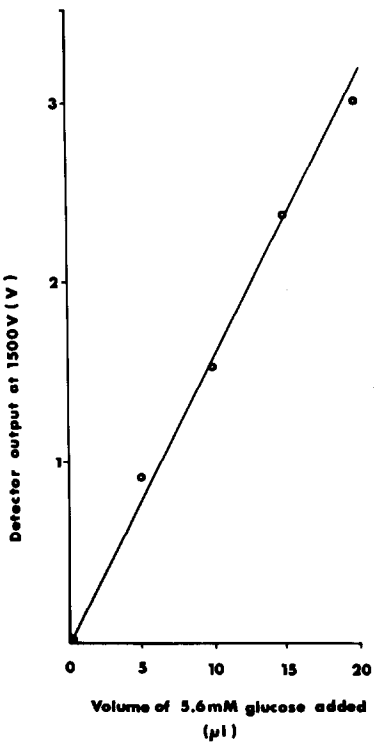

Fig. 3. Calibration curve for solid-phase luminol chemiluminescent assay of glucose (each point is the mean of duplicate determinations).

RESULTS AND DISCUSSION

The response of the two types of photographic film to a series of luminescent peroxide/lucigenin reactions is shown in Fig. 2. A linear relationship between light output and peroxide concentration was obtained in an identical series of reactions in which the light was measured with a photomultiplier tube. Type 47 film had insufficient sensitivity unless exposure times of several minutes were used. Type 410 film developed adequate density after an exposure time of 1 min, and despite its restricted exposure latitude, was chosen as the detection system for the glucose assay with solid-phase reagents.

The calibration curve obtained for the luminescent assay of glucose with solid-phase reagents was linear when a photomultiplier tube was used to monitor the light output. An identical series of reactions, with photographic film used as the detection system, showed (Fig. 2D) the viability of the solid-phase luminescent assay for glucose. However, because of the insufficient exposure latitude of the Type 410 film, discrimination between different amounts of glucose was limited since below 28 nmole the photographic emulsion was unexposed, whilst above this value the emulsion was fully exposed. In its present embodiment the solid-phase assay provides a very sensitive "threshold" type test for the presence or absence of an analyte, in this case glucose. A rapid and quantitative version of the assay would require a fast film (high ASA number) with as wide an exposure latitude as possible. An instant film having this combination of characteristics is not available. The exposure of the film was in some instances, non-uniform, indicating that flow through the filter discs was unsatisfactory when combined with photographic detection techniques. In contrast, the results obtained with an identical analytical system, but measuring the light output by means of a photomultiplier tube, showed much better proportionality to concentration (Fig. 3). This indicates that the fault lies primarily with the flow characteristics of the filter discs rather than with the concept as a whole. The problem of uniform and reproducible sample delivery through thin layers of reagents has been solved by other workers,[2] but the method is proprietary and was thus not available to us.

The prototype solid-phase chemiluminescent analytical system described in this paper has considerable potential for rapid quantitative or semi-quantitative analyses, and would offer a novel departure from current "dipstick" and other "disposable"-type methods. Its future development and exploitation will, however, depend upon the availability of suitable photographic films and a reliable method of delivering sample to the solid-phase reagents.

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DETERMINATION OF TOTAL ARSENIC BY USE OF A ZINC-COLUMN ARSINE GENERATOR

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Summary—A method for the measurement of total arsenic in extracts of environmental materials is described. Arsenic is reduced to arsine with a zinc reductor column, the evolved arsine is decomposed in a heated carbon-tube furnace, and arsenic determined by measurement of its atomic-absorption at 193.7 nm. The detection limit is 0.002 μg/ml and the coefficient of variation is 1.4% at 0.01 μg/ml.

During a study of the chemical speciation of arsenic in the marine environment a method was required for determining the arsenic present in extracts derived from geological and biological material.

Arsenic forms a volatile hydride and this property is widely used to separate arsenic from matrices which might interfere in its determination by atomic-absorption spectroscopy. Various reductant systems such as $Zn/KI/SnCl_2/HCl$,[1] $NaBH_4/HCl$,[2] and $Mg/TiCl_3/HCl$,[3] have been used to reduce inorganic arsenic to its hydride, these being added as solid pellets or solutions. Most hydride separation methods are, however, limited in their application, owing to severe interference from other elements.[2,4,5]

Lichte and Skogerboe[6] have reported the use of a column of zinc for the generation of arsenic hydride, and Knudson and Christian,[7] and Shaikh and Tallman,[8] have used carbon furnaces for the determination of arsenic in its hydride form. This paper describes the coupling of a zinc-column reductor to a carbon furnace for the rapid and fairly interference-free determination of nanogram quantities of arsenic.

EXPERIMENTAL

Apparatus

A Varian–Techtron AA5 atomic-absorption spectrophotometer fitted with a Perkin–Elmer HGA 72 carbon furnace was used for all measurements. The standard carbon tube was replaced with a 10-mm bore carbon tube with a single central gas inlet hole (Fig. 1). The light-source was a Rank Hilger slotted arsenic hollow-cathode lamp, operated at 7 mA; the slit-width was 300 μm; the 193.7-nm resonance line was measured. The zinc-column arsine generator (Fig. 2) was connected into the inert-gas feed line between the HGA 72 control module and the inlet to the furnace.

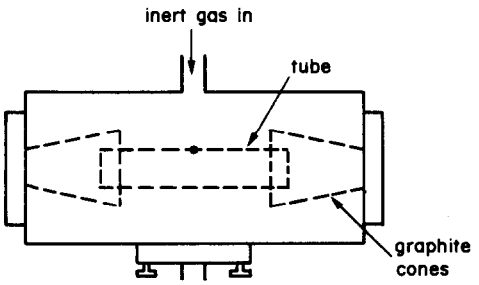

Fig. 1. HGA 72 carbon furnace (not to scale).

Reagents

All chemicals were of analytical-reagent grade. Stock standard solutions (1000 μg/ml) of arsenic(III) and arsenic(V) were prepared by dissolving As_2O_3 and As_2O_5, respectively, in 0.1*M* sodium hydroxide. Aliquots of these solutions were diluted, when required, with demineralized distilled water to give working solutions in the μg/l. range.

Procedure

The sample to be analysed was dissolved in 2*M* hydrochloric acid and sufficient potassium iodide and ascorbic acid were added to reduce all the inorganic arsenic present

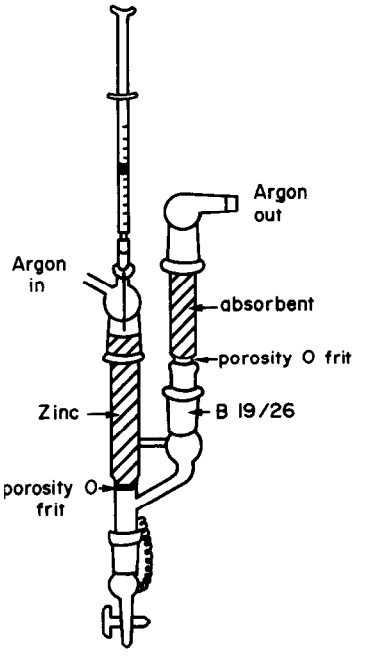

Fig. 2. Zinc-column arsine generator.

to the tervalent form (1 ml of 1*M* iodide/1*M* ascorbic acid solution per 20 μg of arsenic).

Before the injection of 1 ml of solution onto the zinc column the furnace (1700°) and recorder were turned on and a stable base-line was established (this took approximately 10 sec). The solution was injected as quickly as possible and the furnace was turned off when the recorder signal returned to the previously established base-line (approximately 20 sec).

RESULTS AND DISCUSSION

Zinc-column arsine generator

Two forms of zinc were tested in the column, namely zinc filings (<2 mm) from zinc sticks, and zinc shot (16–30 mesh) which was activated by removing the surface oxide coating by immersion in 0.5*M* hydrochloric acid for several minutes. Zinc shot gave delayed arsine generation, and the coefficient of variation for the signal for five successive injections of a 0.1-μg/ml As(III) standard was larger (4%) than that obtained with the zinc filings (2.7%). Zinc filings were therefore used in all further work.

Water vapour must be prevented from entering the carbon tube, as the molecular absorption of water and acid vapour interferes with the arsenic absorption signal. Moreover, the operational life of the carbon tube is drastically reduced. Anhydrous calcium chloride (8–16 mesh) and calcium sulphate (10–20 mesh) were found to be suitable absorbents for water vapour, but both remove small quantities of arsine and therefore before sample solutions are analysed an arsenic standard should be injected into the zinc column to saturate any arsine adsorption sites.

Optimization of operating conditions

All conditions were optimized with a 0.1-μg/ml As(III) standard. The effect of furnace temperature, flow-rate and acid concentration on the arsenic absorption signal (peak height) is shown in Figs. 3, 4 and 5, respectively. A linear relationship between absorbance and concentration was obtained up to a concentration of 70 ng/ml.

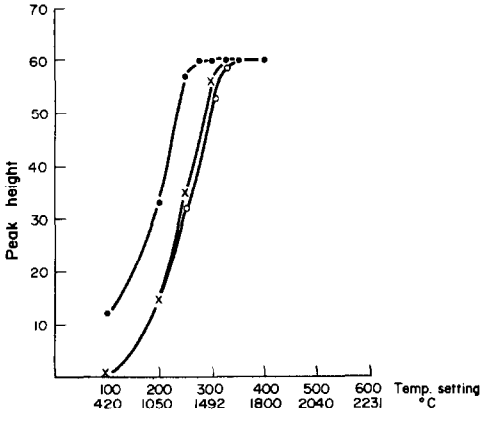

Fig. 3. Effect of furnace temperature on the absorption signal (arbitrary units). Flow-rate setting 5.0; [HCl] 2.0*M*; ● 5-mm tube; × 8-mm tube; ○ 10-mm tube.

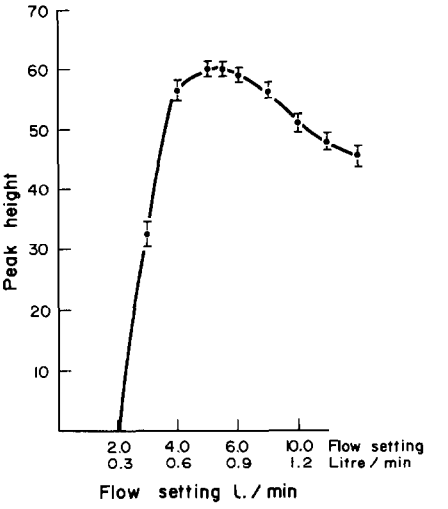

Fig. 4. Effect of flow-rate on the absorption signal (arbitrary units). Temperature setting 350; [HCl] 2.0*M*; 8-mm tube.

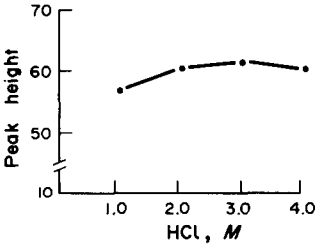

Fig. 5. Effect of hydrochloric acid concentration on the absorption signal (arbitrary units). Temperature setting 350; flow-rate setting 5.0.

Form of arsenic

Preliminary experiments showed that arsenic(V) was not quantitatively reduced to arsine by the zinc column (see Table 1).

The presence of 1 ml of 1*M* potassium iodide/1*M* ascorbic acid solution in a 50-ml sample containing hydrochloric acid and 5 μg of arsenic(V) was sufficient to give complete reduction to arsenic(III). The ascorbic acid was added to prevent oxidation of iodide to iodine; the removal of iodine also shifts the equilibrium of the As(V)/I^- reaction to the right:

$$As(V) + 2I^- \rightleftharpoons As(III) + I_2$$

The time required for the complete reduction of As(V) to As(III) was found to be dependent on the

Table 1. Generation of arsine from As(V)

[HCl], *M*	1.05	2.10	3.15	4.20
Conversion,* %	43 ± 1	49 ± 2	54 ± 1	57 ± 1

* As(V) standard 0.1 μg/ml, three determinations.

Table 2. Effect of acid concentration on time for reduction of As(V) to As(III) as shown by recovery of arsine

[HCl], *M*	2	3	6	12
Reduction time, *min**	45	20	5	5

* Reduction time for complete conversion of 5 μg of As(V) (in 50 ml of solution) into arsine.

hydrochloric acid concentration (Table 2). Up to 20 μg of arsenic(V) can be completely reduced with 1 ml of the reductant solution.

Interferences

The levels at which certain ions interfere are given in Table 3. A number of other ions (Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{6+}, Fe^{3+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, SO_4^{2-}, Sn^{2+}, Zn^{2+}) were tested and showed no significant interference at concentrations up to 500 μg/ml (1.5 mg/ml for SO_4^{2-}).

Precision and detection limit

The precision was estimated from replicate analyses of a 0.01 μg/ml As(III) standard, made up in 2*M* hydrochloric acid that was 1*M* in potassium iodide and 1*M* in ascorbic acid. The relative standard deviation at this concentration was 1.4% (five determinations).

The standard deviation of the blank analyses, based on 5 determinations, corresponded to a concentration of 0.001 μg/ml. The source of the blank (<0.005 μg/ml) was mainly the hydrochloric acid.

Conclusion

The experimental system described in this paper allows the determination of arsenic down to 0.01 μg/ml with a relative standard deviation of less than 2%. The advantages of this system are the rapid throughput of prepared samples (one column injection per 30 sec) and the high concentration of foreign ions which can be present without causing interference. This makes the system particularly suitable for the determination of arsenic in environmental samples, which generally contain a wide range of other trace elements.

Table 3. Effect of inorganic ions on the generation of arsine from 0.1-μg/ml As(III)

Element	Form added	Interference level,* μg/ml
Cu	$CuCl_2$	50
Hg	$HgCl_2$	2.5
Mo	$NaMO_4 \cdot 2H_2O$	400
Sb	$SbCl_3$	25
Se	H_2SeO_3	0.1
Si	Na_2SiO_3	50
V	NH_4VO_3	250

* Interference was deemed to occur if the absorbance changed by 5% or more. Mo enhanced the signal, the other elements all suppressed it.

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SPECTROPHOTOMETRIC DETERMINATION OF ATROPINE, PILOCARPINE AND STRYCHNINE WITH CHLORANILIC ACID

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Summary—In dioxan–chloroform medium the acceptor chloranilic acid forms 1:1 molecular complexes with the alkaloids atropine, pilocarpine and strychnine, with maximum absorption at 535, 527.5 and 535 nm respectively. Conformity with Beer's law allows the use of the complexes for the assay of these drugs.

Molecular complexes are formed by interaction between electron donors and electron acceptors.[1] Donors fall into two major categories, lone-pair donors such as amines (including alkaloids), alcohols, sulphoxides *etc.*, and π-donors such as aromatics, particularly polycyclic systems. Some compounds such as azoaromatics and aromatic amines may behave as lone-pair donors towards some acceptors and π-donors towards others. Acceptors may be of σ- and of π-type. The former include iodine, recently employed in the assay of alkaloids[2–7] and antihistamines.[8] π-Acceptors are aromatic systems containing electron-withdrawing substituents such as nitro, cyano or halogen groups, *e.g.*, *p*-benzoquinone,[9] dichlorobenzoquinone,[10] fluoranil,[11] chloranil,[12] tetracyano-*p*-benzoquinone[13] and 7,7′,8,8′-tetracyanoquinodimethane.[14] *p*-Chloranilic acid has been largely used in the determination of metal ions,[15,16] but not hitherto in the assay of alkaloids.

The present work describes the spectrophotometric determination of atropine, pilocarpine and strychnine by complexation with chloranilic acid. These alkaloids are usually present in pharmaceutical dosage forms in very small amounts (1 mg or less per unit dose). Such small amounts preclude the use of many methods for assay of these alkaloids.

EXPERIMENTAL

Reagents

Alkaloids. Atropine base (BDH), atropine sulphate (Merck), pilocarpine hydrochloride (Merck), strychnine base (Merck), strychnine hydrochloride (Sigma).

Alkaloid dosage forms. Atropine sulphate eye-drops, 1% solution containing 0.002% phenylmercuric nitrate (Evans Medical). Atropine sulphate injections B.P., 0.1% (E.G.Y.T.) and atropine sulphate injections B.P., 0.06% (Evans Medical).

Chloranilic acid solution. p-Chloranilic acid dissolved in 1,4-dioxan to give 0.005*M* and 0.2% solutions, which were kept in the dark when not in use and under these conditions were stable for up to 6 weeks.

Other reagents and solvents were analytical grade and used as such.

Standard solutions

Atropine and strychnine bases. An accurately weighed amount of the base was dissolved in chloroform to give 0.2% and $5.0 \times 10^{-3}M$ solutions.

Pilocarpine hydrochloride. An accurately weighed amount (0.1173 g) was dissolved in about 15 ml of water in a 100-ml separatory funnel. The solution was alkalinized to litmus with a few drops of dilute ammonia solution and extracted with 15, 10, 10 and 10-ml portions of chloroform, each extract being washed with the same 15 ml of water in another separatory funnel. The washed extracts were filtered through anhydrous sodium sulphate in a filter paper in a small funnel, into a 50-ml standard flask. The funnel and paper were washed with 1–2 ml of chloroform and the volume was made up with the same solvent to 50 ml.

A $5.0 \times 10^{-3}M$ solution of pilocarpine base in chloroform was similarly prepared from 0.2447 g of pilocarpine hydrochloride, with final dilution to 200 ml.

Preparation of assay solutions

Atropine sulphate, pilocarpine hydrochloride and strychnine hydrochloride powders. About 0.12 g, accurately weighed, was dissolved in about 15 ml of water in a 100-ml separatory funnel and alkalinized. The bases were extracted with chloroform and collected in a 50-ml standard flask as described for pilocarpine hydrochloride.

Atropine sulphate eye-drops. A 5.0-ml portion was transferred into a 100-ml separatory funnel containing 10 ml of water and alkalinized. The base was extracted with chloroform and collected in a 50-ml standard flask as above.

Atropine sulphate ampoules. The contents of 12 ampoules were pooled in a dry 20-ml conical flask, and mixed; 10.0 ml were measured into a 50-ml separatory funnel, alkalinized to litmus with ammonia and extracted with 4, 2, 2 and 2 ml of chloroform, the extracts being collected in a 10-ml standard flask.

General procedure

An aliquot of assay solution was transferred into a 20-ml standard flask, 15 ml of 0.2% chloranilic acid solution were

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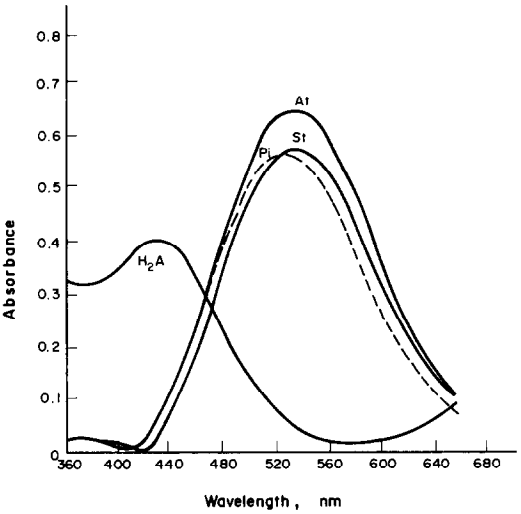

Fig. 1. Absorption spectra of chloranilic acid (H_2A, 0.35 mg/ml) and its complexes with atropine (0.15 mg/ml, At), pilocarpine (0.05 mg/ml, broken line, Pi), and strychnine (0.16 mg/ml, solid line, St) in 1:3 chloroform dioxan mixture.

added, and the solution was mixed and diluted to 20 ml with dioxan. The absorbance was measured at the corresponding λ_{max} against a blank solution prepared by diluting 15 ml of chloranilic acid solution with dioxan to 20.0 ml.

RESULTS AND DISCUSSION

Molecular complex formation

In dioxan–chloroform medium, atropine (**I**), pilocarpine (**II**) and strychnine (**III**) react instantaneously with chloranilic acid (CA) to give purple products having similar spectra (Fig. 1). Chloranilic acid in dioxan–chloroform mixture is golden yellow, with λ_{max} at 428 nm and a minimum near λ_{max} of the complexes. The similarity of λ_{max} for all three complexes is probably due to the reaction mechanisms being the same with the alkaloid as donor and chloranilic acid as acceptor.

Chloranilic acid exists in three forms,[18] the neutral yellow H_2A at very low pH, the dark violet HA^- which is most stable at pH 2, and the pale violet A^{2-}, stable at high pH. It gives a purple colour in water, acetonitrile, dimethylformamide and ammonia. As the reaction products in non-aqueous medium are purple, we conclude that HA^- is the form of chloranilic acid involved in the complexes. Previous reports on analogous systems[14,17,19–21] support this finding.

The colour of the complexes is stable for at least 24 hr if the solutions are kept in the dark. Doubts have been voiced about the utility of chloroform as the solvent[14,22,23] since it may give hydrogen-bonding with some alkaloids. Chloroform did not interfere in the present system and was preferred to dioxan because it gave lower experimental error.

Properties of the complexes

The continuous-variation[24] and molar-ratio[25] methods both showed that 1:1 complexes are formed as expected from the single donor centre in the alkaloids.

The mechanism is similar to that[17] for amino-acid complexes with chloranilic acid.

However, both methods of investigation indicate that a 2:1 donor:acceptor complex is also formed under certain conditions.

Hence for quantitative formation of the 1:1 complexes an excess of chloranilic acid is needed. Addition of 15 ml of 0.2% chloranilic acid solution in dioxan gives maximal complex formation with **I** in the concentration range 0.025–0.200 mg/ml, **II** at 0.01–0.08 mg/ml and **III** at 0.03–0.24 mg/ml (as free base in the final solution) and the solutions conform with Beer's law. Plots of absorbance (A) *vs.* concentration of free base (C) are linear but give small negative intercepts on the absorbance axis.

The accuracy of the method was tested by recovery experiments on known amounts of the salts of the alkaloids (Table 1). The error (95% confidence limits) was between 0.8 and 1.8% relative.

Various atropine dosage forms were also assayed. The phenylmercuric nitrate preservative in some of them (0.002%) did not interfere. The results are pre-

Table 1. Recovery results of alkaloids from their salts

Atropine		Pilocarpine		Strychnine	
Added, *mg/ml*	Recovery, %	Added, *mg/ml*	Recovery, %	Added, *mg/ml*	Recovery, %
0.100	100.1	0.036	99	0.100	100.9
0.120	102.2	0.057	100	0.120	100.7
0.140	100.4	0.063	100	0.140	101.1
0.160	99.6	0.068	100	0.160	100.1
0.185	100.0	0.078	101	0.180	100.7
0.200	100.6	0.078	101	0.200	101.4
Mean	100.5		100		100.8
Standard deviation	±0.9		±0.8		±0.4

Table 2. Assay results for atropine dosage forms

Eye-drops (1%)		Injections (0.1%)		Injections (0.06%)	
Taken,* mg/ml	Recovered, %	Taken,* mg/ml	Recovered, %	Taken,* mg/ml	Recovered, %
0.125	105.3	0.120	105.4	0.108	110.2
0.150	105.7	0.140	102.5	0.114	109.5
0.160	105.1	0.150	102.8	0.120	109.2
0.170	105.0	0.160	101.6	0.126	107.6
0.180	105.2	0.170	101.4	0.132	110.3
0.190	104.3	0.180	100.8	0.138	109.1
0.200	104.2	0.190	102.0	0.144	108.1
0.210	106.5	0.200	104.0	0.150	111.4
0.220	105.5				
0.230	104.3				
Mean	105.1		102.6		109.4
Standard deviation	±0.7		±1.5		±1.2

* According to label claim.

sented in Table 2. The recovery values are based on the amounts found and those calculated to be present from the nominal concentration of the preparations. The reproducibility was rather poorer than that for the pure salts.

Interferences

Substances having no basic centre are not expected to interfere, since extraction of the alkaloid base precedes the colour reaction. However, the method will not differentiate between the alkaloids investigated, and amines will interfere. Nevertheless, the method is useful for routine analysis and quality control, and the non-specificity could be overcome by using a preliminary chromatographic separation.

As the acceptor, chloranilic acid is superior to chloranil, reaction with which is slow[26–28] and gives complexes which are stable only for about 2 hr, whereas chloranilic acid gives immediate formation of a colour which is stable for at least 24 hr.

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ANALYTICAL DATA

MICROSCOPIC DISSOCIATION PROCESSES OF SOME TYROSINE DERIVATIVES

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Summary—The macroscopic and microscopic thermodynamic quantities relating to the dissociation of *o*-, *m*- and *p*-tyrosine, tyrosinol and tyramine have been determined at 25° and $I = 0.2$ (KCl). The effects of the substituents are reflected unambiguously only in the ΔG values and are due to changes in the solute–solvent interaction.

The acid–base properties of polyprotic acids are usually characterized in terms of macroscopic thermodynamic quantities. If two or more groups are of comparable acidity, the dissociation steps overlap, and the thermodynamic macroquantities are composites of the micro-quantities which give an exact description of the acid-base properties. The microscopic acid dissociation constants can be determined if the degree of dissociation of at least one of the two groups can be measured as a function of pH. Several experimental procedures are known.[1–10] However, microscopic enthalpies and entropies have been reported only for cysteine and tyrosine. Coates *et al.*[3,4] calculated micro-enthalpies from the temperature-dependence of the micro-constants, but with rather large errors.

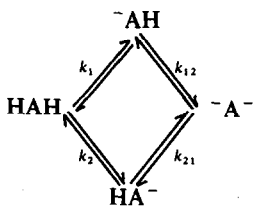
The aim of the present work is to clear up the relationships between the macro and micro-quantities.

The acid–base properties of various tyrosine derivatives are also elucidated.

THEORETICAL

Relationships between the thermodynamic macroscopic and microscopic quantities

The dissociation of a bifunctional acidic molecule can be described by the scheme:



$$H_2A \overset{K_1}{\rightleftharpoons} HA \overset{K_2}{\rightleftharpoons} A$$

where the micro-constants are related to the macro-constants by the expressions:

$$K_1 = \frac{[H]([HA^-] + [^-AH])}{[HAH]} = k_1 + k_2 \quad (1)$$

$$\frac{1}{K_2} = \frac{([HA^-] + [^-AH])}{[H][^-A^-]} = \frac{1}{k_{12}} + \frac{1}{k_{21}} \quad (2)$$

$$K_1K_2 = \frac{[H]^2[^-A^-]}{[HAH]} = k_1k_{12} = k_2k_{21}. \quad (3)$$

It is assumed that in the deprotonation of HAH (or protonation of $^-A^-$) an ideal mixture of ^-AH and HA^- with mole ratios α_1 and $\alpha_2 = 1 - \alpha_1$ is formed. We can define the microscopic free energy (Δg^0) similarly to the macroscopic quantity (ΔG^0):

$$\Delta g^0 = -RT \ln k \quad (4)$$

Accordingly, the following equations can be obtained:

$$\Delta G_1^0 = -RT \ln K_1 = \alpha_1 \Delta g_1^0 + \alpha_2 \Delta g_2^0 + RT \Sigma \alpha_i \ln \alpha_i \quad (5)$$

$$-\Delta G_2^0 = -RT \ln K_2^{-1} = -\alpha_1 \Delta g_{12}^0 - \alpha_2 \Delta g_{21}^0 + RT \Sigma \alpha_i \ln \alpha_i. \quad (6)$$

Substitution of $\alpha_1 = k_1/(k_1 + k_2)$ and equation (4) into equations (5) and (6) and solving for K_1 and K_2 gives equations (1) and (2). Similarly, by adding equations (5) and (6) and solving for K_1K_2, we obtain equation (3). On this basis we assume that the definition in equation (4) is valid.

Since an ideal mixture of ^-AH and HA^- is assumed to be formed, the macroscopic enthalpy changes are given by weighted averages of the microscopic enthalpies:

$$\Delta H_1^0 = \alpha_1 \Delta h_1^0 + \alpha_2 \Delta h_2^0 \quad (7)$$

$$\Delta H_2^0 = \alpha_1 \Delta h_{12}^0 + \alpha_2 \Delta h_{21}^0 \quad (8)$$

In the relationship between the macro- and micro-entropies, however, the entropy of mixing has also to be taken into account:

$$\Delta S_1^0 = \alpha_1 \Delta s_1^0 + \alpha_2 \Delta s_2^0 - R\Sigma\alpha_i \ln \alpha_i \quad (9)$$

$$-\Delta S_2^0 = -\alpha_1 \Delta s_{12}^0 - \alpha_2 \Delta s_{21}^0 - R\Sigma\alpha_i \ln \alpha_i. \quad (10)$$

Similar relations may be written for molecules containing more than two groups of comparable acidity.

Evaluation of micro-quantities

Microscopic dissociation constants. The basic principle of the methods used is that a certain (generally spectroscopic) parameter (molar absorptivity, chemical shift, *etc.*) of at least one of the acidic groups differs from that of its conjugate base, and hence the degree of dissociation of this group can be monitored specifically.

For a bifunctional acidic molecule, the measured values of the parameters for each acidic group at a given stage of titration may be expressed as:

$$p_1 = x^{HAH} p_1^{HAH} + x^{HA^-} p_1^{HA^-} + x^{^-AH} p_1^{^-AH} + x^{^-A^-} p_1^{^-A^-} \quad (11)$$

$$p_2 = x^{HAH} p_2^{HAH} + x^{HA^-} p_2^{HA^-} + x^{^-AH} p_2^{^-AH} + x^{^-A^-} p_2^{^-A^-} \quad (12)$$

The mole fractions are identified by one index, and the parameters by two indices, the upper referring to the protonation state of the two groups, the lower to the individual group. The values of these parameters for the species in which both groups are protonated (p_1^{HAH}, p_2^{HAH}) or fully deprotonated ($p_1^{^-A^-}$, $p_2^{^-A^-}$) can be measured experimentally, whereas their values for the intermediate species ($^-$AH and HA$^-$) cannot be determined directly. It may be assumed, however, that the molar values of these parameters for each individual group are independent of whether the other group is protonated (superscript p) or deprotonated (superscript d):

$$p_1^p = p_1^{HAH} = p_1^{HA^-}$$

and

$$p_1^d = p_1^{^-AH} = p_1^{^-A^-} \quad (13)$$

$$p_2^p = p_2^{HAH} = p_2^{HA^-}$$

and

$$p_2^d = p_2^{^-AH} = p_2^{^-A^-} \quad (14)$$

and

$$x_1^p = x^{HAH} + x^{HA^-},$$

$$x_1^d = x^{^-AH} + x^{^-A^-} \quad (15)$$

$$x_2^p = x^{HAH} + x^{^-AH},$$

$$x_2^d = x^{HA^-} + x^{^-A^-} \quad (16)$$

Substitution of equations (13)–(16) into (11) and (12) gives:

$$p_1^{exp} = x_1^p p_1^p + x_1^d p_1^d \quad (17)$$

$$p_2^{exp} = x_2^p p_2^p + x_2^d p_2^d \quad (18)$$

Substitution of $x_1^p = 1 - x_1^d$ and $x_2^p = 1 - x_2^d$ into (17) and (18) respectively leads to:

$$x_1^d = \frac{p_1^{exp} - p_1^p}{p_1^d - p_1^p} \quad \text{and} \quad x_2^d = \frac{p_2^{exp} - p_2^p}{p_2^d - p_2^p}. \quad (19)$$

The x_1^d and x_2^d values, which represent the fractional dissociation of each group, can be obtained from experimental data. For the scheme above, the fractional dissociation of each group is defined as:

$$x_1^d = \frac{[^-AH] + [^-A^-]}{[HAH] + [^-AH] + [HA^-] + [^-A^-]} = \frac{k_1[H] + k_1 k_{12}}{[H]^2 + (k_1 + k_2)[H] + k_1 k_{12}} \quad (20)$$

$$x_2^d = \frac{[HA^-] + [^-A^-]}{[HAH] + [^-AH] + [HA^-] + [^-A^-]} = \frac{k_2[H] + k_2 k_{21}}{[H]^2 + (k_1 + k_2)[H] + k_1 k_{12}}. \quad (21)$$

Microconstants can be calculated from the fractional dissociation as a function of pH [equations (20) and (21)] by graphical[1,2] or non-linear least-squares curve-fitting methods.[11,12] If the microconstants in equations (20) and (21) are replaced by the macroconstants K_1 and K_2, which are calculated from independent pH data, these equations yield simpler forms. On introduction of equation (1) into the denominator, equations (20) and (21) can be rearranged to linear forms and solved by a linear least-squares method.[13] Further substitution of (3) into (20) and (21) leads to even simpler forms which contain only one refinable parameter. In a given experimental arrangement, the accuracy and reproducibility of measurements of the pH and the characteristic parameter will decide which computational method is the most suitable for evaluation of the experimental data.

If the basic principle is not strictly valid, *i.e.*, the dissociation of at least one of the acidic groups cannot be followed specifically, equation (19) is not suitable for direct calculation. This is quite commonly the case with NMR methods when the two acidic groups are not sufficiently separated. In such cases, the effect of the other group on the parameter of the monitored group can be taken into account as follows:

$$x_1^d = \frac{p_1^{exp} - p_1^p - x_2^d \Delta p_2}{p_1^d - p_1^p - \Delta p_2}$$

and

$$x_2^d = \frac{p_2^{exp} - p_2^p - x_1^d \Delta p_1}{p_2^d - p_2^p - \Delta p_1}. \quad (22)$$

In (22), Δp_1 and Δp_2 are the total changes in the parameters of the monitored groups, caused by dissociation of the other group. The computational method suggested by Sayer and Rabenstein[7] is based on the iterative refining of all parameters (x_1^q, x_2^q, Δp_1 and Δp_2). The set of equations is poorly "conditioned", however, and thus reliable values for the microconstants cannot always be obtained. In another method, the effect of the other group is taken into account by means of suitable model compounds and in this way more reliable values are found[14] for x_1^q and x_2^q.

It often happens that the dissociation of only one of the two acidic groups can be followed (*e.g.*, in the case of molecules containing an –SH or –OH group, the dissociation of the group being monitored by ultraviolet spectroscopy). Then, a single fractional dissociation *vs.* pH curve serves for calculation of all the microconstants. In such cases, the utilization of macroconstant(s) determined by pH titration may be advantageous.

Microscopic dissociation enthalpy and entropy. Microscopic enthalpies cannot be determined calorimetrically. Since the thermodynamic microscopic quantities (Δg^0, Δh^0 and Δs^0) may be defined analogously to the macroscopic quantities (ΔG^0, ΔH^0 and ΔS^0), we have assumed that the same relationships are valid for the micro-quantities as for the macro-quantities. Hence the van't Hoff relation is also valid, *i.e.*, from the temperature-dependence of the microscopic dissociation constants the micro-enthalpies and micro-entropies can be obtained as follows:

$$\ln k_i = -\frac{\Delta h_i^0}{RT} + \frac{\Delta s_i^0}{R}. \qquad (23)$$

EXPERIMENTAL

Chemicals used and experimental conditions

DL-*p*-tyrosine, DL-phenylalanine and phenol (*p.a.*, Reanal), tyramine, 2-phenylethylamine, DL-*o*-tyrosine, DL-*m*-tyrosine and DL-tyrosinol·HCl (Fluka) were used for the experiments. Amino-acids were purified by recrystallization from ethanol–water mixture, and 2-phenylethylamine and phenol by distillation at reduced pressure.

The macro- and micro-constants were determined in a special experimental arrangement, where the dissociation was followed spectrophotometrically and by pH titration simultaneously. Ligand solutions (0.003 and 0.004*M*) were titrated with carbonate-free 0.2*M* potassium hydroxide in a closed system with circulation between the pH titration vessel and a flow-through quartz cell by means of a peristaltic pump. The ionic strength of the solution was adjusted to 0.2*M* with potassium chloride. At each titration point in the pH range 7.5–11.4 (7.5–12.4 in the case of *o*-tyrosine), the pH and the absorbance of the phenolate group (in the range 250–350 nm) were measured.

The measurements were carried out at 15, 20, 25, 30 and 35°. A Radiometer pHM-64 digital pH-meter with G 202 B glass and K401 calomel reference electrodes, and a Beckman ACTA MIV double-beam recording spectrophotometer were used. The dissociation macro-enthalpies were determined at 25° with an LKB 8700-1 solution and reaction calorimeter, by the method described previously.[15]

Calculation

The thermodynamic macroscopic quantities were calculated from the pH and calorimetric data as reported earlier.[15]

From the fractional dissociation curve [equation (21)] of the phenolic hydroxy group, the microscopic dissociation constants were calculated by four different methods:

(*i*) a graphical method;[1]

(*ii*) a linear least-squares method,[13] the K_1 value obtained from separate pH measurements also being used in the calculation;

(*iii*) a non-linear least-squares curve-fitting method,[12] in which all microconstants were iteratively refined;

(*iv*) the direct solution of equation (21), by using the values of K_1 and K_1K_2 determined from separate pH data.

RESULTS AND DISCUSSION

The dissociation macroconstants calculated from the pH titrations, and the macro-enthalpies calculated from the calorimetric measurements, together with the macro-entropies, are listed in Table 1.

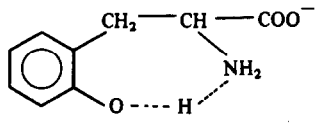
It can be seen that the small but similar inductive effects of the *para* and *meta* hydroxy groups cause similar changes in the thermodynamic quantities of

Table 1. The thermodynamic macro-quantities for dissociation of ligands [25°C; I = 0.2 (KCl)]

		pK	ΔG, *kJ/mole*	ΔH, *kJ/mole*	$-\Delta S$, $J.mole^{-1}.K^{-1}$
	COOH	2.41	13.7	3.6	34
o-tyrosine	pK_1	8.67	49.3	39.0	35
	pK_2	11.01	62.6	28.2	115
	COOH	2.22	12.6	2.6	34
m-tyrosine	pK_1	8.94	50.8	38.5	41
	pK_2	10.03	57.0	28.6	95
	COOH	2.25	12.8	2.5	35
p-tyrosine	pK_1	9.04	51.4	38.2	44
	pK_2	10.08	57.3	28.7	96
tyrosinol	pK_1	8.94	50.8	44.6	21
	pK_2	9.98	56.8	32.0	83
tyramine	pK_1	9.41	53.5	30.6	79
	pK_2	10.45	59.4	47.7	39

the carboxyl group relative to those of phenylalanine (see Table 5). With *o*-tyrosine, however, the sterically favoured interaction between the $-^{+}NH_3$ group and the hydroxy group decreases the electron-attracting effect of the $-^{+}NH_3$ group, so the intramolecular hydrogen-bond between the $-COO^{-}$ and $-^{+}NH_3$ groups is weakened, which results in slightly increased pK and ΔH values for the carboxyl group.

For *m*- and *p*-tyrosine, tyramine and tyrosinol, the macroquantities identified by subscripts 1 and 2 are combined quantities, since the acidities of the phenolic hydroxy and the protonated side-chain amino groups are comparable. For *o*-tyrosine, however, it may be assumed that in the dissociation of $H_2A^{\pm}$ the following molecule, which contains an intramolecular hydrogen-bond, is formed:



Hence, microscopic processes should not be reckoned with in this case.[10]

The microscopic dissociation constants for the phenolic hydroxy and $-^{+}NH_3$ groups were determined by selectively monitoring the dissociation of the phenolic hydroxy group spectrophotometrically. The micro-constants were then calculated from the fractional dissociation curves. The values obtained for *p*-tyrosine with the different computational methods are listed in Table 2.

It was found that the most reliable results could be obtained with method (*ii*) or (*iv*), when the macro-constant(s) determined in a separate pH study were used in the calculation process. The k_1/k_2 values calculated by these methods are in good agreement with most of the data reported earlier.[1,3,9,13] The slightly different results from the graphical method (*i*) are caused by the possible error in the extrapolation,[16] and from the non-linear least-squares method (*iii*) by the larger experimental error in the spectrophotometric titrations and because there are too many refinable parameters. It is noteworthy, however, that when the dissociation of both acidic groups can be independently monitored, the non-linear least-squares method provides excellent results.[14] The fractional dissociation curves were therefore evaluated by use of the separately determined macro-constant(s).

Table 3. Dissociation macro-constants and k_1/k_2 values at different temperatures [I = 0.2 (KCl)]

		15°	20°	25°	30°	35°
o-tyrosine	k_1/k_2	1.00	1.05	1.02	1.00	1.01
	pK_1	8.97	8.79	8.67	8.56	8.45
	pK_2	11.18	11.09	11.01	10.93	10.84
m-tyrosine	k_1/k_2	0.56	0.51	0.45	0.39	0.31
	pK_1	9.17	9.06	8.94	8.83	8.71
	pK_2	10.21	10.12	10.03	9.94	9.85
p-tyrosine	k_1/k_2	0.60	0.58	0.46	0.44	0.41
	pK_1	9.25	9.15	9.04	8.93	8.81
	pK_2	10.25	10.17	10.08	9.97	9.87
tyrosinol	k_1/k_2	0.66	0.53	0.44	0.35	0.31
	pK_1	9.21	9.09	8.94	8.81	8.70
	pK_2	10.17	10.08	9.98	9.88	9.79
tyramine	k_1/k_2	5.52	4.73	3.75	3.17	2.57
	pK_1	9.59	9.50	9.41	9.32	9.22
	pK_2	10.74	10.60	10.45	10.32	10.18

To determine the dissociation micro-enthalpies, the pH–spectrophotometric titrations were performed at five different temperatures. The calculated k_1/k_2 values, together with the macro-constants calculated from the pH titrations, are listed in Table 3. It can be seen that the k_1 and k_2 values for *o*-tyrosine are equal when calculated on the assumption that microscopic processes occur, and their ratio does not depend on temperature. Substitution of $k_1 = k_2$ and $k_{12} = k_{21}$ into equations (1) and (2) leads to $K_1 = 2k_1$ and $K_2 = k_{12}/2$. Spectrophotometric measurements showed that at 25° pk_1 = 8.98 and pk_{12} = 10.70, in accordance with these equalities (*cf.* Table 3). This confirms that HA for *o*-tyrosine has a single structure (obviously that shown above), and that accordingly the microscopic dissociation processes do not occur.

The k_1/k_2 values in Table 3 clearly show the order of acidity of the donor groups: the side-chain ammonium groups are more acidic than the corresponding phenolic hydroxy groups, exception in tyramine, where the absence of the carboxylate group reverses the order of the acidities. This is in agreement with the earlier data.[1–3,9,13]

Table 2. The dissociation micro- and macro-constants calculated for *p*-tyrosine by different computational methods [25°; I = 0.2 (KCl)]

	Graphical method	Linear regression	Non-linear regression	With fixed K_1 and K_1K_2 values
pk_1	9.51	9.54	9.57	9.54
pk_2	9.22	9.20	9.16	9.20
pk_{12}	9.52	9.55	9.42	9.58
pk_{21}	9.82	9.87	9.83	9.92
k_1/k_2	0.50	0.46	0.39	0.46
pK_1	9.04	9.04*	9.02	9.04*
pK_2	10.00	10.04	9.97	10.08*

* Calculated from pH measurements.

Table 4. The thermodynamic micro-quantities for dissociation of ligands [25°; $I = 0.2$ (KCl)]

		1*	2*	21*	12*
m-tyrosine	p*k*	9.45	9.10	9.87	9.52
	Δh, *kJ/mole*†	24.0	45.0	22.1	43.1
	Δs, $J.mole^{-1}.K^{-1}$	−100	−23	−114	−37
p-tyrosine	p*k*	9.54	9.20	9.92	9.58
	Δh, *kJ/mole*†	24.6	44.4	22.5	42.3
	Δs, $J.mole^{-1}.K^{-1}$	−99	−27	−114	−41
tyrosinol	p*k*	9.43	9.11	9.81	9.49
	Δh, *kJ/mole*†	24.0	53.7	22.9	52.6
	Δs, $J.mole^{-1}.K^{-1}$	−99	6	−114	−9
tyramine	p*k*	9.51	10.08	9.78	10.35
	Δh, *kJ/mole*†	24.1	55.1	23.2	54.2
	Δs, $J.mole^{-1}.K^{-1}$	−99	−7	−109	−16

* Subscripts identifying the dissociation processes.
† Uncertainty ±0.5 kJ/mole.

The logarithms of the micro-constants are a linear function of the inverse of the absolute temperature, so the thermodynamic micro-quantities can be obtained by this method. The Δh_i and Δs_i values, together with the pk_i data, are listed in Table 4. Table 5 gives the thermodynamic macroquantities for some reference compounds.

The pk_1 values characteristic of the phenolic hydroxy groups can be compared with the dissociation constant of phenol, and the pk_2 values characteristic of the side-chain ammonium groups can be compared with p*k* for phenylalanine and 2-phenylethylamine. It can be seen that the weak electron-releasing effect of the phenolic hydroxy group is manifested in the pk_2 values, and the powerful electron-attracting effect of the $^+NH_3$ group in the pk_1 values. The substituent effect is also reflected in the Δh_1 and Δh_2 values, although not so definitely as in the p*k* data.

In accordance with this, the electron-releasing effect of the alkyl chain results in the pk_{21} values being larger than the pk_1 values and the p*K* of phenol. On the other hand, because the electron-releasing effect of the phenolate group is stronger than that of the phenolic hydroxy group, the pk_{12} values are larger than the pk_2 values and the p*K* of phenylalanine or 2-phenylethylamine, but the substituent effects are not exhibited in the Δh data. However, it frequently happens that there is no simple, easily interpreted relationship between the enthalpy change and electron shifts within the molecule, this being due to solute–solvent interactions, which also contribute to ΔH.

It can also be seen from Table 4 that the micro-enthalpies for a given acidic group depend slightly on the protonation state of the other group ($\Delta h_1 \sim \Delta h_{21}$ and $\Delta h_2 \sim \Delta h_{12}$); there is a bigger effect on the entropy changes. Thus, the protonation state of the other group affects the hydration conditions of the dissociation process.

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Table 5. Thermodynamic dissociation macro-quantities for some reference compounds [25°; $I = 0.2$ (KCl)]

		p*K*	ΔG, *kJ/mole*	ΔH, *kJ/mole*	ΔS, $J.mole^{-1}.K^{-1}$
phenylalanine[17]	COOH	2.19	12.46	2.5	−33.4
	$^+NH_3$	9.06	51.53	44.8	−22.6
2-phenylethylamine		9.92	56.42	54.3	−7.1
phenol		9.73	55.34	24.6	−103.1

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SPECTROPHOTOMETRIC AND ANALOGUE DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF ULTRAMICRO AMOUNTS OF CADMIUM WITH CATIONIC PORPHYRINS

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Summary—α,β,γ,δ-Tetrakis(1-methylpyridinium-3-yl)porphine [T(3-MPy)P] and α,β,γ,δ-tetrakis(1-methylpyridinium-4-yl)porphine [T(4-MPy)P] have been found to react rapidly with cadmium to give coloured complexes in weakly alkaline media at room temperature. Simple and practical methods for the determination of cadmium at ng/ml levels by conventional and analogue derivative spectrophotometry have been proposed. The analogue method gives higher sensitivity. T(3-MPy)P gives higher sensitivity than T(4-MPy)P. The interference of various foreign cations and anions has also been examined and in many cases eliminated or reduced. Adsorption of the porphyrins and their cadmium complexes onto the glassware, which is usually observed under the conditions of reaction and causes significant errors in the determination, can be suppressed almost completely by addition of fairly large amounts of a salt such as sodium chloride.

Water-soluble meso-substituted porphyrins are very useful as highly sensitive colour-producing reagents for metal ions because they possess Soret bands which have extremely large molar absorptivity (1×10^5–6×10^5 $l.mole^{-1}.cm^{-1}$) and are more easily handled than water-insoluble reagents. However, there are also a few disadvantages. One is that, in general, the complexation reaction of the porphyrin with the metal ion in aqueous medium is very slow at room temperature. Hence several attempts have been made to accelerate it, including (*a*) heating,[1–3] (*b*) addition of an auxiliary complexing agent such as pyridine or imidazole[4] or a reducing agent such as hydroxylamine or ascorbic acid,[5] and (*c*) utilization of the metal-substitution reaction with a cadmium, lead or mercury(II) porphyrin complex,[6] which is very rapid even at room temperature (although in the case of the cadmium complex, addition of pyridine or imidazole is usually necessary to accelerate the complexation[4]). These attempts were found to be quite effective for acceleration of the complexation of the porphyrin (*a*) with many metal ions, (*b*) with cadmium or copper(II), and (*c*) with copper(II), cobalt(II), manganese(II) and zinc. Another disadvantage is that the cationic porphyrin and its metal complexes are readily adsorbed on glass, especially in neutral and weakly alkaline media where the complexation reaction is more rapid than in acid. This causes significant errors in the determination of traces of metals. Therefore, acceleration of the complexation reaction and suppression of the adsorption are important in determinations with porphyrins.

On the other hand, during a series of studies concerning analytical application of porphyrins, it has been found that the cationic porphyrins α,β,γ,δ-tetrakis(1-methylpyridinium-3-yl)porphine [T(3-MPy)P] and α,β,γ,δ-tetrakis(1-methylpyridinium-4-yl)porphine [T(4-MPy)P], (Fig. 1) form complexes directly and rapidly with cadmium, copper(II), mercury(II), lead and zinc at room temperature in neutral and/or weakly alkaline media without addition of any auxiliary complexing agent or reducing agent. Adsorption of these porphyrins and their complexes on glass can be suppressed by addition of a salt such as sodium chloride, nitrate or sulphate.

On the basis of these findings, the spectrophotometric determination of cadmium with T(3-MPy)P and T(4-MPy)P has been studied. The analogue derivative technique[7,8] has been introduced to make the determination more sensitive. Thus simple and practical methods for the determination of cadmium at ng/ml levels have been developed, and these are described in this paper.

EXPERIMENTAL

Reagents

T(3-MPy)P was synthesized by the method described in an earlier paper[2] and T(4-MPy)P by the method of Pasternack, *et al.*[9] All other chemicals used were of analytical-reagent grade. All solutions were prepared with distilled, demineralized water unless otherwise described.

Apparatus

For measurements of the absorbance and the absorption spectrum, a Hitachi 139 spectrophotometer and a Hitachi 556 dual wavelength spectrophotometer respectively, were

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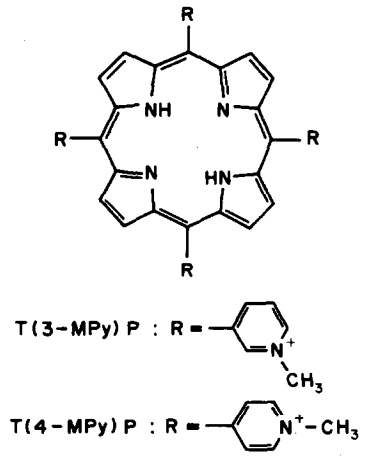

Fig. 1. T(3-MPy)P and T(4-MPy)P.

used, the latter being used as an ordinary double-beam spectrophotometer throughout all the measurements. To obtain the derivative spectra a modified Hitachi 200-0576 derivative unit (composed of two analogue differentiation circuits) was connected between the spectrophotometer output and a Hitachi 057 *X-Y* recorder input. The details of this apparatus and the principles and characteristics of the analogue derivative spectrophotometry are described in earlier papers.[7,8]

Procedures

Spectrophotometry with T(3-MPy)P. Place an aliquot of the sample solution containing less than 8 μg of cadmium in a 25-ml standard flask. Add 1 ml of 0.3*M* sodium tartrate, 1 ml of 0.3*M* sodium citrate, and 1 ml of 10^{-3}% triethylenetetramine solution or 0.5 ml of 1% dimethylglyoxime solution in ethanol as the masking agent, if necessary. After addition of 4 ml of $1 \times 10^{-4}M$ T(3-MPy)P, adjust the pH of the solution to 9.5–11 by adding a sufficient volume of sodium borate–sodium hydroxide buffer solution. Then add 2.5 ml of 4*M* sodium chloride and dilute to the mark with water. After allowing to stand for about 5 min, measure the absorbance at 441 nm against a reagent blank, using 1-cm glass cells.

Spectrophotometry with T(4-MPy)P. Place an aliquot of the sample solution containing less than 12 μg of cadmium in a 25-ml standard flask and treat as described above, but with T(4-MPy)P instead of T(3-MPy)P, and absorbance measurement at 450 nm (this wavelength is that of the absorption maximum when the complex is measured against a reagent blank, and differs slightly from that for measurement against water).

Second derivative spectrophotometry with T(3-MPy)P. Place an aliquot of the sample solution containing less than 380 ng of cadmium in a 25-ml standard flask. Add masking agent as in the spectrophotometric procedure, if necessary. Add 1 ml of $1 \times 10^{-6}M$ T(3-MPy)P solution, 1 ml of 0.1*M* sodium hydroxide and 2.5 ml of 4*M* sodium chloride, and dilute to the mark with water. After allowing the solution to stand for about 5 min, record the second derivative absorption spectrum of the resultant solution, in the Soret region, against a reagent blank, using 1-cm glass cells and the following conditions; circuit No. 6, scan-speed 150 nm/min, and recorder sensitivity $\times 1$. Measure the second derivative value (vertical distance from a peak to a trough or the base-line to a trough) on the chart.

RESULTS AND DISCUSSION

Effect of addition of salts

Suppression of adsorption. As the reason for adsorption of cationic porphyrins and their complexes on glassware is thought to be to the electrostatic interaction between positive charges on the substituents in meso-positions of the porphine ring and negative charges on the glass surface, it was thought that an increase of the cation concentration in the solution by addition of acids, bases or salts would be effective in suppressing the adsorption. In fact, adsorption is not observed in acidic or strongly alkaline medium. However, in acidic medium, the cadmium–porphyrin complex tends to decompose, and in strongly alkaline medium even such metal ions as silver and calcium interfere with the cadmium determination because their complexation reaction with the porphyrin is also accelerated. As salts such as sodium, potassium or ammonium chloride, nitrate or sulphate seemed to minimize the adsorption, in this study sodium chloride was added to give a concentration of about 0.4*M* in the final solution, which made the adsorption negligible. If perchlorate or thiocyanate is present, the cationic porphyrins and their complexes generally tend to precipitate because of formation of ion-pairs. Addition of fairly large amounts of these salts, therefore, should be avoided.

Chloride as an axial ligand. In general, the absorption spectrum of the metal–porphyrin complex is known to shift gradually and slightly with increasing pH or the concentration of a salt such as sodium chloride or thiocyanate. Figure 2 shows a set of the Soret bands for a series of solutions containing identical concentrations of cadmium–T(3-MPy)P complex and sodium sulphate but of varied pH, from which it is seen that the Soret band of the complex gradually shifts to longer wavelengths as the pH rises, and an

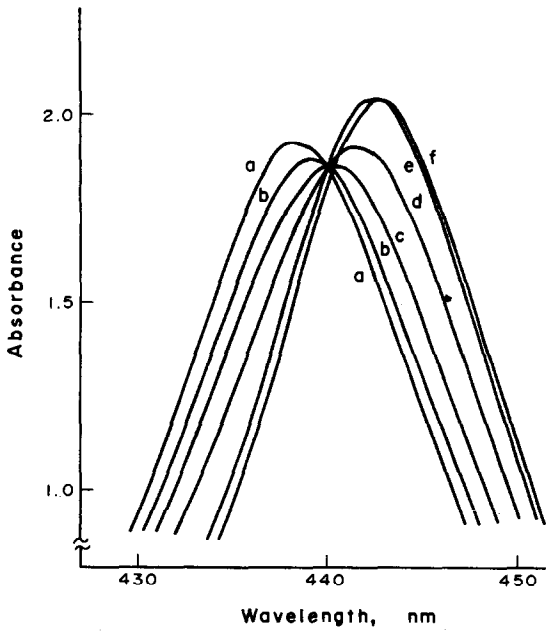

Fig. 2. Absorption spectra of Cd-T(3-MPy)P complex as a function of pH. [Cd-T(3-MPy)P] = $5.7 \times 10^{-6}M$; [$1Na_2SO_4$] = 0.5*M*; reference, water; pH—a 7.6, b 11.0, c 11.3, d 11.8, e 13.1, f 13.6.

isosbestic point exists at 440 nm over pH range 7.6–13.1. (In this case, sodium sulphate is added as an adsorption inhibitor which differs from chloride or thiocyanate and does not affect the spectrum of the complex.) A similar result was obtained for the T(4-MPy)P complex. These spectral variations are thought to correspond to dissociation of a proton from a water molecule co-ordinated axially to the central metal ion (*i.e.*, cadmium) of the complex, corresponding to the equations

$$Cd(H_2O)_2P^{4+} \rightleftharpoons Cd(H_2O)(OH)P^{3+} + H^+ \quad (1)$$

$$k_a = \frac{[Cd(H_2O)(OH)P^{3+}][H^+]}{[Cd(H_2O)_2P^{4+}]} \quad (2)$$

where P and k_a represent the non-dissociative part of T(3-MPy)P or T(4-MPy)P and the acid dissociation constant, respectively. Further shift of the Soret band at higher pH (Fig. 2) suggests the deprotonation of a further water molecule co-ordinated to the central metal ion, but this was not studied in detail. However, by analysis of the results shown in Fig. 2 and for the T(4-MPy)P complex, according to the method used by Pasternack *et al.*,[10] the dissociation of a proton from each complex was ascertained and the acid dissociation constants for both complexes were determined (Table 1).

A spectrophotometric titration in the Soret region of the cadmium(II)-T(3-MPy)P complex at pH 9 with sodium chloride was carried out and the result is shown in Fig. 3. The Soret band of the complex shifts gradually and slightly to longer wavelengths as the sodium chloride concentration is increased and an isosbestic point exists at 436.5 nm for the chloride concentration range 0–0.4*M*. This spectral variation is thought to be due to axial co-ordination of chloride (in general, the salt anion, X^-), in accordance with the equations

$$Cd(H_2O)_2P^{4+} + X^- \rightleftharpoons Cd(H_2O)XP^{3+} + H_2O \quad (3)$$

$$k_1 = \frac{[Cd(H_2O)XP^{3+}]}{[Cd(H_2O)_2P^{4+}][X^-]} \quad (4)$$

where k_1 represents the formation constant of the ternary complex. Further shift of the Soret band at a higher chloride concentration region (Fig. 3) suggests the axial co-ordination of another chloride ion. A similar result was obtained for the cadmium-T(4-MPy)P complex. The formation constants were determined in the manner used by Pasternack *et al.*[10] and are also given in Table 1.

Absorption spectra

Figure 4 shows the absorption spectra of T(3-MPy)P, T(4-MPy)P and their cadmium complexes under the conditions of the determination. The Soret, β and α bands of the T(3-MPy)P complex lie at 441, 573 and 613 nm, and those of the T(4-MPy)P complex at 448, 580 and 624 nm, respectively. The Soret bands (which are important for the determination because of their extremely large molar absorptivities) shift slightly to longer wavelengths than those found in the absence of sodium chloride. This suggests interaction between the complexes and chloride ions as described above.

Table 1. pk_a of Cd(II)–porphyrin complexes and log k_1 of Cd(II)–porphyrin–Cl^- complexes at 25°C, μ = 1.5 (Na_2SO_4)

Complex	pk_a	log k_1
Cd–T(3-MPy)P	11.6	1.5
Cd–T(4-MPy)P	11.6	1.7
$Cd(H_2O)_6^{2+}$	9.0*	1.4*

* Data from *Stability Constants*, The Chemical Society, London, 1964: pk_a: 25°C, μ = 3($HClO_4$ + $NaClO_4$); log k_1: 25°C, μ = 2 ($NaClO_4$).

Influence of sodium chloride concentration and selection of analysis wavelength

As already described, addition of sufficient sodium chloride is required to suppress the adsorption, and λ_{max} for the Soret band of the cadmium complex depends on the sodium chloride concentration. The selection of wavelength for the cadmium determination, therefore, needs to take both effects into consideration. Fortunately, the absorbance is practically unaffected over the chloride concentration range 0.2–1.0*M* for measurement at 441 nm in the determination with T(3-MPy)P and the range 0.1–0.5*M* for measurement at 450 nm in that with T(4-MPy)P.

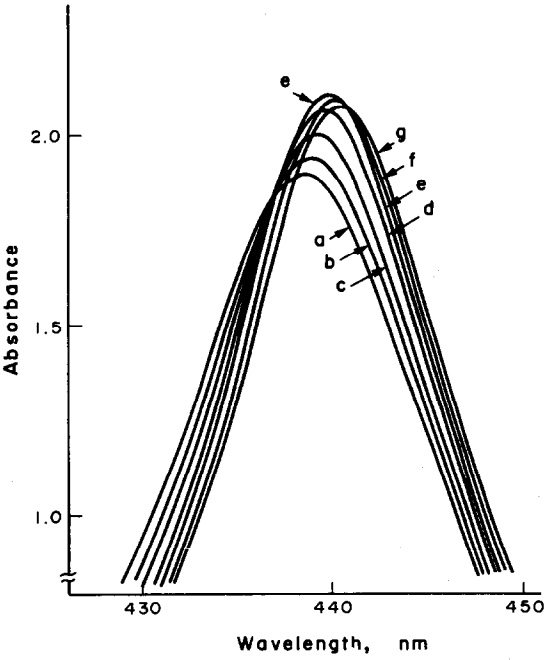

Fig. 3. Absorption spectra at pH 9 of Cd-T(3-MPy)P complex as a function of Cl^- concentration. [Cd-T(3-MPy)P] = 5.7 × 10^{-6}*M*; [Na_2SO_4] = 0.5*M*; reference, water; [Cl^-]—a 0, b 0.01, c 0.03, d 0.1, e 0.4, f 2.0, g 3.0*M*.

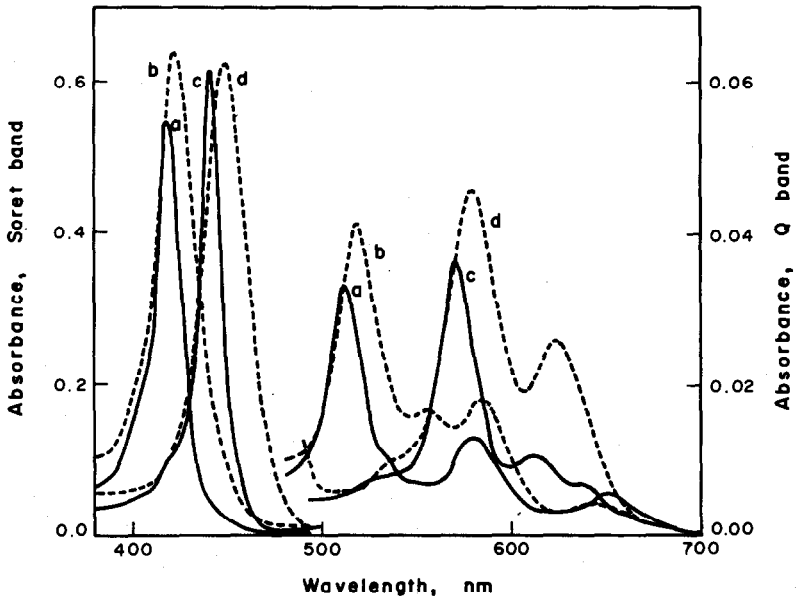

Fig. 4. Absorption spectra of T(3-MPy)P (a), T(4-MPy)P (b) and their cadmium complexes (c, d) in 0.4*M* sodium chloride medium: pH 10.5; reference, water; [T(3-MPy)P]—a, c $1.6 \times 10^{-6}M$, b, d nil; [T(4-MPy)P]—b, d $2.6 \times 10^{-6}M$, a, c nil; [Cd]—a, b nil; c, d $5.3 \times 10^{-6}M$.

Influence of pH

Figure 5 shows that both porphyrins form the cadmium complexes rapidly and give almost constant absorbances in the pH regions 9.3–11.7 and 8.7–11.1 for T(3-MPy)P and T(4-MPy)P respectively, in the presence of 0.4*M* sodium chloride. In these pH regions, cadmium exists as a monohydroxo, $Cd(H_2O)_5(OH)^+$, or a monochloro complex, $Cd(H_2O)_5Cl^+$, or both (depending on pH), before it reacts with the porphyrins, which is thought to be one of the reasons for the fast complexation. Further, the pH ranges where almost constant absorbances are obtained are remarkably broad, whereas in the absence of sodium chloride no such pH regions are found. The reason for this seems to be that the predominant complex formed in such pH regions in the presence of 0.4*M* sodium chloride is $Cd(H_2O)ClP^{3+}$, as may be deduced from the values of pk_a and k_1 in Table 1; further, the deprotonation of $Cd(H_2O)ClP^{3+}$ is negligible because it occurs only in more strongly alkaline media. Thus, the complex species scarcely change in the pH regions where almost constant absorbances are obtained, and the adsorption of the porphyrins and their complexes is considerably suppressed by the presence of the chloride as already described.

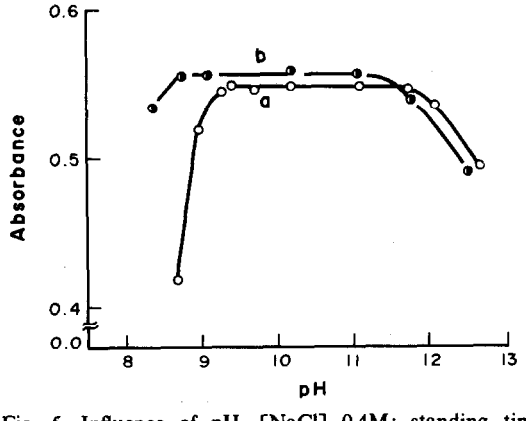

Fig. 5. Influence of pH. [NaCl] 0.4M; standing time 5 min; reference, reagent blank. (a) Cd-T(3-MPy)P system: [Cd] 178 ng/ml; [T(3-MPy)P] $1.6 \times 10^{-5}M$; wavelength 441 nm. (b) Cd-T(4-MPy)P system: [Cd] 284 ng/ml; [T(4-MPy)P] $1.6 \times 10^{-5}M$; wavelength 450 nm.

Influence of porphyrin concentration

The porphyrins were found (by the mole-ratio method) to form a 1:1 complex with cadmium, but about 100% excess of porphyrin is required to complete the complexation rapidly. To prevent decolouration of the porphyrin complex and allow for consumption of porphyrin by other metal ions, however, it is preferable to add fairly large amounts of the porphyrin.

Stability of the complexes to light

One of the present authors has already reported that solutions of the cadmium complex of the anionic porphyrin, α,β,γ,δ-tetrakis(4-sulphophenyl)porphine or α,β,γ,δ-tetrakis(4-carboxyphenyl)porphine, are appreciably decolourized by light.[4] Figure 6 shows that a solution of the cadmium–T(3-MPy)P complex is stable to light for at least 5 hr if excess of T(3-MPy)P is present, whether sodium chloride is also present or not. On the other hand, with excess of cadmium, the absorbance decreases by only about 3% in 5 hr in presence of 0.4*M* sodium chloride, but by about 12%

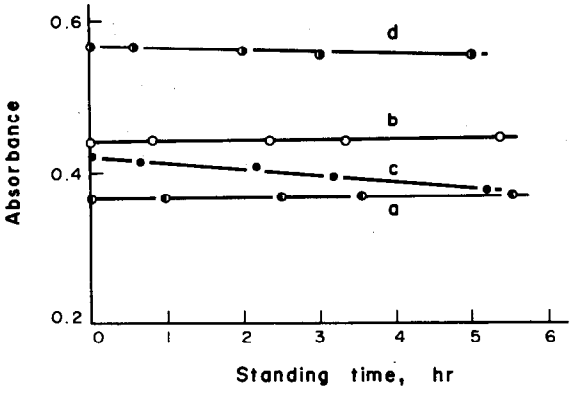

Fig. 6. Influence of light on stability of Cd-T(3-MPy)P complex at pH 10.5. [T(3-MPy)P]—a, b $1.6 \times 10^{-5}M$, c, d $1.6 \times 10^{-6}M$; [Cd(II)]—a, b $1.3 \times 10^{-6}M$, c, d $6.3 \times 10^{-6}M$; [NaCl]—a, c, nil, b, d $0.4M$; wavelength—a, c 443 nm*, b, d 441 nm; reference—a, b reagent blank, c, d, water. *The wavelength of the absorption maximum in sodium chloride medium.

in absence of the sodium chloride. Similar results were obtained for the T(4-MPy)P complex.

Thus the decolouration of the solutions of the complexes, which is attributed to a redox reaction, was found not to be as serious as expected, and it was suppressed almost completely by addition of excess of porphyrin and appreciably by addition of sodium chloride.

Calibration graphs

Linear calibration plots were obtained by the recommended procedures, the weight of cadmium in the sample taken being given by

$$\text{Cd} = 7.7_5A\ \mu\text{g} \quad (5)$$

$$\text{Cd} = 12.7_5A\ \mu\text{g} \quad (6)$$

where A is the absorbance, and equations (5) and (6) refer to T(3-MPy)P and T(4-MPy)P respectively. The optimum ranges for the cadmium determination and the molar absorptivities are summarized in Table 2.

The relative standard deviation for 3.55 μg of cadmium determined by the T(3-MPy)P procedure was 0.4% (16 variates).

Interferences

The possible interference in the determination of 3.55 μg of cadmium by the procedure with T(3-MPy)P, which gives rather high sensitivity, was examined. Cations were added in the form of chlorides, nitrates or sulphates; anions were added as sodium or potassium salts. The limiting value of the concentration of foreign ions was taken as that which caused an error of not more than 3%. The results are summarized in Table 3. Most anions do not interfere even when present in fairly large amounts, but most cations interfere because of their hydrolysis or their complexation with T(3-MPy)P when no masking agent is used. Interferences caused by cations except zinc, however, can be removed or reduced by addition

Table 2. Optimum ranges and molar absorptivities of the recommended procedures

Reagent	Optimum range, μg	Molar absorptivities, $l.mole^{-1}.cm^{-1}$
T(3-MPy)P	0.4–8	3.61×10^5
T(4-MPy)P	0.6–13	2.20×10^5

of sodium tartrate, sodium citrate, triethylenetetramine or dimethylglyoxime, and the tolerance limits thus obtained are those shown in Table 3. The interference of zinc can be reduced by modifying the procedure as follows.

The same volume of sample solution is placed in each of two 25-ml standard flasks. One sample flask is treated by the recommended procedure, and gives absorbance A_1. To the other sample flask 2 ml of $0.1M$ EDTA are added after the cadmium has reacted with T(3-MPy)P to give a full colour, and the mixture is let stand for about 10 min, for the cadmium complex has to decompose completely (the zinc complex remains stable). Then, 2.5 ml of $4M$ sodium chloride are added, the solution is diluted to the mark with water, and the absorbance (A_2) is measured. The cadmium content is obtained by subtracting A_2 from A_1. With this modified procedure, up to 10 μg of zinc will not interfere.

Sensitization by analogue derivative spectrophotometry

We have already reported that derivative spectrophotometry by use of an analogue differentiation circuit is extremely effective for increasing the sensitivity.[7,8] As an example, the second derivative spectrophotometric determination of cadmium with T(3-MPy)P is described here.

Selection of conditions for measurement of the second derivative spectrum. The second derivative spectrum of the analyte is recorded and the vertical distance from a peak to a trough or from the base-line to a trough of the spectrum is measured. Since this distance (D) depends on both the time constant of the analogue differentiation curcuit (our apparatus has 6 circuits, with different time constants) and the scanning speed used, these need to be selected to give a well-resolved large peak (to give good selectivity and higher sensitivity in the determination). This is done on the basis of the breadth of the bands in the ordinary absorption spectrum. In general, a large time constant and/or a fast scanning speed should be used for a broad band in the absorption spectrum. In Fig. 7 the second derivative spectra of the Soret band of a cadmium–T(3-MPy)P complex solution measured with varying circuit number or scanning speed are shown; circuit No. 6 and a scanning speed of 150 nm/min are seen to be preferable for the cadmium determination (the circuit numbers increase with increasing time constant).

Table 3. Influence of foreign ions

Ions added	Tolerance limit
Al(III)[a]*, Ca(II)*, Mg(II)*, V(IV)[a]*, V(V)[a]*	1000 μg
Cr(VI)[a], Fe(II)[a], Ni(II)[a]	500 μg
Fe(III)[a]	100 μg
Cu(II)[d]	50 μg
Ag(I), Co(II)[d], Cr(III)[b], Hg(II)[c]	10 μg
Mn(II)[d], Pb(II)[d]	4 μg
Zn(II)	<4 μg
Br^-*, Cl^-*, NO_3^-*, SO_4^{2-}*, tartrate*, citrate*	100 mg
ClO_4^-	50 mg
I^-, SCN^-	10 mg

Cd(II) taken 3.55 μg.
* Maximum tested.
(a) 1 ml of 0.3*M* sodium tartrate added.
(b) 1 ml of 0.3*M* sodium citrate added.
(c) 1 ml of 0.001% triethylenetetramine solution added.
(d) 0.5 ml of 1% dimethylglyoxime solution (in ethanol) added.

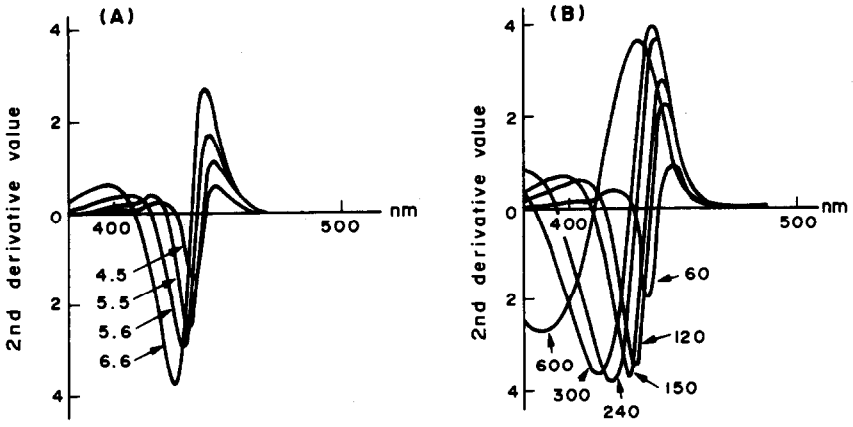

Fig. 7. Influence of (A) circuit number and (B) scan-speed on the second derivative spectrum of the Cd-T(3-MPy)P complex. [Cd(II)] 236 ng/ml; [T(3-MPy)P] $1.2 \times 10^{-6}M$, [NaCl] 0.4*M*, pH 10.5; cells 1 cm, reference water. Numerical values indicate 1st and 2nd differentiation circuit numbers in (A) and scan-speed (nm/min) in (B), respectively. The recorder sensitivity was set so that a derivative value of 1.0 corresponded to 1.0 cm on the chart. In (A) the scan-speed was 150 nm/min; in (B) both circuits were No. 6.

Calibration graphs. Linear plots of *D vs.* weight of cadmium were obtained up to 0.4 μg of cadmium, the equations being

$$\text{Cd} = 400D \text{ ng} \quad (7)$$

for the peak-to-trough measurements, and

$$\text{Cd} = 600D \text{ ng} \quad (8)$$

for the baseline-to-trough measurements, where *D* was the second derivative value obtained with the recorder sensitivity set so that a derivative value of 1.0 gave a signal of 20 cm (*i.e.*, full scale deflection) on the chart. Equation (8), although the sensitivity is lower, is preferable for the analysis of "real" samples because it is less affected by concomitant ions which give T-(3-MPy)P complexes with a Soret band near to that of the cadmium-T(3-MPy)P complex. As a second derivative value of 0.1 corresponds to a signal of 2 cm on the chart, cadmium at the ng/ml level can be readily determined by the proposed method.

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SPECTROPHOTOMETRIC DETERMINATION OF TRACES OF IRON, COPPER, ZINC, ALUMINIUM AND BISMUTH IN LEAD- AND TIN-BASE SOLDERS AND WHITE-METAL BEARING ALLOYS

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Summary—Serious co-precipitation (>60%) of traces of tervalent metal ions has been found to occur in the precipitation of $PbSO_4$. A moderate amount (approximately 11%) of Zn^{2+} and an insignificant amount of Cu^{2+} are also co-precipitated. To deal with this problem, for the determination of Fe (with 1,10-phenanthroline), Al (with oxine–EDTA–KCN), Bi (with diethyldithiocarbamate–EDTA–KCN), and Cu (with 2,2′-biquinoline) in lead- and tin-base solders or white-metal bearing alloys, the $PbSO_4$ is dissolved in tartrate solution. Zinc is determined with dithizone after the $PbSO_4$ has been thoroughly washed with a dilute ammonia solution.

In general, in lead- and tin-base solders and white-metal bearing alloys, tin, antimony and lead are major constituents, with ranges 0.1–99% Pb, 0.5–95% Sn and <20% Sb. Arsenic is a minor constituent (0.01–2%). Iron, copper, zinc, aluminium and bismuth are trace constituents, and are often determined spectrophotometrically, but major interferences come from tin, antimony, lead and arsenic. Tin, antimony and arsenic are usually removed by volatilizing their bromides from perchloric acid or sulphuric acid medium.[1,2] Lead can be separated as lead sulphate[3–5] or chloride[6–8] or masked by complexation with tartrate, citrate or EDTA.[9] Luke,[3] Ota[4] and Karabash *et al.*[5] used sulphuric acid to separate lead, but did not consider co-precipitation of traces of other metals. We have found, however, that there is serious co-precipitation of traces of metals, especially tervalent elements.

In this paper, we report a study on the co-precipitation of iron, aluminium, bismuth and copper by lead sulphate, and suggest rapid and simple spectrophotometric procedures for determination of these elements in lead- and tin-base solders and white-metal bearing alloys, in a single sample solution without separation of lead. We have also studied the elimination of co-precipitation of zinc by lead sulphate, by use of ammonia as a complexing washing agent, thereby obtaining simple spectrophotometric procedures for determining zinc and copper in lead- and tin-base solders and white-metal bearing alloys, in a single sample solution. We have also examined the effect of pH and potassium cyanide on the spectrophotometric determination of aluminium with 8-hydroxyquinoline (oxine).

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EXPERIMENTAL

All chemicals used were analytical reagent grade.

Preparation of sample solutions

(*A*) *For spectrophotometric determination of Fe, Al, Bi, Cu.* Depending on the content of iron, aluminium, bismuth and copper, weigh up to 2 g of sample into a 250-ml beaker. Add 20 ml of hydrobromic acid–bromine mixture (2 ml of bromine and 18 ml of concentrated hydrobromic acid). Cover with a watch-glass and heat gently, avoiding excessive loss of bromine, until dissolution of the sample is complete. When analysing high-tin alloys, it may be necessary to add a few extra drops of bromine to ensure complete dissolution of the sample and oxidation of the tin to the stannic state. When dissolution is complete, add 10 ml of concentrated sulphuric acid and heat under a well-ventilated fume-hood to expel stannic bromide, antimonous bromide and arsenious bromide. Heat until less than 2 ml of sulphuric acid remains. Let cool, then carefully rinse the watch-glass and the wall of the beaker with water. Add 6 g of tartaric acid and neutralize with concentrated ammonia solution until all the lead sulphate has dissolved (pH ~10). Transfer the clear solution to a 100-ml standard flask and dilute to volume. Run a blank through the entire procedure (the "procedure blank"), using the same amounts of all reagents.

(*B*) *For spectrophotometric determination of Zn and Cu.* After dissolving the sample with hydrobromic acid–bromine and volatilizing stannic bromide, antimonous bromide and arsenious bromide from sulphuric acid medium as described above, decant the supernatant liquid through a Whatman No. 40 paper into a 100-ml standard flask. Wash 5–6 times with 0.5*N* sulphuric acid, by decantation. Add 5 ml of ammonia solution (1 + 1) to the beaker, stir vigorously to dissolve co-precipitated zinc and copper(II) by formation of their ammine complexes and again decant the solution into the filter. Repeat the extraction with ammonia, then wash the precipitate with ammonia solution (1 + 20) several times, decanting into the filter each time. Adjust the pH of the filtrate to below 2 and dilute to the mark. Run a "procedure blank", using the same amounts of all reagents.

Determination of iron. Preparation of the calibration graph and the spectrophotometric determination of iron have already been described.[10]

Determination of copper.[11] For the calibration graph transfer 0, 0.50, 1.00, 2.00, 4.00 and 6.00 ml of standard copper solution (10.0 μg/ml) to 50-ml beakers and dilute to 20 ml. Add 1 g of hydroxylamine hydrochloride, 5 ml of 10% tartaric acid solution and 1 g of methenamine. Adjust the pH to approximately 5 with ammonia solution (1 + 1) (pH-meter). Transfer the solution to a separatory funnel. Add 10 ml of 0.02% 2,2'-biquinoline solution in isoamyl alcohol and shake vigorously for 1–2 min. Allow the phases to separate and transfer the alcohol layer to a 1-cm cell through a tuft of glass wool squeezed into the dry stem of the funnel. Measure the absorbance against a reagent blank at 545 nm.

For the determination, apply the same procedure (*A* or *B*) to a 10.00-ml or other suitable aliquot of sample solution and the same sized aliquot of the "procedure blank", prepared as described, and measure the absorbance against that of the "procedure blank".

Determination of zinc.[12,13] For the calibration graph, pipette 0, 2.00, 4.00, 6.00, 8.00 and 10.00 ml of standard zinc solution (1.00 μg/ml), into 60-ml separatory funnels and dilute to 20 ml. Add 10 ml of acetic acid–sodium acetate buffer solution (150 ml of glacial acetic acid and 300 g of anhydrous sodium acetate per litre, pH ~4.8, purified by extraction with dithizone solution in carbon tetrachloride) and 2 ml of 25% sodium thiosulphate solution, then shake vigorously for 2 min with 10.00 ml of 0.001% dithizone solution in carbon tetrachloride. Run the clear carbon tetrachloride extract into a 1-cm cell through a tuft of glass wool squeezed into the dry stem of the funnel. Measure the absorbance against a reagent blank at 535 nm.

For the determination of zinc, take a 10.00-ml or other suitable aliquot of sample solution (*B*) in a 60-ml separatory funnel, add 5 ml of 10% ammonium citrate solution (purified by extraction with dithizone solution in carbon tetrachloride), cool to room temperature, and adjust the pH to approximately 8.5. Add 5 ml of 0.01% dithizone solution in carbon tetrachloride (or 0.05% solution if more copper and other metals reacting with dithizone are present) and shake for 30 sec. If the dithizone solution is green, shake for another 30 sec. Allow the phases to separate and draw off the carbon tetrachloride layer into another separatory funnel. Unless the separated carbon tetrachloride phase is distinctly green, add another 5 ml of dithizone, shake for 30–60 sec, and draw off the organic phase; continue in this manner until the last portion is still green after the shaking. Combine the extracts, and shake for 1 min with 10 ml of 0.02*M* hydrochloric acid. Draw off the carbon tetrachloride into another separatory funnel and shake it vigorously for 1 min with a fresh 10-ml portion of the hydrochloric acid. Combine the two acid extracts, add 2 ml of carbon tetrachloride and draw off to remove any coloured droplets of carbon tetrachloride. Repeat once more with 2 ml of carbon tetrachloride. Transfer the acid solution to a 25-ml standard flask, and make up to the mark. This aqueous solution contains the zinc. The carbon tetrachloride layer contains the copper. Take a 10.00-ml or other suitable fraction of the aqueous extract, and determine the zinc in it by the procedure for the calibration graph, but measure the absorbance against a blank prepared by applying the *entire* procedure to an aliquot of "procedure blank" equal to the aliquot of test solution used.

Determination of aluminium.[14,15] For the calibration graph take 0, 2.00, 4.00, 6.00, 8.00 and 10.00 ml of standard aluminium solution in 0.02*M* hydrochloric acid (10.0 μg/ml) and dilute to 20 ml. Adjust the pH to approximately 10 and add 1 ml of 5% 8-hydroxyquinoline solution in ethanol. Extract with successive 10, 5 and 5-ml portions of chloroform, and dilute the combined extracts to 25.00 ml. Run each extract into a 1-cm cell through a tuft of glass wool squeezed into a dry funnel and measure the absorbance at 395 nm against the reagent blank.

For the determination, to a 10.00-ml or other suitable aliquot of sample solution (*A*) add 1 g of EDTA, and adjust to pH 9.5–10 with ammonia. Add 15 ml of pH 10 ammonia–ammonium chloride buffer solution.[9] Then add 1 g of potassium cyanide and stir to dissolve it. Heat slowly to boiling and boil for 3 min. Cool to room temperature, transfer to a 60-ml separatory funnel and add 1 ml of 5% 8-hydroxyquinoline solution in ethanol. Allow to stand at room temperature for 1 hr. Extract with chloroform and measure the absorbance against that of a blank prepared in the same way from the same size of aliquot of the "procedure blank".

Determination of bismuth.[9] For the calibration graph transfer 0, 1.00, 2.00, 3.00, 4.00, 5.00 and 6.00 ml of standard bismuth solution in 0.17*M* nitric acid (5.00 μg/ml) to 60-ml separatory funnels and dilute to 10 ml with 0.17*M* nitric acid. Add 0.5 g of EDTA and adjust the pH to 8. Then add 0.5 g of potassium cyanide and stir to dissolve it. Finally add 1 ml of 0.2% sodium diethyldithiocarbamate solution. Shake with 10.00 ml of carbon tetrachloride for 1 min. Run the extract into a 1-cm cell through a tuft of glass wool squeezed into the dry stem of the funnel. Measure the absorbance against a reagent blank at 400 nm within 30 min.

For the determination treat a 10.00-ml or other suitable aliquot of sample solution (*A*) as for the calibration graph but measure against a similarly treated "procedure blank" (if the sample contains more copper, add 1 g or more of potassium cyanide).

RESULTS AND DISCUSSION

Selection of high boiling-point acid

When lead- and tin-base solders and white-metal bearing alloys have been dissolved in hydrobromic acid–bromine mixture, the tin, antimony and arsenic are present as stannic bromide (b.p. 202°), antimonous bromide (b.p. 280°) and arsenious bromide (b.p. 221°), which can be volatilized. High boiling-point acids such as sulphuric (b.p. 338°) or perchloric (b.p. 200°) are usually used for this purpose. If perchloric acid is used, a special fume-hood is desirable, but may not be available, so sulphuric acid is commonly used.

Co-precipitation of traces of metals with lead sulphate

Luke[3] studied photometric determination of aluminium in lead, tin- and lead-base solders with aluminon; he suggested that lead should be removed as sulphate, but did not consider co-precipitation of aluminium with lead sulphate. His results showed that the amount of aluminium found was 8–20% lower than the amount taken. Ota[4] studied spectrophotometric determination of iron in tin and lead alloys with 1,10-phenanthroline, and also separated lead as lead sulphate with no consideration of co-precipitation of iron. Karabash *et al.*[5] reported a chemical–spectroscopic method for determining impurities in lead. Lead sulphate was precipitated in hot, dilute nitric acid medium, set aside for 3 hr at room temperature, then separated by decantation and heated with 6*M* nitric acid for 2 hr. They claimed that twenty metals, including iron, aluminium, bismuth, zinc and copper, at concentrations of 10^{-1}–10^{-6}% in the lead, were not co-precipitated with the lead sul-

Table 1. Adsorptive co-precipitation of traces of iron, aluminium, bismuth, zinc and copper on lead sulphate*

	Amount added, *mg*	Amount found, *mg*	Co-precipitated, %	%, average	Spectrophotometric method
	1.00	0.32	68		
Fe	1.00	0.40	60	64	1,10-Phenanthroline
	1.00	0.35	65		
	0.50	0.21	58	60	8-Hydroxyquinoline
Al	0.50	0.19	62		
	1.50	0.60	60	62	Diethyldithiocarbamate
Bi	1.50	0.55	63		
	0.100	0.091	9		
Zn	0.100	0.091	9	11	Dithizone
	0.100	0.086	14		
	0.50	0.51	—		
Cu	0.50	0.49	2	1	2,2'-Biquinoline
	0.50	0.49	2		

* The amount of lead was 1 g. The lead sulphate precipitate was washed more than 10 times with 0.5*N* sulphuric acid, by decantation.

Table 2. Determination of iron by the 1,10-phenanthroline method, with trien as masking agent for copper in sample solution (A)

Sample	NBS values, % Cu	NBS values, % Fe	Fe found, %
NBS 54d tin-base bearing metal	3.62	0.027	0.026 0.026
NBS 53e lead-base bearing metal	0.054	<0.001	0.001 0.0009
NBS 127b solder (Sn40, Pb60)	0.011	—	0.002 0.002

phate, but this claim was in contradiction with the large errors in their results.

In contrast, our results display loss of trace constituents by co-precipitation with a large mass of lead sulphate (Table 1). The higher the charge on the cation, the more serious is the co-precipitation, in accordance with the general adsorption rule. Insignificant amounts of copper(II) and only about 11% of the zinc were co-precipitated, but more than 60% of the tervalent ions, aluminium, bismuth and iron (Table 1).

Determination of Fe, Al, Bi and Cu in sample solution (A)

To eliminate co-precipitation of iron, aluminium and bismuth, the lead sulphate precipitate was dissolved with tartaric acid as complexing agent at pH 10. Determination of iron, aluminium, bismuth and copper according to the procedures given above gave values (Tables 2–5) in good agreement with the certified values or the amounts added.

Table 3. Determination of bismuth in sample solution (A) by the diethyldithiocarbamate method

Sample	NBS value or Bi added, %	Bi found, %
NBS 127b solder (Sn40, Pb60)	0.06	0.061 0.062
NBS 54d tin-base bearing metal	0.044	0.046 0.045
NBS 53e lead-base bearing metal	0.052	0.053 0.052
Synthetic sample (large mass of Pb and traces of Bi, Fe, Cu, Al, Zn)	0.144 0.144	0.145 0.146

Table 4. Determination of copper in sample solution (A) by the 2,2'-biquinoline method

Sample	NBS value or Cu added, %	Cu found, %
NBS 127b solder (Sn40, Pb60)	0.011	0.011 0.011
NBS 54d tin-base bearing metal	3.62	3.59 3.65
NBS 53e lead-base bearing metal	0.054	0.053 0.053
Synthetic sample (large mass of Pb and traces of Cu, Fe, Al, Bi, Zn	0.050 0.050	0.049 0.050

Table 5. Determination of aluminium in sample solution (A) by the 8-hydroxyquinoline method

Sample	NBS value or Al added, %	Al found, %
Synthetic sample	0.050	0.051
(large mass of Pb	0.050	0.048
and traces of Al,	0.050	0.052
Fe, Cu, Zn, Bi)	0.050	0.048
NBS 127b solder	—	0.0083
(Sn40, Pb60)		0.0086
NBS 54d tin-base	—	0.0042
bearing metal		0.0043
NBS 53e lead-base	—	0.013
bearing metal		0.014

Elimination of co-precipitation of zinc

Sample solution (A) is unsuitable for determination of zinc by the dithizone method, because of the competitive reaction of lead, which is present as a major constituent. The lead must be separated first, and if this is done by precipitation as the sulphate, even though the amount of zinc co-precipitated is much smaller than that of iron(III), aluminium and bismuth (Table 1), it still cannot be tolerated. To recover co-precipitated zinc from the lead sulphate, after its separation and washing with 0.5*N* sulphuric acid by decantation the precipitate is again washed with ammonia solution to remove the zinc as the $Zn(NH_3)_4^{2+}$. The data in Table 6 confirm the effectiveness of this method. Hence sample solution (B) can be used for determination of zinc by the dithizone method. Because any co-precipitated copper will also be removed as the ammine complex, $Cu(NH_3)_4^{2+}$, sample solution (B) can be used for determination of both zinc and copper (with different aliquots).

Effect of pH on determination of aluminium with 8-hydroxyquinoline

Gentry and Sherrington[16] reported that extraction of aluminium with 1% 8-hydroxyquinoline solution in chloroform is complete in the pH range 8–11.5; however, our results (Table 7) for a synthetic sample containing trace aluminium, a minor amount of iron, and EDTA and potassium cyanide showed that extraction was incomplete at pH less than 9 and higher than 12, but almost complete in the pH range of about 9.5–11 (controlled with ammonia–ammonium chloride buffer solution) when 8-hydroxyquinoline was added to the aqueous solution after adjustment of the pH. This effect is presumably a consequence of complexation with EDTA (which is strongest at pH 5.0) at pH <9.5, and co-precipitation at pH >12.0 on the ferric hydroxide produced on decomposition of the

Table 6. Determination of zinc in sample solution (B) by the dithizone method

Sample	Use of NH_3 as complexing washing agent	NBS value or Zn added, %	Zn found, %
Synthetic sample (1 g of		0.0100	0.0091
Pb and traces of Zn,	No	0.0100	0.0091
Fe, Cu, Al, Bi)		0.0100	0.0086
	Yes	0.0100	0.0098
		0.0100	0.0098
NBS 127b solder	Yes	—	0.0006
(Sn40, Pb60)			0.0006
NBS 54d tin-base	Yes	—	0.0035
bearing metal			0.0034
NBS 53e lead-base	Yes	—	0.0016
bearing metal			0.0015

Table 7. Effect of pH on spectrophotometric determination of aluminium by the 8-hydroxyquinoline method

pH	Iron added, *mg*	Aluminium added, *mg*	Aluminium found, *mg*	Relative error, %
8.5	0.20	0.050	0.004	−92
9.0	0.20	0.050	0.036	−28
9.5	0.20	0.050	0.049	−2
10	0.20	0.050	0.049	−2
11	0.20	0.050	0.049	−2
12	0.20	0.050	0.013	−74
13	0.20	0.050	0.001	−98

Table 8. Effect of amount of potassium cyanide on spectrophotometric determination of aluminium by the 8-hydroxyquinoline method

Sample	Aluminium added, *mg*	Iron added, *mg*	Potassium cyanide added, *mg*	pH of aqueous solution	Colour of chloroform layer	Aluminium found, *mg*	Relative error, %
Synthetic sample I	0.050	0.10	10	8–8.5	Yellow–black	0.059	+18
(large mass of Pb	0.050	0.10	10	8–8.5	Yellow–black	0.060	+20
and traces of Al, Fe, Bi, Cu, Zn)							
Synthetic sample II	0.050	0.20	50	8–8.5	Yellow–black	0.058	+16
(traces of	0.050*	0.20	50	8–8.5	Yellow–black	0.023†	—
Al and minor Fe)	0.050*	0.20	50	8–8.5	Yellow–black	0.030†	—
	0.050	0.20	1000	8.5	Very pale yellow	0.004	−92
	0.050	0.20	1000	10	Yellow	0.049	−2
	0.050	0.20	3000	12	Pale yellow	0.013	−74

* Approximately 100 mg of ammonium fluoride added before 8-hydroxyquinoline solution.
† Amount of aluminium equivalent to iron blank.

Fe–EDTA complex by boiling at high pH. Thus, as shown by Table 7, the optimum pH for determining aluminium by the 8-hydroxyquinoline method by the procedure given is about 10.

Elimination of interference of iron in determination of aluminium

Iron(III) reacts with 8-hydroxyquinoline to form the green-black ferric 8-hydroxyquinolate even in the presence of EDTA. To eliminate the resulting iron interference in the determination of aluminium, Pigott[15] and Claassen *et al.*[14] suggested masking small amounts of iron (<50 mg) by adding 1 g of EDTA and 3 g of potassium cyanide (*i.e.*, at least 8 times as much cyanide as needed to react with the amount of iron in the sample taken), and gently boiling the solution (pH >8) for 15–20 min; the ferricyanide formed was said to be reduced to non-interfering ferrocyanide. Our results show that besides pH, the amount of potassium cyanide is also important (Table 8). At the iron level examined, even 50 mg of potassium cyanide proved insufficient to mask 0.1 mg of iron(III), and positive errors were obtained for aluminium. An attempt was made to apply a correction for the iron by masking the aluminium with fluoride, but the correction was not reproducible, owing to either variation in the amount of iron(III) complexed or incomplete masking of the aluminium (or both). Addition of a large amount (3 g) of potassium cyanide caused the Fe–EDTA complex to decompose and yield ferric hydroxide during heating, resulting in adsorptive co-precipitation of aluminium by the ferric hydroxide. Addition of 1 g of potassium cyanide was optimum, at pH 10 (ammonia–ammonium chloride buffer), giving complete masking of the iron and avoiding its hydrolysis.

Pigott[15] assumed that on boiling, the hydroxide ions from hydrolysis of the cyanide reduced the ferricyanide to ferrocyanide:

$$2Fe(CN)_6^{3-} + 2OH^- \rightleftharpoons 2Fe(CN)_6^{4-} + H_2O_2$$

However this explanation seems untenable in view of the redox potentials, and also because ferricyanide is more stable than ferrocyanide and so should not interfere anyway. Moreover, we made direct tests for ferrocyanide and found that practically none was produced even with boiling for 20 min.

Effect of amount of hydroxylamine hydrochloride on development of the copper(I) 2,2′-biquinoline complex

Our results show that even 0.5 g of hydroxylamine hydrochloride was not enough for complete reduction of copper(II) and colour development in sample solutions, even though ample for traces of copper in pure solution. When 1 g of hydroxylamine hydrochloride was added, complete reduction and colour development were obtained. The most likely explanation is formation of an inert tartrate-bridged copper(II)–tartrate–aluminium(III) complex,[17] or an analogous complex with iron(III).

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PERFORMANCE OF THE ORION 97-70 TOTAL RESIDUAL CHLORINE ELECTRODE AT LOW CONCENTRATIONS AND ITS APPLICATION TO THE ANALYSIS OF COOLING WATERS

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Summary—The performance characteristics of the Orion 97-70 total residual chlorine electrode have been determined and the electrode has been found to give a near-theoretical response down to chlorine concentrations in the range 1–5 μg/l. Within-batch relative standard deviations are about 6 8% at concentrations above 50 μg/l. and 10–15% at lower levels. The method is virtually free from interferences (only strong oxidizing agents such as permanganate interfere) but large variations in salinity affect the calibration by changing the conditional standard potential. The best performance at low concentrations (<50 μg/l.) is achieved only if the manufacturer's recommended procedure is changed, namely by using a more dilute iodide reagent, stirring constantly, adding the iodide reagent before the buffer solution and using chloramine-T as a standard.

Residual chlorine concentrations are measured by the Central Electricity Generating Board so that the chlorination of saline cooling water can be controlled, to prevent mussel settlement in the culverts leading to the condensers. Cooling waters at inland power stations are chlorinated intermittently to prevent the formation of bacterial slimes on the condensers themselves. A recent EEC directive[1] setting a limit of 5 μg/l. for chlorine in fresh waters "needing protection or improvement in order to support fish life" makes it prudent to be able to measure at this level in cooling tower discharges, although there is at present no obligation to make such measurements.

Methods for determining residual chlorine have been reviewed by Midgley.[2] The most commonly used method is colorimetry with *N*,*N*-diethyl-*p*-phenylenediamine (DPD) as reagent,[3] but this is insufficiently sensitive for low chlorine levels in discharges. Amperometric titration has adequate sensitivity, but is time-consuming, requires expensive apparatus and is not capable of continuous operation.

A potentiometric method using the Orion 97-70 residual chlorine electrode has shown considerable sensitivity in synthetic fresh-water solutions,[4] but the apparatus would be unsuitable for analysis *in situ*. Since chlorinated waters are chemically reactive, samples sent to the laboratory for analysis will often give low results because of decay of the residual chlorine. Jenkins and Baird[5] determined residual chlorine at high levels in waste-water, and Scarano and Saroglia[6] have measured chlorine in sea-water down to about 10 μg/l. This paper describes a study of the use of the Orion 97-70 electrode for measurements at low levels of total residual chlorine in both fresh and saline natural waters used for industrial cooling. The factors limiting the performance of the electrode are investigated and the dependence of the e.m.f. on the iodide concentration is demonstrated both theoretically and experimentally.

THEORY

Electrode potential

The total residual chlorine electrode consists of a platinum electrode and a silver iodide membrane electrode mounted on a common stem. Iodide is added to the sample solution to cause the reduction of all forms of residual chlorine, including chloramines, bromine, bromamines, *etc*., with the formation of iodine.

$$\text{oxidant} + 2I^- \rightarrow I_2 + \text{reduced oxidant}$$

The cell potential is given by

$$\begin{aligned} E &= E_{Pt} - E_{AgI} \\ &= E^0_{Pt} + \frac{2.3RT}{2F}\log\frac{[I_2]}{[I^-]^2} - E^0_{AgI} \\ &\quad + \frac{2.3RT}{F}\log[I^-] \\ &= E^0 + \frac{2.3RT}{2F}\log[I_2] \end{aligned} \qquad (1)$$

Since the iodide terms from the two electrode potentials cancel, leaving iodine as the only concentration variable, the neglect of activity coefficients in this treatment is justified. Equation (1) implies that the

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potential is independent of iodide concentration (provided that enough is added to reduce all the residual chlorine), but this is misleading because of the chemical equilibria discussed below.

Electrode calibration

The equation for the electrode calibration graph is of the form

$$E \simeq \text{constant} + k \log [\text{TRC}] \quad (2)$$

where [TRC] is the total residual chlorine concentration.

Equations (1) and (2) are related through

$$[I_2]/[\text{TRC}] = \alpha \quad (3)$$

$$k \simeq \frac{2.3RT}{2F} \quad (4)$$

$$E^0_{Pt} - E^0_{AgI} + k \log \alpha \simeq \text{constant} \quad (5)$$

Hence

$$E \simeq E^0 + k \log \alpha + k \log [\text{TRC}) \quad (6)$$

Although α depends on the total residual chlorine concentration and on the iodide concentration, the experimental conditions can usually be adjusted to make it virtually constant. In such circumstances, equations (5) and (6) are valid within the limits of experimental error.

Iodine complexes

Iodine forms complexes with halide ions in solution.[7]

$$I_2 + I^- \rightleftharpoons I_3^-; \quad \beta_1 = 724 \quad (7)$$

$$I_2 + Br^- \rightleftharpoons I_2Br^-; \quad \beta_2 = 15 \quad (8)$$

$$I_2 + Cl^- \rightleftharpoons I_2Cl^-; \quad \beta_3 = 1.7 \quad (9)$$

Thus the total residual chlorine concentration is

$$[\text{TRC}] = [I_2] + [I_3^-] + [I_2Br^-] + [I_2Cl^-] = [I_2](1 + \beta_1[I^-] + \beta_2[Br^-] + \beta_3[Cl^-]) \quad (10)$$

In fresh waters the concentrations of bromide and chloride are negligible compared with that of the iodide added during analysis and equation (10) can be simplified to

$$[\text{TRC}] \simeq [I_2](1 + \beta_1[I^-]) \quad (11)$$

From equations (3) and (11)

$$\alpha \simeq 1/(1 + \beta_1[I^-]) \approx 1/\{1 + \beta_1(T_A - 3[\text{TRC}])\} \quad (12)$$

where T_A is the total concentration of iodide added. Substituting for α in equation (6) gives

$$E \simeq E^0 - k \log\{1 + \beta_1(T_A - 3[\text{TRC}])\} + k \log [\text{TRC}] \quad (13)$$

Equation (12) shows the dependence of α on the total residual chlorine concentration. If, however, $\beta_1 T_A \ll 1$ or $T_A \gg 3[\text{TRC}]$, α is effectively constant, enabling a near-theoretical response to be obtained from the electrode.

In estuarine and marine waters, where the bromide and chloride concentrations cannot be neglected as above, we have from equations (3) and (10)

$$\alpha = 1/(1 + \beta_1[I^-] + \beta_2[Br^-] + \beta_3[Cl^-]) \quad (14)$$

At the normal level of chlorination of sea-waters, $[\text{TRC}] \ll [Br^-] \ll [Cl^-]$ and therefore, $[Br^-] \simeq T_B$ and $[Cl^-] \simeq T_C$ where T_B and T_C are the total concentrations of bromide and chloride respectively. Moreover, the ratio $\gamma = T_B/T_C$ is virtually constant. Hence we can write

$$\beta_2[Br^-] + \beta_3[Cl^-] = [Cl^-](\beta_3 + \gamma\beta_2) \simeq \beta'_3 T_C$$

where $\beta'_3 = \beta_3(1 + \gamma\beta_2/\beta_3)$. Substituting in equation (14) gives

$$\alpha \simeq 1/(1 + \beta_1[I^-] + \beta'_3 T_C)$$

In practice, however, $\beta_1[I^-] \ll \beta'_3 T_C$ and $\alpha \simeq 1/(1 + \beta'_3 T_C)$. Substituting for α in equation (6) gives

$$E \simeq E_0 - k \log(1 + \beta'_3 T_C) + k \log[\text{TRC}] \quad (15)$$

Equation (15) shows that increasing salinity (or T_C) causes a reduction in e.m.f. and the electrode apparently indicates a lower total residual chlorine concentration than expected from a calibration in demineralized water. The calibration slope at different salinities will, however, be unchanged.

In brackish water the condition $\beta_1[I^-] \ll \beta'_3 T_C$ may not be met and equation (15) may not be a good approximation. The trend in e.m.f. produced by increasing salinity will, however, be the same.

EXPERIMENTAL

Apparatus

Orion 97-70 total residual chlorine electrodes (MSE Scientific Instruments, Manor Royal, Crawley, England) were used for all experiments. Measurements in the laboratory were made with a Corning 110 digital pH-meter reading to 0.1 mV. Measurements in the field were made with a Corning 610 portable analogue pH-meter (smallest division 1 mV on expanded scale). Colorimetric measurements were made with a Lovibond 1000 Comparator (The Tintometer Ltd., Salisbury) and spectrophotometric measurements with a Pye–Unicam SP6-500 spectrophotometer. Solutions were stirred with a magnetic stirrer in the laboratory and a battery-powered LPL Vibro-agitator in the field.

Reagents

Distilled water was continuously circulated through a nuclear-grade mixed-bed ion-exchange column (Elga Spectrum). The chlorine demand of this water was in the range 0.5–5 μg/l., and usually less than 2 μg/l.

Acetate buffer solution, pH 4, was prepared by dissolving 243 g of sodium acetate trihydrate and 480 g of acetic acid in water and diluting to 1 litre; 1 ml of this solution was added per 100 ml of sample.

The iodide reagent solutions were 0.5, 0.125, 0.025 or 0.005*M* potassium iodide, and usually contained sodium hydroxide (1 g/l.) but in the preliminary investigations this was sometimes omitted or replaced by sodium hydrogen carbonate (2.1 g/l.). Chlorine standards were prepared (*a*) by adding small volumes of potassium iodate solution (1.006 g/l. ≡ 1 g/l. of Cl_2) to a mixture of 1 ml each of buffer and iodide reagent solutions, stirring for 2 min and diluting to 102 ml, (*b*) from sodium hypochlorite solution (B.D.H. laboratory reagent) standardized iodometrically, and (*c*) from chloramine-T (sodium salt of *p*-toluenesulphochloramide trihydrate, "Baker Analyzed" reagent).

Unless otherwise specified, analytical-reagent grade materials were used.

Procedure

To 100 ml of standard or sample solution 1 ml of iodide reagent solution and 1 ml of acetate buffer solution were added in that order. The electrode was rinsed and then immersed in the solution. Use of the 0.025*M* potassium iodide reagent is referred to below as the "high-level" procedure for the chlorine range 50–1000 μg/l. and that of the 0.005*M* reagent as the "low-level" procedure for the chlorine range 1–100 μg/l. The solutions were stirred gently throughout.

In the known-addition experiments the initial chlorine concentration was approximately doubled in the addition step.

Any variations from the procedures described above are detailed, as they occurred, in the next section.

PRELIMINARY INVESTIGATIONS

Calibration

The calibration graph of e.m.f. against the logarithm of the chlorine concentration was linear only over the range 0.2–10 mg/l. when the reagents supplied with the electrode were used with iodate standards according to the manufacturer's instructions.[8]

With attention to the factors discussed below, a linear calibration graph could readily be obtained for concentrations down to 50 μg/l. and with care the range could be extended down to 1 μg/l. The age, pH and concentration of the iodide reagent and the use of chloramine-T standards were the most important factors in this improvement in performance, and the final procedure differed in several ways from the Orion method.

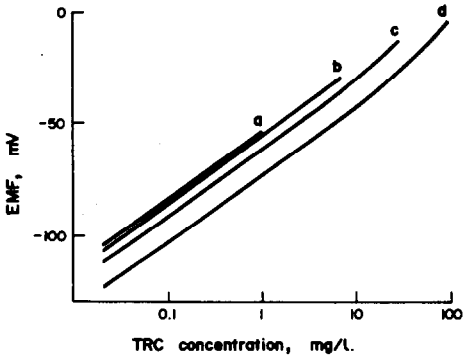

Fig. 1. Calculated effect of variation of the concentration of iodide reagent on calibration; 1 ml of reagent solution added per 100 ml of chlorine solution. Reagent concentration (*M*): (a) 0.005; (b) 0.025; (c) 0.125; (d) 0.50.

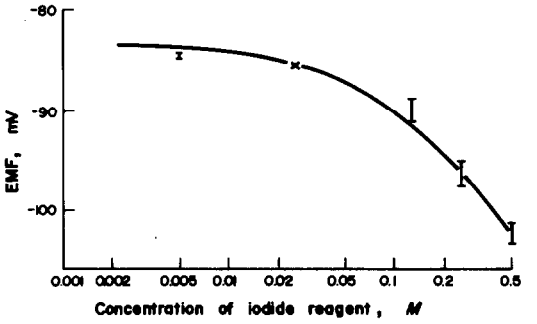

Fig. 2. Effect of variation of iodide concentration at constant total residual chlorine concentration (1 ml of reagent solution added per 100 ml of chlorine solution). Full line is response calculated relative to 0.025*M* KI as reference (×). Vertical error bars show observed ranges.

Iodide reagent concentration

In accordance with equation (13), increasing the iodide concentration produces a more negative e.m.f., corresponding to a lower concentration of free iodine, $[I_2]$. Calculated calibration curves for different reagent concentrations are shown in Fig. 1.

At TRC concentrations below about 1 mg/l. the e.m.f. is linearly related to log[TRC] and there is no theoretical reason why the calibrations should not be extrapolated downwards indefinitely. At higher concentrations deviations from linearity occur, because there is no longer a sufficient excess of iodide to keep the ratio $[I_2]/[I_3^-]$ virtually constant, so the proportion of molecular iodine increases. Eventually there is insufficient iodide to reduce all the residual chlorine; the upper extremities of the curves in Fig. 1 mark (approximately) these limiting TRC concentrations.

At a constant chlorine concentration, the rate of change of e.m.f. with iodide concentration increases as the iodide concentration increases, as shown in Fig. 2. In consequence, if the random errors in preparing and dispensing the iodide reagent are practically constant in absolute magnitude, there will be larger variations in e.m.f. and hence larger analytical errors, with the concentrated (0.5*M*) reagent recommended by Orion than with the more dilute reagents favoured in this work.

In practice, deviations from linearity were observed at low TRC concentrations (Fig. 3) where they would not be expected from the theory. These deviations increased with the concentration of iodide added, and for measurements in the range applicable to power station cooling waters (<1 mg/l.), the lower reagent strengths would be preferred. The deviations at low TRC concentrations were attributed to the presence of iodine in the reagent from oxidation of iodide by atmospheric oxygen. On standing, the reagent solutions developed the yellow colour typical of iodine; in the case of the solutions without added base, this occurred within a few hours, but for solutions containing sodium hydrogen carbonate several days were required. Solutions with sodium hydroxide remained colourless for even longer periods.

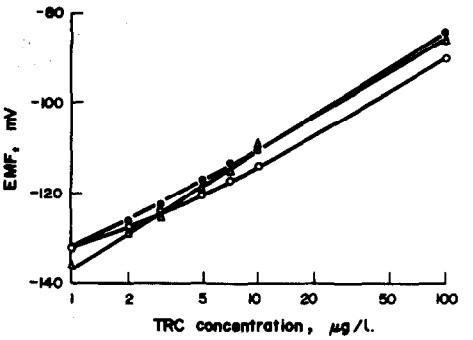

Fig. 3. Observed effect of variation of iodide reagent concentration on calibration; 1 ml of reagent solution added per 100 ml of chlorine solution. [KI]: ○ 0.125*M*; ● 0.025*M*; △ 0.005*M*.

The deviations from the extrapolated linear responses in Fig. 3 indicated that 0.0017% of the iodide in the freshly-prepared 0.5*M* solution (with added sodium hydroxide) had been oxidized to iodine. Further investigations into reagent oxidation are described below.

Age and pH of iodide solutions

Calibration with the 0.5*M* potassium iodide reagent prepared in different background media and stored in various ways was repeated over a period of time. Solutions were prepared in demineralized water, 0.025*M* sodium hydrogen carbonate, and 0.025*M* sodium hydroxide. Solutions were stored in both full and half-full bottles, and in the dark and in the light.

Potassium iodide solution was added (0.25 ml per 100 ml of standard solution of chlorine concentration 2000, 200, 50 and 20 µg/l.) and the e.m.f. values were recorded. The test was repeated at weekly intervals for four weeks. During this time, all the solutions except those containing sodium hydroxide developed the yellow iodine colour. The most negative e.m.f. values, *i.e.*, those indicating least iodine, were always obtained with solutions containing sodium hydroxide and the least negative always from solutions with no base added. The results were the same whether the solutions were stored in light or in the dark, and whether the bottles were full and unopened before use or half-full and opened several times over the period of the test. These results imply that high pH of the potassium iodide solution is the most important factor in minimizing oxidation to iodine, but although the trend in change in e.m.f. was always the same ($NaOH < NaHCO_3 <$ no base) over the four-week period, the differences were small and did not exceed $2\sqrt{2}\sigma_w$, where σ_w is the within-batch standard deviation. Over a period of four weeks from preparation, therefore, the method of preparation itself is not very critical for measurements at chlorine levels above about 20 µg/l., although a solution containing sodium hydroxide is to be preferred and such solutions were used for all further tests.

Twelve weeks after preparation of the solutions, the tests were repeated. Solutions containing sodium hydroxide showed no deterioration in performance but the other solutions (stored in the light) gave apparently lower sensitivities (21–23 mV per decade instead of 26–27 mV per decade) over the chlorine range 20–200 µg/l. The solutions containing sodium hydrogen carbonate and those with no added base that had been stored in the dark deteriorated less than those stored in the light (sensitivity ~25 mV per decade). For solutions containing sodium hydroxide, storage in the dark made no difference, and the sensitivity remained at about 27 mV per decade for the chlorine range 20–200 µg/l.

The reagent solutions supplied with the electrodes had a pH of 7.1 and were deep yellow. Their ages were unknown. With 1 ml of these solutions added per 100 ml of sample, the calibration started to deviate from linearity at about 200 µg/l., which is inconveniently high for the analysis of cooling water. Fresh solutions made 0.025*M* in sodium hydroxide were used for all further tests.

Standard chlorine solutions

Potassium iodate is a primary standard material and is recommended by Orion for calibration of the electrode. In this work, potassium iodate was found to be suitable for calibration at TRC levels down to 10 µg/l. when 0.025*M* potassium iodide reagent was used, but at lower concentrations of iodate or iodide, the contact time of 2 min recommended by Orion was insufficient to allow full conversion of iodate into iodine. Rigdon *et al.*[4] came to similar conclusions, although their concentration limits were different.

Sodium hypochlorite standards allowed calibrations to be made down to 1 µg/l. and reaction was complete even with the most dilute (0.005*M*) iodide reagent tested. The hypochlorite solution, however, is unstable and itself needs standardizing iodometrically by titration with thiosulphate.

Chloramine-T solutions allowed calibration down to 1 µg/l. with 0.005*M* iodide reagent. At higher levels the calibration coincided with that obtained with potassium iodate. Rigdon *et al.*[4] also found chloramine-T to be the most useful standard and chloramine-T solutions were used for almost all further work. The chloramine-T stock solution (chlorine equivalent 1000 mg/l.) was found to be stable for at least 4 months.

Stirring

The manufacturers recommend measurement without stirring of the test solution, but this was found to be unsuitable for measurements at levels below about 50 µg/l. At concentrations below 50 µg/l. the e.m.f. was too low (compared with the extrapolated Nernstian response) and drifted unsteadily to higher values without reaching equilibrium in 10 min. Without stirring there was no discrimination between concentrations of less than 10 µg/l. With stirring, however, steady e.m.f. values could be obtained at concentrations down to 1 µg/l., in accordance with the Nernstian

Table 1. Precision of measurements in fresh waters by the high-level procedure (up to 1 mg/l. TRC, 0.025*M* potassium iodide reagent)

Concentration, μg/l.	Mean e.m.f., *mV*	Relative standard deviations in concentration, %		
		Within-batch	Between-batch*†	Total†
1000	−55.4	5.7	9.5	11.2
500	−64.0	6.3	N.S.	6.3
200	−75.1	4.6	N.S.	7.3
100	−83.2	7.2	N.S.	10.3
50	−91.8	7.3	N.S.	8.7
20	−102.1	7.2	N.S.	10.4

* Between-batch standard deviations were not significant (N.S.) at the $P = 0.05$ level, except for the result at 1000 μg/l.

† Calculated from the raw data for the 1000-μg/l. solution. Other within-batch and total standard deviations calculated from normalized data.

Table 2. Precision of measurements in fresh waters by the low-level procedure (1–100 μg/l. TRC, 0.005*M* potassium iodide reagent)

Concentration, μg/l.	Mean e.m.f., *mV*	Relative standard deviations in concentration, %		
		Within-batch	Between-batch*†	Total†
50	−94.9	8.9	N.S.	10.9
20	−106.0	10.5	N.S.	11.6
10	−114.8	15.5	N.S.	15.5
5	−123.4	13.4	N.S.	19.7
2	−134.6	20.4	N.S.	22.2
1	−144.1	89.8	N.S.	110.3

* N.S. = not significant at the $P = 0.05$ level.

† Calculated from raw data for the 50-μg/l. solution, other between-batch and total standard deviations from normalized data.

response at higher concentrations. Gentle stirring was used in all the other experiments reported in this work.

PERFORMANCE CHARACTERISTICS

Calibration

The e.m.f. was linearly related to the logarithm of the total residual chlorine concentration over the range tested (1 μg/l.–1 mg/l.), provided that the necessary precautions were taken in the preparation of the iodide solution. The slope was 95–98% of the theoretical (Nernstian) value of 29.58 mV per decade at 25°.

Precision

Direct potentiometry. The precision of measurements in fresh waters was estimated by analysing solutions of chloramine-T in demineralized water according to both the high- and low-level variants of the procedure. Duplicate measurements were made at each concentration in each batch, and six batches of measurements were made for each procedure, spread over three or four days. The results are given in Tables 1 and 2. The between-batch and total standard deviations were obtained after normalizing the results with respect to the mean e.m.f. for the most concentrated solution in each batch. The raw data for the most concentrated solutions were processed directly. A similar, but less extensive, series of tests was run with saline samples (0.6*M* sodium chloride in demineralized water). The results are shown in Tables 3 and 4.

The between-batch standard deviations of the normalized data were non-significant, with one possibly significant exception (1 μg/l. in saline water, Table 4). Such an absence of variation indicates that the slope factor is constant (within the range tested) over the period tested.

The between-batch standard deviations of the raw data were also non-significant with one possibly significant exception (1000 μg/l. in fresh water, Table 1), indicating that the standard potential did not vary significantly over the period of the test.

Known-addition potentiometry. Solutions of residual chlorine in demineralized water were analysed with five measurements made at each level. The standard deviations at concentrations of 150, 50 and 10 μg/l. were 9.5, 6.9 and 2.1 μg/l. respectively.

Effect of temperature

Calibrations were carried out according to the high- and low-level procedures at temperatures of 15, 25 and 35°, with the results shown in Fig. 4. With the

Table 3. Precision of measurements in saline water by the high-level procedure (0.025*M* potassium iodide)

Concentration, μg/l.	Mean e.m.f., *mV*	Relative standard deviations in concentration, %		
		Within-batch	Between-batch*†	Total†
500	−71.7	8.4	N.S.	8.4
200	−82.2	9.5	N.S.	9.5
50	−98.4	7.1	N.S.	7.5
20	−109.3	7.1	N.S.	12.1

* N.S. = not significant at the $P = 0.05$ level.
† Calculated from raw data for the 500-μg/l. solution, otherwise from normalized data.

Table 4. Precision of measurements in saline water by the low-level procedure (0.005*M* potassium iodide)

Concentration, μg/l.	Mean e.m.f., *mV*	Relative standard deviations in concentration, %		
		Within-batch	Between-batch*†	Total†
20	−106.8	11.9	N.S.	14.1
5	−125.2	15.0	N.S.	16.3
2	−134.9	22.2	N.S.	43.6
1	−144.0	13.9	24.9	29.3

* Between-batch standard deviations were non-significant (N.S.) at the $P = 0.05$ level except for that at 1 μg/l.
† Calculated from raw data for 20-μg/l. solution, otherwise from normalized data.

high-level procedure, the standard cell potential shifted by 0.6–0.7 mV/deg and the calibration slope changed in proportion to the absolute temperature (as would be expected for a Nernstian slope).

The low-level calibration shows a steeper slope than expected from theory, especially at the higher temperatures; this was thought to be caused by consumption of the residual chlorine by organic matter in the water used to prepare the solutions.

Response time

The response of the electrode was generally complete in less than 15 sec for increases in concentration and 30 sec for decreases in concentration. At concentrations below 10 μg/l., the response was apparently slower, but at such concentrations it is possible that the signal changed slowly because of concentration changes arising from the chlorine demand of the water. The response times quoted are for a rinsed electrode newly immersed in a fresh sample solution to which the reagents have already been added.

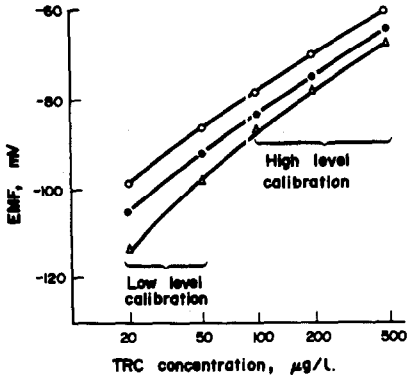

Fig. 4. Effect of temperature: ○ 15°; ● 25°; △ 35°.

Interferences

Interference tests were carried out at four levels of total residual chlorine, with permanganate, iron(III) and (the common contaminants occurring at highest concentration) calcium and magnesium. The effect of large concentrations of chloride is considered below under the heading of salinity changes and the effect of pH is also considered separately.

The results in Table 5 show that permanganate gave a positive interference. Although the metal ions caused consistently low readings, none of the differences exceeded two standard deviations and no significance can be attached to them. The following were also tested and shown to have no significant effect at the 1-mg/l. chlorine level: BrO_3^- (10 mg/l.); $Cr_2O_7^{2-}$ (1 mg/l.); Cu^{2+} (10 mg/l.); Pb^{2+} (1 mg/l.); Br^- (10 mg/l.); EDTA (10 mg/l.); NTA (10 mg/l.).

In a further test, the possibility of interference by iodate in sea-water was investigated. Solutions containing 0.6 mole of sodium chloride and 50 μg of chlorine per litre were analysed in the presence and absence of iodate (60 μg/l.). The readings with iodate present were about 6% high, but this was less than one relative standard deviation.

Table 5. Interferences

Interferent	Concentration, mg/l.	Change in TRC reading, μg/l. 10*	50*	100†	1000†
MnO_4^-	0.05	+56	+58	+60	+60
	0.01	+10	+10	+8.6	+25
Fe^{3+}	100	−0.85	−6.4	−3.3	−139
	20	−0.9	−5.0	−3.3	−56
$Ca^{2+} + Mg^{2+}$	100 + 100	−0.8	−3.9	−3.3	−16

* TRC concentration, μg/l., analysed by low-level procedure.
† TRC concentration, μg/l., analysed by high-level procedure.

Table 6. Effect of salinity

	Change in e.m.f.* compared with value in demineralized water, mV		
NaCl concentration, M	0.1	0.3	0.6
Calculated with $\beta_1 = 3.6$	−7.4	−17.9	−28.3
Calculated with $\beta_1 = 1.7$	−1.7	−4.6	−8.0
Observed†	−2.0 ± 0.15	−4.3 ± 0.3	−6.6 ± 0.2

* Using high-level procedure (0.025M KI)
† Mean of 6 determinations in range 20–1000 μg/l.

Salinity changes

The high- and low-level procedures were carried out in the presence of 0.0, 0.1, 0.3 and 0.6*M* sodium chloride, and the results are shown in Fig. 5. In both cases the e.m.f. values become more negative as the chloride concentration increases, but the calibration slopes stay the same. The shifts are caused by the formation of the I_2Cl^- complex, which diminishes the concentration of free iodine, I_2, and hence, from equation (1), the e.m.f. becomes more negative. Values of the stability constant, β_1, given in the literature[7] are grouped around two values, 3.6 and 1.7. Calculation of the shifts in e.m.f. with salinity gave the results in Table 6. The observed results are in good agreement with the calculated effect for the smaller stability constant of 1.7.

The known-addition method is not significantly affected by changes in salinity.

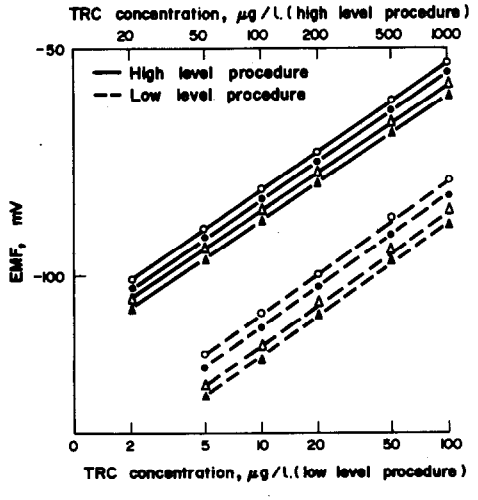

Fig. 5. Effect of salinity. [NaCl]: ○ nil; ● 0.1*M*; △ 0.3*M*; ▲ 0.6*M*.

Effect of pH

If the acetate buffer added to the samples fails to reduce the pH to about 4, the reaction between iodide and residual chlorine will proceed slowly and may not reach completion, *i.e.*, less than the stoichiometric amount of iodine will be formed and the electrode will indicate a residual chlorine lower than the true value. Figure 6 shows how much the calibration deviates from the Nernstian value even at pH 5. The pH affects the reaction and not the electrode itself: varying the pH of a solution after it has been allowed to react at pH 4 changes the e.m.f. by about 1 mV over the pH range 3–6.

For most natural waters, the addition of 1 ml of the acetate buffer solution should be adequate for adjustment of the pH to about 4. Before the technique is applied to highly alkaline waters, however, the effectiveness of the pH adjustment should be tested and more buffer used if necessary.

Effect of light

The effect of light on the e.m.f. was tested at 20 and 50 μg/l. by the low-level procedure and at 50 and 200 μg/l. by the high-level procedure. The maximum difference observed for any solution was 1.5 mV between dark and daylight conditions. The e.m.f. values in tungsten-lamp and fluorescent light were intermediate between the two extremes. The e.m.f. changes were reversible and independent of concentration.

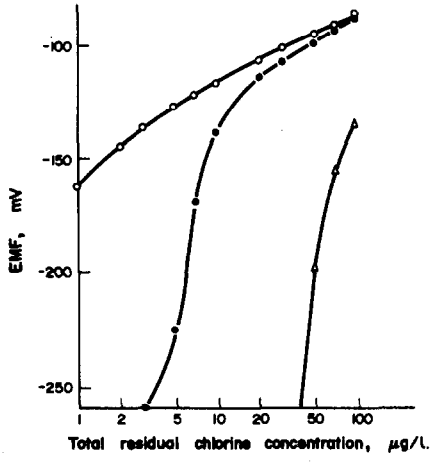

Fig. 6. Effect of pH: ○ pH 4; ● pH 5; △ pH 6.

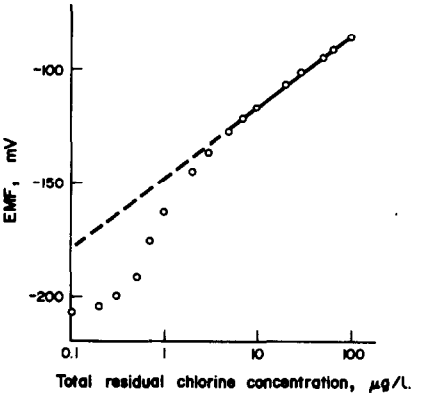

Fig. 7. Electrode calibration at low chlorine levels: 1 ml of 0.005*M* KI added per 100 ml of chlorine solution.

Order of reagent addition

Sodium hypochlorite was added to synthetic sea-water (0.6*M* NaCl + 65 mg/l. Br^-) and to a dechlorinated sample from Southampton Water. Measurements were made after adding reagents in the order:

(*a*) buffer first, then potassium iodide within 15 sec;
(*b*) potassium iodide first, then buffer within 15 sec;
(*c*) buffer first, then potassium iodide after 5 min;
(*d*) potassium iodide first, then buffer after 5 min.

The results in Table 7 show that, contrary to the recommendations of Orion, the potassium iodide reagent should be added *first* if loss of TRC from the sample is to be avoided, and this is particularly important in waters which have a chlorine demand. With a 5-min delay between additions, the losses are significant even in synthetic sea-water; the likely cause of this further loss of TRC is volatility of bromine and hypobromous acid, especially as the ratio of the concentrations of these species to hypobromite increases as the pH falls. Analysis of chlorinated samples from Southampton Water when buffer was added first gave results about half those indicated by the DPD colorimetric method.

Limit of detection

The limit of detection was not determined in a formal manner[9] because once the problem of molecular iodine in the iodide reagent had been minimized, the lower end of the calibration range was dominated by negative deviations, *i.e.*, the electrode indicated less residual chlorine than had been added. Figure 7 shows the typical shape of a calibration curve extending to low concentrations. The concentration at which the curve deviated from linearity varied between batches of water from the same source but was the same for two different electrodes. Figure 4 previously showed that the response deviated more from the theoretically expected values as the temperature increased.

Possible reasons for the presence of less iodine than expected from the quantity of chlorine added are (*a*) loss of iodine by volatilization, (*b*) incomplete reaction of the residual chlorine with the iodide reagent, (*c*) reaction of the residual chlorine or iodine with organic matter in the water or trace impurities in the buffer solution.

The volatilization of iodine should increase with temperature, which would agree with the results in Fig. 4, but should be independent of pH in the range tested and of the source of water. Moreover, in a system at equilibrium the relative loss of iodine, and, therefore, the effect on the e.m.f., should be independent of concentration. Although the apparatus used in these tests was open to the atmosphere, conditions were such that the relative loss by volatilization was not expected to depend significantly on concentration.

Table 7. Order of reagent addition

First reagents	Delay between reagents	E.m.f. readings for 100 µg/l. Cl_2*	
		Synthetic sea-water	Sea-water
Buffer	<15 sec	−88.4 (−3)	−98.2 (−58)
KI	<15 sec	−88.0 (0†)	−88.5 (−4)
Buffer	5 min	−94.3 (−4.2)	−115.2 (−90)
KI	5 min	−89.1 (−9)	−91.3 (−24)

* Figures in parentheses give percentage loss of TRC in sample compared with highest reading.
† Reference for percentage loss of sample.

Incomplete reaction of iodide with the residual chlorine should be less likely at high temperatures, which does not accord with the results in Fig. 4, nor should it depend on the water used to prepare the solutions. Increasing the pH should slow the reaction down, but at pH ≥ 5 the calibration curve had large negative deviations (Fig. 6). In very dilute solutions ($Cl_2 < 1$ μg/l.) at pH 4, it is possible that the reaction does not reach completion, but this does not account for the spasmodic occurrence of deviations at chlorine concentrations above 1 μg/l.

Reaction of the residual chlorine or iodine with organic matter in the water should increase with temperature, in accordance with Fig. 4, and would depend on the source of water. The effect of pH on these reactions is uncertain and would depend on the organic species present.[10] The water used in our laboratory had a chlorine demand of 0.5–5 μg/l. (usually 1–2 μg/l.), as calculated from the deviations of the electrode response from the Nernstian value.

It is unlikely that the response limit of the electrode itself has been approached in this work, and the problems encountered are probably not those of preparing and preserving pure standard solutions. Such problems are common to all methods of residual chlorine determination and it was concluded that for manual analyses the electrode could be calibrated directly with standard solutions down to 50 μg/l. TRC and in most cases down to 5 μg/l., depending on the quality of the water available for preparing the solutions. Since a Nernstian response can be obtained at a level of 1 μg/l. it is reasonable to calibrate at higher levels and extrapolate to 1 μg/l.

Analysis of chlorinated waters

The simplest method for a succession of samples with the same temperature (within 1°) and salinity (within 0.05*M* chloride) is direct potentiometry. If, however, samples vary in temperature or salinity, or if standard solutions matching the samples in temperature and salinity cannot be readily prepared, known-addition potentiometry is usually more convenient and accurate, since within fairly wide limits it allows for changes in equilibria caused by variations in conditions.

Results. Samples were analysed *in situ* at power stations on Southampton Water and the River Trent by using known-addition potentiometry with a portable pH-meter and checking the concentration by means of the DPD colorimetric method with a Lovibond Comparator having a 40-mm cell.

Samples from these locations were returned to our laboratory, chlorinated, and analysed in the laboratory, with use of a digital pH-meter for the potentiometric readings and a spectrophotometer for the colour method. Analyses were also done in the laboratory on the tap-water and drinking water, and on water from the Skegness sea-well which had been stored in tanks at the Central Electricity Research Laboratories. The results of the various analyses are shown in Table 8.

Some of the measurements referred to levels too low to be determined by colorimetry (<20 μg/l.), but at which the electrode was still capable of discrimination. The water from the Trent had such a high content of suspended solids (in addition to a natural yellow colour) that unfiltered samples could not be analysed spectrophotometrically. It was also evident from potentiometric measurements at CERL that the water from the Trent had a chlorine demand that prevented accurate measurements from being made. When the e.m.f. values were displayed on a chart-recorder, no stable reading could be obtained, since any chlorine added (up to at least 500 μg/l.) was

Table 8. Analyses of chlorinated waters (Cl_2, μg/l.)

		DPD	
	Potentiometry	Free residual	Total residual
Sea (Southampton Water), *in situ*	340 ± 23	200–250	250–300
	245 ± 5	200–250	200–250
	195 ± 9	100–150	100–150
	108 ± 3	20–40	20–40
Sea (Southampton Water), at CERL	222 ± 11	—	219*
	34.0 ± 0.5	—	53*
	9.8 ± 1.0	—	13*
Skegness sea-water, at CERL	104 ± 13	—	93*
	17.8 ± 0.4	—	9*
	5.8 ± 1.3	—	0*
River Trent water, *in situ*			
Discharge to river	<1†	<20	<20
Cooling tower	7.1 ± 0.6	<20	<20
Condenser outlet	171 ± 3	150–200	350
Tap-water at CERL	38 ± 1	—	<100‡
Drinking water at CERL	111 ± 7	—	100‡
River Trent water, at CERL	150†	—	130

* Measured with a spectrophotometer.
† Direct potentiometry—see text.
‡ 1-cm cell.

consumed in minutes. The method adopted with these samples was to insert the electrode in the sample, note the highest e.m.f. obtained and read the concentration from a calibration graph. For measurements *in situ*, the less sensitive portable pH-meter had to be used and the consumption of chlorine was not detected at concentrations above about 5 μg/l. At very low concentrations, however, such as in the discharge to the river, the chlorine added in the known-addition method was consumed before the electrode could indicate a change in e.m.f., and direct potentiometry had to be used in this case also. Jenkins and Baird[5] had similar problems and observed continuous drift for waste-water samples dosed with chlorine up to 3 mg/l., when the B.O.D. exceeded 50 mg/l.

In the analysis of Trent water, the electrode tended to become fouled by silt, particularly on the silver iodide membrane. With fouled electrodes, the results had a negative bias because of the chlorine demand of the silt. Wiping with a paper tissue adequately removed the fouling.

DISCUSSION

Analytical performance

When suitable precautions are taken, the slope of the electrode calibration agrees with the theoretical Nernstian value down to chlorine concentrations of about 1 μg/l. As this is equivalent to a concentration of less than $10^{-7}M$, this Nernstian limit is probably lower than has been demonstrated for any other ion-selective electrode in dilute solutions of determinand.[11] In this work the Nernstian response of the TRC electrode has been observed at lower concentrations than before,[4,6] probably because the more dilute iodide solution used introduced less trace iodine to bias the calibration. The precision of the measurements (7–10% relative standard deviation) is typical of electrodes used in manual analysis without special control of temperature.[12]

These characteristics, together with the electrode's rapid response and freedom from interferences, make this one of the best ion-selective electrodes. For measurements at low levels, however, considerable care must be taken over the method as a whole and it should be noted that the procedure recommended by the manufacturers differs in several respects from that found necessary in this work. In particular, the Orion iodide reagent is too concentrated in iodide and insufficiently alkaline; the order of addition of the reagents should be iodide first, then buffer; stirring is essential at low concentrations; potassium iodate is not a suitable standard at low concentrations.

Operational control of chlorination

The electrode has adequate sensitivity for measurement of residual chlorine at the 0.2-mg/l. level recommended for power station operations. The precision of such measurements should be at least as good as that obtainable by the method currently in use (colorimetry with DPD). In analysis by direct potentiometry, the dependence of the response on salinity is a possible source of error at estuarine power stations, but the known-addition method eliminates this problem.

Since this work started, the Orion 1770 continuous on-line monitor that uses this type of electrode has become available.

Analysis of environmental samples

The electrode has proved capable of making measurements at concentrations as low as those proposed for environmental protection,[1] *e.g.*, 5 μg/l. When used with a portable pH-meter and stirrer, the electrode can be used for measurements in the field. The known-addition method is recommended for such measurements unless the salinity and temperature of the samples are known to be constant, but for waters with an unsatisfied chlorine demand, measurement by the known-addition method is liable to error and direct potentiometry is preferred. Even so, the accuracy of measurements in such waters will be unpredictable and comparatively poor, but this would probably be true of any method.

Comparison with other methods

Methods for determining residual chlorine have recently been reviewed.[2,13] Earlier reviews[3,14,15] dealt mainly with colorimetric methods.

The concentrations measurable by the Orion electrode are considerably below those measurable by direct amperometry or colorimetry. Residual chlorine levels below 20 μg/l. have been determined by a time-consuming amperometric titration and by an indirect method using differential-pulse polarography, but neither method is suitable for field work and both involve considerably more expensive apparatus.

The DPD colorimetric method is suitable for field work when used with a colour comparator, but can measure only down to about 20 μg/l., and the precision is limited by the number of steps on the comparator disc. Even with a spectrophotometer, DPD is incapable of much better sensitivity and is prone to error from turbidity and natural colour in the sample.

The characteristics determined for the use of the Orion electrode in discrete analyses in this work indicate that the potentiometric method would probably be at least as suitable as direct amperometry for continuous on-line monitoring of residual chlorine. Comparative tests of the potentiometric and amperometric methods have not been carried out, except by Helz *et al.*,[13] whose results are of limited value since the model of Orion monitor used (SLeD) was suitable only for pure waters such as drinking water. No results for the later model 1770 monitor have been found in the literature.

CONCLUSIONS

The Orion 97–70 electrode is suitable for the determination of total residual chlorine down to levels of

1–5 μg/l., but accurate and precise measurement at μg/l.-levels depends on use of a procedure different in several ways from that recommended by the manufacturer.

At high total residual chlorine concentrations, the variation of e.m.f. with iodide concentration is shown to agree with that calculated from the effect of dissociation of the I_3^- ion, but at concentrations below 20–50 μg/l., the e.m.f. values also indicate production of iodine by atmospheric oxidation of the iodide reagent. The use of dilute, alkaline, iodide reagent solutions minimizes the bias from this source.

Salinity changes affect the response of the electrode but use of the known-addition method avoids analytical errors from this source. The only species found to cause significant interference was permanganate. Other oxidizing agents such as bromate, iodate, dichromate and copper(II) had no significant effect under the conditions tested. The electrode may be used for the analysis of fresh-water and sea-water samples in the laboratory or, with a portable pH-meter, in the field. A continuous on-line monitor that uses the same electrode is now commercially available. The potentiometric method is at least as sensitive as any other currently available and is cheaper and more convenient than other methods of comparable sensitivity and precision. In particular, the potentiometric method is more sensitive than the widely used DPD colorimetric method and is capable of better precision than the version of the DPD method in which a colour comparator is used.

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EFFECT OF CATIONIC SURFACTANT ON THE FORMATION OF FERRON COMPLEXES

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Summary—In the spectrophotometric determination of aluminium and iron with ferron (7-iodo-8-quinolinol-5-sulphonic acid, H_2L), the addition of cationic surfactants greatly improves the linearity of the calibration curve and widens the useful pH range. The effect of cetyltrimethylammonium chloride (CTMAC) on the stepwise stability constants (K_1, K_2 and K_3) of the ferron complexes of aluminium and iron (ML^+, ML_2^- and ML_3^{3-}) and on the acid-dissociation constants (K_{a1} and K_{a2}) of ferron has been studied in connection with the role of the surfactant. CTMAC greatly increases the value of K_3 while exerting little effect on K_1 and K_2, thus rendering ML_3^{3-} the predominant species even at very low concentration of free L^{2-}. It also has some effect on the acid-dissociation constants of ferron, but sometimes it acts to decrease the free L^{2-} concentration. It is therefore concluded that the improvements due to addition of surfactant should be attributed to the increased K_3 value. The presence of surfactant micelles is not essential, because the surfactant has a favourable effect when present at well below its critical micelle concentration, and because the continuous variations plots show a peak at a point corresponding to the composition M:L:Q (Q = cationic surfactant) = 1:3:3.

Malát[1,2] found that the colour reaction between tin(IV) and Pyrocatechol Violet is greatly sensitized by addition of gelatin. Since then a number of spectrophotometric methods have been proposed in which cationic surfactants are used in conjunction with metallochromic reagents.[3–23]

Aluminium can be determined spectrophotometrically with ferron, but the method has several disadvantages. Ferron shows considerable absorbance at the wavelength of maximum absorption of the aluminium–ferron complex and the absorbance of the reagent varies considerably with pH. In addition, the calibration curve is non-linear unless very high reagent concentrations are used. These disadvantages can be drastically reduced by addition of a cationic surfactant, cetyltrimethylammonium chloride (CTMAC).[18]

Although iron(III) can be determined successfully with ferron in the absence of cationic surfactants, the addition of the latter is recommended because it extends the useful pH range down to 1.5.

Cationic surfactants frequently shift the absorption peak of metal–dye complexes to longer wavelengths. The shift is usually accompanied by increased molar absorptivity. The most widely accepted explanation for these effects is that the positive charge on the surfactant micelles enhances the acid dissociation of organic dyes so that the formation of higher ligand: metal ratio complexes is made easier.[14,24]

The acid dissociation of ferron, however, is not always enhanced by cationic surfactants. CTMAC suppresses it in neutral to alkaline medium when the ionic strength is high. Shijo and Takeuchi[11] also report that although the acid dissociation of Chrome Azurol S (CAS) is enhanced by addition of CTMAC at low pH, it is suppressed at pH > 12. Apparently other factors may be responsible for the improvements obtained by addition of cationic surfactants.

The present investigation was undertaken to obtain clearer insight into the role of cationic surfactants. The stepwise stability constants of the ferron complexes of aluminium and iron(III) were determined in the absence and presence of CTMAC. The results indicated that, of the three stepwise stability constants of the ferron complexes, the third is greatly increased by addition of CTMAC.

A study was also made of the composition of the ferron complexes formed in the presence of CTMAC. The results indicated the stoichiometry metal:ferron: surfactant = 1:3:3. It was also found that the presence of surfactant micelles is not of primary importance in the complex formation.

EXPERIMENTAL

Reagents

Standard aluminium solution, 2.5×10^{-3}M. Dissolve 0.2965 g of aluminium potassium sulphate 12-hydrate in a small volume of water containing 1 ml of concentrated sulphuric acid and dilute to 250 ml with water.

Standard iron(III) solution, 2.5×10^{-3}M. Dissolve 0.3014 g of ferric ammonium sulphate 12-hydrate in a small volume of water containing 1 ml of concentrated sulphuric acid and dilute to 250 ml with water.

Ferron solution, 2.5×10^{-3}M. Dissolve 0.2195 g of ferron in 250 ml of water.

CTMAC solution, 5.0×10^{-2}M. Dissolve 3.994 g of cetyltrimethylammonium chloride (CTMAC) in 250 ml of water.

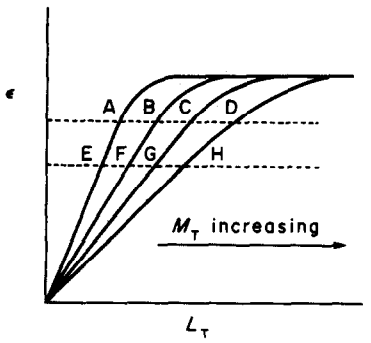

Fig. 1. Method of corresponding solutions for determination of $[L]_T$ and $\bar{n}$. ϵ as a function of L_T for different M_T's at constant pH.

Buffer solution (pH 4.5). Dissolve 6.804 g of sodium acetate trihydrate in 200 ml of water, adjust the pH to 4.5 with concentrated hydrochloric acid and dilute to 250 ml with water.

All reagents used were the purest grade commercial products.

Apparatus

A Hitachi 340 spectrophotometer with matched 10-mm cells was used. The Toa HM-20B digital pH-meter used was frequently calibrated with standard potassium hydrogen phthalate and phosphate buffer solutions. Conductivities were determined with a Toa CM-15A digital conductivity meter.

Determination of stability constants

Stability constants of metal ion–dye complexes are usually calculated from the absorbance *vs.* pH data for constant total concentration of metal ion in the presence of a large excess of the dye. For this method to be applied to complexes of hydrolysable metal ions, however, the hydrolysis constants of the metal ions must be known. The method of corresponding solutions[25] was therefore used because it permits stability constants to be calculated from the data obtained at constant pH, where the degree of hydrolysis is constant if the metal concentration is below the level at which the metal ion can polymerize. Thus, even when no reliable data are available about the hydrolysis equilibrium, conditional stability constants can be calculated.

Because the method of corresponding solutions is rarely used, a brief description of this method might be necessary.

Consider complex formation between a hydrolysable metal ion, M^{3+}, and ferron (H_2L), assuming that no polynuclear species exist in solution and that the pH is constant throughout.

We first define a measurable quantity ϵ as

$$\epsilon = \frac{A/l - \epsilon_L L_T}{M_T} \tag{1}$$

where A is the absorbance at the wavelength chosen (λ), l the optical path-length, ϵ_L the apparent molar absorptivity of ferron at the specified pH and λ, and L_T and M_T are the total concentrations of L and M, respectively.

Theoretically ϵ is given by

$$\epsilon = \frac{\epsilon_0 + (\epsilon_1 - \epsilon_L)K_1'[L]_T + (\epsilon_2 - 2\epsilon_L)K_1'K_2'[L]_T^2 + (\epsilon_3 - 3\epsilon_L)K_1'K_2'K_3'[L]_T^3}{1 + K_1'[L]_T + K_1'K_2'[L]_T^2 + K_1'K_2'K_3'[L]_T^3} \tag{2}$$

where ϵ_0 is the apparent molar absorptivity of the metal ion at the specified pH and λ, $[L]_T$ the total concentration of L not bound to M, ϵ_n the molar absorptivity of $ML_n^{(3-2n)+}$ at λ, and K_n' is the conditional stepwise stability constant, defined as

$$K_1' = [ML]/[M]_T[L]_T$$
$$K_2' = [ML_2]/[ML][L]_T$$
$$K_3' = [ML_3]/[ML_2][L]_T$$

where $[M]_T$ is the total concentration of metal ion not bound to ferron and charges are omitted for simplicity.

The average number, $\bar{n}$, of L bound to M is related to the stability constants and $[L]_T$ by the equation

$$\bar{n} = \frac{K_1'[L]_T + 2K_1'K_2'[L]_T^2 + 3K_1'K_2'K_3'[L]_T^3}{1 + K_1'[L]_T + K_1'K_2'[L]_T^2 + K_1'K_2'K_3'[L]_T^3} \tag{3}$$

The term "corresponding" means "having the same value of ϵ". Since ϵ is a function of only $[L]_T$, as will be seen from equation (2), corresponding solutions have the same value of $[L]_T$ and hence the same value of $\bar{n}$, because $\bar{n}$ is also a function of only $[L]_T$.

The technique is as follows: prepare a series of solutions having the same M_T and pH but different L_T values and measure the absorbance of each solution. Calculate the values of ϵ and plot them against L_T. Make similar plots for different values of M_T, obtaining a graph like that shown in Fig. 1. Then cut the curves with horizontal lines drawn at several points. The intersections of the straight lines and the ϵ *vs.* L_T curves, *e.g.*, A, B, C, D in Fig. 1, represent a series of corresponding solutions because they have the same value of ϵ. The points E, F, G, H represent another series of corresponding solutions.

Plot L_T against M_T for a series of corresponding solutions, obtaining a straight line (Fig. 2), the slope of which is $\bar{n}$ for the series. Obtain $[L]_T$ by extrapolating to $M_T = 0$. Establish the relationship between $\bar{n}$ and $[L]_T$ by repeating the procedure for different sets of corresponding solutions. Then find the conditional stability-constant values which minimize the sum of the squares of the differences between calculated and observed values of $\bar{n}$.

Under the conditions of the present investigation, where hydrolysis equilibria other than

$$M^{3+} + H_2O = MOH^{2+} + H^+$$
$$K_h = \frac{[MOH^{2+}][H^+]}{[M^{3+}]}$$

($pK_h = 2$ and 5 for Fe^{3+} and Al^{3+}, respectively) are not important, the stepwise stability constants defined by

$$K_n = [ML_n]/[ML_{n-1}][L]$$

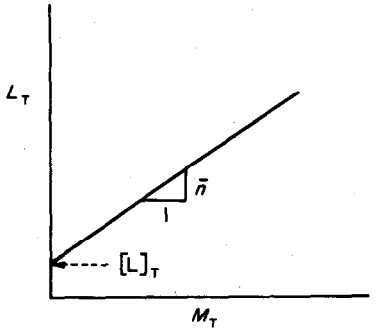

Fig. 2. Method of corresponding solutions for determination of $[L]_T$ and $\bar{n}$. L_T as a function of M_T for constant ϵ.

are related to the conditional stability constants by the equations

$$K_1 = K'_1\alpha_{L(H)}([H^+] + K_h)/[H^+]$$

$$K_2 = K'_2\alpha_{L(H)}$$

$$K_3 = K'_3\alpha_{L(H)}$$

where $\alpha_{L(H)} = 1 + [H^+]/K_{a2} + [H^+]^2/K_{a1}K_{a2}$, the quantities K_{a1} and K_{a2} being the first and second acid dissociation constants of H_2L.

Procedure

An appropriate volume of standard aluminium solution was placed in a 50-ml beaker. An appropriate volume of potassium chloride solution was added to adjust the ionic strength to 0.1, and then 0.25–5.0 ml of ferron solution and 5.0 ml of acetate buffer solution were added. For the study of the effect of CTMAC, 5 ml of CTMAC solution were added. The contribution of CTMAC to the ionic strength was neglected. The pH was adjusted to 4.30 ± 0.02 with hydrochloric acid or sodium acetate solution. The mixture was transferred into a 25-ml standard flask and diluted to the mark and equilibrated at 25°. The absorbance of the solution was measured and the stability constants were calculated as described above. Equilibrium was reached within 1 hr in the absence of CTMAC. In the presence of CTMAC, equilibrium was attained rather slowly, so the absorbance was measured after about 40 hr. The pH was checked again at the end of each run. Formation of aluminium–acetate complexes was neglected.

The stability constants of iron–ferron complexes were determined in much the same way, except that pH 2.00 was used, adjusted with hydrochloric acid and sodium hydroxide without a buffer. Equilibrium was attained within 1 hr both in the presence and absence of CTMAC.

The wavelengths used for absorbance measurements were 370 nm (Al–ferron system), 385 nm (Al–ferron–CTMAC system), 610 nm (Fe–ferron system), and 600 nm (Fe–ferron–CTMAC system).

RESULTS AND DISCUSSION

Calibration curve

The calibration graph for aluminium in the presence of CTMAC is linear up to $1 \times 10^{-4}M$, but nonlinear in its absence. The difficulties associated with the use of ferron are greatly reduced by addition of CTMAC. When the concentration of ferron is $5.0 \times 10^{-4}M$, the reagent blank absorbance is reduced from 0.2 to 0.05 by addition of CTMAC. The reduced reagent blank is of special advantage because the reagent blank changes remarkably with pH. In addition, the sensitivity is slightly increased.

The calibration graph for iron is linear at higher pH, but not at pH lower than 3.0 in the absence of CTMAC. In the presence of CTMAC, a linear calibration graph is obtained up to $2 \times 10^{-4}M$ iron even at a pH as low as 1.5.

Table 1. pK_{a1} values of ferron (25°C)

μ (KCl)	CTMAC	
	None	0.01*M*
0.1	2.44	1.34
0.5	2.37	1.30

μ = ionic strength.

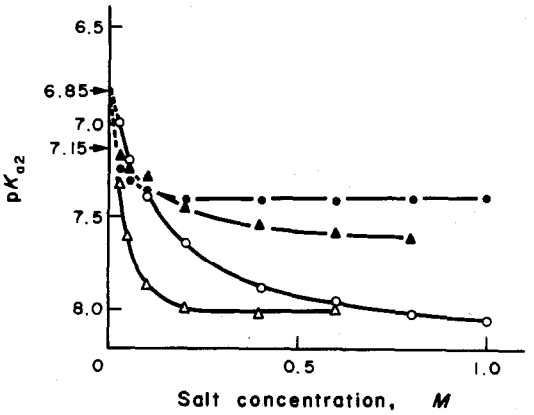

Fig. 3. The effect of salts on the pK_{a2} of ferron. CTMAC: —●— —▲— 0, —○— —△— $1.0 \times 10^{-2}M$; salt: —●— —○— KCl, —▲— —△— KNO_3.

Effect of CTMAC on the acid dissociation of ferron

Ferron dissociates in two steps, the zwitterion proton first and then a proton from the phenolic group. The first and second acid-dissociation constants, K_{a1} and K_{a2}, determined spectrophotometrically, are shown in Table 1 and Fig. 3.

The K_a values were determined at an ionic strength of 0.1 (KCl), except the value of K_{a1} in presence of CTMAC, which is so large that measurements had to be made at low pH, and this could not be attained without increasing the ionic strength to above 0.1. Thus it was calculated from the value obtained at $\mu = 0.5$, by means of the Debye-Hückel equation. The ion-size parameter for HL^- was assumed to be 8×10^{-8}.[26] As seen from Table 1, the value of K_{a1} is increased about tenfold by addition of CTMAC.

The effect of some strong electrolytes on the value of pK_{a2} in the presence and absence of CTMAC is shown in Fig. 3, which clearly shows that pK_{a2} is decreased by CTMAC at low ionic strengths, but increased at higher ones. This increase is lower for potassium nitrate than for potassium chloride as inert electrolyte.

Extrapolation to zero electrolyte concentration yields $10^{-7.15}$ and $10^{-6.85}$ for the thermodynamic K_{a2} values in the absence and presence of 0.01*M* CTMAC, respectively.

When potassium nitrate was added to a solution of CTMAC, a slight increase in viscosity was noticed at a nitrate concentration of about 0.2*M*. At nitrate concentrations higher than 0.6*M*, the solution became highly viscous. If potassium iodide was added instead of potassium nitrate, the solution became turbid at an iodide concentration as low as 0.025*M*. The turbidity was observed even when the concentration of CTMAC was below its critical micelle concentration (cmc). On the other hand, no apparent change was noticed on addition of potassium chloride. Appar-

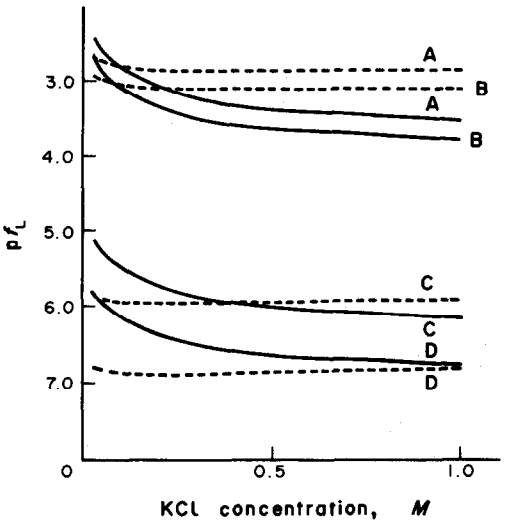

Fig. 4. Effect of KCl on f_L. CTMAC: ----- 0, —— $1.0 \times 10^{-2}M$; pH: A, 4.55; B, 4.30; C, 2.00; D, 1.50.

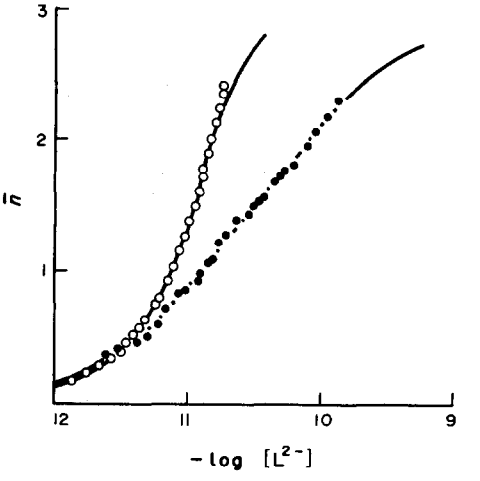

Fig. 6. Formation curve for Fe(III)–ferron complexes. Calculated from data obtained at pH 2.0 and $\mu = 0.1$ (KCl). CTMAC: —●— 0, —○— $1.0 \times 10^{-2}M$; ●○: observed, —— calculated.

ently there are specific interactions between the surfactant cation and added anions.

It is the fully deprotonated ferron which combines with M^{3+} to form the complexes ML^{+}, ML_2^{-} and ML_3^{3-}. The combined effect of CTMAC and inert electrolyte on K_{a2} will therefore alter the conditional stability constants of the complexes by changing the fraction of ferron present as L^{2-} (f_L). This effect was calculated for different pH values and is shown in Fig. 4. At pH 2.0, used for the iron–ferron investigation, CTMAC increases f_L at chloride concentrations $<0.4M$, but decreases it at higher chloride concentrations. The value of f_L is increased by a factor of nearly 5 by addition of CTMAC at pH 1.5 and ionic strength 0.1 (KCl), the conditions used for the calibration graph for iron. The aluminium–ferron system was investigated at pH 4.3, where the second acid dissociation of ferron is suppressed by addition of CTMAC if the chloride concentration is $>0.1M$. At pH 4.55 and ionic strength 0.8 (KCl), the value of f_L is decreased by a factor of about 4 by addition of CTMAC, but the linearity of the calibration graph for aluminium is greatly improved by addition of CTMAC. Apparently factors other than the enhanced dissociation of ferron should be taken into consideration to explain the role of CTMAC.

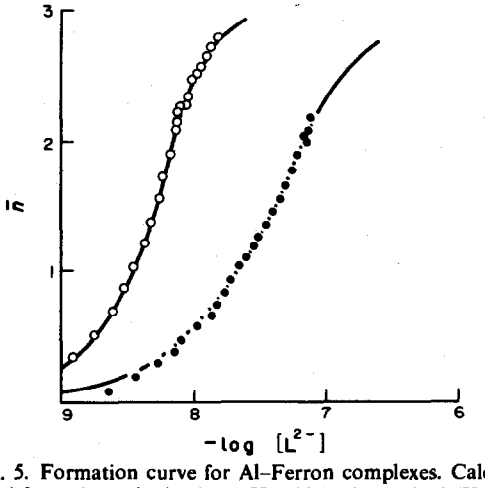

Fig. 5. Formation curve for Al–Ferron complexes. Calculated from data obtained at pH 4.30 and $\mu = 0.14$ (KCl). CTMAC: —●— 0, —○— $1.0 \times 10^{-2}M$; ●○: observed, —— calculated.

Effect of CTMAC on the stability constants of ferron complexes

Figure 5 shows the relationship between $\bar{n}$ and $[L^{2-}]$ for aluminium–ferron complexes at pH 4.3 in the absence and presence of CTMAC and Fig. 6 shows that for iron–ferron complexes at pH 2.00. Available data for the hydrolysis constants indicate that there is no polymerization of aluminium and iron(III) at these pH values. Best fits to the observed $\bar{n}$ and $[L^{2-}]$ data were obtained with the values of stability constants listed in Table 2. In the presence of CTMAC, the value of K_2 is very small in comparison with K_1 and K_3 and any value smaller than a certain limit will fit the experimental data if a proper value is assigned to the product K_2K_3. The curves in Figs. 5 and 6 for the presence of CTMAC were drawn with maximum possible values of K_2 ($10^{5.8}$ for aluminium–ferron and $10^{9.7}$ for iron–ferron).

In 1961, Langmyhr and Storm[27] reported values of the stability constants for the aluminium–ferron complexes. Their values, however, do not agree with ours. Close examination of Langmyhr and Storm's paper shows that their constants were based on tacit but incorrect assumptions. First they did not take hydrolysis into account, and then they calculated the stability constants by a method applicable only to a system for which $K_1 \gg K_2 \gg K_3$.

Table 2. Stepwise stability constants of ferron complexes (25°C)

Ion	μ	CTMAC	log K_1	log K_2	log K_3	log K_2K_3
Al	0.14 (KCl)	None	7.9	7.2	7.2	14.4
		0.01*M*	8.5	<5.8	>10.8	16.6
Fe(III)	0.1 (KCl)	None	11.5	10.5	9.7	20.2
		0.01*M*	11.5	<9.7	>12.2	21.9

μ = Ionic strength.

It is interesting that CTMAC increases the apparent value of K_3 while decreasing that of K_2. This may indicate that ML_3^{3-} combines more strongly than ML_2^- with surfactant cations.

To visualize more clearly the effect of CTMAC on the formation of ferron complexes, the distribution of metal ion between various complexes as a function of $[L^{2-}]$ was calculated with a maximum value assigned to K_2. The results (Figs. 7 and 8) show that in presence of CTMAC ML_2^- is not formed in appreciable quantities, but the domain where ML_3^{3-} is the predominating species is extended to lower $[L^{2-}]$. If enhanced acid-dissociation of ferron were the only cause for formation of the higher complexes, the distribution curves would have simply been moved to the left by addition of CTMAC to the system.

Both HL^- and L^{2-} have high absorptivities at the wavelength where the absorption spectrum of AlL_3^{3-} has a peak. Therefore it is not advisable to make the L^{2-} level sufficiently high for formation of only AlL_3^{3-} (*e.g.*, by adding large quantities of ferron or raising the pH). On the other hand, addition of cationic surfactants makes the AlL_3^{3-} species predominant even at low concentrations of L^{2-}. It also greatly reduces the absorptivity of the reagent itself at the absorption maximum of AlL_3^{3-}, so higher concentrations of ferron can be used.

For the determination of iron, addition of surfactants is not absolutely necessary, because FeL_3^{3-} is more stable than AlL_3^{3-} (so FeL_3^{3-} is the predominant iron species over a wide range of $[L^{2-}]$) and ferron has negligible absorptivity at the absorption peak of FeL_3^{3-} (so high concentrations of ferron can be used anyway). However, the addition of CTMAC may have merit on some occasions because it extends the useful pH range down to 1.5 (see Figs. 9 and 10).

Composition of the complex formed in the presence of CTMAC

In the discussion, CTMAC was regarded as one of the components of the medium and not treated as a complex-forming species, simply to avoid the difficult computation of so many equilibrium constants.

However, because it seemed very likely that CTMAC takes part in the complexation, a study was made of the composition of the iron(III)–ferron complex formed in the presence of CTMAC. The method of continuous variations was used. The absorbances of a series of solutions having the same total concentration but different individual concentrations of iron(III), ferron and CTMAC were

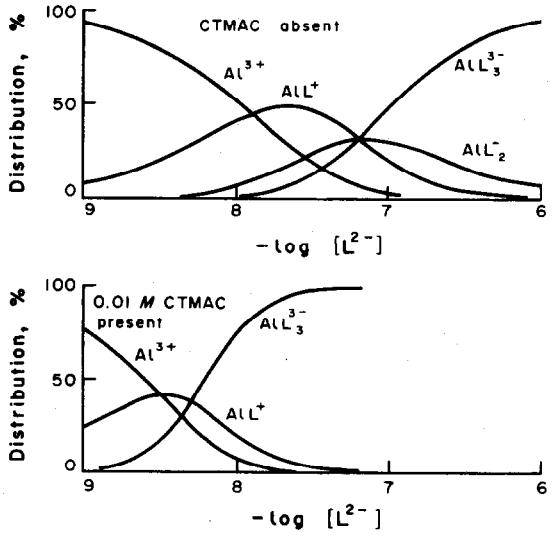

Fig. 7. Distribution of aluminium between various ferron complexes (calculated with maximum possible value of K_2).

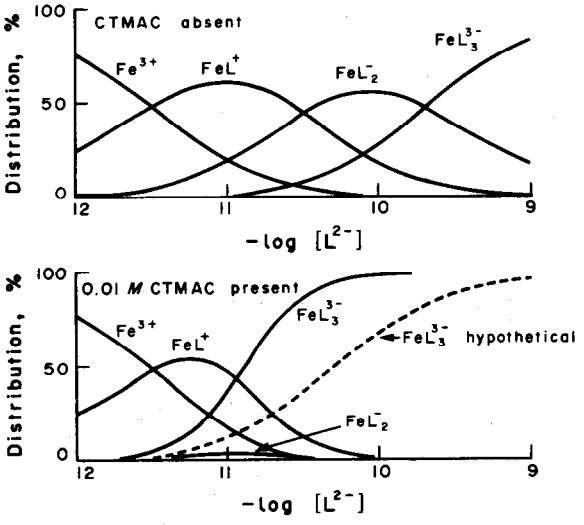

Fig. 8. Distribution of Fe(III) between various ferron complexes (calculated with maximum possible value of K_2).

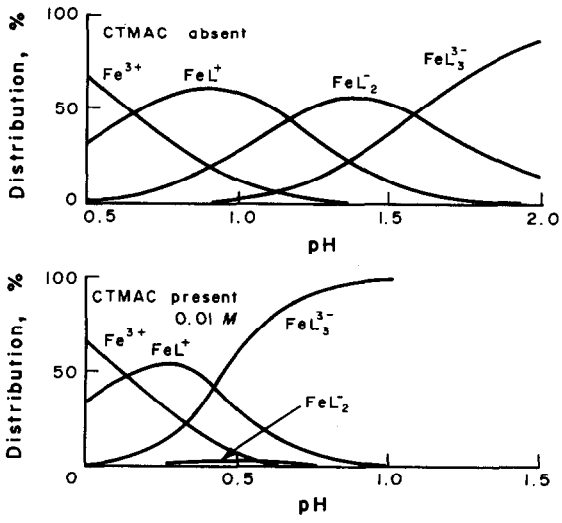

Fig. 9. Distribution of various Fe(III)–ferron species as a function of pH. $[H_2L] + [HL^-] + [L^{2-}] = 1.0 \times 10^{-3}M$. Formation of $Fe(OH)^{2+}$ neglected.

measured and plotted on a triangular diagram (Fig. 11), which shows that the absorbance is maximum at a point corresponding to the composition FeL_3Q_3 (Q = CTMAC). The same composition was found for the corresponding aluminium complex.

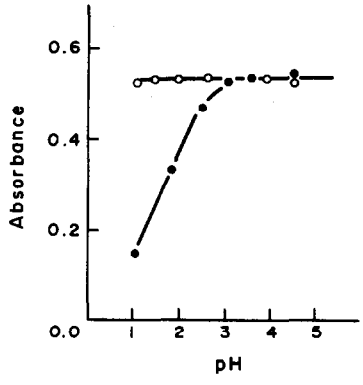

Fig. 10. Effect of pH on the formation of the Fe(III)–ferron complex in the presence and absence of CTMAC. Fe(III): $1.0 \times 10^{-4}M$; ferron: $1.0 \times 10^{-3}M$; CTMAC: —●— 0, —○— $1.0 \times 10^{-2}M$; Wavelength 600 nm; reference water.

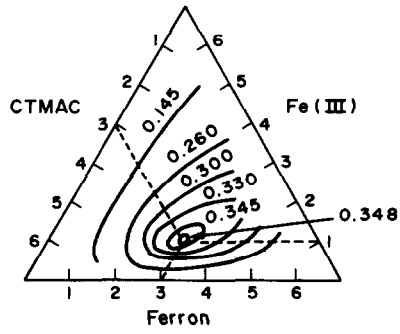

Fig. 11. Continuous-variations plot showing the stoichiometry Fe(III):ferron:CTMAC = 1:3:3. Fe(III) + ferron + CTMAC = $7.0 \times 10^{-4}M$; pH 1.53; ionic strength 0.1 (KCl); wavelength 600 nm; reference water.

Nature of the complex formation

The favourable effects of cationic surfactants on complexation reactions are usually attributed to the unusual environment on the surface of surfactant micelles,[9,14,24] but the simple stoichiometry of the complex described above casts doubt on this explanation.

It was therefore interesting to study whether the favourable effects are observed at surfactant concentrations below the critical micelle concentration (cmc).

The cmc values obtained for CTMAC and cetyltrimethylammonium bromide (CTMAB) by various investigators[8,12,28,29] are shown in Table 3 together with ours. Agreement between the results is good except for the one reported by Bailey *et al.*,[8] who used a spectrophotometric method with Rose Bengal Extra. As pointed out by Mukerjee and Mysels,[30] the spectrophotometric method tends to give lower results than other methods; these authors postulate that the spectral change occurs when just a few surfactant cations have combined with the dye.

Hiskey and Downey[31] have shown that the colour change of Methyl Orange with increasing concentration of octadecyltrimethylammonium chloride can be accounted for by assuming a combining ratio of dye to quaternary ammonium cation of 1:1. Our preliminary investigation indicated that the spectral change of the ferron–CTMAC system at about pH 4.5

Table 3. Critical micelle concentration of CTMAC and CTMAB

	cmc, *M*	Temp. °C	Method	Reference
CTMAC	1.2×10^{-3}	40	Conductivity	Tamamushi *et al.*[28]
	1.3×10^{-3}	30	Conductivity	Ralston *et al.*[29]
	1.03×10^{-3}	20	Surface tension	Shijo and Takeuchi[12]
	2.0×10^{-3}	25	Conductivity	Present investigation
CTMAB	4.0×10^{-6}		Dye	Bailey *et al.*[8]
	1.0×10^{-3}	60	Conductivity	Klevens[28]
	$0.8–0.9 \times 10^{-3}$			Kuwamura[28]
	0.88×10^{-3}	25	Conductivity	Present investigation

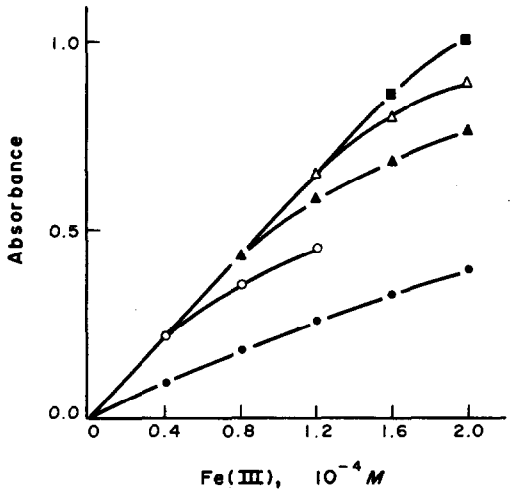

Fig. 12. Calibration curve for Fe(III) in the presence of different concentrations of CTMAC. pH: 1.5; ferron: $1.0 \times 10^{-3}M$; ionic strength: 0.1 (KCl); CTMAC: —●— 0, —○— $2.0 \times 10^{-4}M$, —▲— $4.0 \times 10^{-4}M$, —△— $6.0 \times 10^{-4}M$, —■— $8.0 \times 10^{-4}M$.

can be interpreted in terms of the equilibrium

$$Q^+ + HL^- = QHL \quad (\log K \text{ approx. } 3.7).$$

Thus there are good reasons for considering that the value reported by Bailey *et al.* is incorrect, and adopting a value of around $10^{-3}M$ as the cmc of CTMAC.

Figure 12 shows the effect of various concentrations of CTMAC on the shape of the calibration curve for iron at pH 1.5. Although the concentration of CTMAC did not exceed $10^{-3}M$, its presence increased the slope of the calibration curve.

At low iron concentrations, all the calibration curves obtained with CTMAC present were identical and linear. The length of the linear portion increased linearly with increasing concentration of CTMAC, and the deviation from linearity began when the CTMAC:iron(III) ratio fell below 5. This indicates that a very small amount of CTMAC is sufficient to obtain good results if iron is present at low concentration, and that surfactant micelles are not important.

Takeda *et al.*,[32] who studied the absorption spectrum of pinacyanol chloride–sodium dodecylsulphate mixture by a stopped-flow technique, showed that the first step in solubilization of the pinacyanol chloride was the formation of a dodecyl sulphate salt of the pinacyanol cation, which was then solubilized in the micelle phase.

In a practical application of the ferron–CTMAC procedure for the determination of iron and aluminium, it is recommended to add ferron and CTMAC in concentrations much higher than those used in the present study, to avoid slight changes in the absorption spectrum of the reagent blank with minor changes in the amount of CTMAC added and to make the method applicable to higher concentrations of the metal ions. Under these circumstances, CTMAC apparently does form micelles, but it is very likely that their role is merely to solubilize the electrically-neutral slightly soluble species QHL and ML_3Q_3.

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UNTERSUCHUNGEN ZUR ATOMSPEKTROSKOPISCHEN SPURENANALYSE IN A^{III} B^{V}-HALBLEITERMIKROPROBEN—VI*

UNTERSUCHUNGEN ZUR SCHICHTABTRENNUNG, PROFIL- UND SPURENANALYSE AN InSb-MATERIALIEN

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Zusammenfassung—Es wird ein Verfahren zur Profil- und Spurenanalyse in InSb-Materialien beschrieben. Zur Schichtabtrennung im nm-Bereich wird die Methode der anodischen Oxidation (galvanostatische Arbeitsweise) verwendet. Die Parameter der Schichtabtrennung wurden optimiert, die Messung der Schichtdicke der abgetrennten Schichten erfolgte ellipsometrisch. Die analytische Bestimmung der Spurenelemente erfolgte durch AES- und AAS-Messungen. Die erreichbaren Nachweisgrenzen liegen bei einer Oberfläche von 1 cm^2 und einer Schichtdicke von 60 nm in Bereich von 10^{17} Atome/cm^3. Verteilungsprofile für Mg und Te in InSb werden gezeigt.

Für die Profilanalyse von halbleitenden Materialien ist es erforderlich, über reproduzierbar arbeitende, gut auflösende Schichtabtrennungsverfahren und nachweisstarke, für Mikroproben einsetzbare Bestimmungsverfahren zu verfügen. Unsere ersten Untersuchungen zu dieser Problematik führten wir am GaAs durch.[1] Hinsichtlich der Tiefenauflösung sind die Verfahren des Ätzens nach anodischer Oxidation oftmals am günstigsten.[1–9] Für die Bestimmung der Spuren sind neben atomspektroskopischen auch elektrochemische Methoden und die Massenspektroskopie geeignet.

Ziel dieser Arbeit ist es, die am GaAs durchgeführten Untersuchungen auf den $A^{III}B^{V}$-Halbleiter InSb zu erweitern. Das betrifft sowohl die Verfahren zur hochauflösenden Schichtabtrennung als auch die Charakterisierung analytischer Bestimmungsmöglichkeiten.

Untersuchungen zur anodischen Oxidation mit dem Ziel der Herstellung von Dielektrika wurden bereits durchgeführt. Dewald[10] oxidierte InSb galvanostatisch (I = 0,2 mA/cm^2 in 0,1M Kalium hydroxid) Es wurde festgestellt, daß unmittelbar an der Oberfläche nur Indium vorliegt, weil SbO_x im gewählten Elektrolyten aufgelöst wird. Bereits in einer Tiefe von 20 nm ist das In:Sb Verhältnis gleich 1. Daraus kann man schlußfolgern, daß in dieser Tiefe kein Sb–KOH-Kontakt erfolgt. Diese Methode wurde auch von anderen Autoren zur Herstellung dicker Oxidschichten verwendet.[11–16]

Zur Verminderung der Auflösung der Schicht wurde als Elektrolyt 0,1M Borax-Lösung eingesetzt.[17,18] Einige Autoren stellen einen Zusammenhang zwischen der Oxidationszeit und der sich einstellenden Spannung fest.[11,12,17,18] Tschaikin und Mitarbeiter[19,20] fanden, daß die Schichtdicke anfangs proportional mit der Zeit (bis zu 0,1–0,2 μm) danach langsamer wächst. Ein anderes Resultat wird von Sakurai *et al.* angegeben.[12] Hier wurde Proportionalität zwischen Schichtdicke und Oxidationszeit bis zu 2,4 μm festgestellt (I = 3 mA/cm^2). Die Endspannung betrug nur 40 V. Bei Anwendung eines Impulsbetriebes[20] wurde ebenfalls ein linearer Zusammenhang für die Schichtdicken von 2 μm gefunden.

Festgestellt wurde, daß die Orientierung (111, 211, $\overline{2}11$) Einfluß auf die anodische Oxidation hat.[9,11,12,19–21] Neben wäßrigen, wurden auch

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nichtwäßrige Elektrolytlösungen als Medium verwendet: 2%iges Kaliumnitrit in Tetrahydrofurfurylalkohol (THF);[14] 5% Borax, 5% Wasser, 90% Ethylenglykol und Kaliumhydroxid in Tetrahydrofurfurylalkohol;[21] $KMnO_4$ in Aceton.[22]

Bei Einsatz der Dewald-Methode[9] wurden Oxidschichten bis zu 120 nm (KOH/THF, I = 1 mA/cm^2) bzw. bis zu 500 nm (Ethylenglykol, 1,5 mA/cm^2) erreicht.[21]

Die in einer früheren Veröffentlichung[1] formulierten Forderungen für die Anwendung der anodischen Oxidation zur Profilanalyse—gleichmäßige Oxidation der Komponenten und Oberfläche, keine Lösung des oxidierten Materials durch den Elektrolyten und reproduzierbare Beziehungen zwischen elektrischen Parametern und Schichtdicke—behalten volle Gültigkeit. Die galvanostatische Arbeitsweise ist aus diesen Gründen besser als die potentiostatische. Elektrolyt und elektrische Parameter sind experimentell zu prüfen.

EXPERIMENTELLER TEIL

Substanzen

(*a*) InSb—Einkristall-Blättchen, Orientierung 211 und $\overline{211}$.

(*b*) InSb—Na-dotiert (1–6 × 10^{17} At/cm^3), Orientierung 111 und $\overline{111}$.

(*c*) InSb vom *n*-Typ mit Trägerkonzentration 10^{14} (T = 180°C), Orientierung 111 und $\overline{111}$.

Elektrolyt. Ammoniumpentaborat—2 g/l. in H_2O, und 8 g/l. in Ethylenglykol–H_2O (1:1)[26] für InSb.

Apparatur

Amperostat, Eigenbau, UdSSR (0–100 mA, Stabilität ± 3%); Kathode 10 cm^2 Pt; Anodenhalter Ta (anodisch oxydiert); Stromdichte *ca.* 1 mA/cm^2 für InSb.

Jarrell-Ash Atomabsorptionsspektrometer 811, Beckman Graphitrohrküvette 1268, Untergrundkompensation nach der Zweilinienmethode. Perkin–Elmer Atomabsorptionsspektrometer 303 mit Graphitrohrküvette HGA 74, Untergrundkompensation mit Hilfe der Deuteriumlampe.

Schichtdickenmessung. Ellipsometrisch und chemisch. Letztere erfolgt durch Flammen-AAS am AA-Spektrometer 303 (Perkin–Elmer) mit C_2H_2–Luft-Flamme. Die Umrechnung erfolgt nach

$$d = \frac{M}{\varrho A}$$

A = Oberfläche-Areal; M = Masse InSb; ϱ = Dichte; InSb = 5,78 g/cm^3.

Verfahren der anodischen Oxidation

Die Oberflächen der Materialien wurden mechanisch bis zur Klasse 14 poliert. Ätzen mit einer Mischung von Milchsäure und Salpetersäure (10:1) hatte auf die Experimente keinen Einfluß. Die nicht zu oxidierende Seite wurde mit Paraffin bedeckt. Dann wurde das Material in die Ta-Halterung geklemmt und anodisch oxidiert. Die oxidierte Schicht wurde mit Flußsäure (1:10) aufgelöst. Die Lösung wurde aufgesaugt und analysiert. Beliebig viele Wiederholungen waren möglich.

Arbeitsweise der Spurenbestimmung

Die Flußsäure-haltigen Analysenlösungen werden eingedampft und in 50 µl 1*M* Salpetersäure aufgenommen. Die mechanisch abgetrennten Mengen können in 50–100 µl 1*M* Salpetersäure nach Einwaage gelöst werden. Bezogen auf 1 cm^2 werden bei einer Schichtdicke von 60 nm 35 µg InSb gelöst. Bei höheren Dotierungen erfolgt eine entsprechende Verdünnung. Probevolumina von 10 bis 20 µl werden in die Graphitrohrküvette gegeben und programmiert, getrocknet, verascht und atomisiert.

ERGEBNISSE

Optimierung des Abtrennungverfahrens

Es wurden Stromdichten zwischen 0,5 und 5 mA/cm^2 überprüft. Bei Anwendung des wäßrigen Elektrolyten war das In:Sb—Verhältnis in der oxidierten Schicht gleich 1, (s.a. Abb. 2), bei Verwendung des gemischten Elektrolyten war es <1, d.h. hier wurde Indium teilweise gelöst. In Wasser, Aceton, Ethanol und wäßrigem Elektrolyt trat während 24 Stunden keine Veränderung der Oberflächeninterferenz ein, d.h. es wurde nichts abgelöst. Hinsichtlich dieses Kriteriums konnten die obigen Stromdichten als gleichwertig angesehen werden.

In der Tabelle 1 wurden die relativen Standardabweichungen der Schichtdicken auf einer und von mehreren Oberflächen zusammengefaßt. Aus dieser Tabelle geht hervor, daß die relative Standardabweichung mit zunehmender Schichtdicke besser wird. Ein Vergleich mehrerer Proben weist bei 32 nm Dicke eine Toleranz von 0,08 aus.

In der Abb. 1 wurde die Abhängigkeit der sich mit der Oxidation einstellenden Spannungsdifferenz ΔU von der Oxidationszeit bei unterschiedlichen Stromdichten dargestellt. In der Anfangsphase ergibt sich in jedem Fall direkte Proportionalität. Ab einer bestimmten Spannungsdifferenz ($\Delta U_{\ddot{U}}$) ergibt sich eine Verlangsamung des Anstieges der Spannungsdifferenz. Dieser $\Delta U_{\ddot{U}}$-Wert ist von der Stromdichte abhängig. Bei ΔU_{max} wächst die Spannungsdifferenz mit zunehmender Oxidationszeit nicht mehr. Im Unterschied zu unseren früheren Untersuchungen[1] am GaAs hängt beim InSb die sich ausbildende Spannungsdifferenz von der Stromdichte und der Orientierung ab. Dies wurde auch von anderen Autoren gefunden.[11,12,17,19] Dieses Ergebnis ist jedoch schwer zu erklären. Man kann annehmen, daß die Erscheinung verbunden ist mit der Abhängigkeit der Raumladung von der Stromdichte. Trotz dieser Schwierigkeiten gelingt es

Tabelle 1. Reproduzierbarkeit der Schichtdicke bei anodischer Oxidation des InSb

Bedingung	Schichtdicke h, *nm*	Relative Standardabweichungen, s_r, %
jeweils eine	18	5
Schicht	28	3
(n_1 = 10–15)	92	2
mehrere	32	8
Schichten	75	5
(n_2 = 15)		

n_1 = Zahl der Messungen auf einer Schicht.
n_2 = Zahl der Schichten, die jeweils einmal vermessen werden.

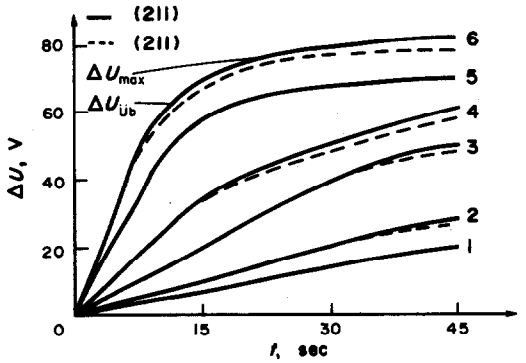

Abb. 1. Kinetische Kurve der anodischen Oxidation des InSb in Abhängigkeit von der Stromdichte: *1*—0,5 mA/cm²; *2*—2 mA/cm²; *3*—4 mA/cm²; *4*—10 mA/cm²; *5*—15 mA/cm²; *6*—20 mA/cm².

auch beim InSb im Gebiet bis zu $\Delta U_{Ü}$ oxidierte Schichten zu erhalten deren ΔU-Wert eine Funktion der Schichtdicke ist (vgl. Abb. 2). Es zeigte sich, daß bei diesen Bedingungen die Dicke der Oxidationsschicht, die bei gegebenem ΔU abgelöst wird, nicht von der Geschwindigkeit der Oxidation abhängt. Jedoch vermindert sich mit abnehmender Stromdichte die Streuung der Werte der Schichtdicke. Deshalb ist es günstig, bei niedrigen Stromdichten zu arbeiten. Infolge der Unlöslichkeit des Oxides im Elektrolyt ist das möglich. In der Abb. 3 wurde die maximal mögliche Schichtdicke, h_{max}, bis zu der eine lineare Abhängigkeit zwischen h und dem ΔU-Wert besteht, in Abhängigkeit von der Stromdichte aufgetragen.

Unter diesen Bedingungen verhalten sich die Flächen 211 und 111 gleich. Zur laufenden Kontrolle des Prozesses der anodischen Oxidation wurden als Vergleichsmaterial Schichten in Abstufung von 0,01 bis 0,005 hergestellt. Die Interferenzfarben, die auch von anderen Autoren beobachtet wurden,[11,12,17,19]

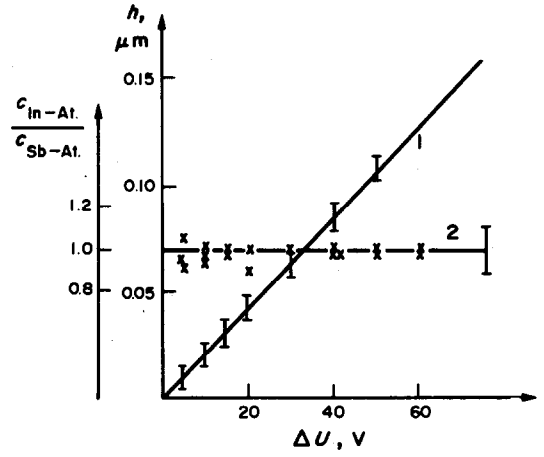

Abb. 2. Abhängigkeit der Schichtdicke der oxidierten Schicht (Kurve 1) und des In/Sb-Atomverhältnisses (Kurve 2) von der sich ausbildenden Spannungsdifferenz (ΔU). *1*—Mittelwert mit statistischer Sicherheit $P = 95\%$, *2*—Einzelwerte.

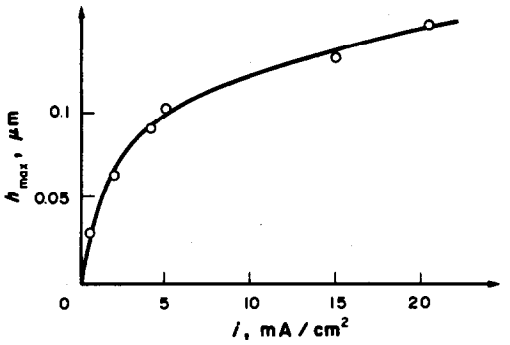

Abb. 3. Abhängigkeit der maximal möglichen Schichtdicke des oxidierten InSb von der Stromdichte.

sind sehr hell, so daß diese farbigen Interferenzen als Meßgröße (Eichskala) für die Dicke der Oxidationsschicht dienen können. Die Oxidationsschichten können mit Flußsäure (1:10 bis 1:20) schnell gelöst werden.

Mechanische Abtrennung von Schichten

Für die Abtrennung größerer Schichtdicken (> 1 μm) ist die anodische Oxidation zu zeitaufwendig. In diesem Fall ist es möglich, auf mechanischem Wege mit Hilfe eines Hartschnittmikrotoms in 1 μm-Schritten Schichtabtrennungen vorzunehmen. Die Methode wurde früher beschrieben.[1] Für ionenimplantierte Materialien hat das Verfahren wegen des zu geringen Auflösungsvermögens keine Bedeutung. Notwendig ist jedoch dieses Verfahren, wenn Elemente bestimmt werden sollen, die leicht flüchtige Fluoride bilden (z.B. Ge) oder deren analytische Bestimmung sehr durch Fluorid gestört werden kann (z.B. Ga).

Die Spuren- und Dotierungselemente wurden durch Emissionsspektrographie (AES) und Atomabsorptionsspektrometrie (AAS) bestimmt.

AES. Die Bestimmung der Spuren in den mit Flußsäure abgelösten Oxidationsschichten mit der AES wurde mehrfach beschrieben.[1,2,7,28] Die optimalen Arbeitsgrenzen, die in den In-haltigen Matrices erhalten wurden, entsprechen denen, die in GaAs erhalten wurden.[1,28] Bei der Bestimmung ist die Flußsäure-Lösung auf 20 mg C-Pulver (0,4% Natriumfluorid) zu geben und zu trocknen. Das homogenisierte Material wird im Gleichstrombogen angeregt. Da Indium—ähnlich wie Natrium—auch ein leichtionisierbares Element ist, treten systematische Fehler auf, wenn die Indiumkonzentration in C-Pulver >0,1% ist. Bei einer Oberfläche von 1 cm² können 60–70 nm dicke Schichten oxidiert, gelöst und auf 20 mg-C-Pulver gegeben werden. Dickere bzw. größere Schichten müssen auf eine entsprechend größere Menge C-Pulver gegeben werden.

AAS. Die Ergebnisse des Verfahrens sind in der Tabelle 2 dargestellt. Aus der Tabelle 2 geht hervor, daß Bestimmungen bei den gewählten Bedingungen bis zu Atomkonzentrationen von 10^{17} At/cm³ möglich sind. Dies entspricht dem normalen Dotierungsbereich.

Tabelle 2. Nachweisgrenzen von Spurenelementen in In-Materialien bei Bestimmung durch AAS mit elektrothermischer Atomisierung

Spurenelement	Wellenlänge, *nm*	Nachweisgrenzen absolut, *pg*	Nachweisgrenzen relativ,* At/cm^3
Be	234,9	10	5×10^{17}
Cd	228,8	3	1×10^{16}
Ga	294,4	60	4×10^{17}
Ge	265,2	100	7×10^{17}
Mg	202,2	50	1×10^{18}
Mg	285,2	2	4×10^{16}†
Pb	263,3	80	2×10^{17}
Sn	224,6	80	3×10^{17}
Te	214,3	100	4×10^{17}
Zn	307,6	10,000	$7{,}5 \times 10^{19}$
Zn	213,9	10	$7{,}5 \times 10^{16}$†

* Relative Nachweisgrenzen bezogen auf: 60 nm Schichtdicke, 1 cm^2 Oberfläche; aufgenommen in 0,1 ml; Analysenprobe 0,02 ml.
† Wert wurde extrapoliert. Infolge der Allgegenwartskonzentration sind Bestimmungen nur bis etwa 10^{17} At/cm^3 möglich.

Ergebnisse zur Profilanalytik

In der Abb. 4 ist die Verteilung von Magnesium im InSb (211) dargestellt. Die Dicke der abgetrennten Schichten betrug 60 nm. Die Messung erfolgte bei der empfindlichen Mg-Linie (285,2 nm). Die Herstellung des Materials erfolgte durch Ionenimplantation.

In Abb. 5 ist die Verteilung von Tellur im InSb, welches durch Epitaxie gewonnen worden war, dargestellt. Die Dicke der abgetragenen Schichten betrug 200 nm. Damit verbessert sich die relative Nachweisgrenze auf etwa 10^{17} At/cm^3. In dem gewählten Material lagen die Tellurgehalte (vgl. Tab. 3) im Bereich 10^{18} bis unterhalb 10^{17} At/cm^3. Wegen des Grenzbereiches des Nachweises erfolgte die Bestimmung auch auf inversvoltammetrischem Wege: Elektrolyse in 0,3*M* Salzsäure bei 0,8 V: danach wird Antimon bei −0,2 V gelöst (10 min): danach erfolgt die Bestimmung.

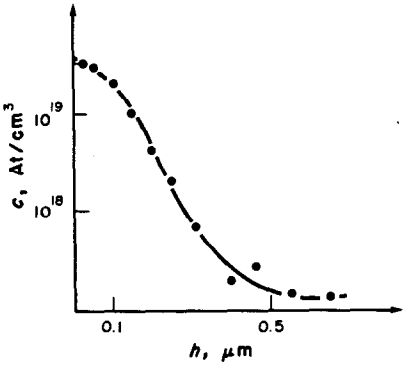

Abb. 4. Profil der Verteilung von Mg in InSb (211).

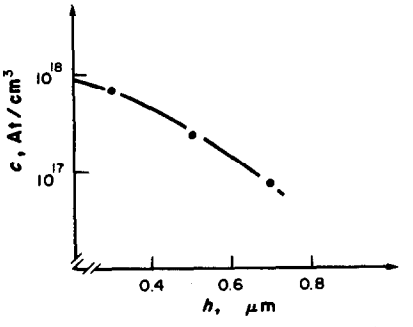

Abb. 5. Profil der Verteilung von Te in einer Epitaxieschicht des InSb.

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Summary—A method for the determination of distribution profiles and traces in InSb-materials is described. Stripping of the layers of several nm thickness was achieved by anodic oxidation (galvanostatic conditions). The stripping method was optimized, and the thickness of the layers stripped was determined by ellipsometric measurements. Traces of elements were determined by atomic emission and flameless atomic-absorption spectrometry. Detection limits in the range of 10^{17} atoms/cm^3 for a 1-cm^2 surface layer 60 nm thick were observed. Distribution profiles for Mg and Te in InSb are shown.

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SEPARATION OF LEAD-203 FROM CYCLOTRON-BOMBARDED THALLIUM TARGETS BY ION-EXCHANGE CHROMATOGRAPHY

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Summary—A simple method is presented for the separation of lead-203 from copper-backed thallium cyclotron targets. The procedure involves cation-exchange chromatography in hydrochloric acid and hydrochloric acid–acetone mixtures. Further purification involves anion-exchange chromatography in nitric acid–hydrobromic acid mixtures. A cation-exchange column containing 3.0 g of resin can handle as much as 15 g of thallium and 160 mg of copper. An anion-exchange column containing 3.0 g of resin can separate lead from up to 200 mg of thallium and 10 mg of copper. Separations are extremely sharp and less than 0.1 μg of thallium and less than 0.1 μg of copper remain in the lead-203 fraction.

Lead-203 is the only isotope of lead suitable for use as a tracer in the field of nuclear medicine, because of its reasonable half-life (52.1 hr), gamma-ray emission (0.28 MeV) in the region most useful for *in vivo* counting and scanning, and absence of radioactive decay products. It has been used for imaging bleomycin in tumours,[1] for skeletal imaging,[2] and as a label for red blood-cells.[3] It has also been used as its ethylenediaminetetra-acetate chelate for cisternography.[4,5] Other applications of ^{203}Pb are in investigations on the effects of lead pollution, entailing biochemical, biological and ecological studies.[6]

Lead-203 is usually produced in a cyclotron by proton or deuteron bombardment of a thallium target, the nuclear reactions being ^{203}Tl(p,n)^{203}Pb, ^{205}Tl(p,3n)^{203}Pb, ^{203}Tl(d,2n)^{203}Pb, ^{205}Tl(d,4n)^{203}Pb. In an effort to obtain higher yields of ^{203}Pb, and also to eliminate some difficulties encountered in the radiochemistry, Chackett *et al.*[7] investigated the reaction ^{203}Tl(^{3}He,3n)^{203}Pb with a 29-MeV external ^{3}He beam. The ^{203}Bi produced (half-life 11.8 hr) decays to the desired ^{203}Pb. Their method of separation consisted of adding sodium hydrogen phosphate to precipitate the ^{203}Bi with 1–2 mg of bismuth (added as nitrate solution) as bismuth phosphate, thus separating the bismuth from the thallium. The bismuth solution was sorbed on an anion-exchange resin, and 24 hr later the ^{203}Pb produced *in situ* was eluted from the column with 0.3*M* hydrochloric acid, while the bismuth was retained on the column. Qaim *et al.*[8] suggested a separation scheme consisting of precipitation of ^{203}Pb with ferric hydroxide and removal of the carrier iron from the ^{203}Pb by anion-exchange chromatography. Neirinckx[9] also precipitated ^{203}Pb with ferric hydroxide but separated the carrier iron by solvent extraction. Ramamoorthy and Watson[10] separated the ^{203}Pb from the thallium by ion-exchange chromatography on hydrous zirconium oxide. The recovery of ^{203}Pb was about 75% and the thallium impurity level was below 10 ppm. Bonardi[11] separated ^{203}Pb and thallium by a combination of precipitation and anion-exchange. Levin *et al.*[12] separated ^{203}Pb from the thallium by dissolving the latter in sulphuric acid and isolating ^{203}Pb from the solution by co-precipitation. Bajo and Wyttenbach[13] separated the ^{201}Pb by solvent extraction with diethyldithiocarbamic acid in chloroform. Thallium-201 was separated from the mother activity (^{201}Pb) by solvent extraction with the same reagent. Toribara and Koval[14] removed the thallium from a dilute acid chloride solution by continuous extraction with ethyl ether. Essentially all of the thallium and almost none of the lead is removed in a 3-hr extraction. In most of the published methods only a few hundred mg of thallium target material had to be separated from the ^{203}Pb.[7,8,11,13]

A method of separating gram amounts of thallium from micro amounts of many other elements, such as lead, indium and copper by cation-exchange chromatography has been developed by Strelow and van der Walt.[15] The other elements are retained on Bio-Rad AG50W-X4 cation-exchange resin from 0.1*M* hydrochloric acid containing 0.05% chlorine, while the thallium passes through the column and can be eluted quantitatively with 0.1*M* hydrochloric acid containing 40% acetone. The other elements are then eluted with 3*M* hydrochloric acid or 3*M* nitric acid. Less than 10 μg of thallium remain in the elements separated from

10 g of thallium. This method has been adapted for the separation of ^{203}Pb from thallium cyclotron-target material. The general eluting agent (3*M* hydrochloric acid) is replaced by 0.1*M* hydrochloric acid containing 82% acetone, which elutes lead very selectively. Copper, which is used as a backing for the thallium target and may be present in milligram amounts, and most other elements are retained on the resin column. The chlorine (0.05%) is replaced by bromine (0.05%) reagent as oxidant for the thallium, since bromine is easier to use, serves as self-indicator for completion of the oxidation, and is more efficient in equilibrating the column.

This paper describes the modified cation-exchange separation. A second separation step on a column of Bio-Rad AG1-X4 anion-exchange resin is also described; it can be used to remove remaining traces of copper and thallium from the ^{203}Pb when an extremely pure product is required.

EXPERIMENTAL

Reagents and apparatus

The chemicals used were of analytical-reagent grade. Thallium metal (99.999%) was obtained from Fluka A. G., Buchs, Switzerland. Water was distilled and passed through an Elgastat demineralizer. Bio-Rad AG50W-X4 sulphonated polystyrene resin (100–200 mesh) and AG1-X4 quaternary amine resin (200–400 mesh) were used. The columns were made in borosilicate glass tubes (20.0 mm bore, 110 mm long) with a B19 female joint at the top, and at the other end a tube (14.4 mm bore, 200 mm long), fitted with a fused-in No. 1 porosity glass sinter and a burette tap at the bottom.

A Varian–Techtron AA-5 atomic-absorption spectrometer was used. A slotted quartz tube[15] was used in the beam-path over the burner head, as described by Watling,[16] to obtain better sensitivity for lead and thallium.

A 4096-channel gamma-spectrophotometer coupled to a Ge(Li) detector was used to measure the ^{203}Pb activity.

Elution curves

Thallium, copper and lead on the AG50W-X4 resin. The column was filled with a slurry of AG50W-X4 resin (100–200 mesh) to a mark at 13.0 ml. The resulting resin bed was about 80 mm in length and was equilibrated by passage of 50 ml of 0.1*M* hydrochloric acid containing 0.05% bromine (v/v) (solution A). A solution of about 10 g of thallium(III), 130 mg of copper(II) and 5 mg of lead(II) in 200 ml of 0.1*M* hydrochloric acid containing 0.05% (v/v) bromine was passed through the resin bed. The elements were washed onto the resin with small portions of solution A (about 40 ml altogether). The following sequence of reagents was used for elution: four 10-ml portions of 0.1*M* hydrochloric acid containing 60% acetone for thallium(III); 120 ml of 0.1*M* hydrochloric acid containing 82% acetone for lead(II); 100 ml of 3.0*M* hydrochloric acid for copper(II). A flow-rate of 3.5 ± 0.3 ml/min was used throughout. Fractions (10 ml in volume) were collected with an automatic fraction collector from the beginning of the sorption step. The amounts of thallium, copper and lead were determined in each fraction. Small amounts of the elements were determined by atomic-absorption spectrophotometry, with an air–acetylene flame and the 217.0, 278.6 and 324.8 nm lines for lead, thallium and copper, respectively. Larger amounts of the elements were determined by titration with EDTA. Copper was titrated at pH 5.6 ± 0.2 with Xylenol Orange as indicator in the presence of 1,10-phenanthroline, and thallium(III) in the presence of ammonium tartrate at pH 8.8 ± 0.2, with Methylthymol Blue as indicator. The results are shown in Fig. 1.

Thallium, copper and lead on AG1-X4 resin. The column was filled with a slurry of Ag1-X4 (200–400 mesh, chloride form) to a mark at 8.7 ml. The resulting bed was about 52 mm in length and was equilibrated by passage of 100 ml of 0.2*M* hydrobromic acid–0.1*M* nitric acid (solution B). A solution of about 100 mg of thallium(III), 10 mg of copper(II) and 2 mg of lead(II) in 25 ml of 0.2*M* hydrobromic acid–0.1*M* nitric acid was passed through the resin bed. The elements were washed onto the resin with small portions of solution B (30 ml altogether). The resin was eluted with the following sequence of reagents: 75 ml of solution B; 170 ml of 2.0*M* nitric acid–0.03*M* hydrobromic acid. Fractions (10 ml in volume) were collected from the beginning of the sorption step. The flow-rate was 2.4 ± 0.3 ml/min throughout. The amounts of thallium, copper and

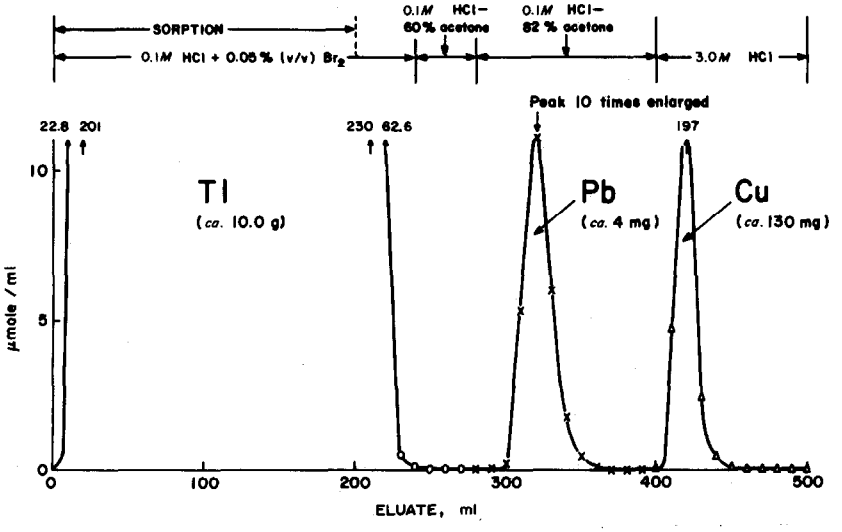

Fig. 1. Elution curve for Tl(III) (*ca.* 10.0 g), Cu(II) (*ca.* 130 mg) and Pb(II) (*ca.* 4 mg) with 0.1*M* HCl + 0.05% Br_2 and HCl–acetone mixtures. Column 13.0 ml (3.0 g) of AG50W-X4 (100–200 mesh), length 80 mm, diameter 14.4 mm. Flow-rate 3.5 ± 0.3 ml/min.

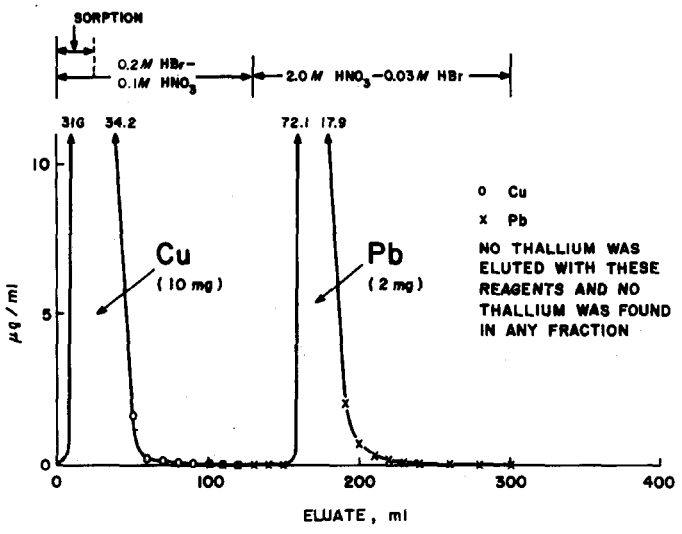

Fig. 2. Elution curve for Tl(III) (*ca.* 100 mg), Cu(II) (*ca.* 10 mg) and Pb(II) (*ca.* 2 mg) with nitric acid–hydrobromic acid mixtures. Column 8.7 ml (3.0 g) of AG1-X8 (200–400 mesh), length 52 mm, diameter 14.4 mm. Flow-rate 2.4 ± 0.3 ml/min.

lead were determined in each fraction by atomic-absorption spectrophotometry as above. The results are presented in Fig. 2.

Separation of lead-203 from thallium target material

Thallium metal (99.999%) was melted onto a water-cooled copper plate to give a cyclotron target with length 230 mm, width 10 mm and thickness 0.35–0.38 mm. The target was bombarded with 16-MeV deuterons for 42 min at a beam-current of 37 µA, and "cooled" for a few hr to let the short-lived isotopes decay. The thallium was milled from the copper plate to a depth of 0.35 mm, giving about 9.7 g of thallium target material, which was dissolved in 30 ml of 2*M* nitric acid. The nitrates were converted into chlorides by repeated evaporation with 10*M* hydrochloric acid, with bromine added dropwise to oxidize the thallium(I) to thallium(III). After a final evaporation of the solution to incipient dryness, the residue was dissolved in 50 ml of 0.4*M* hydrochloric acid, with dropwise addition of bromine until a clear solution was obtained. The resulting solution was boiled until light yellow in colour, diluted to 200 ml with demineralized water and cooled to room temperature. Saturated bromine water (3 ml) was added with stirring (solution C).

Cation-exchange separation of ^{203}Pb from thallium and copper on AG50W-X4

Solution C was passed through the resin bed (prepared and equilibrated as described above). The elements were washed onto the resin with small portions of 0.1*M* hydrochloric acid containing 0.05% v/v bromine (40 ml altogether), each portion being drained to the top of the bed before the next was added. The eluate was collected from the beginning of the sorption and the elution was completed with 60 ml of 0.1*M* hydrochloric acid containing 60% acetone, this eluate being collected separately. The ^{203}Pb was then eluted with 80 ml of 0.1*M* hydrochloric acid containing 82% acetone, and a further 20 ml of the same eluting agent, the two eluates being collected separately. Finally, the copper was stripped from the resin column with 80 ml of 3.0*M* hydrochloric acid. All the eluates were evaporated, or diluted with demineralized water, to give a volume of 50 ml (a 100-ml beaker being used). The ^{203}Pb activity was measured for all fractions, the 400-ml beaker used for collecting the thallium eluate, and the resin column. All results were corrected for decay and are presented in Table 1.

Anion-exchange separation of ^{203}Pb from the remaining micro amounts of thallium and copper impurities

The first ^{203}Pb fraction from the cation-exchange separation was evaporated to dryness and the residue treated twice with 2 ml of concentrated nitric acid, with evaporation to dryness each time, to convert the chlorides into nitrates. The residue was treated with 2 ml of concentrated nitric acid and 1 ml of concentrated hydrobromic acid and evaporated to incipient dryness. The salts were dissolved in 2.0 ml of 1.0*M* nitric acid, cooled, and diluted with 13 ml of demineralized water, then 5 ml of 0.8*M* hydrobromic acid were added and the solution was mixed. The solution was passed through the resin bed (prepared and equilibrated as described above). The elements were washed onto the resin with small portions of 0.2*M* hydrobromic acid–0.1*M* nitric acid (30 ml altogether) and the copper was completely eluted with a further 50 ml of this acid mixture. The eluate was collected from the beginning of the sorption. The ^{203}Pb was eluted with 70 ml of 2.0*M* nitric acid–0.03*M* hydrobromic acid and a further 30 ml of the same eluting agent, the eluates being collected separately. All the eluates were evaporated to 20 ml (a 50-ml beaker being used). The ^{203}Pb activity was measured for all fractions, the two beakers used for collecting the copper eluate and the first lead eluate, and the resin column. All the results were corrected for decay and are presented in Table 2.

RESULTS AND DISCUSSION

Figures 1 and 2 show that a sharp separation of lead from thallium and copper is obtained. No thallium (*i.e.*, less than 0.01 ppm) was found in any of the fractions collected from the anion-exchange resin column. The thallium can be eluted with 75 ml of 1.0*M* ammonia solution followed by 200 ml of 0.5*M* nitric acid—0.1*M* thiourea.[18]

The method described provides an excellent means

Table 1. Separation of ^{203}Pb from thallium cyclotron-target material on the cation-exchange resin AG50W-X4

Fraction	^{203}Pb activity,* *counts/200 sec*	Recovery, %
Main thallium fraction (eluted with 0.1*M* HCl + 0.05% Br_2)	87	0.08
Second thallium fraction (eluted with 0.1*M* HCl–60% acetone)	16	0.02
400-ml beaker used for collecting the main thallium eluate	0	0
First ^{203}Pb fraction (eluted with 80 ml of 0.1*M* HCl–82% acetone)	106,541	99.89
Second ^{203}Pb fraction (eluted with additional 20 ml of 0.1*M* HCl–82% acetone)	0	0
Resin column	0	0

* All measurements were carried out on a volume of 50 ml in a 100-ml beaker, except those on the 400-ml beaker and the resin column.

for the separation of lead from up to 15 g of thallium and up to 160 mg of copper. When only the cation-exchange separation was used, less than 15 μg of thallium was found in the lead fractions when 15 g of thallium were present originally. Only 3–8 μg of copper were found in the lead fractions when 130 mg of copper were present originally. When both cation- and anion-exchange columns were used in the separation of 10 g of thallium and 160 mg of copper from μg amounts of lead, less than 0.1 μg of thallium or copper was found in the lead fractions.

The method is well suited for the separation of ^{203}Pb from cyclotron-irradiated thallium target material and a carrier-free product of high purity is obtained. Very little ^{203}Pb activity is lost in the separation as can be seen from Tables 1 and 2. The recovery of ^{203}Pb activity was better than 99%. When a small thallium cyclotron target (less than 200 mg of thallium, containing less than 10 mg of copper) is used, the separation can be carried out either on the cation-exchange or the anion-exchange resin. The method can easily be adapted for hot-cell operation, with a lead shield, and can be carried out relatively rapidly, especially when only the cation-exchange separation is used.

According to the distribution coefficients published by Strelow *et al.*[17] the following elements should also be separated from lead by the cation-exchange procedure described above: uranium, gold, mercury, the platinum metals, arsenic, tungsten, molybdenum, iron, gallium, calcium, magnesium, manganese, cobalt and vanadium. Zinc and indium should accompany lead because of their similar distribution coefficients.[17] When the cation-exchange separation is followed by the anion-exchange procedure, zinc and indium should accompany copper because of their low distribution coefficients under these conditions.[18,19]

After the ion-exchange separations have been carried out, the eluates can be evaporated to dryness and the ^{203}Pb dissolved in a suitable solvent, providing a carrier-free product of high purity.

The method is also suitable for the separation of ^{201}Tl from the mother isotope ^{201}Pb by either the cation-exchange or the anion-exchange separation.

Table 2. Separation of ^{203}Pb from thallium and copper impurities on the anion-exchange resin AG1-X4

Fraction	^{203}Pb activity,* *counts/200 sec*	Recovery, %
Copper fraction (eluted with 0.2*M* HBr–0.1*M* HNO_3)	29	0.04
First ^{203}Pb fraction (eluted with 70 ml of 2.0*M* HNO_3–0.03*M* HBr)	76,264	99.90
Second ^{203}Pb fraction (eluted with additional 30 ml of 2.0*M* HNO_3–0.03*M* HBr)	6	0.01
150-ml beaker used for collecting the copper eluate	0	0
100-ml beaker used for collecting the first ^{203}Pb eluate	36	0.05
Resin column	0	0

* All measurements were carried out on a volume of 20 ml in a 50-ml beaker, except those on the 150-ml beaker and the resin column.

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SELECTIVE SEPARATION AND DIFFERENTIAL DETERMINATION OF ANTIMONY(III) AND ANTIMONY(V) BY SOLVENT EXTRACTION WITH *N*-BENZOYL-*N*-PHENYLHYDROXYLAMINE AND GRAPHITE-FURNACE ATOMIC-ABSORPTION SPECTROMETRY USING A MATRIX-MODIFICATION TECHNIQUE

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Summary—If copper is used as a matrix modifier for the determination of antimony, the ashing temperature for antimony in aqueous solution and a BPHA–$CHCl_3$ extract can be raised to 1300° and 1100°, respectively. A selective procedure for separating antimony(III) from antimony(V) by extraction with BPHA in chloroform is described, along with the conditions for preserving trace antimony in water samples. The recommended method has been applied satisfactorily to the determination of antimony(III) and antimony(V) in various types of water at sub-ng/ml levels.

It is well recognized that the toxicity and physiological behaviour of antimony depend on its oxidation state. According to European Community Standards,[1] the maximum admissible level of antimony in surface and drinking water is 10 ng/ml. As Sb occurs in the environment at very low concentrations (<1 ng/ml), the development of methods for determination of antimony(III) and antimony(V) has received considerable attention. Although graphite-furnace atomic-absorption spectrometry (AAS) has been widely used for the determination of trace antimony in a variety of samples, it seems that there are only a few studies on the differential determination of antimony(III) and antimony(V) reported in the literature. With reference to industrial hygiene, Eller and Haartz[2] have presented a comprehensive review of sampling and analytical methods for antimony and its compounds. Kambara *et al.*[3] have described the extraction of antimony(III) with BPHA (*N*-benzoyl-*N*-phenylhydroxylamine), counting the activity of Sb(III) in the organic phase and of Sb(V) in the aqueous phase with a scintillation counter, but no detailed study of the extraction behaviour of antimony with BPHA was given. A procedure consisting of extraction of antimony(III) from atmospheric dusts into benzene and determination by hydride generation has also been suggested.[4] Recently, Nakashima[5] used zirconium(IV) to suppress the conversion of antimony(V) into stibine in hydrochloric acid medium (<2*M*) for selective determination of antimony(III) in the presence of antimony(V). No interference from antimony(V) was reported for Sb(V)/Sb(III) ratios up to 4:1, at an antimony(III) level of 50 ng/ml. Total antimony was determined after reduction of antimony(V) to antimony(III) with potassium iodide. The difference between the two determinations gives the concentration of antimony(V). Subramanian and Meranger[6] studied the solution conditions affecting the ammonium pyrrolidinedithiocarbamate–methyl isobutyl ketone (APDC–MIBK) extraction system for graphite-furnace atomic-absorption determinations of As(III), As(V), Sb(III), Sb(V), Se(IV) and Se(VI) in detail, and applied the method for the determination of antimony in polluted water. After comparing the extraction behaviour of antimony(III) and antimony(V) with APDC, diethyldithiocarbamate and dithizone, Kamada and Yamamoto[7] developed a method for selective extraction of antimony(III) and differential determination of antimony(III) and antimony(V) with the APDC–MIBK extraction system in conjunction with the use of EDTA to mask interferences from many metal ions. The detection limit for both the Subramanian and Meranger and the Kamada and Yamamoto methods is 1 ng/ml, and the methods are suitable for toxicity studies.

Unfortunately, all these studies were carried out with larger concentrations of antimony than occur in nature.

The present investigation describes the determination of antimony in aqueous solution and in BPHA–$CHCl_3$ extracts by graphite-furnace AAS, with copper as matrix modifier. Antimony(III) can be readily separated from antimony(V) by BPHA–$CHCl_3$ extraction over a wide range of acidity, and antimony(V) can be reduced to antimony(III) with potassium iodide. By this method, antimony can be

readily determined in its different oxidation states at sub-ng/ml levels.

EXPERIMENTAL

Apparatus

A Perkin–Elmer model 503 atomic-absorption spectrophotometer, equipped with an HGA-72 graphite furnace and a Hitachi model 56 chart-recorder, was employed for all absorption measurements of antimony at 217.6 nm under "argon stop" conditions. The Perkin–Elmer antimony electrodeless discharge lamp was operated under the conditions recommended by the manufacturer. A deuterium background corrector was used throughout.

For measurement of the appearance temperature of atomization, the absorbance–time profile for antimony and the temperature–time profile for the HGA-500 graphite surface were obtained simultaneously by using a laboratory-made photoconductive device and a double-pen recorder (Hitachi QD-25). The temperature scale was established by using the temperature programme on the HGA-500 power supply, and a linear response was obtained over the temperature region 1000–2500 K. The heating-rate setting of the atomizer was 0.10 K/msec. The appearance temperature T_{app} was determined by a method similar to that suggested by Sturgeon *et al.*[8]

Reagents

Standard antimony(III) solution, 1000 μg/ml. Prepared by dissolving 0.2742 g of potassium antimonyl tartrate (analytical-reagent grade) in demineralized water, adding 10 ml of 10% tartaric acid solution and diluting to 100 ml with demineralized water.

Standard antimony(V) solution, 10 μg/ml. Prepared by the procedure suggested by Kamada and Yamamoto.[7]

*N-Benzoyl-N-phenyhydroxylamine solution in chloroform, 0.10*M. Prepared by dissolving 2.13 g of BPHA (laboratory synthesized, m.p. 121°) in 100 ml of chloroform.

Saturated potassium iodide solution. Freshly prepared from the analytical-grade reagent and demineralized water just before use.

All other chemicals used were analytical-reagent grade.

Study on stabilizing effect of copper

A 20-μl portion of 0.050 μg/ml aqueous standard antimony solution was injected into a graphite tube with an Eppendorf microlitre pipette fitted with disposable polypropylene tip, along with the same volume of 1000-μg/ml aqueous copper solution. After drying the residue was ashed at various temperatures for 40 sec and atomized at 2600° for 5 sec, the absorption signals of antimony in the presence and absence of copper being measured and plotted against ashing temperature to assess the loss of antimony during the preatomization stages and the stabilizing effect of copper. The same procedure was used for the determination of antimony in the BPHA–$CHCl_3$ extract except that the organic extract was injected *after* 50 μl of 400-μg/ml copper solution had been injected and dried.

Extraction of antimony(III) and antimony(V) from water samples

All water samples were filtered through 0.45-μm Millipore filters after collection, if there was any suspended matter or sediment. A 50-ml water sample was then taken in a 100-ml separatory funnel, 4.5 ml of concentrated hydrochloric acid and 2 ml of 0.10*M* BPHA solution (in chloroform) were added, and antimony(III) was extracted by shaking for 1 min. The chloroform phase was transferred into a dry quartz centrifuge tube fitted with a quartz stopper. To the remainder of the aqueous phase 1 ml of saturated potassium iodide solution was added, the solution was thoroughly mixed and let stand for 10 min for reduction of antimony(V) to antimony(III), then 2 ml of 0.10*M* BPHA solution in chloroform were added and the extraction procedure was carried out as already described. Then 50 μl of 400-μl/ml copper solution were injected into the graphite furnace and dried, and 20 μl of the BPHA extract were injected and dried gently at 100°. The residue was ashed at 1000° for 40 sec and atomized at 2600° for 5 sec, and the antimony absorbance was measured.

RESULTS AND DISCUSSION

Stabilizing effect of copper

Antimony is one of the relatively volatile elements, so a certain loss of antimony may occur during the preatomization stages, and the ashing loss will increase with temperature and holding time. To prevent such losses, nickel has been applied as a matrix modifier.[9] From comparing the stabilizing effect of nickel, platinum and copper, it was found that copper is the most effective matrix modifier for antimony. In the presence of copper, the critical ashing temperature for antimony in aqueous solution and the chloroform extract can be raised to 1300° and 1100°, respectively. The sensitivity for antimony is improved by a factor of 1.8 for aqueous solution and 2.2 for the organic solution. The results are shown in Fig. 1.

The stabilizing effect of copper depends on the copper concentration. The absorbance for antimony increases over the range 0–12 μg of copper, and at higher amounts begins to level off. So addition of 20 μg of copper was employed for the remainder of experiments in this study.

The stabilizing effect of copper in the antimony determination has been attributed to the formation of more stable alloys or solid solutions.[10] The temperature–time profile for the graphite-tube surface at a heating rate of 0.10 K/msec and the absorbance–time profile for antimony in the absence and presence of matrix modifier are shown in Fig. 2. It can clearly be seen that the appearance temperature for antimony in the presence of copper is increased.

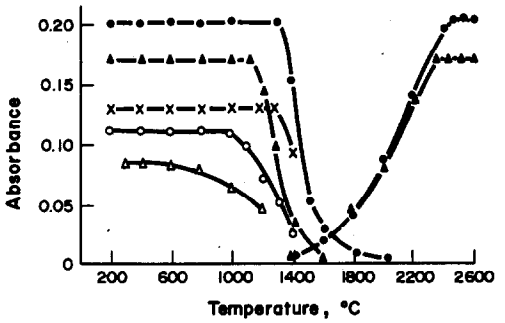

Fig. 1. Effect of ashing temperatures on the absorbance of antimony(III) in the presence and absence of matrix modifiers. (○) 1.0 ng of Sb(III) in 0.01% tartaric acid solution. (×) 1.0 ng of Sb(III) + 20 μg of Ni. (●) 1.0 ng of Sb(III) + 20 μg of Cu. (△) 1.0 ng of Sb(III) in BPHA–$CHCl_3$ extract. (▲) 1.0 ng of Sb(III) in BPHA–$CHCl_3$ extract + 20 μg of Cu.

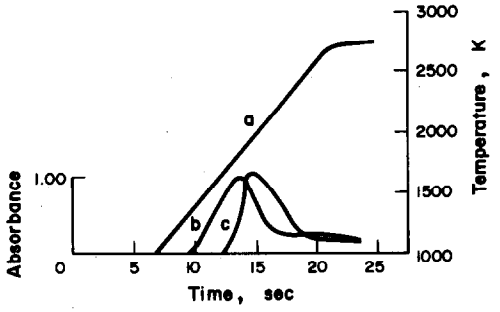

Fig. 2. Absorbance–time profile of antimony(III) in the presence and absence of copper as a matrix modifier, and temperature–time profile of the graphite-tube surface heated at 0.10 K/msec. (*a*) Temperature–time profile. (*b*) Absorbance–time profile for 25 ng of Sb(III). (*c*) Absorbance–time profile for 25 ng of Sb(III) + 25 μg of Cu.

Effect of diverse ions

It has been reported that addition of a small amount of mercury(II) can reduce the interferences from some metals in the determination of antimony.[11] In the present method much higher concentrations of diverse ions can be permitted, since the ashing temperature that can be used is high enough for any complex matrix present in the water samples to be readily burnt off. Direct determination of antimony at a concentration of 40 ng/ml showed no interferences from Te(IV) (20 μg/ml), Se(IV) (25 μg/ml), As(III) and Sn(II) (30 μg/ml), Ni, Sr, Al, Au(III), Cd, Cr(VI), Co(III), Fe(III), Li, Mg, Mn(II), Pb, Ti(III), V(V), Ag, Hg(II), Ba, Bi, Ca, Mo(VI), Zn, K, Na, silicate, phosphate, bromate, chloride, EDTA, ascorbic acid, tartrate and oxalic acid (all at least 50 μg/ml). It must be noted that the permissible amount of selenium, arsenic and tin is relatively low. The reason for this is that copper can also stabilize these elements, presumably by a similar mechanism to that for antimony. Therefore, some competing reactions may occur if these elements are also present in the heated graphite tube, since a certain amount of copper is consumed in stabilizing them. Generally, however, the concentrations of these elements in water samples are lower than these tolerance limits. Furthermore a certain degree of separation of antimony(III) from diverse ions can be achieved by extraction, so no interferences are expected.

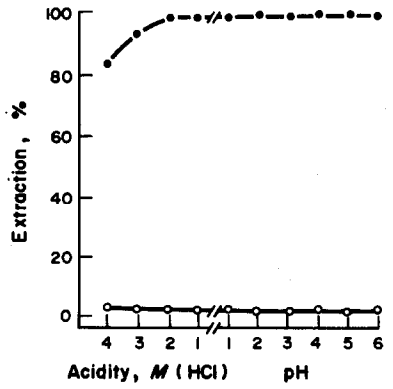

Fig. 3. Dependence of extraction of antimony(III) and antimony(V) on the acidity of the aqueous solution. Extraction conditions: aqueous phase and organic solvent both 10 ml, original concentration of Sb(III) or Sb(V) in aqueous solution 50 ng/ml. (●) Sb(III). (○) Sb(V).

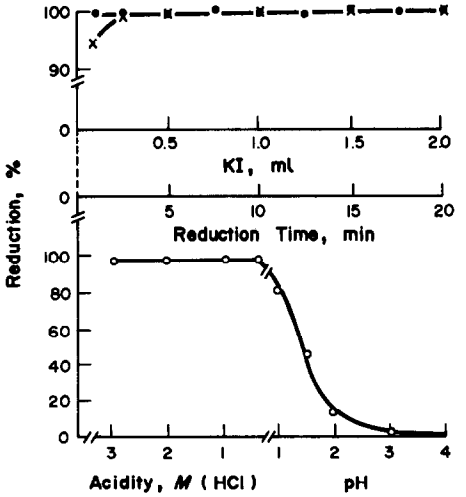

Fig. 4. Relationship between the reduction efficiency of antimony(V) and acidity of the aqueous solution, amount of KI added and reduction time. Extraction conditions: aqueous phase and organic solvent both 10 ml, original concentration of Sb(V) 50 ng/ml. (○) Effect of acidity (1 ml of saturated KI solution, 10 min reduction time). (●) Effect of amount of KI (1*M* HCl medium, 10 min reduction time). (×) Effect of reduction time (1*M* HCl, 1 ml of KI solution).

Selective extraction of antimony(III) and antimony(V)

The solution conditions affecting the selective extraction of antimony(III) and antimony(V) with the BPHA–$CHCl_3$ system were studied, including the acidity range, phase-volume ratio, conditions for reducing antimony(V) to antimony(III), and stability of the organic extract. All the results are shown in Figs. 3 and 4.

Figure 3 shows that antimony(III) can be extracted quantitatively over a wide range of acidity, from pH 6 to 2*M* hydrochloric acid, but there is virtually no extraction of antimony(V) over this acidity range, so reduction of antimony(V) to antimony(III) is a prerequisite for its successful determination. If the acidity of the aqueous solution is in the range 0.5–3*M* hydrochloric acid, addition of 0.1–2 ml of saturated potassium iodide solution and standing for 5–20 min will give quantitative reduction of antimony(V) to antimony(III). The optimum reduction conditions are: 1*M* hydrochloric acid, 1 ml of saturated potassium iodide solution, and a reducing time of 10 min.

To investigate the effect of phase-volume ratio, 5 ml of BPHA–$CHCl_3$ solution were used for the extraction of antimony from various volumes of aqueous phase containing 0.25 μg of antimony. The absorption signal obtained was constant up to an aqueous:organic phase-volume ratio of 25:1. Since

the solubility of the Sb(III)–BPHA complex in water is extremely low, the Sb(III) is quantitatively extracted into the organic layer. During and after the extraction fine droplets of chloroform containing the chelate may be dispersed in the water layer, but this does not affect the concentration of the chelate in the chloroform phase.

Varying the concentration ratio of antimony(III) to antimony(V) from 1:10 to 10:1 at a level of 11-ng/ml total antimony and determination of the antimony(III) or antimony(V) showed there was no mutual interference over this range of concentration ratios.

For the analysis of large numbers of samples, it is of prime importance that the chloroform extracts should remain stable for a sufficient period of time to permit precise determination. The absorption signals of the extract were found to remain almost unchanged for at least 48 hr (extracts kept at room temperature).

The main advantage of the proposed method using the BPHA–$CHCl_3$ extraction system in combination with matrix modification, over the APDC–MIBK method,[6,7] is that lower concentrations of antimony in water samples (down to 0.2 ng/ml) can be determined.

Stabilizing effect of tartaric acid on storage of antimony(III) and antimony(V) in water samples

Of great importance in the differential determination of antimony(III) and antimony(V) is the question of their interconversion during sampling, transport and analysis. Laboratory experiments were designed for studying this question. To a lake water or waste-water sample, in which no antimony could be detected by the present method, appropriate quantities of standard antimony(III) or antimony(V) solution were added to make the antimony concentration 2 ng/ml, and the concentration of antimony was determined at regular intervals to assess the stab-

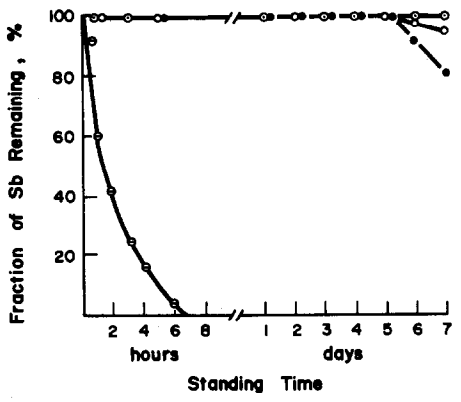

Fig. 5. Stabilizing effect of tartaric acid on antimony(III) and antimony(V). Antimony(III) or antimony(V) 2 ng/ml, concentration of tartaric acid 1%. (⊖) Water sample + Sb(III). (●) Water sample + Sb(III) + tartaric acid. (○) Water sample + Sb(V). (⊙) Water sample + Sb(V) + tartaric acid.

Table 1. Determination of antimony(III), antimony(V) and total antimony in water samples*

Sample	Determination of Sb(III)			Determination of Sb(V)			Determination of total antimony		
	[Sb(III)] in sample, *ng/ml*	[Sb(III)] added as spike, *ng/ml*	Total [Sb(III)] found, *ng/ml*	[Sb(V)] in sample, *ng/ml*	[Sb(V)] added as spike, *ng/ml*	Total [Sb(V)] found, *ng/ml*	[Sb(III)+ Sb(V)] in sample, *ng/ml*	[Sb(III)+ Sb(V)] added as spike, *ng/ml*	[Total Sb(III)+ Sb(V)] found, *ng/ml*
Sea-water 1	ND	0.20	0.18	0.30	0.40	0.70	0.30	0.60	0.85
Sea-water 2	ND	0.20	0.20	—	—	—	—	—	—
Lake water 1	ND	0.20	0.20	ND	0.20	0.21	ND	0.40	0.40
Waste-water 1	ND	0.20	0.18	ND	0.20	0.18	ND	0.40	0.35
Waste-water 2	ND	0.20	0.16	0.30	—	0.30	—	—	—
Waste-water 3	ND	0.20	0.20	0.60	1.0	1.5	—	—	—
Waste-water 4	ND	0.20	0.19	0.70	—	0.70	—	—	—
Waste-water 5	ND	1.0	1.0	1.6	1.0	2.5	1.5	2.0	3.6
Waste-water 6	0.30	1.0	1.3	2.1	2.0	4.0	2.5	3.0	5.8
Waste-water 7	0.90	1.0	1.8	6.6	—	6.6	—	—	—

* Averages of two replicates.
ND = not detected.

ility of the two oxidation states. Figure 5 shows the results. Antimony(III) in lake water or waste water appears to be unstable, since none could be detected after standing for 6 hr, presumably because of conversion into antimony(V) by some oxidants present in water samples. However, if tartaric acid was added to give a 1% concentration in the samples, the antimony(III) concentration remained unchanged for at least 5 days. Antimony(V) is relatively stable anyway, and no significant stabilizing effect of tartaric acid on antimony(V) during storage was observed.

The Kamada and Yamamoto method was also used, with the APDC–MIBK system applied for the extraction of antimony(III) at pH 6 and of total antimony at pH 1. The results confirmed that no antimony(III) was extracted at pH 6 in the absence of tartaric acid, but an equivalent amount of antimony(V) was found by extraction at pH 1 when only standard antimony(III) was originally added. This led us to the conclusion that a significant change in species distribution took place rapidly in the absence of tartaric acid. The decrease in absorbance of antimony(V) after a standing period of 6 days is probably due to adsorption of antimony(V) on the walls of the container.

The rate of conversion of antimony(III) into antimony(V) decreases with increasing acidity of the water samples. Addition of tartaric acid prevents the conversion and may be employed in the practical analysis of water samples.

Analysis of water samples

Although no pronounced interferences occur from diverse ions when the matrix modification technique is used in combination with the solvent extraction procedure, the standard-addition method was applied for the analysis of water samples to prevent unpredictable interferences due to the extreme complexity of industrial effluents. The results of some water samples are summarized in Table 1. The results refer only to the free extractable antimony(III) and reducible antimony(V). No attempt was made to recover other antimony species.

The relative standard deviation was found to be 3% for 9 replicate determinations at an average antimony level of 3.5 ng/ml. The characteristic concentration of the method for 1% absorption was found to be 0.04 ng/ml.

Tests for release of antimony from the Millipore filters,[1,12] failed to detect any.

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SOME REMARKS ON THE QUANTITATIVE EXPRESSION OF THE SELECTIVITY OF AN ANALYTICAL PROCEDURE

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Summary—The selectivity of an analytical method may be defined as expressing the degree to which a component can be determined in the presence of other but similarly behaving components without interference. The method itself will usually serve for determination of the other components, under slightly different conditions. The selectivity is closely related to the resolution of the method and also to the resolution of the instrument used for obtaining the signals. A simple formula is suggested for expressing the percentual degree of selectivity of an analytical procedure, on the basis of signal overlap caused by the interfering components, and can be used quite generally. A distinction is made between the terms analytical selectivity and selectivity of an analytical procedure, and between selectivity and specificity.

The term selectivity is widely used among analysts for characterization of a method or a reagent with respect to the species which is to be determined. The term is always concerned with the determination (or detection) of one component in the presence of other constituents. The more selective the method (or reagent), the less is the interference of the other components in the determination (or detection).

In a broader sense, the term is sometimes used by chemists to express the preferential reaction of a reagent with one component rather than another. For example the reaction between an optically active reagent and two enantiomers may be selective for one of them. If the free-energy changes of the two diastereomer formation reactions differ, *i.e.*, the equilibrium constants are not the same, the difference may be used as a quantitative expression of the selectivity.

Similarly we can say an ion-exchange resin is selective, if the free-energy changes for sorption of two similar ions with the fixed ionic groups of the resin are different from each other under the given conditions.

Since the characterization of an analytical method needs quantitative specification, many attempts have been made to give a quantitative formulation of selectivity. The first concept was by Belcher,[1] who proposed a "selectivity index", and was further developed by Belcher and Betteridge,[2–4] but only as a qualitative expression. Kaiser[5] gave a mathematical formula by which the selectivity of a method that is capable of determining several species may be expressed quantitatively. Both concepts have been criticised by Wilson.[6] The main objection to Kaiser's proposal was that it was not suitable for practical purposes. Pszonicki[7] proposed the use of what he called non-specifity coefficients, by which he could express quantitatively the interference of other components in the determination of one particular component. We think that Pszonicki was right in not calling these terms selectivity coefficients. Specificity is not the same as selectivity, since it is concerned with a reagent that reacts with only one component (or one group of components), and does not deal with the relative reactivity of more than one component.

In Gottschalk's concept[8] the selectivity is defined simply as the extent of non-interference, and the degree of selectivity is expressed by the threshold amount of an interferent that will produce a detectable effect on the main reaction. We think that this concept is too general, and includes too much in the term selectivity.

Recently Fujiwara *et al.*[9] successfully used an extended formula for quantitative expression of the selectivity of atomic-absorption and emission spectroscopic procedures in the determination of trace metal constituents in different matrices. They introduced into the original Kaiser equation the concentration of the analyte and the concentrations of the interferents, and also terms expressing the interaction effects of the interferents.

The intention of the present paper is no more than to support the concept just mentioned, and to suggest a slightly modified form for the general expression of the selectivity of an analytical procedure.

The following basic assumptions are made.

(1) We speak of the selectivity of a procedure only when more than one of the components present can give a response to the analytical system used.

(2) The selectivity of a procedure refers only to systems where components of similar behaviour and having similar physical and chemical properties are present. We would not speak of selective determination of manganese, if no other metal ions were

present in the system, even though interfering substances such as proteins might be present. This restriction of the term corresponds both to traditional analytical usage and the concept of Kaiser.[5] What is meant by similarity of the components depends, of course, on the actual analytical problem. Acetaldehyde can be determined selectively in a solution containing other types of aldehydes, but all primary amines can be selectively determined (as a group) in a solution in which secondary and tertiary amines are also present.

(3) Selectivity is concerned only with cases where the analytical method is suitable (or at least potentially suitable) for determination of more than one component of similar behaviour and giving similar signals. If the method serves for the determination (or detection) of only one component it is said to be specific, not selective. By extension, however, if the method gives identical response to a substituent group, irrespective of the matrix to which the group is attached, but to no other group, it can again be regarded as specific (for the group concerned).

(4) The quantitative expression of the selectivity will refer only to a given procedure with given concentration conditions.

(5) Analytical methods suitable for determination of different but similar components (elements, compounds, phases or groups of these) can be represented diagrammatically with two co-ordinates. One co-ordinate (let us call it z) identifies the components, the other (x) their amounts. Table 1 gives some examples. Selectivity is concerned with the resolution of the method along the z co-ordinate.

For example, a mixture of two acids can be analysed by titration with alkali in aqueous medium, with suitable acid–base indicators, if the logarithms of the dissociation constants differ by 4 or more. If a pH-meter is used instead of an indicator, the acids can be titrated selectively if their pK values differ by not less than 3, because the uncertainty of the end-point is lower. However, this rule only holds if the higher pK value does not exceed 9. If the pK values of the acids are too close to each other the selectivity can be increased by using a different solvent (*e.g.*, acetic acid if both acids are strong) or by adding a reagent which will make one of the acids appear stronger, *e.g.*, mannitol for boric acid, or copper for acetylacetone.[10]

Again, lead(II) and thallium(I) have half-wave potentials very close to each other, but thallium can be selectively determined polarographically if the half-wave potential of the lead is shifted to more negative values by use of complexing agents. If the half-wave potentials differ by at least 0.2 V, the determinations can be carried out selectively.

If two metal ions are to be determined spectrophotometrically in presence of each other and the absorption curves overlap extensively, we cannot selectively determine them directly, however good the resolution of the instrument. We have to use a selective reagent which will shift the absorption curves so that no overlap occurs.

Further, in a chromatographic separation compounds of similar behaviour can only be determined in presence of each other if there are no overlapping bands, or no components have the same retention volume. For completely successful determination of all the components the following requirements must be fulfilled. The ratios of the distribution coefficients of pairs of contiguous components (the separation factors, or selectivity factors) should be sufficiently different from unity; the column used should have adequate resolution (this determines the peak width); the absolute values of the distribution coefficients should be in a suitable range. If the distribution coefficients of two components are 10^{-1} and 10^{-2} (a separation factor of 10) they cannot be separated and determined individually, because they will both be eluted in the dead volume, without appreciable retention. If, however, we use another column with a different and more suitable stationary phase, we may be able to separate them. For chromatographic determination of all the components present a universal detector (with a cell size corresponding to the column resolution) must be used, but if only one component or group of components is to be determined a specific detector can be used. Thus chromatography may be selective or specific.

From these examples the following conclusions can be drawn.

(1) Analytical methods used for determination (or

Table 1. Two co-ordinate analytical methods

Method	z	x
Gravimetry	Solubility scale	Mass of precipitate
Acid–base titration	pH	Volume of titrant
Complexometric titration	pM	Volume of titrant
Redox titration	Redox potential	Volume of titrant
Spectrophotometry	Wavelength	Absorbance
NMR	Frequency	Peak area
Polarography (d.c.)	Voltage	Wave height
Chromatography	Volume, time	Peak area
Thermogravimetry	Temperature	Change in mass
Electrogravimetry	Electrode potential	Mass deposited

detection) of compounds similar to each other in behaviour can be characterized by the resolution along the z co-ordinate.

(2) This resolution depends on the following factors.

(*a*) The chemical and physical properties of the substances to be determined;

(*b*) The chemical and physical properties of the system used, *e.g.*, the solvent used for acidimetric titrations, the stationary phase for chromatographic separation.

(3) The resolution can be altered at will by means of physical and chemical factors, for all analytical methods.

(4) The selectivity is acceptable if the error caused by incomplete resolution in the z direction is less than that of the measurement itself (in the x direction).

(5) We may speak of selective determination of several compounds by use of the same method, but the number of such components which can be selectively determined when present together will depend on the informational power of the method used. Thus, theoretical considerations show that in aqueous medium only 3 or 4 acids at most can be titrated selectively in presence of each other, and even then only if their pK values are optimally spread. In methyl isobutyl ketone medium six or more acids can be titrated selectively. In chromatography, depending on the resolution of the column and on the components themselves, the number of selectively determinable components can be very large. Indeed, the information power of this technique is one of the highest of all analytical systems, if the components are distributed uniformly over the chromatogram.

(6) Selectivity, with respect to a pair of components, depends on the following factors.

(*a*) The thermodynamic (or kinetic) properties of the two components under the given conditions. In titrimetric determination of acids the corresponding quantities are the pK values, in spectrophotometry the electronic transition energies, in chromatographic separations the distribution coefficients; for separations based on differences in diffusion rates or mobilities, kinetic parameters are used instead of thermodynamic. For selective determinations both the ratios (or differences) of the parameters and their absolute values are important.

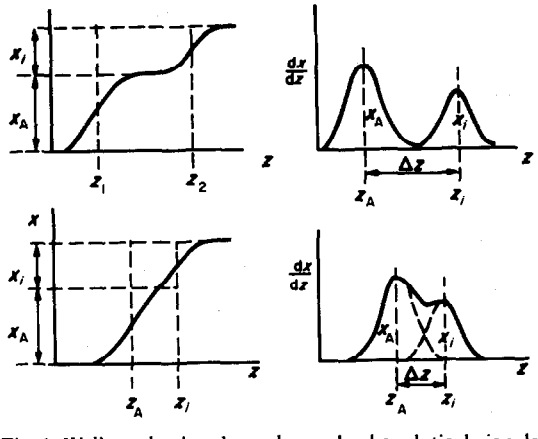

Fig. 1. Well resolved and poorly resolved analytical signals, and their derivatives.

(*b*) The resolution ability of the analytical system along the z co-ordinate. Figure 1 shows well resolved and poorly resolved signals for two similar components (A and B). If the signals are Gaussian the resolution R can be expressed in the usual way:

$$R = \frac{\Delta z}{2(\sigma_A + \sigma_B)}$$

where Δz is the difference in position of the two peaks, and σ_A^2 and σ_B^2 are the variances of the peaks. If $R > 1$, the determinations of both components are selective.

(*c*) The resolution power of the instrument used, along the z co-ordinate. This is usually expressed as $\delta z/z$. For selectivity to be obtained the following inequality must hold:

$$\delta z < \Delta z.$$

That is, the width of the "window" of the instrument must be smaller than the distance between the two adjacent signals. If this is not the case, the instrument cannot see separately the signals of the components.

This concept is in accordance with the selectivity term used for telecommunication systems, where the frequency width is the quantity responsible for the resolution.

On the basis of the discussion above the following definition is proposed for numerical representation of the selectivity of a procedure.

The *degree of non-selectivity* (Δ_A) will be defined as the ratio of the total signal from a given amount of interfering components to the signal for the component to be determined:

$$\Delta_A = 100 \frac{\Sigma \gamma_i c_i}{\gamma_A c_A} \%$$

where

$$\gamma_A = \partial x_A / \partial c_A \text{ and } \gamma_i = (\partial x_i / \partial c_i)_{c_A = 0}$$

are the sensitivities for the analyte A and the interfering (*i.e.*, similarly behaving) component i under given instrumental conditions (x_A and x_i are the signals, and c_A and c_i the concentrations). The selectivity (S_A) is then given by $S_A = (100 - \Delta_A)\%$.

In the simple case (Fig. 2) when the signals are Gaussian and the determination is based on the peak area T_A, if T_i is the area of overlap of x_A and x_i,

$$\Delta_A = 100 \frac{\Sigma T_i}{T_A} \%$$

Intercomponent effects may be taken into account by means of a term $\Sigma \mu_i c_i$ (see Fujiwara *et al.*[9]):

$$\Delta_A = 100 \frac{|\Sigma \gamma_i c_i + \Sigma \mu_i c_i|}{\gamma_A c_A} \%$$

where

$$\mu_i = \left(\frac{\partial x_A}{\partial c_i}\right)_{c_A}$$

A small problem arises with the sign of the numerator in this expression for Δ_A. To obtain a useful and practical expression for selectivity, the absolute value of the algebraic sum of the terms in the numerator should be taken used.

Example. In the paper by Fujiwara *et al.*,[9] in the determination of cadmium in sea-water by atomic-absorption and atomic-emission spectrometry, the sensitivity and concentration values summarized in Table 2 were found. From these data, the selectivities of the two procedures for determination of cadmium up to 0.5 mg/l. in the sea-water used are as follows.

For the atomic-absorption procedure

$$S_{Cd} = 100\left[1 - \frac{10500 \times 0.0009 + 1350 \times 0.0035 + 400 \times 0.0085}{0.5 \times 322}\right] = 89.1\%$$

For the atomic-emission procedure:

$$S_{Cd} = 100\left[1 - \frac{|-0.0011 \times 10500|}{0.5 \times 1480}\right] = 98.4\%$$

The corresponding values calculated by means of Kaiser's original formula are 2.5×10^4 and 1.35×10^6, and by means of the Fujiwara formula[9] 8.42 and 66.3 respectively.

Factors affecting selectivity

(1) We can choose conditions which will increase the resolution of the method by increasing Δz to decreasing the peak-width σ. The use of masking reactions for interfering (similarly behaving) components can either shift the positions of their signals (to increase Δz) or decrease their sensitivities. In both cases the overlap will be smaller and therefore the selectivity increased. If the sensitivity for the interfering components is sufficiently reduced, the method will become specific.

In chromatographic methods, both Δz and σ can be influenced by use of different stationary or mobile phases and column parameters.

(2) It was mentioned above that the resolution of the instrument must be higher than that of the method. Thus two components with adjacent light-absorption peaks cannot be selectively determined by colorimetry (*i.e.*, photometry with broad-spectrum polychromatic radiation). By means of a suitable band filter, however, selective determination can be realized unless the absorption bands overlap, in which case selectivity can only be achieved by using nearly monochromatic radiation. In the last case Δz will be lower but so will the quantity of light arriving at the detector.

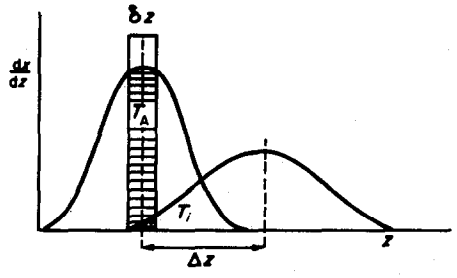

Fig. 2

Table 2. Concentration and sensitivity values for atomic-absorption and atomic-emission determination of cadmium in sea-water, with the 228.8 nm analytical line; data from the paper by Fujiwara *et al.*[9]

Metal	Concentration, *mg/l.*	Sensitivities (γ) AAS	AES
Cd	0.5	322	1480
Na	10500	0.0009	−0.0011
Mg	1350	0.0035	0
Ca	400	0.0085	0
Cu	0.5	0	0
Zn	0.5	0	0
Fe	0.5	0	0
Mn	0.5	0	0

Remarks

The proposed used of Δ_A values to define the degree of selectivity is very similar to that of the terms standard deviation and systematic error, which are accepted for the expression of precision and accuracy respectively.

The proposed percentual non-selectivity value ($\Delta_A\%$) should not be confused with the cross-contamination (overlap) factor used in chromatography, though in a few cases they are formally similar.

Since the selectivity of the method (or procedure) depends strongly on the conditions used, we may also speak of selective determination of a certain compound or element in the presence of specified amount (*e.g.*, 1000-fold ratio) of another (similarly behaving) component. In this case, the signals for calculation of the degree of selectivity are those corresponding to the specified ratio of the components, and this condition must be stated unambiguously.

It can happen that Δ_A exceeds 100%. In such cases, of course, there is no selectivity at all.

The parameters (selectivity coefficients, ΔpK values, *etc.*) affecting analytical selectivity play an important role in analytical chemistry, but are not in themselves sufficient to give quantitative information on the selectivity of an analytical method or procedure.

According to the basic assumptions—mentioned earlier—the term selectivity is restricted to systems of components that behave similarly. Therefore the interference of phosphate ions in the flame photometric determination of calcium is not a selectivity problem, but only a common interference problem (akin to turbidity in spectrophotometric methods), the

phosphate not being "similar" to calcium. Although phosphate can be determined indirectly by flame photometry, the principle of the method is not similar to that for the calcium determination.

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CONVENTIONAL FLUORESCENCE SPECTROMETRY OF POLYNUCLEAR AROMATIC HYDROCARBONS IN SHPOL'SKII MATRICES AT 77 K*

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Summary—A simple fluorimeter assembled from commercial components and its use for the study and application of the Shpol'skii effect on polynuclear aromatic hydrocarbons (PAHs) in n-alkane matrices at 77 K are described. The correlation between the dimensions and geometries of PAHs and their corresponding Shpol'skii solvents is considered. Analytical figures of merit have been evaluated, and the power of the Shpol'skii technique with a conventional fluorimeter in the direct qualitative and quantitative determination of 11 PAHs in mixture is demonstrated. Comparisons with conventional room-temperature fluorescence and laser-excited Shpol'skii spectrometry are also commented upon.

The Shpol'skii effect was reported in 1952 by Shpol'skii *et al.*[1] Some aromatic compounds, when dissolved in selected n-alkane solvents and then frozen in liquid nitrogen (b.p. 77 K), exhibited extremely narrow-banded and line-rich "quasi-linear" fluorescence spectra. During the following two decades, several reports were published concerning the Shpol'skii effect, as well as many publications which described its application to both qualitative and quantitative determinations of real samples, as reviewed by Kirkbright and deLima.[2] In a series of three papers, Kirkbright and co-workers[2–4] reported an extensive study of the quasi-linear luminescence emission spectra of 23 PAHs in n-pentane, n-hexane, n-octane and n-decane, and the luminescence excitation spectra of 12 PAHs at 77 K in hexane, heptane and octane, and demonstrated the fingerprinting ability of quasi-linear emission and excitation spectra for qualitative and quantitative determination of a mixture of 8 PAHs without prior separation. Colmsjo and Stenberg illustrated the solvent effect and the improvement in spectral characteristics obtained by lowering the temperature from 77 to 63 K; the peaks become better resolved and better defined in position, which facilitates identification of PAHs in mixtures.[5,6] A library of 33 reference spectra of common PAHs was used to identify by comparison PAHs in fractions collected in the chromatography of a gasoline automobile exhaust sample; however, only 10 reference fluorescence spectra were shown.[7] The need to expand the library of reference spectra for the identification of the remaining peaks in well-resolved Shpol'skii fluorescence spectra from chromatographic fractions was again remarked on later.[8]

The application of laser excitation to low-temperature Shpol'skii luminescence spectra and determination of PAHs was pioneered by Vo-Dinh and Wild,[9] Abram *et al.*[10] and Winscom *et al.*,[11] who used commercially available lasers to enhance the selectivity and sensitivity of the method. In 1980, Fassel *et al.*[12] reported the use of a nitrogen-laser-pumped tunable dye-laser for the selective excitation of 4 PAHs in a solvent-refined coal liquid sample and a shale oil sample. Selected excitation wavelengths within the response curve of a single dye were employed. Recently, the same authors have presented the use of site-specific laser-excited Shpol'skii spectroscopy for the direct qualitative and quantitative characterization of complex mixtures of PAHs, including multialkylated isomers, at a temperature of 15 K obtained with a closed-cycle helium refrigerator.[13]

Laser-excited Shpol'skii spectroscopy seems to have great analytical potential, but the extremely high investment cost of tunable dye-lasers and pump-lasers and their complex and time-consuming operation hamper the use of this powerful technique. In this paper, a reliable double-monochromator spectrofluorimeter employing a xenon-arc continuum source is described. This system allows rapid and reproducible acquisition of quasi-linear luminescence emission spectra and rapid change of selected excitation wavelength for the analysis of multicomponent mixtures of PAHs. The correlation between the linear dimensions and molecular geometries of PAHs and their corresponding Shpol'skii alkane solvents is explicitly presented for 23 typical PAHs. Such a comprehensive survey should be a helpful guide in choosing the solvent that gives the best-defined, best-resolved and narrowest peaks for the unambiguous identification of other PAHs. Limits of detection obtained with the present system are evaluated; these values are the basis for comparison of the detection power for different PAHs. A table of excitation and emission maxima is also given which allows selection of optimal wavelengths for direct qualitative and quantitative determination of 23 PAHs in mixtures, as well as gener-

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ation of a data base of reference spectra. In addition, the linear dynamic range and the upper limit of analytically useful concentration are discussed. Finally, results for the application of conventional fluorimetry with a Shpol'skii solvent (n-heptane) to the quantitative analysis of a synthetic mixture of 11 PAHs is presented.

EXPERIMENTAL

Apparatus

A simple spectrofluorimeter was assembled using an Eimac high-intensity xenon-arc lamp (300 W) (Varian/Eimac Division, San Carlos, CA) as the light-source, a Heath monochromator with wavelength-control unit as the dispersing element for excitation wavelengths, a commercial Dewar-flask cell compartment to hold liquid nitrogen and the quartz sample tube (3 mm i.d., 5 mm o.d.), a GCA/McPherson EU-700-77 scanning monochromator and an EU-701-30 photomultiplier tube as the dispersing element and detector for emission wavelengths (GCA/McPherson Co., Acton, MA), a low-noise nanoammeter[14] to process the photomultiplier current, and an Omniscribe B5217-51 recorder (Houston Instrument, TX).

Reagents

n-Pentane, n-hexane, n-heptane and n-nonane were distilled-in-glass grade solvents obtained from Burdick & Jackson Laboratories Inc. (Muskegon, MI). n-Octane was 99 + % gold-label grade obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Standard PAHs were of different commercial brands,[22,23] and were recrystallized 3 times from ethanol, as necessary.

Procedure

High-resolution excitation and emission spectra were recorded for solutions in n-alkanes at 77 K. Each PAH solution (200 μl) was rapidly frozen in the quartz sample cell. To scan the luminescence emission spectrum, a 2-mm slit-width was used on the excitation monochromator (corresponding to a spectral band-pass of about 4 nm) and a 0.1-mm slit-width was used on the emission monochromator (corresponding to a spectral band-pass of 0.2 nm); and to scan the excitation spectrum, these monochromator slit-widths (and spectral band-passes) were interchanged. The emission monochromator was equipped with a programmable filter assembly (EU-700-56) which provided filters for broad-band isolation of given spectral regions to reduce stray light and scatter. The emission spectrum was scanned at a rate of 6.0 nm/min, and an RC-filter time-constant of 0.5 sec was used on the nanoammeter.

The internal reference method was used for calibration; the luminescence intensity of the internal standard was concurrently measured along with that of the analyte emission. The internal standard compensated for variation of experimental conditions such as the position of the sample cell in the optical path, the rate of cooling and hence the inhomogeneity of the sample surface, and the source intensity.[2,12,13] For quantitative work, a combined standard addition–internal standard procedure was used to compensate for any "inner filter" effect or the effect of energy transfer between the analyte and other molecules present.[2] The internal standard in this case could be another PAH in the sample instead of an externally added reference, since in analysis of "real" samples, it might be difficult to find a substance having a quasi-linear luminescence emission spectrum did not overlap that of the analyte(s) and whose excitation spectrum did overlap that of the analyte(s).

Table 1. Half-widths (*nm*) of fluorescence bands of PAHs in n-alkane matrices at 77 K

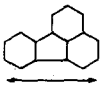
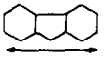
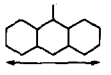
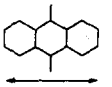
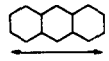
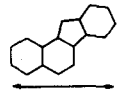
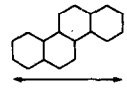
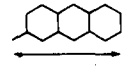
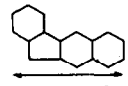
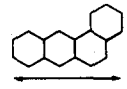
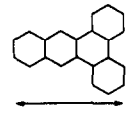
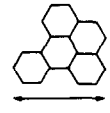
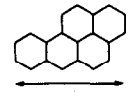
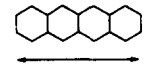
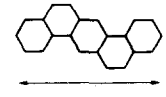
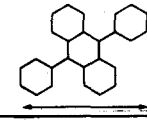
PAH	Formula	Length, Å*	n-Pentane Ex	n-Pentane Em	n-Hexane Ex	n-Hexane Em	n-Heptane Ex	n-Heptane Em	n-Octane Ex	n-Octane Em	n-Nonane Ex	n-Nonane Em
Acenaphthene[a]		4.8	+ 1.6	+[f] UD	+ 2.1	+ UD[g]	+ 2.1	+ 1.7				
Naphthalene[a]		4.8	+ 2.1	+ 2.2	0 3.4	+ 2.3	0 3.1	+ 2.3				
Fluoranthene[b]		6.6	0 2.8	0 UD	+ 1.5	+ UD	+ 1.8	0 UD				
Fluorene[b]		6.8	+ UD	0 UD	++ 1.0	+ UD	+ 1.5	+ UD				
Phenanthrene[b]		6.0	+ 2.4	++ 1.0	+ 2.0	++ 1.2	+ 1.9	+ 1.4				
9-Methylanthracene[b]		7.2	+ 2.0	+ 1.6	+ 1.3	+ 1.8	0 2.7	0 2.6	0 2.9	0 3.3		
9,10-Dimethylanthracene[b]		7.2	0 3.6	0 8.2	+ 1.4	+ 1.6	+ 2.3	0 2.5				
Pyrene[b]		6.0	0 2.4	++ 0.9	0 2.2	++ 0.7	0 2.3	++ 1.1				
1-Methylpyrene[b]		6.2	0 3.4	++ UD	0 3.4	++ 0.7	0 2.7	+ UD				

Table 1—*cont.*

PAH	Formula	Length, Å*	n-Pentane Ex	n-Pentane Em	n-Hexane Ex	n-Hexane Em	n-Heptane Ex	n-Heptane Em	n-Octane Ex	n-Octane Em	n-Nonane Ex	n-Nonane Em
Anthracene[c]		7.2			+ 2.5	+ UD	++ 1.1	++ 0.9	+ UD	+ UD		
Perylene[c]		7.0[h]			+ 1.9	++ 0.9	+ 1.6	++ 1.0	+ 1.8	+ 1.8		
1,2-Benzofluorene[c]		8.5			0 3.0	+ UD	0 2.4	+ UD	0 3.0	0 UD		
Chrysene[c]		8.4			0 4.9	+ 1.4	0 4.1	+ 0.7	0 3.2	0 UD		
2-Methylanthracene[d]		8.6					0 4.8	0 UD	+ 2.1	0 2.9	0 3.8	0 5.9
2,3-Benzofluorene[d]		8.5					0 UD	+ UD	+ 1.1	++ 1.0	+ 1.2	+ 1.3
1,2-Benzanthracene[d]		8.4			+ UD	+ 1.4	+ UD	+ UD	+ 3.0	++ 1.2	+ 2.9	+ 1.8
1,2:3,4-Dibenzanthracene[d]		8.4					0 5.4	++ 0.7	0 4.9	++ 0.5	0 5.3	+ 2.2
1,2-Benzopyrene[d]		7.0[h]					+ 1.7	++ 1.2	+ 1.6	++ 0.8	+ 2.2	+ 1.5
1,12-Benzoperylene[d]		7.0[h]					+ UD	++ 0.9	+ UD	++ 0.9	+ 1.9	+ 1.2
3,4-Benzopyrene[d]		8.4					+ 1.8	+ UD	++ UD	++ 0.5	+ 2.5	+ UD
Naphthacene[e]		9.6					0 UD	0 UD	0 3.9	+ 1.9	+ 1.4	++ 1.2
1,2:5,6-Dibenzanthracene[c]		11.1					0 4.0	+ UD	0 4.3	+ UD	0 3.5	++ 1.0
9,10-Diphenylanthracene[i]		9.8			0 8.7	0 11.0	0 8.2	0 11.7	0 8.2	0 11.8	0 8.1	0 12.7

* Bond lengths used in the calculation of the lengths of the PAHs were: [25]C–C aromatic = 1.4 Å, C–C aromatic to aliphatic = 1.54 Å, C–C biphenyl = 1.48 Å.

[a] n-Pentane being the Shpol'skii solvent.

[b] n-Hexane being the Shpol'skii solvent.

[c] n-Heptane being the Shpol'skii solvent.

[d] n-Octane being the Shpol'skii solvent.

[e] n-Nonane being the Shpol'skii solvent.

[f] + some Shpol'skii effect, + + extensive Shpol'skii effect, 0 no Shpol'skii effect.

[g] Fwhm could not be determined because of overlap of peaks.

[h] Molecule has more than two layers of aromatic rings.

[i] No solvent had a clear-cut advantage as Shpol'skii solvent.

RESULTS AND DISCUSSION

Correlation between linear dimensions and molecular geometries of PAHs and their corresponding Shpol'skii alkane solvents

In Table 1, a qualitative picture is shown of the relationship between the size and shape of PAHs and the trend of the Shpol'skii effect with respect to n-alkane solvent type. PAHs having the same alkane as their "Shpol'skii solvent" are grouped together. The "Shpol'skii solvent" is taken as being that alkane which gives the best spectral resolution (*i.e.*, the luminescence emission and excitation spectral bands from the frozen crystalline matrix have the narrowest band-widths). It is often the case that use of the Shpol'skii solvent also results in the simplest luminescence spectra. The effective band-width depends on the number of "sites",[13] *i.e.* the number of different orientations of the molecules in the polycrystalline matrix, and the capability of the fluorimeter system to resolve them, yet, with a conventional fluorimeter, it is not possible to apply site selection, and hence the narrowest "band-width" has been used as the criterion for choosing the Shpol'skii solvent. It is evident that there is a general relationship between the dimensions and geometries of PAHs and the corresponding Shpol'skii solvents. Shpol'skii,[15] and more recently Brown *et al.*,[16,17] have mentioned that the degree of broadening of vibronic absorption linewidths caused by the specific orientation effect, depends on the degree of similarity between the molecular dimensions of the PAH and the n-alkane. The crystalline structures of n-pentane, hexane, heptane and octane have been well-characterized by X-ray methods.[18–21] The n-pentane structure is orthorhombic, with 4 molecules per unit cell and a volume of 543 Å^3. Both n-hexane and n-octane are triclinic with one molecule per unit cell and volumes of 165 and 208 Å^3, respectively; n-heptane is triclinic, with 2 molecules per unit cell and a volume of 382 Å.^{3} Santoni and Mandon have stated that the most favourable conditions for the appearance of quasi-linear spectra are equality of length of the alkane and PAH molecules and similarity of geometrical form.[24] Thus n-pentane would be the Shpol'skii solvent for naphthalene, n-heptane for anthracene, and n-nonane for naphthacene, as illustrated in Fig. 1. Such ideal matches do not exist for PAHs which are not straight chains of fused rings or which have alkyl groups at some position in the molecule. However, the effective lengths of these PAHs can be measured along an axis through the PAH molecule which, when placed parallel to the major axis of the n-paraffin, permits the PAH molecule to have maximum contact with the alkane. Their values are given in Table 1. Basically, it can be seen that the effective lengths serve as a reliable indication to the Shpol'skii solvents for the PAHs, failing only when the PAH molecule has more than two layers of aromatic rings in its structure, such as perylene, 1,2-benzopyrene and 1,12-benzoperylene, when the breadth of the molecule also comes into effect. In general the results in Table 1 agree with those of Kirkbright *et al.*[2,4] and Colmsjo *et al.*,[5,7] with a few contradictions which probably arise because of different opinions on the definition of "Shpol'skii solvents".

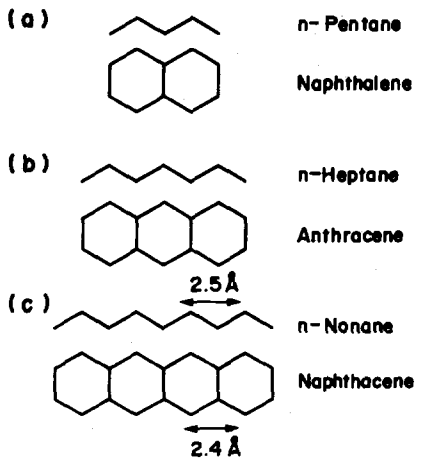


Fig. 1. Conditions favourable for the Shpol'skii effect: equality in dimensions and similarity of geometrical form between (a) n-pentane and naphthalene, (b) n-heptane and anthracene, and (c) n-nonane and naphthacene.[23]

Limits of detection and linear dynamic range of conventional Shpol'skii luminescence at 77 K

The detection power of the present system has been evaluated by calculating the limits of detection from the peak heights both in the excitation spectrum and the emission spectrum of individual PAHs at a concentration of about 1 ppm in their Shpol'skii solvents. Note that measurements were not always made at the most intense excitation and/or emission wavelengths; if the excitation and emission maxima turned out to be too close together, the next most intense excitation or emission peak was chosen in order to avoid extensive scatter; in such cases, the ratio of the height of the measured peak to that of the most intense peak in the same spectrum is given in parentheses after the wavelength of the measured peak. A second wavelength was also chosen if the overlapping region between the excitation and emission spectra was to be scanned and the most intense peak happened to fall inside that region. It can be seen from Table 2 that, in general, the system used results in limits of detection of about 10 ng/ml. The last column in Table 2 includes the limits of detection by conventional room-temperature fluorimetry.[22] The present method is about three orders of magnitude less sensitive than room-temperature fluorimetry. One reason is that much smaller spectral band-passes (4 and 0.2 nm) are used, compared to the 12 nm for the room-temperature work.[22] Another reason is that with a frozen crystalline alkane matrix, snow is formed, so only the front surface is irradiated instead of the entire volume.[23]

The standard calibration curve for 1-methylpyrene

Table 2. Limits of detection by conventional Shpol'skii luminescence excitation and emission at 77 K[a]

PAH	Shpol'skii solvent	Excit. maximum, *nm*	Excitation LOD *ng/ml* (λ_{em}, *nm*; %)[b,c]	Emiss. maximum, *nm*	Emission LOD, *ng/ml* (λ_{ex}, *nm*; %)[c,d]	RTF LOD, *ng/ml*[e]
Acenaphthene	Pentane	306.3	30 (334.4; 36%)	318.9	30 (291.4; 84%)	0.04
Naphthalene	Pentane	289.3	50 (334.8)	334.8	40 (289.3)	0.2
Fluorene	Hexane	301.9	10 (317.3)	317.3	10 (301.9)	0.006
Fluoranthene	Hexane	289.3	40 (465.2; 72%)	434.4	40 (289.3)	0.02
Phenanthrene	Hexane	294.5	20 (345.3)	345.3	20 (294.5)	0.07
9-Methyl-anthracene	Hexane	387.6	2 (412.8; 43%)	387.8	4 (259.1; 40%)	0.003
9,10-Dimethyl-anthracene	Hexane	399.6	2 (426.3; 31%)	399.6	3 (263.0; 25%)	0.006
Pyrene	Hexane	338.1	2 (391.7; 51%)	371.3	8 (335.0; 17%)	0.04
1-Methyl-pyrene	Hexane	346.4	0.5 (374.7)	374.7	0.2 (346.4)	0.02
Anthracene	Heptane	380.5	4 (402.1; 56%)	380.5	20 (248.0; 10%)	0.002
Perylene	Heptane	415.2	3 (443.9)	443.9	9 (400.0; 13%)	0.001
1,2-Benzo-fluorene	Heptane	265.0	7 (363.3; 31%)	346.1	2 (265.0)	0.003
Chrysene	Heptane	270.9	7 (360.7)	360.7	3 (267.0; 52%)	0.01
2-Methyl-anthracene	Octane	382.9	10 (410.5; 55%)	387.6	8 (257.6; 81%)	0.003
2,3-Benzo-fluorene	Octane	340.1	3 (353.9; 32%)	338.8	2 (266.7; 39%)	0.003
1,2-Benz-anthracene	Octane	291.5	4 (384.1)	384.1	2 (291.5)	0.008
1,2: 3,4-Dibenz-anthracene	Octane	291.0	20 (397.0; 11%)	374.8	2 (291.0)	0.006
1,2-Benzo-pyrene	Octane	334.0	30 (409.4; 18%)	388.4	20 (335.6; 12%)	0.07
1,12-Benzo-perylene	Octane	387.9	20 (445.7; 17%)	419.7	7 (303.8; 62%)	0.04
3,4-Benzo-pyrene	Octane	388.4	0.8 (402.7)	402.7	1 (299.0; 54%)	0.008
Naphthacene	Nonane	479.1	7 (513.7; 10%)	479.0	7 (277.4; 32%)	0.001
1,2; 5,6-Dibenz-anthracene	Nonane	300.6	6 (394.4)	394.4	3 (300.6)	0.006
9,10-Diphenyl-anthracene		396.2	10 (425.8; 46%)	402.7	20 (256.0; 28%)	0.003

[a] LODs were calculated as the concentration (ng/ml) of PAH that gave a signal 3 times the background noise, the latter being equal to $N_{p-p}/5$.

[b] Excitation LODs were determined at the excitation maximum, using the emission wavelength in parentheses; excitation slit-width = 0.2 nm, emission slit-width = 4 nm.

[c] Height of peak used for emission/excitation as percentage of height of the emission/excitation maximum; if no % is given, then percentage is 100%.

[d] Emission LODs were determined at the emission maximum, using the excitation wavelength in parentheses; emission slit-width = 0.2 nm, excitation slit-width = 4 nm.

[e] Reference 22 for RTF (conventional room-temperature fluorimetry).

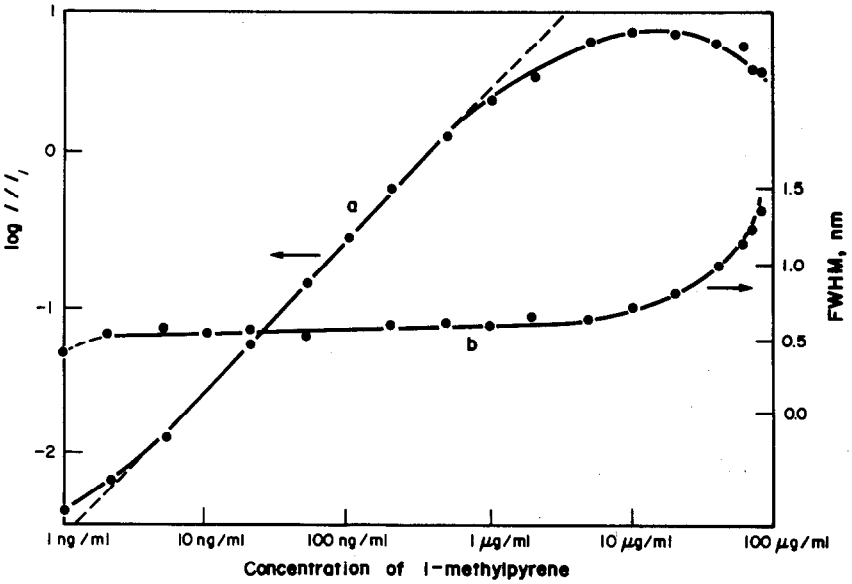


Fig. 2. (a) Analytical calibration curve for 1-methylpyrene (λ_{ex} = 346.2 nm, $\lambda_{em,measured}$ = 374.7 nm) using 3,4-benzopyrene (λ_{em} = 402.4 nm) as the internal standard, and (b) dependence of full-width at half-maximum of the 374.7-nm luminescence emission peak on concentration of 1-methylpyrene.

is shown in Fig. 2 as an example. The linear dynamic range of 3 orders of magnitude deviates from a slope of unity at about 1 μg/ml and reverses slope at concentrations above 10 μg/ml. This reversal of slope can probably be accounted for by the observation that the full-width at half-maximum increases dramatically at 10 μg/ml and higher concentrations, the PAH molecules in the host alkane matrix then being concentrated enough to allow site-to-site energy transfer.[17] Consequently Shpol'skii fluorescence is best utilized in the sub-μg/ml region.

Direct qualitative and quantitative analysis of mixtures of PAHs by conventional Shpol'skii luminescence at 77 K

The sharp-line absorption spectra exhibited by

Table 3. PAHs in order of increasing maximum excitation wavelength (n-heptane solutions)

PAH	Excit. max. *nm* (FWHM, *nm*)		Emiss. max., *nm* (FWHM, *nm*)	
2-Methylanthracene	257.6	(2.7)	388.7	(2.6)
1,2-Benzofluorene	265.0	(2.4)	346.1	(UD)
2,3-Benzofluorene	266.7	(UD)	339.9	(UD)
Chrysene	270.9	(4.1)	360.7	(0.7)
Naphthacene	277.4	(UD)	475.3	(UD)
Fluoranthene	288.6	(1.8)	434.8	(UD)
Naphthalene	289.2	(3.1)	334.0	(2.3)
1,2;3,4-Dibenzanthracene	290.6	(5.4)	375.7	(0.7)
1,2-Benzanthracene	291.6	(UD)	383.3	(UD)
Phenanthrene	295.0	(1.9)	346.0	(1.4)
1,2;5,6-Dibenzanthracene	300.1	(4.0)	393.1	(UD)
Fluorene	301.5	(1.5)	317.0	(UD)
Acenaphthene	306.6	(2.1)	319.1	(1.7)
Pyrene	321.0	(2.3)	371.6	(1.1)
1,2-Benzopyrene	335.6	(1.7)	386.7	(1.2)
1-Methylpyrene	346.2	(2.7)	374.3	(UD)
9-Methylanthracene	368.9	(2.9)	388.7	(2.6)
9,10-Dimethylanthracene	378.5	(1.8)	399.6	(1.6)
Anthracene	380.5	(1.1)	402.1	(UD)
1,12-Benzoperylene	388.2	(UD)	419.1	(0.9)
3.4-Benzopyrene	389.2	(1.8)	402.8	(UD)
9,10-Diphenylanathracene	396.2	(8.2)	425.8	(UD)
Perylene	415.2	(1.6)	443.9	(1.0)
Anthanthrene	433.4	(UD)	459.7	(UD)

UD = Fwhm could not be determined because of overlap of peaks.

PAHs in frozen Shpol'skii solvents facilitate selective excitation for the qualitative and quantitative analysis of mixtures.[13] Interference by weak emission from other components, because of partial overlap of absorption lines at the excitation wavelength, can be resolved by choosing a characteristic emission wavelength of the compound of interest for detection purposes. Selection of the excitation wavelengths that exhibit minimal spectral overlap is greatly facilitated by comparison of the excitation spectra of the individual compounds. For this reason, we strongly recommend potential users to collect a reference file of quasi-linear luminescence spectra obtained with their own instruments. In Table 3, a tentative scheme is presented in which the 24 PAHs are arranged in increasing order of excitation-maximum wavelengths. The third column shows that an emission wavelength can generally be found for selective detection even when the excitation maximum is overlapped by the excitation bands of neighbouring PAHs in the table. This table should be of help in analysing mixtures of PAHs, but should not be regarded as providing the only answer, as other emission wavelengths are sometimes used for resolving interfering components, especially when in analysis of "real" samples, or to reduce the analysis time and effort.

To test the power of the Shpol'skii technique with our fluorimeter system, a synthetic mixture of 11 PAHs was prepared in n-heptane, the concentrations ranging from 50 to 470 ng/ml. n-Heptane was chosen as a compromise among the five alkane solvents, as suggested by Fassel *et al.*[13] A combined standard addition–internal standard procedure was adopted, the internal standard being one of the components of the sample mixture.[12] This internal–standard approach was found to be quite feasible; 1,2-benzanthracene was chosen as the internal standard for the determination of 1,2-benzopyrene and 3,4-benzopyrene because it also emitted when the sample solution was excited at 292.2 nm for the determination of 1,2-benzopyrene, and 3,4-benzopyrene was used as the internal standard for the determination of the other PAHs because its strong emission at 402.8 nm lay in a region isolated from most emissions from other PAHs, and because almost any excitation wavelength would cause emission. With only 6 excitation wavelengths, all 11 PAHs could be identified and their concentrations determined. The results are shown in Table 4. Note that some excitation wavelengths were not those given in Table 2; 292.2 nm was the most selective excitation wavelength for 1,2-benzopyrene in the mixture, 361.6 nm was used for anthracene because the emission at 380.5 nm could then be used for its selective detection (its emission maximum at 402.1 nm was overwhelmed by the intense fluorescence from 3,4-benzopyrene), and 392.2 nm was used instead of 415.2 nm for perylene because only then could we observe the emission of 3,4-benzopyrene at 402.8 nm and use it as the internal standard for determination of perylene. As can be seen from Table 4, the average error was about 9%; the major sources of error could have been the inhomogeneity in orientation of the molecules in the matrix, the inherent uncertainty in fluorimetry, and penetration into the non-linear region on the high concentration side of the standard calibration curve if too large a standard addition were made.

Although quasi-linear peak widths became narrow with decrease in temperature,[6] 77 K was found to be low enough for satisfactory identification of 11 PAHs in the present study. A liquid–nitrogen bath is cheaper, simpler and easier to use than freezing nitro-

Table 4. Quantitative analysis of 11-component PAH mixture in n-heptane by conventional Shpol'skii luminescence at 77 K

λ_{ex}, nm	λ_{em}, nm	PAH	Taken, μg/ml	Found, μg/ml	Error, %
289.2	321.9	Naphthalene	0.05	0.05	5
	375.6	1,2;3,4-Dibenzanthracene	0.41	0.46	13
292.2	371.8	1,2-Benzopyrene*	0.38	0.40	5
	402.8	3,4-Benzopyrene	0.37	0.37	1
295.0	346.0	Phenanthrene	0.41	0.36	11
	360.6	Chrysene	0.45	0.49	8
306.6	319.3	Acenaphthene	0.08	0.08	5
	371.0	Pyrene	0.41	0.47	16
361.6	380.5	Anthracene	0.40	0.46	13
	383.9	1,2-Benzanthracene	0.48	0.44	8
392.2	443.9	Perylene	0.08	0.08	7

* Standard addition was done on two portions of the same sample solution. One portion was used for the determination of 1,2-benzopyrene and 3,4-benzopyrene, with 1,2-benzanthracene (λ_{em} = 383.9 nm) as the internal standard. Another portion was used for the determination of the other PAHs, with 3,4-benzopyrene (λ_{em} = 402.8 nm or 426.6 nm) as the internal standard.

gen or lower temperatures requiring liquid hydrogen or liquid helium. The limits of detection can be improved by using dye-laser excitation, but apparently by no more than 2 orders of magnitude, judging from the analytical curve for benzo[*a*]pyrene shown by Yang *et al.*[13] Moreover, the complications involved in changing dyes and tuning the frequency doubler when working at excitation wavelengths shorter than 360 nm can result in considerable consumption of time and effort; in addition, the initial cost and maintenance cost of lasers and the use of gated detection electronics must be considered. With the introduction of commercial spectrofluorimeters similar to the one described in this work, conventional Shpol'skii luminescence could develop into a routine analytical technique in any laboratory.

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APPLIED POTENTIAL AND RADIOTRACER STUDIES ON POLY(VINYL CHLORIDE) MATRIX ION-SELECTIVE ELECTRODE MEMBRANES

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Summary—This paper reports the effect of applied potentials on PVC matrix membranes containing (*i*) the barium ion-sensitive barium–Antarox C0880 complex and 2-nitrophenyl phenyl ether solvent mediator, and (*ii*) the calcium ion-sensitive Orion 92-20-02 phosphate-based calcium liquid ion-exchanger. Platinum electrodes were placed in solutions on each side of the membranes. The barium ion-sensitive membranes are unable to maintain stable current flows but the calcium ion-sensitive membranes are characterized by stable current flows over prolonged periods even after successive polarity reversals. Results are presented, from radiotracer experiments for permeation of ions through the membranes with and without an applied potential. No evidence was found for significant permeation of barium-133 ions through the barium ion-sensing membranes into an initially inactive solution, but it was found that barium-133 ions were incorporated into the membranes after removal of the applied potential. Permeation of sodium-22 ions through the calcium ion-sensing membranes occurred only to a limited extent in the presence of an applied potential and not at all in its absence, confirming electrode selectivity trends for calcium and sodium. Calcium-45 ions did not permeate the calcium ion-sensing membranes into an inactive counter-solution against the potential gradient, but on reversal of the polarity, permeation occurred to a far greater extent than in the absence of an applied potential. These differences in behaviour are compatible with the more complicated membrane pathways of the barium ion-sensing membranes, imposed by the complexing of barium ions by the ethyleneoxy units of Antarox C0880 in a tight helical conformation. The calcium ion-sensing membranes are much less constrained, thus permitting more facile replacement of the calcium ions in the membrane by ions from solution.

Radiotracer diffusion studies have been useful in establishing relationships between the availability of membrane pathways for ion transport and the potentiometric selectivity characteristics of PVC-matrix ion-selective electrodes containing calcium bis-dialkylphosphate ion-sensors.[1–3] Similar studies[4] have revealed that the availability of such membrane pathways is not important in determining the characteristics of electrodes containing the tetraphenylborate of the barium complex of a nonylphenoxypoly(ethyleneoxy)ethanol, $C_9H_{19}C_6H_4O(CH_2CH_2O)_{n-1}C_2H_4OH$ (Antarox C0880, in which $n = 30$). These studies implied that for these membranes an important role should be assigned to the ability of the membranes to incorporate and hold ions by complexation.

Studies of the effect of applied potentials and currents have been of use in other work[5–8] in leading to a better understanding of ion-transport phenomena within membranes. This paper reports a study of the effect of applied d.c. potentials on PVC-matrix membranes containing (*i*) the barium ion-sensitive barium–Antarox C0880 complex and 2-nitrophenyl phenyl ether solvent mediator, and (*ii*) the calcium ion-sensitive Orion 92-20-02 calcium liquid ion-exchanger, based on a calcium dialkylphosphate ion-sensor and dioctylphenyl phosphonate solvent mediator. Results are presented for potential-induced and zero-potential permeation of ions through these membranes, obtained by using appropriate radiotracers.

EXPERIMENTAL

Membranes

PVC-supported membranes were prepared as previously described[9,10] from PVC (0.17 g) and (*i*) a solution of the tetraphenylborate of the barium complex of Antarox C0880 (0.04 g) in 2-nitrophenyl phenyl ether (0.36 g) (barium ion-sensitive membranes) and (*ii*) Orion 92-20-02 calcium liquid ion-exchanger (0.40 g) (calcium ion-sensitive membranes).

Applied potential experiments

The cell (Fig. 1) enclosed in an air thermostat at 35° was charged with appropriate solutions (10 ml) on either side of the membrane. Fixed potentials were applied between platinum electrodes in the solutions on either side of the membrane, with a purpose-built potentiostat (up to 20 V). Currents flowing through the membranes were monitored by using a scaler (MS310) to count the charge passing between the two electrodes.

The barium ion-sensitive membranes and membranes containing only 2-nitrophenyl phenyl ether (NPPE) solvent mediator were used between barium chloride solutions ($10^{-3}M$), and the calcium ion-sensitive membranes between calcium chloride solutions ($10^{-3}M$); in some experiments the membranes were also used between volumes of doubly demineralized water.

Radiotracer studies

The radiotracer studies were performed by labelling one of the solutions in the cell with the relevant radioisotope. Permeation of sodium through calcium ion-sensitive membranes was followed by replacing one of the calcium chloride solutions by $10^{-3}M$ sodium chloride labelled with sodium-22.

Diffusion of radiotracers through a membrane was monitored[4] by periodically removing 20-μl (^{133}Ba) or 10-μl

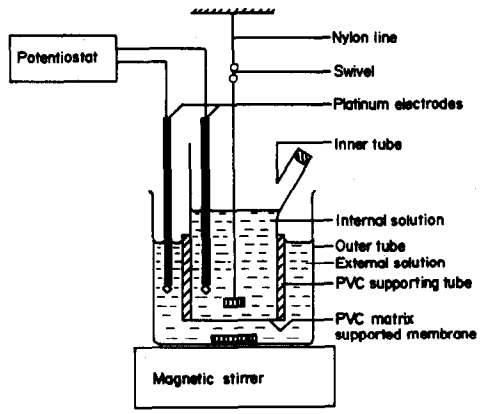

Fig. 1. Apparatus used.

(^{45}Ca, ^{22}Na) samples with a Lang-Levy auto-zero micropipette, placing them on filter paper (Whatman No. 1, 2.1 cm diameter), and counting with a Geiger-Müller mica end-window tube.

The activity of each membrane at the end of the experiment was monitored by (*i*) slicing the membrane from its supporting PVC tubing, counting each side of the membrane in turn with the Geiger-Müller tube, and using the data to calculate C_{act}/C_{inact} (C_{act} refers to the membrane surface in contact with the labelled solution, C_{inact} to the other surface), and (*ii*) dissolving the membrane in tetrahydrofuran (10 or 5 ml) and counting the activity of 10–60 μl samples of this solution, spotted onto filter papers, in order to determine the uptake (the activity contained by the membrane at the experiment conclusion as a percentage of the total initial activity in the radioactive solution).

All radiotracer counts were taken for 300 sec and corrected for background (*ca.* 170 counts). Count-rates of *ca.* 8, 133 and 50 cps were obtained from 10-μl samples for ^{133}Ba, ^{45}Ca and ^{22}Na respectively. The half-lives of the radioisotopes were long enough for the effect of decay during the experiments to be negligible.

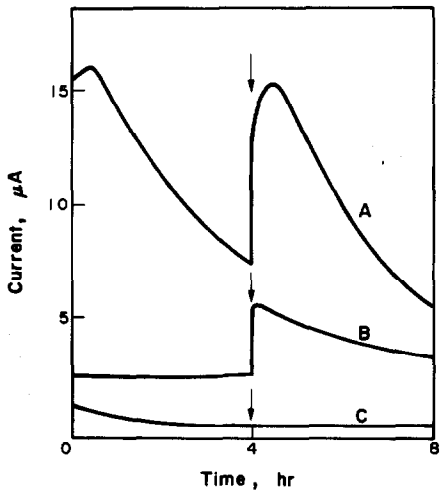

Fig. 2. Current-flow patterns for a potential of 2 V applied across membranes containing the barium–Antarox C0880 adduct, separating $10^{-3}M$ barium chloride solutions (A), or water (B), and across a membrane containing only 2-nitrophenyl phenyl ether (C) with reversal of the applied potential polarity after 4 hr (↓).

Materials

The Antarox C0880 was a gift by GAF (Great Britain) Ltd., Manchester. Metal chloride solutions were prepared from BDH Analar grade materials. Radioactive solutions were prepared by addition of (*i*) 1.4 ml of $6.39 \times 10^{-5}M$ barium chloride (*ca.* 0.08 mCi/ml barium-133 activity) to 100 ml of $10^{-3}M$ barium chloride, (*ii*) 0.2 ml of $1.1 \times 10^{-3}M$ calcium chloride (*ca.* 2.2 mCi/ml calcium-45 activity) to 100 ml of $10^{-3}M$ calcium chloride, and (*iii*) 0.3 ml of $1.9 \times 10^{-5}M$ sodium chloride (*ca.* 0.2 mCi/ml sodium-22 activity) to 100 ml of $10^{-3}M$ sodium chloride.

RESULTS

Membranes containing barium–Antarox C0880 complex

Current measurements. The patterns of current flow observed when a potential of 2 V was applied across membranes containing barium–Antarox C0880 complex are shown in traces A and B of Fig. 2, for barium chloride solutions and doubly demineralized water respectively. Trace C shows the pattern of current flow for a membrane containing only the solvent mediator, NPPE, separating barium chloride solutions. In all these experiments the polarity of the applied potential was reversed after 4 hr.

Trace A shows that the current flowing through these barium sensitive membranes decayed rapidly, to *ca.* 50% of the initial maximum value after ~3 hr, and that reversal of the polarity of the applied potential caused rapid recovery of the current flowing through the membrane to ~95% of the initial maximum value, followed by rapid decay of the current. There was no evidence for any significant recovery of the current upon further reversals of the polarity. Attempts to revive the limited current-bearing capacity of the membranes by immersion in $1M$ barium chloride for 18 or 72 hr before application of further cycles of potential also proved unfruitful. The current flow was found to be sensitive to temperature variations. Thus, the small initial increase in current shown in trace A corresponded to the time taken for the temperature within the air thermostat to reach 35°. Such initial current increases were eliminated by letting the temperature equalibrate before application of the potential. A similar pattern of current-flow decay and recovery was obtained with several membranes of this type for an applied potential of 2 V. When the potential was applied for 48 hr before reversal of the polarity, the current decayed to 1–2 μA after 24 hr and recovered to ~80% of its initial maximum value on reversal of the polarity. The initial maximum observed varied in the range 15–20 μA for different membranes, because of variations in membrane thickness and composition.

Similar patterns were also noted for applied potentials of 4 and 5 V, with intial maxima of 35 and 55 μA, respectively. For an applied potential of 10 V an initial maximum of 90 μA was observed, but the polarity could be reversed more than six times before the

current failed to recover; the maximum current decreased with each reversal of polarity.

Trace B shows that with a potential of 2 V across a barium-sensitive membrane separating two lots of demineralized water, the initial current was 2–3 μA (the same order as for demineralized water without the dividing membrane). It also shows that the current decayed more slowly, at $\sim$0.1 μA/hr and that reversal of the polarity caused an increase to 5–6 μA, followed by decay at 0.4–0.5 μA/hr.

Trace C shows that applying 2 V across a membrane containing only NPPE solvent mediator and separating barium chloride solutions gave an initial current of $\sim$1.0 μA, decaying to $\sim$0.1 μA, and that reversal of the polarity caused no significant recovery.

Barium-133 permeation studies. The activity measurements made at the conclusion of experiments are presented in Table 1. In experiments 1 and 2 the membranes were removed from contact with the solutions immediately after removal of the applied potential, but in experiments 3–5 they were not removed until at least 48 hr after removal of the applied potential. In all these experiments the polarity was reversed at least three times and the membranes were in contact with the solutions for at least 148 hr.

These studies showed no evidence that significant amounts of radioactive ions permeated through the membranes into the initially inactive solutions. The values of C''/C' in Table 1 refer to the end of the experiment, but, samples extracted at various times during a run never gave a ratio exceeding 0.02.

The values of C_{act}, C_{inact}, and the percentage uptake all show that barium-133 was incorporated into the membranes to a far greater extent after removal of the applied potential.

Control experiments already described[4] suggest that the value of C_{act}/C_{inact} would be $\sim$4 if all the incorporated barium-133 were concentrated at or near the active solution/membrane interface. Thus, the data in Table 1 show that while there was some permeation of radioactive ions into the membranes, there was not an equilibrium distribution of the radioactive ions within the membranes, (which would lead to a C_{act}/C_{inact} value of $\sim$1).

Membranes containing Orion 92-20-02 calcium ion-exchanger

Current measurements. The current-flow patterns observed are shown in Fig. 3. The traces shown were obtained with the same membrane. The polarity of the applied potential was reversed after 24 hr. The traces indicate that pseudo-stable current-flows were established within an hour of application of the potential, and that they decayed at $\sim$0.1 μA/hr. When the potentials were applied for longer than 24 hr there was no evidence for significant further decay in the current, *e.g.*, for an applied potential of 5 V the current was 4.4, 4.5, 4.7 and 4.9 μA at 25, 48, 96 and 120 hr, respectively, after the initial application of the potential.

Table 1. Radioactive count data for PVC membranes containing barium and calcium ion-sensors

Expt. no.	Membrane materials of in 0.17 g of PVC	Nature of applied potential	No. of polarity reversals	Duration of expt., *hr*	Active solution*	Inactive solution*	C''/C'	C_{act}†	C_{inact}†	C_{act}/C_{inact}†	Uptake, %
Reference expt.[4]		Zero potential	—	4	$BaCl_2$	$BaCl_2$	0	17442	8209	2.12	2.0
1§			5	192	$BaCl_2$	$BaCl_2$	0.02	4447	3201	1.39	0.5
2§			3	148	$BaCl_2$	$BaCl_2$	0.02	3122	1661	1.94	0.5
3	0.04 g of barium sensor + 0.36 g of NPPE	2 V with several polarity reversals	6	172	$BaCl_2$	$BaCl_2$	0.02	47183	20174	2.34	3.8
4			8	444	$BaCl_2$	$BaCl_2$	0.02	40895	29720	1.38	4.9
5			3	148	$BaCl_2$	$BaCl_2$	0.02	36487	25822	1.41	4.4
6§	0.40 g of Orion 92-20-02 calcium liquid non-exchanger	5 V with inactive solution +ve	0	54	$CaCl_2$	$CaCl_2$	0	293660	209795	1.40	11.9
7§		5 V with inactive solution −ve	0	148	$CaCl_2$	$CaCl_2$	1.80	38509	178479	0.21	9.4
8§			0	148	NaCl	$CaCl_2$	0.05	28229	68406	0.41	1.7
9		Zero potential	—	148	$CaCl_2$	$CaCl_2$	0.27	253104	169022	1.50	9.8
10			—	148	NaCl	$CaCl_2$	0.01	2236	1428	1.57	0.2

* Solutions all 10^{-3} *M*.

† Direct comparison of ^{45}Ca, ^{22}Na and ^{133}Ba values is not possible because of the different natures and concentrations of the radiotracers used.

§ Membranes removed from contact with the solutions immediately after removal of the applied potential.

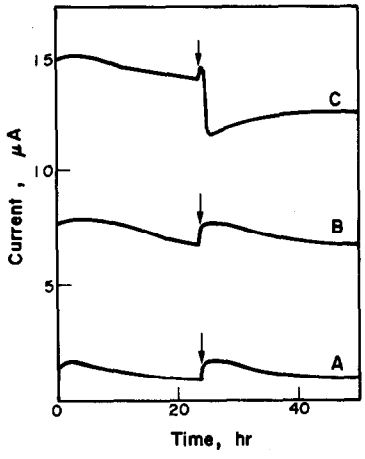

Fig. 3. Current-flow patterns for potentials applied across a membrane containing Orion 92-20-02 calcium liquid ion-exchanger separating $10^{-3}M$ calcium chloride solutions, with reversal of applied potential polarity after 24 hr (↓). Applied potential: 2 V (A), 10 V (B) and 20 V (C).

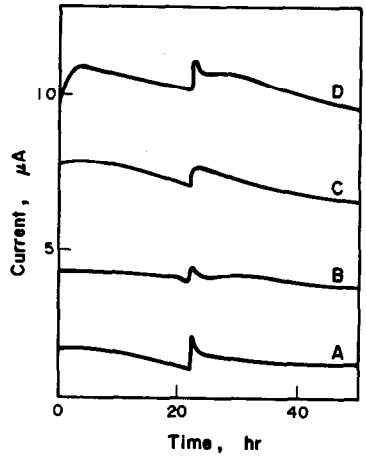

Fig. 4. Current-flow patterns for potentials applied across a membrane containing Orion 92-20-02 liquid ion-exchanger separating water, with reversal of applied potential polarity after 24 hr (↓). Applied potential: 2 V (A), 5 V (B), 10 V (C) and 15 V (D).

Though the current flowing under similar conditions of applied potential varied considerably from membrane to membrane of this type, *e.g.*, pseudo-stable currents of ~15 and 25 μA were obtained with two such membranes at a potential of 20 V, an individual membrane gave essentially the same current-flow pattern over several cycles of polarity reversal, changes in the applied potential, and replacement of the calcium chloride solutions by new solutions, although the current did eventually decrease if the changes were continued.

Replacement of the calcium chloride solutions by demineralized water caused no significant changes in the current-flow patterns (Fig. 4).

Ion permeation studies. In all the applied-potential experiments, current flows of ~5 μA were obtained.

The C''/C' data (Table 1) for the end of the experiment show that sodium-22 ions did not permeate these membranes in the absence of an applied potential, and only to a very limited extent in the presence of an applied potential. They show further that calcium-45 ions did not permeate the membrane into the inactive counter-solution against the potential gradient, that is, when the positive electrode was immersed in the inactive solution, but with the opposite polarity (experiment 7) the calcium-45 permeated into the inactive counter-solution to a far greater extent than in the absence of an applied potential (experiment 9).

Figure 5 shows plots of C''/C' *vs.* time. Traces A–D refer to experiments 7–10, respectively. These traces confirm the behaviour of sodium-22 permeation through the membranes. The traces for calcium-45 show an induction period which is shortened by application of a potential with the correct polarity. These traces also confirm the far greater extent of permeation of calcium-45 ions through the membranes under an applied potential of suitable polarity. The slopes of the linear regions on these plots provide a measure of the relative rates of permeation of the ions through the membranes. These slopes had values of 6×10^{-7} and 40×10^{-7} sec^{-1} for calcium-45 permeation in the absence and presence of an applied potential, and 0 and 1×10^{-7} sec^{-1} for sodium-22 permeation in the absence and presence of an applied potential.

The activity contained by the membranes at the end of the experiments shows that calcium-45 entered the membranes much more readily than sodium-22 did, and that the extent of incorporation of cal-

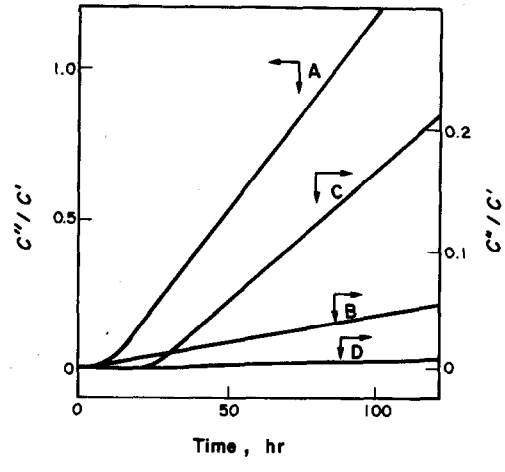

Fig. 5. Plots of C''/C' *vs.* time for the permeation of calcium-45 i (A) and sodium-22 (B) at an applied potential of 5 V, and for permeation of calcium-45 (C) and sodium-22 (D) at zero potential, through membranes containing Orion 92-20-02 calcium liquid ion-exchanger.

cium-45 ions by the membranes was about the same whether a potential was applied or not, whereby the extent of incorporation of sodium-22 was increased by an applied potential. The C_{act}/C_{inact} ratios show that considerable permeation of the incorporated radioactive ions occurred within the membranes. Control experiments already described[4] indicated that this ratio would be ~33 for calcium-45 and ~4 for sodium-22 if all the incorporated radioactive ions remained concentrated at or near the active solution/membrane interface, but the ratios obtained in experiments 7 and 8 suggest that these radioactive ions were concentrated near the inactive solution/membrane interface.

DISCUSSION

The results demonstrate that the application of d.c. potentials across these two different membrane systems causes markedly different current-flow behaviour. Radiotracer diffusion studies[1–4] have shown that primary ion transport through the membranes into the inactive counter-solutions occurs much more readily for the calcium ion-sensitive membranes based on calcium dialkylphosphate sensors than for the barium ion-sensitive membranes based on the barium–Antarox C0880 complex. The results of the present study favour the assignment of an important role to such differences in explaining the different behaviour of these membrane systems when potentials are applied across them.

The inability of the barium ion-sensitive membranes to maintain stable current-flows is due to the charge-carrying species being displaced faster from the membranes by application of a potential than they can be replaced by permeation of barium ions,[4] causing a rapid increase in the membrane resistance from the initial value of *ca.* 0.2 MΩ. Indeed, the very low values for percentage uptake of barium-133 in experiments 1 and 2, where the membranes were removed from the solutions immediately after removal of the applied potential, shows that even normal barium ion incorporation by the membranes is inhibited during the application of potentials. Thus, the overall effect of applying a potential across these membranes is to increase the membrane resistance, and if the potential is applied for lengthy periods the resistance approaches that of a membrane containing only the solvent mediator, NPPE, *i.e.*, ~10 MΩ with a consequent current of ~0.1 μA.

The fact that the current flow with these membranes recovers on potential-reversal only if there is still a significant current flow through the membrane, is probably due to total barium depletion of the barium-sensitive membranes causing changes in the membrane structure which make the uptake of barium ions from solution by the normal barium ion-exchanger sites within the membrane even more difficult. Thus, though barium ions are quite readily incorporated into the membranes after removal of the applied potential, (*cf.* uptake data for experiments 3–5), the fact that current-flow cannot be restored for these membranes, even by storage in concentrated barium chloride solutions between potential application cycles, suggests that this uptake of barium is not due to interaction with the normal ion-exchanger sites within the membranes. The stability of the barium–Antarox C0880 adduct is attributed[11] to 12 ethyleneoxy units assuming a tight helical conformation with a radius of ~1.3 Å in which a barium ion is held by a cage of 12 oxygen atoms (6 in each loop) through ion-dipole interaction, and the removal of barium ions from the membranes under an applied potential probably causes collapse of the helical loops, thus removing the pathways for permeation through the membrane, which are in any case more constrained that for the calcium ion-sensitive membranes.

The stable current-flows established through the calcium ion-sensitive membranes containing Orion 92-20-02 calcium liquid ion-exchanger suggest that calcium ions displaced from the membranes by application of a potantial are readily replaced by calcium ions from solution. This facile replacement is consistent with the ready permeation of calcium ions through these membrane systems under zero-potential conditions.[1–2] Indeed, application of an applied potential with the correct polarity increases both the rate and extent of radioactive calcium permeation through these membrane systems (*cf.* traces A and C in Fig. 5). The non-permeation of the calcium-45 into the inactive solution in experiment 6 is not really surprising, since it is the positive electrode that is immersed in this solution. However, the uptake data show that this unfavourable potential gradient does not prevent the uptake of the radioactive ions by the membrane itself, and indeed suggest that the total uptake is actually increased, because the polarity of the applied potential prevents the release of radioactive calcium ions into the inactive solution. Table 1 also shows that the application of a potential of suitable polarity only changes the distribution of the radioactive ions within the membrane and not the extent of incorporation of these ions.

The value of $d(C''/C')/dt$ obtained for the permeation of calcium ions through the membraness in the absence of an applied potential (6×10^{-7} sec^{-1}) is less than values previously reported[1,2] (18×10^{-7}, 11×10^{-7} and 22×10^{-7} sec^{-1}). The values vary widely between different batches of Orion 92-20-02 liquid ion-exchanger.[1,2] The use of a different batch of the ion-exchanger may also account for the extent of calcium-45 ion incorporation by the membranes in this study being much greater than that previously reported.[2] but the difference is much more likely due to the fact that in the previous study no account was taken of the self-absorption of activity by the membrane. Control counting experiments showing that the placing of a non-active membrane between a sample of calcium-45 and the counter reduced the count by a factor of ~33 suggest that ignoring the effect of mem-

brane thickness could cause considerable error. Thus, the present estimate of the uptake of calcium-45 is much more reliable than the previous one.

The current flows obtained through these membranes are consistent with membrane resistances of ~ 1 MΩ, similar to those reported for PVC membranes[7] and Millipore filters[8] containing Orion 92-20-02 calcium ion-exchanger. Variations in the currents flowing through these membranes on application of different potentials simply reflect Ohm's law and the varied resistances of the membranes.

Sodium-22 permeates through the membranes into the inactive counter-solution only to a very limited extent, even when a potential of suitable polarity is applied across the membrane. On the other hand, the extent of incorporation of sodium-22 into the membrane increases significantly on application of a potential, in agreement with the hypothesis[7,8] that the deterioration of the selectivity characteristics (relative to sodium) of Orion calcium liquid ion-exchanger membrane systems, upon application of potentials, is due to the greater extent of incorporation of sodium ions into the bulk of the membrane under these conditions.

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SHORT COMMUNICATIONS

IRON(III) AS ACTIVATOR FOR CATALYTIC FLUORIMETRIC MICRODETERMINATION OF V(V)

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Summary—The activation of the vanadium(V)-catalysed aerial oxidation of sodium 4,8-diamino-1,5-dihydroxyanthraquinone-2,6-disulphonate by iron(III) is discussed. The oxidation product is intensely fluorescent and allows fluorescence-monitoring of the slow reaction, which is preceded by an induction period. On the basis of this investigation, an accurate method for determination of vanadium(V) at the 1–10 ng/ml level has been developed.

Extremely low concentrations can be determined by means of catalytic analysis, but the selectivity of catalytic determinations is often rather lower than that of other methods for trace analysis. However, the specificity and selectivity of an analytical procedure depend on the specificity offered by both the chemical reaction and the monitoring technique and fluorescence-monitoring in catalytic methods of analysis has the additional advantage of improving the analytical characteristics of such methods.[1] Wilson and Ingle[2,3] have recently discussed the advantages of these methods.

The most sensitive catalytic methods are generally associated with the development of the theory and practice of activation and inhibition of catalytic reactions. Very interesting discussions of such effects has been given by Bontchev[4] and Hadjiioannou.[5]

A catalytic determination will be selective if either the individual catalyst shows very different catalytic activities under different reaction conditions, or a variety of catalysts react according to different reaction mechanisms. The selectivity of a catalytic method can often be improved by proper choice of pH, reagent concentration, temperature, complexing agents and monitoring technique, or by use of an activator to increase the rate of a given catalytic reaction in presence of a certain catalyst.[6]

This paper presents the catalytic fluorimetric determination of vanadium(V), based on activation by iron(III). The method allows the determination of V(V) at the 1–10 ng/ml level. This activation technique not only improves the sensitivity of the indicator reaction[7] used, but also makes the method more selective.

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EXPERIMENTAL

Apparatus

A Perkin–Elmer fluorescence spectrophotometer, model MPF-43A, was used, equipped with an Osram XBO 150-W xenon lamp, excitation and emission grating monochromators, 1 × 1 cm quartz cells, an R-508 photomultiplier and a Perkin–Elmer 023 recorder. A standard "bar" of Rhodamine B (conc. $1 \times 10^{-7} M$) gave a scale reading of 60 units, with the following parameters: $\lambda_{ex} = 480$ nm, $\lambda_{em} = 570$ nm, excitation and emission wavebands 5 nm, sensitivity 10 coarse and 7 fine, temperature 25°. The variation of fluorescence intensity with time was recorded at a chart-speed of 2 cm/min with fixed excitation and emission wavelengths.

Reagents

Solutions were made of sodium 4,8-diamino-1,5-dihydroxyanthraquinone-2,6-disulphonate ($1 \times 10^{-2} M$), vanadium(V) ($1 \times 10^{-3} M$, from ammonium vanadate and standardized gravimetrically) and iron(III) [$1.79 \times 10^{-2} M$ from $Fe(NO_3)_3 \cdot 9H_2O$ and standardized gravimetrically], and diluted as required.

Doubly distilled and demineralized water and analytical grade reagents were used.

Procedure

Place 5 ml of $10^{-4} M$ sodium 4,8-diamino-1,5-dihydroxyanthraquinone-2,6-disulphonate and 5 ml of $2M$ hydrochloric acid in a dry 25-ml standard flask. Add enough demineralized water to give a final volume of 25 ml when all the reagents are present. Finally add the volumes of premixed vanadium(V) and iron(III) solution needed to give final concentrations of 1–10 ng/ml for vanadium and 5 μg/ml for iron. Measure fluorescence intensity as a function of time, starting 60 sec after addition of the vanadium–iron solution, with $\lambda_{ex} = 524$ nm, $\lambda_{em} = 582$ nm. All the solutions should be at 28°.

RESULTS AND DISCUSSION

In an attempt to increase the sensitivity of the proposed method for determination of V(V),[7] an examin-

ation was made of the activating action of certain substances that had previously been employed for this purpose in the indicator reactions of V(V)-catalysed oxidation of aromatic amines.[4] No activation of the reaction was observed when phthalate, phosphate, citric acid, phenol, hydroquinone, oxalate or ethanol was added. However, the presence of Fe(III) accelerated the V(V)-catalysed overall reaction.

The kinetic curves obtained in presence of Fe(III), of V(V) and a mixture of both, under identical conditions, are shown in Fig. 1. As observed, when both Fe(III) and V(V) are present in the medium, the slope of the curve increases and the length of the induction period decreases.

Iron(III) acts in a similar manner to V(V), by catalysing the aerial oxidation of the disulphonate reagent, giving rise to the same fluorescent product. However, under the optimum experimental conditions for the catalytic action of V(V), the effect of Fe(III) is slight. The kinetic data are summarized in Table 1.

The action of one-electron oxidizing agents on arylamines or phenols is based on an oxidation process which proceeds by a homolytic mechanism. This includes the subtraction of one electron from the organic substrate as the first step,[8] with formation of free radicals.

$$ArNH_2 - e^- - H^+ \rightarrow ArNH \quad (1)$$

$$ArOH - e^- - H^+ \rightarrow ArO \quad (2)$$

Iron(III) or V(V) can act in a similar manner on the disulphonate reagent, the free radicals formed reacting with atmospheric oxygen (electron acceptor) with formation of hydrogen peroxide in a subsequent step. The peroxide could cause regeneration of the catalyst according to equations (3) and (4):

$$2Fe^{2+} + H_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + 2H_2O \quad (3)$$

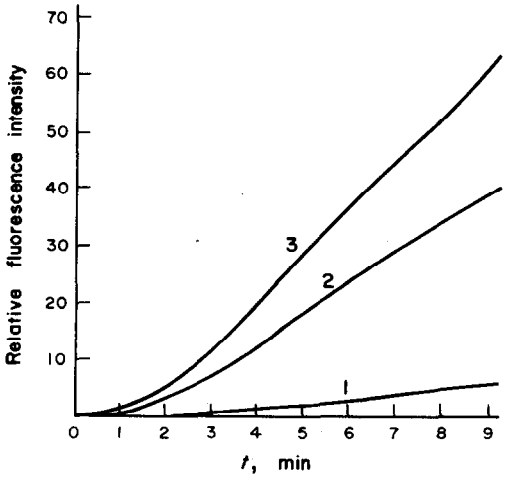

Fig. 1. Fluorescence *vs.* time curves for: 1, 5 ppm Fe(III); 2, 0.1 ppm V(V); 3, 5 ppm Fe(III) and 0.1 ppm V(V). [DADHADS] = $2 \times 10^{-5}M$. [HCl] = $0.4M$; λ_{ex} = 524 nm; λ_{em} = 582 nm.

Table 1. Reciprocal induction-periods and initial rates for iron(III)–DADHADS reaction ([DADHADS] = $2 \times 10^{-5}M$, [HCl] = $0.4M$)

Fe(III), *ppm*	$1/T$, cm^{-1}	log (100 tan α)
2	0.10	0.49
5	0.14	0.96
10	0.20	1.30
15	0.27	1.57

$$2VO^{2+} + H_2O_2 \rightarrow 2VO_2^+ + 2H^+ \quad (4)$$

On the other hand, studies on the nature of the reaction product reveal it is a diquinone type compound, which according to previous studies[7] results from the hydrolysis of the corresponding imine compound. Consequently, in going from the amino-hydroxy compound to the imino-quinone compound two electrons are involved. During the time necessary to complete the induction period, formation of the amino-quinone compound takes place, the accelerated step being characterized by formation of the imine-quinone and subsequent hydrolysis.

In order to understand the action of Fe(III) on the V(V)-catalysed oxidation, the kinetic curves in presence of 0.1 ppm V(V) and various concentrations of Fe(III) were recorded (Fig. 2). As shown, when both iron and vanadium are present in the reaction medium, the rate of oxidation increases, *i.e.*, the consumption of the reagent is speeded up. This could occur through a reaction sequence such as that represented by equations (5)–(8):

$$RH_2 + VO_2^+ + H^+ \rightarrow RH + VO^{2+} + H_2O \quad (5)$$

$$2VO^{2+} + H_2O_2 \rightarrow 2VO_2^+ + 2H^+ \quad (6)$$

$$RH_2 + Fe^{3+} \rightarrow RH + Fe^{2+} + H^+ \quad (7)$$

$$2Fe^{2+} + H_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (8)$$

If the assumption is made that equation (7) is slower than equation (5), then the following reaction could occur

$$VO_2^+ + Fe^{2+} + 2H^+ \rightarrow VO^{2+} + Fe^{3+} + H_2O \quad (9)$$

with a decrease in the rate of the accelerated step

$$VO_2^+ + RH + H^+ \rightarrow R + VO^{2+} + H_2O \quad (10)$$

$$Fe^{3+} + RH \rightarrow R + Fe^{2+} + H^+ \quad (11)$$

This is manifested in Fig. 2 by the rate values being lower than those obtained by adding the velocities of the reactions catalysed by V(V) and Fe(III) considered individually, the difference increasing with the concentration of iron. However, the primary step would occur more extensively (activation) if both vanadium and iron were present in the medium, with the nett result of decreasing the length of the induction period.

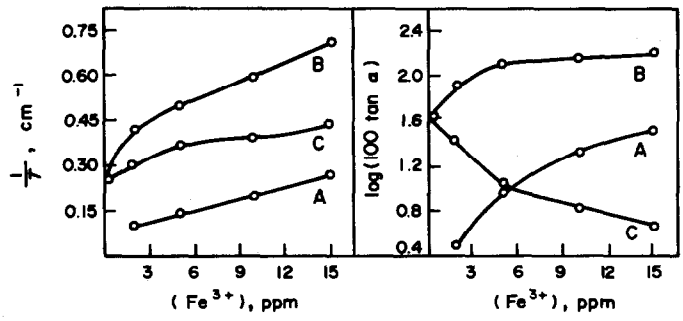

Fig. 2. Induction period and initial rate *vs.* iron concentration. A, [V(V)] = 0; B, [V(V)] = 0.1 ppm; C, difference (B-A): [DADHADS] = $2 \times 10^{-5}M$; [HCl] = $0.4M$: λ_{ex} = 524 nm: λ_{em} = 582 nm.

Studies on the variables affecting the fluorescence development

The characteristics of the fluorescence excitation and emission spectra and the procedure for optimization of the acidity ($0.4M$ HCl) and reagent concentration ($2 \times 10^{-5}M$) have been described in a previous paper.[7]

The Fe(III) concentration was optimized by recording the fluorescence–time curves at different Fe(III) concentrations, in the presence and absence of V(V). Plots of the the initial rate and reciprocal of the induction period *vs.* concentration of Fe(III) provide the optimum values of this variable.

Figure 2 shows the effect of Fe(III) concentration on the reaction rate. For a fixed concentration of V(V), a diminution in the Fe(III) concentration increases the induction period and the time necessary for full oxidation of the reagent. For Fe(III) concentrations above 5 ppm, the initial rate is virtually constant. For concentrations lower than this, the length of the induction period cannot be evaluated accurately, since the completion of the induction period and the accelerated reaction do not occur sharply, but only gradually, according to the amount of the imine compound formed.

Thus, an Fe(III) concentration of 5 ppm has been taken as optimum, since it ensures a small blank reaction, while still making it possible to determine V(V) with adequate sensitivity.

Table 2. Reciprocal induction periods and initial rates for activated V(V)–DADHADS reaction ([DADHADS] = $2 \times 10^{-5}M$, [HCl] = $0.4M$, [Fe(III)] = 5 ppm)

V(V), *ng/ml*	1/T, cm^{-1}	log (100 tan α)
1	0.26	1.34
2	0.29	1.38
3	0.32	1.40
5	0.38	1.43
8	0.48	1.45
10	0.53	1.47

Characteristics of the kinetic methods

The fluorescence–time curves for different amounts of V(V) and constant concentration of Fe(III) have been obtained. The results are summarized in Table 2.

The data obtained after analysis of such curves by the induction period and initial rate methods are summarized in Table 3. The effect of foreign ions on the determination of V(V) by the induction period method is summarized in Table 4. It is noteworthy that the selectivity of the method in absence of Fe(III)[7] is notably improved in presence of the activators. The tolerance ratios (foreign ion:vanadium) increased for the majority of the interferent ions, except for Ce(IV) and Hg(II), for which the ratio was lower, as a result of the oxidizing character of these ions.

Table 3. Characteristics of the kinetic methods

Method	Range of applicability, *ng/ml*	Taken, *ng/ml*	Found($\bar{x}$), *ng/ml*	*n*	*S*, *ng/ml*	*W*,%	*G*,%
Induction period	1–10	5	4.86	11	0.15	3.1	2.1
Initial rate	1–10	5	4.81	11	0.32	6.6	4.6

$\bar{x}$ = mean value; *n* = number of determinations: *S* = standard deviation: *W* = coefficient of variation; *G* = mean relative error ($= 100tS/\bar{x}\sqrt{n}$, where *t* is Student's *t* for 95% confidence).

Table 4. Effect of diverse ions on the determination of 5 ng/ml V(V)

Ion added	Tolerance ratio* [ion]/[V(V)]
Cd(II), Cr(III), W(VI), Br^- Li(I), Pd(II), Mn(II), Zn(II)	4000
Cu(II), Co(II), Th(IV), Mo(VI), PO_4^{3-}, EDTA	1000
Ni(II), Al(III), As(III), F^-	400
Hg(II), Au(III)	1
Fe(II), I^-	0.8
Ce(IV)	0.2

*For <2% relative error.

Application

The method was applied to determination of non-volatile vanadium in two crude oils. The sample was evaporated to dryness on a hot-plate and then heated for 15 min at 600–700° in a muffle furnace. The cooled residue was dissolved in a minimum of nitric acid (1 + 1) and diluted with water to volume in a 50-ml standard flask. A 1-ml aliquot was analysed by the procedure given.

The values obtained were 8.3 ± 0.4 μg/ml for oil A (atomic absorption spectrometry gave 7.1 μg/ml) and 14.2 ± 0.3 μg/ml (AAS gave 12.5 μg/ml). The values are averages of 6 replicates for the fluorescence method and 5 for the AAS.

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SPECTROPHOTOMETRIC DETERMINATION OF OSMIUM WITH 1,5-DIPHENYLCARBAZIDE

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Summary—The formation of the bluish violet osmium–diphenylcarbazide complex in weakly acidic solution is utilized for the determination of osmium by spectrophotometry. When measurements are made at 560 nm, after extraction of the complex into isobutyl methyl ketone, Beer's law is obeyed up to 150 μg of osmium. Relatively few ions interfere, and these can be masked with EDTA and fluoride.

Spectrophotometric methods using thiourea[1] and thiocyanate[2,3] are generally employed for the determination of osmium. These procedures lack adequate sensitivity and selectivity and necessitate the separation of osmium by extraction[3] or distillation[4] before the determination.

Although many reagents[5–7] have been proposed for osmium, their sensitivities do not approach that provided by 1,5-diphenylcarbazide, the osmium complex of which ($\epsilon = 1.5 \times 10^5$ $1.mole^{-1}.cm^{-1}$)[8] was formed by heating for 5 min at 65° in a medium made 1.4*M* and 1.2*M* with respect to acetic acid and perchloric acid respectively. This determination was completed after extraction of the blue-violet diphenylcarbazide complex into chloroform. Interference from many elements was overcome, to some extent, by prior extraction of osmium tetroxide into chloroform and reaction with the diphenylcarbazide (DPCI) in the organic phase,[9] but this caused considerable diminution of the sensitivity ($\epsilon = 3.1 \times 10^4$ $1.mole^{-1}.cm^{-1}$), and the reaction took 2 hr to go to completion in the organic phase. It seemed to us, however, that further investigation of this colour reaction could yield interesting results. Detailed examination disclosed that the extraction of the complex formed in weakly acidic solution into isobutyl methyl ketone (IBMK) can form the basis of a rapid, simple and reliable method for determination of osmium. The method, though slightly less sensitive ($\epsilon = 2.7 \times 10^4$ $1.mole^{-1}.cm^{-1}$) can be rendered sufficiently selective by use of EDTA and fluoride for application to samples containing various metal ions, including noble and base metals.

EXPERIMENTAL

Reagents

Osmium solution, 10 μg/ml. Weigh an ampoule containing ~1 g of osmium tetroxide, then break it under water in a glass-stoppered bottle. Make up the solution and washings to volume in a 250-ml standard flask, collecting, drying and weighing the glass fragments. Find the amount of OsO_4 dissolved from the difference in weight. Prepare a working solution (osmium 10 μg/ml) by suitably diluting the stock solution with water.

Diphenylcarbazide solution, 1%. Dissolve 1 g of reagent in 100 ml of high-purity acetone.

Buffer solution, 0.5M. An equimolar mixture (0.5*M*) of acetic acid and sodium acetate solution, adjusted to pH 5.0.

Procedure

Place a sample solution containing not more than 150 μg of osmium in a 60-ml separatory funnel, and add 5 ml of buffer solution and 1 ml of DPCI solution. Extract with two 10-ml portions of IBMK, shaking for 1 min. Collect the organic phases in a 25-ml standard flask and make up to the mark with IBMK. Measure the absorbance in 10-mm cells at 560 nm against a reagent blank carried through the procedure. Prepare a calibration graph with 0.5–15 ml of standard 10-μg/ml osmium solution.

RESULTS AND DISCUSSION

Preliminary studies showed that the reaction proceeds smoothly in solutions buffered to pH 4.0, to give a bluish violet precipitate which can be dissolved by the addition of acetone. However, it takes at least 30 min for the absorbance to reach its maximum even in the presence of excess of reagent. It was later found that the reaction can be made to go to completion rapidly by extracting the precipitate into an organic solvent such as IBMK, chloroform, amyl acetate, isoamyl alcohol, benzene, carbon tetrachloride, cyclohexane, cyclohexanol and cyclohexanone. Though IBMK and isoamyl alcohol were found to be the most suitable, the former was preferred for further studies.

Absorption spectrum

The absorption spectrum of the osmium complex extracted into IBMK (Fig. 1) showed that the complex absorbed maximally over the range 555–570 nm. The reagent blank under similar conditions showed no absorption over the wavelength range 450–700 nm. For further studies 560 nm was chosen as the optimum wavelength.

Optimal conditions and spectral properties

Variation of the pH of the reaction medium revealed that the complex formation remained maxi-

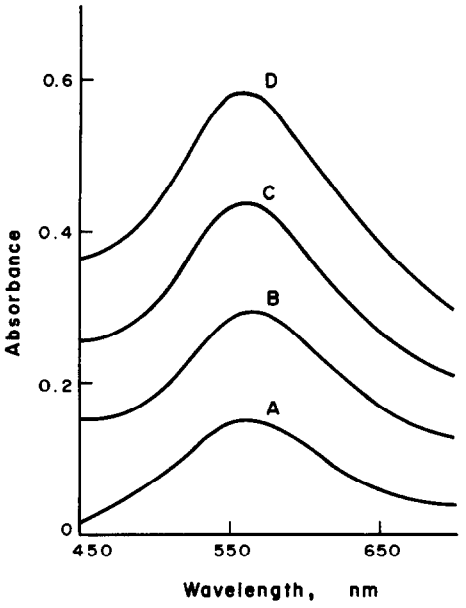

Fig. 1. Absorption spectra: (A) 25 μg of osmium (VIII) + 1 ml of 1% DPCI solution + 5 ml of pH-5 buffer, extracted with 2 × 10 ml of IBMK and extract made up to 25 ml; (B, C and D) as in (A) but with 50, 75 and 100 μg of osmium(VIII).

mal and constant over the pH range 4.0–6.0 (Fig. 2). Though the addition of 8 mg of DPCI to solutions containing 25 μg of osmium and buffered to pH 5.0 in a final volume of 25 ml was found to be sufficient, amounts up to 20 mg did not affect the absorbance. It was found that the use of an IBMK solution of DPCI was ineffective, and that equilibration for 1 min was sufficient for maximum extraction of osmium. An aqueous to organic phase volume-ratio of up to 3:1 did not affect the absorbance.

Under optimum conditions, Beer's law was obeyed over the range 5-150 μg of osmium and an apparent molar absorptivity of 2.7×10^4 l.mole^{-1}.cm^{-1} was obtained.

Ten replicate analyses of standard solutions each containing 25 μg of osmium gave an average recovery of 100.5% with a relative standard deviation of 1.2%.

The composition of the complex was established as 1:1 Os:DPCI by the continuous-variation, mole-ratio and equilibrium-shift methods.

Interference studies

The effect of mg amounts of several ions on the determination of 10 μg of osmium was studied. No interference was noticed in the presence of NO_3^-, NO_2^-, Br^-, SCN^-, SO_3^{2-}, AsO_3^{3-}, AsO_4^{3-}, Li^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Al^{3+}, Tl^+, Pb^{2+}, Sb^{3+}, Bi^{3+}, Ce^{4+}, UO_2^{2+}, Se^{4+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Rh^{3+}, Ni^{2+}, Pt^{4+}, MoO_4^{2-} and WO_4^{2-}. The presence of Fe^{3+}, Ru^{3+}, VO_4^{3-}, CrO_4^{2-}, Pd^{2+}, Cu^{2+} and Co^{2+} gave high recoveries while Ir^{4+}, Zr^{4+}, Ti^{4+}, La^{3+} and Be^{2+} caused low recoveries.

The interference due to Fe^{3+}, Zr^{4+}, Ti^{4+}, Be^{2+} and Ir^{4+} (500 μg) was overcome by the addition of 1 ml of 2% sodium fluoride solution, and the addition of 1.5 ml of 0.1*M* EDTA eliminated the interference due to La^{3+}, Co^{2+}, Cu^{2+}, Pd^{2+}, VO_4^{3-} and Ru^{3+} (250 μg). Addition of 1 ml of saturated sodium chloride or sodium sulphate solution had no effect on the recovery of osmium.

Difficulties were encountered with acidic sample solutions which required adjustment of pH to the desired range, if this was done before the addition of DPCI, the results being low by 7, 20 and 37% for solutions which had stood for 5, 10 and 15 min respectively. No such effect occurred, however, when the pH adjustments were made in the presence of DPCI, even for samples that stood for 30 min before the extraction.

As the masking agents were found to be ineffective for destroying the interfering metal–DPCI complexes after the extraction with IBMK, care must be exercised to mix the solution thoroughly before the extraction.

Recovery studies

Table 1 furnishes the results for recovery of osmium from synthetic mixtures to which EDTA and fluoride were added before the DPCI. The data clearly show that the method works satisfactorily in the presence of noble and base metals. As no standard samples were available for testing the validity of the method for the analysis of real samples, the method was applied to synthetic mixtures corresponding to osmiridium (syserkite) and iridio-osmium (nevjanskite) samples. Appropriate metal solutions were mixed in the ratios given by Mellor[10] then treated with 1 ml of 5% hydrazine sulphate solution and heated gently almost to dryness. The residue was mixed with 3 g of an 8:1 mixture of potassium hydroxide and nitrate and heated at 800°C for 30 min.[11] The cooled melt was leached with water, acidified with about 10 ml of concentrated hydrochloric acid and made up to volume in a 100-ml standard flask. Aliquots of the solution

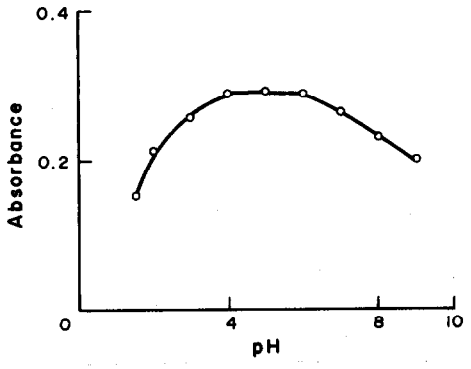

Fig. 2. Effect of pH: 50 μg of osmium(VIII) + 1 ml of 1% DPCI, adjusted to different pH values, extracted with 2 × 10 ml of IBMK and extract made up to 25 ml; measured at 560 nm against solvent in 10-mm cells.

Table 1. Recovery studies (10 μg of osmium)

Sample	Composition	Individual results, μg
1	Fe^{3+} (5 mg) + Zn^{2+} (4 mg) + Cu^{2+} (1 mg)	10.1, 10.0, 10.2
2	Zn^{2+} (5 mg) + Pb^{2+} (2 mg) + Mn^{2+} (2 mg) + Ni^{2+} (1 mg)	10.0, 9.8, 10.1
3	Cu^{2+} (5 mg) + Zn^{2+} (2 mg) + Ni^{2+} (2 mg) + Fe^{3+} (1 mg)	10.0, 10.2, 10.0
4	Cu^{2+} (5 mg) + Zn^{2+} (2 mg) + Ni^{2+} (2 mg) + Co^{2+} (1 mg)	10.0, 10.0, 10.1
5	Pt^{4+} (2.5 mg) + Pd^{2+} (2.5 mg)	10.1, 10.0, 10.0
6	Pt^{4+} (0.25 mg) + Pd^{2+} (0.25 mg) + Rh^{3+} (1 mg) + Ru^{3+} (0.25 mg)	10.0, 10.0, 10.0
7	Ru^{3+} (1.5 μg) + Ir^{4+} (2.5 μg) + Pt^{4+} (0.05 μg)	10.1, 10.0, 10.0
8	Pt^{4+} (10 μg) + Ru^{3+} (1 μg) Ir^{4+} (60 μg) + Rh^{3+} (2 μg)	10.0, 10.1, 10.1

Table 2. Analysis of synthetic samples after oxidative fusion

Sample composition,[10] (%)	Composition of synthetic mixture, μg	Aliquot taken, ml	Os found, μg	Recovery, %
Osmiridium or syserkite				
1. Colombia				
Ir (57.8), Pd (0.63),	Ir (330), Pd (4),	10	19.8	99.0
Ru (6.37), Os (35.1)	Ru (36), Os (200)	20	40.4	101.0
2. California				
Ir (53.5), Pd (2.6),	Ir (500), Pd (24),	5	20.0	100.0
Ru (0.5), Os (43.4)	Ru (5), Os (400)	10	39.8	99.5
3. Urals				
Pt (0.62), Ir (43.28),	Pt (8), Ir (540),	4	20.0	100.0
Pd (5.73), Ru (8.49),	Pd (75), Ru (110),	8	40.4	101.0
Os (40.11)	Os (500)			
4. Borneo				
Pt (0.15), Ir (58.27),	Pt (2), Ir (750),	4	19.8	99.0
Pd (2.64), Os (38.94)	Pd (35), Os (500)	8	40.0	100.0
5. Australia				
Ir (58.13), Pd (3.04),	Ir (350), Pd (20)	10	20.0	100.0
Ru (5.22), Os (33.46)	Ru (32), Os (200)	20	40.0	100.0
6. South Africa				
Pt (0.2), Ir (17),	Pt (1.5), Ir (125)	4	20.0	100.0
Ru (8.9), Os (69.9)	Ru (65), Os (500)	12	59.8	99.7
Iridio-osmium or nevjanskite				
7. Columbia				
Pt (0.1), Ir (70.4),	Pt (1.5), Ir (1025)	10	24.8	99.2
Pd (12.3), Os (17.2)	Pd (180), Os (250)	20	50.0	100.0
8. Urals				
Pt (1.1), Ir (77.2),	Pt (10), Ir (740),	10	20.0	100.0
Pd (0.5), Ru (0.2),	Pd (5), Ru (2),			
Os (21)	Os (200)	20	39.8	99.5
9. South Africa				
Pt (0.1–3.1),	Pt (12), Ir (450),	10	25.0	100.0
Ir (46.8–77.2),	Ru (2), Os (250),	20	50.3	100.6
Ru (0–0.5), Os (21–49.3)	Rb (30)			
Rh (0.5–7.7)				

were analysed by the described procedure, with fluoride and EDTA added to overcome interference. The results in Table 2 show that the recoveries are satisfactory. The method would therefore be of value in the rapid analysis of osmium-containing samples, as it does not require a prior separation of osmium by distillation.

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DETERMINATION OF MOLYBDENUM BY EXTRACTION OF ITS THIOCYANATE INTO ETHYL METHYL KETONE

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Summary—A simple, sensitive and selective spectrophotometric method for determination of molybdenum is described. A solution containing 100 μg of Mo in 2.5*M* hydrochloric acid is treated with ascorbic acid and ammonium thiocyanate and after standing for 8 min is shaken with an equal volume of ethyl methyl ketone for 30 sec. The absorbance of the complex is measured at 465 nm against a reagent blank. The complex is stable for 1 hour. There is no interference from Re(VII), SO_4^{2-}, Cl^-, CH_3COO^-, PO_4^{3-}, NO_3^-, $C_2O_4^{2-}$, citrate or tartrate, and at least 5 mg of U(VI), 10 mg of Cr(III, VI), Th, or Ni, and 20 mg of W(VI) can be tolerated. Vanadium(V) interferes at the 500 μg level, and fluoride slightly decreases the absorbance.

Stannous chloride[1–6] is often used to reduce molybdenum for formation of the Mo(V) thiocyanate complex, but the molybdenum may be further reduced, which lowers the sensitivity of the method. Other elements, *e.g.*, rhenium, vanadium, tungsten, uranium, chromium, which in their lower oxidation states form thiocyanate complexes are also reduced and interfere in the determination of molybdenum. Hence, a milder reductant which reduces molybdenum quantitatively to Mo(V) is desirable, and ascorbic acid has been recommended.[7,8] We have found that use of this reagent, coupled with extraction into ethyl methyl ketone, makes the method highly sensitive.

EXPERIMENTAL

Materials

A stock solution of molybdenum was prepared from sodium molybdate dihydrate, standardized gravimetrically by the oxine method,[9] and diluted to give a working solution with molybdenum concentration about 10 μg/ml. Solutions of other metal ions (mg/ml level) were made from commonly available salts.

Ethyl methyl ketone was distilled and the fraction distilling at 79–80° collected for use.

Procedure

Place the sample solution, containing about 100 μg of molybdenum, in a 100-ml separatory funnel, add 5 ml of concentrated hydrochloric acid, 2 ml of 10% ascorbic acid solution and 2 ml of 20% ammonium thiocyanate solution, dilute to 20 ml with distilled water, mix and let stand for 7–10 min. Then shake with 20 ml of ethyl methyl ketone for 30 sec. Transfer the organic phase to a 25-ml standard flask and make up to the mark with the pure solvent. Measure the absorbance at 465 nm against a similarly prepared reagent blank.

If the sample solution also contains iron(III) and/or copper(II), strip these from the extract by shaking it with 20 ml of 2% stannous chloride solution (in dilute hydrochloric acid). If ruthenium(III) is present, strip it similarly with 2% thiourea solution.

RESULTS AND DISCUSSION

In acid solution, molybdenum forms an orange complex with thiocyanate in presence of ascorbic acid; the complex is quantitatively extractable into ethyl methyl ketone, giving a golden yellow colour. The absorbance is a function of various parameters.

For 20 ml of solution containing 100 μg of Mo, 3 ml of 10% ascorbic acid solution and 2 ml of 20% ammonium thiocyanate solution, the absorbance varies with acidity, and is maximal and constant over the hydrochloric acid concentration range 2.25–3.5*M*.

The absorbance also depends on the ascorbic acid concentration, and for 2.5*M* hydrochloric acid medium (20 ml) containing 2 ml of 20% ammonium thiocyanate solution, the absorbance is maximal and constant if 1.5–5.0 ml of 10% ascorbic acid solution are present and a 7–10 min colour development time is allowed.

The absorbance is similarly dependent on the ammonium thiocyanate concentration and is constant and maximal if 1.8–4.0 ml of 20% thiocyanate solution are present per 20 ml of aqueous phase.

Isoamyl alcohol, isobutyl methyl ketone, isoamyl acetate, or 1% tribenzylamine solution in chloroform can also be used for the extraction but give an absorbance only about 90% of that obtained with ethyl methyl ketone. Chloroform itself does not extract the complex. The extraction is complete if the shaking time is at least 30 sec.

The conditions given in the procedure are based on these data and are selected as giving wide tolerances.

The absorbance at 465 nm remains constant for an hour, and the molar absorptivity is 8.03×10^3

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Table 1. Comparison of the proposed method with the existing thiocyanate methods

Method		Sandell sensitivity, $\mu g/cm^2$	Ref.
Aqueous phase	Organic phase		
Mo(VI), 3*M* HCl, SCN^-, I^-, SO_3^{2-}	—	0.031	13
Mo(VI), 1*M* HCl, Fe^{2+}, SCN^-, Sn^{2+}	isoamyl alcohol	0.018	1
Mo(VI), 2*M* HCl, $N_2H_4.H_2SO_4$, SCN^-	tribenzylamine–chloroform	0.016	14
Mo(VI), HCl–H_2SO_4, tartaric acid, Fe^{3+}, Cu^{2+}, SCN^-, Sn^{2+}	isobutyl methyl ketone	0.015	2
Mo(VI), HCl, SCN^-, ascorbic acid, Cu^{2+}	tributyl phosphate–chloroform	0.015	15
Mo(VI), HCl, ascorbic acid, SCN^- (proposed method)	ethyl methyl ketone	0.012	

$l.mole^{-1}.cm^{-1}$. Beer's law is obeyed up to a molybdenum concentration of 6 μg/ml in the extract.

The continuous-variation[10,11] and mole-ratio[12] methods show that the ratio of Mo(V) to thiocyanate in the complex is 1:3.

Interferences

At the aqueous-phase concentrations (mg/ml) shown in brackets, sulphate (50), chloride (25), nitrate (25), acetate (25), tartrate (5), citrate (5), phosphate (10), oxalate (2.5), W(VI) (0.1), Cr(III, VI) (0.5), U(VI) (0.25), V(V) (0.025), Th (0.5), Ni (0.5) have no effect, but fluoride slightly decreases the absorbance. The interference due to Fe(III) (0.05) and Cu(II) (0.1) is eliminated by washing the organic extract with 2% stannous chloride solution, and Ru(III) is similarly removed with 2% thiourea solution. Re(VII) does not interfere.

Conclusion

The method compares favourably with existing thiocyanate methods for determination of molybdenum (Table 1). The complex formed is sufficiently stable and the results are reproducible. It is a sensitive and selective method. The validity of the method has been tested by the analysis of several synthetic samples, some having compositions representative of different molybdenum alloys (Table 2), and of some steels.

Table 2. Analysis of samples by the proposed method

Sample*	Composition of sample		Mo found, μg
	Other elements†	Mo, μg	
I	V(0.5)	40	41
II	Cr(10)	100	100
III	U(5), Ni(10)	50	50
IV	Fe(0.02), Ni(1.26), Cr(0.4), Cu(0.13), W(0.04), Al(0.02), Mn(0.02)	100	100
V	Fe(1.45), Ni(0.375), Cr(0.5), Co(0.0025), Mn(0.05), Si(0.025), P(0.00125)	100	102
VI	Fe(1.32), W(0.34), C(0.07), Cr(0.2)	50	50
BCS 219/4		Mo 0.58%	Mo 0.60, 0.58%
BCS 261/1		Mo 0.11%	Mo 0.09, 0.10%
BCS 406/1		Mo 1.00%	Mo 0.96, 0.95%

* Synthetic samples equivalent to: IV, cristite-I; V, stainless steel; VI, steel CA-15.

† Figures in brackets indicate the amount in mg.

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A SERIAL ASCII KEYBOARD–PRINTER

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Summary—The construction of a simple keyboard–printer is described. It is intended for use with single-board microcomputers such as the KIM and SYM which have a 20-mA loop ASCII interface.

It is now common to interface microcomputers with many types of instruments. Many inexpensive 8-bit single-board microcomputers (SBC) can be purchased for less than $300. Most SBCs do not have a printer, video display, or full alphanumeric keyboard but usually provide ASCII serial interface hardware and software. The hexadecimal keyboard and display on many SBCs is adequate only for entry and display of machine language codes. To use assembler–editors or higher level languages such as BASIC, a full ASCII keyboard and display unit is required for initial programming and for interaction of the operator with the experiment. However, if many SBCs are used as stand-alone units with various instruments in a laboratory, as in ours, the cost of purchasing a separate commercial teletype or CRT terminal plus printer combination for each SBC becomes prohibitive. These commercial units are 2–10 times the cost of the SBC.

This paper is concerned with the construction of a keyboard–printer which costs about $300 in parts. It is constructed with commercially available sub-units and requires the construction of only one small interface board. A printer was chosen for the display unit instead of a video display, because it is less expensive and because hardcopy is desired in most experiments. The keyboard–printer was constructed specifically for the KIM (MOS Technology, Norristown, PA) and SYM (Synertek Systems Corp., Santa Clara, CA) SBCs used in our laboratory, but it can be used with any SBC with a standard ASCII serial interface. The keyboard–printer has no local copy capability, however, and the printer responds only to characters from the keyboard that are echoed by the serial interface of the SBC or to characters generated directly by the SBC.

The keyboard–printer was constructed with a commercially available keyboard and electrostatic printer [George Risk (George Risk Industries, Kimball, NE) model 753 ASCII encoded keyboard kit ($50), Panasonic (Panasonic Company, Secaucus, NJ) model EUY10E011L line printer ($85) and a Panasonic model EUYPUD7001L parallel ASCII decoder/printer driver board ($115)]. The printer uses 60-mm wide electrostatic paper, and either 16 or 32 characters per line may be selected (other models provide longer line length). The printing speed is two lines/sec and the decoder board has a one-line memory buffer.

Both the commercial keyboard and printer are designed to work with parallel TTL level ASCII data. The KIM TTY (teletype) line, however, delivers 20-mA current-loop serial ASCII. The UART (universal asynchronous receiver and transmitter) board shown in Fig. 1 was constructed to transform the parallel ASCII from the keyboard into serial ASCII, and the serial ASCII from the KIM into parallel ASCII for the printer.

The UART circuit (Fig. 1) operates as follows. When a key is struck on the keyboard, it triggers IC2 to deliver a 15-μsec logic 0 pulse to pin 23 of IC1, after which, on the positive edge the 7-bit parallel ASCII character is strobed into IC1. The 555 timer (IC3) then shifts these character data out of IC1 at pin 25, along with the two stop bits provided by the UART at 1.7 kHz (106 baud). IC8, T2, and T3 then transform these TTL level data with optical isolation into 20-mA current-loop data for the KIM TTY interface.

Serial ASCII from the KIM TTY interface enters IC9 and T4 in 20-mA current-loop form and is translated into TTL level serial ASCII. It then enters pin 20 of IC1 and is shifted into the second half of IC1 at 1.7 kHz. When the 7 bits of the ASCII character are received, IC1 (pin 19)—through T1—delivers a logic 0 level STROBE signal to the printer interface. The interface senses the STROBE level, latches the ASCII character data into a buffer, and sends out a logic 0 acknowledge (ACK) pulse which resets the STROBE level. The UART will continue to process further ASCII characters even if an ACK pulse is not received, in which case the STROBE line remains low. During the print cycle, ACK pulses are not generated and the ASCII character data from IC1 are not accepted by the printer interface.

In some cases, pins 6–12 of IC1 could be directly connected to the ASCII parallel port of a printer interface. However, the particular printer interface board chosen uses 8-bit negative true parallel ASCII logic and translates ASCII codes with the MSD (most significant digit or HEX representation of ASCII bits

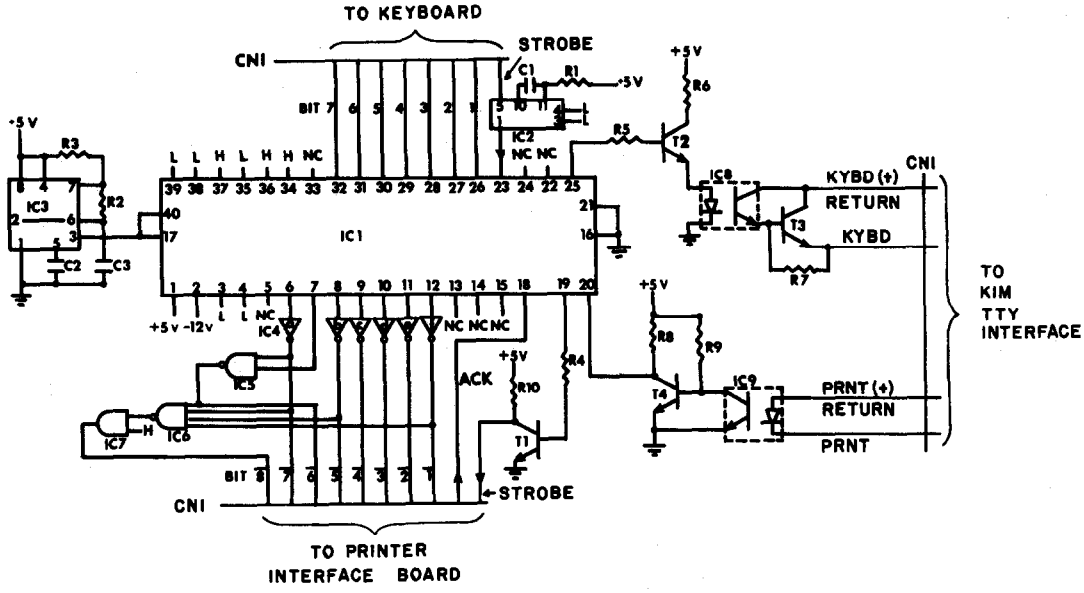

Fig. 1. UART keyboard/printer interface. IC1, AY5013A UART; IC2, 74121 one-shot; IC3, 555 timer; IC4, 7404 hex inverter; IC5, IC7, 7400 NAND gate (2 gates for IC7); IC6, 7420 NAND gate; IC8, IC9, TI111 opto isolator; T1–T4, 2N3904; R1, 2.2 kΩ; R2, 39 kΩ; R3, 27 kΩ; R4, R5, R8, 10 kΩ; R6, 220 Ω; R7, 100 Ω; R9, 270 kΩ; C1, 0.01 μF ceramic; C2, 0.02 μF ceramic; C3, 0.01 μF tantalum; CN1, 30-pin edge-connector on UART interface board.

5–8) equal to 0 as a CR–LF and with the MSF equal to 1, 6–9, E, and F, into a SPACE. Thus upper-case letters, numbers, and symbols are unaffected (the MSD equals 2–5). However, lower-case letters (bits 6 and 7 high or MSD = 6 or 7) and control characters (bits 6 and 7 low or MSD = 0 or 1) with bit 5 high are printed as a SPACE and with bit 5 low are translated as a CR–LF (PRINT).

Therefore IC4 inverts the ASCII data bits from the UART for logic compatibility, while IC5, IC6 and IC7 act as decoding circuitry to transform certain ASCII control codes into spaces (NULL and LF in particular), and all ASCII letters into upper case. This decoding logic works in the following manner (an ASCII code chart is helpful in understanding the following).

The lower case letter problem is solved by "NANDing" ASCII bits 6 and $\overline{7}$. If ASCII bit 7 is high, the character just received by the UART is a letter, and the output of IC4a is low, forcing the output of IC5 high regardless of the status of ASCII bit 6. Hence, ASCII bit $\overline{6}$ (output of IC5) which goes to the printer interface is high, and ASCII bit 6 is translated by the printer as being low even if it is high, so a lower-case letter (ASCII bits 7 and 6 high) will be printed as an upper-case letter (ASCII bits 7 and 6, high and low respectively).

The control characters are decoded by "NANDing" bits $\overline{7}$, $\overline{6}$, $\overline{5}$ and $\overline{1}$ so that a CR–LF or PRINT occurs only once at the end of a line rather than after certain control characters which the KIM transmits after a line. If a control code with bit 5 low is transmitted to the UART, bits 5, 6 and 7 will be low, forcing three inputs of IC6 high. If ASCII bit 1 is high, the fourth input of IC6 will be low, causing its output to be high, and bit $\overline{8}$ to the printer will remain high, resulting in a CR–LF. This is the case for all odd ASCII control codes (such as CR). Any control code with bit 5 low reaching the UART with bit 1 low, however, causes the output of IC6 to go low, causing bit $\overline{8}$ to the printer to go low, and resulting in a SPACE being printed. ASCII control codes with bit 1 low include NULL and LF. Control characters with bit 5 high will also cause a SPACE to be printed although none of these is transmitted by the KIM. Thus the decoding prevents certain control characters from causing a CR–LF when not desired.

The KIM transmits each line of data as DATA, CR, LF and then outputs six NULLs. A CR will cause the printer to print the data line in the buffer. The LF and six NULLs (translated into seven SPACES by the UART), which are also transmitted, will not be accepted by the printer while the printer is printing. Inclusion of seven spaces (translated LF and NULLs) in this KIM data transmission allows the printer enough time to finish printing before any real data from the next line arrive, privided that the transmission rate is $\leqslant 110$ baud, preventing the loss of some of these data.

The UART interface board, along with the keyboard, the Panasonic printer interface unit, and a line printer were encased in an aluminium box along with a dedicated power supply delivering +5 V at 800 mA, −12 V at 400 mA, and −24 V at 1.5 A (circuit diagram available from the authors). Both +5 V and −12 V are used by the keyboard, UART board, and printer interface board, and the printer interface board also requires −24 V.

Although this keyboard–printer was designed for use with a KIM, it can be used with other microcomputers, in some cases with modification. Other keyboard–printer units have been interfaced to SYMs by using the SYM 110-baud TTY lines. The KIM synchronizes to any baud rate while the SYM TTY interface is constructed to accept only exactly 110 baud. For keyboard–printer units interfaced to SYMs, R2 in Fig. 1 was replaced by a 10-turn 50 kΩ pot to allow fine tuning of the frequency or IC3 was replaced by a baud-rate generator (MC14411) and a 1.8432-MHz crystal. The circuitry connected to pins 25 and 20 of IC1 in Fig. 1 can be replaced by standard circuitry for TTL to RS232 interconversion for microcomputers that use RS232.

The UART board has been used with other printers and keyboards. Keyboard–printer units have been constructed with capacitive touch-switch keyboards, including ones made by Tasa (Santa Clara, CA) and RCA (model VP-601) (Lancaster, PA) which are similar in cost to the George Risk keyboard. More expensive printers such as the Axiom EX801 (Glendale, CA) have been used in place of the Panasonic printer and interface card. For this particular unit, IC4-IC7 and T1 in Fig. 1 are eliminated and direct connection is made to IC1.

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SPECTROPHOTOMETRIC DETERMINATION OF URANIUM(VI) WITH 2-(3,5-DIBROMO-2-PYRIDYLAZO)-5-DIETHYLAMINOPHENOL IN THE PRESENCE OF ANIONIC SURFACTANT

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Summary—A highly sensitive method for spectrophotometric determination of uranium has been devised. The method is based on formation of a red-violet 1:2 (metal:ligand) complex from the reaction of uranium(VI) with 2-(3,5-dibromo-2-pyridylazo)-5-diethylaminophenol (3,5-diBr-PADAP) in the presence of an anionic surfactant, sodium lauryl sulphate. Its molar absorptivity is found to be 9.1×10^4 l.mole^{-1}.cm^{-1}. The absorbance is constant in the range pH 8.4–9.9 Beer's law is obeyed for 0–1.4 μg/ml concentrations of uranium. In the presence of DCTA the method is selective for uranium, and can be used for the determination of trace amounts of uranium in water samples.

Florence and co-workers described the use of 2-(2-pyridylazo)-5-diethylaminophenol (PADAP)[1] and 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP)[2] for the spectrophotometric determination of uranium(VI) and reported the practical molar absorptivities to be 6.59×10^4 and 7.4×10^4 l.mole^{-1}.cm^{-1} respectively. However, as these methods require the presence of 45% of ethanol or acetone in the final solution, routine analysis for uranium would require a lot of ethanol or acetone.

We have studied the spectrophotometric characteristics of the complexes formed by some metal ions with the dibromo derivative of PADAP, 2-(3,5-dibromo-2-pyridylazo)-5-diethylaminophenol (3,5-diBr-PADAP), in the presence of an anionic surfactant, sodium lauryl sulphate (SLS), and devised a method for spectrophotometric determination of trace silver[3] based on this reaction. We have now extended this approach and worked out a sensitive spectrophotometric method for uranium. The practical molar absorptivity is 9.1×10^4 l.mole^{-1}.cm^{-1} at pH 9.3 and 576 nm, making the reaction one of the most sensitive known for uranium(VI). Furthermore, the method needs no ethanol or acetone. If DCTA is used as masking agent the colour reaction is highly selective for uranium(VI). The method has been applied to the direct determination of uranium in several water samples.

EXPERIMENTAL

Reagents

Standard uranium solution, 1.00 × 10^{-3}M. Dilute the solution as required, before use.

3,5-DiBr-PADAP solution in ethanol, 5.00 × 10^{-4}M. Dissolve 0.020 g of 3,5-diBr-PADAP, synthesized by the procedure described previously,[3] in 100 ml of ethanol.

SLS solution, 4.0 × 10^{-2}M. Dissolve 5.76 g of sodium lauryl sulphate (C.P.) in 500 ml of warm water.

DCTA solution, 0.1M. Suspend 8.65 g of 1,2-diaminocyclohexane-*N*,*N*'-tetra-acetic acid in about 200 ml of water, neutralize to pH 8.5 with sodium hydroxide, then dilute to 250 ml with water.

Buffer solution, pH 9.0. Adjust 0.1*M* sodium borate to pH 9.0 with hydrochloric acid.

Unless otherwise stated, all reagents used were of analytical-reagent grade, and demineralized water was used throughout the investigation.

Recommended procedure

Place an accurately measured volume (not more than 18 ml) of nearly neutral sample solution, containing less than 35 μg of uranium, in a 25-ml standard flask. If a larger sample volume is desired, it should be evaporated to about 10 ml and then transferred to the flask. If the sample is strongly acidic, take an equal volume and determine the volume of 1*M* sodium hydroxide required for its neutralization. Add, in the following order and with mixing between additions, 1.25 ml of DCTA solution, 2.0 ml of pH-9.0 buffer, the volume of 1*M* sodium hydroxide required for neutralization, 2.0 ml of SLS solution and 1.25 ml of 3,5-diBr-PADAP solution. Dilute to volume with water and mix. Unstopper the flask and place it in a water-bath at 70–80° for 2 min. Cool the flask in running water for 1.5 min, let it stand at room temperature for about 5 min, then measure the absorbance at 576 nm against a reagent blank, in a cell of suitable path-length.

RESULTS AND DISCUSSION

Absorption spectra

In the presence of SLS, 3,5-diBr-PADAP dissolves in water quite well, and exhibits an absorption maximum at 462 nm. Its uranyl complex is red-violet in colour and exhibits two absorption peaks, at 570 and 600 nm. With excess of 3,5-diBr-PADAP present, the optimum wavelength for measurement is 576 nm. The absorption spectra for the reagent and its uranyl complex are shown in Fig. 1. Figure 2 shows that the absorbance is practically constant when the colour is developed over the pH range 8.4–9.9.

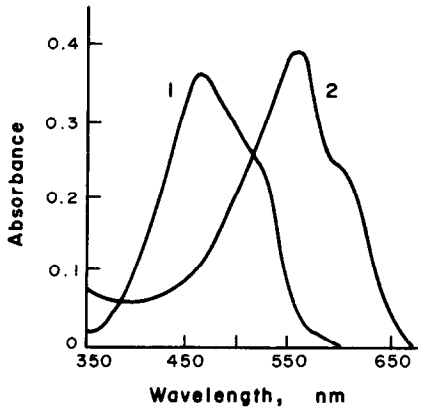

Fig. 1. Absorption spectra: (1) 3,5-diBr-PADAP ($1.00 \times 10^{-5}M$)–SLS ($4.0 \times 10^{-3}M$); (2) 3,5-diBr-PADAP ($1.00 \times 10^{-5}M$)–SLS ($4.0 \times 10^{-3}M$)–U(VI) ($5.00 \times 10^{-6}M$), pH 9.3; measured in 1-cm cells against water.

Effect of reagent concentration

For ~35 μg of uranium(VI) in 25 ml of solution, the optimum reagent concentrations of the final solution were found experimentally to be: $2.00–4.50 \times 10^{-5}M$ 3,5-diBr-PADAP, $3.5–5.0 \times 10^{-3}M$ SLS and $1.0–6.0 \times 10^{-3}M$ DCTA. Higher concentrations of DCTA decrease the absorbance of the uranyl complex.

The effect of ethanol on the absorbance was also tested. The absorbance gradually decreases with increasing amount of ethanol, and there should not be more than 10% v/v of ethanol in the final solution, a difference from the methods of using PADAP and Br-PADAP described by Florence *et al.*[1,2]

Effect of time and temperature for colour development

Uranium(VI) appears to react sluggishly at room temperature with 3,5-diBr-PADAP in the presence of SLS and DCTA, and the maximal absorbance is not reached even on standing for 45 min, but heating in a water bath at 70–80° for 1.5–2 min give complete colour development. The absorbance at 576 nm then remains constant for at least 48 hr.

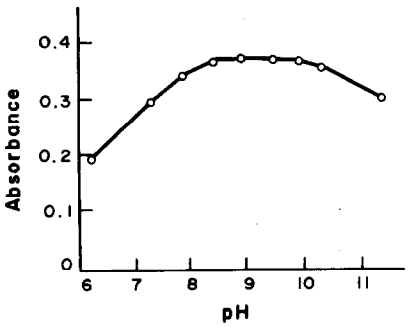

Fig. 2. Effect of pH. The composition of each solution was $4.20 \times 10^{-6}M$ U(VI), $5.0 \times 10^{-3}M$ DCTA, $4.0 \times 10^{-3}M$ SLS, $2.5 \times 10^{-5}M$ 3,5-diBr-PADAP. Measured at 576 nm, in 1-cm cells, against reagent blank.

Adherence to Beer's law

The calibration graph prepared according to the recommended procedure is linear and passes through the origin, the colour system obeying Beer's law for uranium concentrations up to 1.4 μg/ml. The practical molar absorptivity is 9.1×10^4 $l.mole^{-1}.cm^{-1}$ at 576 nm.

Composition of complex

The nature of the complex was investigated by the continuous variation and molar ratio methods, with a fixed concentration of SLS, and the molar ratio of uranium(VI) to 3,5-diBr-PADAP in the complex was found to be 1:2.

Comparison of solubilizing agents

The colour reaction of uranium(VI) with 3,5-diBr-PADAP in the presence of different solubilizing agents, namely ethanol, tetradecyldimethylbenzylammonium chloride (Zeph), cetylpyridinium bromide (CPB), polyoxyethylene sorbitan mono-oleate (Tween 80) and SLS, was examined (Table 1). It can be seen that SLS gives the highest sensitivity.

Effect of diverse ions

The selectivity of the method was investigated by the determination of 25 μg of uranium in the presence of various ions, examined individually. An ion was considered not to interfere if it caused a change of less than 2% in the absorbance obtained for uranium alone. The amounts found to be tolerable were as follows: 5 mg of zinc, 2.5 mg each of aluminium, barium, cadmium, calcium, chromium(VI), copper(II), iron(III), lead, magnesium, manganese(II), mercury(II), nickel, tungsten(VI) and yttrium, 0.75 mg each of bismuth, cobalt(II), niobium, thorium and zirconium, 0.5 mg each of arsenate, carbonate, chromium(III) and phosphate, 0.025 mg each of silver (pH adjusted with nitric acid instead of hydrochloric) and vanadium(V). The tolerance levels for many of the metal ions can be increased by adding more DCTA as masking agent, but the concentration of the excess of DCTA must not exceed 0.006*M* in the final solution, or it will cause low results for uranium.

This was tested with 25 mg of calcium chloride or magnesium sulphate, as follows. After addition of the

Table 1. Comparison of solubilizing agents*

Solubilizing agent	λ_{max}, nm	Molar absorptivity, 10^4 $l.mole^{-1}.cm^{-1}$
Ethanol, 45%	600	5.6
Tween 80, 0.02%	600	7.1
Zeph, 0.1%	600	3.9
CPB, 0.05%	600	6.9
SLS, 0.1%	576	9.1

*Uranium 1.0 μg/ml, DCTA $5.0 \times 10^{-3}M$, 3,5-diBr-PADAP $2.5 \times 10^{-5}M$; pH 9.3; measured in 1-cm cells against reagent blanks; solubilizing agent concentrations in final solution as shown in Table).

Table 2. Uranium analyses

Sample*	Sample volume, *ml*	U(VI) added, *μg*	U(VI) found,† *μg*	Recovery, %
Reservoir water	50.0	0.	0	
	50.0	5.0	4.9	98
	50.0	8.0	7.6	95
	50.0	10.0	9.9	99
Waste-water	50.0	0	0.2	
	50.0	5.0	5.0	96
	50.0	10.0	9.8	96
	50.0	15.0	14.5	95
Well water	15.0	0	3.6	
	15.0	5.0	8.5	98
	15.0	15.0	18.6	100
	15.0	25.0	28.6	100

*The samples were preliminarily digested with nitric acid.
†Mean of two parallel determinations.

3,5-diBr-PADAP to the test solution according to the recommended procedure, 0.1*M* DCTA was added dropwise until the colour of the solution changed from red to orange yellow, then one or two drops in excess. This proved satisfactory, but for larger amounts of the two salts the sensitivity of the colour reaction for uranium was decreased.

The effect of high concentrations of sodium chloride and sulphate was also investigated, and the results showed that less than 30 mg of the chloride or 25 mg of the sulphate caused no interference, but larger amounts decreased the absorbance of the uranium complex (*e.g.*, by about 10% with 125 mg of the sulphate and about 25% with 1.25 g). However, the absorbance of the complex still obeyed Beer's law.

Bromide and iodide were also found to be well tolerated, but only the 2.5-mg level was tested.

Determination of uranium in water samples

The uranium contents in a reservoir water, a waste-water from a copper refinery and water from a well containing copper uranite ore were examined by the recommended method and with addition of standard uranium(VI) solution. The recovery of uranium was 95–100% (Table 2). Nine analyses of the well water gave an average uranium concentration of 0.238 mg/l., with a relative standard deviation of 3.8%.

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ANALYTICAL DATA

EFFECT OF INTERACTION OF THENOYLTRIFLUOROACETONE WITH NEUTRAL OXO-DONORS IN THE SYNERGISTIC EXTRACTION OF TERVALENT ACTINIDES

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Summary—The interaction of HTTA with TBP, DOSO, DBBP and TOPO in xylene has been studied by a spectrophotometric method. The complex species formed is HTTA.S (where S is a neutral donor) and the equilibrium constants for the formation of the species follow the order TOPO > DBBP > DOSO > TBP. After application of a correction for the HTTA–S interaction, the free HTTA and S concentrations in the organic phase were calculated. Plots of log *D* *vs.* log [HTTA] for the tervalent actinides Am, Cm, Bk and Cf gave straight lines with a slope of 3 only after application of the interaction correction; otherwise curves with slopes varying from 3 to 2 were found. The equilibrium constants of the organic phase synergistic reactions of the tervalent actinides are found to be ~10% higher after application of the HTTA–S interaction correction.

The synergistic extraction of metal ions by mixtures of chelating agents (HA) and various neutral oxo-donors (S) has been much studied.[1–3] If the equilibrium constant for a synergistic extraction of a metal ion is to be determined, it must be shown that HA and S do not react with one another, unless the association constant for HA and S is already known, in which case the free concentrations of HA and S can be calculated. There are only a few reports[4–8] of the interaction of neutral donors with chelate extractant molecules. The interaction of thenoyltrifluoroacetone (HTTA) with tributyl phosphate (TBP) in benzene[4] and kerosene,[5] has been reported, but no data are available on the interaction of HTTA with dioctyl sulphoxide (DOSO), dibutylbutyl phosphonate (DBBP) or trioctylphosphine oxide (TOPO) in the organic phase. The present study concerns the interaction of HTTA with TBP, DOSO, DBBP and TOPO in xylene. The equilibrium constants for organic-phase reactions have been calculated. Irving and Edgington[9] considered that the variable slope they found for the plot of log *D* *vs.* log [HTTA] in the system Th(IV)–HTTA–TBP was caused by interaction of HTTA with TBP, but they did not attempt to apply numerical corrections for the interaction. The importance of this interaction correction in the study of the synergistic extraction of the tervalent actinides is illustrated here for the system M(III)–HTTA–TOPO (where M is Am, Cm, Bk or Cf).

EXPERIMENTAL

Reagents

The HTTA used was Merck *pro analysi*. TBP (BDH, laboratory grade) was distilled under reduced pressure after an initial treatment with dilute sodium hydroxide solution. DOSO was kindly supplied by Dr. M. S. Subramanian of this laboratory; its purity was checked by measurement of the melting point. DBBP (K & K) was purified by distillation under reduced pressure after treatment with sodium hydroxide. TOPO (Eastman Kodak) was used as received. Xylene (BDH "AnalaR") was the solvent. The tervalent actinides Am, Cm, Bk and Cf were purified and radioassayed as described previously.[10] All other reagents used were of analytical-reagent grade.

Equilibration of neutral oxo-donors with HTTA

Organic phases (2 ml) containing mixtures of HTTA (at a fixed concentration of 0.02*M*) and varying concentrations of neutral oxo-donors (TBP, DOSO, DBBP 0.0–0.3*M* and TOPO 0.0–0.4*M*) were equilibrated with the same volume of 0.13*M* hydrochloric acid for 5 hr. After centrifugation, 1-ml aliquots of the aqueous phase were taken and diluted to 10 ml and the absorbance measured at 292 nm (Cary-14 spectrophotometer) against 0.13*M* hydrochloric acid. From the known molar absorptivity (7.88×10^3 l.mole^{-1}.cm^{-1})[11,12] at 292 nm, the aqueous-phase HTTA concentrations were determined. Free neutral oxo-donor concentrations in the organic phase were calculated from values for the distribution coefficient (*D*) for HTTA with and without neutral oxo-donors present.

HTTA variation experiments

Aliquots (1 ml) of xylene solutions of varying HTTA concentration and a fixed initial concentration of TOPO

($3.0 \times 10^{-4}M$) were equilibrated for 1 hr with an equal volume of a chloroacetate buffer (pH 2.70, $I = 0.005$) spiked with the tracers of various tervalent actinides at $30 \pm 0.1^{\circ}$. After the solutions had been left to settle for 30 min, aliquots of both phases were taken for radioassay.

Calculation of the HTTA–S interaction equilibrium constant (K)

The distribution coefficient (D) for HTTA is given by

$$D_{HTTA} = [HTTA]_{org}/[HTTA]_{aq}. \quad (1)$$

The equilibrium between HTTA and the neutral oxo-donors (S) may be written as

$$[HTTA]_{org} + n[S]_{org} \rightleftharpoons [HTTA.nS]_{org} \quad (2)$$

for which the equilibrium constant, K, is given by

$$K = [HTTA.nS]_{org}/[HTTA]_{org}[S]^{n}_{org}. \quad (3)$$

The distribution coefficient for HTTA in the presence of neutral oxo-donors may be expressed by

$$D = \frac{[HTTA]_{org} + [HTTA.nS]_{org}}{[HTTA]_{aq}}. \quad (4)$$

Substituting for $[HTTA.nS]_{org}$ from equation (3) gives

$$D = \frac{[HTTA]_{org} + K[HTTA]_{org}[S]^{n}_{org}}{[HTTA]_{aq}} \quad (5)$$

or

$$D = D_{HTTA}(1 + K[S]^{n}_{org}). \quad (6)$$

Values for the free neutral donor concentrations $[S]_{org}$ can be calculated by assuming that $n = 1$ in equation (4), as it can be easily shown that,

$$[S]_{org} = \text{Initial}\,[S]_{org} - (D - D_{HTTA})\frac{0.02}{D + 1} \quad (7)$$

where 0.02 is the initial value of $[HTTA]_{org}$ in all cases.

A graph of D *vs.* $[S]_{org}$ (equilibrium concentration of the free neutral oxo-donors) should give a straight-line plot if $n = 1$ in the systems studied. In the above treatment, hydration of HTTA, S and HTTA.S has been ignored, and it is assumed that changes in the activity coefficients over the concentrations used are not significant. As seen from Fig. 1, straight lines are observed when D is plotted *vs.* [S], so n has a value of one in all the systems studied. The equilibrium constants for HTTA–S species are evaluated from the slopes and intercepts of these straight lines.

RESULTS AND DISCUSSION

Table 1 gives values of the distribution coefficient for HTTA with various neutral oxo-donors and Fig. 1 shows the least-square straight line plots of D *vs.* equilibrium concentrations of the free neutral oxo-donors. The values of K calculated from the slopes and intercepts of these lines, along with values for equilibrium constants for some similar systems, are given in Table 2. From Fig. 1 it can be seen that the D-values for TOPO at a concentration greater than a $0.2M$ fall much below the line for the lower TOPO concentrations. This lowering of D-values at higher TOPO concentrations could result from some form of non-ideal behaviour of concentrated TOPO solutions. Cox and Davis[5] in their studies of the interaction of HTTA and TBP in kerosene suggested that a complex formed by hydrogen bonding of TBP to the keto-

Table 1. Variation of the distribution coefficient, D, of HTTA with neutral donor concentrations

Initial $[HTTA]_{org} = 0.02M$, $D_{HTTA} = 37.28$

Initial [Neutral donor]$_{org}$, M	Free [Neutral Donor]$_{org}$, M	D
	TBP	
0.050	0.049	39.9
0.100	0.097	44.0
0.150	0.145	51.5
0.200	0.193	56.3
0.250	0.243	59.6
0.300	0.292	63.3
	DOSO	
0.050	0.047	42.8
0.100	0.094	53.3
0.150	0.143	58.3
0.200	0.192	63.3
0.250	0.241	70.7
0.300	0.290	75.9
	DBBP	
0.050	0.043	56.3
0.100	0.091	70.7
0.150	0.139	86.3
0.200	0.187	100.5
0.250	0.236	120.2
0.300	0.286	130.6
	TOPO	
0.025	0.019	55.2
0.050	0.041	69.0
0.075	0.063	89.0
0.100	0.087	104.0
0.125	0.111	116.0
0.150	0.136	130.6
0.175	0.160	141.9
0.200	0.185	149.4
0.300	0.284	184.2
0.400	0.384	209.5

hydrate may be present. In the light of this, equation (4) may be extended to

$$D = \frac{[HTTA_{enol}]_{org} + [HTTA.H_2O]_{org} + [HTTA.H_2O.n\text{TBP}]_{org}}{[HTTA.H_2O]_{aq}}.$$

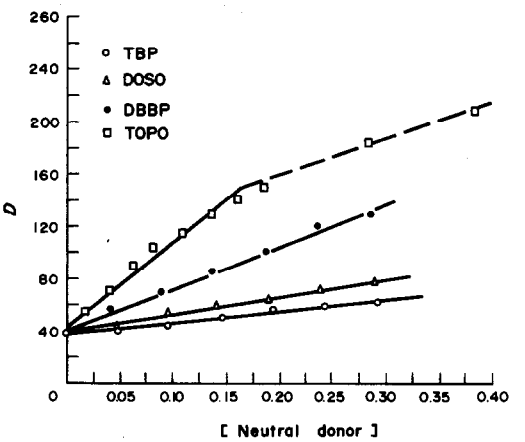

Fig. 1. Variation of the distribution coefficient of HTTA with neutral oxo-donor concentrations.

Table 2. Equilibrium constants of the adducts formed between synergistic extractants

Equilibrium	Solvent	log K	Ref.
HTTA + TBP $\rightleftharpoons$ HTTA.TBP	benzene	0.46	4
	xylene	0.41	Present work
HTTA.H_2O + TBP $\rightleftharpoons$ HTTA.H_2O.TBP	kerosene	0.54	5
HTTA + DOSO $\rightleftharpoons$ HTTA.DOSO	xylene	0.56	Present work
HTTA + DBBP $\rightleftharpoons$ HTTA.DBBP	xylene	0.94	Present work
HTTA + TOPO $\rightleftharpoons$ HTTA.TOPO	xylene	1.24	Present work
HTTA + TOA $\rightleftharpoons$ HTTA.TOA	benzene	3.15	8
HTTA + TOA + H^+ + Cl^- $\rightleftharpoons$ HTTA.TOA.HCl	benzene	5.32	8
HBPHA + TBP $\rightleftharpoons$ HBPHA.TBP	CCl_4	1.24	6
	benzene	1.11	6
HBPHA + TOPO $\rightleftharpoons$ HBPHA.TOPO	CCl_4	2.41	6
	benzene	2.32	6
HDEHP + TBP $\rightleftharpoons$ HDEHP.TBP	toluene	2.00	7

Table 3. Distribution coefficients for M–TTA–TOPO systems at varying HTTA concentrations

Initial [TOPO] = $3.0 \times 10^{-4} M$

[HTTA], M	After correction, [TOPO], $M \times 10^{-4}$	log D experimental		log D^* corrected	
		Am(III)	Cm(III)	Am(III)	Cm(III)
0.016	2.35	−0.512	−1.0273	−0.512	−1.027
0.024	2.12	−0.047	−0.581	0.034	−0.500
0.040	1.77	0.478	−0.698	0.698	0.153
0.056	1.52	0.787	0.241	1.125	0.580
0.064	1.42	0.898	0.355	1.291	0.748
0.080	1.26	1.087	0.530	1.577	1.019

* log D values normalized for [TOPO] = $2.35 \times 10^{-4} M$

Cox and Davis[5] found $n = 1$, as found in the present work for all the systems studied.

From Table 2, it may be seen that K follows the order TBP < DOSO < DBBP < TOPO, which is also the order of basicity of these neutral oxo-donors. A similar order of the equilibrium constants (TBP < TOPO) was observed by LeRoux and Fouche[6] in studies of the interaction of *N*-benzoyl-*N*-phenylhydroxylamine (HBPHA) with TBP and TOPO. In the literature, K values for HTTA–TBP in benzene (2.9)[4] and in kerosene (3.5)[5] have been reported which agree well with our value (2.6) in xylene.

From the K values (Table 2) the free concentrations of S were calculated for the extraction of tervalent actinides (*i*) at fixed TOPO and varying HTTA concentrations and (*ii*) at fixed HTTA and varying TOPO concentrations. As an example Table 3 gives values of the distribution coefficient for the M(III)–HTTA–TOPO system (M = Am, Cm) at fixed TOPO and varying HTTA concentrations before and after applying the correction for the HTTA–TOPO interaction. It can be seen from Table 3, column 4, that the corrected log D^* values (details on obtaining this value are given in Refs 13 and 14) are much higher than the experimental log D values. Also it is observed that $\Delta \log D$ (log D^* − log D) value increases

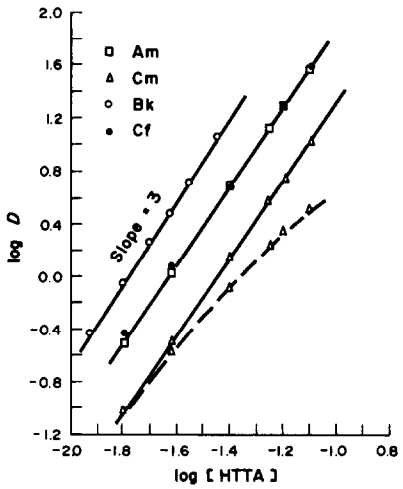

Fig. 2. Variation of the distribution coefficient of tervalent actinides as a function of HTTA concentration at a fixed TOPO concentration.

with increasing HTTA concentration at a fixed TOPO concentration. The graph (Fig. 2) of log D (for Cm) *vs.* log [HTTA] is a curve (broken lines) from which three straight lines with slopes 2.8, 2.4 and 2.0 could be drawn, but log D^* *vs.* log [HTTA] is a single straight line with a slope of 3 (continuous lines), as expected from the known composition of the species extracted, $M(TTA)_3.nTOPO$ (n = 1 and 2, M = Am, Cm, Bk and Cf). Similar straight lines with slopes equal to 3 are obtained for Am, Bk and Cf. This shows the importance of applying the correction. Most previous authors have not applied such corrections, which is obviously unsatisfactory. The equilibrium constants for the organic phase synergistic reactions for the tervalent actinides were found to increase by $\sim 10\%$ when the HTTA–S interaction correction was applied.

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FORMATION CONSTANT AND STOICHIOMETRY OF THE ACETONITRILE:18-CROWN-6 COMPLEX BY NUCLEAR MAGNETIC RESONANCE, RAMAN AND INFRARED SPECTROSCOPY

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Summary—Crown ethers are increasingly used in a variety of chemical applications. While crown ether complexes with alkali-metal cations have been extensively studied, relatively little is known about their complexes with neutral molecules to form so-called host: guest complexes. The use of NMR is reported for the determination of the formation constant (2.1 ± 0.1) for the acetonitrile:18-crown-6 complex. The suitability of Raman spectroscopy for studies of the solid-phase complex is demonstrated and limitations in the use of infrared spectroscopy are discussed.

Crown ethers are of considerable importance because of their ability to complex cations, especially alkali-metal cations, by ion–dipole interaction. This mechanism permits solvation of ionic salts in non-aqueous solvents, a phenomenon frequently utilized in phase-transfer catalytic systems. In addition, crown ethers provide good model systems for biochemical and biological membrane transport agents, such as the macrocyclic antibiotics, one of which, valinomycin, is the principal intracellular transport agent for potassium. Complete understanding of the interaction of crown ethers with traditional alkali-metal cation complexants requires that other effects be recognized and understood. We have recently reported the spectroscopically evidenced changes of dichromate, oxalate, and permanganate anions in the presence of their crown ether–cation complexes.[1] The current study probes specific effects by looking at a specific complex of 1,4,7,10,13,16-hexaoxacyclo-octadecane (18-crown-6) with acetonitrile (MeCN).

Considerable interest has been generated in recent years concerning neutral complexes comprised of crown ethers (termed the donor or host molecule) and small organic guest molecules. The driving force in these types of interaction is formation of hydrogen bonds between the *n*-donor oxygen atoms of the crown ether and the acidic hydrogen atoms of the CH or NH groups in the guest molecule.[2] This phenomenon has been termed "host–guest complexation"[3] and is germane to understanding the interactions that occur in biological systems, such as enzyme catalysis.

The complex that forms between 18-crown-6 and MeCN has been recognized since at least 1973,[4] but its formation constant has not hitherto been reported. In this paper, we report the stability constant determined by nuclear magnetic resonance techniques. Because of the inherent instability of the complex, spectroscopic investigations of its nature are difficult. Infrared, Raman, and nuclear magnetic resonance methods are considered below with respect to the 18-crown-6:acetonitrile complex.

EXPERIMENTAL

Instrumentation

The NMR studies were performed with a Bruker WM250 NMR spectrometer operating at 250 MHz for protons. Raman spectra were run on a Spex 1401 Raman spectrometer interfaced to a Nicolet 1180 data-acquisition system. A Perkin-Elmer 180 infrared spectrophotometer was used to obtain infrared spectra.

Reagents

All solvents were reagent grade and were dried over 8–12 mesh activated molecular sieves (Fisher Scientific). 18-Crown-6 (Parish) was purified as described below. 18-Crown-6/MeCN complex was obtained from Parish and was also prepared by the methods described below.

Purification of 18-crown-6

18-Crown-6 was purified by a modification of the method used by Cram *et al.*[5] It was dissolved with heating (but not boiling) in enough acetonitrile to effect solution.

The solution was allowed to cool slowly to room temperature, and was then further cooled in an acetone/ice slurry (at between -15 and $-20°$). The resulting crystals (18-crown-6/MeCN complex) were rapidly vacuum-filtered. It was essential to filter rapidly in order to prevent the crystals from sequestering atmospheric water. The crystals were then transferred to a round-bottomed flask and left under reduced pressure (about 15 mmHg) for about 6 hr. This treatment completely removed all MeCN present as solvent and in the complexed form. The purified crown ether was a fine, hygroscopic white powder, m.p. 38–40°.

Preparation of 18-crown-6/MeCN complex

The complex obtained from Parish appeared "wet". Therefore, during the course of this work two methods

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were used to synthesize the complex in order to make comparisons with the Parish product. In the first method, the Parish product was first subjected to vacuum to remove MeCN, leaving the 18-crown-6. Then the MeCN was replaced by passage of a stream of nitrogen saturated with MeCN over the 18-crown-6 for 6 hr. The product of this treatment exhibited a Raman spectrum that did not differ significantly from that of the original Parish complex, but the material did not have the "wet" appearance of the Parish complex. Formation of the complex was evidenced by the Raman spectrum and by marked increase in the melting point to 73–78°.

The second method consisted of the procedure used to purify the 18-crown-6, but stopping after collection of the complex. This procedure yielded a product which exhibited the "wet" appearance of the Parish complex. However, all three preparations of the complex gave rise to the same Raman spectral features and to identical melting point ranges.

RESULTS AND DISCUSSION

Purification of 18-crown-6

When 18-crown-6 was used as received, non-reproducible results were obtained in the stability constant studies (*vide infra*). The crown ether was therefore purified by the procedure described above, and the product was found to be highly satisfactory. The manufacturer apparently packs the crown ether in the liquid form into the glass bottle, and upon cooling a single mass is formed. When this clump is fractured, the crystals appear "wet" and exhibit a hydroxyl vibrational intensity in the 3500 cm^{-1} region of the infrared spectrum. In addition, a wide melting point range is observed: 34–38°. After purification, the crown ether is in the form of a fine white powder which exhibits negligible hydroxyl vibrational intensity and melts in the range 38–40°, in excellent agreement with the literature value of 39–40°.

Nuclear magnetic resonance spectroscopy

Owing to the nature of the hydrogen bonding between 18-crown-6 and MeCN in the complex, a rather weak overall interaction is expected. Indeed, even passing a stream of dry nitrogen over the crystalline complex is sufficient to remove the MeCN completely. This characteristic behaviour prompted a search for a method to determine the stability constant of weakly associated species.

The method selected was that of Foster and Fyfe.[7] A series of solutions was prepared, containing guest molecule (MeCN, concentration 5 m*M*) and varying concentrations of the host molecule (18-crown-6) chosen so that the host was present in great excess. Carbon tetrachloride was used as the solvent because it is considered not to form a complex with 18-crown-6. The experimental NMR parameter observed was the chemical shift (*vs.* tetramethylsilane, TMS, internal standard) of the MeCN methyl protons as a function of host concentration. MeCN is ideal for this type of study since it exhibits a single sharp resonance near 2.0 ppm, which is not obscured by the 18-crown-6 proton resonance at 3.55 ppm.

The relationship between the chemical shift of the MeCN methyl protons and the donor concentration is given in equation (1).

$$\Delta/[H] = -K\Delta + \Delta_0 K \qquad (1)$$

where $\Delta = \delta^G_{obs} - \delta^G_o$, $\Delta_o = \delta^G_{GH} - \delta^G_o$, [H] = host concentration, K = formation constant, δ^G_{obs} = observed chemical shift of guest (MeCN) protons in the presence of host (18-crown-6), δ^G_o = chemical shift of guest protons in the absence of host, and δ^G_{GH} = chemical shift of guest protons in "pure host-guest complex".

A plot of $\Delta/[H]$ *vs.* Δ is linear, with slope equal to $-K$. Typical data are shown in Table 1. Three determinations yielded an average value of 2.1 (standard deviation 0.1) at 298 K. These plots exhibited good linearity, with high correlation coefficients, suggesting the existence of a 1:1 complex [equation (1) was derived on the assumption of 1:1 stoichiometry; see reference 6]. The MeCN methyl protons shift downfield with increasing 18-crown-6 concentration, an observation consistent with a hydrogen-bonded system undergoing rapid exchange.[8,9]

An attempt was made to determine the stoichiometry of the solid complex. A 0.1*M* solution of the complex in carbon tetrachloride was examined by NMR and the integrated peak intensities were used to calculate the relative concentrations of 18-crown-6

Table 1. Effect of 18-crown-6 concentration on the chemical shift of acetonitrile present at 0.005*M* in CCl_4 at 298 K

18-Crown-6 concentration [H], *M*	MeCN methyl proton chemical shift *vs.* TMS, *Hz*	Δ	$\Delta/[H]$
0	488.70	0	—
0.202	493.68	4.98	24.7
0.257	494.73	6.03	23.4
0.307	495.34	6.64	21.7
0.399	496.59	7.89	19.8
0.602	497.62	8.92	14.8
0.700	498.66	9.96	14.3
0.800	499.49	10.79	13.5

For explanation of Δ and $\Delta/[H]$ see text. The data constitute a representative set for which the calculated *y*-intercept, slope, and correlation coefficient are 35.8, −2.14 and −0.98, respectively.

and MeCN present in solution. Repetitive analysis of the same sample failed to give consistent values for the stoichiometric factors. Values ranged from as high as 2.4:1 (MeCN:18-crown-6) to as low as 1.4:1 for replicate analyses. This is undoubtedly a manifestation of the instability of the complex (volatility of MeCN present in the complex). Furthermore, the method of preparation of the complex affects the apparent stoichiometry, the complex formed in solution having higher ratios of MeCN to 18-crown-6 than the complex formed by adsorption of MeCN from MeCN-saturated nitrogen gas (*vide supra*). This type of complication seems to have resulted in a good deal of confusion in the literature concerning the nature of the solid crystalline complex. For example, reported stoichiometric values include 1:1,[10] 2:1,[4] and 1.6:1[11] (MeCN:18-crown-6).

Infrared and Raman spectroscopy

A definitive Raman spectroscopic study of the complex was reported in 1974 by McLachlan.[4] This study demonstrated that the 18-crown-6 molecule exists in approximate D_{3d} symmetry when present in the solid crystalline complex. Little was said regarding the orientation of the MeCN with respect to the crown ether. The MeCN may reasonably be postulated to be oriented perpendicularly to the plane of the 18-crown-6 with the methyl protons directed toward three of the 18-crown-6 oxygen atoms. This type of orientation is analogous to that found in the complexes which form between some alkylammonium ions and 18-crown-6,[12] although the interaction is of course much weaker in the complex with MeCN.

The Raman data obtained were generally consistent with those reported by McLachlan, with the exception of one rather striking feature. The CN stretching frequency in the solid crystalline complex appears at 2243 cm^{-1}, a shift of 10 cm^{-1} from the CN stretching frequency in pure liquid MeCN. This shift was confirmed by repetitively running pure MeCN and the solid complex in succession and comparing their respective Raman spectra (Fig. 1). This shift has not previously been reported in the literature. It may be rationalized by postulating a weakening of the force constant of the CN band by the increased electron density acquired by inductive effects through the methyl protons. However, hypotheses of this type can never be certain; final confirmation would require a normal co-ordinate analysis, a task which poses a formidable computational problem and remains to be done.

Infrared data for comparison with the Raman data are of course desirable. However, difficulties were encountered during attempts to record infrared spectra of the complex. These problems are a direct result of the inherent instability of the complex and the nature of 18-crown-6. Alkali-metal halide cell materials are not recommended for use when analysing species containing 18-crown-6. The crown ether will attack these materials (especially potassium salts), rendering the infrared data obtained questionable or even invalid in regions where spectral changes occur on alkali-metal cation complexation. This limitation is especially important when analysing solutions, since the crown ether will cause solvation of the cell material, resulting in formation of potassium: 18-crown-6 complex; this complex is known to be very stable.

Infrared spectra of the solid complex could not be obtained, owing to the instability of the complex. Any perturbation applied to the complex is sufficient to destroy it. For example, a perturbation as gentle as passing a stream of dry nitrogen over the complex volatilizes the MeCN and is evidenced by marked changes in melting point before and after such treatment. Standard procedures used to obtain infrared spectra of solids generally employ much harsher treatments, such as pressure (to form an alkali-metal halide pellet) and heating (to form a film). Results obtained by utilizing these methods, a number of which are extant in the literature, should be treated with caution. Despite the growing use of crown ethers in research and catalysis, the problems associated with infrared analysis of crown ether complexes have not always been recognized.

While the lack of complementary infrared spectroscopic data for this system is unfortunate, Raman spectroscopy provides a nearly ideal alternative since it is non-intrusive and overcomes the need for halide salt plates.

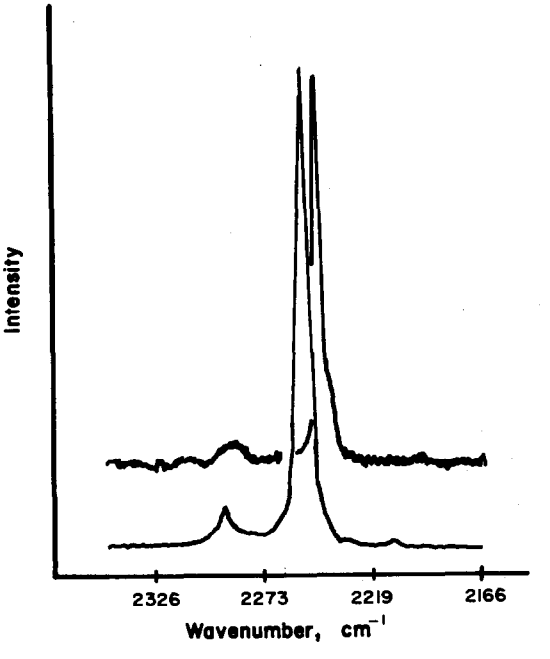

Fig. 1. C–N stretching region in Raman spectra of acetonitrile (MeCN); in the complexed (solid) state, upper and as pure liquid MeCN, lower curve. The relative intensities are arbitrary.

Conclusion

The widespread use of crown ethers requires that the details of crown ether complexation be investigated. While a good deal of effort has been directed towards complexes with alkali-metal cations, little attention has been directed towards complexes with neutral organic molecules. We have demonstrated the applicability of NMR methods to the determination of the formation constant of a representative very weak host–guest complex. This method is applicable to the entire class of complexes. The spectral shift evidenced in the Raman spectra has not been reported before, but is definitely indicative of formation of the crystalline MeCN:18-crown-6 complex. While the lack of infrared data is unfortunate, the considerable effort directed towards trying to obtain satisfactory infrared spectra has resulted in identification of problems inherent in the infrared analysis of crown ethers. These limitations have not always been clearly stressed in the literature. NMR is thus seen to be a valuable probe for determining weak formation constants of compounds in the liquid state, while Raman spectroscopy constitutes a near-ideal non-intrusive probe for solid-state complexes of crown ethers.

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ANNOTATIONS

THE EFFECT OF HEATING TIME IN THE DETERMINATION OF PHOSPHORUS BY WET DIGESTION

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Summary—It is shown that the length of heating during wet digestion is a critical factor in the determination of phosphorus in some seeds and nuts. For each sample a certain minimum heating time at a chosen temperature is necessary.

The interest in trace elements, whether as essential constituents in biological systems, or a potential source of danger to life, or on account of their physicochemical properties, has led to the publication of many analytical procedures for their determination.[1–4] An essential preliminary step in the determination of mineral elements in ecological materials is the decomposition of the organic matter, either by wet- or dry-ashing. Although the choice of procedure is still largely a matter of personal preference, for phosphorus wet digestion with mixed acids is preferred,[5] to minimize volatilization losses. In the last decade efforts have been directed towards reducing the deficiencies of the conventional digestion procedures. Thus, Adrian[6] proposed a wet digestion performed under pressure at low temperature. John[7] has reported an automated digestion method which is superior to dry-ashing for most elements.

The new digestion methods often show no advantage in precision or accuracy over the conventional methods, but are known to be easy, fast and safe, and require virtually no equipment.[6]

However, many of the published procedures are rather vague about the heating conditions during the wet digestion stage. For instance, we consider that instructions that digest mixtures be heated initially at moderate heat, with a later increase,[5] or until white fumes of sulphuric acid are evolved,[8] are inadequate. In the course of our study of the phosphorus content of the edible parts of certain seeds and nuts we have therefore examined systematically the effect of heating time on the amount of phosphorus found.

EXPERIMENTAL

Reagents

All reagents were of analytical reagent grade. Phosphorus standard stock solution was prepared by dissolving 0.4393 g of dry potassium dihydrogen phosphate in 1 litre of distilled water. Ammonium molybdate–sulphuric acid reagent was prepared by dissolving 25.0 g of ammonium paramolybdate tetrahydrate in 200 ml of warm distilled water, filtering the solution into 680 ml of 70% v/v sulphuric acid, and diluting to 1 litre; the solution was stored in the dark. Stannous chloride solution was prepared immediately before use by dissolving 0.5 g of stannous chloride dihydrate in 250 ml of 2% v/v hydrochloric acid.

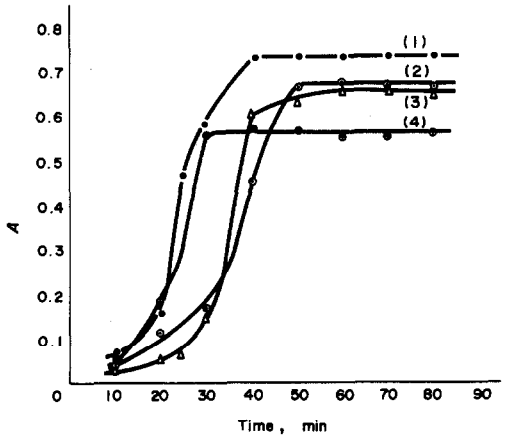
Procedure

A 0.25 g sample was placed in a 100-ml digestion flask, and 1 ml of 60% perchloric acid, 5 ml of 70% nitric acid and 0.5 ml of 98% sulphuric acid were added.[5] Two variations of the digestion procedures were then used. In one (P1), the flask was heated at 65° for 10 min, then the mixture was allowed to cool and total phosphorus was determined by the molybdenum blue method. The procedure was repeated for each sample, for periods of 20, 25, 30, 40, 50, 60, 70 and 80 min. In each case excessive charring of samples was avoided, and the mixture always contained free liquid.

In the second procedure (P2), the sample was heated initially at "moderate heat", starting at a temperature below 65°, with gentle swirling of the flask. The heat was gradually increased, and digestion was stopped 15 min after the appearance of white fumes.[5] About 25 min of heating were required. Blank determinations were also made.

RESULTS AND DISCUSSION

Figure 1 shows the variation of absorbance with heating time for eight seeds and nuts, studied by procedure P1. A characteristic curve is obtained for each sample. Although the chemistry of the reactions involved in wet digestion is still not well understood, it would appear that the reactions go to completion after a time interval which depends on the material being digested. It is of interest that different completion times are observed for the apparently similar samples used in this study. This would indicate that generalizations of methods should be done with due care. The initial slopes of the curves shown are prob-



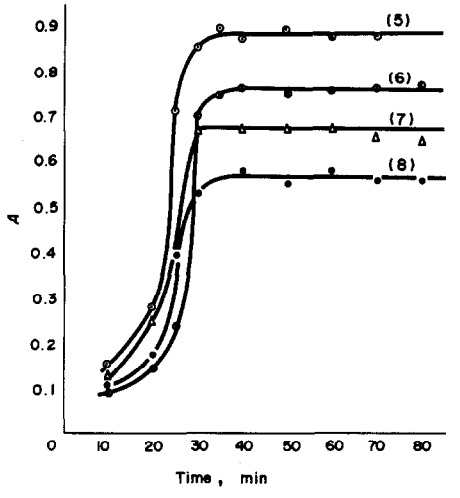


Fig. 1. Variation of absorbance with time for (1) Elaeis guineansis, (2) Arachis hypogaea, (3) Sorghum bicolor, (4) Voandzeia subterranea, (5) Cucumis melo, (6) Phaleolus vulgaris, (7) Tamarindus indica, (8) Irvingea gabonensis.

ably a reflection of the resistance of the organic matter in the samples to decomposition.

The maximum phosphorus contents estimated by procedure P1 are compared in Table 1 with the values obtained by the conventional heating procedure (P2). The P2 values are generally lower than the P1 values, suggesting that procedure P2 gave incomplete decomposition. It was also observed that increasing the heating temperature in procedure P1 did not significantly alter the maximum phosphorus value found, but did reduce the heating needed to reach it. However, heating too rapidly should be avoided.

Table 1. Results for the determination of phosphorus (%)* by procedures (P1) and (P2)

Sample	P1	P2
Elaeis guineansis	0.24	0.22
Arachis hypogaea	0.22	0.14
Sorghum bicolor	0.21	0.08
Voandzeia subterranea	0.17	0.04
Cucumis melo	0.29	0.14
Phaleolus vulgaris	0.25	0.21
Tamarindus indica	0.22	0.08
Irvingea gabonensis	0.18	0.10

*Average of at least three determinations.

CONCLUSION

It is evident that it is essential to ensure that digestion reactions proceed to completion to avoid underestimation of the desired element. The requirement that digestion be done at "moderate heat" until the evolution of white fumes of sulphuric acid appears liable to produce errors. For an accurate determination, preliminary runs should be carried out to determine the minimum heating time necessary for each sample. Furthermore, the lower the temperature that can be used, the less the risk of apparent loss through polymerization or volatilization of phosphorus compounds.

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HOMOGENEITY OF SOLIDS: A PROPOSAL FOR QUANTITATIVE DEFINITION

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Summary—A simple formula has been deduced to predict the detectability of chemical homogeneity of solid substances by use of an analytical method with known spatial resolution. The formula includes the ratio of the spatial resolution of the method to the spatial distribution of the component to be determined, and the standard deviation of the method.

Investigations of the chemical uniformity of solid substances are becoming more and more important. Solid raw materials or products used in electronics or in metallurgy must be, in many cases, rigorously analysed, since their chemical homogeneity decisively influences their use and the physical properties of the product.

Danzer *et al.*[1] recently published a paper in which, using statistical criteria, they deduced a formula by which the homogeneity of a solid sample could be evaluated. The resolution and the standard deviation of the analytical procedure were included in the formula. Another approach is described here, in which the effect of the spatial resolution of the analytical method on the detectability of inhomogeneity is also included.

Principles of homogeneity

The concentration distribution of an element in the surface layer of a solid sample can be readily followed in the direction of the two co-ordinates by a scanning analytical method, assuming that the concentration change in the direction of the co-ordinates is relatively high and the distances between areas of different compositions are easily measurable. Thus, the local concentration of the components in a very small area on the surface can be determined precisely. If, however, the diameter of the spot analysed is comparable with the distance between the areas of high and low concentrations, or the standard deviation of the method is comparable with the difference between the high and low concentrations, reliable information on the homogeneity or the concentration distribution of an element cannot be obtained.

For surface-spot analysis many new high-efficiency scanning methods are now available, *e.g.*, laser-generated atomic-emission spectroscopy, electron-beam microanalysis, secondary ion mass spectrometry, *etc.* Their spatial resolution varies between 1 and 100 μm.

It is clear that the extent of information on homogeneity depends on the following factors.

(1) The amount or volume of the subsample which is directly analysed. The smaller this is, the higher will be the geometric resolution of the scanning process, and concentration changes over very short distances can be recognized.

(2) The statistical error of spot analysis. Concentration differences can be detected only if they are significantly greater than the statistical error of the analysis.

(3) The distance between the points of high and low concentrations of the components in the direction of the space co-ordinates.

(4) The mean value of the concentration differences of the elements to be determined, in the direction of the three co-ordinates.

It is easy to see that four factors are not independent. The higher the geometric resolution and precision of the analytical method, and the greater the distance and concentration difference between the points of low and high concentrations, the more reliable will be the results of the scanning process, or the information on the inhomogeneity of the solid sample. If, however, the geometric spatial resolution is poor, and the standard deviation is high, inhomogeneity of the sample cannot be detected.

Deduction of the formula proposed

For calculation of the approximate limits of detection, or determination of inhomogeneity, the following assumptions are made.

(1) For simplification the solid sample is considered to be analysed only in the direction of the z co-ordinate.

(2) The change in signal caused by a change in concentration of the component of interest, in the direction z, can be approximated by a sine curve, which can be characterized by an average amplitude and wavelength. The amplitude is denoted by $\Delta x/2$, and the wavelength by Δz.

(3) The "window" for spot analysis is characterized

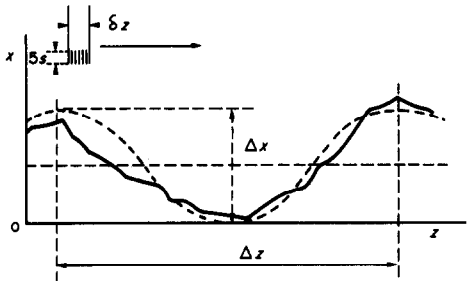

Fig. 1. Concentration distribution of an element in a solid. The resolution of the instrument is high compared to the concentration change. The error is also very low. ($\Delta z \gg \delta z$; $\Delta x \gg s$). The vertical lines indicate the uncertainty of the measurement; the standard deviation s can be taken as a fifth of the range of the signals for a given level.[2]

by a diameter, or, in the direction of z, a linear width which is denoted by δz.

(4) The standard deviation of the analytical measurement is denoted by s. This is used in connection with the determination of signal difference Δx.

The reader may wonder why a sine wave is chosen rather than, for example, square waves of random phase and amplitude. We believe that the wave function selected to describe the concentration profiles does not significantly influence the formula deduced, but the use of the sine wave makes the whole deduction much simpler.

In Fig. 1 the concentration change of a component in the direction of the z co-ordinate in a solid sample, and the sine wave used for approximation of the changing signal are shown. The frequency of the change is relatively low compared to the "window" or spatial resolution of the profiling methods, and the amplitude is quite high compared to the standard deviation of the analytical measurement ($\Delta z \gg \delta z$; $\Delta x \gg s$). This is the most favourable case, where the change of concentration can be determined quite rigorously. Figure 2 presents the case of a solid sample with an inhomogeneity occurring with higher frequency, analysed by a method of low spatial resolution (*i.e.*, with large window width), and higher standard deviation; it can immediately be seen that the presence of inhomogeneity cannot be verified ($\delta z > \frac{1}{2}\Delta z$; $s > \Delta x'$). This occurs because the large window acts as a filter (moving window average[3]) giving the average composition of the substance, integrated over the distance δz. The signal difference observed will be lower than in the case of high resolution, *i.e.*, the observed amplitude will be lower than the true value ($\Delta x' < \Delta x$).

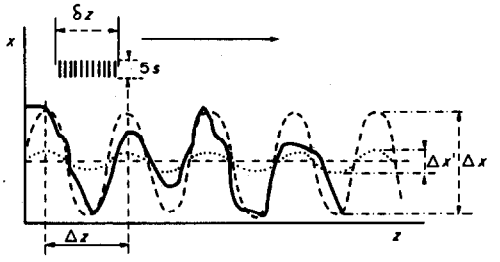

Fig. 2. Concentration distribution of an element in a solid. The spatial resolution of the instrument is low compared to the concentration changes. The error is also considerable. Because of the integrating effect, the signal difference observed will be $\Delta x'$ rather than Δx. See caption to Fig. 1 for note on range = 5s, and on uncertainty of measurement.

For a concentration change in the direction of the z co-ordinate to be detected with acceptable confidence, the following condition must be fulfilled:

$$\Delta x' > 3s, \tag{1}$$

i.e., the signal difference observed between the points of low and high concentration should be at least three times the statistical error (or noise) of the measurement. This signal difference is connected with the "window" of the single analytical measurement and with the distance between the points of low and high concentration. The relationship can be expressed easily, if we consider spot analysis as giving an integrated value of the composition of the substance over the distance δz.

Using the transfer function of a boxcar window with a width of δz, we obtain

$$\frac{a'}{a} = \frac{\sin \pi f \delta z}{\pi f \delta z} \tag{2}$$

where a is the original amplitude, a' is the amplitude of the smoothed sine wave, and f is its frequency. Since in this case $f = 1/\Delta z$ and $2a = \Delta x$, $2a' = \Delta x'$, we can write:

$$\frac{\Delta x'}{2} = \frac{\Delta x}{2} \frac{\sin(\pi \delta z/\Delta z)}{\pi \delta z/\Delta z} \tag{3}$$

from equations (1) and (3) we obtain the formula

$$\Delta x > \frac{3s\pi \delta z/\Delta z}{\sin(\pi \delta z/\Delta z)} \tag{4}$$

For the presence of inhomogeneity to be detectable equation (4) must be satisfied. Taking into consideration the concentration difference, Δc, corresponding to Δx, and the standard deviation in concentration units (s_c), the following condition must hold for the verification of homogeneity:

$$\Delta c(\Delta z, \delta z, s_c) < \frac{3s_c\pi \delta z/\Delta z}{\sin(\pi \delta z/\Delta z)} \tag{5}$$

The solid sample appears homogeneous if the concentration differences Δc at distances $\Delta z/2$ are lower than the quantity on the right-hand side, and the measurements are made with a window δz and standard deviation s_c.

A similar formula can be obtained if the Fisher significance test is used instead of the 3s criterion.

Thus the system appears homogeneous if

$$\frac{\sigma^2}{s^2} < F(95) \tag{6}$$

where $F(95)$ is the critical value for the F-test, σ^2 is the apparent variance of the changing concentration of the component, and s^2 is the square of the standard deviation of the measurements.

The variance of the sin or cos function is $a^2/2$, where a is the amplitude. From equation (3):

$$\sigma^2 = \tfrac{1}{2}\left[\frac{\Delta x \sin(\pi\delta z/\Delta z)}{2\pi\delta z/\Delta z}\right]^2 \tag{7}$$

From (6) and (7) we get the condition

$$\Delta x < \sqrt{8}\, s \sqrt{F(95)}\left[\frac{\pi\delta z/\Delta z}{\sin(\pi\delta z/\Delta z)}\right] \tag{8}$$

for homogeneity.

The critical value of $F(95)$ for $n_1 = n_2 = 20$ degrees of freedom (easily obtainable by means of the usual methods) is 2.12.

With $\sqrt{8}\sqrt{2.12} = 4.12$, Δc instead of Δx, and s_c instead of s, condition (8) becomes:

$$\Delta c(\Delta z, \delta z, s_c) < 4.12\, s_c\left[\frac{\pi\delta z/\Delta z}{\sin(\pi\delta z/\Delta z)}\right] \tag{9}$$

If we treat the spatial resolution problem more rigorously, *i.e.*, the real composition change along the z co-ordinate is decomposed into cosine-wave-components (by the Fourier method), we come to the conclusion that the concentration difference is detectable only when the amplitude ($\Delta x/2$) and frequency ($1/\Delta z$) of the components satisfy the conditions given in equation (5) or (9). All other components with higher frequency or lower amplitude contributing to the real spatial distribution will be filtered out and not be observable.

Remarks. For the sake of simplicity only one-dimensional changes were considered in the deduction of the formula. However, the pattern in the direction of the other co-ordinates can be treated in a similar manner.

Examples. (1) A perfectly mixed 1:1 (particle ratio) mixture of chromium oxide and manganese oxide is examined by laser-generated emission spectroscopy. The average size of the particles of both components is the same, 25 μm. A circular spot with a diameter of *ca.* 100 μm can be analysed by the laser-generation system. The relative standard deviation of the determination for chromium is 5%. Can heterogeneity of the mixture be detected by laser emission spectroscopy?

The chromium concentration difference between the particles (between chromium oxide and manganese oxide) is $\Delta c = 68 - 0 = 68\%$; $s_c = 68 \times 0.05 = 3.4\%$; $\Delta z = 50\,\mu$m; $\delta z = 100\,\mu$m; (Δz is the average distance between centres of chromium oxide particles).

Using formula (5) we obtain

$$68 \ll 3 \times 3.4 \times \frac{2\pi}{\sin 2\pi}$$

The mixture appears completely homogeneous.

(2) From the same chromium oxide and manganese oxide another perfectly mixed sample is prepared, but with a 1:40 composition. The mixed sample is carefully spread on a glass plate, and chromium is determined in the surface monolayer. Does the mixture appear homogeneous or not?

The dilution is to be taken into consideration when obtaining the Δc value:

$$Ac = \frac{68}{40} - 0 = 1.7\%$$

Since the concentration is much lower than before, the relative standard deviation of the determination will be taken as 10%:

$$s_c = 1.7 \times 0.10 = 0.17\%$$

$\delta z = 100\,\mu$m as before, but Δz is now $\approx 150\,\mu$m. Hence

$$1.7 > 3 \times 0.17 \times \frac{\pi\frac{100}{150}}{\sin \pi\frac{100}{150}} = 1.2$$

The mixture will behave as a heterogeneous one.

Conclusions

By taking into consideration the spatial resolution of the analytical method and the spatial distribution of the elements in the surface of solid materials, and using the formula for filtering by moving window average, it is possible to deduce a simple formula for the prediction of homogeneity.

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THE USE OF OUTER-SPHERE COMPLEX FORMATION REACTIONS IN ION-EXCHANGE CHROMATOGRAPHY

SEPARATION OF MALEATE AND FUMARATE IONS

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Summary—Outer-sphere complex formation reactions have been used to increase the selectivity of ion-exchange separation of maleate and fumarate ions. The stability constants of the maleate and fumarate complexes of tris(ethylenediamine)cobalt(III) and hexa-amminecobalt(III) have been determined at different ionic strengths from the elution volumes and the parameters of the ion-exchanger bed, and the values at $I = 0$ obtained by extrapolation. They are: $\log K\,[Co(en)_3^{3+} + Ma^{2-}] = 3.33$; $\log K\,[Co(NH_3)_6^{3+} + Ma^{2-}] = 3.77$; $\log K\,[Co(en)_3^{3+} + Fu^{2-}] = 1.19$; $\log K\,[Co(NH_3)_6^{3+} + Fu^{2-}] = 1.99$. The two anions can be separated quantitatively.

In a previous paper[1] we reported the use of outer-sphere complex formation reactions for enhancing the selectivity of separation of oxalate and sulphate ions.

We now report on the changes in the retention of maleate and fumarate ions on an anion-exchanger column when cationic cobalt(III) complexes [such as tris(ethylenediamine)cobalt(III) and hexa-amminecobalt(III)] are added to the eluent.

The stability of an outer-sphere complex strongly depends on the possibility of hydrogen-bond formation.[2–8] Therefore, because of their *cis–trans* isomerism, fumarate and maleate ions form outer-sphere complexes of different stability with cobalt(III) complexes,[9] and the selectivity of the maleate–fumarate separation is enhanced by the presence of these complexing agents. The stability constants of the outer-sphere complexes have been calculated from the elution volumes and the column parameters.

EXPERIMENTAL

Reagents

Analytical grade potassium chloride, maleic and fumaric acid were used. Tris(ethylenediamine)cobalt(III) chloride and hexa-amminecobalt(III) chloride were prepared from cobalt(II) chloride,[10] recrystallized from aqueous solution and dried, after washing with ethanol, at 105°. The column was packed with 100–200 mesh strong anion-exchanger (Dowex AG1X4 in chloride form). A weak cation-exchanger was used for the binding of the complex cations (Bio-Rex 70, 100–200 mesh).

Elution experiments

A 172 × 4.0 mm column was packed with the anion-exchanger (bed volume 2.16 cm^3). The column was equilibrated with potassium chloride, tris(ethylenediamine)-cobalt(III) chloride or hexa-amminecobalt(III) chloride solution, as required for the experiments. The chloride concentration of the eluents was determined by argentometric titration. The pH was adjusted to between 8.0 and 10.0. A 30–50 μl portion of 0.1*M* maleic or fumaric acid solution (previously adjusted to pH 8.0–9.0 with potassium hydroxide) was injected with a Hamilton microsyringe for the elution experiments. The absorbance of the eluate at 207 nm was monitored in a flow-cell. Since the absorbance of the cobalt(III) complexes in the eluent is high at this wavelength, a weak cation-exchanger column was connected between the anion-exchanger column and the flow-cell to remove the cobalt(III) complexes. Column dead volume was determined by injecting 100 μl of 4*M* potassium chloride. The eluents contained various concentrations of potassium chloride, tris(ethylenediamine)cobalt(III) chloride and hexa-amminecobalt(III) chloride. The chloride concentration was varied between 0.1 and 0.22*M*.

All the elution experiments were repeated with each eluent. The retention volumes were determined and the distribution coefficients were calculated.

Separation of anions

An anion-exchanger column was used for the separation of maleate and fumarate ions. A 290 × 6.5 mm cation-exchanger column was connected after the anion-exchanger column. The procedure was identical with that used for the elution experiments. Elution was carried out first with 0.13*M* potassium chloride at pH 8.88 [Fig. 2(*a*)], then with 0.13*M* tris(ethylenediamine)cobalt(III) chloride solution at pH 8.55. The separation was repeated with the hexa-amminecobalt(III) eluent [Fig. 3(*b*)]. An 80-μl sample was injected in both cases. The test sample was 0.05*M* in both maleic and fumaric acid.

DISCUSSION

The distribution coefficient of an ion can be determined from the data obtained from elution experiments:[11]

$$V_{max} = V_N + V_0 \tag{1}$$

$$D = \frac{V_N}{X} \tag{2}$$

where V_{max} is the elution volume, V_N the adjusted elution volume, V_0 the dead volume, D the volumetric

distribution coefficient, and X the volume of the ion-exchanger bed.

The distribution coefficient can also be calculated, with a good approximation, from other data collected during the ion-exchange separation:[11]

$$D = K_x Q^Z/[B]^Z \quad (3)$$

where K_x is the concentration equilibrium constant of the ion-exchange reaction, Q is the capacity of the resin, Z the charge of the anion, and $[B]$ the concentration of the counter-ion in the eluent.

If the anion examined participates in side-reactions with positively charged ions (*e.g.*, in complex formation, protonation reactions, *etc.*) then the conditional distribution coefficient is:

$$D' = K_x Q^Z/\alpha_L[B]^Z \quad (4)$$

where α_L is the side-reaction coefficient.

If outer-sphere complex formation is the only possible side-reaction and the complex formed is a 1:1 species, then the stability constant of the outer-sphere complex can be derived from equations (2)–(4):

$$\log D - \log D' = \log \alpha_L \quad (5)$$

$$\log \alpha_L = \log (1 + [M(X)_n]K_1 \quad (6)$$

where $[M(X)_n]$ is the concentration of the inner-sphere complex ion which participates in the side-reaction and K_1 is the stability constant of the 1:1 outer-sphere complex formed with it in the side-reaction.

Table 1. Calculated distribution coefficients of the maleate anion at various chloride concentrations

Eluent	$[Cl^-]$, M	V_N, ml	D*	D'†
KCl	0.100	50.2_2	23.2_5	—
	0.106	37.5_8	17.4_0	—
	0.106	43.2_3	20.0_1	—
	0.125	28.9_6	13.4_1	—
	0.157	15.6_2	7.23	—
	0.157	15.8_5	7.34	—
	0.197	10.2_1	4.73	—
	0.197	10.7_0	4.95	—
	0.212	9.3_3	4.32	—
	0.212	9.1_7	4.24	—
$[Co(en)_3]Cl_3$	0.091	18.7_6	—	8.68
	0.100	13.1_5	—	6.09
	0.122	10.7_0	—	4.95
	0.122	9.0_3	—	4.18
	0.129	7.9_7	—	3.69
	0.150	7.0_8	—	3.28
	0.164	5.8_9	—	2.73
	0.200	3.4_7	—	1.60
$[Co(NH_3)_6]Cl_3$	0.090	13.1_2	—	6.07
	0.116	7.7_6	—	3.59
	0.124	6.8_5	—	3.17
	0.144	5.8_7	—	2.72
	0.148	5.3_3	—	2.47
	0.199	2.8_1	—	1.30
	0.215	2.9_5	—	1.35

*D = distribution coefficient with KCl as eluent.
†D' = distribution coefficients with cobalt(III) complexes as eluents.

Table 2. Calculated distribution coefficients of the fumarate anion at various chloride concentrations

Eluent	$[Cl^-]$, M	V_N, ml	D*	D'†
KCl	0.118	64.4_1	29.8_2	—
	0.125	54.4_5	25.2_1	—
	0.161	32.8_0	15.1_8	—
	0.165	31.5_5	14.6_1	—
	0.210	19.0_9	8.84	—
	0.212	19.9_1	9.22	—
	0.221	18.8_6	8.73	—
$[Co(en)_3]Cl_3$	0.122	52.1_9	—	24.1_6
	0.149	33.7_9	—	15.6_4
	0.150	33.3_3	—	15.4_3
	0.159	27.5_6	—	12.7_6
	0.179	23.4_4	—	10.8_5
	0.211	16.8_4	—	7.79
$[Co(NH_3)_6]Cl_3$	0.098	71.2_9	—	33.0_0
	0.116	52.9_5	—	24.5_1
	0.144	34.6_2	—	16.0_3
	0.148	31.1_5	—	14.4_2
	0.187	18.5_2	—	8.57
	0.196	18.7_3	—	8.67

*D = distribution coefficient with KCl as eluent.
†D' = distribution coefficients with cobalt(III) complexes as eluents.

The maleate and fumarate ions were eluted with potassium chloride, tris(ethylenediamine)cobalt(III) chloride and hexa-amminecobalt(III) chloride solutions, each at different chloride concentrations. The distribution coefficients were determined by means of equation (2) from the adjusted elution volumes (Tables 1 and 2). Their logarithms are plotted in

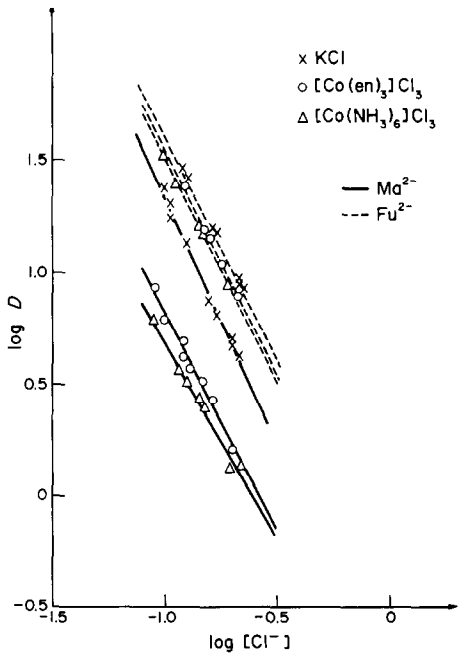

Fig. 1. Distribution coefficients of maleate and fumarate as functions of the logarithm of the chloride concentration.

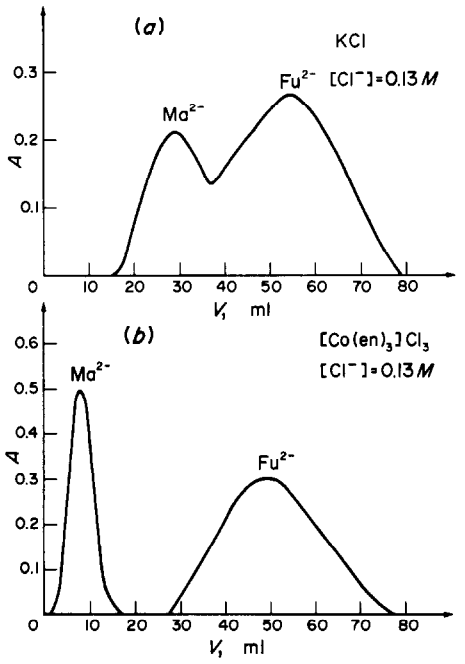

Fig. 2. Chromatograms of the separation of maleate and fumarate with potassium chloride and tris(ethylenediamine)cobalt(III) chloride as eluents. (*a*) [Cl^-] 0.13*M*, eluent KCl; (*b*) [Cl^-] 0.13*M*, eluent $[Co(en)_3]Cl_3$.

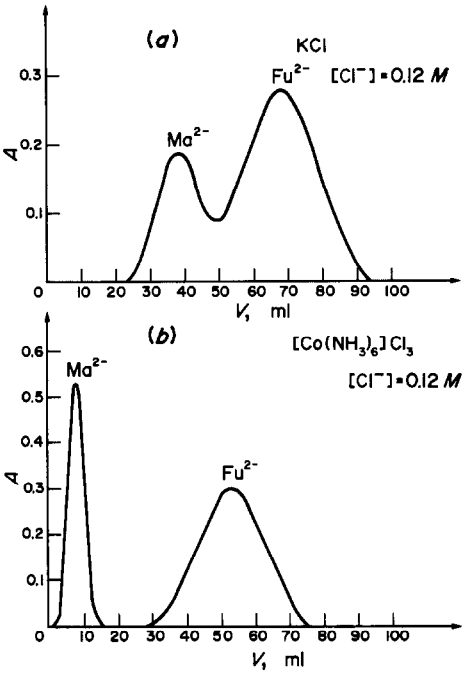

Fig. 3. Chromatograms of the separation of maleate and fumarate with potassium chloride and hexa-ammine-cobalt(III) chloride as eluents. (*a*) [Cl^-] 0.12*M*, eluent KCl; (*b*) [Cl^-] 0.12*M*, eluent $[Co(NH_3)_6]Cl_3$.

Table 3. Data for calculation of the stability constants of the outer-sphere complexes of maleate and fumarate and various cobalt(III) complex cations

Complex	[Cl^-], *M*	[Cation], $10^{-3}M$	*I*	log *D**	log *D*'†	log α	log *K*
$[Co(en)_3]Ma^+$	0.100	3.33	0.20	1.332	0.825	0.507	1.82
	0.125	4.17	0.25	1.118	0.634	0.484	1.69
	0.150	5.00	0.30	0.944	0.479	0.465	1.58
	0.175	5.83	0.35	0.796	0.347	0.449	1.49
	0.200	6.67	0.40	0.668	0.233	0.435	1.41
	0.225	7.50	0.45	0.555	0.133	0.422	1.34
	0.250	8.33	0.50	0.454	0.043	0.411	1.28
$[Co(NH_3)_6]Ma^+$	0.100	3.33	0.20	1.332	0.689	0.643	2.01
	0.125	4.17	0.25	1.118	0.517	0.601	1.85
	0.150	5.00	0.30	0.944	0.376	0.568	1.73
	0.175	5.83	0.35	0.796	0.257	0.539	1.62
	0.200	6.67	0.40	0.668	0.154	0.514	1.53
	0.225	7.50	0.45	0.555	0.063	0.492	1.45
	0.250	8.33	0.50	0.454	−0.018	0.472	1.37
$[Co(en)_3]Fu^+$	0.100	3.33	0.20	1.601	1.550	0.051	0.57
	0.125	4.17	0.25	1.408	1.350	0.058	0.53
	0.150	5.00	0.30	1.249	1.187	0.063	0.49
	0.175	5.83	0.35	1.116	1.049	0.067	0.45
	0.200	6.67	0.40	1.000	0.930	0.070	0.42
	0.225	7.50	0.45	0.898	0.825	0.073	0.39
	0.250	8.33	0.50	0.806	0.731	0.075	0.35
$[Co(NH_3)_6]Fu^+$	0.100	3.33	0.20	1.601	1.503	0.098	0.88
	0.125	4.17	0.25	1.408	1.310	0.098	0.78
	0.150	5.00	0.30	1.249	1.152	0.097	0.70
	0.175	5.83	0.35	1.116	1.018	0.098	0.64
	0.200	6.67	0.40	1.000	0.902	0.098	0.58
	0.225	7.50	0.45	0.898	0.800	0.098	0.53
	0.250	8.33	0.50	0.806	0.709	0.097	0.48

**D* = distribution coefficient with KCl as eluent.
†*D*' = distribution coefficients with cobalt(III) complexes as eluents.

Fig. 1 against the logarithms of the chloride concentration. The slopes of the lines are described by equations (3) and (4). It can be concluded from Fig. 1 that the distribution coefficients of maleate and fumarate differ more when the eluent contains tris(ethylenediamine)cobalt(III) chloride or hexa-amminecobalt(III) chloride, than when it contains potassium chloride. Thus, a higher selectivity can be achieved and the two anions separated from each other.

Quantitative separation can be achieved with either 0.13*M* tris(ethylenediamine)cobalt(III) chloride or 0.12*M* hexa-amminecobalt(III) chloride [Figs. 2(*b*) and 3(*b*)] but not with potassium chloride solutions at the same chloride concentrations [Figs. 2(*a*) and 3(*a*)].

It is apparent from Fig. 1 that the distribution coefficients of the two anions change significantly with change in eluent. The stability constants of the outer-sphere complexes formed at various ionic strengths can be calculated by means of equations (5) and (6) from the distribution coefficients calculated at identical chloride concentrations from the regression lines (Table 3).

The logarithms of the stability constants determined at various ionic strengths are plotted against the cube root of the ionic strength of the solutions in Fig. 4. The stability constants at $I = 0$ can be obtained from the intercepts. They are:

$$\log K\,[Co(en)_3^{3+} + Ma^{2-}] = 3.33$$

$$\log K\,[Co(NH_3)_6^{3+} + Ma^{2-}] = 3.77$$

$$\log K\,[Co(en)_3^{3+} + Fu^{2-}] = 1.19$$

$$\log K\,[Co(NH_3)_6^{3+} + Fu^{2-}] = 1.99$$

Figure 4 is in agreement with the cube-root law of ionic strength described in the literature.[12]

The log *K* values of the maleate complexes are remarkably higher than those of the fumarate complexes, in accordance with the *cis* and *trans* structures of these anions, *i.e.*, their complex-forming tendency.

The complexes formed with hexa-amminecobalt(III) are more stable than those formed with tris(ethylenediamine)cobalt(III). This agrees with the order found earlier[1] and also corresponds to the order of polarizability of these complex cations.

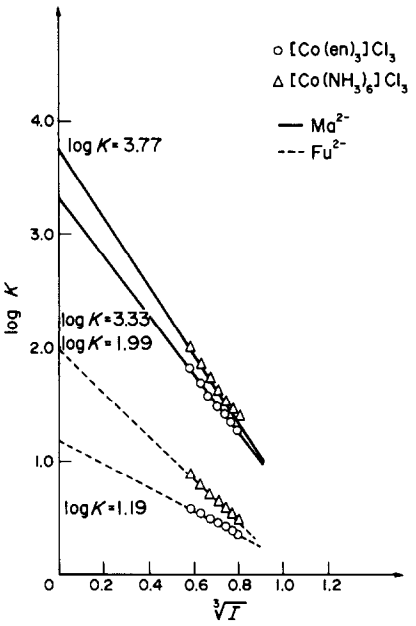

Fig. 4. Extrapolation of the stability constants to $I = 0$. The slopes, intercepts and regression coefficients are tabulated below.

Complex	Intercept	Slope	Regression coefficient
$[Co(NH_3)_6]Ma^+$	3.77 ± 0.04	−3.03 ± 0.06	−0.9990
$[Co(en)_3]Ma^+$	3.33 ± 0.04	−2.58 ± 0.05	−0.9989
$[Co(NH_3)_6]Fu^+$	1.99 ± 0.03	−1.89 ± 0.04	−0.9988
$[Co(en)_3]Fu^+$	1.19 ± 0.02	−1.04 ± 0.03	−0.9983

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STUDIES OF PRETREATMENTS IN THE DETERMINATION OF Zn, Cd, Pb, Cu, Sb AND Bi IN SUSPENDED PARTICULATE MATTER AND PLANKTON BY DIFFERENTIAL-PULSE ANODIC-STRIPPING VOLTAMMETRY WITH A HANGING MERCURY DROP ELECTRODE

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Summary—The determination of Zn, Cd, Pb, Cu, Sb and Bi by differential-pulse anodic-stripping voltammetry has been applied to samples of plankton and suspended particulate matter after decomposition of organic matter by two methods: low-temperature ashing with microwave-activated oxygen and wet-ashing in pressurized Teflon crucibles. The loss of these elements, and contamination, were studied with a standard reference material. The relative merits of these oxidation techniques are discussed.

Determination of trace elements in environmental science raises two difficult problems concerning contamination during sampling and analysis. The first problem has been solved with the help of an appropriate sampling apparatus.[1]

A recent paper has described the direct and simultaneous determination of Zn, Cd, Pb, Cu, Sb and Bi in sea-water by differential-pulse anodic-stripping voltammetry (DPASV).[2] In this paper the application of this technique has been extended to the determination of the same elements in solid matrices such as suspended matter and plankton. We have adopted this technique for various reasons: the equipment is simple and inexpensive, and the method exhibits high sensitivity, which offers the possibility of analysing the small quantities of matter collected on the filters used.

Analysis for trace elements in solid samples (suspended particulate matter, plankton, sediment) includes decomposition of the organic matter followed by a dissolution step leading to a solution for subsequent analytical determinations. This ashing may be achieved by various "classical" or more recently developed procedures, each of which has particular advantages and disadvantages.[3,4] It appears that at the ultratrace level very promising results can be obtained with low-temperature ashing[5] or by pressurized acid decomposition.[6] The latter technique offers an excellent approach for elements such as Hg, As, Se, Sb.

EXPERIMENTAL

Apparatus

An E 310 Brucker polarograph was used with a controlled-temperature Metrohm polarographic cell (25 ± 1°) fitted with a saturated calomel electrode (SCE), a platinum counter-electrode and a hanging mercury drop electrode (Metrohm E 410).[2]

The dry ashing was performed with microwave-activated oxygen in an apparatus manufactured by Trapelo Division, Mass, U.S.A.

A home-made Teflon digestion bomb was employed for the wet-ashing.[7] It is shown in Fig. 1. It is hermetically sealed with a cover and an O-ring made of dense Teflon. The vessel (35 ml) fits into a duralumin container covered by a plate fastened to it by means of 4 screws. The cover is machined so that it presses on the Teflon lid, providing a simple and effective seal. The whole device can be brought to the required temperature for mineralization either by warming in an oven or on a hot-plate, with constant stirring. The life-time of the O-ring depends on the number of digestions. It generally has to be replaced after 8–10 digestions.

Reagents

Standard metal stock solutions (10^{-2}–$10^{-3}M$) were prepared by weighing out the pure metal, dissolving it in a few ml of nitric or hydrochloric acid, and making up to volume. They were further diluted daily as necessary.

The water was demineralized and purified with a Millipore "Milli-Q" water system.

Hydrochloric, perchloric and nitric acids were all Merck "Suprapur".

Sampling and storage of the sample

Suspended particulate matter. The samples were filtered through a preweighed 0.45-μm Millipore filter (47 mm diameter), dried at 60° and stored in individual plastic Petri dishes until further treatment ashore. After drying at 60°, the filters were weighed again with the same accuracy (0.05 mg). The mass of total suspended matter was determined by difference.

The complete sampling procedure is described elsewhere.[1]

Plankton. The plankton was collected with a 100-μm mesh nylon net and washed several times with ultrapure water in order to remove all the sea-water salts. The samples were then frozen at −20° in polyethylene bottles, for analysis.

In the laboratory, the samples were lyophilysed (Leybold-Heraeus GT 2 apparatus) for 24 hr. The dry residue was very heterogeneous, so it was ground in a mechanical corundum mortar to pass a 50-μm sieve. The samples were stored in hermetically stoppered vials. As shown by Duyckaerts and Gillain,[5] by application of the Fisher–Snedecor test, the samples prepared in this way can be considered as homogeneous if their weight exceeds 300 mg.

Decomposition of the sample

Low-temperature ashing (LTA). The filter with the suspended particulate matter or 300 mg of plankton is decomposed in a stream of oxygen excited by a radiofrequency discharge. Low temperatures (70–150°) can be maintained by using low excitation power.

We have shown[5] that the LTA method does not introduce any noticeable losses of Zn, Cd, Pb and Cu. The ashing was conducted under the following conditions:

power 200 W
oxygen pressure 1 mmHg
oxygen flow 40 ml/min
time 10–15 hr

Pressurized acid digestion. The material collected on the filter or approximately 300 mg of plankton (accurately weighed) was transferred to the Teflon vessel in the bomb, 4 ml of concentrated nitric acid and 4 ml of 30% hydrogen peroxide solution were added, then the bomb was closed and heated at 150° for 6 hr. After cooling to room temperature, the bomb was opened and the acid digest evaporated to dryness so that the most suitable supporting electrolyte could be used for the DPASV. Purified nitrogen was passed over the acid to accelerate the evaporation and to avoid contamination by dust. A blank solution containing 4 ml each of the nitric acid and the hydrogen peroxide was prepared in the same way.

Analysis of the residue

The residue from the LTA or from the Teflon bomb was dissolved in 1 ml of "Suprapur" concentrated hydrochloric acid. The solution was then diluted to volume with purified water in a 100-ml standard flask. After both digestion techniques, a Rhodamine test proved that antimony was at least partly oxidized to the quinquevalent state. To reduce Sb(V) to electroactive Sb(III) sulphur dioxide was bubbled through the solution for 1 hr. The excess of sulphur dioxide was removed by a flow of purified nitrogen.[8]

The metal concentrations in the particulate matter and plankton were determined by the method of standard additions. The following procedure is recommended: the sample solution (pH 1, 30 ml) is introduced into the electrolysis cell and adjusted to 2*M* sodium chloride concentration by addition of the high-purity solid (*e.g.* Merck "Suprapur"). The sample is stirred (magnetic stirrer, 450 rpm) and oxygen removed by passage of pure nitrogen for 30–40 min. Electrolysis is done at a potential of −1.200 V (*vs.* SCE) for 1–5 min, with a flow of nitrogen through the space above the surface of the solution. Stirring is stopped 1 min before the end of the electrolysis, and the current–potential curve is then recorded from −1.200 to −0.350 V. The potential is held at −0.350 V, with stirring, for 3–8 min. At the end of this plating time, the stirring is again stopped and the resulting current–potential curve is recorded down to a potential of 0 V, with an appropriate current sensitivity.

The sensitivities for Cu and Sb are different because of the number of electrons exchanged (1 for Cu and 3 for Sb), and because the pre-electrolysis potential lies on the plateau of the $i_p = f(E_p)$ curve for Sb but not for Cu.[2] From calibration curves obtained after a systematic parametric study of the analysis of Cu, Sb, Bi mixtures, we found that the Cu and Sb give the same peak current when the copper concentration is about 100 times that of antimony.

Table 1. Recovery of Zn, Cd, Pb, Cu, Sb and Bi from a standard (NBS 1571) by the LTA and pressurized wet methods

Element	Recovery %		Concentration, *ug/g**
	LTA	Wet method	
Zn	95–105	92–112	25.0 ± 3.0
Cd	84–112	90–115	0.11 ± 0.01
Pb	86–108	88–107	45.0 ± 3.0
Cu	98–108	90–115	12.0 ± 1.0
Sb	25–35	88–115	2.90 ± 0.30
Bi	90–114	85–120	(0.1)†

*Dry weight of material.
†Value not certified.

RESULTS AND DISCUSSION

The LTA method minimizes sample handling, is simpler to perform and gives very low blank values because it avoids the use of reagents (Table 1).

Nevertheless, the mineralization of a standard sample (NBS No. 1571) under the conditions described, has shown that losses of antimony can occur (Table 2), chiefly in the presence of chloride. Because of the dependence of temperature and oxidation rate on the applied power, different powers and ashing times were investigated (Fig. 2), with a standard solution of Sb(III). We found that quantitative recovery of antimony is obtained only with a low power used for a very short time.

The conclusion is that volatilization losses cannot be prevented under the conditions previously cited as necessary for complete mineralization of marine samples (power 200 W, time 10–15 hr). Consequently, low-temperature ashing appears to be acceptable only when non-volatile compounds are dealt with.

A definite advantage of the wet-ashing in a Teflon bomb is that no significant loss of antimony is observed after treatment for 6 hr at 150°. Recoveries were satisfactory as shown in Table 2.

For the pressurized acid digestion, we determined the volume of acid (nitric or a 1:1 nitric–sulphuric mixture) necessary to dissolve completely in 6 hr, a

Table 2. Blanks obtained after LTA and pressurized acid decomposition

Element	Blank, *μg/g**	
	LTA	Wet method
Zn	0.030	0.12
Cd	0.003	0.01
Pb	0.020	0.12
Cu	0.020	0.06
Sb	n.d.†	n.d.
Bi	n.d.	n.d.

*Dry weight ashed.
†n.d. = not detected.

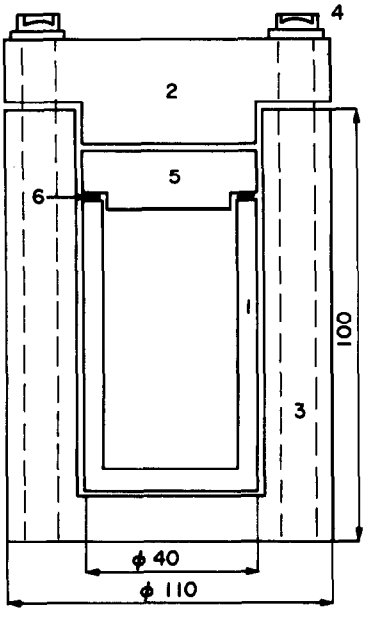

Fig. 1. Cross-section of the closed bomb (dimensions in mm). 1, Teflon vessel; 2, duralumin cover; 3, duralumin body; 4, screws; 5, Teflon cover; 6, Teflon O-ring.

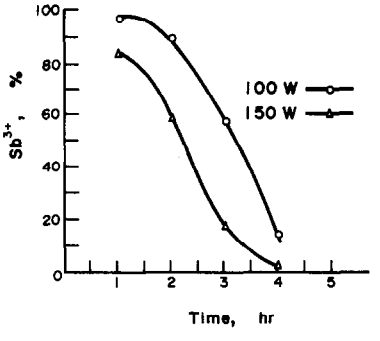

Fig. 2. Recovery of Sb(III) with ashing time for two power levels (100 and 200 W).

300-mg sample[5] at a temperature that would give a pressure lower than 10 atm in the bomb. From these experiments it was concluded that samples with a high organic content were easily decomposed but not completely mineralized when these acids were used. Consequently, the use of the nitric acid–hydrogen peroxide mixture in the bomb was deemed necessary.

Table 3. Average levels and ranges of concentration (μg/l.)* of Zn, Cd, Pb, Cu, Sb and Bi found in suspended matter (Southern Bight, Belgian Coast)†

	Zn	Cd	Pb	Cu	Sb	Bi
Mean	10.3	0.027	1.12	0.52	0.015	0.017
Lowest	0.7	0.010	0.20	0.16	<0.010	<0.010
Highest	15.0	0.050	5.80	1.90	0.030	0.030

*Per litre of sea-water sample.
†A Du Pont E310 curve resolver[2] was used for analysis of the stripping curve, for determination of Sb.

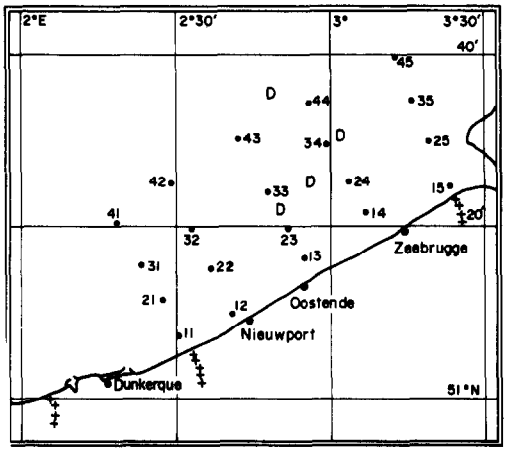

Fig. 3. Location of sampling in the North Sea (Belgian Coast). D = dumping.

The pressurized acid decomposition method has the additional advantage of reducing the digestion time from 15–20 hr to 5–6 hr. Nevertheless the blank values are somewhat higher than those obtained by LTA (Table 1).

The results obtained by both methods show good agreement and appear to be within the limits of precision achievable by DPASV. The general levels and distribution of Zn, Cd, Pb, Cu, Sb and Bi in Belgian coastal water are shown in Table 3 and Fig. 3. The dominant sources of material controlling trace-metal levels in this selected area are coastal discharges, dumping and rivers (mainly the Scheldt). The values increase towards the coast. High levels of these elements occur chiefly in the vicinity of the coasts and the river Scheldt. The highest levels are found at station 14, and are mainly due to the considerable

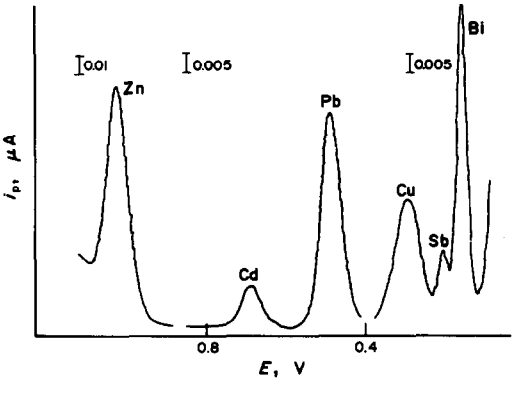

Fig. 4. Voltamperograms of Zn, Cd, Pb, Cu, Sb and Bi in particulate matter (sample from station 45, at pH 1 and 2*M* chloride concentration). Deposition times 2 min (Zn, Cd, Pb) and 5 min (Cu, Sb, Bi). Pulse amplitude 35 mV (Zn, Cd, Pb) and 10 mV (Cu, Sb, Bi); scan-rate 2 mV/sec (Zn, Cd, Pb) and 0.5 mV/sec (Cu, Sb, Bi). The concentrations of metals in the sample (station No. 45) used for Fig. 4 were (μg/l.): Zn, 1.21, Cd 0.019, Pb 0.41, Cu 0.22, Sb, 0.006, Bi 0.030.

Table 4. Average levels and ranges of concentration (μg/g)* of Zn, Cd, Pb, Cu, Sb and Bi in plankton (Southern Bight, Belgian Coast)

	Zn	Cd	Pb	Cu	Sb	Bi
Mean	1000	1.85	29	63.6	0.04	0.09
Lowest	84	0.13	5	34.0	0.02	0.03
Highest	3418	4.40	64	177.0	0.10	0.20

*Dry weight lyophilysed.

and continuous suspension of material near the harbour of Zeebrugge.

The values found for the Cd, Pb and Cu concentrations are of the same order of magnitude as those reported by Mart[9] for the same area.

Results for plankton are given in Table 4. The biological concentration factors for a given element may be expected to vary over a range of five or ten times the mean value. Copper and especially zinc show remarkably high concentration factors in plankton.

Further investigations are being made to establish a more detailed geographical picture of the metal distribution, detect any seasonal cycles involving metals, determine a possible relationship between the particulate and dissolved phases, and study the interrelation between the metals and dissolved organic matter (speciation).

The DPASV/HMDE method is convenient for the simultaneous determination of Zn, Cd, Pb, Cu, Sb and Bi in suspended matter and plankton (Fig. 3). Zn has no effect on the copper stripping peak: the intermetallic Zn–Cu compounds were found only at relatively high concentration of both elements in the electroanalysis solution (Zn 500 μg/l., Cu 80 μg/l.). In this case, the problem of interaction can easily be overcome by the addition of gallium.[7]

CONCLUSION

In the course of this work, we have tried to demonstrate that both decomposition techniques perform equally well. The choice of the one rather than the other depends only on the general trends of the research work in hand. The Teflon bomb is most convenient for the determination of volatile metals such as Hg, Sb, As, Se, and LTA will yield very good results in the determination of less volatile heavy metals.

The blanks for both pretreatments are very low and the recovery levels obtained under routine conditions are generally good, considering the problems inherent in this type of trace analysis.

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DIFFERENTIAL-PULSE POLAROGRAPHIC DETERMINATION OF *N*-NITROSOPROLINE IN UNCOOKED MEAT

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Summary—Ham and bacon have been analysed for *N*-nitrosoproline by differential-pulse polarography. Quantitative polarograms without interfering peaks were obtained from ethanol extracts prepared after extensive clean-up which included freeze-drying and column chromatography. Differential-pulse polarography proved sufficiently sensitive to give peaks corresponding to *ca.* 100 μg of *N*-nitrosoproline per kg in several commercial samples of cured meat.

N-Nitroso compounds are well-known as carcinogens, mutagens, and teratogens.[1–4] Study of these compounds and their effects has been hampered by lack of suitable analytical methods. The analytical problems have been discussed recently.[5] In general, the identification and determination of *N*-nitrosamines are considered scientifically acceptable if satisfactory gas chromatography/mass spectroscopy results can be obtained. The GC–MS method is time-consuming and expensive and useful only for volatile compounds. Non-volatile compounds are mainly dealt with by preparation of volatile derivatives. Recently Fine *et al.* have applied high-pressure liquid chromatography to analysis for non-ionic non-volatile *N*-nitroso compounds in foodstuffs.[6] Walters *et al.* have determined *N*-nitrosarcosine on powdered cornflakes by a method involving denitrosation with hydrogen bromide.[7] Hasebe and Osteryoung have systematically studied the application of differential-pulse polarography (DPP) to the determination of various *N*-nitrosamines.[8,9] These results, together with those of other workers,[10] have demonstrated that for most *N*-nitrosamines DPP gives detection limits of about $10^{-7}M$ and therefore this technique is comparable to GC–MS when coupled with appropriate sample clean-up procedures. Because of the difficulty involved in analysis of complex materials for non-volatile (*i.e.*, non-steam-distillable) *N*-nitrosamines and because of the recognized importance of the problem of *N*-nitrosoproline (NOPro) and other non-volatile *N*-nitrosamines in cured meats, we elected to develop procedures for determination of NOPro in bacon and ham.

The focus of this work is not on qualitative identification of NOPro. It has been amply demonstrated that NOPro occurs in raw cured meats, and that it is there in substantially higher concentration than other *N*-nitrosamines. We have confirmed the identity of NOPro by the R_f value and by DPP peak potential. This combination of information is adequate for our purposes because of the substantial amount of information which has been accumulated on NOPro in cured meats.[5] We were more interested in demonstrating the applicability of the DPP method for NOPro, which was developed with standards,[8] to an analytical problem involving a difficult matrix. The objective was to devise sample clean-up procedures, using previous work as a guide, which would prove appropriate for use with the electrochemical analysis technique.

EXPERIMENTAL

Materials

Chloroform, n-hexane, n-heptane, and methylene chloride were redistilled in glass. Absolute ethanol (ACS grade) and methanol (analytical reagent grade) were used without further purification. *N*-Nitrosoproline crystals were prepared according to a modification of the method of Lijinsky *et al.*[8,11] Column separation was performed on Sephadex LH-20 in ethanol.

Apparatus

Polarographic data were obtained with a Model 174 Polarographic Analyzer (Princeton Applied Research Corporation, Princeton, N.J.) and Model 174/70 Drop Timer. Polarograms were recorded on an Omnigraphic Model 2000 X-Y recorder (Houston Instrument Company, Austin, Texas). The polarographic conditions employed were those described before.[8] A Sorvall superspeed automatic refrigerated centrifuge, Model RC-2-B, a macro homogenizer, Model 45 VirTis 16-200, and a freeze-drier, Freeze Mobile, VirTis, were used.

RESULTS AND DISCUSSION

The experimental work was done in the following sequence. First, a procedure was developed for the isolation and concentration of NOPro, and was tested with NOPro standards. Next, several clean-up methods reported for samples containing non-volatile

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N-nitrosamines were examined for their utility in the DPP analysis. The significance of this step lies in the fact that clean-up requirements for DPP are not necessarily the same as those for GC. The examination of these procedures led to development of a suitable scheme for the DPP analysis. Finally, samples of bacon and ham were analysed for NOPro.

Isolation and concentration procedure

Following the suggestion of Kushnir *et al.*[12] the initial isolation and concentration procedure was carried out as follows.

A weighed 50-g sample of bacon or ham was ground and blended with 100 ml of water for 20 min in the homogenizer, then the material was centrifuged at 10,000 rpm for 20 min. The supernatant liquid was decanted and kept, and the blending step was repeated. The two supernatant liquids were combined and then decanted through glass wool to remove any remaining particles. The liquid (pH 5.2–5.5) was adjusted to pH 7.5 with 5*M* sodium hydroxide and stored in a refrigerator overnight to facilitate removal of fat and lipid. The cold aqueous solution was then separated from the solids by decanting and extracted twice with a total volume of 400 ml of methylene chloride. The resulting solution is referred to below as the clarified aqueous solution and is nominally free from fat and lipids.

Re-examination of modification of the activated carbon adsorption method

It has been reported that NOPro can be quantitatively adsorbed by activated carbon.[13] The clarified aqueous solution was stirred for 4 hr with granular activated carbon (*ca.* 2 g per 100 ml). After the residual liquid had been decanted, the carbon was washed with two 10-ml portions of distilled water, and then refluxed with ethanol for about 3 hr to desorb the *N*-nitrosamines. After removal of the solvent from the ethanolic extract by use of a rotary vacuum evaporator, the residue was dissolved in 0.1*M* hydrochloric acid. Examination of the resulting solution by DPP revealed a large background current caused by failure of the clean-up procedure to remove other compounds of the matrix. The interfering compounds are undoubtedly lipids, which give rise to a capacity current due to adsorption at the electrode surface. This problem seemed so serious that this procedure was not studied further.

Re-examination of a modification of the solvent extraction method

Solvent extraction methods for sample clean-up were based on the work of Kawabata.[14] Clarified aqueous solutions obtained as described above were made acidic with 6*M* hydrochloric acid. NOPro was extracted from the homogenate with two portions (each twice the aqueous phase volume) of methylene chloride or of ethanol–methylene chloride mixture (1 : 3 v/v). The combined extract was dried over small amounts of anhydrous sodium sulphate in a dried Erlenmeyer flask fitted with a screw cap. The solvent was evaporated from the extract with a rotary vacuum evaporator. Residual material was treated several times with a total volume of 10–20 ml of 0.1*M* hydrochloric acid. Differential-pulse polarograms gave a large background current at potentials near −0.8 V *vs.* SCE, which is the reduction potential of NOPro in 0.1*M* hydrochloric acid.[8] Therefore, attempts to clean up the supernatant by using solvent extraction also gave poor results.

Modification of the freeze-drying method using Sephadex

For the isolation of NOPro from raw bacon, Kushnir *et al.* have developed a procedure which uses freeze-drying.[12] The subsequent analysis involves derivative-formation and GC. We examined a variation of this procedure[15] to test its utility in the DPP determination.

The clarified aqueous solution obtained as described above was freeze-dried. The residual material obtained was extracted three times with a total volume of 45 ml of ethanol. Small solids were separated by passing the extract through glass wool. The clarified extract was concentrated to 0.20–0.25 ml by evaporation with prepurified nitrogen gas and its volume measured accurately. A known volume of the concentrated liquid was dissolved in 0.1*M* hydrochloric acid (as supporting electrolyte), and then analysed by DPP. This procedure gave a well-defined DPP peak for NOPro. However, compounds giving rise to currents at more positive potentials interfered. The freeze-drying approach appeared promising, so attempts were made to modify it by providing an additional stage of clean-up.

The original procedure used[12] included a silica-gel clean-up stage. In agreement with Wasserman[12] we found that that step gave poor recoveries, and (at least in our hands) was not reproducible. Use of silica gel was especially difficult under our ambient conditions, which exhibited large changes and large rates of change in relative humidity.

Sephadex column chromatography has been used for clean-up of fish extracts for analysis for pesticide residues and for the clean-up of cutting fluids for analysis for nitrosamines.[16,17] We tried that approach to purify further the freeze-dry residue. An LH-20 column was prepared by soaking 15 g of dry LH-20 overnight in ethanol. The gel was slurried into a 55 × 1.2 cm bore column and compressed to *ca.* 45 cm during assembly of the column. The solution resulting from elution at about 1 ml/min was collected in fractions and each fraction was evaporated in a tared vial with prepurified nitrogen. The vial was rinsed out with 0.1*M* hydrochloric acid, the resulting solution accurately made up to 5 ml, then analysed by DPP. If air was used instead of prepurified nitrogen, the NOPro decomposed during the evaporation, and

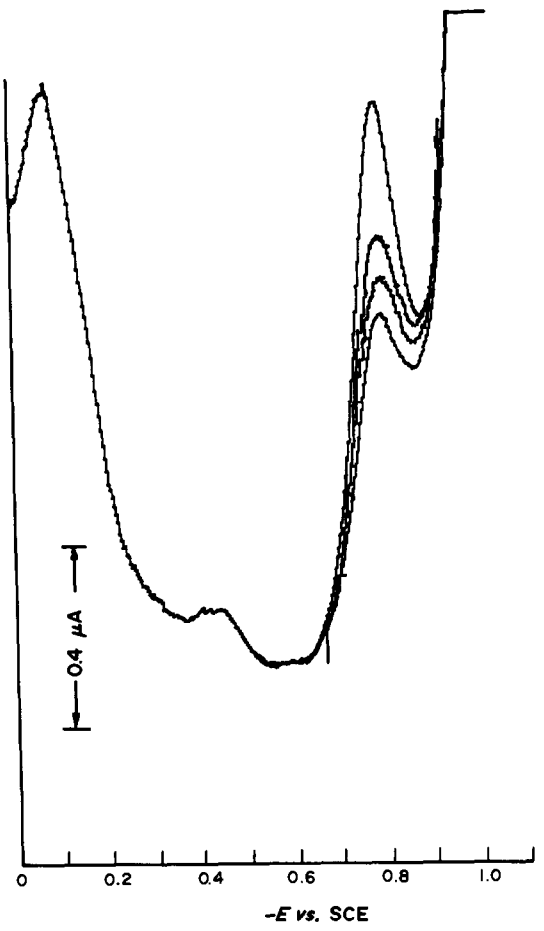

Fig. 1. DPP of raw bacon sample. Scan-rate 2 mV/sec; drop time 2 sec; pulse amplitude −50 mV. The lowest curve is for the sample in 5 ml of 0.1 *M* HCl and the successively higher curves correspond to the sample after addition of 10, 20 and 50 μl respectively of 21.5 μg/ml NOPro solution.

the DPP peak for NOPro disappeared. Polarograms obtained from these solutions are shown in Fig. 1.

Examination of 10-ml fractions showed that essentially all the NOPro is eluted with 40–60 ml of eluent under these conditions. Some interfering substances are eluted in the earlier fractions, and in some samples elution of higher molecular-weight interferents begins at about 60 ml. The optimum procedure appeared to be to use the 40–55 ml fraction for analysis.

Thin-layer chromatographic characterization of the concentrated liquid was carried out on silica gel 60 *F*-254 (Merck) plates developed with chloroform–methanol (9:1 v/v). NOPro was clearly separated from associated interfering materials, the maximum R_f of which was about 0.10. The R_f of NOPro was about 0.26.

Proposed procedure

The work described above resulted in the following procedure. Take 50 g of uncooked bacon or ham. Mince, grind and blend with 150 ml of water for 15 min. Centrifuge the resulting pap-like material at 10,000 rpm for 20 min. Decant the supernatant liquid and keep it. Repeat the blending with another 150 ml of water, centrifuge, and decant. Filter the combined liquid extract through glass wool. Adjust the pH of this liquid to about 7.5, and then refrigerate overnight. Remove the coagulated material from the supernatant. Extract the aqueous solution with two 200-ml portions of methylene chloride. Freeze-dry the clarified aqueous solution and extract the residual material three times with a total of 45 ml of ethanol. Filter the resulting solution through glass wool and then concentrate it to 0.25–0.20 ml by passing prepurified nitrogen through it, and measure the volume accurately (in a 1-ml graduated tube). Chromatograph a known volume of the concentrate on a Sephadex LH-20 column with ethanol. Evaporate the 40–55 ml fraction of eluate to dryness with prepurified nitrogen. Take up the residue in 0.1*M* hydrochloric acid and make up to 5 ml, with the same acid, and analyse by DPP. The total time required to analyse a sample is 2 days or less.

Recovery of NOPro from aqueous solution

Recoveries of NOPro by both the solvent extraction and freeze-drying methods were tested with aqueous solutions of standards. It has already been established[12] that good recoveries are obtained for NOPro added to raw bacon and taken through the freeze-drying step. This appears to be sufficient evidence that the steps leading to the defatted, lipid-free solution, which is the starting point for the clean-up methods investigated, yield satisfactory recoveries of NOPro. It should be noted that the problems with recoveries experienced by Kushnir *et al.*[12] arose in the silica-gel clean-up step, which we did not employ. We used aqueous solutions of standards to represent the defatted, lipid-free solution. Results were unaffected by addition of vegetable oils.

To study recoveries in the solvent extraction method, *ca.* 0.2*M* hydrochloric acid solutions containing various amounts of NOPro were extracted for 10 min each time with two portions of methylene chloride (each twice the aqueous phase volume). There was no significant difference in the DPP peak heights obtained with and without use of sodium chloride as a salting-out agent, although its use was recommended for improving the extractability.[14] After drying with a small amount of anhydrous sodium sulphate, the combined extract was evaporated with a rotary vacuum evaporator, and the residue extracted several times with a total of 10 ml of 0.1*M* hydrochloric acid. Recoveries determined by DPP are presented in Table 1.

To test recoveries in the freeze-drying method, similar solutions were freeze-dried. The resulting residue was extracted several times with a total of 10 ml of 0.1*M* hydrochloric acid. Recoveries as determined by

Table 1. Recovery of *N*-nitrosoproline from aqueous solution

Method	Added, μg	Recovery, %
Solvent-extraction	2.15	95
	4.30	97
	6.45	99
Freeze-drying	5.0	90
	10.0	92
	20.0	99

DPP are also shown in Table 1. Both the solvent extraction and freeze-drying methods give satisfactory recoveries of NOPro from pure water.

In the Sephadex clean-up step, conditions were adjusted so that recoveries were 100%, within the experimental error, determined from elution of standard samples of NOPro from the column and analysis by DPP with use of both standard curves and standard additions.

Determination of NOPro in commercially available cured meat

Ham and bacon samples obtained from a local major chain supermarket were analysed according to the procedure outlined above. The quantitative analysis for NOPro was done by the standard-addition method as illustrated in Fig. 1. From this figure it is apparent that addition of the analyte changes the background current (which is due to catalytic reduction of protons, probably with NOPro as catalyst), and therefore the faradaic current is estimated by drawing for each current–voltage curve an artificial base-line in the form of a straight line across the bottom of the peak, tangential to the upward curve before and after the peak. The standard-addition graph from the data in Fig. 1 has an intercept of 0.31 μA, slope (m) of 2.00 $\mu A.ml.\mu g^{-1}$, and standard deviation about the line (s_m) of 0.02 μA. Thus the unknown amount of NOPro in the cell is 0.78 μg, and the detection limit estimated from $ts_m/m = 0.04$ μg/ml at 95% confidence, where t is the Student-t statistic. This corresponds to a concentration of about 0.2 μM in the cell, which is reasonable considering the complexity of the sample. Expressed in terms of a sample size of 50 g, this corresponds to a detection limit of 4 μg/kg. If a single fixed base-line is used, the amount of NOPro estimated from Fig. 1 is 0.73 μg; the difference is not regarded as significant. Results for NOPro in three different ham samples were 0.17, 0.20 and 0.07 mg/kg, and for one bacon sample 0.27 mg/kg. These values are in reasonable agreement with those for NOPro in uncooked bacon, as found by Wasserman.[15] The elaborate efforts required to establish a laboratory standard sample were not undertaken. Because the degree of homogeneity of individual samples is not known, it is not possible to state a standard deviation for the procedure as a whole with confidence. However, on the basis of the estimate of the detection limit given above we infer that the precision of these results is about 5–10%.

This procedure has the advantages that it avoids the silica gel clean-up step and also avoids the esterification step essential for the GC determination. It therefore decreases both the analysis time and the amount of work required for the determination. Also, from this evidence and the experience gained with other sample types,[18] Sephadex column chromatography for sample clean-up appears to be a generally effective procedure for complex samples intended for *N*-nitrosamine determination by DPP.

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FLOW-INJECTION ANALYSIS OF SILICATE ROCKS FOR TOTAL IRON AND ALUMINIUM

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Summary—A flow-injection method is described for the spectrophotometric determination of total iron and aluminium in silicate rocks. Rock samples are opened up by fusion with a mixture of lithium carbonate and boric acid, the melt is taken up in 1*M* hydrochloric acid and the resulting solution is used for the determination of both iron and aluminium. The flow system for the determination of iron needs no particular reagents, involving simply measurement of the absorbance of the chloro-complex of iron(III) at 335 nm. The system for aluminium consists of the reduction of iron(III) to iron(II), colour development with Xylenol Orange (XO), destruction of XO-chelates other than that of aluminium by addition of EDTA and subsequent measurement of the absorbance of the aluminium–XO complex at 506 nm. The systems permit semi-automatic, rapid analysis of silicate rocks for iron and aluminium. Results obtained for standard rocks were in good agreement with the recommended values. The precision ranged from 0.1 to 0.9% for iron and from 0.3 to 0.7% for aluminium.

Geochemical research and the ceramic industries require vast number of chemical analyses of silicates, and the methods employed should yield a high productivity per analyst. In this respect, instrumental techniques, particularly emission spectroscopy, X-ray fluorescence spectroscopy, atomic-absorption spectrophotometry, fast-neutron activation analysis, *etc.*, have made considerable progress and are becoming capable of answering the need for accurate, rapid rock analyses on a routine basis.[1,2]

Chemical methods involving "complete analysis" and individual determinations have also been fully developed and established on a firm foundation.[1,3] Because of the capabilities of flow-injection analysis,[4–9] as developed by Růžička and Hansen and by Stewart, it is of interest to assess its value for silicate analysis. The automatic nature of FIA appears to ensure large throughputs and to serve to reduce the need for highly competent and well organized analysts in the chemical analysis of silicates. However, FIA needs liquid samples to work with, so for highest efficiency as many elements as possible should be determined by FIA of separate portions of a sample solution obtained from a single decomposition, since it is the decomposition step that is the most labour-intensive, and has the lowest throughput.

On the basis of these considerations we have developed methods for the continuous spectrophotometric determination of total iron and aluminium in silicate rocks by FIA.

EXPERIMENTAL

Reagents

Standard solutions. A stock solution of iron (1 mg/ml) was prepared by dissolving about 1 g of iron(III) chloride hexahydrate in 1*M* hydrochloric acid and diluting to 200 ml with the same acid and standardized complexometrically. Working standards were prepared by diluting the stock solution with 1*M* hydrochloric acid. A stock solution of aluminium (1 mg/ml) was prepared by dissolving about 0.9 g of aluminium chloride hexahydrate in 1*M* hydrochloric acid and making up to 100 ml with the same acid, and complexometrically standardized. Working standards were obtained by appropriate dilution with distilled water or 0.1*M* hydrochloric acid, so that they were 0.1*M* in hydrochloric acid.

Masking solutions. A 0.1% ascorbic acid solution and 0.02*M* EDTA.

Xylenol Orange–buffer solution. One g of Xylenol Orange was dissolved in 1 litre of 1*M* acetic acid–1*M* sodium acetate buffer solution (pH 4.3).

All chemicals used were of analytical-reagent grade.

Apparatus

The flow diagrams for the determination of iron and aluminium are shown in Fig. 1. The peristaltic pumps, sample-injection valve, spectrophotometer, flow-through cell (volume 31.4 μl, light-path 10 mm) and data-processor were the same as described previously.[10,11] The flexible silicone rubber tubes (bore 4.0 mm, length 20 cm) were connected immediately after the pumps to damp the pulsed stream generated by the pumps. The systems were assembled from 1.0-mm bore Teflon tubing [except for the back-pressure coils (bore 0.5 mm) and the dampers] and connectors. Cylindrical mantle heaters were used for heating the mixing coils to control the reaction of aluminium with Xylenol Orange and to enhance the rate of reduction of iron(III) with ascorbic acid and the EDTA destruction of the iron(II)–Xylenol Orange complex.

Dissolution of silicate rocks

About 50 mg of powdered rock sample (accurately weighed) is placed in a platinum crucible and mixed with 0.15 g of anhydrous lithium carbonate and 0.15 g of boric acid. The mixture is fused in a muffle furnace by heating gently for 3 min and then strongly for 12 min at ~950° to yield a clear melt.[12] The contents are swirled occasionally

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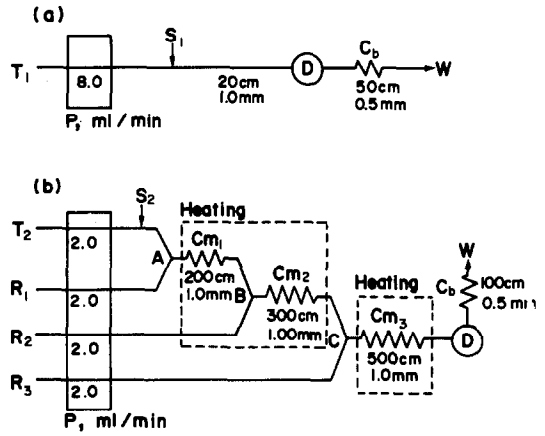

Fig. 1. Flow diagrams. Systems (*a*) and (*b*) are used for the determination of iron and aluminium respectively. T_1, carrier (1*M* HCl); S_1, sample (318 μl); T_2, carrier (0.1*M* HCl); S_2, sample (125 μl); R_1, reducing solution (0.1% ascorbic acid); R_2, Xylenol Orange–buffer solution; R_3, masking solution (0.02*M* EDTA); P, peristaltic pumps; D, spectrophotometer; W, waste; Cm_1, Cm_2, Cm_3, mixing coils; C_b, back-pressure coil; A, B, C, confluence points. The numerals under the coils and tubing refer to their lengths in cm and internal diameters in mm. The parts of the manifold enclosed by the dotted line are placed in the mantle heaters and kept at about 140°.

to ensure complete oxidization of ferrous iron. After cooling the melt is dissolved in 20 ml of 1*M* hydrochloric acid, with magnetic stirring. It should take less than 20 min to achieve the dissolution. The clear solution thus obtained is made up to volume in a 100-ml standard flask with 1*M* hydrochloric acid. The resulting solution is used as such to determine iron. For the determination of aluminium a ten-fold dilution is made with distilled water.

Flow-injection analysis

Determination of iron. Carrier solution T_1 (1*M* hydrochloric acid) is pumped into the analytical line at a flow-rate of 8.0 ml/min with a peristaltic pump, Fig. 1(*a*). The sample solution (318 μl) is introduced into the carrier stream by a six-way loop-valve injector and the absorbance is monitored in the flow-through cell at 335 nm against distilled water as reference.

Determination of aluminium. The flow system illustrated in Fig. 1(*b*) is used.[11] The sample solution (125 μl) injected into the carrier stream T_2 of 0.1*M* hydrochloric acid (flow-rate 2.0 ml/min) is merged into the ascorbic acid solution R_1 (flow-rate 2.0 ml/min) at the point A, and iron(III) is reduced to iron(II) in the mixing coil Cm_1 (bore 1.0 mm, length 200 cm). At the point B the sample zone is mixed with Xylenol Orange–buffer solution R_2 (flow-rate 2.0 ml/min). The colour-forming reactions of aluminium, iron(II) *etc.* with Xylenol Orange proceed in the mixing coil Cm_2 (bore 1.0 mm, length 300 cm), and are accelerated by placing the coils Cm_1 and Cm_2 in a mantle heater (inside temperature ~140°). After the sample slug meets the EDTA masking solution R_3 (flow-rate 2.0 ml/min) at the point C, Xylenol Orange complexes other than the aluminium chelate are completely destroyed by the EDTA, in the mixing coil Cm_3 (bore 1.0 mm, length 500 cm) heated to ~140° (inside the mantle heater). The temperature at the outlet of Cm_3 is ~50°. The absorbance of the aluminium–Xylenol Orange chelate is measured at 506 nm against a reagent blank. The pH of the waste is 4.05.

For calibration a series of working standard solutions is injected into the analytical line before and after the sample runs. All solutions are analysed in triplicate.

RESULTS AND DISCUSSION

Lithium metaborate fusion has been advocated by Ingamells and widely adapted for spectrometric, colorimetric[13] and atomic-absorption analysis,[14,15] where the sample must be brought into solution. Dissolution of the cooled melt obtained with the 1:1 mixture of lithium carbonate and boric acid used in this work is rather slow but can be accelerated by magnetic stirring (a Teflon-coated stirring bar should be used). It usually takes less than 20 min to complete the dissolution. Polymerization of silica appears to proceed slowly, as judged from successive spectrophotometric determinations of silica by molybdate method, but no deposition of silica was found. We found that letting the solution stand for up to a week or more does not affect the determination of aluminium with Xylenol Orange to any great extent.

Determination of iron

The absorption of ultraviolet radiation by solutions of iron(III) in hydrochloric acid has already been applied for the determination of iron[16,17] and the detection of iron in liquid chromatography.[18] Iron(III) in 0.1–4.2*M* hydrochloric acid exhibits an absorption maximum at 330–345 nm, and the sensitivity increases with increasing concentration of hydrochloric acid (Fig. 2). With 6*M* hydrochloric acid there are absorp-

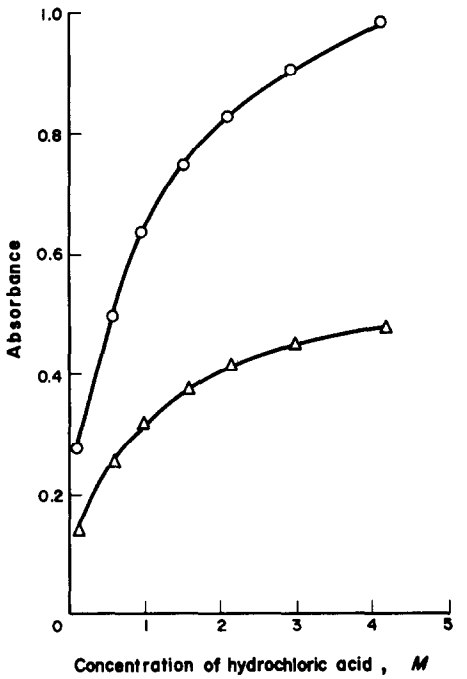

Fig. 2. Effect of concentration of hydrochloric acid on absorbance of iron(III). Fe(III) concentration: 19.3 μg/ml (○); 9.65 μg/ml (△).

Table 1. Effect of various ions on the determination of iron [250 μg/25 ml]*

Ion†	Fe(III) found, μg
SiO_3^{2-} 6.5 mg	251
Ti^{4+} 250 μg	248
Al^{3+} 3.6 mg	252
Mg^{2+} 2.6 mg	250
Ca^{2+} 2.8 mg	249
PO_4^{3-} (added as NaH_2PO_4) 790 μg	248
Li^+ 97 mg	246
BO_3^{3-} (added as H_3BO_3) 49 mg	248

*Equivalent to a sample solution from 12.5 mg of rock.
†Each metal added as chloride. SiO_3^{2-} obtained through fusion with a mixture of lithium carbonate and boric acid (1:1).

tion maxima at 325–330 and 365 nm. If 1*M* hydrochloric acid medium is used, measurement at 335 nm (molar absorptivity 1.84×10^3 $l.mole^{-1}.cm^{-1}$) is satisfactory, although the sensitivity is low and the dependence on chloride concentration is rather severe. However, preparation and use of a large enough batch of 1*M* hydrochloric acid minimizes the effect of variation of acid concentration, giving better precision. Beer's law is obeyed from 0.9 to 60 μg/ml.

This simple system is based on the injection of sample solution (318 μl) into the carrier stream pumped at 8.0 ml/min and allows a very rapid continuous analysis by direct monitoring of the absorbance, throughput being as high as 280 measurements per hour.

The effect of foreign ions on the determination of iron(III) at the 10 μg/ml level (equivalent to 2.86% Fe_2O_3 in rocks) was investigated. Table 1 indicates that neither the major elements (amounts equivalent to 41% SiO_2, 3.3% TiO_2, 53% Al_2O_3, 35% MgO, 32% CaO, 2.8% P_2O_5) nor the borate flux used interfere.

Routine runs for determination of total iron in eight standard rock samples gave the results in Table 2, which agree well with the recommended values. The iron content ranged from 2.2 to 13.4% Fe_2O_3 in these rocks and could be determined with a relative standard deviation of 0.1–0.9% for three measurements.

Determination of aluminium

In previous work[11] we found that Xylenol Orange could be successfully used for the selective spectrophotometric determination of aluminium in brasses and aluminium bronze when suitable conditions were chosen. The absorption maximum of the aluminium–Xylenol Orange complex is at 506 nm at pH 3.8–5.5.

The flow system used for analysis of silicate rocks is essentially the same as that described previously[11] except for the back-pressure coil and heating of coil Cm_3 (Fig. 1). Both iron(III) and iron(II) react with Xylenol Orange to give coloured chelates,[21,22] with strong absorption at the wavelength used for measuring the aluminium absorbance, so an effective masking system must be incorporated in the flow system. The system chosen consists of reduction of iron(III) to iron(II) with ascorbic acid and destruction of the iron(II)–Xylenol Orange chelate by EDTA. In static tests, the colour of the iron(II) chelate (iron at the 0.4 μg/ml level) was found to fade slowly after the addition of EDTA, being masked completely within 12 min at room temperature, whereas the absorbance of the aluminium chelate (aluminium 0.4 μg/ml) remained almost unchanged with time. In the continuous flow system, the iron is rapidly and completely masked by heating the coil Cm_3 or using a stopped-flow technique to ensure long enough reaction time. The stopped-flow technique, however, gives lower sample throughput. The heating conditions were optimized by varying the temperature of coil Cm_3 over the range 35–140° (internal temperature of mantle heater), coils Cm_1 and Cm_2 being kept at 140°. The results are shown in Table 3. The effect of iron(III) can be eliminated completely by heating at a temperature of about 80° or above.

Table 2. Determination of total iron and aluminium in standard samples of silicate rocks by flow-injection analysis

Sample	Fe_2O_3, %		Al_2O_3, %	
	Found*	Recommended value[19,20]	Found*	Recommended value[19,20]
G-2†	$2.60 \pm 0.00_3$	2.65	$15.34 \pm 0.07_2$	15.40
GSP-1†	$4.23 \pm 0.02_1$	4.33	$15.29 \pm 0.07_7$	15.25
AGV-1†	$6.69 \pm 0.04_6$	6.76	$17.33 \pm 0.09_1$	17.25
PCC-1†	$8.30 \pm 0.03_3$	8.35	—	0.74
DTS-1†	$8.80 \pm 0.01_5$	8.64	—	0.24
BCR-1†	$13.28 \pm 0.04_8$	13.40	$13.99 \pm 0.03_6$	13.61
JG-1§	$2.08 \pm 0.01_8$	2.19	$14.38 \pm 0.10_5$	14.23
JB-1§	$8.86 \pm 0.05_0$	8.97	$14.60 \pm 0.08_6$	14.51

*Mean ± std. devn.
†Provided by the U.S. Geological Survey. G-2, granite; GSP-1, granodiorite; AGV-1, andesite; PCC-1, peridotite; DTS-1, dunite; BCR-1, basalt.
§Provided by the Geological Survey of Japan. JG-1, granite; JB-1, basalt.
—Not determined because of lack of sensitivity of the method.

Table 3. Effect of heating temperature of mixing coil Cm_3

Temperature, °C	Absorbance	
	Al solution*	Al–Fe solution†
35	0.142_4	0.147_2
50	0.142_9	0.145_0
80	0.143_2	0.144_6
130	0.144_0	0.143_2
140	0.142_8	—

*4.55 μg/ml.
†4.55 and 3.33 μg/ml, respectively.

The effect of various ions, including those of some minor elements that give stable Xylenol Orange complexes, was then examined. The results obtained are summarized in Table 4. Major components of rocks, including SiO_2, MgO and CaO in much larger amounts than the sample weight in the aliquot used (1.25 mg), have no effect on the determination of aluminium (174 μg, equivalent to 26.3% Al_2O_3 in rocks), assuming a tolerance of ±3.5 μg (relative error ±2.0%). This is also the case for iron (up to 260 μg, equivalent to 30% Fe_2O_3). Titanium (2.3% as TiO_2), manganese (54% as MnO), and phosphate (47% as P_2O_5) do not interfere. Vanadium(V), lanthanum, cerium, zirconium and thorium (~3200 ppm levels), which all form Xylenol Orange complexes, give rise to no serious errors for the rock types usually encountered.

Table 4. Effect of various ions on the determination of aluminium [174 μg/25 ml]*

Ion†	Al(III) found, μg
SiO_3^{2-} 7.0 mg	176
Ti^{4+} 17 μg	174
Fe^{3+} 85 μg	172
130 μg	172
260 μg	171
400 μg	169
660 μg	167
900 μg	167
1300 μg	164
2600 μg	155
Mn^{2+} 530 μg	172
Mg^{2+} 6.2 mg	175
Ca^{2+} 5.4 mg	172
PO_4^{3-} (added as NaH_2PO_4) 390 μg	173
Zr^{4+} 3.9 μg	176
La^{3+} 4.0 μg	174
Ce^{3+} 4.0 μg	174
Th^{4+} 4.0 μg	175
V(V) 3.5 μg	174
Li^+ 5.1 mg	174
BO_3^{3-} (added as H_3BO_3) 4.5 mg	173

*Equivalent to a sample solution from 1.25 mg of rock.
†Each metal added as chloride. See Table 1 for SiO_3^{2-}.

The recommended procedure was applied to the determination of aluminium in eight standard rocks and the results are summarized in Table 2. They are in good agreement with the recommended values. The precision for three measurements is satisfactory (0.3–0.7% relative standard deviation) and the throughput of 50 determinations per hour is sufficiently high. For both the iron and the aluminium systems, the precision of measurement, the base-line stability, and the linearity of the calibration graph are all satisfactory. Flow systems for determination of other elements in the same sample solution basis are being developed.

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CHLOROFORM EXTRACTION OF ETHYL XANTHATE COMPLEXES FROM SULPHURIC ACID MEDIA

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Summary—The chloroform extraction of 30 elements (Fe, Co, Ni, Zn, Cd, Ge, Sn, V, As, Sb, Bi, Cu, Ag, Au, Mn, Re, Ga, In, Tl, Se, Te, Cr, Mo, U, Pt, Pd, Rh, Ir, Ru and Ce) from 0.1–8*M* sulphuric acid in the presence of potassium ethyl xanthate has been studied. Pd(II), Bi, As(III), Sb(III), Se(IV) and Te(IV) are completely extracted and Au(III) is largely extracted over the range of acid concentration investigated. Fe(II), Tl(I), Rh(III) and Cr(VI) are only slightly extracted and Se(VI), Te(VI), Ru(III), Cr(III), Mn(II), Zn, Ce(IV), Ir(IV) and Ge(IV) are not extracted at all. Depending on the acid concentration, the remaining elements are all partly extracted. Results are compared with those obtained in an earlier study of the extraction of xanthate complexes from hydrochloric acid media. The processes involved in the formation of some xanthate complexes and potential analytical separations are discussed.

Recently, one of the authors published a review[1] on the solvent extraction, characteristics and analytical uses of ethyl xanthate complexes and a study of the chloroform extraction of 32 elements from 0.1–10*M* hydrochloric acid in the presence of potassium ethyl xanthate.[2] The information obtained from this study led to the development of spectrophotometric and atomic-absorption spectrophotometric (AAS) methods—based on xanthate extraction separation schemes—for the determination of minor and trace amounts of tellurium,[3] arsenic,[4] selenium,[5] bismuth,[6] antimony[7] and molybdenum[8] in diverse ores, concentrates and other materials. This study also suggested that the extraction of some species, notably antimony(III), molybdenum(VI), bismuth and silver, from fairly concentrated hydrochloric acid media is probably inhibited by the formation of chloro-complexes, and that antimony(V) is extracted as a chloro-complex. The extraction of selenium(VI) and tellurium(VI) was considered to be due to their reduction by chloride ions, xanthate ions, or both. Most of the published information[1] on the solvent extraction of xanthates is based on extractions from hydrochloric acid media. Consequently, it was considered that a study of the extraction of xanthate complexes into chloroform from sulphuric acid media would be of use to analytical chemists and would yield additional useful information on the processes involved in the formation of some complexes.

EXPERIMENTAL

Reagents

Solutions of thallium(I), antimony(III), manganese(II), uranium(VI), rhenium(VII), chromium(VI) and silver were prepared by dissolving $(Tl)_2SO_4$, $KSbOC_4H_4O_6$, $MnSO_4.H_2O$, $UO_2SO_4.3H_2O$, NH_4ReO_4, $K_2Cr_2O_7$ and Ag_2SO_4, respectively, in water. A solution of antimony(V) was prepared by treating some of the antimony(III) solution with sulphuric acid and *aqua regia*.[7] After evaporation of the solution to ~1 ml, potassium hydroxide solution and sufficient tartaric acid to give a final concentration of ~0.1% were added. Subsequently, the pH of the solution was adjusted to ~7 with dilute sulphuric acid, then sufficient concentrated sulphuric acid was added for the final solution to be 1*M* in sulphuric acid. A solution of chromium(III) was prepared by treating some of the chromium(VI) solution with hydrogen peroxide plus sufficient concentrated sulphuric acid to give a final concentration of 1*M* and then boiling the solution to decompose the excess of hydrogen peroxide.

Solutions of cerium(IV), selenium(IV), gallium and iron(II) were prepared by dissolving $Ce(NH_4)_4(SO_4)_4.2H_2O$, H_2SeO_3, Ga_2O_3 and $(NH_4)_2Fe(SO_4)_2.6H_2O$, respectively, in water containing sufficient concentrated sulphuric acid for the final acid concentration to be 1*M*. Ascorbic acid was added to the iron(II) solution to prevent aerial oxidation. Solutions of molybdenum(VI), arsenic(III), vanadium(V) and germanium were prepared by dissolving the oxides in dilute sodium hydroxide solution and adjusting the pH to ~7 with dilute sulphuric acid. A solution of arsenic(V) was prepared by treating a part of the arsenic(III) solution with nitric acid, hydrogen peroxide and sufficient concentrated sulphuric acid for the final acid concentration to be 1*M* and then evaporating the solution to fumes of sulphur trioxide.

Nickel, zinc, cobalt and cadmium solutions were prepared by dissolving the metals in dilute sulphuric acid followed by evaporation of the solutions to dryness and dissolution of the salts in water. An iron(III) solution was prepared by dissolving the metal in water plus sufficient concentrated sulphuric acid for the final concentration to be 1*M*, then oxidizing the iron(II) with hydrogen peroxide and destroying the excess of peroxide by boiling. Indium, bismuth, copper and tellurium(IV) solutions were prepared by dissolving the respective metals and TeO_2 in nitric acid plus the volume of concentrated sulphuric acid required for a 1*M* solution and evaporating the solution to fumes of sulphur trioxide. Palladium(II) and platinum(IV) solutions were prepared in a similar manner after dissolution of the metals with *aqua regia*.

Iridium(IV) and ruthenium(III) solutions, 1*M* in sulphuric acid, were prepared by dissolving $(NH_4)_2IrCl_6$ and $(NH_4)_2RuCl_5$, respectively, in water containing the required volume of concentrated sulphuric acid and evaporating the solutions to fumes of sulphur trioxide. A gold(III) solution was prepared by dissolving the metal in *aqua regia* plus the required volume of concentrated sulphuric acid and ~1 ml of concentrated perchloric acid and evaporating the solution to fumes of perchloric acid. A rhodium(III) solution was prepared by dissolving $(NH_4)_3RhCl_6 . 1\frac{1}{2}H_2O$ in dilute sulphuric acid and evaporating the solution to dryness. The residue was dissolved in dilute potassium hydroxide solution, the pH adjusted to ~7 with dilute sulphuric acid, and sufficient concentrated sulphuric acid added for the final acid concentration to be 1*M*.

A stock solution of tin(IV) was prepared by dissolving the metal in the volume of concentrated sulphuric acid required for a 5*M* solution. Hydrogen peroxide was added to oxidize the tin and the solution was evaporated to fumes of sulphur trioxide to destroy the excess of peroxide. A working solution, 1*M* in sulphuric acid, was prepared by fivefold dilution of this solution.

In all these working solutions the concentration of the element concerned (assuming the starting materials were nominally 100% pure) was 100 μg/ml. Analytical-reagent grade chloroform was used without further purification.

General extraction procedure

Five-ml aliquots of working solution (*i.e.*, 500 μg of the element concerned) were added to a series of 125-ml separatory funnels containing sufficient 10*M* sulphuric acid to provide a final acidity in the range 0.1–8*M*, then each solution was diluted to 50 ml with water (Note 1). Ten ml of chloroform and 1 ml of freshly prepared 20% potassium ethyl xanthate solution were added to two solutions at a time and, after ~30 sec, the solutions were shaken for 1 min. Each chloroform phase was drained into a 150-ml beaker (Note 2). Each aqueous phase was extracted twice more, in a similar manner, with 5-ml portions of chloroform and 1 and 0.5 ml of xanthate solution, then washed by shaking for ~30 sec with 5 ml of chloroform. Except for certain tests (Note 3), the combined extracts were treated with 10 ml of nitric acid (1 + 1) and chloroform was removed by evaporation in a hot water-bath. Depending on the volatility and other properties of the element investigated and the method used for its determination, the resulting solutions were either evaporated to dryness (Note 4) or to fumes of perchloric acid or dryness after treatment with suitable acids (Note 5) or *aqua regia* (Note 6).

The amount of the element in the chloroform extract was determined by the methods listed in Table 1. The percentage of the element extracted was then calculated from the initial amount of the element and that extracted.

Notes

1. Tests with selenium, tellurium and thallium in their highest oxidation states were carried out after oxidation of these elements with 0.5% potassium permanganate solution in 1*M* sulphuric acid medium before adjustment of the acid concentration of the solution with 10*M* sulphuric acid. In tests with platinum and gold, 5-ml aliquots of the solutions in 1*M* sulphuric acid were treated in a similar manner to ensure that these elements were in their highest oxidation states before the extraction step.

2. In tests with arsenic(III) and arsenic(V), each chloroform phase was drained into a 125-ml separatory funnel and the arsenic(III) in the extract was oxidized to arsenic(V) with bromine–carbon tetrachloride solution. Arsenic was then stripped into water and determined by the molybdenum blue method.[4]

3. Extracts obtained during tests with ruthenium(III) were evaporated to dryness with hydrochloric acid to prevent loss of ruthenium as the volatile tetroxide. In tests with iridium(IV) the extracts were treated in a similar manner except that the salts were subsequently treated with *aqua regia*, followed by evaporation to dryness, to ensure that any iridium present would be in the oxidized condition required for complex formation with *o*-dianisidine.

4. Solutions obtained during tests with germanium, chromium, cerium, rhenium and tellurium were evaporated to dryness as described.

5. Solutions obtained during tests with molybdenum, manganese, iron, copper, bismuth, silver, cobalt, nickel, zinc, cadmium, gallium, indium and thallium were treated with perchloric acid and evaporated to dryness. Solutions obtained during tests with selenium and vanadium were treated similarly but evaporated to fumes of perchloric acid. Solutions obtained during tests with antimony were treated with perchloric and sulphuric acids, evaporated to fumes of perchloric acid and treated with *aqua regia* to convert any unreactive antimony species present into antimony(V) species. After the removal of *aqua regia* by evaporation of the solutions almost to dryness, the salts were dissolved in dilute potassium hydroxide solution and antimony was determined by the iodide method.[7] Solutions obtained during tests with tin or uranium were treated with sulphuric, hydrochloric and perchloric acids and evaporated to dryness before the determination of tin by AAS[9] or of uranium with azide. In tests with rhodium, the solutions were treated with hydrochloric acid and evaporated to dryness, the residues were dissolved in dilute potassium hydroxide solution and the resulting solutions were acidified with hydrochloric acid before the AAS determination of rhodium.

6. Solutions obtained during tests with platinum, gold and palladium were treated with *aqua regia* and evaporated to dryness. Gold and platinum were determined by AAS after dissolution of the residues with *aqua regia*.

RESULTS

The degree of extraction of a number of elements into chloroform, as ethyl xanthate complexes, from 0.1–8*M* sulphuric acid is given in Table 2 and Figs. 1–4. Extraction from >8*M* sulphuric acid was not investigated because the density of these solutions is greater than that of chloroform, which precludes the use of a simple triple extraction. The acid concentrations shown are the initial concentrations of the solutions before the addition, in three successive extraction steps, of a total volume of 2.5 ml of 20% potassium ethyl xanthate solution. The oxidation states of the elements shown are those in which they were initially added. Tests with arsenic, antimony, thallium, selenium and tellurium were also carried out on the highest oxidation states because of the known reducing action of xanthates.[1] Tests with platinum, palladium, rhodium, ruthenium and iridium were done on their most common oxidation states. The extraction of osmium and lead was not investigated because a suitable osmium compound was not immediately available and because lead sulphate is formed in sulphuric acid medium. Tests with some of the elements listed and with other metal ions (not shown) showed that manganese(II), zinc, selenium(VI), tellurium(VI), cerium (IV), chromium(III), ruthenium(III), iridium(IV) and germanium(IV) are not extracted from 0.1–8*M* sulphuric acid.

Table 1. Analytical methods used to determine the degree of extraction

Element	Method*	Wavelength, *nm*
Fe	LAS as thiocyanate	478
Co	AAS	240.7
Ni	AAS	232.0
Zn	AAS	213.9
Cd	AAS	228.8
Ge	LAS with ammonium molybdate	830
Sn	AAS	235.4
V	LAS as phosphotungstovanadate	410
As	LAS with ammonium molybdate	845
Sb	LAS as iodide	425
Bi	LAS as iodide	460
Cu	AAS	324.8
Ag	AAS	328.1
Au	AAS	242.8
Mn	LAS as permanganate	546
Re	LAS as thiocyanate	425
Ga	AAS	294.4
In	AAS	303.9
Tl	AAS	276.8
Se	LAS with 3,3'-diaminobenzidine hydrochloride	420
Te	LAS with thiourea	330
Cr	AAS	357.9
Mo	LAS as thiocyanate	460
U	LAS with sodium azide	360
Pt	AAS	266
Pd	LAS as iodide	408
Rh	AAS	343.5
Ir	LAS with *o*-dianisidine	530
Ru	LAS with thiourea	620
Ce	LAS after oxidation to cerium(IV)	350

*LAS = light-absorption spectrophotometry.
AAS = atomic-absorption spectrophotometry.

Table 2. Extraction of ethyl xanthate complexes into chloroform after a triple extraction

Species	Extraction from 0.1–8*M* sulphuric acid, %										
	0.1*M*	0.5*M*	1*M*	1.5*M*	2*M*	3*M*	4*M*	5*M*	6*M*	7*M*	8*M*
Fe(II)	3.0	0	0	0	0	0	0	0	0	0	0
Fe(III)	36.6	9.4	5.6	1.8	0.6	0	0	0	0	0	0
Co(II)	98.4	34.7	11.1	4.2	2.6	1.0	0.6	0.4	0.5	0.2	0.1
Ni(II)	98.8	31.0	9.8	7.0	4.2	1.8	0.1	0	0.3	0.2	0
Cd(II)	85.3	8.9	3.0	1.3	1.2	0.4	0.2	0.1	0.1	0.1	0.1
Ga(III)	8.0	0.5	0	0	0	0	0	0	0	0	0
In(III)	100	100	96.9	80.6	52.7	26.0	15.6	14.7	12.9	9.9	5.8
Tl(I)	4.1	0.3	0	0	0	0	0	0	0	0	0
Tl(III)	25.6	5.5	3.5	3.5	1.4	0.1	0	0	0	0	0
Sn(IV)	94.6	82.8	67.8	47.2	21.2	10.7	7.4	6.3	6.2	6.0	3.4
Cu(II)	69.2	75.2	80.4	82.0	79.6	75.8	79.6	80.0	79.6	83.8	90.2
Ag(I)	55.2	53.2	65.6	66.8	61.6	47.2	58.0	54.8	56.4	53.6	51.6
Au(III)	93.2	86.8	93.6	97.0	96.4	93.8	96.6	94.6	93.2	95.6	82.0
Pt(IV)	44.7	33.7	36.1	33.1	40.3	34.2	41.3	46.9	56.1	62.2	59.8
Pd(II)	100	99.1	98.7	99.4	100	100	100	99.4	100	100	99.6
Rh(III)	2.1	1.4	1.0	3.3	3.2	0.8	3.8	1.6	6.2	2.1	8.8
V(V)	50.8	9.2	1.6	0.4	0	0	0	0	0	0	0
U(VI)	5.2	8.0	7.8	6.4	9.0	6.2	7.0	7.6	6.6	4.4	4.6
Re(VII)	0.8	1.4	1.4	2.0	3.0	11.8	27.2	29.4	41.2	45.2	88.8
Cr(VI)	2.3	0.7	0.3	0.3	0.1	0.2	0.1	0.2	0.1	0.2	0.2
Se(IV)	100	100	99.5	99.7	99.7	99.3	99.7	100	100	100	99.6
Te(IV)	98.6	99.6	100	100	100	100	99.2	100	100	98.2	100

Iron, cobalt, nickel and cadmium

Table 2 shows that iron(II), iron(III), cobalt(II), nickel and cadmium are partly extracted as ethyl xanthate complexes at low sulphuric acid concentrations, particularly in the range 0.1–1*M*. This is consistent with the extraction of these ions from hydrochloric acid media[2] except that more iron(III) is extracted from 0.1*M* sulphuric acid than from 0.1*M* hydrochloric acid, probably because it forms a weaker sulphato-complex than chloro-complex.

Gallium, indium and thallium

Gallium(III) xanthate is only slightly extracted from 0.1*M* sulphuric acid. More is extracted from 0.1*M* hydrochloric acid.[2] The extraction of indium(III) xanthate is better from sulphuric acid media. The colourless thallium(I) complex, which is of limited solubility in non-polar solvents,[10] is only slightly extracted from either 0.1*M* sulphuric or hydrochloric acid. The yellow thallium(III) complex is partly extracted from 0.1–2*M* sulphuric acid. Previous work[2] showed that it is more readily extracted from hydrochloric acid media.

Tin, copper and silver

Tin(IV), which is probably reduced to tin(II) by xanthate,[11] is appreciably extracted as a yellow complex from 0.1–2*M* sulphuric acid. This is consistent with its extraction from hydrochloric acid solutions except that the complex is more readily extracted from sulphuric acid medium, probably because of the more feeble complexing action of sulphate ions.

Copper(II) is largely extracted over the whole range of acid concentration investigated. However, as in previous work involving its extraction from hydrochloric acid media,[2] the results obtained were erratic. This is because of the low solubility in chloroform of the copper(I) ethyl xanthate complex, which is formed by the decomposition of the copper(II) complex.[12]

Silver is appreciably extracted over the whole range of sulphuric acid concentrations investigated. Earlier work[2] showed that it is not extracted from >3*M* hydrochloric acid, which suggests that it forms a stronger chloro-complex than xanthate complex. The degree of extraction is influenced by the limited solubility of the yellow complex in chloroform, since, as found previously, some of the precipitate remained at the interface after the extraction step.

Gold, platinum and palladium

Gold(III) and platinum(IV) are both appreciably extracted from 0.1–8*M* sulphuric acid. The erratic results obtained for these elements are consistent with those reported for their extraction from hydrochloric acid media.[2] In tests with gold, a yellow colour was produced in the aqueous phase on the initial addition of xanthate solution. This colour rapidly disappeared when the solution was mixed or after it was shaken with chloroform. As found previously,[2] this suggests that gold reacts to form a gold(III) complex that rapidly decomposes to a gold(I) complex of limited solubility. Presumably, the yellow platinum complex is a platinum(II) complex formed by the reduction of platinum(IV) to platinum(II) by xanthate.[13] In tests with platinum, a red-brown precipitate, insoluble in chloroform, formed in the aqueous phase on standing.

Palladium(II) is completely extracted from 0.1–8*M* sulphuric acid. Complete extraction is also obtained from 0.1–10*M* hydrochloric acid.[2]

Rhodium

Table 2 shows that rhodium(III) is slightly extracted from 0.1–8*M* sulphuric acid. It is not extracted from 0.1–10*M* hydrochloric acid.[2] An additional test carried out at ~pH 3 showed that, at the 1-mg level, ~15% of the rhodium(III) present was extracted into chloroform when the aqueous phase was allowed to stand for ~1 hr after the addition of xanthate solution. This suggests that the rate of formation of the complex is slow. The formation of a yellow rhodium(III) xanthate complex has been reported previously.[14]

Vanadium and uranium

The extraction of vanadium and uranium from sulphuric acid media is consistent with that reported for hydrochloric acid media.[2]

Arsenic, antimony and bismuth

Figure 1 shows that arsenic(III) can be completely extracted into chloroform from 0.1–8*M* sulphuric acid and, as found previously,[2] from 0.1–10*M* hydrochloric acid. However, the extraction of arsenic, initially present as arsenic(V), from sulphuric acid solutions is not entirely consistent with that found for hydrochloric acid solutions. From sulphuric acid solutions, more arsenic is extracted at low acidities, and less from ~5–8*M* solutions. Previously,[2] it was considered that the increase in extraction from ~5–10*M* hydrochloric acid was caused by the reduction of

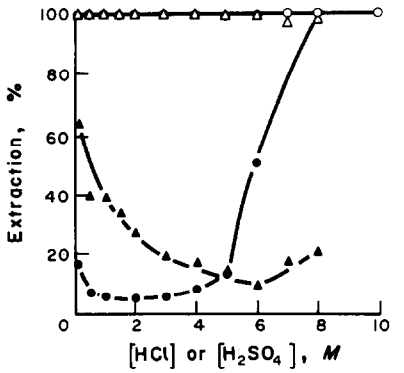

Fig. 1. Extraction of As(III) (open symbols) and As(V) (filled symbols) as a function of the HCl (circles) or H_2SO_4 (triangles) concentration, respectively.

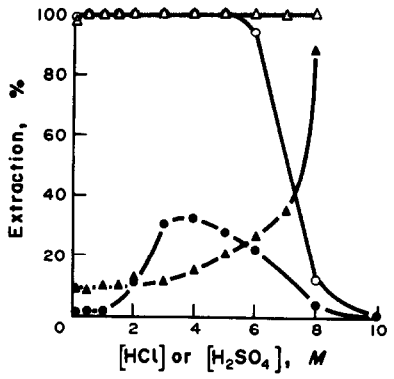

Fig. 2. Extraction of Sb(III) (open symbols) and Sb(V) (filled symbols) as a function of the HCl (circles) or H_2SO_4 (triangles) concentration, respectively.

arsenic(V) by chloride ions and its subsequent extraction as either arsenic(III) xanthate or as the chloro-complex. The extraction profile shown in Fig. 1 for arsenic(V) extracted from sulphuric acid solutions definitely suggests that the increase in extraction from the ~ 6–$8M$ acid (*i.e.*, in the absence of chloride ions) can only be caused by the extraction of arsenic(III) xanthate resulting from the reduction of arsenic(V) to arsenic(III) by xanthate. Tests showed that no arsenic(V) was extracted from sulphuric acid solutions in the absence of xanthate. However, the fact that considerably more arsenic is extracted from ~ 5–$8M$ hydrochloric acid than from 5–$8M$ sulphuric acid suggests that, in hydrochloric acid media, the increase is most probably caused by the reduction of arsenic(V) by both chloride ions and xanthate. The degree of extraction at both low hydrochloric and sulphuric acid concentrations must also be due to partial reduction of arsenic(V) by xanthate. Possibly xanthate reduces arsenic(V) more readily in both dilute and strongly acidic media than at intermediate acidities in the range ~ 2–$5M$.

Figure 2 shows that antimony(III) can be extracted quantitatively as the ethyl xanthate complex from 0.1–$8M$ sulphuric acid, whereas, in hydrochloric acid media, complete extraction is only obtained from 0.1–$5M$ solutions.[2] The decrease in extraction from $\geq 6M$ hydrochloric acid is probably caused by the formation of stronger chloro-complexes. Previously,[2] it was considered that the increase in the extraction of antimony, initially present as antimony(V), from ~ 2–$6M$ hydrochloric acid was caused by the extraction of antimony(V) as the chloro-complex. However, as shown in Fig. 2, antimony present as antimony(V) is also extracted from sulphuric acid solutions and the degree of extraction increases considerably from $\geq 6M$ sulphuric acid. This shows that antimony(V) is partly reduced to antimony(III) by xanthate in sulphuric acid media. Consequently, in hydrochloric acid solutions, the increase in the extraction from ~ 2–$6M$ hydrochloric acid is probably due both to the partial extraction of antimony(V) as the chloro-complex and to the extraction, as the xanthate, of some antimony(III) produced by reduction of antimony(V) with xanthate. However, the fact that very little antimony, present as antimony(V), is extracted from $8M$ hydrochloric acid compared with that extracted from $8M$ sulphuric acid suggests strongly that the decrease in extraction at high hydrochloric acid concentrations is probably due to the formation of unreactive hydrolysis products of antimony(V).[15]

Figure 3 shows that bismuth is completely extracted into chloroform as the xanthate over the whole range of sulphuric acid concentration investigated. However, in hydrochloric acid media, the extraction is only quantitative from ~ 0.1–$3M$ hydrochloric acid.[2] The decrease in extraction from $> 3M$ hydrochloric acid is considered to be due to the formation of stronger chloro-complexes.

Molybdenum, rhenium and chromium

The extraction profiles for the extraction of molybdenum(VI)—which is reduced to molybdenum(V) by xanthate[1]—from both sulphuric and hydrochloric acid media[2] are shown in Fig. 4. The anomalous results obtained for sulphuric acid solutions were confirmed in several additional tests. The "hump" in the extraction profile at $\sim 4M$ sulphuric acid and the increase in the degree of extraction at $\sim 8M$ sulphuric acid are considered to be caused by the formation of

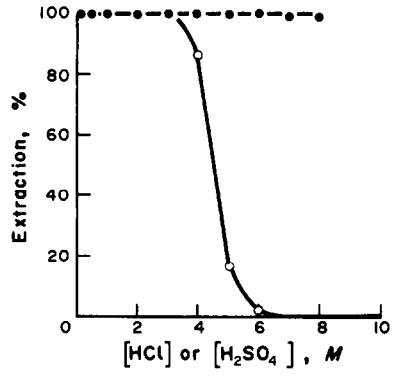

Fig. 3. Extraction of Bi as a function of the HCl (○) or H_2SO_4 (●) concentration.

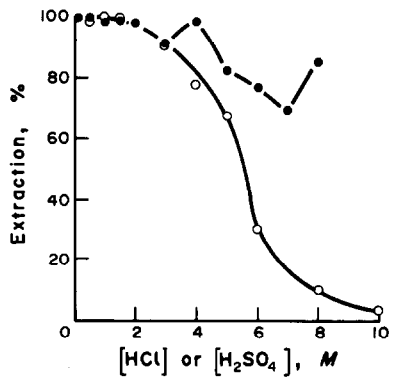

Fig. 4. Extraction of Mo(VI) as a function of the HCl (○) or H_2SO_4 (●) concentration.

different xanthate complexes, since there is a change in the colour of the extracts. Those from 0.1–2*M* sulphuric acid were reddish-purple, and those from 4 and 8*M* solutions were brownish-red and bluish-red, respectively. Changes in the colour of the extract were not observed in earlier work involving the extraction of molybdenum from hydrochloric acid media.[2] Previous investigators have reported that molybdenum forms different xanthate complexes.[16, 17] The lower degree of extraction from $>3M$ hydrochloric acid than from sulphuric acid is considered to be due to the formation of stronger chloro-complexes.

Table 2 shows that the extraction of rhenium, initially present as rhenium(VII), increases from $\sim$2–8*M* sulphuric acid. This is reasonably consistent with its extraction from hydrochloric acid solutions.[2] Because it is rhenium(III) that is considered to react with xanthate,[2] the results obtained for sulphuric acid media suggest that rhenium(VII) is reduced to rhenium(III) by xanthate. Rhenium is not extracted from either sulphuric acid or hydrochloric acid solutions in the absence of xanthate.

Previously[2] it was found that neither chromium(VI) nor chromium(III) is extracted as a xanthate from hydrochloric acid solutions. However, Table 2 shows that small amounts (μg-quantities) of chromium(VI) are extracted from dilute sulphuric acid solutions. Chromium(III) is not extracted. Because chromium(VI) and, particularly, chromium(III) react very slowly with xanthate in weakly acidic solutions,[18] and at hydrochloric acid concentrations above about 2*M*, because chromium(VI) is reduced to chromium(III) by chloride, neither of these ions would be expected to be extracted from hydrochloric acid media.

Selenium and tellurium

The results in Table 2 show that both selenium(IV) and tellurium(IV) are completely extracted as xanthate complexes from 0.1–8*M* sulphuric acid. These findings are consistent with those reported previously for hydrochloric acid media.[2] However, although in hydrochloric acid solutions, the extraction of these elements, when present as selenium(VI) and tellurium(VI), gradually increases with an increase in the hydrochloric acid concentration to $\sim$100% from $\geq 8M$ and $\geq 10M$ hydrochloric acid, respectively, no extraction of these ions occurred from sulphuric acid solutions. Consequently, the increase in extraction from hydrochloric acid must be due to the reduction of selenium(VI) and tellurium(VI) to selenium(IV) and tellurium(IV) by chloride ions—not by xanthate ions.

DISCUSSION

The results obtained in this work and those obtained previously[2] have led to a better understanding of the processes involved in the extraction of various elements as ethyl xanthate complexes from sulphuric and hydrochloric acid media, particularly those elements that form strong chloro-complexes. The results also show that, in some instances, it may be more advantageous to extract from sulphuric acid solutions than from hydrochloric acid solutions, and *vice versa*. The separations of selenium(IV) and tellurium(IV) from selenium(VI) and tellurium(VI), respectively, are possible by extracting quadrivalent selenium and tellurium from $\sim$0.1–8*M* sulphuric acid. However, a strongly acidic hydrochloric acid medium, or a mixture of hydrochloric and sulphuric acids, is preferable for the separation of total selenium and tellurium, because the chloride ions present function as the reductant.[3, 5] Bismuth and antimony(III) can be quantitatively extracted as the xanthates over a much wider range of sulphuric acid concentration ($\sim$0.1–8*M*) than hydrochloric acid concentration ($\sim$0.1–3*M* and $\sim$0.1–5*M*, respectively). For the separation of arsenic(III), 10*M* hydrochloric acid is preferable to 8*M* sulphuric acid because, in general, fewer other elements are co-extracted from hydrochloric acid solutions. In dilute acid solutions, less gallium, thallium(I) and thallium(III) will be extracted from sulphuric acid than from hydrochloric acid of the same concentration. The group separation of cobalt, nickel, indium, tin, palladium, molybdenum, bismuth and possibly antimony(III) and arsenic(III)—after suitable reduction of antimony(V) and arsenic(V)—should be possible by extraction from $\sim$0.1*M* sulphuric acid. As mentioned previously,[2] the co-extraction of iron(III) and copper(II) can be avoided by reduction with ascorbic acid and complexation with thiourea, respectively. Tin can be prevented from extraction by complexing it with hydrofluoric acid[7] and the extraction of thallium(III) can be minimized by reducing it to thallium(I) with sulphurous acid.[6]

In this work, tests showed that neither ruthenium(III) nor iridium(IV) is extracted from 0.1–8*M* sulphuric acid. Previous work[2] showed that they are also not extracted from 0.1–10*M* hydrochloric acid. However, in an additional test with ruthenium at $\sim$pH 3 in the presence of tartaric acid to keep it in solution, a green extract was obtained when the aqueous phase was allowed to stand for $\sim$1 hr after the addition of xanthate solution. This extract contained $\sim$18% of the ruthenium initially added (500 μg). This shows that ruthenium forms a xanthate complex and that the rate of formation of the complex is slow. Recently, ruthenium has been determined by AAS after the extraction of the ruthenium(III) xanthate complex into methyl isobutyl ketone at pH 7–8.[19] No iridium(IV) was extracted in a similar test at $\sim$pH 3.

As mentioned previously,[2] care should be taken in applying the results given in this paper to specific analytical problems, because the extraction profiles of the elements may change somewhat with factors such as the amount of the species to be extracted, the time allowed for complex formation, the amount of matrix elements present, the presence of complexing agents and the amount of potassium ethyl xanthate and the volume of chloroform used for extraction.

It should be emphasized that because of the toxic nature of xanthates, weighing of xanthate compounds, extractions and all other operations involving their use should be prepared in a fume hood. Xanthate solutions should be added by pipette with the aid of a suction bulb or by using a graduated or marked medicine dropper. The aqueous phase remaining after the extraction and any excess of xanthate solution should be treated with concentrated nitric acid and boiled vigorously to decompose the xanthate before disposal of the solution. Recent studies of the health of workers in industrial plants involved in the production of xanthates and other organic sulphur compounds, in which the air contains carbon disulphide and xanthate vapour, have shown that prolonged exposure to these vapours causes a high rate of respiratory illnesses, dermatitis, headaches, fatigue, insomnia, marked changes in liver functions, disorders of the nervous and cardiovascular systems and other related disorders.[20–23] The authors can attest to allergic reactions such as severe sneezing and nasal congestion.

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VARIABILITY OF SELECTIVITY COEFFICIENTS OF SOLID-STATE ION-SELECTIVE ELECTRODES

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Summary—The generalized model for the selectivity mechanism of solid-state ion-selective electrodes has been experimentally verified. The experimental parameters investigated were the concentration of interfering ion, temperature and stirring. Among the systems studied were electrodes sensitive to chloride (bromide, iodide), bromide (chloride, iodide), iodide (chloride, bromide), silver (copper, lead), copper (silver, lead) and lead (silver, copper), the species given in brackets being considered as the interferents. The model has been confirmed except for cases where the concentration of ions formed at the electrode surface by metathesis is too small to be the factor that dictates the electrode potential.

The literature data and our own measurements of the selectivity coefficients of a number of ion-selective electrodes have pointed out discrepancies which should be explainable on the basis of an adequate model of electrode function and mechanism of interference. Such differences were found for both liquid-state and solid-state electrodes. The latter were studied in this work.

In previous papers[1,2] we have reported an important role of diffusion processes which are a consequence of metathesis at the interface between the electrode material and the electrolyte solution. In the processes a significant part is played by the nature of the products formed as the result of an exchange reaction, and by their activity or inactivity in the electrode phenomena. From consideration of both the diffusion processes and the thermodynamic equilibrium we have proposed a generalized model of electrode interferences.[3]

The principal assumptions of the generalized model are (*a*) zero-current conditions, (*b*) total electroneutrality, (*c*) rapidity and reversibility of the ion-exchange processes, (*d*) constancy of the appropriate chemical standard potentials, (*e*) constancy of the diffusion coefficients in the solution.

For a reasonable mathematical description of the processes, the following simplifications have been introduced: (*a*) electrochemical potentials change only in the direction perpendicular to the macroscopic membrane surface, (*b*) concentrations of ions change linearly within the diffusion region, (*c*) steady-state distribution exists at the membrane interface, (*d*) activities are equal to concentrations, (*e*) other processes, such as redox, adsorption, *etc.*, are negligible.

The treatment presented below deals with solid-state electrodes for which it can be assumed that the mobility of interfering ions in the membrane phase is negligible. The opposite case has previously been considered,[3,4] a typical example being attack of hydroxide on the fluoride electrode.

In the case of the electrodes considered the assumptions mentioned have led to an equation describing the electrode potential in a system where the following metathetic reaction occurs:

$$AB + C \rightleftharpoons AC + B$$

where B and C denote the main and interfering ions, respectively, and A the active membrane site.

At this point it must be noted that the product of the exchange reaction, depending on the conditions of its formation, may be electrode-active (mixed phase formation) or electrode-inactive (dissolution–precipitation).

The electrode potential is generally described by the equation:[3]

$$E = \text{constant} \pm \frac{RT}{F} \ln \frac{K_{B,C}}{S\frac{D_C}{D_B} + (1 - S)K_{B,C}} \left([B] + \frac{D_C}{D_B}[C]\right) \quad (1)$$

where D_B and D_c are the diffusion coefficients of B and C, respectively, $K_{B,C}$ is the equilibrium constant of the metathetic reaction and S is the apparent coverage factor. S may be evaluated from equation (1) by using measured potential values and literature data for the thermodynamic equilibrium constants and diffusion coefficients (Table 1). These data are mostly reliable, but discrepancies in data found in the literature indicate that in some cases their accuracy may be questioned. The sign preceding the logarithmic term depends on whether the electrode is cation or anion selective.

With proper approximations the generalized equation is reduced to that given by the total equilibrium model of Pungor and Tóth.[5]

$$E = \text{constant} \pm \frac{RT}{F} \ln([B] + K_{B,C}[C]) \quad (2)$$

Table 1. The constants used in the calculations

Compound	pK_{so}	Ref.	Ion	D_x, $10^{-5}cm^2/sec$	Ref.
Ag_2S	49.2	6	Ag^+	1.65	8
CuS	37.5	7	$\frac{1}{2}Cu^{2+}$	0.71	8
PbS	26.6	6	$\frac{1}{2}Pb^{2+}$	0.92	8
AgI	16.1	6	I^-	2.05	8
AgBr	12.3	6	Br^-	2.08	8
AgCl	9.8	6	Cl^-	2.03	8

based on mixed-phase formation, for which the selectivity coefficient is the ratio of the solubility products of both solid phases. Otherwise the generalized equation is reduced to that postulated by the diffusion model suggested previously:[1,2]

$$E = \text{constant} \pm \frac{RT}{F} \ln\left[\frac{K_{B,C}}{K_{B,C} + D_C/D_B}\left([B] + \frac{D_C}{D_B}[C]\right)\right] \quad (3)$$

which considers only the dissolution–precipitation mechanism, for which the selectivity coefficient is the ratio of the corresponding diffusion coefficients when $K_{B,C} \gg 1$ or equal to the ratio of the solubility products when $K_{B,C} \ll 1$. These two models assume invariable values of the selectivity coefficients, while in the generalized model the values are variable, which can explain a number of discrepancies found among the literature data.

The aim of this study was to confirm the validity of our model for several electrode systems. It was expected that under properly chosen experimental conditions it would be possible to find values of selectivity coefficients which correspond to the two limiting cases as well as to the intermediate range, in which the selectivity coefficients are time-dependent.

Among the systems studied were: chloride electrode with bromide and iodide interferences, bromide electrode with chloride and iodide interferences, iodide electrode with chloride and bromide interferences, silver electrode with copper and lead interferences, copper electrode with silver and lead interferences, and lead electrode with silver and copper interferences.

EXPERIMENTAL

The electrodes used were the Cl 101 chloride electrode (Mera, Poland), OP-Br-7111-C bromide electrode (Radelkis, Hungary), OP-I-7112-C iodide electrode (Radelkis), 94-16 silver electrode (Orion, U.S.A.), 94-29 A copper electrode (Orion), and 94–82 A lead electrode (Orion).

All reagents used were of analytical grade. Solutions were always made in 1*M* potassium nitrate (in doubly distilled water). The measuring cell temperature was controlled to within ±1°.

The selectivity coefficients were evaluated by the method of separate solutions.

RESULTS AND DISCUSSION

Two main cases were considered, according to whether the equilibrium constant for the exchange reaction was larger or smaller than unity. As an example of the former case the results for iodide interference with the chloride electrode are discussed in detail.

The effect of changing the iodide concentration in the range from 10^{-4} to $10^{-1}M$ indicates that the lowest concentration reflects the diffusion model only and even over a period of several hours the selectivity coefficient is close to the ratio of the diffusion coefficients of both ions. The highest concentration results in a nearly immediate potential drop, the final value corresponding to a selectivity coefficient expressed by the ratio of the solubility product of AgCl to that of AgI. Intermediate concentrations produce, after a diffusion-controlled period, a drop in potential to that for the total equilibrium state (Fig. 1). Experimental conditions such as temperature and stirring influence

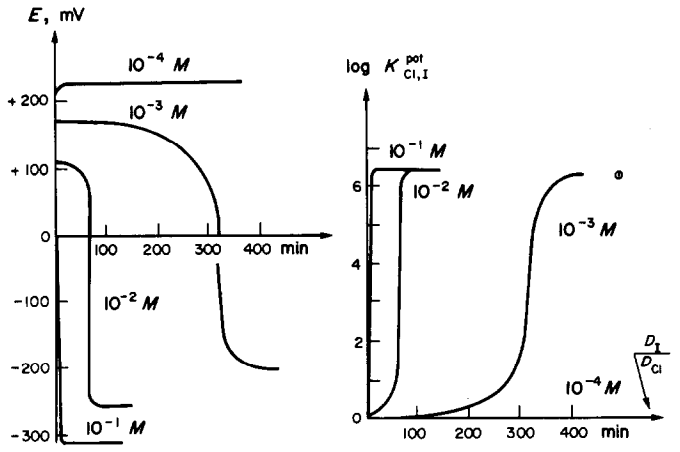

Fig. 1. The effect of concentration of interfering iodide on the potential response of the chloride-selective electrode and on the selectivity coefficient. Temperature 25°C; unstirred solution. The point ⊙ indicates the value of the equilibrium constant calculated on the basis of data given in Table 1.

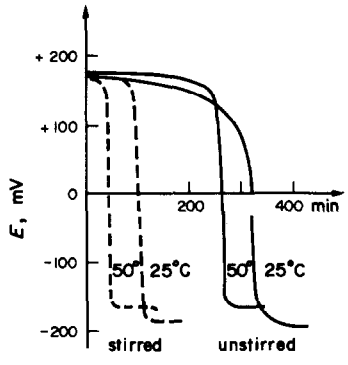

Fig. 2. The effect of stirring and temperature on the potential response of the chloride-selective electrode in presence of $10^{-3}M$ iodide.

this behaviour, shortening (for a given concentration) the diffusion-controlled period (Fig. 2).

Similar behaviour has been observed in other cases, such as the chloride electrode in bromide solution,[3] bromide electrode in iodide solution, copper electrode in silver solution and lead electrode in silver and copper solutions (Fig. 3).

It is obvious that in all instances the selectivity coefficients evaluated fall within the limits defined by both models. However, in some systems the limiting values indicated on the figures are not reached during the experiment. This fact may be interpreted, according to the present model, as due to a slow approach towards the total equilibrium state. On the other hand, it could be assumed that equilibrium is really attained but the equilibrium constants for the given experimental conditions are not known with sufficient accuracy.

As an example of the equilibrium constant being smaller than unity the iodide electrode in bromide solution will be discussed in more detail (Fig. 4). At a bromide concentration of $0.1M$, the equilibrium is rapidly established and corresponds to an iodide concentration of about $10^{-4}M$ at the membrane surface.

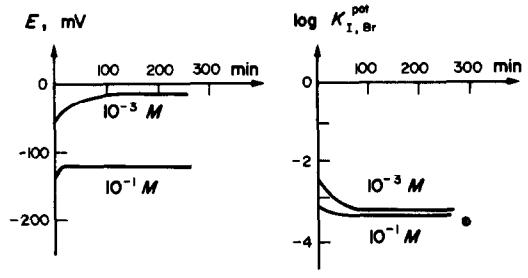

Fig. 4. The effect of concentration of interfering bromide ion on the potential response and selectivity coefficient of the iodide-selective electrode. Temperature 25°C; unstirred solutions. Point ⊙—see Fig. 1.

When the bromide concentration is $10^{-3}M$ the iodide concentration after equilibration is about $10^{-6}M$. At this level the equilibrium is established rather slowly[9] and some parasitic processes may occur. However, the final potential value reflects the expected equilibrium state fairly well. As before, even a small uncertainty in the solubility products may result in the selectivity coefficient changing by up to one order of magnitude. If this is allowed for, the correlation with the expected final value of the selectivity coefficient is quite good.

Similar behaviour was found in the case of chloride attack on the bromide membrane.[3] Such a potential response is due to the exchange reaction proceeding according to the dissolution–precipitation mechanism, for which the selectivity coefficient is given by the ratio of the two solubility products.

The behaviour of the iodide electrode in chloride solution should result in a selectivity coefficient equal to the solubility product ratio, but this is not quantitatively the case, because the iodide concentration in $10^{-1}M$ chloride solution is close to $10^{-7}M$, which corresponds to the range of the calibration curve where parasitic processes may influence the electrode response.[9] We have noted, for example, a significant dependence of the evaluated selectivity coefficient on the electrode pretreatment (Fig. 5).

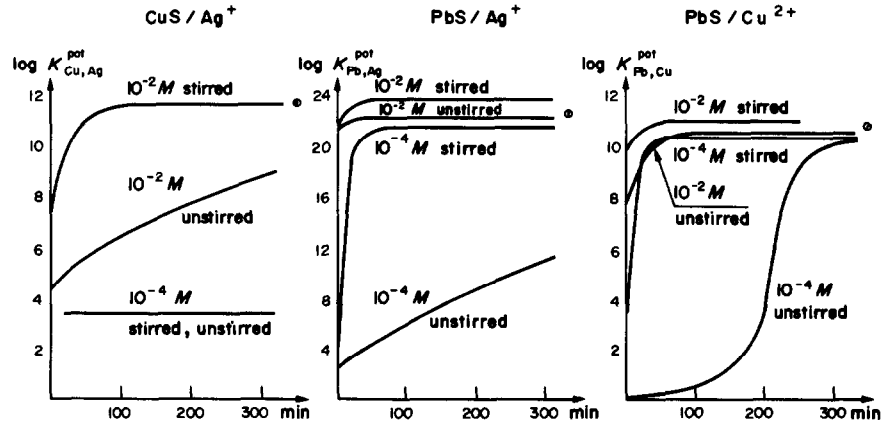

Fig. 3. The effect of concentration of interfering ion and stirring on the selectivity coefficient of metal-selective electrodes. Temperature 25°C. The point ⊙—see Fig. 1.

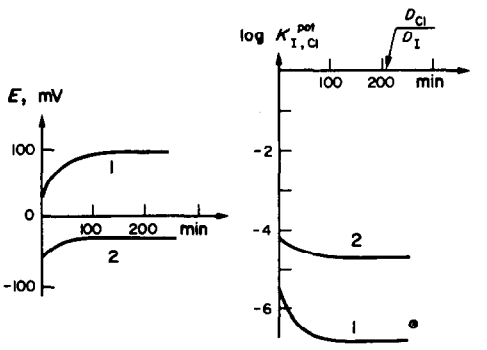

Fig. 5. The effect of iodide-selective electrode pretreatment on the potential response and selectivity coefficient in presence of $10^{-1}M$ chloride. Temperature 25°C; unstirred solutions. 1—Electrode soaked 10 min in $0.1M$ $AgNO_3$, 2—electrode soaked 10 min in $0.1M$ KI. Point ⊙—see Fig. 1.

When the equilibrium constant is significantly smaller, as happens for the attack of lead ions on the copper or silver electrode, as well as for copper attack on the silver membrane, the main ion concentration at the electrode surface is much smaller than that which corresponds to the practical limit of detection. This means that our model cannot then be applied to interpretation of the electrode behaviour because a number of other factors define the electrode potential.[10] Those factors may be adsorption, redox reactions or the level of uncontrolled trace contaminants. Selectivity coefficients can be formally calculated but they have no physical meaning in our model.

We are convinced that the examples presented cover all practical situations that may be met in discussing the selectivity coefficients of solid-state electrodes.

CONCLUSIONS

The investigations presented indicate that in evaluation of selectivity coefficients a number of precautions should be kept in mind. Hitherto, these were intuitively obvious to many workers experienced in the field of ion-selective electrodes. Obtaining reasonable numerical values for selectivity coefficients requires compliance with such experimental conditions as proper ion concentrations, temperature, and stirring. The numerical values obtained fall within the limits given by the limiting models of interference based on the two mechanisms described as dissolution–precipitation and mixed-phase formation. Such a treatment enables us to explain at least some of the existing discrepancies in the selectivity coefficient data in the literature.

We are, however, aware that the model is in some respects an idealized one, and that it does not take into account some of the more complicated effects such as non-ideal miscibility of solid phases, occurrence of chemical reactions more complicated than simple metathesis, irreversibility of processes and slowness of reactions, non-linearity of transport phenomena, mutual interactions of diffusion processes, effect of the microscopic membrane surface and the existence of other parasitic processes.

Quite apart from the detailed considerations of physical and chemical processes it should be firmly stated that thermodynamics and kinetics both contribute to the selectivity of ion-selective electrodes.

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ATOMIC-ABSORPTION SPECTROPHOTOMETRIC DETERMINATION OF ANTIMONY, ARSENIC, BISMUTH, TELLURIUM, THALLIUM AND VANADIUM IN SEWAGE SLUDGE

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Summary—Electrothermal atomic-absorption spectrophotometry (AAS), by use of a graphite furnace, in conjunction with sample pretreatment by homogenization, was evaluated as a rapid method for the determination of bismuth, thallium and vanadium in sewage sludge. This method was compared with use of flame, electrothermal and hydride-generation (for bismuth) AAS in conjunction with conventional acid digestion and dry-ashing pretreatments and was found to be applicable to this type of sample. Comparisons were also made between flame and hydride-generation AAS in conjunction with an acid digestion pretreatment for the determination of antimony, arsenic and tellurium in sewage sludge. The hydride-generation technique was considered the better for waste-water samples because of its greater sensitivity.

The disposal of sewage sludge on agricultural land is a common practice in the U.K. Application of the sludge to agricultural land may have beneficial effects,[1,2] but there is a risk of toxic elements accumulating in the soil.[3] This has caused concern because of the potential hazards associated with the contamination of soil, crops and ground water, leading to mobilization of toxic elements in food-chains.[4]

The behaviour of certain toxic elements in waste-water treatment processes has been studied only to a limited extent.[5–7] Concentrations of arsenic,[8,9] bismuth[10] and antimony[8,9] in sewage sludge may be of the order of 30 μg/g, vanadium concentrations as high as 400 μg/g,[9,10] and tellurium and thallium, which have rarely been reported, occur at much lower concentrations.[8]

Various methods have been employed for the determination of elements in waters, waste-waters and sewage sludges;[11–13] atomic-absorption spectrophotometry (AAS) is probably the most widely applicable for environmental samples.[14] Flame AAS is considered the traditional mode of analysis for most elements.[12] The hydride-generation technique is applicable to those elements producing hydrides (which include arsenic, antimony, tellurium and bismuth),[12] especially when they are present at low concentrations. Flameless AAS in conjunction with a rapid pretreatment method has recently been developed for routine metal determination in waste-water samples.[14]

Most analytical methods require a liquid sample; a pretreatment is therefore necessary to dissolve the metal species and destroy the organic matter. Various methods have been used, including ashing procedures and acid digestions.[15]

Sulphuric acid and hydrogen peroxide have been used to digest fish tissue[16] and organic material[17] for analysis for arsenic, and in the determination of vanadium in biological tissue.[18] Nitric acid, sulphuric acid and hydrogen peroxide have been employed to digest seaweed[19] and organic material[20] before arsenic determination. A nitric and perchloric acid digestion for soil and plants[21] and for sludges[22] has also been reported. Nitric, sulphuric and perchloric acids have been employed in the digestion of biological tissue for tellurium, antimony and arsenic determinations.[23] A nitric–sulphuric acid mixture is recommended for antimony in organic matter.[24] A perchloric–hydrofluoric acid mixture has been used for vanadium in geological samples[25,26] and a nitric–perchloric–hydrofluoric acid mixture for vanadium in sediments.[27] Dry ashing has been reported for vanadium determinations in biological tissue.[28]

The experiments reported here were undertaken in order to compare the applicability of flame, electrothermal and hydride-generation AAS, in conjunction with various sample pretreatments, for the determination of arsenic, antimony, bismuth, tellurium, thallium and vanadium in sewage sludge.

EXPERIMENTAL

Instrumental

A Perkin–Elmer model 5000 atomic-absorption spectrophotometer fitted with standard burner heads was used for all flame atomic-absorption determinations. The same spectrophotometer, fitted with a Perkin–Elmer HGA 500 heated graphite atomizer, and a model 603 atomic-absorption spectrophotometer fitted with an HGA 400, were used for flameless determinations. A Perkin–Elmer MHS-1 hydride-generation system was used in conjunction with the model 603 spectrophotometer for the determination of the

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Table 1. Parameters for atomic-absorption spectrophotometry

Element	Wavelength, *nm*	Slit-width, *nm*	Flame AAS	Electrothermal AAS* Step 1	Step 2	Step 3	Hydride generation
V	318.4	0.7	N_2O–C_2H_2 reducing (rich—red)	120°C† 30–60 sec	1000°C† 30–60 sec	2900°C‡ 7 sec	—
Bi	223.0	0.2	Air–C_2H_2 oxidizing (lean—blue)	120°C 30–60 sec	600°C 30–60 sec	2300°C 5 sec	Programme I§
Tl	276.8	0.7	—	120°C 30–60 sec	700°C 30–60 sec	2700°C 5 sec	—
As	193.7	0.7	Air–C_2H_2 oxidizing (lean—blue)		—		Programme I§
Sb	217.6	0.2	Air–C_2H_2 oxidizing (lean—blue)		—		Programme I§
Te	214.3	0.2	Air–C_2H_2 oxidizing (lean—blue)		—		Programme II‖

* Uncoated graphite tube used for Tl, Bi; pyrolytically coated tube for V (see text).
† Ramp time 10 sec; held for 30–60 sec according to volume injected (20–50 μl).
‡ Ramp time 0 sec for V, 1 sec for Bi, Tl.
§ Argon purge 30 sec; carrier-gas stream 40 sec.
‖ Argon purge 45 sec; carrier-gas stream 40 sec.

hydride-forming elements (arsenic, antimony, bismuth and tellurium). The use of electrodeless discharge lamps (EDLs) has been reported to be of benefit in terms of sensitivity and lamp life.[29] EDLs were used for arsenic, bismuth, antimony, tellurium and thallium. A hollow-cathode lamp was used for vanadium. Instrumental parameters are detailed in Table 1.

In-situ *pyrolytic coating of graphite tubes*

For flameless atomic-absorption determinations of vanadium, pyrolytically coated graphite tubes were used. The coating was performed *in situ* by passage alternately of argon and of a methane–argon mixture (1:9 v/v) under temperature and gas flow conditions optimized to ensure uniform pyrolysis of the methane, thus producing an even coating. The conditions are summarized in Table 2. Pyrolytic coating extended the tube life, improved sensitivity and reduced memory effects, the last being the most pertinent in the determination of vanadium.

Reagents

The following reagents were all BDH "Aristar" grade: nitric acid (s.g. 1.42), sulphuric acid (s.g. 1.84), hydrochloric acid (s.g. 1.18), perchloric acid (s.g. 1.54), hydrofluoric acid (s.g. 1.15) and hydrogen peroxide (100 volume).

Standard solutions

Stock solutions were prepared from the following analytical-grade chemicals: arsenic trioxide, antimonyl potassium tartrate, tellurium, thallium(I) nitrate and ammonium metavanadate. The bismuth stock solution was a commercially available atomic-absorption standard (BDH).

Working standards were prepared by serial dilutions of stock solutions, and contained 1% v/v nitric acid.

Glassware

Borosilicate glassware was used throughout, and was cleaned by soaking for 24 hr in 5% v/v Decon 90 detergent and then 24 hr in 10% v/v nitric acid. PTFE beakers and polypropylene apparatus were similarly cleaned.

Sampling

Sludge samples were collected in polyethylene jerry-cans (cleaned as for glassware) and immediately acidified to a concentration of 1% v/v nitric acid.[30]

Homogenization

The sludge was diluted tenfold with 1% v/v nitric acid. An Ultra Turrax (Scientific Instrument Co., London), fitted with a titanium shaft was used to homogenize the samples as described previously.[31]

Table 2. Conditions for *in-situ* pyrolytic coating of graphite tubes

Step	1	2	3	4	5	6	7	8
Temperature, *°C*	500	1950	20	500	1950	20	500	1950
Ramp time, *sec*	1	16	1	1	16	1	1	16
Hold time, *sec*	20	230	100	20	230	100	20	230
Internal flow, *ml/min*	300		300	300		300	300	
Internal alternative flow,* *ml/min*		150			150			150

* Alternative gas, 10% methane in argon.

Decomposition procedures

Nitric acid–sulphuric acid digestion. A modified version of a recommended digestion[11] was used, as reported before.[32] To undiluted sludge were added 30 ml of concentrated sulphuric acid and 50 ml of concentrated nitric acid; the mixture was heated at 120° and successive 20-ml portions of nitric acid were added until digestion was complete. The digest was filtered and then made up to volume in a 100-ml standard flask.

Nitric acid–hydrogen peroxide digestion. A modification of a previous method[33] was used. Preliminary oxidation with nitric acid ensured a controlled reaction.[34] An addition of 75 ml of nitric acid was made to 50 ml of sludge in a PTFE beaker; further 25-ml portions of nitric acid were added, followed by 10-ml additions of hydrogen peroxide until the digestion was complete. The digest was filtered and made up to volume in a 100-ml standard flask.

Nitric acid–perchloric acid digestion. The recommended method[11] was used with some modification. PTFE beakers each containing 50 ml of sludge were heated at 120°, with several 30-ml additions of nitric acid. Perchloric and nitric acids were added to the cooled reaction mixture, which was then heated to about 200°. Successive additions of perchloric and nitric acids were made until completion of the digestion. The digest was filtered before being made up to 100 ml.

Nitric–perchloric–hydrofluoric acid digestion. The method used was similar to that previously employed[27] and is an extension of the nitric–perchloric acid digestion described above, hydrofluoric acid being added as a final stage, before filtration and making up to 100 ml.

Dry ashing. The ashing procedure adopted was as reported previously.[32] The sludge (50 ml) was charred in covered Pyrex beakers at 200° for 1 hr in a muffle furnace. The temperature was then increased to 450° and kept there for 14 hr. After cooling and the addition of 5 ml of concentrated nitric acid, the ashing was continued for a further hour. The residue was boiled with extraction acids before filtration and making up to 100 ml.

Nitric acid–sulphuric acid–hydrogen peroxide digestion. The method was essentially that described elsewhere.[20] The sludge was charred with sulphuric acid; the digestion was continued with nitric acid and was completed by the addition of hydrogen peroxide. The digest was then filtered and made up to 100 ml.

Prereduction for tellurium, arsenic and antimony

Before hydride generation, reduction of the sample is sometimes required to ensure that the determinand is in the correct oxidation state.[35] To convert all tellurium into tellurium(IV), boiling for 2 min with concentrated hydrochloric acid[35] or boiling for a longer period with *aqua regia*[36] have been proposed. The latter method was used; equal volumes of *aqua regia* (3:1 v/v hydrochloric acid–nitric acid) and digested sample were boiled together for 15 min. Standard solutions were treated in the same manner.

Similarly, the oxidation states of arsenic and antimony affect the sensitivity of the hydride-generation method.[35] Arsenic(V) gives lower sensitivity than arsenic(III).[35] Potassium iodide[21] and sodium iodide[23] have been used to ensure that the arsenic is in oxidation state (III).

Antimony is also reduced with potassium iodide[35] or sodium iodide.[24] Antimony(V) gives only half the sensitivity given by antimony(III).[35] As the reduction is instantaneous, the determination should be performed immediately; delay allows the formation of iodine from the acid solution, which may cause interference in hydride generation.[35]

Statistical treatment

For each pretreatment (acid digestion, dry ashing and homogenization) five replicates and two blanks were used in order to validate the statistical analysis. The results were statistically treated; mean values, within-group relative standard deviation and analysis of variance by the *F*-test were obtained.[37] Tukey's test[37] was used to indicate which means were statistically different at the 0.05 (5%) significance level.

RESULTS AND DISCUSSION

Bismuth

Flame atomic-absorption was used to determine bismuth concentrations in the acid-digested and dry-ashed samples. Electrothermal atomic-absorption was used to analyse the homogenized sludge samples for bismuth, by direct comparison with standards. The acid-digested and dry-ashed samples were also analysed for bismuth by the hydride-generation technique. The values obtained and the statistical analysis of the results are shown in Table 3, and indicate that no significant difference was found between treatments, except for flame atomic-absorption of the perchloric acid digest, and hydride-generation from the hydrogen peroxide digest, the first giving low and the second high results. No results were recorded for the

Table 3. Comparison of bismuth concentrations in sewage sludge, from analysis of acid-digested and dry-ashed samples by flame atomic-absorption (F) and hydride generation (H), and from analysis of homogenized samples by electrothermal atomic-absorption (E)

Pretreatment	Analytical method	Mean concentration,* μg/ml	Relative standard deviation, %
Homogenization	E	0.49a	7
HNO_3–H_2O_2	F	0.55a	12
HNO_3–$HClO_4$	F	0.28b	14
NHO_3–$HClO_4$–HF	F	0.54a	7
Dry ashing	F	0.43a	4
HNO_3–H_2O_2	H	0.70c	4
HNO_3–$HClO_4$–HF	H	0.56a	10
Dry ashing	H	0.41a	12

* Means followed by the same letter do not differ at the 0.05 level of significance.

Table 4. Comparison of vanadium concentrations in sewage sludge, by electrothermal atomic-absorption analysis (E) of homogenized samples and flame atomic-absorption analysis (F) of acid-digested and dry-ashed samples

Pretreatment	Analytical method	Mean concentration,* μg/ml	Relative standard deviation, %
Homogenization	E	14.4a	15
HNO_3–H_2O_2	F	14.9a	3
HNO_3–H_2SO_4	F	13.0ab	3
HNO_3–$HClO_4$	F	11.3b	4
HNO_3–$HClO_4$–HF	F	13.2ab	8
Dry ashing	F	11.9ab	3

* Means followed by the same letter(s) do not differ at the 0.05 level of significance.

nitric–sulphuric acid digests, because of severe background interference, but the limit of detection was approximately 0.1 μg/ml.

In the hydride-generation method, prereduction was considered unnecessary since bismuth(III) is virtually the only oxidation state occurring naturally.[35]

The detection limits of the electrothermal and hydride-generation methods were comparable, about 1 ng/ml.

Vanadium

Vanadium in the acid-digested and dry-ashed samples was determined by flame atomic-absorption. The standard-addition method was used to determine vanadium in the homogenized sludge samples by electrothermal atomic-absorption since reproducibility was poor for direct comparison with standards. The results and statistics are shown in Table 4. The relative standard deviations for the flame AAS determinations were all lower than that for the electrothermal determination. However, the results showed agreement statistically, with the exception of the nitric–perchloric acid digest, which gave lower vanadium recoveries than any other pretreatment.

Owing to difficulties in obtaining reproducible signals from electrothermal atomic-absorption for vanadium, and because the relative standard deviations obtained were higher than those of other analytical methods, the reproducibility was examined by having three analysts determine vanadium in five replicate subsamples independently. The comparison in Table 5 indicates that the relative standard deviations differed slightly but not significantly.

Table 5. Influence of change of operator on the reproducibility of vanadium determination in homogenized samples by electrothermal atomic-absorption

Analyst	Mean concentration,* μg/ml	Relative standard deviation, %
1	14.4	15
2	14.7	12
3	14.3	13

* The means do not differ at the 0.05 level of significance.

The results for dry ashing and flame atomic-absorption were rather low, probably owing to incomplete mineralization at the temperature used. Ashing at 650° has beeen recommended for vanadium determination.[28] The low recovery from the nitric–perchloric acid digestion could be due to loss during the boiling of acids or interference caused by perchloric acid in the flame atomic-absorption.

Thallium

Thallium concentrations in the homogenized sludge samples were determined by electrothermal AAS with direct comparison with standards and by a standard-additions method. Electrothermal AAS was also employed to determine thallium in the acid-digested and dry-ashed samples (by the standard-additions method) since flame AAS was insufficiently sensitive to detect any thallium. The results are presented in Table 6. No values above the detection limit for thallium were obtained for the dry-ashed samples, possibly because of volatilization of thallium during the heating at 450°. Wet oxidation has been reported as a generally more reliable decomposition method than dry ashing.[15] Although the recovery of thallium from the homogenized samples was comparable with the recoveries from the nitric acid–sulphuric acid, nitric acid–perchloric acid–hydrofluoric acid and nitric acid–perchloric acid digestions, but the nitric acid–hydrogen peroxide digestion gave higher recoveries. It is unlikely that this digestion would cause dissolution of more metal than perchloric or hydrofluoric acid would, and the higher recovery may have been due to high background signals, caused by incomplete destruction of the matrix.

Arsenic

The sludge sample obtained contained arsenic at a concentration too low to be determined by flame

Table 6. Comparison of results for thallium in sewage sludge, obtained by electrothermal atomic-absorption analysis of homogenized, acid-digested and dry-ashed samples

Pretreatment	Mean concentration,* μg/ml	Relative standard deviation, %
Homogenization (calibration curve)	0.070a	4
Homogenization (standard additions)	0.098ab	11
HNO_3–H_2SO_4 (standard additions)	0.069a	23
HNO_3–$HClO_4$–HF (standard additions)	0.084ab	36
HNO_3–$HClO_4$ (standard additions) additions)	0.129b	22
HNO_3–H_2O_2 (standard additions)	0.174b	25
Dry ashing (standard additions)	<0.01c	—

* Means followed by the same letter do not differ at the 0.05 level of significance.

atomic-absorption. To enable this method to be included for comparison, the sludge was spiked with a standard solution of arsenic to give a concentration which would favour all the analytical methods equally. The spiked sludge, digested by the nitric acid–sulphuric acid–hydrogen peroxide method, was analysed for arsenic by flame AAS and the hydride-generation technique. The recovery of arsenic was good and the analytical methods essentially yielded the same result, as expressed in Table 7. Electrothermal AAS of the homogenized sludge samples produced high results (approximately 4 μg/ml) which, if correct, should have been obtained by flame AAS (sensitivity 0.8 μg/ml) analysis of acid-digested samples, but were not. Electrothermal atomic-absorption has been employed previously for the determination of arsenic, although with difficulty.[38] It has been suggested that matrix modification can eliminate depression or enhancement caused by sample components,[39] but it seems that the nature of the homogenized sludge caused interference that could not be compensated by matrix modification and gave erroneously high results. Such molecular spectral interference has already been reported.[40]

No pretreatment of arsenic was thought necessary, because of the agreement between the results of the analytical methods, indicating that all the arsenic was already in the oxidation state (III) needed for hydride generation. Other possible reasons for the concordance of the results include the pH being sufficiently low to ensure that both arsenic(III) and (V) gave the same response,[20] or that the sodium borohydride concentration was high enough to produce an arsenic(V) signal equivalent to 90% of an arsenic(III) signal.[36]

Antimony

Antimony concentrations in the sludge sample were too low to be determined by flame atomic-absorption. To permit a comparison of flame AAS with hydride generation, the sludge was spiked with antimony, as for arsenic. The values obtained are shown in Table 8. A sulphuric acid–hydrogen peroxide digestion has previously been reported to give good recoveries for antimony.[17]

The hydride-generation technique gave better results than flame atomic-absorption. Owing to the virtually total recovery of antimony by the hydride method, no prereduction of antimony was deemed necessary. When sodium iodide was used as a prereductant for antimony the signal response was decreased, possibly because of iodine formation.

Table 7. Comparison of results for arsenic in spiked sewage sludge, obtained by flame atomic-absorption (F) and hydride-generation (H) analysis of acid-digested samples (H_2SO_4–HNO_3–H_2O_2)

Analytical method	Concentration added, μg/ml	Mean found,* μg/ml	R.S.D., %	Mean recovery, %	Range of recovery, %
H	12.5	12.1	4	96	92–100
F	12.5	12.0	3	96	93–100

* The means do not differ at the 0.05 level of significance.

Table 8. Comparison of results for antimony in spiked sewage sludge, obtained by flame atomic-absorption (F) and hydride-generation (H) analysis of acid-digested samples (H_2SO_4–HNO_3–H_2O_2)

Analytical method	Concentration added, μg/ml	Mean found,* μg/ml	R.S.D., %	Mean recovery, %	Range of recovery, %
H	7.6	7.6	8	100	91–113
F	7.6	6.7	8	88	79–95

* The means do not differ at the 0.05 level of significance.

Table 9. Comparison of results for tellurium, obtained from spiked sewage sludge by flame atomic-absorption (F) and hydride-generation (H) analysis of acid-digested samples (H_2SO_4–HNO_3–H_2O_2)

Analytical method	Concentration added, μg/ml	Mean found,* μg/ml	R.S.D., %	Mean recovery, %	Range of recovery, %
F	17.1	15.5	1.8	90	88–92
H	17.1	14.2	17	83	74–107
H	17.1	15.2†	22	89	70–116

* The means do not differ at the 0.05 level of significance.
† *Aqua regia* also added in digestion.

Tellurium

For the same reasons as for arsenic and antimony, the sludge sample was spiked before digestion with nitric and sulphuric acids and hydrogen peroxide. The values obtained from flame atomic-absorption and hydride generation are presented in Table 9.

The mean recoveries from both analytical methods suggest incomplete recovery or loss during the digestion, although previously a similar digestion was demonstrated to be applicable to tellurium.[17]

As the hydride method produced a little lower mean recovery than the flame AAS method did, the oxidation state of the tellurium was investigated. Treatment of digests with *aqua regia* slightly increased the mean recovery, but the range of values obtained was similar to that obtained without this treatment, and statistical analysis showed that there was no significant difference between the results of flame AAS or hydride-generation AAS, with or without prereduction.

CONCLUSIONS

From the comparison of flame, electrothermal and hydride-generation AAS for the determination of bismuth in sewage sludge, the most suitable method appeared to be electrothermal AAS of the homogenized samples by direct comparison with standard solutions.

For vanadium determination, electrothermal AAS was best, but the standard-additions method was required. The same applies to determination of thallium.

For arsenic, antimony and tellurium, flame AAS has been shown to be insufficiently sensitive to detect environmental concentrations. Hydride generation is, therefore, the method of choice, but is subject to many interferences,[41,42] which may have contributed to the high relative standard deviations, *e.g.*, for tellurium. This effect was limited by using a large ratio of acid volume (20 ml of 3% v/v hydrochloric acid) to digest volume (20–100 μl). Since volatilization of the tellurium hydride separates it from the majority of the matrix components, except other hydride species, interferences are then expected to be small.[23] It has been reported that residual nitric acid and perchloric acid do not cause interference,[23] the major cause being incompletely oxidized organic matter.

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SHORT COMMUNICATIONS

TITRATION OF VANADIUM(IV) WITH CERIUM(IV) SULPHATE, WITH FERROIN AS INDICATOR, IN AQUEOUS ALCOHOL AS A FACILE REACTION MEDIUM

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Summary—A new procedure for the titration of vanadium(IV) with cerium(IV) sulphate, with ferroin as indicator, in aqueous alcohol medium, is reported. The visual titration gives accurate results but potentiometric titration fails in this medium; this failure is attributed to the sluggish indicator electrode behaviour. Experimental conditions for preliminary quantitative photochemical reduction of vanadium(V) with alcohol have been established.

The titration of vanadium(IV) with cerium(IV) sulphate, with ferroin as indicator, is not possible in dilute sulphuric acid medium because of the sluggish ferriin–vanadium(IV) reaction but the potentiometric titration gives good results.[1] We have reported earlier that the titration with ferroin as indicator is satisfactory if the titration medium contains acetic acid[2] or acetone[1] in addition to mineral acid; under these experimental conditions the end-point can also be detected potentiometrically. The failure of potentiometric titration of vanadium(IV) with cerium(IV) sulphate in the presence of even a small amount of ethanol, however, has prompted us to investigate the use of ferroin in aqueous alcohol as titration medium.

EXPERIMENTAL

Reagents

Vanadium(IV) sulphate solution (0.05*M*) and cerium(IV) sulphate solution (0.05*M*) were prepared in 1*N* sulphuric acid and standardized as described by Rao and Dikshitulu.[3] Sodium vanadate solution (0.05*M*) was prepared by treating the requisite amount of ammonium metavanadate with a slight excess of sodium carbonate solution, boiling until free from ammonia, then making up to the desired volume; it was standardized with ferrous ammonium sulphate solution by the procedure of Walden *et al.*[4] A 0.01*M* ferroin solution was used. All chemicals were of analytical reagent grade.

Apparatus

A quinhydrone electrode in 1*M* sulphuric acid (E_0 0.696 V *vs.* NHE),[5] bright platinum electrodes, and a salt-bridge consisting of an inverted U-tube with porcelain discs at the ends and filled with 1*M* sulphuric acid, were used in the potentiometric investigation.

Potentiometric investigation

Vanadium(IV) can be titrated potentiometrically with cerium(IV) sulphate solution in dilute sulphuric acid medium, but the electrode equilibration is slow, taking 5–6 min in the vicinity of the equivalence point (though only 1 min earlier in the titration). The potentiometric titration fails to give accurate results if an alcohol is present, although there is no difficulty in attaining an apparently stable potential with the indicator electrode used. The effect of varying alcohol content is shown graphically in Fig. 1 for methanol and ethanol. Isopropyl alcohol behaves similarly but more is needed to affect the result adversely.

Visual titration

In the titration of vanadium(IV) with cerium(IV) sulphate in dilute sulphuric acid, ferroin gives a premature end-point because reduction of the oxidized indicator by vanadium(IV) is slow. However, in a medium of 0.5–2.5*N* sulphuric acid or 1*M* hydrochloric acid containing 40–60% of alcohol, the titration is satisfactory (the indicator reaction is sluggish at other alcohol concentrations).

Procedure

A solution containing 2.5–25 mg of vanadium(IV) and enough 10*N* sulphuric acid to give an overall final acidity of 0.5–2.5*N* was diluted with water to 25 ml and 20–25 ml of alcohol were added. The mixture was cooled to room temperature, 2 drops of 0.01*M* ferroin were added and the solution was titrated with 0.05*M* cerium(IV) sulphate at the usual speed until near the end-point, a 10-sec delay then being allowed between additions. At the end-point the colour changed sharply from orange-red to bright yellow and was permanent for 3–4 min. An indicator correction of 0.02 ml of 0.05*M* cerium(IV) was needed. Typical results are given in Table 1.

Interferences

Amounts of 55 mg of iron(III), 25 mg of chromium(III), 200 mg of cerium(III), 30 mg of vanadium(V), 300 mg of zinc, 70 mg of nickel, 60 mg of copper and 160 mg of manganese(II) were found not to interfere.

The reverse titration under similar conditions was unsuccessful, premature end-points being obtained.

Differential titration of iron(II) and vanadium(IV)

By combination of the procedure above with that of Walden *et al.*,[4] iron(II) and vanadium(IV) can be determined differentially, with ferroin as indicator. One aliquot is titrated with cerium(IV) sulphate in 10–12*N* sulphuric acid medium to obtain the iron(II) content. Another aliquot is titrated as described for vanadium(IV) to obtain the

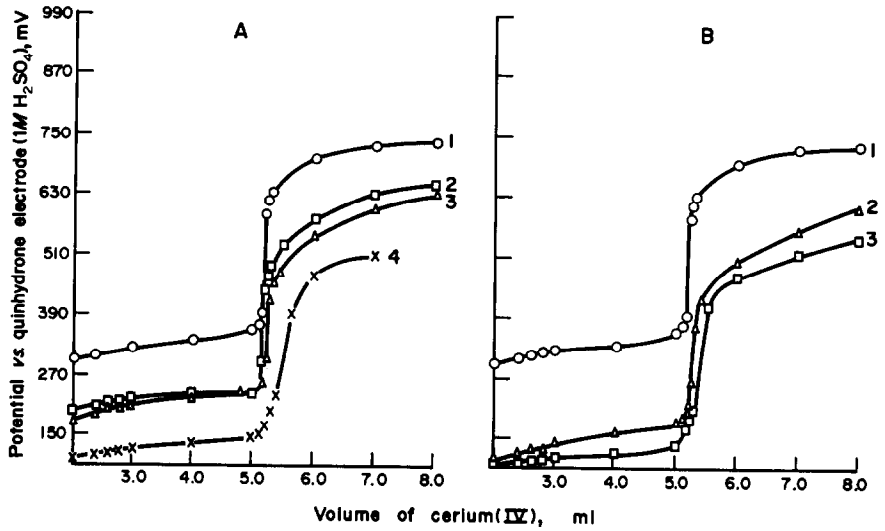

Fig. 1. Potentiometric titration with cerium(IV) sulphate at 28°C: 5.0 ml of 0.052*M* vanadium (IV) in 50 ml of 1*N* H_2SO_4 containing (A) *x*% v/v methanol; (B) *x*% v/v ethanol. (A) 1, O——O $x = 0$; 2, □——□ $x = 2$; 3, △——△ $x = 5$; 4, ×——× $x = 50$.
(B) 1, O——O $x = 0$; 2, △——△ $x = 2$; 3, □——□ $x = 10$.

total of iron(II) and vanadium(IV). Typical results are given in Table 2.

Spectrophotometric investigation

The absorption spectra of vanadium(IV) and (V) in 1*N* sulphuric acid without and with methanol (50% v/v) present were recorded against reagent blanks and are shown in Fig. 2. The methanol had only a small effect on the vanadium(IV) spectrum but a large effect on that of vanadium(V).

Photochemical reduction of vanadium(V)

Preliminary experiments showed that reduction of vanadium(V) by alcohol is quantitative in 2–3*N* sulphuric acid containing 60–70% v/v alcohol on exposure to bright sunlight for about 90 min. The vanadium(IV) can be titrated with cerium(IV) to a visual end-point (the potentiometric end-point gives an inconsistently high result).

Procedure

A solution containing 5–25 mg of vanadium(V) was treated in a 100-ml iodine flask with enough 10*M* sulphuric acid and methanol/ethanol to make it 2–3*N* in sulphuric acid and 60–70% v/v in alcohol when diluted to 40 ml. The flask was stoppered and the solution exposed to bright sunlight for 90 min. During this time the colour changed from bright yellow to blue. The solution was diluted to 50 ml with water and titrated as already described. Typical results are given in Table 3.

Determination of vanadium in ferrovanadium

About 1.0 g of sample (accurately weighed) is treated with 80 ml of 4*M* sulphuric acid and 40 ml of nitric acid (1 + 1) in a 600-ml beaker. When the reaction has abated, the solution is evaporated to dense white fumes, cooled, diluted and again heated to dissolve the salts. The solution is cooled, transferred to a 250-ml standard flask, made up to the mark with distilled water, and titrated by one of the following methods.

Reduction with iron(II). A 5.0-ml aliquot is treated with 5 ml of ~0.1*M* iron(II) and the unreacted iron(II) is titrated with cerium(IV) sulphate in 5*M* sulphuric acid to a ferroin end-point. Enough water and alcohol are then added to make the solution 2*N* in sulphuric acid and 40–50% in alcohol content. The red colour of ferroin is restored, and the solution is titrated with 0.05*M* cerium(IV) sulphate. The second titration gives the content of vanadium.

Reduction with alcohol. A 5.0-ml aliquot is mixed with 3 ml of concentrated sulphuric acid and 8 ml of alcohol and is heated for 10 min on a boiling water-bath, then cooled to room temperature; 20–25 ml each of water and alcohol and 2 drops of 0.01*M* ferroin are added and the mixture is

Table 1. Determination of vanadium

	Vanadium(IV), *meq*	
Alcohol	Taken	Found
Methyl	0.269	0.269
	0.373	0.373
	0.467	0.466
	0.143	0.144
Ethyl	0.164	0.164
	0.273	0.272
	0.375	0.375
	0.448	0.448
Isopropyl	0.140	0.141
	0.238	0.238
	0.313	0.312
	0.440	0.439

Table 2. Determination of iron(II) and vanadium(IV) in mixtures

	Iron(II), *meq*		Vanadium(IV), *meq*	
Alcohol	Taken	Found	Taken	Found
Methyl	0.192	0.192	0.470	0.471
	0.463	0.462	0.230	0.229
Ethyl	0.369	0.370	0.284	0.284
	0.142	0.143	0.450	0.451
Isopropyl	0.160	0.161	0.350	0.349
	0.467	0.468	0.168	0.168

Table 3. Determination of vanadium(V) after photochemical reduction

Alcohol	Vanadium(V), *meq*	
	Taken	Found
Methyl	0.173	0.172
	0.440	0.442
	0.467	0.467
Ethyl	0.212	0.213
	0.360	0.361
	0.478	0.478

titrated with 0.05*M* cerium(IV) sulphate. The mean of five analyses of EURO-Standard 577-1 (V 50.16%) was 50.2 by the iron(II) method and 50.1% by the alcohol method.

Analysis of a vanadium complex

Bis(1,10-phenanthroline)oxovanadium(IV) sulphate, prepared as described by Selbin and Holmes,[8] was analysed by the following procedure.

About 0.1 g of complex (accurately weighed) is mixed with 15 ml of 1.5*M* sulphuric acid and heated on a boiling water-bath to assist dissolution, then cooled to room temperature; 20 ml of alcohol are added, followed by 2 drops of 0.01*M* ferroin. The mixture is titrated with 0.05*M* cerium(IV) sulphate.

The average of three determinations for the VO content, 13.1%, was in good agreement with the value of 12.80% calculated for $VO(C_{12}H_8N_2)_2SO_4$. The phenanthroline was found not to interfere, which greatly simplified the procedure, since there was no need to destroy the organic moiety.

DISCUSSION

The possible cerium(IV)–alcohol reaction does not seem to affect the vanadium(IV)–cerium(IV) titration in aqueous alcohol medium, with ferroin as indicator; presumably the vanadium(IV)–cerium(IV) reaction is much faster, and the vanadium(V)–alcohol reaction is very slow. The failure of the potentiometric titration of vanadium(IV) with cerium(IV) sulphate in the presence of methanol or ethanol or isopropyl alcohol is, however, interesting. Such behaviour was not observed for titrations in aqueous acetone[1] or acetic acid[6] medium. The delayed end-point was originally thought to be due to the simultaneous oxidation of alcohol, but the ferroin end-point gives correct results, so this cannot be the case. Even when the potentiometric titration is done with ferroin present there is no change in the results, *i.e.*, the ferroin end-point is correct while the potentiometric end-point is erroneous. Evidently the platinum of the electrode does not catalyse oxidation of the alcohol. Moreover, if the erroneous potentiometric result is caused by abnormal electrode behaviour, ferroin does not correct this even though it is a good electron-transfer catalyst.[7] The effect is also not related to the presence of vanadium since alcohol also causes high results for iron(II) when the titrant is cerium(IV). However, a fresh platinum electrode introduced just after the ferroin end-point has been reached registers a potential high enough to indicate the presence of excess of cerium(IV), and ferroin added at this stage is oxidized. The error in the potentiometric result must therefore be attributed to non-Nernstian behaviour of the electrode response to the cerium(IV)/cerium(III) couple in alcohol medium.

Figure 1 shows that there is a decrease in the vanadium(V)/vanadium(IV) standard potential when alcohol is present. This indicates formation of a more stable complex of alcohol with vanadium(V) than with vanadium(IV), which is confirmed by the effect of alcohol on the absorption spectra (Fig. 2).

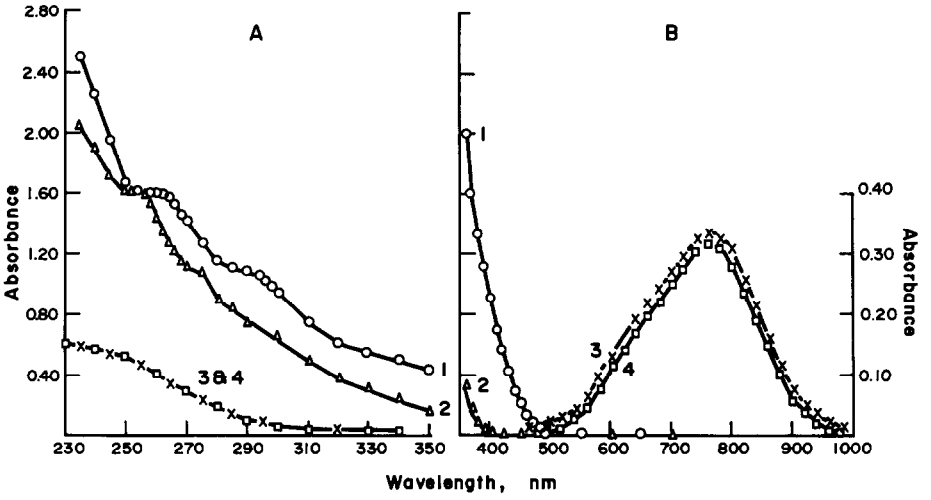

Fig. 2. Absorption spectra. (A) 1, O——O 1.06 × 10^{-3}*M* vanadium(V) in 1*N* H_2SO_4 containing 50% v/v methanol; 2, △——△ 1.06 × 10^{-3}*M* vanadium(V) in 1*N* H_2SO_4; 3, □——□ 2 × 10^{-3}*M* vanadium(IV) in 1*N* H_2SO_4 containing 50% v/v methanol; 4, ×——× 2 × 10^{-3}*M* vanadium(IV) in 1*N* H_2SO_4.

(B) 1, O——O 5 × 10^{-3}*M* vanadium(V) in 1*N* H_2SO_4 containing 50% v/v methanol; 2, △——△ 5 × 10^{-3}*M* vanadium(V) in 1*N* H_2SO_4; 3, ×——× 2 × 10^{-2}*M* vanadium(IV) in 1*N* H_2SO_4 containing 50% v/v methanol; 4, □——□ 2 × 10^{-2}*M* vanadium(IV) in 1*N* H_2SO_4. For curves 3 and 4 in (B), the absorbance scale is given on the right-hand side.

The reaction between vanadium(V) and alcohol in dilute sulphuric acid medium is slow but can be accelerated by exposing the reaction mixture to bright sunlight. Ethanol or methanol is recommended as the reductant because the reaction with isopropyl alcohol is rather slow even in the presence of sunlight.

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SEQUENTIAL TITRATION OF IRON(II) AND VANADIUM(IV) MIXTURES WITH CERIUM(IV) SULPHATE, WITH FERROINS AS INDICATORS

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Summary—The titration of vanadium(IV) with cerium(IV) sulphate, with nitroferroin as indicator, is proposed. Unlike ferroin, the indicator does not need a catalyst in this system. By suitable choice of experimental conditions iron(II) can be titrated first to a ferroin end-point and then vanadium(IV) to a nitroferroin end-point.

The analysis of vanadium or chrome–vanadium steels usually involves preliminary oxidation of the alloying elements to their highest oxidation states, followed by titration with iron(II) in acid solution. It is convenient to do the analysis with a single sample and this is possible if the vanadium(IV) and the excess of iron(II) can be determined sequentially with one titrant. Cerium(IV) is a suitable titrant. Smith and Getz[1] proposed a potentiometric titration in 8*M* perchloric acid medium; Furman,[2] and Dikshitulu and Rao,[3] used dilute sulphuric acid medium, though under different experimental conditions. There seem to be no visual end-point methods for such a sequential titration. We report here the use of ferroin and 5-nitroferroin as indicators for detection of the iron(II) and vanadium(IV) end-points respectively.

The vanadium(IV)–cerium(IV) reaction is considered to be slow, needing elevated temperature when the end-point is detected potentiometrically.[2,4] Rechnitz and Rao,[5] however, inferred from kinetic studies that the reaction is fast and the apparent sluggishness arises in the end-point detection system, *e.g.*, from slow potential equilibration at the platinum electrode. Recently some visual indicator methods[6–8] have been proposed for this titration, with use of a suitable solvent medium or a catalyst. Most of the procedures do not work if the concentration of sulphuric acid in the titration medium exceeds 0.5*M*. Obviously, this places a restriction on extending these procedures to the determination of vanadium(IV) in a test solution containing iron(II). Under the conditions prescribed for the titration of vanadium(IV), iron(II) also reacts with the oxidant, so the sum of the two is obtained. However, the iron(II) alone can be titrated with cerium(IV) sulphate in 5.0*M* sulphuric acid medium with ferroin as indicator, as the vanadium(V)/vanadium(IV) conditional standard potential is then too high for competitive titration of the vanadium. For determination of the vanadium(IV), the acidity must be lowered and a catalyst added. However, the titration is not satisfactory because of the sluggishness of the indicator reactions. In the method reported here, the titration of vanadium(IV) can be completed with 5-nitroferroin as indicator if the solution is diluted with about an equal volume of water.

EXPERIMENTAL

Reagents

Iron(II), vanadium(IV) and cerium(IV) sulphate solutions were prepared and standardized as described earlier.[7] The ferroin and 5-nitroferroin solutions were both 0.01*M*.

Procedures

Titration of vanadium(IV). Between 8 and 30 mg of vanadium(IV) is treated with enough 10*M* sulphuric acid to give an acid concentration of 0.5–2.5*M* on dilution to 45 ml. The mixture is titrated with 0.05*M* cerium(IV) sulphate after addition of one drop of 5-nitroferroin indicator. Near the end of the titration, the titrant must be added dropwise with a 10–15 sec wait after each addition, to allow return of the nitroferroin colour. As the titration proceeds, the masking of the red colour of the indicator by vanadium(IV) decreases. At the end-point the colour changes from red to greenish yellow. Some typical results are given in Table 1.

Amounts of 28 mg of iron(III), 25 mg of chromium(III), 33 mg of manganese(II), 200 mg of zinc, 17 mg of Ag, 875 mg of Cl^- or 700 mg of PO_4^{3-} (examined separately) did not interfere in the titration. Larger quantities of phosphate, however, retarded the indicator oxidation, resulting in overstepped end-points; in the presence of tungstic acid, the colour change is not sharp.

The transition potential of 5-nitroferroin was found to be 1.28–1.25 V under the conditions of the titration (1–2*M* sulphuric acid).

Sequential titration of iron(II) and vanadium(IV). The titrand, containing 8–30 mg each of iron(II) and vanadium(IV), is treated with 10*M* sulphuric acid to give a concentration of 5*M* at the iron(II) end-point. The solution is diluted to 45 ml and a drop of ferroin is added. The solution is titrated with 0.05*M* cerium(IV) sulphate solution till the red colour of ferroin is completely discharged.

Table 1. Determination of vanadium

Vanadium(IV), *mg*	
Taken	Found
8.82	8.84
13.15	13.16
16.67	16.63
23.41	23.33
28.70	28.61

Table 2. Determination of iron(II) and vanadium(IV) in mixtures

Taken, *mg*		Found, *mg*	
Fe(II)	V(IV)	Fe(II)	V(IV)
23.63	10.56	23.58	10.53
14.95	20.59	14.92	20.54
12.71	23.36	12.71	23.26
18.28	18.98	18.24	18.92
9.69	23.05	9.66	22.95

The solution is then diluted with 50–60 ml of water and cooled, 1 or 2 drops of 5-nitroferroin solution are added and the titration is continued to the vanadium(IV) end-point. Some typical results are given in Table 2.

Analysis of ferrovanadium

About 1.0 g of the ferrovanadium sample (accurately weighed) is treated with 40 ml of sulphuric acid (1 + 1) and 40 ml of nitric acid (1 + 1) and heated. When the reaction has abated, the solution is evaporated to dense white fumes, cooled to room temperature and diluted to the mark with water in a 250-ml standard flask. A 5.0-ml aliquot is treated with 10 ml of 0.05*M* iron(II) solution and analysed for excess of iron(II) and the vanadium(IV) content according to the procedure described above. Typical results are given in Table 3.

Table 3. Determination of vanadium in ferrovanadium

Sample	Certified value, %	Found, %
EURO-standard 577-1	50.16	50.17

DISCUSSION

The titration of iron(II) with cerium(IV) sulphate in 5*M* sulphuric acid with ferroin as indicator is free from interference by vanadium(IV). The vanadium(IV) can then be titrated in 0.5–2.5*M* sulphuric acid with 5-nitroferroin as indicator. Ferroin is useless for the latter titration because its redox potential is too low and there is a premature end-point, and the indicator reaction is slow.

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SIMULTANEOUS DETERMINATION OF BROMIDE AND CHLORIDE IN NATURAL WATERS BY ION-EXCHANGE CHROMATOGRAPHY AND DIRECT POTENTIOMETRY WITH AN ION-SELECTIVE ELECTRODE

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Summary—A method has been developed for the sequential determination of bromide and chloride in natural waters by use of ion-exchange chromatography and an ion-selective electrode. Hydrous zirconium oxide has successfully been used as the ion-exchanger.

Titrimetric methods using ion-selective electrodes for end-point detection have been applied for the determination of halides in naturally occurring samples.[1–3] Recently, an ion-selective electrode was used as a detector in chromatography.[4] We have reported a method for determination of chlorine and bromine in silicate rocks by ion-exchange chromatography followed by direct potentiometry with an ion-selective electrode,[5,6] but the sequential determination of the two elements was not successful because of the peculiar response behaviour of the silver halide electrode. In the present work, this problem is overcome by reversing the elution order of the halide ions by using an inorganic ion-exchanger, *viz.* hydrous zirconium oxide.[7,8] The method is then suitable for sequential determination of bromide and chloride in natural waters.

EXPERIMENTAL

Reagents

Redistilled water and guaranteed reagent-grade inorganic chemicals were used throughout. The hydrous zirconium oxide was prepared according to the literature[7,8] and conditioned by immersion for one day in 0.5*M* sodium nitrate at about pH 2 before use.

Apparatus

An Orion Model 94-17 silver chloride electrode, Model 94-35 silver bromide electrode, Model 94-53 silver iodide electrode and Model 90-02 double-junction reference electrode were used, and an Orion Model 701 digital pH-meter combined with a Shimadzu R-111M type recorder. The chromatographic column was 6 mm in bore and 11 cm long. The apparatus for ion-exchange chromatography has already been described.[5]

Preparation of the sample solution

A suitable volume of sample solution is taken in a 50-ml standard flask, and 5.0 ml of 5.0*M* sodium nitrate and 1.0 ml of 0.7*M* nitric acid are added to adjust the ionic strength and pH, and the sample solution is diluted to volume. A standard solution is prepared in the same way, with a known amount of bromide and chloride solution.

Procedure

About 5 ml of the sample solution are injected into the column by syringe. Bromide and chloride are eluted in that order with 0.5*M* sodium nitrate adjusted to about pH 2 at a flow-rate of 0.55 ml/min. Bromide and chloride are determined by comparing the peak heights of the chromatograms of the sample and the standard.

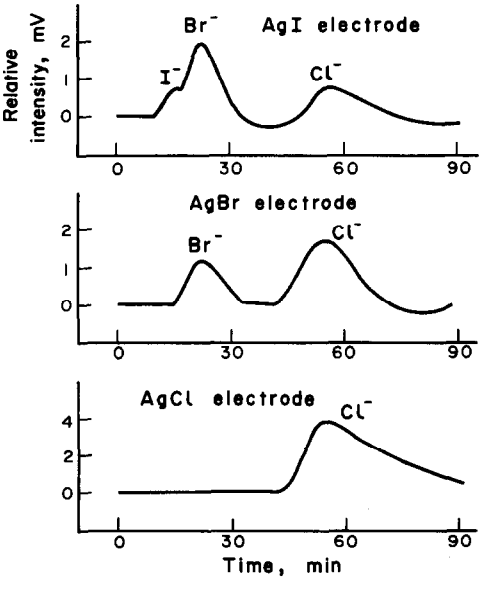

Fig. 1. Chromatograms of halide standards: I^-, 5.0 ng/ml; Br^-, 50 ng/ml; Cl^-, 2.0 μg/ml.

Table 1. Accuracy tests

Sample	Calibration curve method		Standard addition method	
	Br^-, *ng/ml*	Cl^-, *μg/ml*	Br^-, *ng/ml*	Cl^-, *μg/ml*
Pond water (Gunma Univ.)	27	8.6	27	9.0
Pond water Kezouji park)	31	19.3	35	18.5
Tap water (Nitta Town)	—	38.5	—	39.0
Tap water (Gunma Univ.)	—	9.5	—	9.0

RESULTS AND DISCUSSION

Since hydrous zirconium oxide acts as an anion-exchanger in acidic solution,[7] the pH of both the eluent and the sample solution is adjusted to *ca.* 2. Under the conditions described, bromide and chloride can be quantitatively separated, and the separation is not affected by variation of the pH in the range 1.5–2.5.

The response of the three silver halide electrodes in the chromatography of a mixture of iodide, bromide and chloride is shown in Fig. 1. The silver chloride electrode gave poor response to iodide and bromide, and so did the silver bromide electrode to iodide. Although the silver iodide electrode responded to all three halides, the peaks are not sufficiently resolved and they are asymmetric. Further, there was a drift of the base-line after detection of a halide ion which was not a component of the electrode, and this drift caused disturbance in the following peak. This electrode behaviour was the reason for our failure to determine bromide and chloride sequentially by separation with a gel-type ion-exchange resin:[5,6] the bromide could not be detected with the silver chloride electrode, and when the bromide electrode was employed, the bromide peak height was influenced by the preceding peak of the chloride eluted preferentially from the anion-exchanger. This difficulty has now been eliminated by using hydrous zirconium oxide instead of the anion-exchange resin for the chromatography, since it reverses the elution order for halide ions. The silver bromide electrode is then the most suitable as the detector for both bromide and chloride. Iodide up to 200 ng/ml does not interfere.

The calibration graphs obtained by plotting peak height against concentration are linear over the range of 10–100 ng/ml for bromide and 1.0–9.0 μg/ml for chloride, as expected theoretically,[9] but at higher concentrations tend towards Nernstian response, *i.e.* peak height $\propto \log [X^-]$.

Reproducibility was tested by using a pond water sample from the Gunma University campus, and average values of 27 ng/ml bromide and 8.6 μg/ml chloride were obtained from 5 determinations, the coefficients of variation being 3.6 and 1.6% respectively.

The standard-additions method was used for accuracy tests. Table 1 shows very good agreement of the values obtained by the calibration method with those from the addition method. The results show that the method is satisfactory for the sequential determination of bromide and chloride in natural water samples.

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SOME APPLICATIONS OF LIGAND-EXCHANGE—II

SEPARATION OF PHENOLIC COMPOUNDS*

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Summary—The separation of phenol, 2-nitrophenol, 2,4-dichlorophenol and pentachlorophenol by ligand-exchange chromatography was studied with Chelex 100 resin in the Fe^{3+} form as stationary phase and sodium hydroxide solution (pH 7.5, 11.5, 12) as the mobile phase.

Ligand-exchange chromatography[1] is a technique used to separate compounds which can form complexes with metal ions. Siegel *et al.*[2] have described such a method for the determination of amino-acids in sea-water, Carunchio *et al.*[3] have applied the technique for determination of non-ionic surfactants, and a recent paper[4] describes the quantitative trapping of phenolic compounds by a ligand-exchange process involving a chelating resin in the iron(III) form. The phenols retained may be eluted with sodium hydroxide solution, because of differences between the strength of the metal-binding to the phenol and to hydroxide ion.

The purpose of the present work is to apply the ligand-exchange reaction to the separation of phenols retained on the exchanger, by exploiting differences in the stabilities of iron–phenol complexes. Since the stability constants of some iron(III)–phenol complexes are unknown and the resin matrix determines the strength of the binding of a particular ligand,[5] we have studied the effect of pH (sodium hydroxide concentration) on the distribution coefficients of some monohydric phenols.

EXPERIMENTAL

Reagents

Standard solutions (1 mg/ml) of phenol, 2-nitrophenol, 2,4-dichlorophenol and pentachlorophenol were prepared by dissolving weighed amounts of the pure phenols in acetone. Portions of these solutions were then diluted with distilled water to give the test solutions. All chemicals were analytical grade.

Chelex resin in the iron(III) form. An exchanger of the polystyrene–iminodiacetic acid type (Chelex 100, Bio-Rad) was used. The resin (100–200 mesh) in the sodium form was converted into the iron(III) form by mechanical shaking for 12 hr with 100 ml of 1*M* iron(III) chloride. After equilibration was complete the solution was decanted, and the resin was washed with distilled water until the washings were free from iron(III).

Analytical procedures

Rate of sorption. Weighed quantities of dried resin (3 g) in the iron(III) form were placed in 300-ml glass bottles. To each bottle 100 ml of water containing 0.5 μmole of a phenol were added. The bottles were stoppered and rotated in a thermostat at 20°. At intervals of 1, 2, 3, 5, 10, 20 and 30 min the aqueous phase was separated and the concentration of the phenol in it was determined from its absorbance at 270 nm (phenol), 283 nm (2,4-dichlorophenol), 277 nm (2-nitrophenol) or 250 nm (pentachlorophenol), measured with a Perkin–Elmer 320 spectrophotometer and 1.0-cm cells.

Effect of hydroxide concentration on adsorption. Three g of resin in the iron(III) form and 100 ml of water containing 0.5 μmole of a phenol were placed in a glass bottle and equilibrated for 20 min. The aqueous phase was then decanted and the resin was washed with distilled water and equilibrated with 100 ml of sodium hydroxide solution. The resin was filtered off on a sintered-glass filter and the concentration of the phenol in the aqueous phase determined spectrophotometrically as before. The pH of the aqueous phase was also measured.

The distribution coefficient K_d was calculated from

$$K_d = \frac{\text{mg of phenol in resin–iron(III) phase/g of dry resin}}{\text{mg of phenol in solution phase/ml of solution}}$$

The experiment was repeated with various concentrations of sodium hydroxide.

Separation of phenols. An aqueous solution containing 50 ng of each phenol per ml was passed through a column of Chelex resin in iron(III) form. The phenols retained on the exchanger were sequentially displaced by passing through the column solutions of sodium hydroxide at increasing pH values (appropriate to the K_d values), at a flow-rate of 1 ml/min. The phenols obtained by this stepwise elution were determined spectrophotometrically in successive 10-ml fractions of eluate and also by gas chromatography. Portions of the eluate were acidified and extracted with methylene dichloride, then 1-μl samples of the extracts were injected together with *o*-cresol or 2,3,4,5-tetrachlorophenol as internal standards into a 9-m persilaned glass capillary coated with SP 2100, the injector temperature being 300°. The carrier gas was hydrogen and a flame-ionization detector was used.

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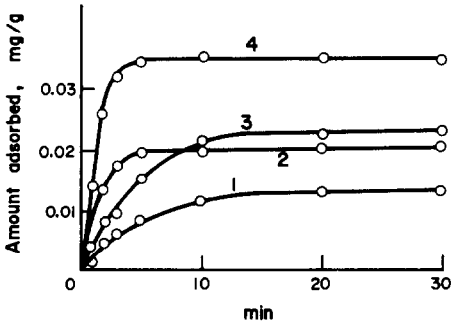

Fig. 1. Rate of adsorption of phenols from aqueous NaOH at 20°: 1, phenol; 2, 2-nitrophenol; 3, 2,4-dichlorophenol; 4, pentachlorophenol. Resin, Chelex 100, Fe^{3+} form; solvent: NaOH at pH 7.5.

RESULTS AND DISCUSSION

Results for sorption equilibria are shown in Fig. 1, which shows that at pH 7.5 2-nitrophenol and pentachlorophenol are adsorbed faster than phenol and 2,4-dichlorophenol. From Fig. 1 the capacity of the resin can be determined; a value of 0.1 μmole/g was obtained for all the phenols tested.

Figure 2 shows that the K_d values for the various phenols decrease with increasing pH. The elution order for these phenols (phenol > 2-nitrophenol > 2,4-dichlorophenol > pentachlorophenol) is independent of pH. This behaviour is in disagreement with the values of the formation constants reported by Sillén and Martell:[6] iron(III)–phenol (log K = 7.78) and iron(III)–2-nitrophenol (log K = 5.59); the first-named is retained less strongly on the resin in the Fe^{3+} form. However, it may be roughly correlated with the pK_a values for the phenols.

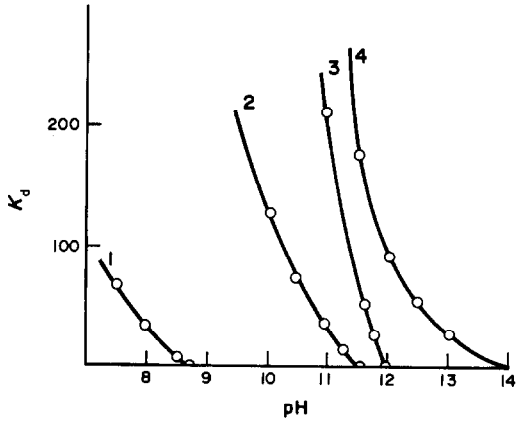

Fig. 2. Effect of pH (NaOH concentration) on the distribution coefficients of phenols: 1, phenol; 2, 2-nitrophenol; 3, 2,4-dichlorophenol; 4, pentachlorophenol. Resin, Chelex 100, Fe^{3+} form.

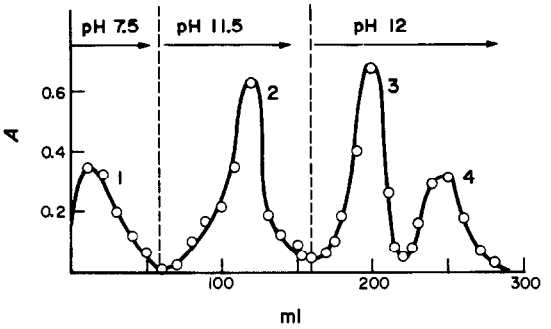

Fig. 3. Elution curves: 1, phenol; 2, 2-nitrophenol; 3, 2,4-dichlorophenol; 4, pentachlorophenol. Column 10 × 70 mm; resin Chelex 100, Fe^{3+} form; eluent NaOH solution; flow-rate 1 ml/min.

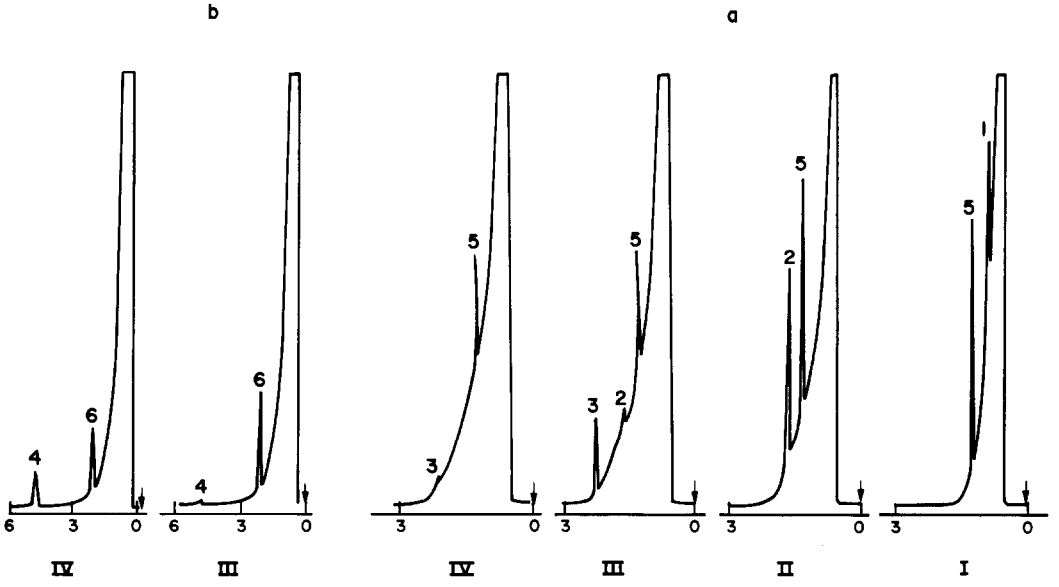

Fig. 4. Gas chromatograms of extracts from NaOH eluates. I = 60 ml, pH 7.5; II = 100 ml, pH 11.5; III = 60 ml, pH 12; IV = 70 ml, pH 12. 1, Phenol; 2, 2-nitrophenol; 3, 2,4-dichlorophenol; 4, pentachlorophenol; 5, *o*-cresol (internal standard); 6, 2,3,4,5-tetrachlorophenol (internal standard); (*a*) column temperature 60°, $\bar{u}$ = 37.5 cm/sec; (*b*) column temperature 130°, $\bar{u}$ = 37.5 cm/sec.

The separation factor calculated from the K_d values of phenol and the substituted phenols is large enough to permit quantitative separation of phenol in the pH range 7.5–10. When sodium hydroxide solution (pH 7.5) is passed through the column at a flow-rate of 1 ml/min, 2-nitrophenol, 2,4-dichlorophenol and pentachlorophenol are quantitatively retained, while phenol passes into the eluate. For pH values > 11, the complex Fe^{3+}–hydroxide in the resin becomes more stable than the complexes iron(III)–2-nitrophenol, iron(III)–2,4-dichlorophenol and iron(III)–pentachlorophenol. The difference in the three K_d values is not large, but it is possible to carry out the separation (Figs. 3 and 4) with different portions of sodium hydroxide solution at pH 11.5 and 12 as eluents.

CONCLUSIONS

The ligand-exchange technique, using a Chelex resin in the iron(III) form, can be applied to trap trace amounts of phenolic compounds from water, and the retained phenols can be separated with an appropriate eluent, provided that there is a sufficiently large difference in the stability of the complexes.

From the distribution coefficients of the phenols between the resin phase and the eluent, it is possible to predict the course of the separation and the optimum conditions.

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TITRIMETRIC DETERMINATION OF CHLORAMINE-T AND SOME ALDOSES BY AMPLIFICATION REACTIONS

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Summary—A titrimetric method with amplification has been worked out for the determination of chloramine-T and certain aldoses. It is based on Leipert determination of iodide produced in the case of chloramine-T by reduction of the determinand with excess of iodide, and extraction and subsequent reduction of the iodine liberated. In the other case, the aldoses are oxidized with iodine, the surplus oxidant is extracted, and the residual iodide is determined. The method is applicable to chloramine-T in the range 0.01–3 mg, with a relative error between −3.0 and −0.4% and a relative standard deviation of 0.6–0.8%, depending on the amount present, and to 0.05–1 mg of glucose, galactose or arabinose, or 0.1–2 mg of lactose or maltose, with a relative standard deviation of 1.4% for >0.5 mg of aldose.

We have recently reported[1] an application of the so-called Leipert amplification reaction[2] in which an iodine solution in chloroform was used for oxidation of chloral hydrate, and after extraction of the surplus iodine, the iodide produced was determined by the Leipert procedure. We have now extended this technique to determination of some aldoses, and developed a variant of it by which we can determine chloramine-T.

In the new variation, the chloramine-T is reduced by excess of iodide, and the iodine produced is extracted into chloroform, then reduced back to iodide, which is determined by the Leipert procedure with either 6- or 36-fold amplification.[3]

EXPERIMENTAL

Reagents

Chloramine-T solution (*1 mg/ml*). Prepared in distilled water. The chloramine-T was freed from possible contamination with dichloramine-T by washing it several times with carbon tetrachloride, and was dried in a vacuum desiccator over calcium chloride.[4] The purity of the compound was determined iodometrically,[5] on the basis of its active chlorine content. Less concentrated solutions were prepared by dilution.

Iodine solution. Prepared weekly by dissolving 0.3 g of pure iodine in 250 ml of pure dry chloroform, and stored in an amber bottle.

Iodide solution, 1 mg/ml.

Sodium thiosulphate solutions, 0.01 and 0.001*N*. Standardized against potassium iodate solutions of similar normalities.

Sodium sulphite solution, 1%. Prepared fresh daily.

Procedure for chloramine-T

In a 50-ml separating funnel, place 0.2–3 ml of chloramine-T solution (0.01–3 mg), 5 ml of iodide solution, 1 ml of 2*M* hydrochloric acid, and distilled water to make a total volume of 10 ml. Extract the liberated iodine with two 10-ml portions of chloroform. Collect the extracts in another funnel, and shake them with 10 ml of water containing 0.5 ml of 1% sodium sulphate solution to reduce the iodine to iodide. Transfer the aqueous (upper) layer to a 100-ml Erlenmeyer flask, and determine the iodide by one of the following methods. Run a blank determination.

6-Fold amplification. To the iodide solution obtained, add 2 ml of saturated bromine water. Stopper the flask and stir the solution for 5 min. Destroy the excess of bromine by adding 5 ml of 80% v/v formic acid solution, shaking till colourless. Add about 0.5 g of potassium iodide and titrate the liberated iodine with thiosulphate in the usual way.

36-Fold amplification. Transfer the iodine, formed as for the 6-fold amplification, to a 50-ml separating funnel. Extract the iodine with four 10-ml portions of chloroform. Collect the extracts in a 100-ml separating funnel. Wash the combined extracts with 5 ml of water to remove any iodide carried over. Shake the iodine solution with 10 ml of water containing 1 ml of 1% sodium sulphite solution, to reduce iodine to iodide. Transfer the aqueous (upper) layer, containing the iodide, to a 100-ml conical flask, then oxidize it with bromine water and complete the determination in the same way as for the 6-fold method.

Procedure for aldoses

In a 50-ml separating funnel, place a suitable volume (1–2 ml) of the aldose solution (0.05–2 mg of aldose) and dilute to about 10 ml with distilled water. Add the iodine solution (0.3 ml for each 0.1 mg of aldose compound), then add, with shaking, 0.01*M* sodium hydroxide (0.5 ml for each 0.1 mg of the aldose compound). Allow the flask to stand for 15 min (20 min for lactose and maltose) with occasional shaking, then acidify with 0.5 ml of 1*N* sulphuric acid. Extract the excess of iodine with two 10-ml portions of chloroform. Transfer the aqueous phase quantitatively to a 100-ml conical flask, add 3 ml of saturated bromine water and stir for 5 min. Destroy the excess of bromine with 4 ml of 80% v/v formic acid solution. Add about 0.5 g of potassium iodide and titrate the liberated iodine with 0.01*N* thiosulphate, using starch as indicator (for less than 0.5 mg of aldose, use 0.001*N* thiosulphate). Run a blank determination.

1 ml of 0.01*N* thiosulphate ≡ 150.1 μg of glucose or galactose, 125.1 μg of arabinose or 300.3 μg of maltose or lactose.

Table 1. Accuracy and precision of the method

Amount of CAT taken, *mg*	Relative error, % 6-fold	36-fold	Relative standard deviation, % 6-fold	36-fold
0.01	—	−3.0	—	0.8
0.05	−1.5	−1.8	0.6	0.7
0.10	−0.4	−1.2	1.0	0.5
1.00	−0.2	−3.0	0.1	0.6
3.00	−0.9	—	0.9	—

RESULTS AND DISCUSSION

Chloramine-T

The oxidation of iodide to iodine by chloramine-T is a function of many parameters, which have to be optimized. The conditions reported refer to 1 mg of chloramine-T.

Effect of acidity. The reaction is known to proceed rapidly and quantitatively in acidic medium. Experiment showed that the reaction was instantaneous and quantitative in 0.05–0.4*M* hydrochloric acid medium. Higher concentrations of the acid give a high blank because the iodide becomes sensitive to aerial oxidation, and lower concentrations of the acid result in incomplete reaction. Sulphuric acid can be used instead of hydrochloric.

Amount of iodide. For quantitative reaction, for 1 mg of chloramine-T, at least the stoichiometric amount of potassium iodide is needed and up to 5 mg can be safely added. Higher amounts of iodide do not affect the determination, but result in a high blank owing to aerial oxidation or iodide.

Aqueous phase volume. The optimal volume is 10–12 ml. Larger volumes give low recoveries because of the phase volume ratio effect.

Solvent. Chloroform is preferred; although benzene is more efficient, it is regarded as a potential toxic hazard. Two 10-ml portions of chloroform are sufficient for complete extraction of iodine in the 6-fold method, but four are required for the 36-fold procedure; amounts of chloramine-T larger than 1 mg can be determined provided the amount of iodine liberated can be extracted with the volumes recommended.

Formic acid. As this acid serves both for destroying the excess of bromine and for the iodate/iodide reaction in the final step, 5 ml of its 80% solution are used.

Accuracy and precision of the method

The results in Table 1 indicate that the method is satisfactory for determination of small amounts of chloramine-T.

For amounts of chloramine-T below 0.05 mg and for the blank, titrate with 0.001*N* thiosulphate. The conversion factors are:

1 ml of 0.01*N* thiosulphate
≡ 205.8 μg of CAT (6-fold amplification)
≡ 36.0 μg of CAT (36-fold amplification)

The procedures described here are more sensitive than previously published methods.[5,8–12] The 6-fold procedure is faster but the 36-fold method is more sensitive.

Aldoses

The conventional method for determination of aldose compounds involves oxidation with excess of aqueous iodine solution and back-titration with thiosulphate:[6]

$$RCOH + I_2 + 3NaOH \rightarrow RCOONa + 2NaI + 2H_2O$$

The equation shows that two iodine atoms are reduced by one molecule of aldose to two iodide ions which can then be dealt with by the Leipert 6-fold amplification procedure. Hence one molecule of aldose is equivalent to 12 iodine atoms, instead of the 2 in the normal reaction, so the overall amplification is 6-fold.

Preliminary studies confirmed that the amount of sodium hydroxide must be carefully controlled[7] to prevent conversion of part of the iodine into iodate. For 1 mg of sample, 5 ml of 0.01*N* sodium hydroxide will give the optimum concentration. For lower

Table 2. The iodometric determination of aldoses

Compound	Weight, μg Taken	Found*	Coeff. of variation, %
Glucose	50	49.0	2.3
	500	495	0.6
	1000	983	1.2
Galactose	50	48.9	2.4
	500	494	0.6
	1000	983	1.4
Arabinose	50	48.7	2.1
	500	494	0.7
	1000	984	1.3
Lactose	100	97.0	2.0
	500	493	0.5
	2000	1964	1.0
Maltose	100	97.8	1.8
	500	496	0.8
	2000	1972	1.1

*Average of 5 determinations.

amounts of aldose, proportionately smaller amounts of hydroxide are used. The second factor is the amount of iodine, and a 1.5–4-fold excess has been found essential for rapid and quantitative oxidation. Larger excesses of iodine should be avoided in order to decrease the number of extractions necessary, since these increase the risk of mechanical loss of aqueous phase, which would cause low results.

The working procedure developed has been applied successfully to the analysis of 0.05–2 mg amounts of aldose compounds. For sample weights lower than 50 μg, inconsistent errors (about ±6%) were obtained, which may be attributed to mechanical losses in the extraction, separation and transfer processes. On the other hand, for sample weights greater than 1 mg, low recoveries were obtained and may be due either to volatilization of some of the iodine produced in the amplification reaction, or to mechanical loss of the aqueous iodide solution during the extraction.

Table 2 shows the recoveries obtained for 5 aldose compounds. They range between 97.0 and 99.2%.

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EXTRACTION CHROMATOGRAPHY WITH MACRORETICULAR POLYMER BEADS IMPREGNATED WITH MONOTHIODIBENZOYLMETHANE SOLUTION

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Summary—Extraction chromatography using macroreticular ethylstyrene–divinylbenzene beads impregnated with monothiodibenzoylmethane (SBB) solution has been investigated. Of the solvents used as the stationary phase, heptan-1-ol showed the highest rate of metal extraction, and loading with 0.5 ml of the solvent per g of resin was found to be the optimum. A column packed with such loaded beads can be used for the separation of nickel(II), iron(III) and cobalt(II).

In extraction chromatography, selection of the support is very important in connection with the function of the stationary phase. Although many polymeric substances, including silica,[1] fluorine polymers,[2] polyethylene,[3] polyurethane,[4] polystyrenes[5] and celluloses,[6] have been used as supports, these have individual advantages and disadvantages. We have been interested in macroreticular (MR) polystyrene-based co-polymer beads for use as supports because beads with different physical properties are easily prepared by varying the composition of the monomers and the diluent. Parrish[7] has reported extraction chromatography with commercially available MR polymer beads impregnated with liquid ion-exchanger and pointed out the consequence of hydrophilicity of the supports in the extraction system. We have also reported an extraction chromatographic system based on MR co-polymer beads and a dibutyl phthalate solution of dithizone, and shown that effective metal extraction is markedly dependent on the physical properties of the beads.[8] An analogous extraction system for the separation of inorganic and organic mercury was later reported by Howard and Arbab-Zavar.[9] In these methods using dithizone as the extractant, the most serious disadvantage is the instability of dithizone.

In this paper, an extraction system based on ethylstyrene and divinylbenzene co-polymer beads and monothiodibenzoylmethane (which is more stable than dithizone and shows high affinity for some heavy metals) is investigated. The proposed system is applied to the separation of some metals.

EXPERIMENTAL

Reagents and apparatus

Commercial divinylbenzene (DVB) solution (nominally about 50% weight of DVB isomers, the remainder being ethylstyrene) was used after removal of the phenolic inhibitor. Monothiodibenzoylmethane (SBB) was prepared and purified by the method reported by Yokoyama *et al.*[10] Radioisotopes, ^{59}Fe, ^{60}Co, ^{65}Zn, ^{109}Cd and ^{203}Hg (as chloride or nitrate) were purchased from the New England Nuclear Corp., U.S.A., and the Radiochemical Centre, Amersham, England. All other reagents were reagent-grade materials and were used without further purification. The radioactivity was determined with a scintillation counter equipped with a well-type sodium iodide crystal detector. Copper(II) and nickel(II) were determined by atomic-absorption.

Polymer beads

Spherical beads of MR ethylstyrene–DVB co-polymer were prepared as reported previously.[8] The 35–60 mesh fraction was collected, washed with benzene and dried *in vacuo*. The physical properties of the beads were as follows: specific surface area 378 m^2/g, pore volume 1.44 ml/g, average pore diameter 15.2 nm, water regain[11] 1.40 ml/g, hexane regain[11] 1.46 ml/g.

Coating the beads

The polymer beads (6 g) were immersed for 1 hr in an appropriate amount of solvent and 30 ml of ethyl acetate containing the required amount of SBB, and the ethyl acetate was then evaporated by means of a rotary evaporator at 30–40°. The beads thus obtained contain 4 μmole of SBB per 100 mg of the solvent-loaded beads, and can be stored for at least one week without deterioration.

Batch operation

One hundred mg of the beads impregnated with the stationary phase containing 4 μmole of SBB were put in a glass-stoppered tube (18 × 180 mm), and 10 ml of metal-ion solution (at various pH values) were added. After a given period of mechanical agitation (320 strokes/min with an amplitude of 40 mm), the concentration of metal ion in the aqueous phase was determined.

Column operation

Beads (3 g) impregnated with the stationary phase were packed into a column. The column (10 × 100 mm) was conditioned with 50 ml of 1*M* perchloric acid and then washed with water until the effluent was neutral. Then 2 ml of sample solution [a mixture of equal volumes of $1 \times 10^{-3}M$ iron(III), cobalt(II) and nickel(II) spiked with iron and cobalt radioisotopes] were added to the column and the metals were eluted with buffer, acid solution or organic solvent at a flow-rate of 1 ml/min.

RESULTS AND DISCUSSION

Thiosubstituted β-diketone derivatives such as thiothenoyltrifluoroacetone (STTA) and monothiodibenzoylmethane (SBB) are efficient reagents in inorganic analysis and have been widely used for the extraction and spectrophotometric determination of many metals. Generally, SBB is more stable than STTA in acidic media,[12] and can be stored in the crystalline state for long periods at room temperature. We thought that the stability of the extracting agent was a dominant factor, and therefore employed SBB in this investigation. On the other hand, Parrish[7] pointed out that a certain minimum surface area and pore diameter of the support were necessary in extraction chromatography, and Amberlite XAD-7 (polyacrylic ester) having high water regain was favourable as the support. The MR DVB–ethylstyrene co-polymer beads prepared in our laboratory have fairly high water regain (1.40 ml/g), so the beads are expected to be an efficient support.

The extraction of metal ions at different pH by batch operation is shown in Fig. 1. Dibutyl phthalate (DBP) was used as the solvent in the stationary phase, because it is a stable high-boiling solvent and has poor solubility in aqueous media. Mercury(II) and copper(II) are extracted effectively in the lower pH region. In terms of $pH_{\frac{1}{2}}$ for extraction of metals, the order of extraction is mercury(II) > copper(II) > iron(III) > zinc(II) > cadmium(II) > cobalt(II) > nickel(II). However, cobalt(II) seems to be extracted as the cobalt(III) complex in the extraction system, as described later. This order corresponds essentially to the magnitude of the stability constants and the behaviour of the SBB complexes in liquid–liquid extraction.[13,14] When the beads hold only SBB without any solvent, the metal extraction is markedly decreased. In the proposed extraction system, the solvent plays a very important role. Beads impregnated with only solvent, without any SBB, showed no metal extraction below pH 7.

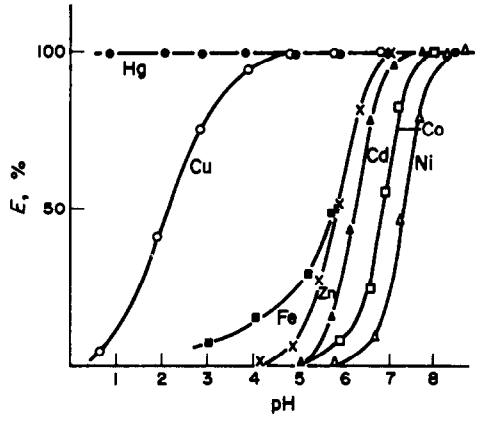

Fig. 1. Extraction of metal ions with SBB–DBP beads. Beads containing stationary phase 100 mg; metal-ion solution 4 × $10^{-5}M$; shaking time 3 hr; solvent loading 0.5 ml/g.

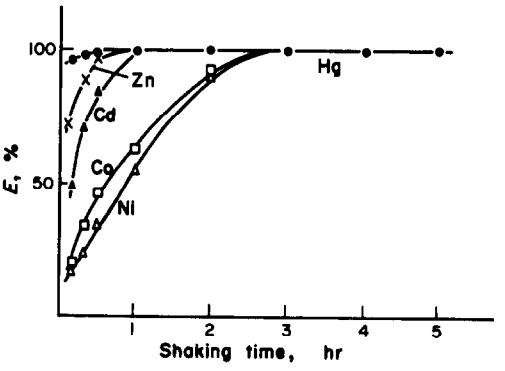

Fig. 2. Effect of shaking time on the extraction of metal ions at pH 8 with SBB–DBP beads. Conditions as for Fig. 1.

The extraction rate for the selected metal ions at pH 7 was determined, and the result is shown in Fig. 2. The extraction of mercury(II), zinc and cadmium reached equilibrium within 30–60 min, but that of cobalt(II) and nickel required about 180 min in the case of SBB–DBP beads. As pointed out by Chaston and Livingstone[15] and by Honjo and Freiser,[14] the slow rate of establishment of extraction equilibrium of the nickel chelate may be due to the change from an octahedral monochelated species $[Ni(H_2O)_4SBB]^+$ to a square-planar species $[Ni(SBB)_2]$.

Although DBP is an interesting solvent to use in the experiment above, its main disadvantage is lack of hydrophilicity. The use of hydrophilic solvents should be more effective in the extraction of metals. The effect of other solvents in this extraction system was investigated and the results are shown in Table 1. To minimize loss of solvent by evaporation during the coating process, solvents having relatively high boiling points were chosen. In addition, solvents giving

Table 1. Effect of solvent on extraction of zinc with SBB–solvent beads

Solvent	Loading, *ml/g*	Extraction, %
Dibutyl phthalate	50	17
Di-isobutyl ketone	50	23
Polyethylene glycol mono-*p*-nonylphenyl ether (n = 2)	50	19
Pentan-1-ol	50	41
Hexan-1-ol	50	42
Heptan-1-ol	12.5	24
	25	43
	50	49
	75	39
	100	35
Octan-1-ol	50	32
Nonan-1-ol	50	30
Decan-1-ol	50	29

Zinc solution 4 × $10^{-6}M$, pH 7 (0.5M acetic acid–sodium acetate), shaking time 2 min.

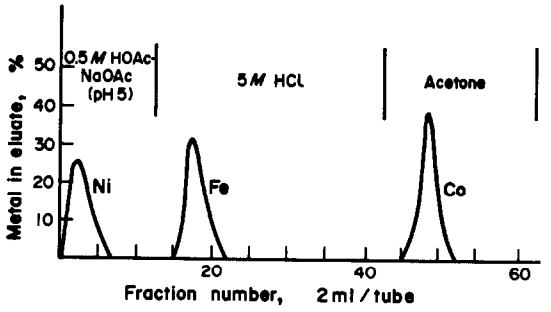

Fig. 3. Separation of iron(III), cobalt(II) and nickel with a SBB–heptan-1-ol bead column.

lower swelling of the beads were used in order to utilize the characteristic physical properties of the beads. Alcoholic solvents showed higher efficiency for metal extraction than the other solvents. Among the alcohols used, heptan-1-ol was the most effective in the extraction of zinc. In the present extraction system, alcohols seem to contribute to increasing the hydrophilicity, which in turn is related to the extraction kinetics.

The effect of the loading on the extraction of the metal ion was also examined, with heptan-1-ol as the solvent (Table 1). A loading of 0.5 ml/g gives the best results. With lower loading, the surface of the bead is not fully covered with solvent, but with too high a loading the advantage of the characteristic physical nature of the MR beads is lost.

For extraction with the SBB–heptan-1-ol (0.5-ml/g loading) beads, the $pH_{\frac{1}{2}}$ values for extraction of iron(III), cobalt(II) and nickel were 4.4, 4.9 and 6.7, respectively, and nickel was not extracted below pH 5 under the conditions indicated in Fig. 1. Separation of the three metals is shown in Fig. 3. Each metal ion is separated quantitatively by using stepwise elution, in which cobalt is washed out with acetone as well as the extraction system with STTA[16] because cobalt(II) reacts with SBB to form the stable SBB–cobalt(III) complex.[17]

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POLLUTION-FREE METHOD FOR THE DETERMINATION OF IRON IN IRON ORE

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Summary—A method for the determination of total iron in iron ores and concentrates is described which avoids the use of mercuric chloride. The sample is decomposed either by an acid attack or by fusion with sodium peroxide. The hot sample solution in about 6*M* hydrochloric acid is treated with hot 10% stannous chloride solution till pale yellow, followed by addition of a slight excess of 2% titanous chloride solution; the excess is then oxidized with perchloric acid (1 + 1). The solution is rapidly cooled in ice-water, and the iron (II) is titrated with potassium dichromate (sodium diphenylsulphonate as indicator). The results show the same degree of precision, accuracy, and degree of interference as those obtained by the standard stannous chloride–mercuric chloride method.

Iron ore ranks second only to crude oil as a commodity in commerce and industrial use. A shipment of iron ore may weigh as much as 100,000 tons and its evaluation may be based on just a few analytical samples. The accurate determination of iron in iron ore is therefore of considerable economic importance.

No instrumental method has been found accurate enough for the determination of iron in iron ores, although many routine iron determinations are carried out by XRF.

Two titrimetric methods have been recommended by ASTM and ISO for the determination of total iron in iron ores and concentrates.[1–3] They differ only in minor details. The ASTM methods are based on a dry sample, and the ISO methods prescribe an "as received" sample and a separate moisture determination.

One of the methods recommended by both ASTM and ISO uses hydrogen sulphide as reductant, the other uses stannous chloride. In the latter case, a solution of mercuric chloride is used to oxidize the excess of stannous chloride. Both methods therefore involve reagents which in many countries are increasingly rejected for environmental reasons.

The search for pollution-free methods has led ISO Committee TC-102/SC-2 to form two task forces. One of these[4] is considering the use of silver as the reducing agent.[5] Some objections that have been made have prevented this method from being more widely used. One is of a practical nature; the reductor must be prepared, cared for and regenerated, a fact which makes the method less attractive for large-scale routine determinations. The second objection involves the possible formation of hydrogen peroxide. There is also uncertainty regarding possible interference from copper.

The other task force is investigating the reduction of most of the iron(III) with stannous chloride, followed by addition of a slight excess of titaneous chloride. The excess of titanium(III) is removed by a delicate procedure requiring a precise titration (with Indigo Carmine solution as indicator) prior to the titration of the iron(II) with potassium dichromate.[6]

The procedure proposed here avoids the titration of the excess of titanium (III); instead the titanium(III) is oxidized in boiling solution with perchloric acid (which is reduced to chloride). This reaction has been previously used for the determination of perchlorate,[7] but not, to our knowledge, for the oxidation of titanium(III).

Under the conditions described in the procedure, the iron(II) is not oxidized.

EXPERIMENTAL

Reagents

Ferrous ammonium sulphate solution (approx. 0.1*N*). Dissolve 40 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 400 ml of sulphuric acid (5 + 95). Transfer to a 1000-ml standard flask, dilute to volume with the same acid, and mix.

Perchloric acid, 70% diluted 1:1.

Phosphoric acid–sulphuric acid mixture. Pour 150 ml of concentrated phosphoric acid into 400 ml of water, with stirring. Add 150 ml of concentrated sulphuric acid, with stirring. Cool and dilute to 1000 ml with water.

Potassium dichromate solution 0.1N. Pulverize about 6 g of standard-grade reagent in an agate mortar, dry in an oven at 105° for 3–4 hr, and cool to room temperature in a desiccator. Dissolve 4.904 g of the dry reagent in 300 ml of water, transfer to a 1000-ml standard flask, dilute to volume, and mix.

Potassium permanganate solution, 25 g/l.

Sodium diphenylaminesulphonate indicator solution. Dissolve 0.2 g of the reagent in water and dilute to 100 ml.

Stannous chloride solution, 10%. Dissolve 20 g of $SnCl_2 \cdot 2H_2O$ in 40 ml of concentrated hydrochloric acid by warming. Dilute to 200 ml with water. Prepare fresh as needed.

Titanium(III) chloride solution, 2%. On a steam-bath warm 1 g of titanium sponge with about 30 ml of concentrated hydrochloric acid until dissolved. Dilute to 50 ml with water. Prepare fresh as needed. If preferred, dilute 1 volume of commercial titanous chloride solution (about 15% w/v) with 7 volumes of hydrochloric acid (1 + 1).

Preparation of sample solution

(*a*) *Acid decomposition.* Transfer 0.4 g of dried 100-mesh sample (weighed to 0.1 mg) to a 400-ml beaker and decompose with hydrochloric acid with addition of a small amount of stannous chloride solution. Filter off and wash the residue, ignite it, then treat the cooled product with hydrofluoric and sulphuric acids. Take up the residue with hydrochloric acid and add to the filtrate, which is then ready for reduction and titration. For details of the acid decomposition step see references 1 and 2. If preferred, analyse the sample on an "as received" basis and determine the hygroscopic water on a separate portion of the sample.[7] This method is limited to samples containing less than 0.1% vanadium or molybdenum.

(*b*) *Fusion decomposition.* Fuse 0.4 g of sample in a zirconium or vitreous carbon crucible with 1.3 g of sodium carbonate and 2.7 g of sodium peroxide, in a muffle furnace at 700° until decomposed. Place the cold crucible in a 400 ml beaker, add about 20 ml of water to the crucible and cover the beaker with a watch-glass. After the reaction has ceased, wash the contents of the crucible into the beaker. If the sample contains more than 0.1% of vanadium and/or molybdenum, filter the alkaline solution and dissolve the iron hydroxide off the filter with hydrochloric acid. If the sample contains less than 0.1% vanadium and/or molybdenum, carefully add 20 ml of concentrated hydrochloric acid to the beaker and mix to dissolve the ferric hydroxide. Rinse the crucible with hot hydrochloric acid and add the washings to the beaker. Evaporate the solution to about 50 ml. For more details see reference 6.

Reduction of iron (III)

Add to the sample solution 1 ml of potassium permanganate solution and boil for about a minute. To the hot solution add 10% stannous chloride solution dropwise until only a light yellow colour remains. It is essential that some iron(III) is left unreduced. If all the iron is inadvertently reduced, reoxidize a little with a drop of permanganate solution.

Reduce the remaining iron(III) by adding 2% titanous chloride solution dropwise until the solution is colourless, then add an additional 3–5 drops.

Rinse the walls of the beaker with water and heat to near boiling. Remove from the source of heat and immediately add—all at once—5 ml of perchloric acid (1 + 1). Mix well by swirling for 5 sec. Cool rapidly in ice-water. Rinse the cover and the walls of the beaker. Add 25 ml of phosphoric acid–sulphuric acid mixture and 0.25 ml of sodium diphenylaminesulphonate solution.

The volume at this stage should be between 100 and 125 ml. Titrate with 0.1*N* potassium dichromate to a deep violet.

Determine the blank, using the same procedure and the same amounts of all reagents used in analysis of the sample, but in the reduction step use only 3–5 drops of the titanous chloride solution. To the cold solution thus prepared add 1.0 ml of 0.1*N* iron(II) solution. Titrate with the 0.1*N* dichromate solution (B_2 ml). In another 400-ml beaker place the same volume of hydrochloric acid (1 + 9) as the volume of the blank solution. Add 1.0 ml of 0.1*N* iron(II) solution, 25 ml of the acid mixture and 0.25 ml of indicator solution. Titrate with the 0.1*N* dichromate solution (B_1 ml). The difference between B_2 and B_1 is the blank value for the reagents.

Calculation of iron content

$$\text{Fe}\,\% = \frac{(V + B_1 - B_2)f}{m} \times 0.005585 \times 100$$

where V ml is the volume of $f \times 0.1N$ dichromate required for titration of the iron in m g of sample.

DISCUSSION

Oxidation of excess of $TiCl_3$ with perchloric acid

Six iron solutions were prepared by decomposition of 0.4000-g portions of NBS No. 690 (66.85% Fe), three by acid decomposition and three by fusion, as outlined in the procedure. The iron(III) was partially reduced with stannous chloride, then titanous chloride solution was added in slight excess.

To test the rate and completeness of the oxidation of titanium(III) by perchloric acid, the excess of titanous chloride solution added was varied from 1 drop to 2 ml. In all six cases, the volume of 0.1*N* potassium dichromate solution required to oxidize the iron was identical (within ±0.1 ml) with that needed for control titrations of solutions obtained by using stannous chloride as sole reductant and mercuric chloride as oxidant for the excess of stannous chloride.[2] This indicates that titanium(III) is efficiently oxidized by 5 ml of perchloric acid (1 + 1). It was further found that this oxidation is almost instantaneous if the perchloric acid is added to the solution at or near the boiling point. At 75° the oxidation is more sluggish. Rapid oxidation of the titanium(III) is important, since the subsequent titration of the iron(II) with potassium dichromate should be carried out expeditiously.

Stability of the iron(II) solution

After the oxidation of the titanium(III) with perchloric acid, the hot solution containing the iron(II) is rapidly cooled. During initial tests, the solutions were cooled under a blanket of carbon dioxide generated by the addition of a saturated sodium bicarbonate solution.

When the cooling and standing period was varied from 4 to 25 min, no change in the volume of titrant needed was noted, thus indicating that the iron(II) was stable under the experimental conditions used. When the addition of bicarbonate was omitted, a slight degree of aerial oxidation of the iron(II) was noted after half an hour of standing, amounting to about 0.05–0.1 ml (total titration ~47 ml) of 0.1*N* potassium dichromate solution.

Verification of procedure

The proposed method was applied to the determination of iron in a variety of iron ores of known composition. The results, along with those obtained by the conventional stannous chloride–mercuric chloride method, are presented in Table 1. The results

Table 1. Determination of iron in standard iron ore samples

Sample	Certified Fe value, %	Iron found, % $SnCl_2$–$HgCl_2$ procedure	Iron found, % $SnCl_2$–$TiCl_3$–$HClO_4$ procedure
NBS-690*	66.85	66.79	66.80
(Canada)		66,76	66.83
NBS-692*	59.58	59.57	59.58
(Labrador)			59.65
NBS-693*	65.11	65.16	65.17
(Nimba)			65.07
NBS-692†	59.58	59.53	59.53
		59.56	59.51
NBS-693†	65.11	65.11	64.98
		65.00	64.94
NBS-27b†	68.23	68.07	67.91
(Sibley)		68.03	68.06
Savage River†	67.2§	66.93	66.95
		67.10	67.12

*Acid decomposition.
†Fusion.
§Suggested value.

Table 2. Results obtained by U.S. Steel Corp.

Sample	Certificate Fe value %	Iron found %
U.S.S.		65.96
Q.C.M. No. 8	66.11	66.23
NBS-690	66.85	66.73
		66.73
NBS-270	64.96	64.86
		65.18
NBS-27E	66.58	66.31
		66.31
NBS-27C	65.00	64.96
		64.95
German Std.	36.21	36.21
629-1		36.03
German Std.	60.78	60.90
632-1		60.72
BSC-301	24.70	24.71
		24.82
NBS-692	59.58	59.61
		59.58
		59.35
	By ISO Method	
Canadian Std.	65.73	65.83
being certified	66.00	65.95
(Bottle 402)	66.15	65.87
		65.84

of a previous round-robin testing programme[2] indicated that the relative standard deviation of the stannous chloride procedure is approximately 0.15%.

The method was applied by U.S. Steel, Monroeville, Pa., to the determination of iron in a number of iron ore samples, with use of the fusion technique. Mr. J. Selvaggio of U.S. Steel obtained the results in Table 2.

Table 3. Results obtained by Andrew S. McCreath & Sons, Inc.

Fe % $TiCl_3$ procedure	Fe % $SnCl_2$ procedure	Fe % $TiCl_3$ procedure	Fe % $SnCl_2$ procedure
66.16	66.03	63.88	63.85
64.63	64.63	61.87	61.93
66.20	66.21	65.96	66.03
64.66	64.62	67.37	67.36
68.52	68.55	64.90	64.77
65.96	65.97	64.58	64.61
65.99	65.97	65.99	65.96
64.66	64.62	13.15	13.17
65.96	65.97	2.95	2.91
68.99	68.92	3.27	3.35

The method was also examined by Andrew S. McCreath & Sons, Inc., Harrisburg, Pa. Mr. F. A. Pennington, Jr. submitted the set of averages of duplicate results in Table 3 for a variety of iron ores (the acid-decomposition procedure was used).

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DETERMINATION OF THIURAM DISULPHIDES AND ANALYSIS OF THIURAM DISULPHIDE–DITHIOCARBAMATE MIXTURES

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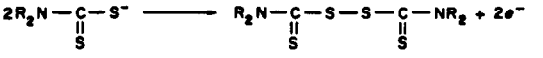
Summary—Potassium trithiocarbonate is used as reductant for the titrimetric determination of thiuram disulphides in dimethylformamide–water media. The method is based on reductive cleavage of the disulphide linkage to yield the corresponding dithiocarbamates. In visual titrations, the end-point is marked by appearance of a yellow colour with the first drop of reagent added in excess. The method is simple, accurate, reliable and of wide applicability. It has been successfully extended to the analysis, in the same sample solution, of thiuram disulphide–dithiocarbamate mixtures.

Thiuram disulphides (II) are the products of mild oxidation of dithiocarbamates (I).



(I) (II)

The lower tetra-alkyl thiuram disulphides are among the most important thiocarbonyl compounds. They are used in pesticide formulations, most often as fungicides. They also find applications as accelerators in rubber vulcanization. These compounds also possess biological activity.

The methods commonly used for determination of thiuram disulphides are based on their acid hydrolysis, or abstraction of a sulphur atom from the disulphide linkage, or reduction to dithiocarbamates.

Acid hydrolysis, leading to formation of the parent amine and evolution of carbon disulphide is the key reaction used for the analysis of thiuram disulphides,[1] but the methods are time-consuming and require special apparatus. Moreover, the stoichiometry of the reaction is not well established.

The sulphur abstraction methods are based on the reaction of thiuram disulphides with potassium cyanide.

(III)

The thiocyanate formed is determined either by titration with silver nitrate[2,3] or by oxidation with bromine to cyanogen bromide, which is determined iodometrically.[4] However, the reaction mixture must be shaken for 30 min to ensure completion of the abstraction reaction, and the thiuram monosulphide (III) must be removed (by extraction with benzene) before the thiocyanate is determined.

Reduction of thiuram disulphides to the corresponding dithiocarbamates is obviously an important approach to the analysis of these compounds. It opens up two pathways for the analysis, one involving reduction followed by determination of the resulting dithiocarbamates, and the other involving direct titration with a suitable reductant. Efforts[5–8] have been made to use the first pathway, but the second appears to have attracted little attention. Scheele and Gensch[9] have described conductometric titrations of tetramethylthiuram disulphide with copper sulphate after reduction with hydroquinone. Lowen[10] attempted to determine tetramethylthiuram disulphide in ferric dimethyldithiocarbamate by potentiometric titration with sodium sulphite as reductant. Potassium fluoride was added to prevent interference from iron(III) during the titration, and a nitrogen atmosphere was used to avoid any oxidation of dithiocarbamate during the titration.

The present communication reports for the first time the use of potassium trithiocarbonate as reductant for the direct titrimetric determination of

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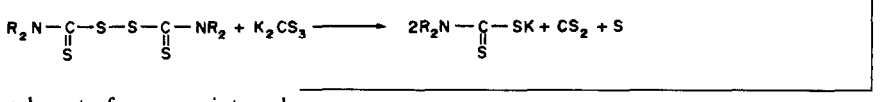
thiuram disulphides, the dithiocarbamates being formed



Since potassium trithiocarbonate forms an intensely yellow solution, the end-point can be detected by appearance of the yellow colour. The equivalence point in potentiometric titrations is marked by a sharp drop in potential. The method can be extended to the analysis, in the same sample solution, of mixtures of thiuram disulphide and dithiocarbamate. The mixture (in dimethylformamide–water medium, in which this group of compounds is readily soluble) is first titrated with potassium trithiocarbonate to determine the thiuram disulphide, and then further titrated with iodine. The solution becomes colourless immediately on addition of the first drop of iodine, and the end-point is signalled by appearance of a yellow colour (due to iodine) in visual titrations, or a sharp jump in potential in potentiometric titrations. The iodimetric titration corresponds to the total amount of dithiocarbamate (the original amount plus that formed in the first titration). The amount of dithiocarbamate originally present is found by difference.

The methods are simple, rapid, accurate and reliable, and have wide applicability.

EXPERIMENTAL

Reagents

Potassium trithiocarbonate, 0.01M. The compound was prepared[11] by saturating an alcoholic solution of potassium hydroxide with hydrogen sulphide and adding an absolute alcohol solution of carbon disulphide, and was collected by filtration and kept in a desiccator. The solution was prepared by dissolving a little more than the calculated amount in doubly distilled water, and standardized iodimetrically, with starch as indicator. A 0.025*M* solution, when stored in tightly closed amber-coloured bottles, retains its titre for about a week.

Iodine, 0.02N. Prepared and standardized as usual.

Thiuram disulphides. The tetramethyl, tetraethyl, tetra-n-propyl, tetraisopropyl and tetrabenzyl compounds were prepared[12] from the respective dithiocarbamates through reaction with iodine, and recrystallized from suitable solvents.

Dithiocarbamates. Sodium diethyldithiocarbamate was recrystallized before use. The sodium dimethyl, potassium di-n-propyl, potassium di-isopropyl and potassium dibenzyl dithiocarbamates were prepared and purified as described earlier,[13] and checked by known methods.

Procedures

Determination of thiuram disulphides. Aliquots of dimethylformamide solutions of each thiuram disulphide were taken in titration vessels, diluted to 35 ml with dimethylformamide and mixed with 15 ml of water, then titrated at room temperature (~25°) visually or potentiometrically with 0.01*M* potassium trithiocarbonate from a microburette till appearance of a yellow colour and/or a sharp drop in potential. The results are recorded in Table 1.

Analysis of mixtures of thiuram disulphide and dithiocarbamate. Aliquots of dimethylformamide solutions of synthetic mixtures containing different ratios of dithiocarbamate and thiuram disulphide were taken in titration vessels and treated as described above for determination of the thiuram disulphide. The titrated solution was then further titrated with 0.02*N* iodine. The solution became colourless immediately on addition of the first drop of iodine solution, and yellow again at the end-point. The end-point was also located potentiometrically (sharp rise in potential). The difference between the amounts of trithiocarbonate and iodine consumed gave the amount of dithiocarbamate in the mixture. The results are recorded in Table 2. In the potentiometric titrations, however, since it is necessary to titrate with trithiocarbonate to beyond the first equivalence point, the surplus trithiocarbonate added must be calculated, and an equivalent volume of iodine deducted from the second titration value, because the excess of trithiocarbonate is also oxidized by iodine.

RESULTS AND DISCUSSION

Potassium trithiocarbonate, on mild oxidation, yields carbon disulphide and sulphur.[14]

$$K_2CS_3 \rightarrow 2K^+ + CS_2 + S + 2e^-$$

It is this reducing property which is responsible for the reductive cleavage of the disulphide linkage of thiuram disulphides. The proposed method, besides being direct, convenient, accurate and reliable, possesses the following advantages.

(*i*) No additional indicator is needed.

(*ii*) The reduction is smooth and rapid at room temperature.

(*iii*) The reaction products do not interfere.

(*iv*) The reagent is easily prepared, and its solution, if stored properly, shows good stability.

(*v*) The potential stabilizes immediately on addition of each increment of reagent and the inflection point is marked by a sharp drop in potential.

The results in Table 1 show that the overall standard deviations calculated from the pooled data for the visual and potentiometric titrations were 0.037 and 0.034 mg respectively. For 10 mg of each compound, the values were 0.063 and 0.060 mg respectively. In the potentiometric titrations, a sharp drop in potential by about 100 mV for addition of 0.05 ml of 0.01*M* reagent was observed at the equivalence point.

The method has been successfully extended to the analysis of mixtures of thiuram disulphide and dithiocarbamate. Since thiuram disulphides are prepared through the oxidation of dithiocarbamates, such mixtures may be commonly encountered. Synthetic mixtures of tetramethylthiuram disulphide and sodium dimethyldithiocarbamate with ratios from 1:4 to 4:1 were analysed, with relative standard deviations of 1% for both components (Table 2) and gave similar precision and accuracy for mixtures of other thiuram disulphides with their parent dithiocarbamates.

Table 1. Determination of thiuram disulphides with potassium trithiocarbonate

Thiuram disulphide	Amount found,*† *mg*		Amount found,†§ *mg*	
	Visual method	Pentiometric method	Visual method	Potentiometric method
Tetramethyl	4.04 (0.03_1)	4.01 (0.02_6)	10.10 (0.06_8)	9.96 (0.06_4)
Tetraethyl	4.02 (0.02_9)	3.98 (0.03_2)	10.06 (0.05_6)	10.04 (0.06_1)
Tetra-n-propyl	3.96 (0.05_1)	3.97 (0.03_7)	9.94 (0.06_2)	10.02 (0.05_3)
Tetraisopropyl	3.98 (0.03_6)	4.02 (0.03_5)	10.04 (0.05_4)	9.95 (0.06_0)
Tetrabenzyl	4.05 (0.03_7)	4.03 (0.04_2)	9.96 (0.07_9)	10.07 (0.06_6)

*Amount taken, 4 mg.
†Mean of 10 results, standard deviation in brackets.
§Amount taken, 10 mg.

Table 2. Determination of sodium dimethyldithiocarbamate and tetramethyl thiuram disulphide

Sodium dimethyldithiocarbamate		Tetramethylthiuram disulphide	
Taken, *mg*	Found,* *mg*	Taken, *mg*	Found,* *mg*
3.00	3.02 (0.03_2)	3.00	3.04 (0.03_5)
3.00	3.04 (0.03_4)	6.00	5.96 (0.06_7)
3.00	3.01 (0.04_1)	9.00	9.06 (0.06_2)
3.00	2.97 (0.03_9)	12.00	12.08 (0.09_1)
6.00	5.92 (0.04_8)	3.00	3.03 (0.03_0)
9.00	8.93 (0.07_0)	3.00	3.01 (0.04_3)
12.00	12.10 (0.06_8)	3.00	2.97 (0.03_8)

*Mean of 10 determinations, standard deviation in brackets.

The proposed method for the analysis of mixtures, besides being simple and accurate, has the added advantage that the same sample solution is used, resulting in saving of time and effort. The potentiometric method is useful when the sample solution is coloured, or for very dilute solutions.

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ANALYTICAL DATA

A PHOTOMETRIC STUDY OF THE COMPLEXATION REACTION BETWEEN ALIZARIN COMPLEXAN AND ZINC(II), NICKEL(II), LEAD(II), COBALT(II) AND COPPER(II)

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Summary—The complexation reaction between Alizarin complexan ([3-*N*,*N*-di(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone; H_4L) and zinc(II), nickel(II), lead(II), cobalt(II) and copper(II) has been studied by a spectrophotometric method. All these metal ions form 1:1 complexes with HL; 2:1 metal:ligand complex were found only for Pb(II) and Cu(II). The stability constants are (ionic strength $I = 0.1$, 20°C):

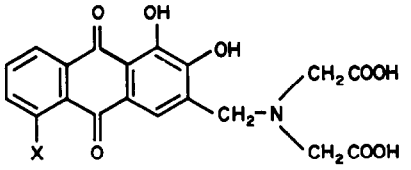
$$Zn^{2+} + HL^{3-} \rightleftharpoons ZnHL^- \quad \log K \pm 3\sigma(\log K) = 12.19 \pm 0.09 \ (I = 0.5)$$
$$Ni^{2+} + HL^{3-} \rightleftharpoons NiHL^- \quad \log K \pm 3\sigma(\log K) = 12.23 \pm 0.21$$
$$Pb^{2+} + HL^{3-} \rightleftharpoons PbHL^- \quad \log K \pm 3\sigma(\log K) = 11.69 \pm 0.06$$
$$PbHL^- + Pb^{2+} \rightleftharpoons Pb_2L + H^+ \quad \log K \approx -0.8$$
$$Co^{2+} + HL^{3-} \rightleftharpoons CoHL^- \quad \log K \pm 3\sigma(\log K) = 12.25 + 0.13$$
$$Cu^{2+} + HL^{3-} \rightleftharpoons CuHL^- \quad \log K \pm 3\sigma(\log K) = 14.75 \pm 0.07$$
$$Cu^{2+} + CuHL^- \rightleftharpoons Cu_2L + H^+ \quad \log K \approx 3.5$$

The solubility and stability of both the reagent and the complexes and the closeness of the values of the stability constants make this reagent suitable for the photometric detection of several metal ions in the eluate from an ion-exchange column.

Two quite different kinds of philosophy may be applied to the choice of reagents used in photometric analysis. By far the most common one is that the reagent should be as selective as possible for the analyte, to allow interference-free determinations.

However, a fairly non-selective reagent can be given a desired selectivity by use of masking agents, and/or pH-control, generally along with extraction of the coloured species formed; the most common reagents used in this way are probably dithizone and oxine. The same reagent may then be used for the determination of many different metal ions. The role played by the reagent is in this case similar to the role of EDTA in complexometry.

Few chromogenic reagents in aqueous solution display selectivity properties similar to those of EDTA. Such a reagent would have to be a chelating agent of structure not far removed from that of EDTA. Examples are Alizarin complexan (Alizarin Fluorine Blue, [3-*N*,*N*-di(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone, AFB)[1] and sulphonated Alizarin complexan (AFBS, [3-*N*,*N*-di(carboxymethyl)-aminomethyl]-1,2-dihydroxyanthraquinone-5-sulphonic acid).[2]



AFB: X = H
AFBS: X = $SO_3^-K^+$

The main use of these two reagents results from their lanthanum or cerium salts giving the only known positive colour reactions with fluoride ions in aqueous solution, and their use as reagents for fluoride is by now well established. However, they form complexes with many other bi- and tervalent metal ions, and could thus be used for selective photometric determination of these ions, *e.g.*, aluminium(III) in the presence of iron(III).[3]

As is well known from complexometry, equilibrium

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calculations are an extremely valuable aid in working out analytical procedures. If all the relevant stability constants are known, the calculations can be done either by the simple concept of conditional constants and side-reaction coefficients introduced by Ringbom[4] or by computer methods such as the HALTAFALL program.[5]

The aim of this work was to provide more data on the equilibria of Alizarin complexan with cobalt(II), copper(II), nickel(II), lead(II) and zinc(II) in order to facilitate such equilibrium calculations. During the course of the work we also studied the influence of several common buffer substances, to exclude the possibility that ternary complexes are formed.

METHOD

The equilibria were studied in 0.1*M* or 0.5*M* sodium nitrate medium spectrophotometrically. Since the complexes are rather stable it was necessary to use competitive complexing agents to make the reaction with Alizarin complexan incomplete. The stability constants of the metal complexes with Alizarin complexan were then evaluated by using Ringbom's concepts,[4] after determination of the conditional constants by plotting A against $(A_L - A)/[M']$, where, A_L is the absorbance of a solution containing only uncomplexed dye at total concentration c_L, A is the absorbance of a solution containing a mixture of dye and complex at a total dye concentration c_L, and $[M']$ is the concentration of metal ions that have *not* reacted with the dye according to the main reaction. The conditional stability constant K' is then obtained as the reciprocal of the slope of the plot.

EXPERIMENTAL

Reagents

Alizarin complexan was purified as described earlier.[6] All other chemicals were of reagent grade. Distilled water was used throughout.

Procedure

Sets of solutions containing equal concentrations of the dye but increasing concentrations of the metal ion in question were buffered to an appropriate pH and the ionic strength was adjusted to 0.1*M* (except for Zn^{2+}, where the ionic strength was 0.5*M*) with sodium nitrate. Complete spectra for the wavelength range 350–700 nm were recorded with a Varian–Cary model 219 recording spectrophotometer. In addition, some of the continuous-variations measurements were made at two different wavelengths in order to ascertain that only one complex was being formed in the solutions. All experiments were done at 20 ± 1°. About ten data points were used for each determination of a stability constant. As an example, a complete set of data is shown in Table 1.

RESULTS

Influence of reagents and buffer substances

Boric acid is known to form a complex with Alizarin complexan.[6] To decide whether any other reagents used as auxiliary complexing agents or buffer substances would influence the absorbance of the dye we made a rough check of the influence of phthalic acid, acetic acid, EGTA [ethyleneglycol-bis(2-aminoethyl ether)tetra-acetic acid] and NTA (nitrilotriacetic acid) at various concentrations up to about 0.1*M*. The spectra recorded showed that, though there may be an influence at very high concentration levels, it is so small as to be within the experimental error at the concentrations and pH ranges used here (pH 5–6).

Stability of the metal complexes

Zinc(II). The continuous-variations investigation confirmed the suggestion by Leonard and West[7] that zinc(II) forms a 1:1 complex with the dye. One of the protons of Alizarin complexan (H_4L) does not take part in the complexation (this is supported by the bathochronic shift occurring on complexation). The reaction may thus be written:

$$Zn^{2+} + HL^{3-} \rightleftharpoons ZnHL^{-}$$

We determined the stability constant for this complex by using thioglycollic acid (TGA) as auxiliary complexing agent. The stability constants for the Zn–TGA complexes and the acid constants of TGA were taken from the work of Anderegg and Malik,[8] recalculated for 0.5*M* ionic strength by use of Debye–Hückel theory. Absorbances were measured at 490 nm.

Table 1 shows the results of the six determinations that were made.

No tendency for zinc(II) to form polynuclear com-

Table 1. Results for determination of the stability constant of the Zn–AFB complex (I = 0.5); each determination consisted of about 10 experimental points with zinc concentrations that varied within the range given

c_{Zn}, *mM*	c_{TGA}, *M*	c_L, *M*	pH	log K'	log $\alpha_{Zn}\alpha_{HL}$	log K_{ZnHL}	No. of points
0.1–15.0	0.426	1.5×10^{-4}	4.2	2.89	9.33	12.22	10
0.1–15.0	0.426	1.5×10^{-4}	4.3	2.90	9.34	12.24	10
0.1–10.0	0.284	1.5×10^{-4}	4.1	3.16	9.02	12.18	9
0.1–10.0	0.284	1.5×10^{-4}	4.3	3.16	9.02	12.18	9
0.1–10.0	0.284	1.0×10^{-4}	4.35	3.13	9.02	12.15	8
0.1–10.0	0.426	1.0×10^{-4}	4.3	2.85	9.33	12.18	8
					log $K \pm 3\sigma(\log K)$ =	12.19 ± 0.09	

Table 2. Results for the determination of the stability constant (I = 0.1) of the Ni–AFB complex (c_L 1.0 × $10^{-4}M$)

c_{Ni}, mM	c_{EGTA}, M	pH	log K'	log $\alpha_{Ni}\alpha_{HL}$	log K_{NiHL}
0.1–10.0	0.010	4.55 ± 0.05	3.64	8.66	12.30
0.1–10.0	0.020	4.35 ± 0.03	3.11	9.14	12.25
0.1–10.0	0.010	4.02 ± 0.03	3.18	9.14	12.32
0.1–5.0	0.010	4.81 ± 0.02	3.42	8.76	12.18
0.1–8.0	0.010	5.00 ± 0.02	3.84	8.31	12.15
0.1–8.0	0.020	5.00 ± 0.03	3.45	8.61	12.06
0.1–10.0	0.020	4.02 ± 0.03	2.82	9.45	12.27
				log $K \pm 3\sigma(\log K)$ =	12.23 ± 0.21

plexes was observed at the concentrations studied. Although the pH range that could be studied was rather narrow, the results seem to confirm that the complexation takes place according to the equation given.

Nickel(II). According to Leonard and West,[7] Ni(II) forms a 1:1 complex with Alizarin complexan, and this was confirmed by a continuous variations plot. The auxiliary complexing agent in this case was EGTA. Ringbom and Saariaho's stability constants[4] were used in the calculations. The data were best explained by the reaction

$$Ni^{2+} + HL^{3-} \rightleftharpoons NiHL^{-}$$

and there was no indication of the formation of other species in the solution within the ranges of pH and concentration studied. The measurements were made at 505 nm, since at this wavelength the absorptivities of both Ni^{2+} and the Ni^{2+}–EGTA complex are zero, whereas the absorptivity of the Ni^{2+}-AFB complex is at its maximum. The results are given in Table 2.

Lead(II). Two different species are formed when lead(II) is added to a solution containing AFB. With the dye in excess, only a 1:1 species is formed, and it can be described as PbHL. At higher lead(II) concentrations, a precipitate is formed. The data may be reasonably well explained by assuming that the precipitate is the uncharged Pb_2L. Moreover, the spectrum of the colloidal solution resembles that of the completely deprotonated dye (Fig. 1), and this seems to support the suggested composition. The auxiliary complexing agent was EGTA, and the stability constants determined by Ringbom and Saariaho[4] were used. The results of the five determinations of the stability constant for the formation of the first complex are given in Table 3, which also shows the pH and concentration ranges employed. The wavelength was 490 nm.

In analytical applications, the dye is always in excess, so the binuclear complex will not normally be formed, except perhaps in an interfering side reaction. We therefore made only a rough determination at

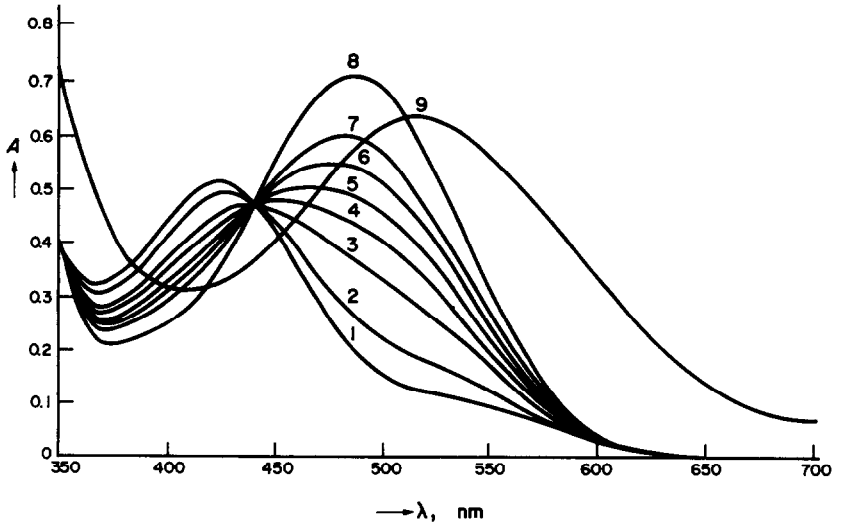

Fig. 1. Absorption spectra of solutions with constant Alizarin complexan concentration, 0.100 mM, and various Pb^{2+} concentrations: 1, 0M; 2, 0.100 mM; 3, 0.300 mM; 4, 0.500 mM; 5, 0.700 mM; 6, 1.00 mM; 7, 1.50 mM; 8, 5.00 mM; 9, 4.00 mM. All solutions except no. 9 had an EGTA concentration of 5 mM. The ionic strength was 0.100M ($NaNO_3$) and the pH was 4.8 (acetate buffer 0.05M).

Table 3. Results of the determination of the stability constant (I = 0.1) of the 1:1 Pb–AFB complex (c_L 1.0 × $10^{-4}M$)

c_{Pb}, mM	c_{EGTA}, M	pH	log K'	log $\alpha_{Pb}\alpha_{HL}$	log K_{PbHL}
0.1–10.0	0.010	4.5	2.64	9.06	11.70
0.1–10.0	0.010	5.0	2.93	8.75	11.68
0.1–10.0	0.020	5.0	2.61	9.05	11.66
0.1–5.0	0.0050	4.2	2.65	9.04	11.69
0.1–5.0	0.0050	4.8	3.16	8.56	11.72
				log $K \pm 3\sigma(\log K)$ =	11.69 ± 0.06

630 nm of the stability constant for the reaction

$$PbHL + Pb \rightleftharpoons Pb_2L + H^+ \quad \log K \approx -0.8.$$

Cobalt(II). Spectra for the system cobalt(II)–AFB–EGTA in acetic acid–acetate buffer were recorded and show that only one complex species is formed. The ratio of metal ion to dye is 1:1 and the data fit the model

$$Co^{2+} + HL^{3-} \rightleftharpoons CoHL^-.$$

EGTA was used as auxiliary complexing agent, and Anderegg's constants[9] were used in our calculations. The maximum absorbances were measured at 490 nm. Six determinations were made, and the results are shown in Table 4.

One of the determinations (the third listed) was made at I = 0.2M and the constant was recalculated to I = 0.1M by using the Debye–Hückel equation.

Copper(II). Copper(II), like lead(II), seems to form two complexes with Alizarin complexan. The second complex is formed in solutions containing excess of copper(II) but, unlike the corresponding lead(II) complex, the copper complex is soluble. However, no further study of this complex was undertaken, since Alizarin complexan will always be present in excess in analytical applications. At first, EGTA was used as auxiliary complexing agent, yielding the first two values for the stability constant shown in Table 5, but the copper(II)–EGTA complex was too strong to allow Alizarin complexan to compete very effectively, so the experiments were continued with NTA as auxiliary complexing agent. Absorbances were measured at 480 nm. As can be seen from Table 5, the results show a slight difference, according to which auxiliary complexing agent is used. This is to be expected since the stability constants of the Cu–

Table 4. Results of the determination of the stability constant of the Co(II)–AFB complex (c_L 1.0 × $10^{-4}M$)

c_{Co}, mM	c_{EGTA}, M	pH	I	log K'	log $\alpha_{Co}\alpha_{HL}$	log K_{CoHL} I = 0.1	I = 0.2
0.1–1.0	0.020	4.4	0.1	3.46	8.76	12.22	
0.1–1.0	0.020	4.95	0.1	3.85	8.42	12.27	
0.1–1.0	0.040	4.73	0.2	3.38	8.60	(12.19)	← 11.98
0.1–1.0	0.010	5.0	0.1	4.22	8.10	12.32	
0.1–1.0	0.020	4.2	0.1	3.34	8.94	12.28	
0.1–1.0	0.020	4.55	0.1	3.56	8.65	12.21	
					log $K \pm 3\sigma(\log K)$ =	12.25 ± 0.13	

Table 5. Results of the determinations of the stability constant (I = 0.1) of the 1:1 Cu(II)–AFB complex (c_L 1.0 × $10^{-4}M$)

c_{Cu}, mM	c_{EGTA}, M	c_{NTA}, M	pH	log K'	log $\alpha_{Cu}\alpha_{HL}$	log K_{CuHL}
0.5–5.0	0.0050		4.07 ± 0.02	1.43	13.20	(14.63)
0.5–5.0	0.0050		4.95 ± 0.02	1.74	12.93	(14.67)
0.1–10.0		0.010	4.97 ± 0.03	3.13	11.65	14.78
0.1–10.0		0.010	4.37 ± 0.03	2.58	12.16	14.74
0.1–10.0		0.010	4.00 ± 0.03	2.20	12.52	14.72
0.1–5.0		0.0050	4.75 ± 0.03	3.22	11.53	14.75
0.1–5.0		0.0050	4.23 ± 0.03	2.76	12.01	14.77
					log $K \pm 3\sigma(\log K)$ =	14.75 ± 0.07

EGTA and the Cu–NTA complexes were determined by different workers by slightly different methods.[9] For EGTA, Anderegg's values[9] were used, and for NTA the values determined by Ringbom and Saariaho.[4]

The results lead us to conclude that copper forms a complex with Alizarin complexan according to

$$Cu^{2+} + HL^{3-} \rightleftharpoons CuHL^{-} \quad \log K = 14.75$$

We determined the order of magnitude of the stability constant for the reaction

$$CuHL^{-} + Cu^{2+} \rightleftharpoons Cu_2L + H^{+} \quad \log K \approx -3.5$$

DISCUSSION

The results presented support the statement in the introduction about the simplicity of Alizarin complexan chemistry. Moreover, the values for the stability constants show that there is potential for use of AFB as a reagent for the detection of several metal ions, *e.g.*, in the eluate from an ion-exchange column. Other workers have used pyridylazo compounds such as PAR for this purpose.[10] In comparison with such reagents, AFB has the advantages of being relatively soluble in water and stable in aqueous solution. Also, the sensitivities of the reactions with different metal ions are similar, which simplifies the calibration procedure. Work is therefore in progress at this laboratory to study the utility of AFB as a reagent in an ion-chromatography detection system.

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ANNOTATIONS

AN EVALUATION OF CELLULOSE AS A SUBSTRATE FOR ROOM-TEMPERATURE PHOSPHORESCENCE*

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Summary—Cotton-linter pulps, wood pulps and several filter papers have been evaluated as substrates for room-temperature phosphorescence. A variety of chemical treatments of one filter paper is discussed in terms of reducing the background phosphorescence of the cellulose and in evaluating possible trace contaminants in cotton fibres. In order to account for uniformity of filter paper used in room temperature phosphorescence, a final evaluation of several different lots of one type of filter paper is presented.

Though room-temperature phosphorescence (RTP) has received considerable attention in drug and polynuclear aromatic hydrocarbon analysis during the past decade, the ultimate success of the method depends on selection of a suitable support material. In recent years, supports such as cellulose, silica gel and sodium acetate have been used in RTP measurements of a variety of organic compounds.[1-3] Of the three major support materials, cellulose appears to offer a considerable advantage because of the wide range of special papers with varied characteristics. The major disadvantage of cellulose is the presence of a broad-band phosphorescence background (at ~400–600 nm).[4-6] We have tested several types of filter paper, but our attempts to minimize the phosphorescence background of the papers by physical and chemical treatments have been unsuccessful.[7,8] In the present study,† several cellulose pulps have been examined as supports to determine whether there is a successful combination of physical/chemical characteristics of cellulose pulp for RTP; treated filter paper has also been examined to find a possible source of the background phosphorescence.

EXPERIMENTAL

Apparatus

All RTP measurements were made with an Aminco-Bowman spectrophotofluorimeter fitted with a Canrad–Hanovia 150-W xenon arc lamp, a laboratory-constructed phosphoroscope[9] for bar-RTP,[10] and a potted Hamamatsu 1P21 photomultiplier tube. An Aminco ratio photometer supplied high voltage to the photomultiplier tube in addition to serving as a d.c. amplifier. All line voltages were regulated with a Sorenson 1001 a.c. regulator.

Reagents and materials

The following companies kindly provided the materials mentioned: Buckeye Cellulose (grade 503 cotton-linter pulp); ITT Rayonier (Cellunier-P wood pulp); Southern Cellulose (grades 270, 277, and 282-R cotton-linter pulps); Eaton-Dikeman (613 and 631 filter papers); Schleicher & Schuell (903 filter paper, lots W01, W02, W12, W92, W93, W94). Diethylenetriaminepenta-acetic acid (DPTA) (Sigma Chemical Co.) was used without further purification. All other reagents were of analytical grade.

Procedure

Following the selected treatments (Table 1), the sheets of S & S 903 were allowed to air-dry in a photographic darkroom for 12 hr. Next, 0.25-in. diameter paper discs obtained with a standard office paper punch were placed under the cover plate of the bar, and the plate was screwed down onto the discs. With a "Micropettor" (Scientific Manufacturing Industries), 5 μl of blank (1*M* potassium iodide/1*M* sodium hydroxide in 50/50 v/v ethanol/water) or 5 μl of analyte [50-ppm *p*-aminobenzoic acid (PABA) in 50/50 v/v ethanol/water] were spotted onto the paper discs. The bar was then placed in the sample compartment where the discs were allowed to dry for 7 min in a flow of dry nitrogen. For each evaluation, 8 or 16 independent measurements were made for both blank ($\lambda_{ex}/\lambda_{em}$, 320/475 nm) and PABA ($\lambda_{ex}/\lambda_{em}$, 296/432 nm). The blank excitation and emission wavelengths were chosen to maximize the signals (which behaved like the substrate background phosphorescence).[6] Blank signals were ~40% lower at the wavelengths used for the PABA.

For the "lot"-analysis (Table 4) of S & S 903 filter paper, all "lots" were soaked in DTPA solution for 24 hr, rinsed for 3 min in water, and allowed to air-dry for 12 hr in a photographic darkroom.

For the handsheet evaluation (Table 2), two handsheets (basis weight 190 g/m² ± 3%, thickness 0.5 mm ± 5%) were made from each source of cellulose pulp. The first

*This research was supported by NIH grant GM-11373-19 and by University of Florida Biomedical Research Support Grant.

†Part of this study contains proprietary information and at the request of the participating companies, no specific details of the manufacture process can be given with respect to each grade of cellulose. For the reader's convenience, an appendix is included to explain terms (used in this article) common to the pulp and paper industry but otherwise unfamiliar to the layman.

Table 1. Summary and results of treatment of S & S 903

Nature of treatment	Time of treatment, *hr*	Change in mean relative signal* Blank	PABA
Dioxan/water (50/50) soak	48	−2	+2
DTPA/water (saturated) soak	48	−1	+2
Ether soak	24	−1	0
Boiling water soak	0.5	0	0
Eimac lamp (250-W) bleach	12	0	−2
Sunlight bleach	24	+2	−1
Sodium hydroxide (3.5*M*, 5°C)	24	0	−1
Periodic acid (0.1*M*) soak	12	†	†

*Mean given for 8 determinations; change in signal relative to untreated S & S 903 (1980 W94 stock).
−2 signal decreased by ≈100%.
−1 signal decreased by ≈50%.
0 no change in signal.
+1 signal increased by ≈50%.
+2 signal increased by ≈100%.
†no net signal observed.

handsheet was made from a sample of pulp as received from the processing plant. The second was made from a pulp sample that had been mechanically beaten for 1 hr.

The sampling procedure for the "lot"-analysis and handsheet evaluation followed the sequence described above.

RESULTS AND DISCUSSION

Background on papermaking

To understand the results obtained, it is necessary to have a basic knowledge of the papermaking process and more specifically, a knowledge of the types of cellulose used in making filter-paper products. A detailed description of papermaking is beyond the scope of this article, but is readily available,[11–14] and so is up to date information on the procedures used in paper analysis.[15–17]

Generally speaking, purified cellulose can be obtained from two major sources—cotton and wood. Wood is comprised mainly of cellulose (~55%) and hemicellulose and lignin (the fractions of each depending on the type of wood). Cellulose is essential for papermaking, and the hemicelluloses can be beneficial; lignin, on the other hand, is undesirable and is removed during chemical pulping and bleaching. The final proportion of cellulose found in wood pulps can reach 90% with certain pulping methods. Cotton fibres are approximately 95% cellulose, with minor amounts of waxes and pectins and very little lignin. Chemical processing of cotton fibres can give yields of >99% cellulose.

Two broad classes of woods of commercial value to the pulp and paper industry are softwoods (pines, spruces, firs and cedars) and hardwoods (oaks, gums, beeches, birches and eucalypti). The major types of fibre found in softwood trees are the spring-wood fibres and the summer-wood fibres. Paper sheets made with a high percentage of spring-wood fibres (flexible fibres with flat surfaces that pack more closely together) are relatively stronger, denser and less porous. The best source of spring-wood fibre is central Canadian softwood pulp (~75% springwood). Hardwoods have much shorter fibres than softwoods and as a result do not bond well (the sheets have low tensile strength), but they do promote good sheet formation (fewer gaps in the sheet).

The two types of fibre found in most varieties of cottonseeds are the lint (staple) fibres and the linters. Lint fibres are used mostly in the textile industry,

Table 2. Results of handsheet evaluation*

Sample	Mean relative signal† Blank H_1	Blank H_2	PABA H_1	PABA H_2	S_A/S_B‡ H_1	S_A/S_B‡ H_2
ITT Rayonier Celluni-P	4.0	5.0	120	180	30	36
Buckeye Cellulose 503	4.5	4.5	180	235	40	52
Southern Cellulose 270	4.8	5.3	190	225	40	42
Southern Cellulose 277	5.7	7.5	310	340	54	45
Southern Cellulose 282–R	8.3	11.0	300	370	36	34

*H_1—1st handsheet—no pretreatment of pulp; H_2—2nd handsheet—pulp beaten for 1 hr prior to handsheet formation.
†Mean relative signal calculated from 8 determinations.
‡Ratio of mean relative signal of PABA (S_A) to mean relative signal of blank (S_B).

while cotton linters are processed into pulp for paper-making or for chemically derived products. Comparisons of the two types of cotton fibre show distinct differences. The lint fibres can grow up to 30 mm in length with cell wall thicknesses of up to 3 μm; cotton linters average 4 mm in length with cell wall thicknesses of up to 10 μm. Lint fibres with thin cell walls and wide lumens collapse when dried and thus add strength and density to a sheet of paper. Cotton linters, on the other hand, with thick cell walls, remain round on drying and impart bulk and porosity to paper. Because of these characteristics, cotton-linter pulp is used extensively for filter paper making.[18]

Diagnostic studies on cellulose products

This study began with a selection of cellulose products with properties appearing to accord with the theories[2] that hydrogen bonding and/or electrostatic interactions give rise to rigid adsorption of organic molecules on the surfaces of solid supports, and that RTP of the compounds can then be observed. The selection included an extremely "pure" wood pulp (Cellunier-P), an equally "pure" cotton-linter pulp (503), additional cotton-linter pulps (270, 277, 282-R) with tailored properties, and several "good" commercially available filter papers (613, 631, and 903).

Background phosphorescence

The first set of experiments (Table 1) was set up to evaluate the background phosphorescence of cellulose. Contrary to popular belief, the vast majority of filter-paper companies marketing products in the clinical fields do *not* add special (luminescent) chemicals (optical whiteners, sizing agents, *etc.*) to their pulps. The desired characteristics of the papers are met through chemical treatment (caustic cooking/extracting and inorganic bleaching followed by extensive washing with purified water) and physical manipulation (refining, beating, *etc*) of the pulps.[19] Thus filter papers are relatively pure, with the cellulose content approaching 100%. However, the question arises of the magnitude of the phosphorescence of "bone-dry" cellulose. [6] Lloyd and Miller[20] have observed that highly purified cotton does not phosphoresce and that any phosphorescence may be attributed to trace contaminants sorbed on the cotton. Similarly, Atalla and Nagel[21] have observed that trace amounts of transition metals incorporated into the crystalline domains of cellulose fibres may be responsible for laser-induced fluorescence in cellulose. More recently, Timell,[22] Huwyler *et al.*,[23,24] Delmer and co-workers,[25–28] Waterkeyn[29] and others[30,31] have pointed out that filter paper cannot be "pure" cellulose because the cotton fibres still contain trace amounts of hemicelluloses and lignin. These hemicellulosic fractions (mainly β-1,3-glucans) increase at the onset of secondary wall formation and contain species that luminesce.

The various soaking treatments (results in Table 1) were designed to deal with trace metals and hemicelluloses and/or lignin in cellulose pulp. The chelating agent DTPA was used in an attempt to remove trace amounts of transition metals that might contribute to the phosphorescence background of the filter paper. Though the background phosphorescence of the blank was reduced only slightly, the analyte signal improved significantly. This treatment gave no improvement for blank levels over past procedures.[8] The effect on the analyte signal is attributed to interaction of the DTPA with PABA, and to the gaps in the paper structure being filled. Unfortunately, the results do not indicate whether trace metals were involved in the phosphorescence background of the cellulose.

Following Millson's work,[4] an Eimac lamp and sunlight were used to try to bleach out "impurities" in the filter paper, but the illumination was actually detrimental, as the analyte signals decreased, possibly because of disruption of the surface structure.

The remaining treatments were used to test for the presence of extractable hemicelluloses (and/or lignin) in cellulose pulp. While none of the treatments unequivocally confirmed the presence of such materials, the soaking with dioxan indicated (by the large decrease in background phosphorescence) that an extractable hemicellulosic (and/or lignin) fraction may have been present. Periodic acid oxidation[29] almost completely destroyed the surface hydroxyl groups (forming aldehyde groups), and no net analyte signal or background was observed. Thus, while hemicelluloses (and/or lignin) appear to be responsible for the background phosphorescence, complete removal of these groups apparently disrupts the surface structure, and then analyte phosphorescence also does not occur.[28]

Fibrillation study and handsheet evaluation

In the fibrillation study (Table 2), two handsheets (one more highly fibrillated than the other) were made from unbeaten and beaten pulps. Beating cellulose pulps over a period of time decreases the average fibre length and increases the average exposed surface area. Therefore, with more hydroxyl groups exposed on the surface, a more complete adsorption of organic molecules on the support material takes place and an enhancement in phosphorescence should be seen. At the same time, however, fibrillation increases the exposure of the hemicellulosic material located in the inner matrix of the cellulose fibres. As a result, a larger phosphorescence background should also be seen. In fact, the results (Table 2) obtained for Cellunier-P, 503, 2570, 277, and 282-R appear to confirm these generalizations. Though the degrees of fibrillation have not been confirmed for Cellunier-P and grade 503, the latter would be expected to be more fibrillated than the former. For the three Southern Cellulose pulps the degree of fibrillation appears to increase in the order grade 270 < grade 277 < grade 282-R.

Table 3. Results of filter paper comparison

Filter paper*	Mean relative signal† Blank	PABA	S_A/S_B
S & S 903	10.7	150	14
S & S 903 (DTPA-treated)	6.8	365	54
Eaton-Dikeman 613	6.4	135 (225)	21 (35)
Eaton-Dikeman 631	6.6	125 (208)	19 (32)

*S & S 903 (basis weight ~ 190 g/m^2· thickness ~ 0.45 mm).
Eaton-Dikeman 613 and 631 (basis weight ~ 70 g/m^2, thickness ~ 0.2 mm).
5-μl volumes used with S & S paper.
3-μl volumes used with Eaton-Dikeman papers.
Numbers in parentheses represent signals that would be obtained by using 5-μl volumes, assuming linearity of determination.
†Mean relative signal calculated from 8 determinations.

Table 4. Results of "lot"-analysis of DTPA-treated S & S 903*

Lot	Blank Mean relative signal	RSD, %	PABA Mean relative Signal	RSD, %	S_A/S_B
W94 (1980)	6.3	3.7	360	3.2	57
W94 (1981)	5.8	4.4	405	2.2	70
W93	5.4	4.3	423	3.5	78
W92	5.2	3.4	408	2.3	78
W12	6.3	3.6	399	2.7	63
W02	5.3	4.7	407	3.3	77
W01	5.2	3.5	400	2.4	77

*Mean relative signals and relative standard deviations calculated from 16 measurements of blank and PABA. S_A (mean relative signal of PABA); S_B (mean relative signal of blank).

Comparison of filter paper

Eaton-Dikeman filter papers 613 and 631 were compared with S & S 903 (see Table 3) to determine whether paper porosity affects the interaction of the cellulose surface groups with analyte molecules. Optimally, the filter paper should allow the bulk of the analyte to remain on the surface, to provide more effective adsorption and/or interaction. Thus "slow" filter papers are the best choices. The 613 and 631 papers gave larger analyte signals than the untreated 903 paper, but the DTPA-treated 903 gave even higher signals. The suggestion that DTPA fills in the gaps of S & S 903 now seems confirmed (by the larger signals), if the analyte molecules are regarded as "trapped" in the DTPA–cellulose matrix.[32]

Comparison of various lots of filter papers

The various lots (see Table 4) of S & S filter paper 903 are consistent in quality for use in RTP applications. On a statistical basis (Duncan's multiple range procedure with $\alpha = 0.01$), the W94 (1980) and W93 lots are significantly different for analyte signals, and the W94 (1980) and W12 lots are significantly different for blank signals. In general, the RSD for blank determinations is larger than for analyte determinations, but no correlations were performed for the signal levels and their respective standard deviations.

CONCLUSION

In view of the results obtained in this study, it is clear that cellulose-based support materials vary considerably; nevertheless, for RTP applications, it is practical to assume that the difference in performance between the poorest paper and the best paper is considerably less than an order of magnitude. Thus, researchers evaluating RTP for analytical studies should select support materials giving the highest signal-to-noise ratio. If filter papers are used (for convenience or because of ready availability), then a pretreatment is recommended to enhance the adsorption characteristics. If the background phosphorescence of cellulose-based products indeed cannot be reduced (and it seems unlikely that it *can* be reduced), time-resolved phosphorimetry may effectively correct for such interferences. We shall shortly evaluate use of the Perkin-Elmer LS-5 for this purpose.

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APPENDIX

Basis weight. The mass per unit area (g/m^2) of paper. In the U.S. paper industry it is generally the weight (lb) of a ream (usually 500 sheets) of paper.

Fibrillation. A pulp-refining technique resulting in loosening of threadlike "elements" from the fibre wall to give greater surface area for forming fibre-to-fibre bonds.

Gap. Voids caused by random fibre orientation during sheet formation.

Handsheet. A sheet of paper made by draining a suspension of fibres in water, on a stationary mould. It is used for testing the physical and chemical properties of a pulp, and is made in accordance with standard procedures.

Hemicelluloses. Various cell-wall polysaccharides, usually not extractable by water or most organic solvents but gradually extracted by dilute ($\sim 10\%$) aqueous alkali.

Lignin. The non-carbohydrate portion of cell walls; it is amorphous, with high molecular weight, predominantly aromatic in nature, and built up of phenylpropane units. It is not a compound, and varies in composition with method of isolation and with species, age, *etc.* of the plant or tree. It is almost completely removed during chemical pulp-processing.

Lint. The ginned cotton textile fibre.

Linters. Short fibres adhering to the cottonseed after ginning.

Pulp. Fibre material, classified according to type (wood, rag, cotton linters, etc.) and manufacturing process.

Thickness. Refers to a single sheet of paper under specific conditions of area and pressure.

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THE SOLVENT EXTRACTION OF Cu(II), Ni(II) AND Co(II) WITH BENZIL MONO(2-QUINOLYL)HYDRAZONE*

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Summary—Benzil mono(2-quinolyl)hydrazone, BmQH, has been studied as an extracting agent for Cu(II), Ni(II), and Co(II). Though the uncomplexed ligand remains undissociated in the pH range 3.5–10, it can lose a proton on complexation with metals, owing to the electron-withdrawing effects of neighbouring groups. The dependence of degree of extraction on pH indicates that complexes of both Cu^{2+} and $Cu(OH)^{+}$ are extracted. $Cu(BmQH)_2$ and Cu(OH)BmQH species are extracted into MIBK, and the Cu(OH)BmQH complex is extracted into benzene. In the vicinity of pH 5.5–6, extraction efficiencies greater than 95% can be achieved with both solvents. Both Ni(II) and Co(II) also show dependence of extraction on pH, but precipitation of both metals in the vicinity of pH 6 limits further studies.

The condensation of α-diketones with 2-aza-aryl hydrazines can produce mono and bis products which can act as multidentate ligands. For example, Lions and Martin showed that biacetyl bis(2-pyridyl)hydrazone could serve as a quadridentate ligand; further studies indicated that deprotonation in the hydrazine moiety could explain the formation of neutral complexes.[2,3] However, if the α-diketone contains phenyl or bulkier groups, the formation of the bis product is hindered. The resulting mono products can serve as either bidentate or terdentate ligands.[4]

As these reagents form deeply coloured complexes with transition elements, analytical applications seem feasible, but very few spectrophotometric methods have been developed. Pflaum determined Co(III) with benzil mono(2-pyridyl)hydrazone in water–ethanol mixtures.[5] Cu(II) has been determined after extraction from a pH-6 phosphate buffer into benzene with benzil mono(2-quinolyl)hydrazone (BmQH).[6]

Though these α-diketone mono(2-aza-aryl)hydrazones appear useful for analytical application, their properties as extracting agents have not been widely investigated. In this work, the ligand benzil mono(2-quinolyl)hydrazone is studied as an extractant for Cu(II), Ni(II) and Co(II).

EXPERIMENTAL

Reagents

Stock solutions of Cu(II), Ni(II) and Co(II) perchlorates were prepared from the hexahydrates.

BmQH was synthesized by refluxing benzil with 2-quinolylhydrazone, for 2 hr, in ethanol acidified with 1 or 2 drops of glacial acetic acid. The resulting yellow-orange crystals were recrystallized from ethanol, washed, and dried. The m.p. was 165–167°. The infrared spectrum indicated the absence of an OH group but had a large peak at 1642 cm^{-1} corresponding to the C=O stretching mode and a peak at 3315 cm^{-1} for the N–H bond. Mass spectral analysis indicated the presence of the mono product.

Buffers and the benzene and MIBK were of reagent grade.

Procedures

Extraction studies. Ten ml of $5 \times 10^{-4}M$ metal ion solution were added to a 100-ml separatory funnel and 10 ml of BmQH solution in the concentration range 4×10^{-3}–$1 \times 10^{-2}M$ in either benzene or MIBK were added. The pH was adjusted by dropwise addition of 1–5% sodium hydroxide or perchloric acid solution. When buffers were used, they were added to the stock solution of the metal. The solution was equilibrated by gentle shaking for 2 hr. After settling, the aqueous phase was collected by filtration and its equilibrium pH was measured.

The metal concentration of the aqueous phase was determined by atomic absorption, and that of the organic phase by difference.

Spectrum of ligand as a function of pH. Ten ml of $5 \times 10^{-4}M$ metal ion solution were added to a 100-ml separatory funnel followed by 10 ml of $1 \times 10^{-2}M$ BmQH in MIBK. The pH was adjusted by dropwise addition of 1% sodium hydroxide solution, and the mixture was equilibrated. The pH of the aqueous phase was then measured and the absorption spectrum of a diluted portion of the organic phase was measured against an appropriate blank.

RESULTS AND DISCUSSION

Absorption spectrum of ligand as a function of pH

The spectrum of the ligand in MIBK is shown in Fig. 1, with the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions at 365

*Presented at the Pittsburgh Conference of Analytical Chemistry, Atlantic City, New Jersey, 12 March 1981.

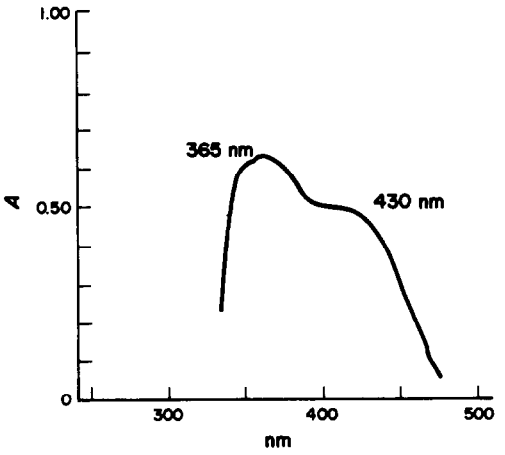
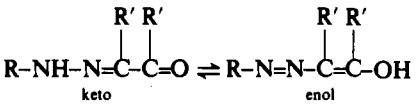


Fig. 1. Spectrum of BmQH *vs.* blank in MIBK.

and 430 nm respectively. The positions and intensities of these two maxima do not change with pH over the range 3.5–10, showing the ligand is a very weak acid which remains in the undissociated form throughout this pH range. In very strongly alkaline medium the ligand will react with hydroxide and form a red colour.

Keto-enol tautomerism has been suggested to occur for these compounds:[4]



However, the absence of an absorption maximum at wavelengths longer than 500 nm and of an OH stretching frequency in the infrared indicates that the enol form is not present.

Extraction of Cu(II) as a function of pH

Figure 2 shows plots of log D *vs.* pH for the extraction of Cu(II) with two different initial concentrations of ligand. At the higher ligand concentration, the extraction is more efficient and starts at lower pH. For a ligand concentration of $1 \times 10^{-2}M$, $pH_{0.5} = 4.1$, and for $7.5 \times 10^{-3}M$ reagent $pH_{0.5} = 4.5$. At pH 5, with $[HL]_0 = 1 \times 10^{-2}M$, an extraction efficiency of 97% is observed, and at the same pH, with $[HL]_0 = 7.5 \times 10^{-3}M$, 84% extraction is found. If the total ligand concentration is kept constant and the copper concentration increased, the slope of the log D *vs.* pH plot varies, but not systematically, so presumably no dimeric copper species are extracted.

Extraction of Cu(II) as a function of $[ClO_4^-]$

As perchlorate salts were used in this work, the effect of perchlorate on the extraction was studied. A positive slope in a plot of log D *vs.* log $[ClO_4^-]$ would indicate the formation of extractable ion-association complexes of perchlorate with cationic metal complexes, but no variation in log D was found, so perchlorate is not involved in the extraction.

Equilibrium of extraction

Because the species extracted must be uncharged and perchlorate is not present in it, the charge on the copper ion must be neutralized by dissociated ligand species (and possibly hydroxide ions). Though the ligand, HL, does not dissociate within the pH range 3.5–10, it is well known that complexation enhances the apparent strength of weak acids. Thus, the extraction reaction may be expressed as follows:

$$M_{aq}^{n+} + n[HI]_0 \rightleftharpoons [M(HL)_n^{n+}]_{aq} \rightleftharpoons [ML_n]_0 + nH_{aq}^+. \quad (1)$$

Since the pH range for extraction is sufficiently basic to allow partial hydrolysis of the metal ion, the following reaction is also possible:

$$[M(OH)^{(n-1)}]_{aq} + m[HL]_0 \rightleftharpoons [M(OH)(HL)_m^{(n-1)+}]_{aq} \rightleftharpoons [M(OH)(L)_{n-1}(HL)_{m-n+1}]_0 + (n-1)H_{aq}^+. \quad (2)$$

The overall equilibrium expressions are

$$K_{ex} = \frac{[ML_n]_0[H^+]_{aq}^n}{[M^{n+}]_{aq}[HL]_0^n} \quad (3)$$

for reaction (1) and

$$K'_{ex} = \frac{[M(OH)(L)_{n-1}(HL)_{m-n+1}]_0[H^+]_{aq}^{n-1}}{[M(OH)^{n-1}]_{aq}[HL]_0^m} \quad (4)$$

for reaction (2). The distribution coefficients are

$$D = \frac{[ML_n]_0}{[M^{n+}]_a} \quad (5)$$

and

$$D' = \frac{[M(OH)(L)_{n-1}(HL)_{m-n+1}]_0}{[M(OH)^{(n-1)+}]_{aq}} \quad (6)$$

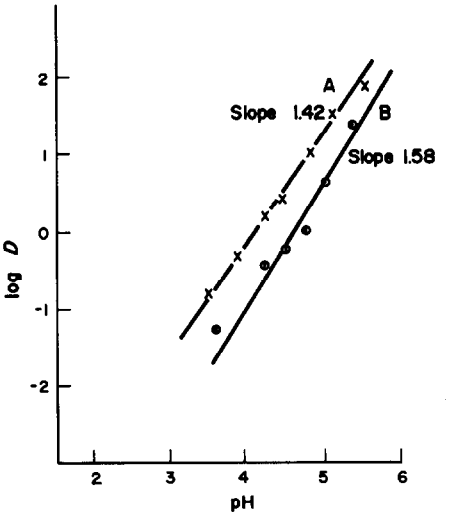


Fig. 2. Plot of log D *vs.* pH for the extraction of Cu^{2+} into MIBK. A: $[HL]_{in} = 1 \times 10^{-2}M$, $[Cu^{2+}]_{in} = 5 \times 10^{-4}M$. B: $[HL]_{in} = 7.5 \times 10^{-3}M$, $[Cu^{2+}]_{in} = 5 \times 10^{-4}M$.

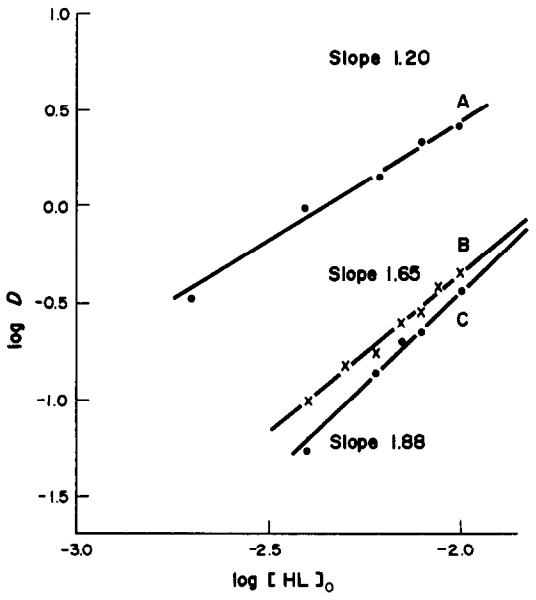


Fig. 3. Plot of log D *vs.* log $[HL]_0$ for extraction of $5 \times 10^{-4}M$ Cu in $0.005M$ biphthalate buffer, pH 5. A: 2×10^{-3}–$1 \times 10^{-2}M$ [HL] in benzene. B: 4×10^{-3}–$1 \times 10^{-2}M$ [HL] in MIBK. C: 2×10^{-3}–$1 \times 10^{-2}M$ [HL] in MIBK.

respectively. Substitution, solving for D and taking logarithms gives:

$$\log D = \log K_{ex} + n \log[HL]_0 + n\text{pH} \quad (7)$$

and

$$\log D' = \log K'_{ex} + m \log[HL]_0 + (n-1)\text{pH}. \quad (8)$$

For Cu(II), slopes of 2 and 1 for log D *vs.* pH would correspond to the extraction of Cu^{2+} and $Cu(OH)^+$, respectively. The slope of 1.4–1.6 (Fig. 2) indicates the extraction of both forms of Cu(II).

Extraction as a function of ligand concentration

Plotting log D *vs.* log $[HL]_0$ at constant pH yields a slope equal to the ligand:metal ratio in the extracted species. Plots for the extraction of Cu(II) into benzene of MIBK are given in Fig. 3. In both instances, the pH was maintained at 5 with a potassium phthalate buffer.

For MIBK, slopes of 1.65 and 1.88 were observed, suggesting extraction of both $[CuL_2]$ and [Cu(OH)L], with the first predominant. For benzene the slope was 1.2, suggesting that the second species predominates in this system, the stoichiometry corresponding to that observed[6] for phosphate-buffered medium at pH 6.

The extraction efficiency is about 2.5 times greater with benzene than with MIBK. This is perhaps because the MIBK is sufficiently polar for the very bulky complex to experience more difficulty in creating "holes" in the MIBK structure than in benzene.

It is also interesting that use of the potassium phthalate buffer diminishes the extraction of copper into MIBK by a factor of 3–5, in comparison with the unbuffered system at the same pH. The phthalate presumably brings about a competing equilibrium which reduces the degree of extraction. Use of unbuffered media and plotting log D *vs.* log $[HL]_0$ (corrected for pH) generated a slope of 1.9, in agreement with that found for the buffered media.

Extraction of nickel and cobalt

Both metals form extractable complexes, but there is precipitation at pH above about 6, so meaningful results can be obtained only at higher acidity than this, and even then there is considerable non-reproducibility. All that can really be said is that both metals would compete with copper in the extraction system.

Conclusion

The implications of these results for practical analysis are (*a*) that the extract could not be used directly for determination of copper through the properties of the complex, since more than one may be formed, (*b*) that the system could be used for isolation of copper, and (*c*) that nickel and copper would interfere.

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DIFFERENTIAL PULSE POLAROGRAPHIC DETERMINATION OF ORTHOPHOSPHATE IN AQUEOUS MEDIA

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Summary—The determination of orthophosphate in aqueous media by differential pulse polarography is described. It is based on determination of the molybdenum in 12-phosphomolybdic acid. High sensitivity is achieved by measuring the polarographic wave due to the catalytic reduction of perchlorate or nitrate in the presence of molybdenum(VI). The method is suitable for samples as small as 3.5 ml which contain as little as 9 ng of phosphorus per ml. The average relative deviation is 3.0% at the 0.045 mg/l. phosphorus level and 1.6% at the 1.2 mg/l. level. Results for the analysis of EPA quality-control water and real surface-water samples are reported.

Various methods for the indirect determination of orthophosphate by measurement of the molybdenum in 12-phosphomolybdic acid have been reported. Atomic-absorption methods[1,2] provide a sensitivity of about 1 mg/l. for phosphorus. A sensitivity of about 20 μg/l. has been achieved by X-ray fluorescence.[3] Bazzi and Boltz[4] developed a d.c. polarographic method with a detection limit of about 1 mg/l. Fogg and Yoo[5] obtained a sensitivity of about 0.1 mg/l. by differential pulse polarography.

Increasingly sensitive methods for polarographic determination of molybdenum have been developed, based on measurement of the polarographic wave resulting from catalytic reduction of perchlorate in the presence of molybdenum.[6–8] By combining the sensitivity of differential pulse polarography with the catalytic effect of molybdenum on the polarographic reduction of perchlorate or nitrate, the procedure developed in this study achieves a sensitivity of 9 μg/l. for phosphorus. Not only does this procedure provide greater sensitivity than previous methods, it also requires less sample and involves fewer steps.

EXPERIMENTAL

Apparatus

Differential pulse polarograms were recorded on a Princeton Applied Research Electrochemical System Model 170 equipped with potentiostatic control and a mechanical drop-detachment device. A dropping mercury electrode working electrode, a saturated calomel (SCE) reference electrode and a platinum-wire counter-electrode were used. The polarographic cell, drop-detachment device and mercury column with its reservoir were placed inside a grounded aluminium cage to prevent pick-up of extraneous electrical signals from the laboratory surroundings. Solutions were deaerated with nitrogen purified by passage through vanadium(II) chloride solution, and a blanket of the nitrogen was kept over the solutions during the recording of polarograms.

Reagents

All chemicals were either analytical or primary standard grade. No further purification was necessary. Demineralized water was used to make all solutions.

Glassware

All glassware was washed with 6*M* hydrochloric acid and rinsed thoroughly with demineralized water. Polyethylene stoppers were washed in 6*M* hydrochloric acid, rinsed with demineralized water, then washed in 8*M* nitric acid and again rinsed with demineralized water.

Standard solutions

Stock molybdenum solution. Prepared monthly by dissolving 17.66 g of ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 1 litre of demineralized water, stored in a polyethylene container, and standardized monthly. Working solutions of lower molybdenum concentration were prepared daily by dilution.

Stock phosphate solution. Prepared monthly by dissolving 1.3609 g of oven-dried (2 hr at 100°) primary-standard grade potassium dihydrogen phosphate, KH_2PO_4, in 1 litre of demineralized water, and stored in a polyethylene container. Working solutions of lower phosphate concentrations were prepared daily by dilution.

Procedures

Standardization of molybdenum solution. A 6 × 1 cm column of freshly amalgamated zinc was activated by passing 20 ml of 1.44*M* hydrochloric acid (12 ml of concentrated acid diluted to 100 ml) through it at a rate of 1 drop/sec. A 0.01*M* molybdenum solution in 1.44*M* hydrochloric acid was prepared by diluting 10.00 ml of stock molybdenum solution and 12.0 ml of concentrated hydrochloric acid to 100.0 ml with demineralized water. Ten ml of iron(III) solution [prepared by dissolving 15.06 g of ferric ammonium sulphate, $FeNH_4(SO_4)_2\cdot 12H_2O$ in 5% v/v sulphuric acid] were diluted to about 100 ml with demineralized water and placed in the receiving beaker. This beaker was covered with a sheet of "Parafilm". The tip of the Jones reductor was placed below the "Parafilm" cover

and the receiving beaker was continuously flushed with nitrogen during the reduction and titration.

Exactly 15.00 ml of the acidic 0.01*M* molybdenum solution were passed through the Jones reductor at a rate of 15 drops/min, and the reductor was rinsed with 10.0-ml of demineralized water. The reduced solution and the rinsings were collected in the receiving beaker and the iron(II) produced was titrated with the 0.1*N* permanganate (previously standardized with arsenic trioxide[9]). A blank solution of 15.00 ml of 1.44*M* hydrochloric acid was treated similarly.

Standardization of molybdenum solutions by differential pulse polarography. Exactly 5.00-ml portions of 4.0*M* perchloric acid and various known microlitre amounts of standard 0.0143*M* molybdenum solution were pipetted into a series of 10.00-ml volumetric flasks and diluted to volume with 0.125*M* sodium hydroxide. The solutions were analysed by differential pulse polarography, after deaeration with nitrogen for 5 min immediately before polarography. The voltage was scanned from +0.20 V to −0.60 V *vs.* SCE. The solutions were maintained at 22 ± 0.5°.

Determination of phosphate in low concentration range. Standard solutions for phosphate concentrations in the range 3×10^{-7}–$3 \times 10^{-6}M$ (P 9–90 μg/l.) were prepared by pipetting 60–600 μl of standard $2.52 \times 10^{-4}M$ phosphate solution into a series of 50-ml standard flasks and diluting to the mark with demineralized water.

Then 3.50 ml of a solution to be analysed were pipetted into a 1.5 × 15 cm Pyrex test-tube fitted with a high-density polyethylene or glass stopper. Next, 500 μl of 4.24% ammonium molybdate solution were added, followed by 1.00 ml of 3.33*M* hydrochloric acid (27.8 ml of concentrated acid diluted to 100 ml). Two min later, 5.00 ml of isobutyl acetate were added, the test-tube was stoppered and the contents were vigorously shaken for 1 min. Then 4.00 ml of the organic (upper) phase (containing the phosphomolybdic acid) were transferred to a 100-ml beaker and the solvent was evaporated by placing the beaker in a laboratory hood providing a strong draught. The residue was dissolved in 5.00 ml of 0.125*M* sodium hydroxide. This solution was then acidified with 5.00 ml of 4.0*M* perchloric acid, transferred to the polarographic cell and analysed by differential pulse polarography. A blank solution containing no phosphate was analysed similarly.

Determination of phosphate in high concentrations. Standard solutions for high phosphate concentrations in the range from 1×10^{-6} to $6 \times 10^{-5}M$ were prepared by pipetting 10–500 μl of 0.01*M* standard phosphate solution into 50-ml standard flasks and diluting to the mark with demineralized water. Then 1 ml of the diluted phosphate solution was placed in the test-tube, followed by 0.50 ml of 0.1*M* molybdate and 1.0 ml of 1.65*M* hydrochloric acid. Two min later, 2.0 ml of isobutyl acetate were added and the tube was shaken vigorously. Exactly 1 ml of the organic phase was transferred into a beaker. The isobutyl acetate was evaporated and the residue dissolved in 5.0 ml of 0.125*M* sodium hydroxide. This solution was acidified with 5.0 ml of 4.0*M* perchloric acid or 1.0 ml of 2*M* sulphuric acid, followed by addition of 4.0 ml of 5.0*M* sodium nitrate. The resulting solution was transferred into a polarographic cell and was analysed for molybdenum content by differential polarography. The magnitude of the catalytic peak was measured and compared with that for the blank.

The amount of phosphate in aqueous samples was determined by extraction of 12-phosphomolybdic acid, and measurement of the catalytic perchlorate current in the presence of molybdenum(VI). Extraction efficiencies were obtained by comparing the peak currents for the extracted 12-phosphomolybdic acid with the peak currents for molybdenum(VI) concentrations corresponding to the theoretical concentrations of 12-phosphomolybdic acid.

Analysis of EPA orthophosphate quality-control sample. The EPA quality-control sample was analysed by taking 3.50 ml of this solution and following the procedure described for phosphate levels below $3.0 \times 10^{-6}M$. A "spiked" EPA sample was prepared by pipetting 100 μl of $2.52 \times 10^{-4}M$ phosphate solution into a 25.00-ml standard flask and diluting the contents to the mark with the EPA sample. A 3.5 ml sample of the spiked EPA solution was analysed in the same manner as the unspiked sample.

Analysis of real surface water. A surface water sample was placed in an acid-washed Nalgene container and immediately cooled on ice. The sample was filtered through a 0.45-μm membrane filter. A spiked surface water sample was prepared by pipetting 10 μl of standard $1.01 \times 10^{-2}M$ phosphate solution into a 10.00-ml standard flask and diluting to the mark with surface water. The spiked surface water was then analysed by the procedure for phosphate levels greater than $3.0 \times 10^{-6}M$.

DISCUSSION AND RESULTS

Phosphate in synthetic samples as well as in EPA and natural water samples is determined by an indirect method utilizing 12-phosphomolybdic acid. In this method, molybdate reduction is monitored by the differential-pulse polarographic method and the peak current is then related to the phosphate concentration in the aqueous solution before the extraction with isobutyl acetate.

The overall procedure is evaluated by measuring peak currents for molybdenum(VI) alone in 2.0*M* perchlorate or 2.0*M* nitrate media and comparing them with the values obtained for known phosphate solutions by extraction and polarographic analysis of 12-phosphomolybdic acid. The results are summarized in Tables 1 and 2. Analysis results for the EPA quality-control sample with very low concentration of phosphate and for natural water samples are presented in Table 3.

Determination of micro amounts of orthophosphate as 12-phosphomolybdic acid involves: (1) formation of the heteropoly acid in the aqueous phase, (2) extraction with isobutyl acetate and (3) detection. The conversion of orthophosphate into 12-phosphomolybdic acid proceeds by the reaction

$$PO_4^{3-} + 12MoO_4^{2-} + 27H^+ \rightarrow H_3PO_4(MoO_3)_{12} + 12H_2O$$

and depends not only on the concentration of acid but also on the ratio of molybdenum(VI) to phos-

Table 1. Peak current as a function of molybdenum(VI) concentration in perchlorate or nitrate solutions*

Molybdenum(VI) concentration, μ*M*	Peak current, *nA*† Perchlorate	Nitrate
0.99	71 ± 5	208 ± 6
1.99	145 ± 7	420 ± 10
4.97	376 ± 10	1004 ± 20
9.95	770 ± 15	1920 ± 25

* Perchlorate concentration is 2.0*M* and that of hydrogen ion 1.94*M*. Nitrate concentration is 2.0*M* and that of hydrogen ion 0.14*M*.

† Polarographic settings are: scan-rate 5 mV/sec, drop-time 1 sec/drop, pulse-amplitude 25 mV, sampling time 15 msec.

Table 2. Current as a function of phosphate concentration*

Amount of phosphate in aqueous phase, *ng*	Peak current,† *nA*	Molybdenum(VI) in polarographic cell, *μM* Found§	Expected
In 2.0*M* perchlorate			
0.00	54 ± 5		
155	200 ± 7	2.5	3.00
310	390 ± 10	5.1	6.00
620	760 ± 15	10.0	12.00
1240	1500 ± 20	20.6	24.00
In 2.0*M* nitrate			
15.5	100 ± 6	0.25	0.30
38.7	190 ± 8	0.70	0.75
77.5	282 ± 10	1.40	1.50
155	570 ± 15	2.75	3.00
310	1060 ± 20	5.40	6.00

* Samples are prepared by taking 1.00 ml of the secondary standard and diluting it with 0.50 ml of 0.1*M* Mo(VI) and 1.0 ml of 1.65*M* HCl. Heteropoly acid is extracted into 2.00 ml of isobutyl acetate and 1.00 ml of the organic phase is analysed.

† Polarographic settings as for Table 1.

§ Molybdenum(VI) concentration is obtained by comparison of the peak currents with those for aqueous molybdate (Table 1).

‡ Obtained by multiplying the 12-phosphomolybdate concentration by 12, assuming conversion and extraction to be complete.

phate. The hydrochloric acid concentration in the aqueous phase is kept to 0.66*M*, which ensures not only rapid formation of 12-phosphomolybdic acid but also enables selective extraction of this acid, thus avoiding the possible interference of silicate and arsenate, which remain in the aqueous phase. In this step, the concentration of molybdate must not be less than 0.02*M*. This provides a molybdate to phosphate ratio

Table 3. Orthophosphate determination in EPA quality-control sample and in surface water*

Determination	Phosphorus in aqueous phase, *μg/l.*	Peak current,† *nA*	Phosphorus found, *μg/l.*
Standards for EPA sample analysis	9.4	206 ± 8	—
	31.2	495 ± 15	—
	40.6	616 ± 15	—
	46.8	700 ± 20	—
	93.6	1201 ± 30	—
EPA sample			
unspiked	—	699 ± 20	45
spiked§	—	1016 ± 25	76
Standards for surface water	626	1560 ± 20	
	1250	2700 ± 25	
	1870	4000 ± 40	
Skokie Lagoon			
unspiked	—	2630 ± 25	1220
spiked§	—	3320 ± 35	1540

*Sample analysed by taking a 3.50-ml aliquot, 0.50 ml of 0.24*M* Mo(VI) and 1.00 ml of 3.33*M* HCl. The 12-phosphomolybdic acid is extracted with 5.0 ml of isobutyl acetate and 4.00 ml of the organic phase are analysed.

† Polarographic settings: scan-rate 5 mV/sec, pulse-amplitude 25 mV, drop-time 1 sec/drop, sampling time 5 msec. Peak currents are the average values of three measurements.

§ EPA quality-sample water is spiked with 0.100 ml of $2.4 \times 10^{-4}M$ phosphate per 25 ml of sample and surface water is spiked with 0.010 ml of 0.0101*M* phosphate per ml of sample.

of 400 or more. Under these conditions, formation of 12-phosphomolybdic acid is rather fast and it can be extracted after 2 min. However, if the molybdate concentration is reduced to 0.01*M*, the extraction efficiency falls to below 50%. Also, if the aqueous-phase phosphate concentration is low, less than the expected amount of molybdenum is recovered in the polarographic cell, and the extraction of phosphomolybdic acid is incomplete. Nevertheless, linear calibration graphs can be obtained even when the aqueous-phase phosphate concentration is as low as $3 \times 10^{-7}M$. Since the amount of phosphomolybdic acid extracted depends on the aqueous-phase phosphate concentration, it is necessary to use standard phosphate solutions that bracket the concentration of the sample. Because of the small amount of free molybdate extracted in the blank, it is also necessary to analyse a blank (for the whole procedure) along with the standard phosphate solutions.

The efficiency of various organic solvents for extracting heteropoly acids has been investigated by Wadelin and Mellon.[10] Their studies showed that organic oxygen-compounds are good extractants. Isobutyl acetate not only selectively extracts 12-phosphomolybdic acid from arsenomolybdic and silicomolybdic acids as reported by Paul[11] but also provides a rather high efficiency for the preconcentration of 12-phosphomolybdic acid.

Before the polarographic analysis, it is preferable to evaporate the isobutyl acetate and it is essential to dissolve the residue in dilute sodium hydroxide solution, followed by acidification with perchloric acid. The dissolution in alkali ensures complete solubilization and recovery of molybdic acid.

The phosphate is then determined indirectly by measuring the catalytic current of perchlorate or nitrate in association with molybdenum(VI). The polarographic behaviour of molybdenum(VI) in various supporting electrolytes has been studied by Boltz *et al.*[12] and Johnson and Robinson.[13]

Molybdenum(VI) is reduced stepwise from Mo(VI) to Mo(V) and from Mo(V) to Mo(III). However, in some of these studies more than two steps have been observed and exact interpretation of these observations is lacking. Recently, Von Henrion *et al.*[14] appear to have elucidated the molybdenum(VI) reduction.

In the presence of perchlorate or nitrate, the second reduction step for molybdenum(VI) is greatly enhanced, as observed by Holtje and Geyer.[6] This behaviour has been investigated further by Stach and Schone,[8] Haight,[7] and Kolthoff and Hondara,[15] and has been used for the determination of small amounts of molybdenum.

The catalytic current is affected not only by the molybdenum(VI) concentration but also by the perchlorate or nitrate concentration. Thus, throughout these experiments the concentration of perchlorate or nitrate is kept constant while the molybdenum(VI) concentration varies directly with the phosphate concentration of the aqueous solution.

The catalytic current increases as the square root of the perchlorate or nitrate concentration, up to approximately 1*M* concentration of these ions. However, with increasing concentration of perchlorate the background noise also increases and perchlorate or nitrate concentrations higher than 2*M* are undesirable.

The hydrogen-ion concentration affects not only the half-wave potential for the catalytic wave, but also (at pH < 1) the magnitude of the catalytic wave, which decreases with increasing pH; for perchlorate medium the hydrogen-ion concentration can be in the range from 0.2 to 2.0*M* while for nitrate medium the optimum range is 0.2–0.4*M*.

Results for the analysis of synthetic EPA and surface water samples show that the current can be reproduced with a relative deviation ranging from 5 to 1% with increasing amount of phosphate. The relative average deviation of the method for the EPA sample is 3.0% at the 0.046 mg/l. phosphorus level and the average recovery for the replicates is 92%. When the phosphate concentration in the starting solutions is less than 30 ng/ml, then the observed values are consistently lower by 6–10% than the expected values. This can in part be attributed to lack of linearity in the calibration graph and to a relatively high contribution from the blank.

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ANALYSIS OF VARIANCE APPLIED TO DETERMINATIONS OF EQUILIBRIUM CONSTANTS

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Summary—The statistical analysis of variance has been applied to the values of the equilibrium constants of the glycinate–proton and glycinate–nickel systems, determined in different laboratories by pH-titration in aqueous solution. The analysis shows how the main part of the error derives from the variability from one titration to another even in the same laboratory. Therefore the data for a single titration (k) must be processed separately, thus yielding a mean value for the equilibrium constant $\overline{\log \beta_{pqr}}(k)$ of the species $M_pH_qL_r$; from these mean values for different titrations in each laboratory l, a within-laboratory grand average, $\overline{\overline{\log \beta_{pqr}}}(l)$, can be calculated; the variance of this grand average measures the experimental error. A further analysis of the data from the different participating laboratories shows that there were no significant differences between laboratories for the constants reported. From these results it can be inferred that all the values of the mean constants $\overline{\log \beta_{pqr}}(k)$ for one species, as determined separately for each titration in four laboratories, belong to the same population. A χ^2 analysis of these populations demonstrates that the stability constants of the species HL, H_2L^+, NiL^+, NiL_2 (with L^- = glycinate) are normally distributed, but not that for NiL_3^-. Therefore, general mean values of the first four constants can be calculated and proposed as reliable standard values at 25° and I = 1.0M Na(Cl): protonation of glycinate, $\log \beta_{011}$ = 9.651(12), $\log \beta_{021}$ = 12.071(26); nickel-glycinate complexes, $\log \beta_{101}$ = 5.615(35), $\log \beta_{102}$ = 10.363(62). These values indicate that the standard deviations are rather higher than those often reported in the literature.

LIST OF SYMBOLS

i = index of one measured point, ml_i, pH_i
k = index of titration
l = index of laboratory
n_k = number of points for species $M_pH_qL_r$ in titration k ($\equiv i_{max}$)
$n_l = \sum_k n_k$ = number of points for species $M_pH_qL_r$ in titrations of laboratory l
$\bar{n}$ = mean value of n_k
m = number of titrations ($\equiv k_{max}$)
m_l = number of titrations in laboratory l ($\equiv k_{max}$ of lth laboratory)
$\bar{m}$ = mean value of m_l
m' = number of laboratories ($\equiv l_{max}$)
β_{pqr} = cumulative formation constant of the species $M_pH_qL_r$
$\log \beta_{pqr}(i,k)$ = log of formation constant calculated from data of point i of kth titration
$\overline{\log \beta_{pqr}}(k)$ = mean value of $\log \beta_{pqr}(i,k)$ for kth titration
$\sigma^2(k)$ = variance of $\log \beta_{pqr}(k)$ as calculated by the computer program
$\sigma_i^2 = \sum_k \sigma^2(k)/m$ = mean variance of $\log \beta_{pqr}$ for any point (estimated)
$\overline{\overline{\log \beta_{pqr}}}$ = average $\overline{\log \beta_{pqr}}(k)$ from one laboratory
σ_{av}^2 = variance of $\overline{\overline{\log \beta_{pqr}}}$
$\overline{\overline{\log \beta_{pqr}}}(l)$ = average of $\overline{\log \beta_{pqr}}(k)$ in laboratory l [$\equiv \log \beta_{pqr}(l)$]
$\overline{\log \beta_{pqr}}(l)$ = $\overline{\overline{\log \beta_{pqr}}}$ in laboratory l
$\sigma^2(l)$ = variance of $\overline{\log \beta_{pqr}}(l)$
σ_{tit}^2 = variance between titrations
$(\overline{\overline{\log \beta_{pqr}}})_{g.av}$ = general grand average of $\overline{\log \beta_{pqr}}(l)$ from m' values
$w(l) = 1/\sigma^2(l)$ = weight of $\overline{\log \beta_{pqr}}(l)$
$\sigma_{g.av}^2$ = variance of $(\overline{\overline{\log \beta_{pqr}}})_{g.av}$
σ_{lab}^2 = variance between laboratories
σ_{pqr}^2 = variance of $\log \beta_{pqr}$ (determined)

The determination of equilibrium constants for aqueous solutions by pH measurements with a glass electrode is widely used.[1,2] It is a recommended and generally accepted usage to perform several titrations in order to average out the errors inherent in single titrations. There is, however, no precise indication as yet whether the processing of the data to get equilibrium constants, particularly by computer programs, should be applied either to single titrations followed by averaging the results or to the whole set of data taken together. It is the purpose of this note to show, by means of analysis of variance, how the right procedure can be identified and followed. This will help to clarify the debate on the assessment of the reliability of the equilibrium constants and their standard deviations.[3]

As to whether the equilibrium constants or their logarithms ought to be handled, we have chosen in principle to use the logarithms because: (*i*) the potentiometric experimental measurements are on the logarithmic scale, *i.e.*, on the free-energy scale, and (*ii*) the logarithms of equilibrium constants are usually re-

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ported in collections of stability constants. At any rate the numerical differences between the averages of the constants and those of their logarithms are negligible in the case at hand. The term "stability constant" in this paper is very often employed to mean "logarithm of stability constant".

Analysis of variance

The experimental data consist of the data-points, i, (ml_i, pH_i), $(i = 1, 2, 3, \ldots, n_k)$ for each titration k $(k = 1, 2, 3, \ldots, m)$. The analytical concentrations of metal, hydrogen ion and ligand are also known. It is therefore possible to determine a value $\log \beta_{pqr}(i, k)$ for each data-point (i, k). These values can be ordered in an array (Table 1) in which the m columns correspond to the titrations, each with n_k points. Each array as a whole is representative of a single formation constant $\log \beta_{pqr}$ and for simplicity of description we assume that only one type of complex, $M_pH_qL_r$, is formed at the measured points.

Usually the values of the cumulative constants

$$\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$$

are refined by computer programs (*e.g.*, MINIQUAD,[4] SCOGS,[5] LETAGROP[6]) by non-linear least-squares methods. The treatment of the data by these programs is implicitly equivalent to forming the array of Table 1. The programs are able to process several arrays at the same time, which means that points representing more than one species can also be handled. Hence the arguments used in the present paper, even if they concern a single species, could be applied to the general case of simultaneous equilibria.

If all the points of every titration belonged to the same population of data, then it should be permissible to calculate the total average by refining all the points together. To establish the validity of this assumption, each set of titration data is refined separately, thus obtaining for each column k a mean value $\overline{\log \beta_{pqr}}(k)$ with variance $\sigma^2(k)$ and n_k-1 degrees of freedom. From m mean values the grand average $\overline{\overline{\log \beta_{pqr}}}$, with variance σ^2_{av} is calculated. If σ^2_{av} is nearly equal (H_0 hypothesis) to the estimated variance between points, σ_i^2/n, the assumption that all the points belong to the same population is proved. If on the contrary some difference between one titration and another exists (H_1 hypothesis), then $\sigma^2_{av} > \sigma_i^2/n$ and the assumption is false (Table 2). The F-test table is used to assess the hypothesis, at a given significance level.[7]

If the H_0 hypothesis is accepted, then the variability between titrations, σ^2_{tit}, is approximately zero, *i.e.*, no real difference exists between the titrations, and the data as a whole can be used as a single batch for the refinement of $\log \beta_{pqr}$. On the other hand, if H_1 is accepted, then $\sigma^2_{tit} > 0$ and the *refinement* must be

Table 2. Analysis of variance as applied to n points* of the kth titration in the same laboratory

$$\sigma^2_{av} = (\sigma_i^2/n) + \sigma^2_{tit}$$

H_0 hypothesis

$$F = (n\sigma^2_{av}/\sigma_i^2) \simeq 1;\ \sigma^2_{tit} \simeq 0$$

H_1 hypothesis

$$F = (n\sigma^2_{av}/\sigma_i^2) > 1;\ \sigma^2_{tit} > 0$$

*The number of points, n, specifies the size of the experiment and it should be constant throughout all the experiments. It is impossible, however, to foresee how many points, n_k, contribute to the definition of $\log \beta_{pqr}$. A mean value, $\bar{n} = 10$, will be assumed in the application.

Table 1. Array of data* for the determination of a constant $\log \beta_{pqr}$ in one laboratory (pqr omitted)

Data point i	Titration k				
	1	2	3	m	
1	$\log \beta(11)$	$\log \beta(12)$	$\log \beta(13)$	.	
2	$\log \beta(21)$	$\log \beta(22)$	$\log \beta(23)$	.	
3	$\log \beta(31)$	$\log \beta(32)$	$\log \beta(33)$	.	
.	.	.	.	.	
.	.	.	.	.	
n_k	.	.	.	.	
$\overline{\log \beta_{pqr}}(k)$	$\overline{\log \beta}(1)$	$\overline{\log \beta}(2)$	$\overline{\log \beta}(3)$	$\overline{\log \beta}(m)$	$\overline{\overline{\log \beta_{pqr}}}$
$\sigma^2(k)$	$\sigma^2(1)$	$\sigma^2(2)$	$\sigma^2(3)$	$\sigma^2(m)$	σ^2_{av}

$\sigma^2(k)$ = variance of $\overline{\log \beta_{pqr}}$ among points of kth titration, as calculated by the computer program.

$\sigma_i^2 = \Sigma\sigma^2(k)/m$ = variance of $\overline{\log \beta_{pqr}}$ among points for any titration, estimated.

$\sigma^2_{av} = \sum_k [\overline{\overline{\log \beta_{pqr}}} - \overline{\log \beta_{pqr}}(k)]^2/(m-1)$ = variance of the grand average

$\overline{\overline{\log \beta_{pqr}}} = \sum_k \overline{\log \beta_{pqr}}(k)/m.$

*One value of $\log \beta_{pqr}(i, k)$ from each data-pair (ml, pH).

done *separately for each titration*. On the assumption that the values of $\overline{\log \beta_{pqr}}(k)$ belong to a normally distributed population, $\overline{\overline{\log \beta_{pqr}}}$ is the central point of the distribution, with variance $\sigma^2_{av} \simeq \sigma^2_{iit}$.

As a further step, when data for the same system are available from different laboratories, a search can be made for differences between laboratories.

Consider the case that $\sigma^2_{iit} \simeq 0$ in all the laboratories. Then an array similar to that of Table 1 can be composed by putting all the

$$n_l = \sum_k n_k$$

points from one laboratory in the same column, each column l ($l = 1, 2, 3, \ldots, m'$) now representing one laboratory. At the bottom of each column we have one laboratory mean value, $\overline{\log \beta_{pqr}}(l)$, with variance $\sigma^2(l)$. The general grand average, $(\overline{\log \beta_{pqr}})_{g.av}$ is calculated for the m' laboratories and, with the same procedure as before, the analysis of variance applied to the function $\sigma^2_{g.av} = (\sigma^2(l)/n) + \sigma^2_{lab}$. In the case $\sigma^2_{lab} > 0$ (remembering that $\sigma^2_{iit} \simeq 0$), differences in apparatus or procedures of different laboratories must be searched for, the data being grouped according to common features and the possible factors of variance being analysed.

On the other hand, consider the case that $\sigma^2_{iit} > 0$ (with $\sigma^2_i/n \simeq 0$). Then the elements in one column of the array can be thought of as m_l mean values $\overline{\log \beta_{pqr}}(k)$ for each titration, with $k = 1, 2, 3, \ldots, m_l$, while each column (l) represents one laboratory. The statistical analysis is then carried out by putting $\sigma^2_{g.av} = (\sigma^2_{iit}/m) + \sigma^2_{lab}$. A mean number of titrations, $\overline{m}$, can be employed instead of m, if the laboratories do not perform equal numbers of titrations. If the H_0 hypothesis holds, then $\sigma^2_{lab} \simeq 0$ and all the $\overline{\log \beta_{pqr}}(k)$ values from each laboratory, obtained by refining each titration separately, belong to the same population and a general mean and variance can be calculated.

If the H_1 hypothesis is accepted both among titrations and among laboratories ($\sigma^2_{iit} > 0$, $\sigma^2_{lab} > 0$), then the differences between laboratories are significant as well as those between titrations, and the search for causes of error, with possible covariances, becomes a very difficult task.

EXPERIMENTAL

The experimental data are taken from the report of the so-called "Nickel–glycine Project". In that project, promoted by the Italian Group of Thermodynamics of Complexes, seven laboratories agreed to determine the equilibrium constants for the glycinate–proton and glycinate–nickel(II) systems.[8] The standard conditions required were the following: (*i*) temperature 25°, (*ii*) ionic strength $I = 1.0M$ Na(Cl), (*iii*) various Ni^{2+} concentrations, and total concentration of the reagents not more than $0.1M$ and (*iv*) other details left to free choice by the laboratories, which generally used their customary procedures and apparatus for determination of equilibrium constants. In this way the methods were varied enough to represent a significant test of the different procedures usually reported in the literature.

The methodological peculiarities of four laboratories out of the seven are reported in Table 3. These four laboratories have been chosen because their data can be treated by the same computer program. The experimental data can be found in the original publication[8] or will be supplied by the authors of this paper upon request.

RESULTS

Application of the analysis of variance

We have reprocessed the data with the computer program MINIQUAD,[4] by refining the constants separately for each titration. The results of the refinement for the glycinate protonation constants are reported in Table 4 and for the Ni(II) complex-formation constants in Table 5.

Table 3. Experimental conditions used in different laboratories

Factor	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4
pH-meter	Radiometer PHM52	Radiometer PHM4	Metrohm E 388	Radiometer PHM52
glass electrode	Radiometer G2025B	Orion 91-01	Metrohm EA 109 T	Ingold 201-NS
ref. electrode	KCl sat. Hg_2Cl_2 Radiometer K401	Ag-AgCl/1M NaCl	KCl sat. Hg_2Cl_2 Metrohm EA 414	KCl sat. Hg_2Cl_2 Ingold 330-NS
liquid junction	porous diaphragm	Wilhelm bridge, 1M NaCl	Wilhelm bridge 1M NaCl	single or double
titrant ([NaOH],M)	0.9456–0.9656	0.8443–0.8482	1.0000	0.5003–0.5014
burette	Metrohm E 415	Metrohm E 415	Microsyringe (Micrometric Inst.)	Radiometer ABU-12
titration	automatic	manual	manual	manual
cell temp. (°C)	25 ± 0.1	25 ± 0.1	25 ± 0.2	25 ± 0.1
pH-range for protonation	1.6–11.7	1.9–11.0	2.4–10.1	1.9–11.0
pH-range for complex formation	2.0–11.5	3.7–11.2	5.1–9.0	3.9–10.4

Table 4. Glycinate protonation constants: mean values, $\overline{\log \beta_{Oqr}}$, refined separately for each titration (k), and grand averages, $\overline{\overline{\log \beta_{Oqr}}}$, for each laboratory ($l$); [in parentheses, estimated standard deviations in units of the last digit(s) of $\log \beta_{Oqr}$]

k \ l	$\overline{\log \beta_{011}}$				$\overline{\log \beta_{021}}$			
	1	2	3	4	1	2	3	4
1	9.649 (1)	9.644 (1)	9.649 (1)	9.669 (3)	12.046 (2)	12.058 (2)	12.076 (3)	12.121 (3)
2	9.637 (6)	9.633 (2)	9.665 (1)	9.640 (3)	12.045 (9)	12.039 (3)	12.077 (1)	—
3	9.662 (1)	9.660 (5)	9.665 (1)	9.639 (4)	12.075 (1)	12.054 (10)	—	—
4	9.666 (1)	—	—	9.643 (4)	12.083 (2)	—	—	12.108 (6)
$\overline{\overline{\log \beta_{Oqr}}}(l)$	9.654 (13)	9.645 (14)	9.660 (9)	9.648 (14)	12.062 (20)	12.050 (10)	12.076 (1)	12.115 (9)

Table 5. Nickel–glycinate complex formation constants: mean values, $\overline{\log \beta_{pOr}}$, refined separately for each titration (k), and grand averages, $\overline{\overline{\log \beta_{pOr}}}$, for each laboratory ($l$); [in parentheses, estimated standard deviations in units of the last digit(s) of $\log \beta_{pOr}$)

k \ l	$\overline{\log \beta_{101}}$				$\overline{\log \beta_{102}}$				$\overline{\log \beta_{103}}$			
	1	2	3	4	1	2	3	4	1	2	3	4
1	5.636 (8)	5.625 (2)	5.618 (3)	5.590 (2)	10.395 (7)	10.329 (13)	10.397 (3)	10.338 (3)	13.824 (13)	14.824 (64)	13.902 (5)	—
2	5.646 (14)	5.601 (3)	5.631 (2)	5.562 (6)	10.408 (14)	10.343 (3)	10.402 (2)	10.269 (8)	13.849 (21)	13.761 (4)	13.909 (3)	—
3	5.614 (15)	5.547 (37)	5.629 (2)	5.561 (2)	10.368 (14)	10.279 (33)	10.403 (2)	10.294 (3)	13.736 (22)	13.575 (57)	13.881 (3)	—
4	5.635 (3)	5.634 (3)	5.632 (2)	5.573 (3)	10.391 (4)	10.311 (23)	10.432 (2)	10.287 (4)	13.811 (25)	14.813 (114)	13.953 (3)	—
5	5.616 (2)	5.604 (13)	5.631 (4)	5.587 (1)	10.366 (6)	10.320 (109)	10.407 (4)	10.329 (2)	—	—	13.967 (7)	—
6	5.629 (8)	5.699 (161)	5.633 (1)	5.599 (2)	10.386 (56)	10.508 (157)	10.423 (1)	10.356 (2)	—	14.448 (285)	13.940 (2)	—
7	5.664 (44)	5.582 (6)	—	5.570 (5)	10.423 (41)	10.308 (6)	—	10.254 (5)	13.965 (56)	13.695 (11)	—	13.504 (10)
8	5.635 (3)	—	—	5.566 (4)	10.376 (4)	—	—	10.271 (6)	13.870 (22)	—	—	13.522 (9)
9	5.609 (9)	—	—	5.615 (6)	10.361 (8)	—	—	10.369 (7)	13.762 (14)	—	—	13.797 (9)
10	5.630 (8)	—	—	5.688 (18)	10.384 (8)	—	—	10.510 (19)	13.794 (14)	—	—	14.263 (29)
$\overline{\overline{\log \beta_{pOr}}}(l)$	5.631 (16)	5.613 (48)	5.629 (6)	5.591 (38)	10.386 (19)	10.342 (76)	10.411 (14)	10.328 (75)	13.826 (71)	14.186 (577)	13.925 (33)	13.772 (354)

Table 6. Analysis of variance within each laboratory, (l); protonation of glycinate, $\log \beta_{Oqr}$

	$\log \beta_{011}$				$\log \beta_{021}$			
l	1	2	3	4	1	2	3	4
σ_i^2 ($\times 10^6$)	9.75	10.0	1.0	12.5	22.5	37.7	2.5	22.5
σ_{av}^2 ($\times 10^6$)	169	196	81	196	400	100	1	81
ϕ_1	3	2	2	3	3	2	1	3
ϕ_2	36	27	27	36	36	27	18	36
$F_{5\%}(\phi_1, \phi_2)$	2.86	3.42	3.42	2.86	2.04	3.42	4.43	2.86
F	173.3	196.0	810.0	156.8	177.8	26.5	4.0	36.0
σ_{tit}^2	>0	>0	>0	>0	>0	>0	>0	>0

$\bar{n} = 10$, assumed number of points; $\phi_1 = m - 1$; $\phi_2 = m(\bar{n} - 1)$; $F = \bar{n}\sigma_{av}^2/\sigma_i^2$.

Table 7. Analysis of variance within each laboratory, (l); nickel–glycinate complex formation constants, $\log \beta_{pOr}$

	$\log \beta_{101}$				$\log \beta_{102}$				$\log \beta_{103}$			
l	1	2	3	4	1	2	3	4	1	2	3	4
σ_i^2 ($\times 10^6$)	265.2	3931	6.33	45.9	545.4	5480.3	3.8	57.7	716.4	16950	17.5	275.8
σ_{av}^2 ($\times 10^6$)	256	2304	36	1444	361	5776	196	5625	4900	332929	900	122500
ϕ_1	9	6	5	9	9	6	5	9	7	5	5	3
ϕ_2	90	63	54	90	90	63	54	90	72	54	54	36
$F_{5\%}(\phi_1, \phi_2)$	2.08	2.25	2.39	2.08	2.08	2.25	2.39	2.08	2.16	2.39	2.39	2.87
F	9.7	5.9	56.9	314.6	6.6	10.5	515.8	974.9	68.4	196.4	514.2	4438.4
σ_{tit}^2	>0	>0	>0	>0	>0	>0	>0	>0	>0	>0	>0	>0

$\bar{n} = 10$, assumed number of points; $\phi_1 = m - 1$; $\phi_2 = m(\bar{n} - 1)$; $F = \bar{n}\sigma_{av}^2/\sigma_i^2$.

Table 8. Interlaboratory general grand averages of equilibrium constants, $(\overline{\overline{\log \beta_{pqr}}})_{g.av}$ for the glycinate–proton and glycinate–nickel systems; [in parentheses estimated standard deviations in units of the last digit(s) of $\log \beta_{pqr}$]

	General grand average
$\overline{\overline{\log \beta_{011}}}$	9.654(3)
$\overline{\overline{\log \beta_{021}}}$	12.076(3)
$\overline{\overline{\log \beta_{101}}}$	5.631(10)
$\overline{\overline{\log \beta_{102}}}$	10.399(10)
$\overline{\overline{\log \beta_{103}}}$	13.907(23)

$$(\overline{\overline{\log \beta}})_{g.av} = \frac{\Sigma w(l) \cdot \overline{\log \beta(l)}}{\Sigma w(l)}; \quad w(l) = \frac{1}{\sigma_{tit}^2}$$

$$\sigma_{g.av}^2 = \frac{1}{m' - 1} \cdot \frac{\Sigma w(l) \cdot [(\overline{\overline{\log \beta}})_{g.av} - \overline{\log \beta(l)}]^2}{\Sigma w(l)}$$

The analysis of variance as applied to the titrations of each laboratory is reported in Table 6 for the protonation constants, and in Table 7 for the complex-formation constants. This analysis shows unequivocally that for every constant the differences between titrations are larger than the variability within one titration, *i.e.*, in every case the H_1 hypothesis is accepted ($\sigma_{tit}^2 > 0$). If it is assumed that the $\overline{\log \beta_{pqr}}(k)$ values are normally distributed, then since the variability within points, σ_i, is much smaller than σ_{tit}, the latter can be considered as the standard deviation of $\log \beta_{pqr}$ from one laboratory.

The correct procedure for refining the equilibrium constants in this case is therefore to refine the constants separately for each titration and then average the values of different titrations.

The analysis of variance has also been applied to the possible differences between laboratories. If weighted averages are considered (Table 8), the differences between laboratories turn out to be insignificant ($\sigma_{lab}^2 \simeq 0$) both for the protonation and the complex-formation constants. The laboratories have been assigned the weightings $w(l) = 1/\sigma_{tit}^2$.

DISCUSSION AND CONCLUSIONS

The main inferences from this analysis are: (*i*) the single experimental points of the titrations do not belong to the same universe and they cannot be treated together in the refinement of the constants, (*ii*) the major source of errors is seen to be the parameters specific for each titration (E_0, standard potential, E_j, liquid-junction potential, concentrations of the reagents, *etc.*), and (*iii*) the mean values of each titration, $\overline{\log \beta_{pqr}}(k)$, belong to the same universe, in whichever laboratory. Hence the true values of the equilibrium constants can be calculated as the mean values among all the titrations of all the laboratories (Table 9). The mean values, as expected, do not differ significantly from the general grand averages of Table 8, but the standard deviations indicate a rather high experimental error. This means, from an analytical point of view, that the concentrations of the species are obtained with a lower precision than is usually assumed.

The sets of values of the equilibrium constants have been analysed to check whether they were normally distributed around the mean. The comparison was done by constructing histograms from the data of Tables 4 and 5, and testing them by means of χ^2-tables with reference to a normal distribution of $\overline{\log \beta_{pqr}}$ with mean μ and standard deviation σ_{pqr} (Table 9). The normality of the distribution has also been confirmed by other tests.[9]

The two protonation constants of glycinate ($L^- = H_2NCH_2COO^-$) and the first two of the complex-formation constants (NiL^+, NiL_2) are normally distributed. The only exception to normality is $\log \beta_{103}$ (NiL_3^-); several suggestions can be put forward to explain this, but only a thorough multifactor analysis of variance could perhaps offer a meaningful explanation.

It can be concluded that the stability constants obtained by the statistical analysis of the experimental data of the "Nickel–glycine Project" can be chosen as reference standard values. They indicate that the error in the equilibrium constants is notably higher than the precision usually reported in the literature. The

Table 9. Population of equilibrium constants from titrations of whatever laboratory; mean, μ, of $\log \beta_{pqr}$ and estimated standard deviations, σ_{pqr}, with normality test (χ^2)

			Normality test					
	μ	σ_{pqr}	n obs.	n intervals	ϕ	$\chi^2(\phi)$ 10%	χ^2	normal
$\log \beta_{011}$	9.651	0.012	14	5	4	7.78	0.86	yes
$\log \beta_{021}$	12.071	0.026	11	5	4	7.78	0.60	yes
$\log \beta_{101}$	5.615	0.035	33	6	5	4.35	4.20	yes
$\log \beta_{102}$	10.363	0.062	33	10	9	14.68	6.28	yes
$\log \beta_{103}$	13.93	0.34	24	9	8	13.36	48.11	no

ϕ = degrees of freedom; $\chi^2 = \dfrac{\Sigma(\text{observed} - \text{expected})^2}{\text{expected}}$

explanation is that the errors usually quoted are actually σ_i^2, whereas since $\sigma_{pqr}^2 = \sigma_i^2 + \sigma_{iit}^2 + \sigma_{lab}^2 + \Sigma$ covariances, a better approximation would be $\sigma_{pqr}^2 \simeq \sigma_{tit}^2$, as shown by the present analysis of variance.

At any rate, these standards offer the researcher the possibility of checking either the improvement of his experimental procedures or the dependability of a new potentiometric system. One possible way towards amendment of the experimental causes of error is to isolate the individual factors. These seem to be: (*i*) the reversibility of the glass electrode over the entire pH range and in high ionic strength solutions, (*ii*) the reproducibility of the liquid-junction potential, (*iii*) the correctness of the model to be refined, *i.e.*, the number and formulae of the species supposed to be present, (*iv*) the precision in reagent concentrations, *etc.* Some of these factors have been critically examined by the authors of the project,[8] but a precise evaluation of their possible effects on the refined constants deserves further endeavour in connection with the correctly assessed values of the standard deviations.

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DIFFUSION COEFFICIENTS AND COMPLEX EQUILIBRIA IN SOLUTION—I

THEORY: DIFFUSION COEFFICIENTS OF COMPLEX SPECIES

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Summary—It is shown that in principle it is possible to obtain values of the formation constants of metal complexes in solution from values of diffusion coefficients. It is also confirmed that, in a system of successively formed complexes, there is a linear relationship between the square root of the diffusion coefficients and the co-ordination numbers of the complexes formed. In some instances such analysis has shown that revision of some previously reported formation constant data is required.

In an earlier paper[1] it was shown that the change in polarographic diffusion current (Δi_d) of a metal ion, induced by the presence of increasing concentrations of a complexing agent ([X]), was related to the mean ligand number ($\bar{n}$) by the relationship:

$$\bar{n} = k\,\Delta i_d \qquad (1)$$

where k is a constant for a given metal–ligand system.

Equation (1) was derived theoretically by considering the effective volume change induced in an aquo-complex by successive replacement of water molecules by other ligands. The consequent change in radii of diffusing particles was related to the change in diffusion coefficient by means of the Stokes–Einstein equation and, in turn, to the change in diffusion current by means of the Ilkovič equation. The relationship was verified for a number of systems by a satisfactory comparison of mean ligand numbers $\bar{n}$, obtained by its means, with those calculated from independently determined values of formation constants covering some six orders of magnitude.

If k is determinable, data pairs $\bar{n}$, [X] become available which can, in principle, lead to estimation of the formation constants of the various complexes formed, if these are mononuclear. Further, diffusion currents are less difficult than half-wave potentials to measure directly and unlike the latter, do not depend on reversibility of the electrode processes. If equation (1) may be exploited, indicator-ion methods are not needed, and formation constants may be evaluated under exactly those voltammetric conditions used for investigating the kinetics of electrode processes. Thus, equation (2), applicable to the wave of a totally irreversible process characteristic of a particular system of complexes, can be used to estimate directly the values of the rate constant, k_e.

$$\frac{i}{i_d - i} = \frac{0.886 k_e t^{1/2}}{D^{1/2}(k_N k_{N-1} \cdots k_{K+1})[X]^{N-K}} \qquad (2)$$

where k_N, *etc.* are the stepwise formation constants for the species with co-ordination numbers N *etc.*, K being the co-ordination number of the species reacting directly with the electrode; t and D are the drop-time of the mercury electrode and the diffusion coefficient of the complex species, respectively.

If a single complex is formed, equation (3) can be used to determine its formation constant:

$$\bar{D} = \frac{D_M + D_{MX}\beta_{MX}[X]}{1 + \beta_{MX}[X]} \qquad (3)$$

where $\bar{D}$, D_M and D_{MX} are respectively the mean diffusion coefficient of simultaneously diffusing "free" (aquo) and complexed metal ions, the diffusion coefficient of the uncomplexed ion, and the diffusion coefficient of the complex; [X] is the free ligand concentration and β_{MX} the formation constant of the complex. Equation (3) is the limiting form of the general equation expressing the mean value of some parameter for simple and complexed species, and which is a function of [X], expressed in terms of the overall formation constants, β_{MX_j}, of a series of complexes, *viz.*

$$\bar{D} = \frac{\sum_0^N D_{MX_j}\beta_{MX_j}[X]^j}{\sum_0^N \beta_{MX_j}[X]^j} = \frac{\sum_0^N D_{MX_j}\beta_{MX_j}[X]^j}{1 + \sum_1^N \beta_{MX_j}[X]^j} = \frac{\sum_0^N D_{MX_j}\beta_{MX_j}[X]^j}{F_0[X]} \qquad (4)$$

For a system for which the β_{MX_j} values are known from independent experiments, and for which $\bar{D}$ values over the experimental ligand concentration range have been determined, equation (4) may be used to estimate values of the individual diffusion coefficients for all species by rearrangement as follows,

$$\bar{D}\left(1 + \sum_{1}^{N}\beta_{MX_j}[X]^j\right) = \sum_{0}^{N} D_{MX_j}\beta_{MX_j}[X]^j$$

$$= D_M + D_{MX}\beta_{MX}[X] + \cdots + D_{MX_N}\beta_{MX_N}[X]^N. \quad (5)$$

The function on the left of equation (5) may be designated $F([X], \bar{D})$ to indicate that it is a function of both the free ligand concentration and of the mean diffusion coefficient. The derived function $F_1([X], \bar{D})$, given as $\left\{\bar{D}\left(1 + \sum_{1}^{N}\beta_{MX_j}[X]^j\right) - D_M\right\}/[X]$, together with the further derived functions $F_2([X], \bar{D}), \ldots, F_N([X], \bar{D})$ may be plotted against [X], and the various products, $D_{MX_j}\beta_{MX_j}$, determined. Such an analysis closely follows that of Leden[5] and DeFord and Hume.[6] With β_{MX_j} known, the diffusion coefficient of each complex species may be estimated, provided that D_M is known.

If equation (1) is valid, there should be a linear relationship between $D_{MX_j}^{1/2}$ and j; this is indeed the case, as will be demonstrated.

In principle, it is possible to use equation (5) in the interrelated forms

$$\bar{D} + \bar{D}\beta_{MX}[X] + \ldots + \bar{D}\beta_{MX_N}[X]^N = D_M + D_{MX}\beta_{MX}[X] + \ldots + D_{MX_N}\beta_{MX_N}[X]^N \quad (6)$$

and

$$(D_M - \bar{D}) = (\bar{D} - D_{MX})\beta_{MX}[X] + \ldots + (\bar{D} - D_{MX_N})\beta_{MX_N}[X]^N \quad (7)$$

to obtain values of $\beta_{MX}, \ldots \beta_{MX_N}$ for a system for which values of $(\bar{D} - D_{MX}) \ldots (\bar{D} - D_{MX_N})$ may be estimated.

There are, however, two obstacles to this procedure. (*i*) The first derived function which follows from equation (7), *viz.*

$$\frac{D_M - \bar{D}}{[X]} = (\bar{D} - D_{MX})\beta_{MX} + \cdots + (\bar{D} - D_{MX_N})\beta_{MX_N}[X]^{N-1}, \quad (8)$$

varies particularly sharply with [X] as $[X] \rightarrow 0$. This makes it very difficult to obtain a reliable intercept from the graph of $(D_M - \bar{D})/[X]$ *vs.* [X]. The steep curvature arises from the fact that the function on the left of equation (8) is dependent not only on [X] but also on the various $(\bar{D} - D_{MX_j})$ terms, which vary sharply at low values of [X]. (*ii*) It is necessary to have some means of reliably assessing the values of the $(\bar{D} - D_{MX_j})$ terms. Since $\bar{D}$ values are determinable experimentally, the problem amounts to the assignment of values for the diffusion coefficients for individual complexes but *independently of data for the formation constants.*

An *approximate* assessment may sometimes be made in terms of the limiting (or near-limiting) value of $\bar{D}$ observed at the highest concentrations of ligand used, if these correspond to the region in which the maximum degree of complexation occurs. However, it is frequently not possible to obtain a limiting value of $\bar{D}$ reliably, the highest complex formed may not be known, and the extent of its formation, even at the highest ligand concentrations accessible, may be small. In other words, the lowest recorded value of $\bar{D}$ may not be assumed to be approaching the value of the diffusion coefficient of the highest complex formed. In any case, it is desirable to develop a method which is not dependent on values of N implied by other techniques or suggested by data for similar systems.

Since $\bar{n} = k\Delta i_d$ for any concentration of ligand, it is possible to write

$$\frac{d \ln F_0[X]}{d \ln [X]} = \bar{n} = k\Delta i_d \quad (9)$$

or

$$\frac{d \ln F_0[X]}{k\, d \ln [X]} = \frac{d \ln F_0'[X]}{d \ln [X]} = \Delta i_d \quad (10)$$

where $F_0[X]$ is the Leden function, given approximately (neglecting the appropriate activity coefficients) by

$$F_0[X] \sim 1 + \beta_1[X] + \ldots + \beta_N[X]^N \quad (11)$$

Since integration of a graph of $\bar{n}$ *vs.* ln[X] (the formation curve) provides $F_0[X]$ data, it is clear that if k can be found, approximate values of formation constants may be determined from the data $(\bar{n}, [X])$. More significantly, if k were known, $\bar{n}_{max}$ (*i.e.*, N) would be implied and, since there is known to be a linear relationship between $D_{MX_j}^{1/2}$ and j, values of the functions $(\bar{D} - D_{MX_j})$ would follow, allowing direct application of equation (7).

Approaches to the evaluation of k

(*i*) If the graph of Δi_d *vs.* [X] *clearly* reaches a limit at high [X] and if $\bar{n}_{max}$ may be inferred, by whatever means, then approximate values of $\bar{n}$ may be assigned to each value of [X]. This is not to be regarded as of general applicability or reliability.

(*ii*) The "pseudo-formation curve", *i.e.*, the graph of Δi_d *vs.* ln[X], may be integrated to provide $F_0'[X]$ data for the range of values of [X]; see equation (10). A series of Leden graphical analyses may then be performed at chosen integral (or intermediate) values of k to establish which gives a sensible family of derived curves. By these means it will be shown that it is possible to establish k to, at least, the nearest half-integral value. Any significant error in k would add to

the inherent cumulative errors involved in the estimation of higher β values. Such analysis, however, is capable of giving a reliable indication of the number of complex species formed.

(*iii*) From equations (9) and (10) it follows that

$$\left[\frac{\mathrm{d}\ln F_0[\mathrm{X}]}{\mathrm{d}\ln[\mathrm{X}]}\right]_{\max} = \bar{n}_{\max} = N \qquad (12)$$

and

$$\left[\frac{\mathrm{d}\ln F_0[\mathrm{X}]}{k\,\mathrm{d}\ln[\mathrm{X}]}\right]_{\max} = \frac{\bar{n}_{\max}}{k} = \left[\frac{\mathrm{d}\ln F_0'[\mathrm{X}]}{\mathrm{d}\ln[\mathrm{X}]}\right]_{\max}. \qquad (13)$$

Thus the limiting slope of the plot of $\ln F_0'[\mathrm{X}]$ *vs.* $\ln[\mathrm{X}]$, at the highest values of [X], provides an estimate of k when $\bar{n}_{\max}$ is known. Further, it indicates the range of values of [X] where $n_{\max}$ is most closely approached, which is also implied by (*ii*).

In this paper attention is confined to the verification and implications of the linear relationship between the square root of the diffusion coefficient of a complex and its co-ordination number with respect to the ligand used.

In subsequent papers of this series the assessment of k values for a range of metal–ligand systems and the direct evaluation of overall formation constants will be described. Such an analysis will be shown to be a necessary preliminary to the exploitation of equations (7) and (8), both graphically and analytically, by means of $(\bar{D} - D_{\mathrm{MX}_j})$ data.

EXPERIMENTAL

In a number of instances, mean diffusion coefficient data estimated from diffusion current values reported in the literature for metal–ligand systems have been used. In other cases, values of diffusion current and capillary characteristics measured by manual polarography at a working temperature of 25° were used. Analytical-grade metal salts (preferably nitrates) were used, and reagent-grade ligands. The metal ion concentration was $5 \times 10^{-4} M$, and the concentration range of the various ligands was dictated by both their strength as co-ordinators and their solubility in water (for benzimidazole 50% v/v aqueous methanol was used as solvent). Potassium nitrate solution (0.1 *M*) was used as supporting electrolyte throughout. Further details of the current and potential measurements were given in an earlier paper.[1]

RESULTS AND DISCUSSION

Determination of diffusion coefficients for individual complexes

Cadmium–benzimidazole. The various functions and derived functions are presented in Table 1. The values of the stability constants quoted are those reported previously,[1] which were calculated from the measured shift in half-wave potential.

Cadmium–thiourea (and substituted thioureas). For these systems the smoothed diffusion current data reported by Lane *et al.*[7] were used in order to estimate the various $\bar{D}$ values for the range of concentrations of ligand. The values of the function

$$\left(1 + \sum_{1}^{N} \beta_j [\mathrm{X}]^j\right)$$

were taken as the $F_0[\mathrm{X}]$ values obtained by Lane from shifts in half-wave potential of the cadmium ion. In some instances the stability constant values used in the estimation of the diffusion coefficients were those obtained by re-analysis of Lane's potential data, and these were rather different from those reported earlier. This point is enlarged upon later.

The linearity of the plots of $D_{\mathrm{MX}_j}^{1/2}$ *vs.* j for the five systems studied is shown in Fig. 1. A comparison of the estimated values of D for the highest complexes formed and those of the limiting values of $\bar{D}$ in each system is worthwhile (Table 2). Whereas for the complexes with the various thioureas these values of D and $\bar{D}$ are a close match, this is clearly not so for benzimidazole. For the latter case, not only is there no *a priori* reason for assuming that $\bar{n}_{\max} = 3$, but the limiting value of $\bar{D}$, in fact, lies much closer to the

Table 1. Analysis for diffusion coefficients of species present in the cadmium–benzimidazole system

[BzIm], *M*	$\bar{D}$, 10^{-6} *cm²/sec*	$1 + \Sigma\beta_j[\mathrm{X}]^j$	$\bar{D}[1 + \Sigma\beta_i[\mathrm{X}]^j]$, 10^{-6} *cm²/sec*	$F_1[\mathrm{X}, \bar{D}]$	$F_2[\mathrm{X}, \bar{D}]$*	$F_3[\mathrm{X}, \bar{D}]$*
0.00	7.20	1	7.20	—	—	—
0.01	6.59	2.15	14.14	694	—	—
0.02	6.25	3.94	24.65	873	16900	—
0.03	6.04	6.49	39.2	1067	17733	77767
0.04	5.89	9.86	58.1	1273	18450	76250
0 05	5.78	14.20	82.1	1498	19260	77200
0.06	5.70	19.53	111.3	1735	20000	76667
0.07	5.64	26.0	146.7	1993	20829	77557
0.08	5.60	33.6	188.4	2265	21625	77813
	Intercept		7.20	535	15400	77209
	β_{MX_j}		1	85	2800	15500
	D_{MX_j}, 10^{-6} *cm²/sec*		7.20	6.29	5.50	4.98
	$D_{\mathrm{MX}_j}^{1/2}$, 10^{-3} *cm/sec*$^{1/2}$		2.68	2.51	2.35	2.23

*The numbers given are those obtained by the arithmetic manipulation of the data, and have not been rounded off to indicate the uncertainty.

Table 2. Comparison of diffusion coefficient values of individual complexes and the limiting value of the mean diffusion coefficient for the various systems

System	D^*_{MX}	$D^*_{MX_2}$	$D^*_{MX_3}$	$D^*_{MX_4}$	$(\bar{D})^*_{lim}$
Cd–BzIm	6.29	5.50	4.98	—	5.60
Cd–TU	6.75	6.37	6.00	5.55	5.57
Cd–MTU	6.00	4.93	3.96	3.13	3.09
Cd–DMTU	5.76	4.54	3.84	2.50	2.53
Cd–ETU	5.71	4.45	3.39	2.43	2.43

*Units 10^{-6} cm^2/sec.

value of D for the complex with co-ordination number 2. This would suggest that at the highest values of ligand concentration, the species MX_2 predominates over MX_3. Calculation of $\bar{n}$ at the highest ligand concentration from *known* stability constants bears this out, a value slightly in excess of 2 being obtained.

A fairly stringent test of the validity of the equations involving $\bar{D}$ would be to estimate the values of $\bar{D}$ expected on the basis of independently determined $F_0[X]$ data. For the systems reported here, $F_0[X]$ data are available from half-wave potentials, since in all cases reversible reductions occur. In turn, recalculations of $F_0[X]$ data from the values of formation constants obtained by graphical analysis give some indication of the validity of the slopes and intercepts judged from the graphical analysis. For the cadmium–benzimidazole system, good agreement (Fig. 2) is obtained between the values of $\bar{D}$ obtained from diffusion currents, and the values estimated from D_{MXj} and $F_0[X]$ data by means of equation (4).

Such good agreement was not found for the cadmium–thiourea system (Fig. 3) when it was treated in this manner; there were large discrepancies, particularly in the ligand concentration range 0.1–0.4*M*. When Lane's values for the formation constants were fed back into the defining equation for $F_0[X]$, there were again large discrepancies (Table 3). This indicates either that the values of some lower constants were in error (and mere inspection suggests that the sequence $\beta_1 = 24$; $\beta_2 = 51$; $\beta_3 = 40$; $\beta_4 = 3590$ reported by Lane is rather unusual) or that the $F_0[X]$ data were in error, which implies significant error in $\Delta E_{1/2}$. The latter would seem unlikely on two counts. (*i*) The overall shifts in half-wave potential reported by Lane are quite large, so significant errors are less likely for this system than for many others; in any case the larger and hence more precisely determinable shifts are always observed for lower ligand concentrations and it is just here that the largest discrepancies occur. (*ii*) The limiting linear section of the $\Delta E_{1/2}$. *vs.* log[X] graph provides a value of 3300 for β_4, which agrees quite favourably with Lane's figure.

Graphical re-analysis of Lane's $\Delta E_{1/2}$ data gave $\beta_1 = 20$; $\beta_2 = 100$; $b_3 = 400$; $\beta_4 = 3000$ (with estimated maximum errors of ± 5; ± 40; ± 100; ± 800 respectively). $F_0[X]$ data recalculated with these revised figures were closer to those obtained experimentally. When these β data, combined with interpolated values of D_{MX_j} and the values of $F_0[X]$ estimated from $\Delta E_{1/2}$, were used to obtain values of $\bar{D}$,

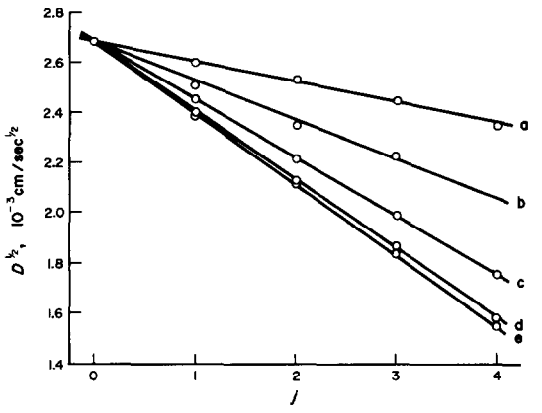

Fig. 1. Graphs of square root of diffusion coefficient of complexes as a function of the number of ligands, *j*, bound to the central metal ion (Cd^{2+}). (*a*) Thiourea; (*b*) benzimidazole; (*c*) methylthiourea; (*d*) dimethylthiourea; (*e*) ethylthiourea.

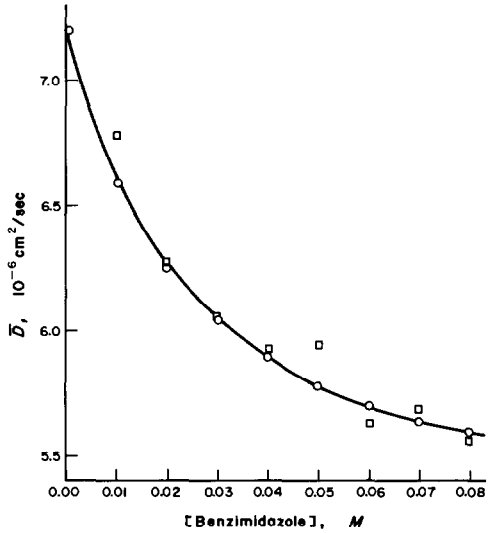

Fig. 2. Comparison of variation of $\bar{D}$ values with concentration of benzimidazole: ○ from observed diffusion currents; □ calculated by means of equation (4).

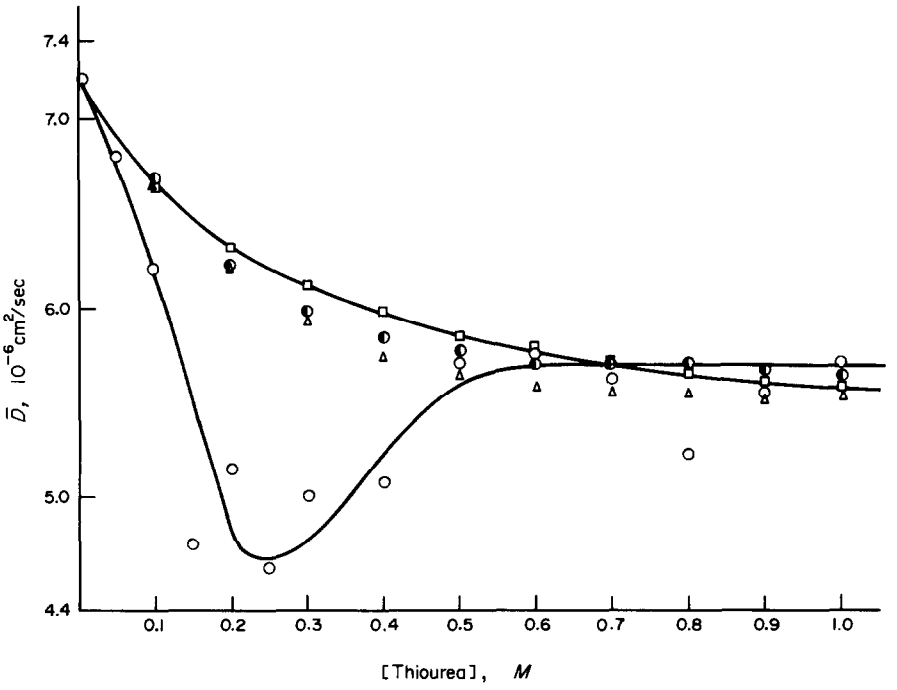

Fig. 3. Comparison of values of $\bar{D}$ for the system cadmium–thiourea, obtained by various means: ○ from F_0 and β data (after Lane *et al.*);[7] △ from F_0 data (after Lane *et al.*)[7] and recalculated β data; □ from diffusion currents measured experimentally; ◑ from F_0 and β data (after Crow).

closer agreement with experimental values was obtained (Fig. 3). It is clear that for the limited number of systems investigated, the stepwise replacement of water molecules in an aquo-complex by other ligands produces a regular decrease in diffusion coefficient. However, to exploit equation (7) for the estimation of formation constants, it is clearly necessary to be able to estimate D_{MX_j} values without prior assumptions regarding the degree of co-ordination. The case of benzimidazole shows how misleading such assumptions might be.

In the most general case where irreversible reductions are encountered and $\Delta E_{1/2}$ data are without thermodynamic significance, only diffusion currents or related parameters are available for manipulation. Clearly it is necessary to obtain at least preliminary values of (*i*) the maximum degree of co-ordination and (*ii*) the *actual* degree of co-ordination at the highest ligand concentrations used experimentally. In essence, this comes down to estimating the value of k [equations (9) and (10)] for given systems; this will be discussed in subsequent papers in this series.

Table 3. Comparison of various values of F_0[X] for the system cadmium–thiourea: 1, from $\Delta E_{1/2}$, 2, calculated from β values due to Lane; 3, calculated from values reassessed by Crow

	F_0[X]		
[TU], *M*	1	2	3
0.00	1.00	1.00	1.00
0.05	2.39	2.35	2.32
0.10	4.66	4.31	4.70
0.15	10.50	7.71	9.2
0.20	16.8	13.9	17.0
0.25	32.2	24.8	30.2
0.30	50.7	43	51.0
0.40	128	113	127
0.50	253	255	274
0.60	492	508	524
0.70	908	919	921
0.80	1638	1543	1514
0.90	2445	2449	2306
1.00	3620	3706	3520

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DIFFUSION COEFFICIENTS AND COMPLEX EQUILIBRIA IN SOLUTION—II

APPROXIMATE EVALUATION OF FORMATION CONSTANTS

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Summary—It is shown, for four systems containing complexes of widely differing stabilities, that a very fair assessment of the values of formation constants is obtainable from the data pairs Δi_d, log [X] (the pseudo-formation curve). This is seen as a preliminary exercise to the more precise analysis which is possible in terms of mean diffusion coefficients which feature in equations reported earlier.

In the previous paper[1] the linear relationship between the square root of the diffusion coefficient of a complex species and its co-ordination number with respect to the ligand used, was verified (at least for the case of uncharged ligands). In this paper attention is focused on the problem of assessing $F_0[X]$ functions from changes in the diffusion current of a metal ion in the presence of increasing amounts of complexing agent.

The four systems analysed have the common feature that nearly reversible reductions take place at the dropping mercury electrode under all conditions used. Thus, values of formation constants for comparison purposes are available from independently determined half-wave potentials. Further, the systems all show the development of a maximum co-ordination number of four but differ very considerably in the *range* of β_4 values, which cover some seven orders of magnitude.

The experimental conditions used were those given in the earlier paper.[1]

The approximate calculation of formation constants by the method described is a necessary preliminary in the more precise analysis which is possible in terms of diffusion coefficients,[1] but requires a reliable indication of the extent of complexation at the highest ligand concentrations used. This makes it possible to assess k in the relationship

$$\frac{\mathrm{d}\log F_0[X]}{k\,\mathrm{d}\log[X]} = \Delta i_d = \frac{\mathrm{d}\log F'_0[X]}{\mathrm{d}\log[X]} = \frac{\bar{n}}{k} \qquad (1)$$

RESULTS AND DISCUSSION

Figure 1 shows the variation of Δi_d with log[ligand] for the four systems investigated. It is the *relative* variation of Δi_d with log[ligand] that reflects the magnitudes of the formation constants characteristic of a given system. This is most clearly shown by comparing the relative positions of the pseudo-formation curves for a lead–thiourea system (showing the weakest complexes) and the cadmium–imidazole system (showing the strongest complexes).

The units in which i_d and Δi_d are measured are quite arbitrary, since the product $k\Delta i_d$ is dimensionless.

Cadmium–thiourea

Figure 2 shows plots of $k\log F'_0[X]$ *vs.* log[X] for integral values of k from 1 to 4, $\log F'_0[X]$ being estimated from integration of the pseudo-formation curve. In the graph for $k = 1$, the limiting slope at log[thiourea] = 0 is ~ 1.1; if it is assumed that at this ligand concentration maximum co-ordination is achieved, then from equation (1) the limiting slope is $\sim n_{max}/k$, so if $n_{max} = 4$, $k \sim 3.6$.

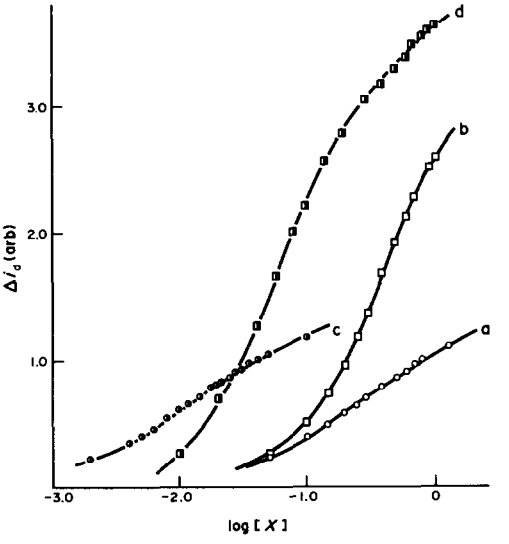

Fig. 1. Variation of Δi_d with log[ligand] (pseudo-formation curves) for the systems: (a) cadmium–thiourea, (b) lead–thiourea, (c) cadmium–imidazole, (d) cadmium–ethylthiourea (arbitrary unit for $\Delta i_d \approx 1$ μA).

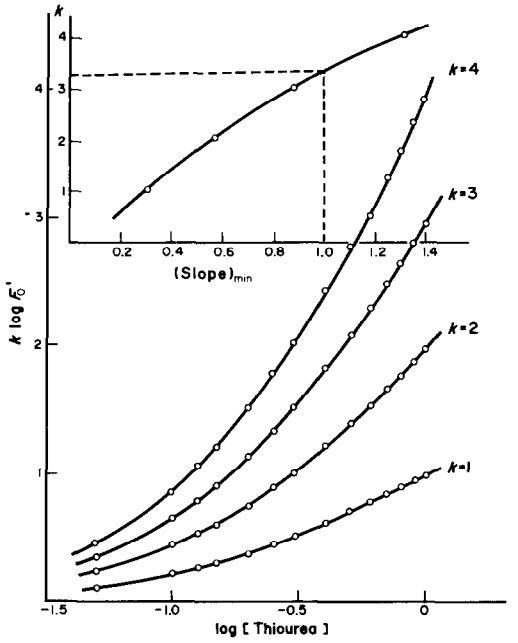

Fig. 2. Variation of $k\log F'_0[X]$ with log[thiourea] for integral values of k from 1 to 4. Inset shows graph of k *vs.* limiting slope at log[thiourea] = −1.3.

If it may be assumed that only the first complex predominates at the lowest ligand concentrations used, then in the region of log[thiourea] ~ −1.3, $\bar{n}$ is ~1. If k is plotted *vs.* the minimum slopes of the four $kF'_0[X]$ plots, the operative k value may be taken as that at which the minimum slope is approximately unity. Such a plot is inset in Fig. 2, and indicates that k is ~3.3. Clearly the consistency of these estimates at least suggests that the value of k lies between 3 and 4.

Consequently, a Leden-type analysis can be made for the $kF'_0[X]$ functions obtained with values of k in the range 3–4; the various derived functions are plotted in Figs. 3–6. At the stage of the F_3 plots (Fig. 5) it is much clearer where the true value of k lies. Here there is increasing positive and negative divergence from linearity on either side of the approximately straight line corresponding to $k = 3.63$. The same

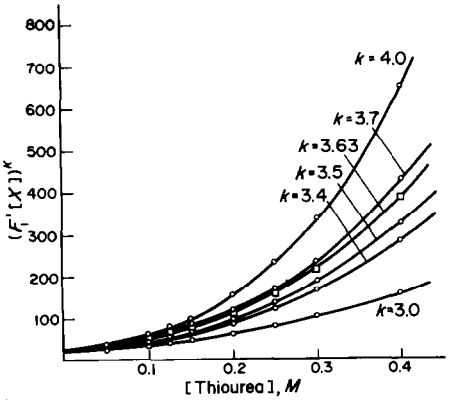

Fig. 3. $(F'_1[X])^k$ *vs.* [thiourea] curves for k values between 3 and 4.

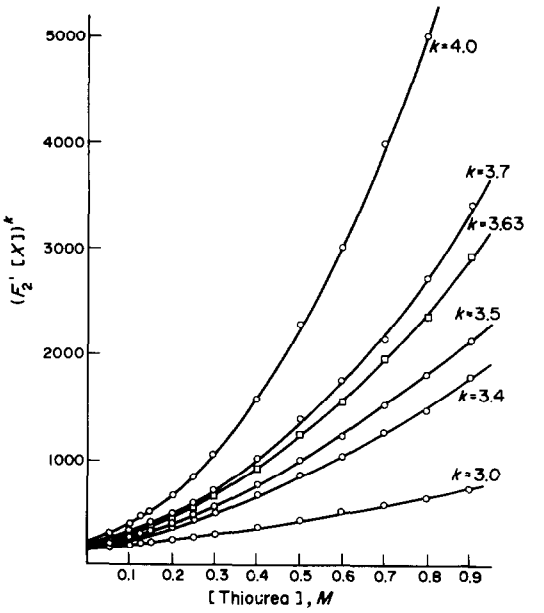

Fig. 4. $(F'_2[X])^k$ *vs.* [thiourea] curves for k values between 3 and 4.

trends are even more clearly seen in the F_4 plots (Fig. 6).

Clearly the assignment of a precise value of k before the Leden analysis is often not possible, but neither is it crucial. Though a rough guide to the value of k is initially helpful, a series of analyses covering a range of values of k is more useful, giving clearer resolution of the more nearly correct k-values and aiding assessment of the relative errors of the β-values obtained. The β-values obtained in this way and those reported in the previous paper[1] (and recalculated from Lane's half-wave potential data[2]) are given in Table 1.

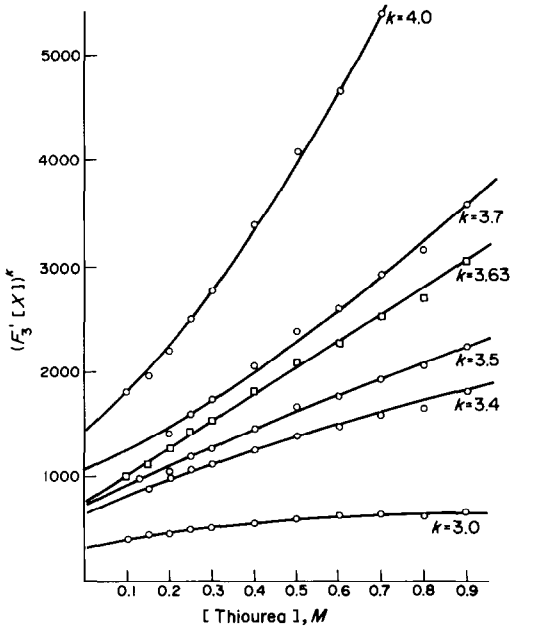

Fig. 5. $(F'_3[X])^k$ *vs.* [thiourea] curves for k values between 3 and 4.

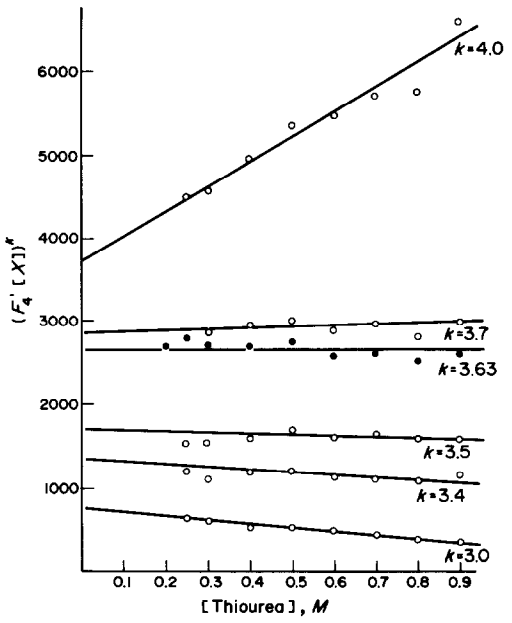

Fig. 6. $(F'_4[X])^k$ *vs.* [thiourea] curves for k values between 3 and 4.

Lead–thiourea

Although the β-values are expected to be considerably lower than those for the corresponding cadmium complexes, as indicated by the very small $\Delta E_{1/2}$ values reported by Lane, the variation in i_d over the same ligand concentration range is almost three times that observed for cadmium (Fig. 1). On the assumption that a maximum co-ordination number of 4 is approached at the highest ligand concentration, a value of 1.44 is estimated for k. The β-values agree favourably with those obtained by Lane (Table 1).

Cadmium–ethylthiourea

This system gave the largest changes in diffusion current, yielding a proportionately smaller value of 1.11 for k. Although the value of β_4 obtained was of the same order as that reported by Lane,[2] the other constants differed considerably from the earlier values. No re-examination of the earlier calculations, using half-wave potential data, has been attempted for this case, but it is perhaps worth comparing the stepwise constants obtained by the two methods (Table 2). The data resulting from the diffusion-current calculations show a more consistent trend.

Cadmium–imidazole

Relatively small current changes were observed, but it is immediately clear from the position of the pseudo-formation curve that the formation constants considerably higher than those of the other systems investigated. The stepwise constants (Table 2) are seen to compare very favourably with those reported by Tanford and Wagner.[3]

CONCLUSIONS

The general validity of this method of analysing pseudo-formation curves has been demonstrated for neutral ligands. The most obvious application is to systems giving irreversible diffusion-controlled polarographic waves and this will be reported on later. Further, the way is now clear to apply the equations[1] based on mean diffusion coefficients. It will be shown that the serious cumulative errors which are inherent in the Leden analysis may be substantially reduced. A practical limitation to this application is the lack of precision in the available diffusion-current data in the literature and indeed in those obtained by the author

Table 1. Values of overall formation constants for the cadmium–thiourea and lead–thiourea systems

System	β_1	β_2	β_3	β_4
Cadmium–thiourea				
present work	20 ± 5	180 ± 20	800 ± 100	2500 ± 500
previous work	20 ± 5	100 ± 40	400 ± 100	3000 ± 800
Lead–thiourea				
present work	6	13	90	109
previous work	4	11	95	110

Table 2. Stepwise formation constants for the cadmium–ethylthiourea and cadmium–imidazole systems

System	log k_1	log k_2	log k_3	log k_4
Cadmium–ethylthiourea				
present work	1.85	1.00	1.30	0.63
previous work	1.40	0.60	1.00	1.49
Cadmium–imidazole				
present work	2.85	2.27	1.66	1.13
previous work	2.80	2.10	1.55	1.17

with conventional methods. This is because many more data points are required—the minimum is 20, covering the range of ligand concentration, particular importance being attached to those at lower ligand concentrations—and it is necessary to measure changes in diffusion current to ± 1 nA. A technique for accomplishing the necessary precision has been devised and will be reported in a later communication.

In this way it is possible not only to obtain results which are clearly superior to those obtained from half-wave potentials but also to obtain results when $E_{1/2}$ data cannot be used because of irreversibility of the electrode process.

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SELECTIVE EXTRACTION OF METAL IONS ASSOCIATED WITH HUMIC ACIDS

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Summary—The ability of a range of electrolyte solutions to release metal ions (Cu, Pb, Cd, Zn) presorbed on two samples of humic acid has been investigated. Though treatment with mineral acid or a chelating agent released a high proportion of the retained metal ion, recoveries were never total. Concentrated salt solutions displaced about 80% of the retained Cd or Zn, and about half of any Cu or Pb held by the organic matter, which indicates that most of the adsorbed metal ion is exchangeable, the extraction efficiency being controlled by competing equilibria. The effect of added clay suspensions was also examined. Analytical procedures for fractionating the total metal content of soils into subgroups have been assessed against the observed extraction behaviour.

Only a fraction of the total metal content of soils and sediments tends to be available for uptake by plants or biota. This fraction is generally associated with the "colloidal" material (*i.e.*, clay minerals, hydrous oxides and organic matter), but views differ on the relative effects of the individual components. Opinions also vary in respect of the most suitable procedure(s) for evaluating "available" levels. A widely adopted approach is extraction with a chemical solution, and a wide variety of active constituents has been proposed, ranging from acids (strong or weak) to complexing agents or salt solutions of different types. Further, as reported in a recent review,[1] the correlations obtained between extraction values and plant uptakes tend to be sensitive to the species of plant grown and type of soil used. There is also uncertainty about whether different reagents release metal ions from the same or from different types of binding sites.

By making arbitrary assumptions about the behaviour of metal ions associated with different components of a sample, when exposed to solutions of varying reactivity, it is possible to propose sequential procedures which theoretically fractionate the total metal content into subcategories. Some investigators have been satisfied with a simple division (*e.g.*, between detrital and non-detrital) while others[2–7] have been more ambitious and have sought to identify several fractions, loosely classified as ion-exchangeable, weakly adsorbed, associated with organic matter, precipitated, retained by hydrous oxides, *etc.* The order of attack adopted varies between authors, owing, in part, to limited understanding of some or all of the competing equilibria involved.

The relative efficiency of various reagents in retrieving Cu, Pb, Cd or Zn ions presorbed onto clay suspension has been investigated.[8] It was found that a few extractants (*e.g.*, EDTA, oxalic acid) recovered all the adsorbed metal ion, but in most of the systems examined the extraction yield varied with the type of clay, the metal ion, and the pH and concentration of the extractant, as well as with the pH during the initial sorption stage.

Recent studies have shown that the adsorption capacity of a common organic component (humic acid) can exceed that of clay minerals. A change in pH can cause marked changes in the uptake of metal ions by such humic acids[9] or humic acid–clay mixtures[10], and we therefore deemed it appropriate to examine the effect of various electrolytes on the organic acid–metal ion equilibria, with a view to clarifying the situation.

EXPERIMENTAL

Humic acid samples

The solids used in the experiments were technical grade products purchased from Fluka AG (HA I) and Aldrich Chemical Co. (HA II). Uptake of hydrogen ions by HA I was small but about 0.8 mole/kg for HA II. Acid washing of HA I (13% ash) and HA II (20%) ash released cations to the following extent (expressed as mmole/kg, HA II values in parentheses): Na^+ 420 (13); Al^{3+} 67 (280); Ca^{2+} 38 (460); Mg^{2+} 20 (80); Fe^{3+} 6 (22); Zn^{2+} 0.2 (3). The adsorptive capacity of the two organic acids for metal ions varied with pH, but at around pH 5 the values (mole/kg) obtained[9] for HA I and HA II respectively were Pb^{2+} 1.0 and 0.7, Cu^{2+} 0.8 and 0.5, Zn^{2+} 0.6 and 0.3, Cd^{2+} 0.5 and 0.25. At pH > 8, the metal–humate complexes of HA I tended to become soluble. Higher pH values were required for dissolution of metal–HA II species.

Extraction studies

Aqueous suspensions of the two humic acids were prepared, and aliquots (5.0 ml, containing about 3 mg of solid) were transferred into clean, acid-washed 30-ml glass vials followed by 10.0 ml of $5 \times 10^{-4} M$ metal nitrate solution and enough doubly demineralized water to give a final volume of 30.0 ml. The vials were sealed and transferred into an end-over-end stirrer device immersed in a water-bath held at 25°. After 24 hr of mixing and equilibration, the contents of the vials were filtered with 25-mm diameter 0.45-μm pore membrane filters. The metal contents of the

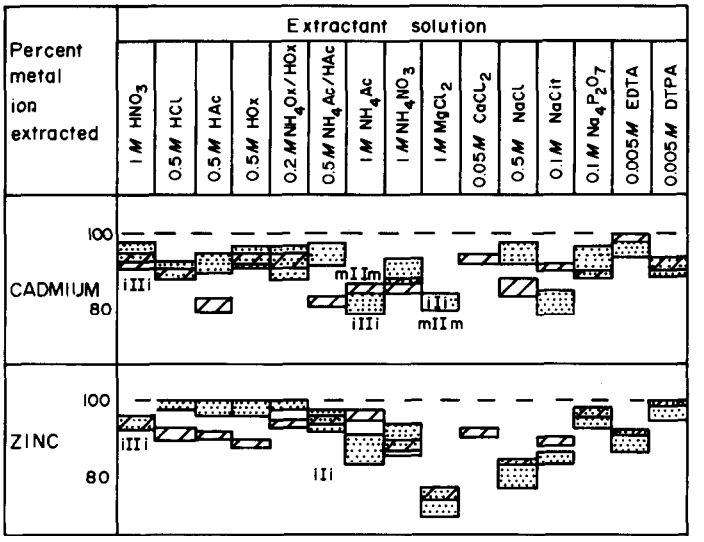


Fig. 1. Percentage of adsorbed Cd or Zn ions extracted from humic acid suspensions by electrolyte solutions. ▨ Humic acid I; ▩ Humic acid II. Symbols indicate mean ± S.D. The lettered entries indicate systems in which addition of clay particles (i = illite, m = montmorillonite) altered the recovery level.

filtrates were then determined (by atomic-absorption spectrometry under appropriate conditions) in order to ascertain the amounts of metals retained by the solid.

The filters were carefully transferred back into the original vials, and 30.0 ml of the chosen extractant were added. The vials were again sealed, transferred to the stirrer and allowed to equilibrate for 24 hr before filtration of the contents with fresh 0.45-μm membranes, Analysis of the filtrates permitted calculation of the percentage of adsorbed material released.

To permit statistical examination of the data, and allow for occasional leakages or losses, replicate studies (generally 10) were made for each of the 16 extractant solutions.

Since the extractants (listed in Figs. 1 and 2) varied greatly in chemical composition and concentration, individual calibration curves were prepared for each extraction system. The atomic-absorption spectrometer used (an updated Varian-Techtron AA5) had background-correction facilities which compensated for some salt effects, but the solution matrix had a significant influence on the slope of Cu and Pb calibration graphs, in particular.

Total loading effects

In another series of studies, the pH of the humic acid suspensions was increased by adding sodium hydroxide. This led to greater uptake of metal ion by the solids in the adsorption stage, as shown in Table 1, but few changes in the degree of extraction by a selected range of solutions. The reverse effect, *i.e.*, smaller total uptake, was achieved by having less metal ion present in solution during the adsorption step, *viz.* by using solutions which were initially 0.5 or 1.0×10^{-4} *M* in metal nitrate, instead of the 1.67×10^{-4} *M* used in the majority of tests.

Clay–humic acid mixtures

Since the adsorptive capacity of clay–humic acid mixtures is not always additive,[10] extraction studies were also made with solid suspensions which contained both components (*i.e.*, 5.0 ml containing 2.5–2.8 mg of humic acid and *ca.* 10 mg of Na^+-form montmorillonite or illite). The presence of the clay increased the time required for filtration, but had little effect on the precision achieved or the percentage of total sorbed metal ion extracted (*cf.* Figs. 1 and 2).

Destructive oxidation

Portions of humic acid (*ca.* 3 mg of solid) and metal nitrate solution (5 μmole of M^{2+}) were treated with 10 ml of 1*M* nitric acid and two successive 5-ml volumes of 30% hydrogen peroxide, with warming to ensure rapid oxidation of the organic matter. The resultant clear solutions were then filtered and analysed for metal content.

RESULTS AND DISCUSSION

Extraction of zinc and cadmium

It can be observed from Fig. 1, that most of the extractants tested displaced more than 80% of the presorbed Zn or Cd ions.

Humic acid I (symbol ▨) retained the sorbed material to a slightly greater extent in acid media, but released marginally greater amounts to the complexing agents. The alkaline complexing solutions (*i.e.*, citrate, pyrophosphate, EDTA, DTPA) appeared to dissolve or colloidally disperse the humic acids, but there was a residual > 0.45-μm fraction which retained a measurable amount of the sorbed ion (this observation is consistent with adsorption/pH trends observed in earlier studies[9]).

No attempt was made to reduce the ash content of the humic acid samples, since drastic purification methods can cause abnormal changes in humate characteristics, and it was believed that most organic acids in their natural environment would be in salt form and associated with other colloidal matter. Though competition from displaced cations may have contributed to the smaller uptake by HA II in the adsorption stage (as shown in Table 1) any residual counter-ions should have had little effect on the metal-ion extraction step.

The two humic acids released different proportions of the adsorbed metal ions into most of the extrac-

Table 1. Effect of pH and metal salt concentration on the amount of metal ion sorbed*

Solid suspension		Initial $[M^{2+}]$, m*M*	Copper		Lead		Zinc		Cadmium	
Humic acid†	Clay type§		pH	% Sorbed	pH	% Sorbed	pH	% Sorbed	pH	% Sorbed
I	—	0.167	4.1	50	4.1	50	4.7	35	4.0	30
	—	0.167	—	—	—	—	5.2	40	4.3	35
	—	0.167	—	—	—	—	—	—	4.9	45
	+ Illite	0.167	5.5	80	5.2	80	6.5	50	5.9	60
	+ Montmorillonite	0.167	4.5	80	4.4	85	6.8	80	6.8	90
II	—	0.050	5.7	95	6.1	90	5.2	50	5.2	65
	—	0.100	5.1	65	5.4	75	5.1	35	5.1	45
	—	0.167	4.7	45	4.6	45	5.5	25	5.4	30
	—	0.167	6.5	85	5.8	80	6.8	40	6.7	50
	+ Illite	0.167	5.8	95	5.8	90	6.2	40	6.7	75
	+ Montmorillonite	0.167	6.2	90	5.8	90	6.6	65	6.5	75

*With $[M^{2+}]$ initially $1.67 \times 10^{-4}M$, 100% adsorption with HA alone corresponds to *ca.* 1.85 mole of M^{2+} per kg of HA. Each 10% increase in sorption due to presence of clay (similar pH) corresponds to *ca.* 45 mmole of M^{2+} sorbed per kg of clay.

†2.6–2.8 mg of HA added to 30 ml of M^{2+} solution.

§Weight of clay present: 10 mg (illite) or 11 mg (montmorillonite).

tants studied (*cf.* Fig. 1 and 2) but these variations have been attributed to differences in chemical structure, that is, the type and spatial location of the functional groups attached to the organic moeity. The chemical reactivity of humic acid samples is usually attributed to constituent carboxylic acid groupings, in particular these located *ortho* or *meta* to phenolic groups or a second carboxylic acid group, with contributions from other functional groups such as $-NH_2$ or –SH. Interaction between metal ions and humic acids has been shown to yield both 1 : 1 species and 1 : 2 complexes.[11–13] In the 1 : 1 forms, the metal ion is either retained through a salt-type linkage (*i.e.*, $RCOO^-M^{2+}\ldots$) or co-ordinated to appropriately

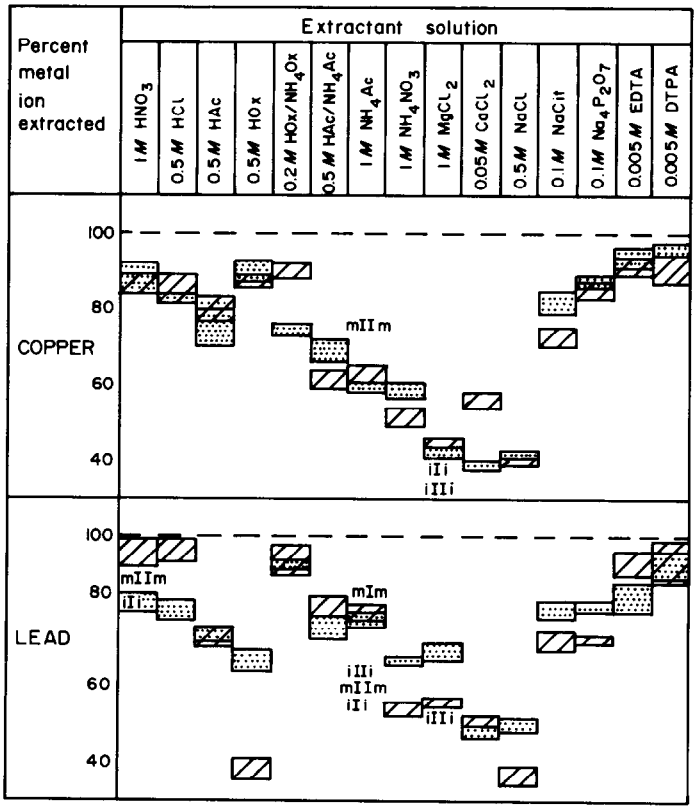
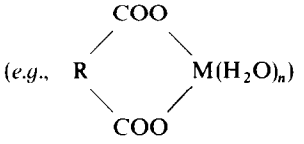


Fig. 2. Percentage of adsorbed Cu or Pb ions extracted from humic acid suspensions by electrolyte solutions. Symbols as for Fig. 1.

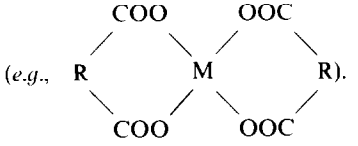
Table 2. Extraction sequences for the subdivision of the total metal content of soils

Cu^2	Zn,Mn,Cu^6	Cd,Co,Cu,Ni,Pb,Zn^5 Fe,Mn	Cu,Zn,Fe,Mn,Mo^7
"Available" ($CaCl_2$)	or "Exchangeable" ($MgCl_2$) (or NaAc)		Sulphides & bound to organic matter (NaOCl)
Weakly bound, specific sites (HAc)		Bound to carbonates (HAc/NaAc)	Adsorbed ions, soluble carbonates (HCl)
Bound to organic matter ($Na_4P_2O_7$)	(H_2O_2)		
	Bound to hydrous oxides of iron and manganese (HOx/NH_4Ox)		($NH_2OH.HCl$)
		($Na_2S_2O_4$/citrate/HCO_3^-)	
		Bound to organic matter (H_2O_2)	
Residual (lattice) components (HF)	Sand (totals) Silt (totals) Clay (totals) Soil (totals) ($HCl/HNO_3/HF$)	Residual solids (HCl/HF)	Silicate minerals ($HNO_3/HClO_4$)

located pairs of functional groups



In the 1:2 complexes, the metal ion becomes fully co-ordinated to functional groups



Because of the polyfunctional nature of the humic materials, extended chains can also develop. Experimental conditions can influence the amount of each type formed, which partially explains why views differ on the relative order of stability for metal humate complexes. Proposed sequences include Cu > Pb ≫ Cd > Zn;[12,14] Pb > Cu > Ni > Cd;[15] and Cu ≫ Zn > Pb.[16]

In an earlier study,[9] which involved reactant ratios similar to those used in this investigation, it was found that every μmole of M^{2+} sorbed by the organic sample released either 1.3 μmole of H^+ (HA I) or 1.1 μmole (HA II). These values imply that the predominant reaction is formation of 1:1 species, a conclusion supported by the effectiveness of salt solutions in displacing the adsorbed metal ions.

As noted above, Cd and Zn humate complexes are less stable than those of Cu and Pb, and comparison of Figs. 1 and 2 shows that this was reflected in the amount displaced by the different reagents. The bonding of the Cd and Zn appears to be predominantly electrostatic (*i.e.*, salt-type linkages), since most of the adsorbed material was displaced by competing cations (*i.e.*, NH_4^+, Na^+, Ca^{2+}, Mg^{2+}). Protons from acid solutions were slightly more effective (owing to their greater affinity for the functional groups involved) and solutions containing chelating agents also gave higher recoveries (partly because of the stability of the metal chelates formed, and partly owing to dissolution of the solid). The marginal differences in the amount of Cd or Zn extracted by many of the displacing reagents imply that Zn was not quite as strongly bonded as Cd.

The total, or near total, recoveries achieved in a limited number of systems demonstrated that the lower values were real, and not an artefact of the experimental procedure. The blocks shown in Figs. 1 and 2 represent the mean value ± the calculated standard deviation (for 8–10 determinations). It was found that the relative standard deviations rarely exceeded 4%, and in nearly two-thirds of the systems studied the precision achieved was better than ± 2%. Careful calibration and manipulation were required to achieve this limited spread, because the procedure involved two filtration steps and analysis of solutions having different chemical compositions.

Extraction of copper and lead

The greater affinity of humic acids for Cu or Pb ions is clearly reflect in the extraction values reported in Fig. 2. In particular, far less of the adsorbed material was displaced by the salt solutions. The amount of lead retrieved by these extractants, and also with the mineral acids and buffer solutions, exceeded the copper recoveries, so copper appears to be more firmly bound than lead on the humic acid samples studied.

As with Cd and Zn, the amount released by chelating agents such as DTPA and EDTA was > 90%, but not total, which again indicates strong bonding of some of the metal ion to residual solids. Increasing

the chelate concentration tenfold (i.e., to 0.05*M* EDTA) had little measurable effect. Behaviour in the presence of acids was somewhat variable, lead recovery in the presence of oxalic acid being quite low. This has been attributed to conditions favouring the formation of sparingly soluble lead oxalate. The addition of ammonium oxalate, however, favoured formation of soluble oxalato-complexes and the lead extraction values then exceeded 90%.

The electrolyte extractants (NH_4NO_3, NaCl, $MgCl_2$, $CaCl_2$) displaced only about half of the sorbed ions (with less from HA I than HA II), which indicates that a higher proportion of Cu and Pb ions become co-ordinated to the organic solid.

Effect of clay suspensions

Natural systems may contain mixtures of clays and organic acids. To assess the effect of greater contamination with silicate material, studies were made with systems in which the clay content was 3–4 times the amount of humic acid added. The extractants used in these comparison studies were nitric acid, ammonium acetate, magnesium chloride and EDTA. Two different types of clay were used, namely montmorillonite (which has a high adsorption capacity and retains metal ions mainly through electrostatic attraction) and illite (with a lower capacity and a tendency to bind some ions specifically, particularly lead).

It was found that in over three-quarters of the systems studied, the amount extracted was similar to that in the absence of the clay. For recoveries differing from that for humic acid alone by > 5% (absolute), the observed values are recorded in Figs. 1 and 2 and indicated by symbols such as m II m (montmorillonite/HA II mixture) or i I i (illite/HA I).

A previous study[10] indicated that interaction between illite and HA I and HA II is limited, and that the adsorption capacity of the mixtures for metal ions is additive, whereas with montmorillonite (or kaolinite) the uptake can be less than the sum of the individual uptakes. It has also been observed[8] that the ability of electrolyte solutions to release metal ions sorbed on clays varies with the metal involved, the type of clay, and reagent used. In summary, chelating agents tend to displace presorbed metal ions totally, mineral acids release > 90%, and ammonium acetate or sodium or calcium chloride solutions displace as little as half of the retained matter.

Most of the variations noted in the clay–humic acid extraction studies can be attributed to extraction values for the clay component being higher, or lower, than those for the humic acids. The highest proportion of varied responses occurred for Pb with ammonium acetate or magnesium chloride solution, these being the two extractants most sensitive to changes in bond strength or bonding mode.

Since the addition of excess of clay only slightly varied the extraction behaviour, the contribution of the inorganic contaminants present in the humic acids used (*i.e.*, the ash fraction) was probably quite small.

Effect of surface loading levels

As shown in Table 1, the amount of metal ion initially retained by the humic acids could be varied by either increasing the system pH, or by reducing the initial metal ion concentrations.

The number and type of metal–humate complexes formed has been reported to be influenced by the total metal: humate ratios, so a few studies were made with HA II to ascertain whether small changes in total loading affected extraction behaviour. It was found that increasing the uptake by increasing the pH led to slightly higher extraction percentages, *i.e.*, proton abstraction appears to create more negative sites, to which metal ions might be electrostatically attracted. Reducing the amount sorbed by using more dilute initial solutions also led to higher extraction values, which implies that formation of 1:1 species with salt-type linkage could be the preferred initial form of bonding. Both these findings indicate that the fraction extracted is influenced by environmental conditions at the time of metal ion retention.

Peroxide oxidation of humic acid

To release metal ions bound to organic matter, destruction of the organic phase has regularly been recommended, and oxidation with hydrogen peroxide has been one of the preferred methods (*c.f.* Table 2). It was found in this study that peroxide treatment of solutions containing metal salt and humic acid yielded 99% recoveries of Zn^{2+}, 97–98% of Cd^{2+} and 95% of the initial Cu^{2+} but only 67% (HA II)–75% (HA I) of the added Pb^{2+}.

It has been noted that though peroxide treatment can make soils incapable of chelating metal ions (*e.g.*, Zn^{2+}), not all the organic matter is necessarily destroyed.[17] Oxalic acid has been identified[18] as the major product formed on treating soil organics with hydrogen peroxide, and the oxalates of calcium,[19] iron and aluminium[20] have been observed in soil samples pretreated with this reagent. It is therefore assumed that the low Pb recoveries noted above are due to the formation of lead oxalate (*cf.* extraction behaviour with oxalic acid, Fig. 2).

Other oxidants may yield different products, and the possible effect of these on metal ion recoveries warrants further investigation.

ANALYTICAL IMPLICATIONS

Hydrochloric acid (0.1*M*),[21] 1*M* ammonium chloride[22] or acetate,[3] and 0.05*M* calcium[2] or 1*M* magnesium chloride[6,23–25] have all been proposed as suitable extractants for the evaluation of the "available" or "exchangeable" fractions of the total metal content of soils.

It may be seen from Figs. 1 and 2, however, that reagents such as these extract differing amounts of metal ion from humic acids, a result similar to that noted earlier for clay suspensions.[8] The preferred re-

agents, magnesium and calcium chlorides, displace, in general, less than the other proposed reactants, but the amount released by each system tends to be quite variable, depending, *inter alia*, on the type of humic acid, surface loading, equilibrium pH, and type of clay present (if any).

Variations between reagent responses may be of minimal concern if the analytical data are intended solely to indicate major differences in soil behaviour (*i.e.*, for comparative studies) and no special significance has to be placed on the assignment terminology used, although the influence of other variables on extraction values probably contributes to the correlation problems associated with plant pot-trials.

However, where sequential extraction steps are to be adopted, with the aim of fractionating the total content into specific categories (*e.g.*, exchangeable, weakly adsorbed, associated with organic matter) improved understanding of the chemical equilibria associated with the extractant–solid interactions becomes highly desirable.

A summary of the sequential approach proposed by four different groups is provided in Table 2, and this can be used to illustrate the complexity of the problem.

It can be seen that the determination of the fraction bound to organic matter occurs at different stages of the various sequences. In one case,[7] the initial step is destruction of organic matter with sodium hypochlorite at pH 9.5. At this pH most types of metal ions released should precipitate as hydroxide species, and retrieval of this group will be deferred to the next stage, namely, acidification with hydrochloric acid (this reagent will also dissolve metal carbonates, displace cations from clay surfaces, *etc.*). Hence any metal ion identified in the alkaline centrifugate may thus provide a poor indication of the material initially associated with the organic matter (or present as sulphides). The source of the analytical result could be colloidal dispersions or metal-organic species not fully oxidised by the reagent used. In the other three systems,[2,5,6] the soil is first treated with calcium or magnesium chloride solution, and as shown in Figs. 1 and 2, this can displace 50–80% of any Cu, Pb, Cd or Zn associated with the humic acid component. Subsequent steps in the sequences (*e.g.*, treatment with acetic acid,[2] or acetate buffer and dithionite/citrate[5]) are capable of displacing further fractions of the metal initially associated with the organic acids. Accordingly, only the fraction most firmly co-ordinated to the organic component will be released on destroying the organic matter with hydrogen peroxide. The use of this reagent can also introduce side-effects, however. For example, low lead recoveries have been noted in this paper, and are thought to be probably due to lead oxalate formation. Any oxalic acid formed in this way could also aid dissolution of hydrous oxide layers (releasing associated metal ions) and addition of peroxide may result in some dissolution of manganese oxide particles.

It may therefore be concluded that if the same soil were subjected to each of the four sequences proposed, the analytical result reported for "bound to organic matter" could be different in each case. It seems likely that development of valid sequential systems may have to await completion of more basic studies, for example, investigation of the extraction of metal ions presorbed on hydrous oxides, or side-effects in oxidation procedures.

Other soil scientists have preferred to determine "non-detrital" or "available" metal ion levels by means of a chelating agent, such as DTPA,[26,27] but the efficiency of these methods can also be influenced by reaction parameters. For example, it has been observed that the amount of metal ion released can be increased by increasing the shaking time,[28,29] extractant concentration,[29] solution pH (from 4.8 to 8.5)[29] or the duration of the initial sample-grinding period.[26]

With organic-rich natural soils some of the metal ions displaced by salt solutions may be present as solubilized metal–humate complexes. The two humic acids studied in this investigation required a high pH for solubilization, but two forest soil acids previously used[9] formed water-soluble complexes at pH > 6 (Cu, Pb) or 7 (Cd, Zn).

In summary, examination of metal ion recoveries from humic acid samples by different chemical extractants has confirmed that in any extraction procedure very careful consideration must be given to the associated chemical equilibria and the impact of competing reactions.

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CHARACTERIZATION AND UTILIZATION OF A LONG-LASTING SESSILE-DROP MERCURY ELECTRODE IN DIFFERENTIAL PULSE ANODIC-STRIPPING VOLTAMMETRIC SUBTRACE METAL ANALYSIS OF NATURAL WATERS

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Summary—Construction details and voltammetric characterization of a long-lasting sessile-drop mercury electrode are reported. The results obtained in the fully automated differential pulse anodic-stripping voltammetric determination of cadmium in a simple purified electrolyte as well as of zinc, cadmium and lead in sea-water samples suggest that this new kind of semi-stationary mercury electrode may be employed in voltammetric trace and ultratrace analysis of natural waters for metal ions.

In recent years, under the thrust of environmental problems, the determination of trace and subtrace (down to 50 ng/l)[1] metal concentrations in natural waters, sea-waters, industrial effluents, biomatrices, *etc.* has received increasing attention. This is mainly because it has been found that some heavy metals (such as cadmium, lead and mercury) have toxicological action, even at very low concentrations, while other metals (such as copper, chromium and zinc) at concentrations below their respective critical threshold levels are essential to man and other organisms.[1–3] Among the various analytical methods currently used in such investigations, electrochemical stripping analysis is of particular interest, because of its intrinsic simplicity, sensitivity and flexibility in operation. Differential pulse anodic-stripping voltammetry (DPASV) is one of the instrumental techniques which appears particularly well suited to the determination of traces of copper, zinc, cadmium and lead. This technique, which requires only simple and inexpensive apparatus, involves cathodic deposition on a stationary or semi-stationary electrode at an appropriate potential and for a given time. The solution is usually stirred in this period to increase the sensitivity of the method, since during the deposition from a solution with a very low bulk concentration of metal, a substantial preconcentration on the working electrode is achieved. At the end of the programmed deposition process, the stirring is stopped and in the following rest period the solution comes to rest so that the mass transfer in the subsequent stripping stage takes place by diffusion only. Finally, an anodic stripping of the deposited metal yields the current signal used for the quantitative determination. In the stripping stage, a sequence of pulses of small amplitude and duration is superimposed on the voltage sweep while the current flowing through the cell is sampled twice, just before the rise and the fall of each pulse.[4–6] Impregnated graphite electrodes,[7,8] glassy-carbon electrodes,[9] noble metal electrodes,[10] mercury-film electrodes,[1,11–13] and Kemula's hanging-drop mercury electrodes[14–21] have been employed as working electrodes. Of these, the mercury-film and the hanging-drop mercury electrode have provided the best results in the determination of trace metals by DPASV. Kemula's electrode, a truly sensitive tool is difficult to manipulate, however, since it must be renewed manually for each determination, with consequent poor repeatability and waste of time. Mercury-film electrodes, which unlike Kemula's electrodes can be operated in a fully automated sequence, require great care in preparation of the surface (where the mercury film will be deposited) as well as long and tedious conditioning before the analysis. Ordinary capillary electrodes with a long drop-time (60–80 sec) have also been employed for the determination of zinc, cadmium and lead in sea-water samples by DPASV.[6,22] However, with such electrodes the deposition time must be quite short (30–40 sec) while the voltage range cannot be larger than 500 mV at a scan-rate of 20 mV/sec.

In a previous paper[23] we have proposed a new kind of semi-stationary mercury electrode: the long-lasting sessile-drop electrode (LLSDME) which allows a longer electrolysis time with more vigorous stirring, a longer rest time to obtain a quiescent solution, a larger potential range at slower scan-rates and lastly, a higher current response. The results obtained in the determination of zinc either in a simple purified electrolyte or in sea-water samples with a semi-automated sequence (the drop was dislodged manually at the end of each anodic scan) and a non-analytical multipolarograph suggested that after optimization of the parameters which affect the sensitivity of the method, the

LLSDME could be suitable for the determination of metals at subtrace levels by DPASV.

In the present paper we report more detail of the construction of LLSDME as well as the results obtained in evaluating the influence of some parameters (namely electrolysis time, rate of stirring, amplitude of superimposed pulse) on the peak current for cadmium in potassium chloride solution. The current was recorded with fully automated equipment, which mechanically dislodged the drop and also controlled both the deposition and rest times. To illustrate the potential analytical applications of the LLSDME, the simultaneous determination of zinc, cadmium and lead in sea-water samples is reported.

EXPERIMENTAL

Construction of the LLSDME

The electrodes were made from ordinary polarographic capillaries (Sargent, drop-time 6–12 sec; Amel, 10–15 sec, 21–22 cm long) as shown in Fig. 1. A 2–3 cm length about 5–6 cm from one end was rotated in a slot-type flame (Fig. 1a) until red-hot and then gently drawn out to reduce its external diameter to about 2 mm (Fig. 1b). The capillary was then cut off about 1–2 cm from the end (Fig. 1c), and the tip ground to a truncated cone (Fig. 1d) with a diamond grindstone. The narrow portion was then bent into a hook, in a small Bunsen flame (Fig. 1e). The capillary was connected to the self-levelling mercury reservoir as shown in Fig. 2, then filled with mercury by means of a vacuum pump or by squeezing the Tygon tubing. When immersed in 0.1*M* potassium chloride (stirred at 800 rpm) with −1.3 V applied in a closed circuit the electrodes gave drop-times of 6–10 min. To avoid blocking, the mercury should be dropping continuously. Some electrodes more than 7 months old are still functioning satisfactorily.

Apparatus

An Amel 472/WR multipolarographic analyser, Amel 862/D X-Y recorder and a synchronized electromagnetic knocker were used. The apparatus was modified to allow the electrolysis and rest times to be varied up to 999 and 100 sec respectively.

Procedure

At the push of a "start" button the mercury drop is dislodged by the knocker, the solution (60–80 ml) is stirred (at 200–800 rpm) while the drop is polarized at a preset potential (*e.g.* −1.20 V for zinc, −0.85 V for cadmium and lead). After 120–360 sec the stirring is stopped, and after a further 12–30 sec rest period the anodic voltamperogram is recorded at 5 or 10 mV/sec. At the end of the scan the knocker dislodges the drop and a new sequence begins automatically. All the determinations were performed at 25° in a Teflon or fused silica cell with an SCE (Ingold) and a platinum ring (Ingold) as reference and counter-electrode, respectively. The reference electrode was filled with saturated potassium chloride solution and connected to the test solution by a tube filled with 1*M* potassium chloride solution and fitted with a sintered-glass disc. The test solution was stirred by a magnetic stirrer and a Teflon-covered iron bar with a Teflon ring fitted over it (Fig. 3). The standard solutions were added from automatic microlitre pipettes (Gilson). The sea-water samples were collected and stored in polyethylene bottles, previously cleaned as described in the literature.[24,25]

Chemicals

Potassium chloride (Merck, Suprapur) and nitric acid, 65% w/w (Merck, Suprapur or BDH, Aristar) were used, as received, to prepare the supporting electrolyte and the cleaning solutions, respectively. The cadmium, lead and zinc standard solutions were prepared by diluting stock solutions of their nitrates (Fluka or BDH, for atomic spectroscopy). Argon (99.99% pure) was used to deoxygenate the solutions. For cleaning purposes and for preparing the standard solutions two different water supply systems were employed. The first was a Millipore Q-system, the second used multiple distillation in glass and then double distillation in quartz. To check the presence of contaminating metals in the mercury used for the tests, voltamperograms were recorded for an anodic voltage scan (from −1.20 to −0.20 V) on the mercury drop in 0.01*M* potassium chlor-

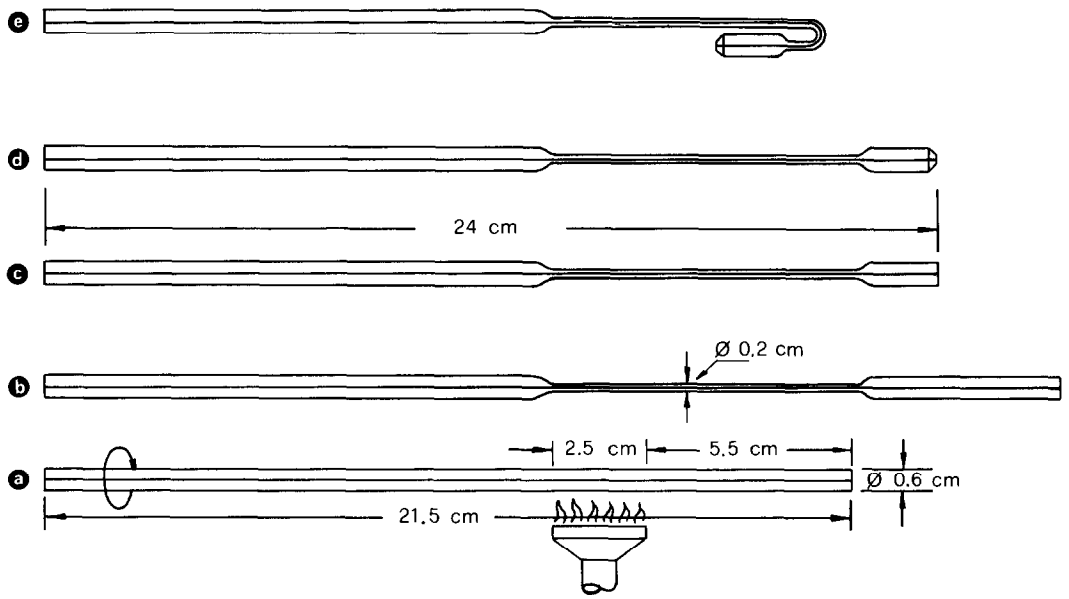


Fig. 1. Details of construction of LLSDME (see text).

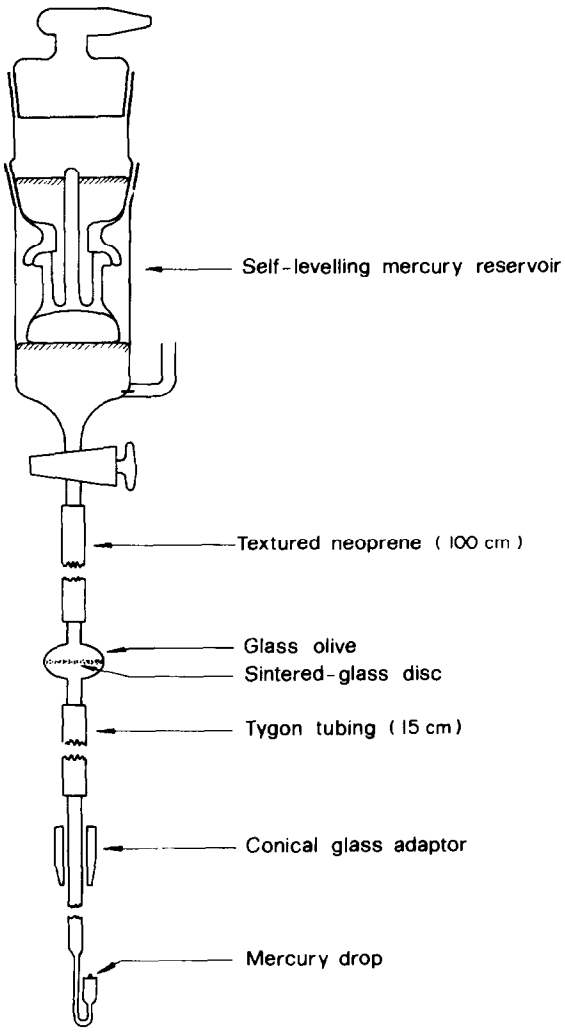


Fig. 2. LLSDME and mercury reservoir assembly.

ide after the drop had grown for 390 sec in open circuit and non-stirred solution. Mercury with a concentration of heavy metals less than our limit of detection was obtained by the following procedure. Mercury (C. Erba, pure for analysis) was scrubbed several times by passing it through a funnel (with a sintered-glass disc in its neck) into a glass tube (100 cm height, 4.5 cm diameter) containing 15% w/w nitric acid; it was then washed with ultrapure water (in a similar column), and dried and distilled under reduced pressure. The intermediate fraction was collected, again scrubbed with 12% v/v nitric acid, washed, dried and lastly redistilled four times under reduced pressure.

RESULTS AND DISCUSSION

Optimization of some parameters affecting the sensitivity

In DPASV, the peak current depends mainly on the electrolysis time, stirring rate, electrode size, pulse amplitude, pulse repetition time and scan-rate. To optimize some of these parameters, we examined the influence of electrolysis time, stirring rate, pulse amplitude and potential scan-rate on the peak current for cadmium in potassium chloride solution (Figs. 4–7). The pulse repetition time was kept constant at 0.1 sec since it is an instrumental characteristic of the polarograph used.

The peak current rises with increase in stirring rate (from 0 to 800 rpm) as shown in Fig. 4a. Thus, vigorous stirring enhances the mass transfer of cadmium ions towards the electrode.

The peak current decreases when the anodic voltage scan-rate is increased (from 5 to 50 mV/sec), as shown in Fig. 4b. This depends on the fact that if the anodic scan-rate is increased the duration of each measurement is reduced and the number of samplings distributed along a voltamperogram decreased (the pulse duration is constant). In other words the number of current differences which add up to give the amplitude of the voltamperogram will also decrease. Moreover, at slow anodic scan-rates a second-order effect becomes evident: though at slow scan-rates the variation of potential during a pulse can be regarded as negligible, at fast scan-rates it can not, and it adds a contribution to the charging current.[6] Therefore, the sensitivity and the resolution of the method will be rather impaired at fast scan-rates since the peaks will be smaller and broader.

Figure 4c shows the influence of the electrolysis time on the peak current, which increases when the deposition time is made longer (from 60 to 360 sec).

Finally, Fig. 4d shows that increasing the modulation amplitude (from 10 to 100 mV) of the superimposed pulse at first increases ($\Delta E \leqslant 80$ mV) and then ($\Delta E > 80$ mV) decreases the peak current.

Thus, to obtain a higher sensitivity in DPASV with an LLSDME a long electrolysis time (300–360 sec), a fast stirring rate (800 rpm), a high pulse modulation amplitude (50–80 mV) and a slow scan-rate (5 or 10 mV/sec) are to be preferred.

Linearity of response and repeatability

The linearity between peak current and concentration was checked by the standard addition method. For this purpose, various volumes of a $10^{-6}M$ standard cadmium solution were added to $0.01M$ potassium chloride. The repeatability was tested at different cadmium concentrations on consecutive mercury drops. The choice of cadmium was suggested by its low abundance in the environment and therefore by the highly unlikely occurrence of accidental pollution of the solutions during the required manipulations.

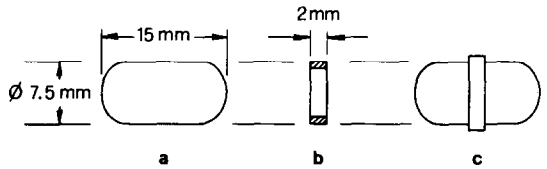
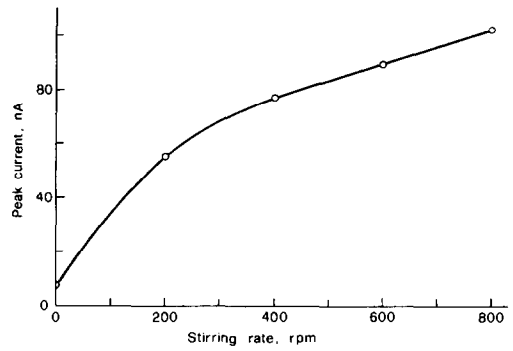
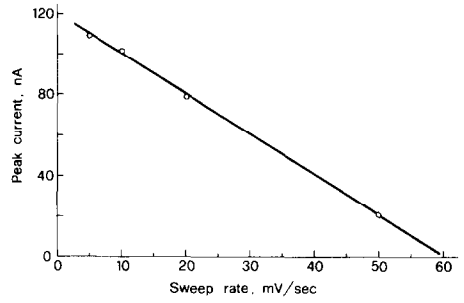


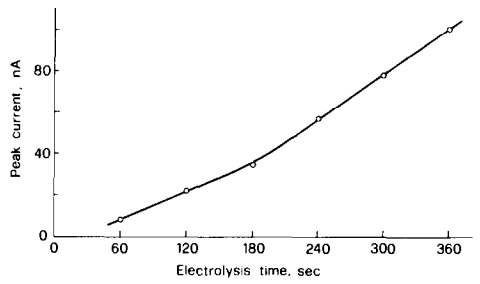
Fig. 3. Magnetic stirring bar (c) obtained by fitting the Teflon ring (b) on the Teflon-covered iron rod (a).



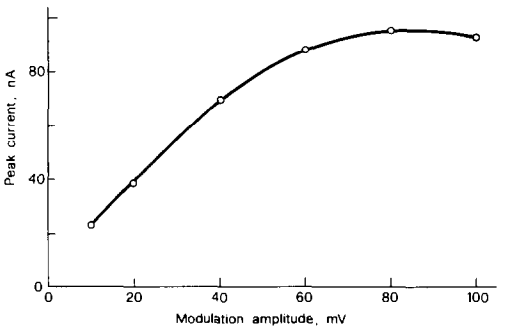
(a)



(b)



(c)



(d)

Fig. 4. Effect of stirring rate (a), anodic sweep-rate (b), electrolysis time (c) and pulse modulation amplitude (d) on peak current for an LLSDME. Solution (84 ml) 1.64 × $10^{-9}M$ Cd in 0.01M KCl; electrolysis potential = −0.85 V; electrolysis time = 300 sec (d) or 360 sec (a, b); stirring rate = 800 rpm (b, c, d); rest time = 30 sec; pulse modulation amplitude = 20 mV or 50 mV (a, c); sweep rate = 10 mV/sec (a, c, d); 25°C.

Typical voltamperograms relative to two (6th and 7th) successive mercury drops are shown in Fig. 5, and the peak currents (i_p) at different cadmium concentrations (C) obtained on seven consecutive drops are reported in Table 1. From Table 1 it follows that over the concentration range examined (10^{-10}–$10^{-9}M$) satisfactory linearity between i_p and C can be obtained. Moreover, from curves a–e and a′–e′ in Fig. 8 or the data reported in Table 1 it is evident that the cadmium peak gives good repeatability (3%, Table 1) even at the lowest concentration examined. Finally, from Table 1 it is possible to calculate by linear regression the concentration of cadmium in the 0.01M potassium chloride solution; it is 1.72 ng/l.

Determination of zinc, cadmium and lead in sea-water

The simultaneous determination of zinc, cadmium and lead in a sea-water sample by DPASV with an LLSDME is shown in Figs. 6 and 7. The sample was

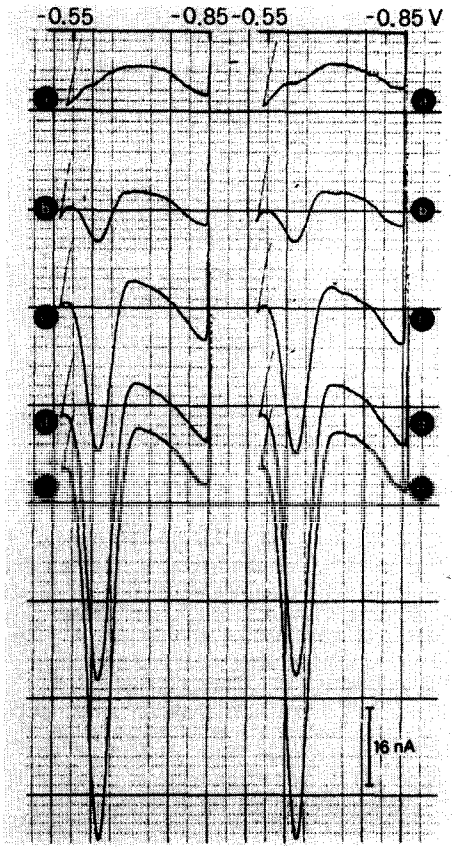


Fig. 5. Anodic stripping voltamperograms of cadmium in KCl solution. (a) 84 ml of 0.01M KCl; electrolysis potential = −0.85 V; electrolysis time = 360 sec; stirring rate = 800 rpm; rest time = 30 sec; pulse modulation amplitude = 50 mV; sweep-rate = 10 mV/sec; mercury head = 57 cm; 25°C; (b–e) as for (a) after addition of (b) 14.1, (c) 56.5, (d) 98.9 and (e) 141.1 ng of cadmium per litre. Voltamperograms a–e and a′–e′ refer to the sixth and seventh mercury drops, respectively (see Table 1). Every voltamperogram is recorded on a "fresh" mercury drop (see text).

Table 1. Correlation between peak currents (nA) and concentration (ng/l.) of cadmium in 0.01*M* KCl*

Cd added	i_p^{I}§	i_p^{II}	i_p^{III}	i_p^{IV}	i_p^{V}	i_p^{VI}	i_p^{VII}	$\bar{i}_p$	Rel. std. devn., %
0.0 (blank)†					very small peak				
14.1	8.56	8.40	8.56	8.00	8.24	8.00	8.00	8.24	3.1
56.5	32.4	32.4	33.0	32.6	32.6	32.2	32.0	32.4	1.0
98.9	57.2	56.4	57.4	56.6	56.6	56.4	56.0	56.6	0.8
141.1	80.2	80.0	79.5	80.0	80.0	79.5	78.4	79.7	0.8
183.4	102.6	102.8	102.1	102.0	102.0	101.4	101.8	102.1	0.5

*Some anodic voltamperograms and the experimental conditions are reported in Fig. 5.
†Concentration of cadmium in original sample = 1.7 ng/l., calculated from the regression line (see text). Concentration of cadmium in the standard solution = $1.06 \times 10^{-6}M$.
§The roman numeral refers to the mercury drop number.

collected by us in the Tyrrhenian sea about 4 miles off the mouth of the harbour-canal of Castiglione della Pescaia (Grosseto, Italy). Two typical explorative voltamperograms are shown in Fig. 6, and some relative to the determination of cadmium and lead are shown in Fig. 7. From curves a–c and a′–c′ in Fig. 7, and curves recorded for the zinc determination, it is evident that every peak gives satisfactory repeatability. From the corresponding i_p–concentration plot (regression line) the concentrations of zinc, cadmium and lead in the sea-water sample were evaluated as 1560, 24 and 366 ng/l., respectively.

Two other sea-water samples were collected by the researchers of the Thalassographic Institute of CNR, Trieste (Italy), in the North Adriatic sea (stations GT 5 and GT 9), during a cruise of the oceanographic ship "Marsili". The samples were stored at low temperature (−5°) and analysed by us after about 4 months. The concentrations of cadmium and lead in the two samples were evaluated as 70 and 510 ng/l. for GT 5, and 17.8 and 418 ng/l. for GT 9.

CONCLUSIONS

The experimental results reported so far, as well as those obtained with some other electrodes of the same kind, suggest that the LLSDME may be employed very satisfactorily for the determination of metals at trace and subtrace levels in natural waters, industrial effluents, biomatrices, *etc.* by the DPASV technique. The LLSDME, easily prepared from an ordinary capillary and usable without precautions over a long period, can be used in fully automated equipment. Moreover, each measurement is performed with a fresh and highly reproducible mercury drop, a condition less likely to be met by stationary electrodes.

Work is in progress in our laboratories to improve the sensitivity (in the work on sea-water reported here we have reached a repeatability of about 3% with a detection limit of about $10^{-10}M$). We intend to modify both the capillary and the polarographic apparatus. A modified capillary design could allow more vigorous stirring of the test solution without detaching the mercury drop, and thus shorter deposition times even for subtrace analyses. Modification of the analyser to allow use of subtractive differential pulse anodic-stripping voltammetry (SDPASV) would cancel the current contribution related to the drop growth. Thus, an appropriate signal recorder could be synchronized with our analysis apparatus so that the current related to the drop growth, recorded for a quiescent solution, is memorized and subtracted from

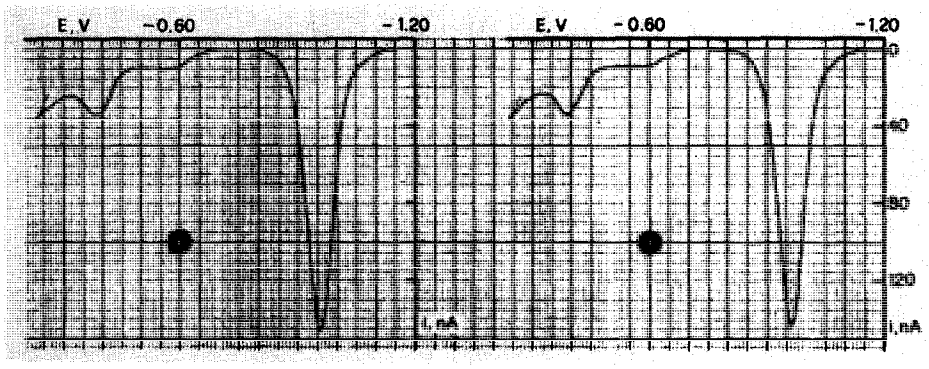


Fig. 6. Explorative voltamperograms from two consecutive mercury drops (a and a′), for a Tyrrhenian sea-water sample (see text). Sample volume = 82 ml; stirring rate = 800 rpm; rest time = 30 sec; pulse modulation amplitude = 50 mV; mercury head = 56 cm; 26°C. Each voltamperogram is recorded on a "fresh" mercury drop (see text). Electrolysis potential = −1.20 V; electrolysis time = 300 sec; rest time = 30 sec; sweep-rate = 10 mV/sec.

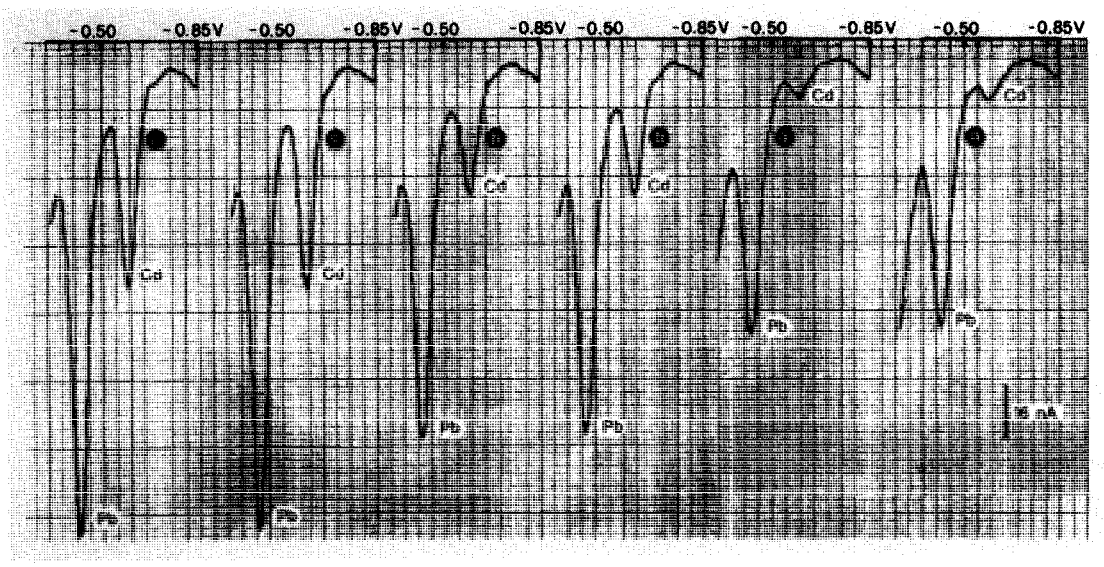


Fig. 7. Determination of cadmium and lead. Electrolysis potential = −0.85 V; electrolysis time = 390 sec; rest time = 30 sec; sweep-rate = 5 mV/sec. (a) Blank; (b) and (c) the same as (a) after addition of 20 μl (b) and 40 μl (c) of $3.56 \times 10^{-6} M$ cadmium and $2.90 \times 10^{-6} M$ lead standard solutions. Curves a′–c′ refer to the results obtained with the next mercury drop.

the currents recorded for subsequent drops in stirred solutions. Therefore, the SDPASV technique may allow improved accuracy and repeatability with an LLSDME.

Finally, we think that this new type of electrode may have many applications in oscillopolarography and in d.c. or a.c. polarography, mainly where decomposition of the medium may occur at very negative potentials, where the performance of Kemula's hanging mercury drop electrodes and the usual dropping mercury electrodes is no longer reliable.

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ANION-EXCHANGE INITIATION OF REACTIONS: DETECTION AND SPECTROPHOTOMETRIC DETERMINATION OF ALIPHATIC AMINES WITH 2,4-DINITROPHENYLHYDRAZINE

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Summary—The role of anion-exchange resin beads in the initiation of reactions of tertiary amines with 2,4-dinitrophenylhydrazine has been explored. This reagent has been used for the selective detection and determination of aliphatic amines. Radiochemical studies have been performed to show that the coloured species is adsorbed but not exchanged on ion-exchange beads. A model for the interaction is proposed.

Ion-exchange resin beads were first used as detection media by Fujimoto.[1] The term "resin spot-test" was later coined by him[2] to describe the detection of a substance by conversion into a coloured species which could be taken up on resin beads. In this way the selectivity and sensitivity of the test were improved and the stability of the colour was enhanced. These tests[3] were mostly used for the detection of inorganic species on the basis of known colour reactions. West *et al.*[4] were the first to develop new resin spot-tests for the detection of organic functional groups such as esters, amides, imides and anilides. In these tests the beads were used as carrier for the ions taking part in the reaction, and could be separated from the reaction medium after the reaction was over.

However, no investigations have so far been reported on whether the uptake of the coloured species by the resin beads is due to ion-exchange or adsorption and no effort has hitherto been made to explore the use of resin beads as a substrate for reactions which apparently do not occur in solution. These aspects are now explored, with reference to detection of amines.

Numerous methods have been proposed for the colorimetric detection and determination of amines. Menzie[5] reported that in non-aqueous media *p*-dimethylaminobenzaldehyde (*p*-DAB) gives interesting colours with amines and heterocyclic nitrogen compounds, but as the colours produced with *p*-DAB are unstable this reaction was not used for the spectrophotometric determination of amines. Qureshi and Khan[6] used *p*-dimethylaminocinnamaldehyde (*p*-DAC) for the detection and determination of primary and secondary aromatic amines, as the colour produced is stable. A colorimetric method for secondary amines was proposed by Umbreit[7] and extended by Karweik and Meyers.[8] Rawat and Singh[9] proposed a sensitive method for the spectrophotometric determination of primary, secondary and tertiary amines. A rather unselective photometric determination of low molecular-weight aliphatic amines was proposed by Korenman and Shermarova,[10] and Toome and Manhart[11] developed a simple simultaneous colorimetric determination of primary and secondary aliphatic amines with fluorescamine.

Nitro compounds are known to give interesting but unstable colours with aliphatic amines.[12] The present study had four objectives: (*i*) to explore the conditions for stabilization of the colour of the nitro-compound/amine reaction products; (*ii*) to develop an ion-exchange method for the selective and sensitive detection of aliphatic amines; (*iii*) to determine aliphatic amines by a simple colorimetric method; (*iv*) to identify the mechanism of the resin-bead reaction. A novel feature was a radiochemical study which showed that the coloured species are adsorbed but not exchanged on the resin surface, so doubt arises whether ion-exchange mechanisms operate in those reactions in which colourless resin beads become coloured[1–4,6,9,13,14] when kept in contact with a coloured solution. The present study shows that in at least some cases no ion-exchange occurs, and these reactions should therefore be reinvestigated.

EXPERIMENTAL

Reagents

Solutions of amines were prepared in conductivity water for detection and in dimethylsulphoxide (DMSO) for determination. A 0.1% ethanol solution of 2,4-dinitrophenylhydrazine (DNPH, Merck guaranteed reagent) was used for detection and a 0.02*M* solution in DMSO for determination.

The resins used were Amberlite IRA-400 (Cl^- form) and Dowex 50W-X8 (Na^+ form).

Procedure for detection

Place 4 or 5 Amberlite resin beads (Cl^- form) in the depression of a white spot-plate. Add 0.01 ml of the amine solution to the beads followed by 0.01 ml of reagent sol-

Table 1. Limits of identification of aliphatic amines at 25°

Substance	Detection limit,* μg
Ammonia	2.3
Methylamine	0.4
Dimethylamine	0.4
Diethylamine	0.7
Trimethylamine	2.4
Triethylamine	3.0
Ethanolamine	1.0
Ethylenediamine	0.5
1,3-Diaminopropane	0.9
Piperidine	1.4
n-Butylamine	0.4

*In the 0.01 ml of solution tested

ution. An immediate green colour on the resin surface confirms the presence of an amine.

Procedure for determination

To 1 ml of solution containing an appropriate amount of the amine in DMSO (see Table 4) add 5 ml of 0.02*M* DNPH in DMSO and make up to volume in a 10-ml standard flask with DMSO. Measure the absorbance in a 1-cm cuvette at 640 nm against a blank prepared in the same way and at the same time as the sample solution. The colour is stable for about 4 hr.

Radiochemical studies

In these exploratory tests, 2 g of Amberlite IRA-400 (Cl^- form) were treated with 0.1*M* chloride-labelled sodium chloride solution. The radio-chloride retained on the resin after repeated washing was measured by β-counting. The labelled resin was then treated with a concentrated solution of the DNPH–amine product, by the batch method. After several hours the liquid phase was tested for radio-chloride. The test was repeated with DMSO, ethanol, aqueous DMSO, aqueous ethanol and ethanolic DMSO as media, for times between 2 and 24 hr, and also with a cation-exchanger (Cs^+ form, γ-counting with a scintillation counter).

Table 2. Tolerance limits for nitrogen-containing foreign substances in detection of 10 μg of piperidine

Foreign substance	Amount added, mg
Aniline	50
Diphenylamine	30
m-Phenylenediamine	10
p-Phenylenediamine	20
β-Naphthylamine	20
N-Phenyl-2-naphthylamine	10
Dimethylaniline	60
Indole	20
o-Toluidine	20
Pyridine	15
p-Dimethylaminobenzaldehyde	5
Succinamide	1
Acetamide	20
Benzamide	10
Nicotinamide	10
Acetanilide	1
Acetonitrile	40
Urea	300
Phenylurea	5
Thiourea	20
Phenylthiourea	2

Table 3. Tolerance limits for non-nitrogen-containing foreign substances in detection of 10 μg of piperidine

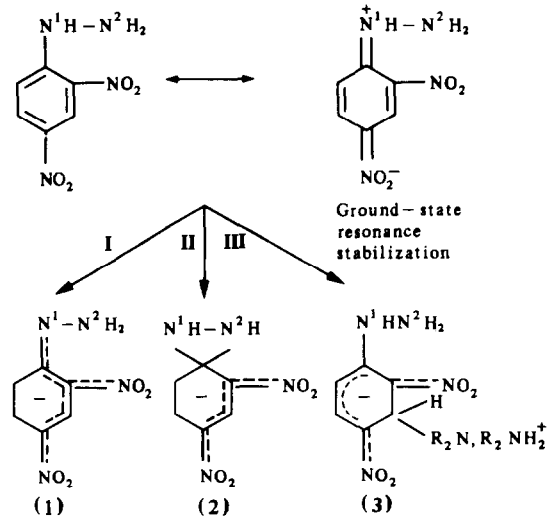
Foreign substance	Amount added, mg
Dioxan	1030
Crotonaldehyde	4
Salicylaldehyde	8
Anisaldehyde	7
Acetaldehyde	10
Acetophenone	0.35
Cyclopentanone	1
Ethyl methyl ketone	12
Methyl propyl ketone	16
Mesityl oxide	10
Phenol	0.5
Resorcinol	1

DISCUSSION

The colours produced when a base is added to the solution of a polynitro-aromatic have been attributed to a variety of interactions. Three noteworthy reviews[15–17] summarize the work up to 1970.

The interaction of 2,4-dinitroaniline with bases was first studied by Crampton and Gold.[18] Proton transfer from the aniline to the base was shown to be responsible for the colour, and this has recently been confirmed by Crampton and Wilson.[19]

Since DNPH is a derivative of 2,4-dinitroaniline it will also probably react by proton loss. *In solution*, however, DNPH apparently does not react with tertiary amines although these would presumably be quite efficient proton acceptors. If proton loss is the major mode of interaction, which proton of DNPH is lost—that from N^1 or N^2 (Scheme 1)?



Scheme 1. The possible reactions between DNPH and aliphatic amines.

Table 4. Spectrophotometric determination of aliphatic amines with DNPH in DMSO at 25°

Substance determined	Range of determination, μg/ml	ϵ_{max}, $l.mole^{-1}.cm^{-1}$	Relative standard deviation, %	No. of detns.
Ammonia	1.7–34	500 ± 44	5.3	6
Methylamine	1.2–12	1974 ± 110	3.5	8
Diethylamine	1.4–23	545 ± 36	7.2	5
Ethanolamine	4.8–48	1320 ± 97	5.0	5
n-Butylamine	1.7–27.2	2500 ± 150	3.2	6
Piperidine	8.5–85	1227 ± 30	3.4	6
Ethylenediamine	2.4–24	2258 ± 140	8.1	5
1,3-Diaminopropane	0.7–74	3950 ± 272	5.2	8

Buncel *et al.*[20] have shown that DMSO stabilizes the conjugate base of polynitroaniline derivatives with respect to the sigma complex with a species such as methanol. We expect a similar effect here, *e.g.*, structure (3) in Scheme 1. This view is supported by Crampton and Wilson.[19] Since our studies were carried out in pure DMSO, there will be a negligible amount of the sigma complex. Buncel *et al.*[20] further state that sigma complexes have two maxima in the visible region while in our case there is only one maximum in the visible region, at 640 nm. Case III therefore seems excluded.

Moreover the stoichiometry of DNPH to amine in (3) would be 1:2, but a Job plot for the stabilized complex shows a stoichiometry of 1:1.

The species (2) is a spiro Meisenheimer complex, the instability of which, according to Bernasconi *et al.*,[21] is governed by two factors, ground-state resonance stabilization and ring strain. In cases I and II the first of these is a common parameter. Furthermore all known spiro Meisenheimer complexes are stable when there is a five-membered ring, and the stability decreases with increase in ring size, which has been recently confirmed by Crampton *et al.*[22] A typical spiro Meisenheimer anion is shown in Fig. 1. In (2) there is a three-membered ring and the strain of such a ring would definitely be prohibitive. This leaves case I as the sole possibility.

There may be two reasons why tertiary amines apparently do not react in solution.

(*a*) The solubility of tertiary amines in DMSO is much lower than that of primary and secondary amines because tertiary amines cannot hydrogen-bond to DMSO.[23]

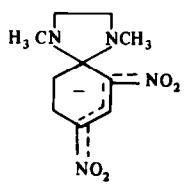


Fig. 1. A typical spiro Meisenheimer complex.

(*b*) There is a marked decrease in the basicity of tertiary amines in DMSO,[24] the sequence being primary > secondary ≫ tertiary.

The results in Tables 1–3 show that the ion-exchange method is selective and sensitive for aliphatic amines. Compounds with similar functional groups, *i.e.*, aromatic amines, amides, anilides, ureas and thioureas, do not interfere. This selectivity arises from the fact that only aliphatic amines form anionic complexes with DNPH. The aromatic amines form charge-transfer complexes which are not as intensely coloured as the reagent and hence do not interfere. The radiochemical studies were performed to pinpoint the role of the resin beads. We had expected exchange to take place and an equivalent quantity of Cl^{-}* or Cs^{+}* to be released, depending on the charge on the coloured species. However, to our surprise, despite repeated efforts no exchange could be detected (results are omitted to save space). It follows that when resin beads become coloured on contact with a coloured solution it cannot be assumed that ion-exchange has necessarily occurred.

The reaction of tertiary amines is interesting. When a tertiary amine is added to the DNPH solution no colouration is observed. However, on the addition of anion-exchange resin beads, the beads are coloured green. The reaction may be explained thus: let us suppose that the tertiary amine (A) reacts with DNPH (B) to form the complex (C), and that the reaction may be represented as

$$A + B \rightleftharpoons C$$

As the solution is not coloured green it is reasonable to assume that initially there is a very small concentration of C, or that C is a transient intermediate. On addition of the resin beads the coloured species is adsorbed by the beads and as a result of this irreversible adsorption the equilibrium shifts to the right and this process continues till the beads are strongly coloured.

In the resin-bead tests this role of the ion-exchanger needs further study. It may, however, be pointed out that this test allows us to distinguish tertiary amines

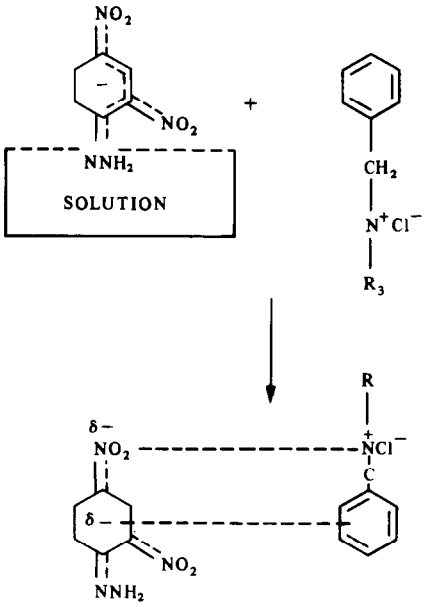


Fig. 2. The model of the interaction of the anionic DNPH–aliphatic amine species with the anion-exchange resin bead.

from primary and secondary amines, as well as from all other organic substances, provided only one species is present. If both the solution and the resin beads are coloured green the solution contains a primary or a secondary amine. On the other hand if the solution is colourless and the resin beads are coloured green then the solution contains only a tertiary amine. In the absence of primary and secondary amines it becomes a specific test for tertiary amines.

A quantitative theory of non-electrolyte sorption by ion-exchangers has not yet been developed. However, sorption of organic non-electrolytes with hydrocarbon groups by ion-exchange resins with hydrocarbon matrices is likely to be affected by London interactions and dipole–dipole interactions.[25] The consequence of such interactions is that the hydrocarbon groups tend to coagulate or to be squeezed out of the polar solution onto a phase boundary. In solution this effect is strikingly demonstrated by soap micelle formation and detergent action. Both the London and the dipole interactions favour local adsorption of the hydrocarbon groups of the solute on the matrix and thus enhance sorption of the non-electrolyte. London forces are specific interactions and depend on the molecular structure of the solute and the matrix. According to the ancient rule "*similia similibus solvuntur*", a high affinity and thus strong sorption may be expected when the chemical configurations of the solute and the matrix are similar. A simple model of the interactions is given in Fig. 2.

DNPH has been very widely used for the detection and determination of aldehydes and ketones, but it has not previously been used for the detection and determination of aliphatic amines. Feigl[26] has presented numerous methods for the detection of amines, but none is selective for aliphatic amines. Also, the sensitivity of these methods is much less (usually 6 μg) than that of the method proposed here.

As DNPH does not form a coloured product with amides, aromatic amines *etc.*, no effort was made to determine aliphatic amines in the presence of such substances, but it appears that there will be practically no interference in the spectrophotometric determination of aliphatic amines by the proposed method.

The coloured species formed by the reaction of DNPH with aliphatic amines is unstable in alcoholic media, but it can be stabilized by replacing the alcohol with DMSO and adding an excess of DNPH.

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EXTRACTION-SPECTROPHOTOMETRIC DETERMINATION OF NIOBIUM WITH DIBENZO-18-CROWN-6 AND THIOCYANATE

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Summary—A method is described for the direct spectrophotometric determination of micro-amounts of niobium by extraction into a benzene solution of dibenzo-18-crown-6 (L) from 3*M* hydrochloric acid containing potassium thiocyanate. The molar absorptivity of the extracted complex is $3.85 \pm 0.03 \times 10^4$ $l.mole^{-1}.cm^{-1}$ (relative standard deviation 0.8%). Co-ordinatively unsaturated complexes of the type $[NbO(SCN)_3]_2L$ and $NbO(SCN)_3L$ are extracted, along with ion-pairs, especially when small amounts of L are used for extraction. The ion-pair complex $[NbOCl_2(SCN)_3][(LK)_2]$ seems to be the main species formed in the organic phase.

Pedersen[1] was the first to report the potential selectivity of crown ethers, L, as ligands complexing most strongly those metal cations having ionic radii which best match the radius of the cavity formed by the polyether ring. The positively charged complex is usually not coloured or fluorescent, although a colorimetric[2] or fluorimetric[3] determination of the cation is possible by solvent extraction of the ion-association species formed by the cationic complex and a coloured or fluorescent organic anion.

We have previously reported the impressive sensitivity and selectivity that can be achieved by using this principle in the fluorimetric determination of potassium with 18-crown-6 and eosin.[4] In continuance of our studies on the analytical application of crown ethers a different approach has been investigated: the use of cationic alkali metal–crown ether complexes for extraction of anionic species containing the cation to be extracted or determined. In this type of extraction, ion-association species L of the type $[LK^+][MX_n^-]$ can be expected in the organic phase.[5,6]

We are also engaged in the search for improved analytical methods for determination of niobium[7–9] and as this element forms anionic thiocyanate complexes[10] its extraction into an organic phase might be accomplished by addition of a cationic crown ether complex, and the extraction followed spectrophotometrically.

The spectrophotometric determination of niobium with thiocyanate, with and without extraction, has become very popular although the reagent concentrations are critical.[11] Affsprung[12] reported that this determination was improved by use of tetraphenylarsonium counter-ions for extraction of the anionic thiocyanatoniobate complex into a chloroform–acetone mixture.

In the present work the potassium complex of dibenzo-18-crown-6 is used to extract the thiocyanatoniobate complexes (from hydrochloric acid medium) into benzene (in chloroform the extracted species precipitate, although partially halogenated hydrocarbons such as 1,2-dichloroethane can be used for the extraction). Investigation of the optimal conditions and the nature of the extracted species has allowed us to establish a new extraction–spectrophotometric determination of niobium and has shown the involved mechanism of this extraction process.

EXPERIMENTAL

Reagents

All reagents used were of analytical-reagent grade.

Nb(V) standard solution (10 μg/ml). Stock solution (200 μg/ml), prepared as described previously,[7] diluted with 2% tartaric acid solution.

Potassium thiocyanate solution, 3*M*. Prepared daily to avoid thiocyanic acid polymerization.

Dibenzo-18-crown-6, solution in benzene, $5 \times 10^{-3}M$.

Tartaric acid solution, 3*M*.

Ascorbic acid solution, 10%. Freshly prepared.

General procedure

Pipette a portion of the sample containing up to 20 μg of niobium into a 100-ml separating funnel, add 5 ml of 6*M* hydrochloric acid and 3 ml of 3*M* potassium thiocyanate, and dilute to 10 ml with redistilled water. Add 10 ml of the crown-ether solution and extract the niobium by mechanical shaking (5 min) to achieve a rapid distribution of the extraction reagent between the two phases. Allow the phases to separate, filter the benzene solution through a dry filter paper and measure the absorbance at 398 nm against benzene.

RESULTS AND DISCUSSION

Spectral characteristics of the complex

The absorption spectra of the extracted species and the reagent blank are given in Fig. 1. The absorbance of the blank is very small at the wavelength of the

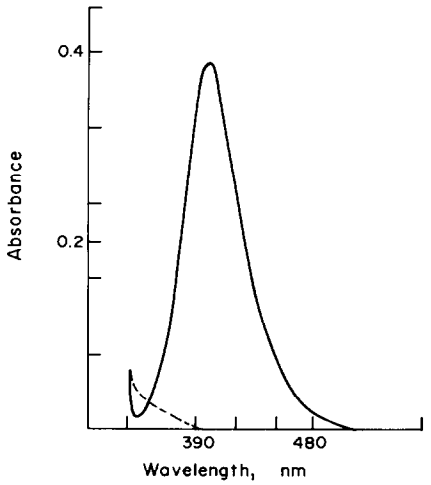

Fig. 1. Absorption spectrum of the niobium complex in benzene (dashed line corresponds to the reagent blank).

absorbance maximum of the complex, so benzene can be used as reference. The calibration graph is linear over the range 1–20 μg of niobium, and the molar absorptivity at 398 nm is 3.85×10^4 l.mole^{-1}.cm^{-1}. The relative standard deviation (10 replicates, 10 μg of niobium) is 0.8%.

Effect of reagent concentrations

The optimization studies were done with a fixed amount of 10 μg of niobium and a single extraction step. Figure 2 shows that the optimum concentrations are 2–4*M* hydrochloric acid and not less than 0.3*M* thiocyanate and 0.004*M* crown ether.

Extraction conditions

The extraction is maximal after 4 min shaking time, and the yellow colour produced remains constant for at least 1 hr. The efficiency of the extraction was determined by stripping the extracted niobium and determining it; a single extraction was found to be 99.4 ± 0.4% complete.

Interference studies

The effect of various metals (those most frequently associated with niobium in steels and niobium ores) on the determination is shown in Table 1. Table 2 gives the influence of some common anions that mask niobium.

As can be seen from Table 1, some elements interfere even at low levels (W, V, Ti, Sn and Cu) but their interference may be reduced by addition of ascorbic acid, tartaric acid or both as shown in Table 1.

Mo(VI) interference can be eliminated by a preextraction step, introduced into the general procedure, consisting of addition of potassium fluoride to the aqueous phase to prevent extraction of Nb(V) while the Mo(VI) is extracted with the crown ether. After this step, boric acid is added to the aqueous phase to demask Nb(V) and allow it to be extracted with a further portion of crown-ether solution.

In general, the extraction is fairly selective, and the method could be especially advantageous for niobium ores containing rare earths.

As shown in Table 2, fluoride is the most efficient masking agent for Nb(V), but the effect of up to 14.7

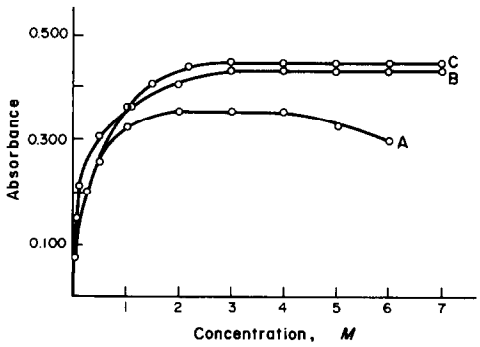

Fig. 2. Extraction efficiency as a function of reagent concentration: (A) molar concentration of HCl in the aqueous phase; (B) molar concentration ($\times 10^3$) of dibenzo-18-crown-6 in the organic phase; (C) molar concentration ($\times 10$) of KSCN in the aqueous phase.

Table 1. Effect of foreign ions on the determination of 10 μg of Nb(V) with DBC and KSCN

Foreign ion	Tolerance limit,* μg: Without masking agent	With masking agent	Masking agent
Th(IV), Zr(IV), U(VI), La(III), Ce(III), Cr(III), Mn(II), Ni(II)	1000		
Co(II)	150		
Fe(III)	50	2000	Ascorbic acid, 10 g/l.
Ta(V)	100	350	Tartaric acid, 150 g/l. + ascorbic acid, 10 g/l.
Cu(II)	10	50	
Sn(IV)	Interfere	20	
W(VI)	Interfere	150	Tartaric acid, 150 g/l.
V(V)	Interfere	50	
Ti(IV)	Interfere	5	
Mo(VI)	Interfere	50†	F^-, 1.5 g/l.

*Level causing an error not exceeding 2% in absorbance for 10 μg of Nb.
†Masking and demasking procedures are given in the text.

Table 2. Effect of some common masking anions on Nb(V) determination

	Tolerance limit, *mg*
Tartrate	1500
Phosphate	80
EDTA	80
Oxalate	2.5
F^- alone	1
F^- (+ boric acid, 8.5 g/l.)	14.7

*Level causing an error not exceeding 2% in the absorbance for 10 μg of Nb.

mg of fluoride in 10 ml of aqueous phase can be eliminated by adding 85 mg of boric acid for demasking.

Nature of the extracted species and mechanism of the extraction

The stoichiometry of the potassium dibenzo-18-crown-6 complex has been clearly established[13–15] as 1:1 (LK^+). As the anionic complexes of niobium and thiocyanate are of the type[10] $NbOX_n(SCN)_m^-$ or $NbO(SCN)_5^{2-}$, the expected Nb/L ratio should be 1:1 or 1:2 for neutralization of the charge.

Experimental results from investigation of the Nb/SCN ratio by the Asmus and equilibrium-shift methods invariably gave a value of 1:3 (Figs. 3 and 4). The experimental Nb/L ratio obtained by the same methods unexpectedly gave a value of 0.5 by the Asmus method. The equilibrium-shift method gave 0.45 at low L concentration but 0.77 at higher L concentration (Figs. 3 and 4).

It seems unlikely that the formation and extraction of any $NbO(SCN)_n$–LK^+ ion-pair could correspond to the Nb/L ratio observed. Thus it appears that neutral adducts of the type[16,17] $Nb_2Cl_{10}L$ and $NbCl_5L$ might be formed.

On this basis, the equilibrium between an aqueous solution containing niobium and thiocyanate, and an organic solution containing a cyclic polyether (L) could be simply expressed as

$$m\mathrm{Nb} + n\mathrm{SCN} + p\mathrm{L}_{org} \overset{K_c}{\rightleftharpoons} [\mathrm{Nb}_m(\mathrm{SCN})_n\mathrm{L}_p]_{org}$$

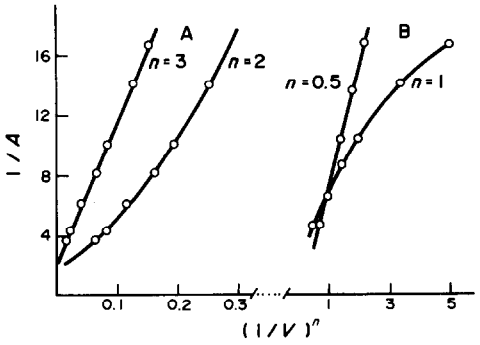

Fig. 3. The Asmus method applied to the Nb–L–SCN system. (A) Relationship Nb/SCN, Nb = 1.08 × $10^{-5}M$, L = 5 × $10^{-3}M$; (B) relationship Nb/L, Nb = 1.08 × $10^{-5}M$, SCN = 0.9M. A = absorbance, V = volume (ml).

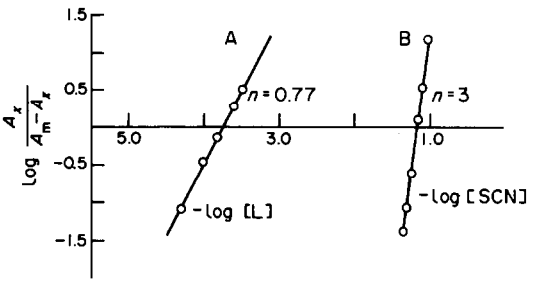

Fig. 4. Equilibrium-shift method applied to the Nb–L–SCN system. Nb = 1.08 × $10^{-5}M$. (A) Relationship Nb/L, SCN = 0.9M; (B) relationship Nb/SCN, L = 5 × $10^{-3}M$. A_x = absorbance of mixture, A_m = saturation absorbance with excess of reagent present.

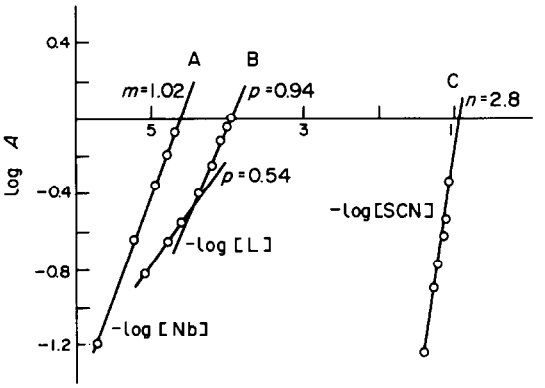

Fig. 5. Determination of molar ratios in the ternary system Nb–L–SCN. (A) L = 5 × $10^{-3}M$, SCN = 0.9M; (B) Nb = 2.15 × $10^{-4}M$, SCN = 1.5M; (C) Nb = 1.08 × $10^{-5}M$, L = 5 × $10^{-3}M$.

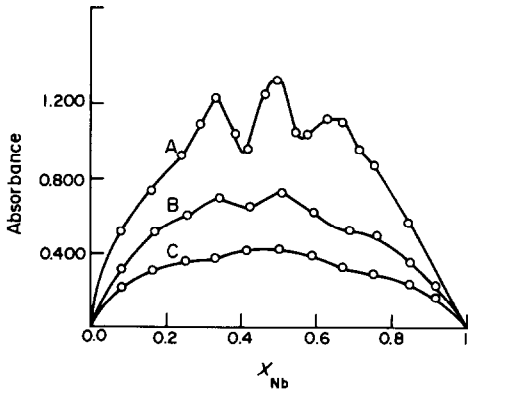

Fig. 6. Continuous-variations study of Nb–L systems. (A) Total concentration of Nb + L = 2.4 × $10^{-4}M$; (B) Nb + L = 1.8 × $10^{-4}M$; (C) Nb + L = 1.2 × $10^{-4}M$; wavelength = 398 nm, 1.00-cm cells.

where the subscript org refers to the benzene phase, and charges are omitted for simplicity. The extraction constant is

$$K_c = [\mathrm{Nb}_m(\mathrm{SCN})_n\mathrm{L}_p]_{org}/[\mathrm{Nb}]^m[\mathrm{SCN}]^n[\mathrm{L}]^p_{org} \quad (1)$$

and assuming that only one species is formed in a given range of reagent concentrations, we may write:

$$[Nb_m(SCN)_nL_p]_{org} = A/\epsilon l \quad (2)$$

where ϵ = molar absorptivity and l = cell path-length. Substituting (2) into (1) and simplifying gives

$$\log A = m\log[Nb] + n\log[SCN] + p\log[L]_{org} + \log K_c\epsilon l$$

It is possible to determine m, n or p by maintaining all concentrations constant except that of the appropriate component. The values found in this way were $m = 1.02$ and $n = 2.8$, but for p two values were obtained, 0.54 at low L concentration and 0.94 at high L concentration (Fig. 5).

Moreover Job plots ([Nb] and [L] varied, their sum being constant) showed three absorption maxima, located at $X_{Nb} = 0.33$, 0.5 and 0.66 (corresponding to 1:2, 1:1 and 2:1 stoichiometries) (Fig. 6).

To get more information about the nature of the extracted complexes, attempts were made to isolate them from the benzene extract. The procedure already described was applied to 0.5 mg of niobium in 10 ml of aqueous phase, and a yellow-orange crystalline compound was obtained. This was filtered off, rinsed with pure benzene to eliminate the excess of LK^+SCN^- and LK^+Cl^- present, and finally dried at 100°. Elemental analysis gave the results in Table 3, which agree with the values calculated for a complex of formula $[NbOCl_2(SCN)_3]^{2-}$ $(LK^+)_2$. This formulation is supported by comparative conductivity measurements on $10^{-4}M$ acetonitrile solutions of this species, L and LK^+Cl^-. The observed specific conductance of the solution of the complex is about 50 times that of the L solution and only slightly lower than that of the $LK^+ \cdot Cl^-$ solution.

DISCUSSION

The results obtained can be explained in terms of an extraction process dependent on the concentration of L. If this concentration is low, the most probable species formed is a neutral adduct $[NbO(SCN)_3]_2L$ analogous to $(NbCl_5)_2L$ (recently demonstrated in the reaction of $NbCl_5$ with L[17] or with cyclic polythiaethers).[16] When excess of L is used, the species $NbO(SCN)_3L$ would also be formed (cf. formation of $NbCl_5L$[17]). Simultaneous formation of both compounds explains the slope of 0.77 obtained for the Nb/L ratio by the equilibrium-shift method (Fig. 4). Under the conditions of the procedure, during the shaking L is mostly changed into the cationic LK^+ complex, which could be responsible for the formation of the ion-pair $NbOCl(SCN)_3^- LK^+$. With a large excess of L, as in the analytical procedure, the species formed in the organic phase could be ion-association complexes having a higher content of L, such as $NbOCl_2(SCN)_3^{2-}(LK^+)_2$, as demonstrated by the analysis of species isolated, supported by the Nb:L ratio of 1:2 found in the continuous-variations experiments.

Table 3. Analysis of the isolated complex

	Experimental, %	Theoretical,* %
C	44.8	45.34
N	3.7	3.69
H	4.2	4.25
Nb	7.8	8.16
Cl	6.6	6.22
K	7.1	6.86

*For $NbOCl_2(SCN)_3^{2-}(LK^+)_2$.

Table 4. Determination of niobium

Sample	Niobium present, %	Niobium found, %			
Stainless steel BCS 261/1	0.91	0.91_3	0.91_4	0.90_7	0.90_9
Pyrochlore OKA-1	0.36	0.34_9	0.35_6	0.34_7	0.35_2

The proton NMR data reported[16,17] for such adducts suggest a weak bond between the Nb(V) and the available oxygen co-ordination sites of the polyether. The low dielectric constant of the organic solvent, its solvating properties and especially the large excess of L (as LK^+) would promote further stabilizing ion-association reactions of such adducts in the organic phase.

Conclusion

The mechanism of extraction anionic metal complexes with cationic crown-ethers becomes quite involved when the metal has great affinity for the oxygen atoms of the polyether. In such cases the species extracted may be neutral adducts of the unsaturated co-ordination type[18] which are extracted along with the expected ion-pair $LK^+MX_n^-$.[5,6]

For the system studied here, it seems that further reaction can take place in the organic phase between the excess of L and the ion-association species containing the cation to be determined, so that species such as $NbO(SCN)_3Cl_2(LK)_2$ are the final species responsible for the absorption properties of the organic extract.

To assess the reliability and utility of the method, a stainless steel (B.C.S. 261/1) and a niobium pyrochlore ore from OKA (Canada) were analysed. The steel was dissolved by the method proposed by Sanz-Medel *et al.*[19] and the ore sample by a modification of Faye's method.[9] The results agreed very well with the expected values (Table 4).

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A NEW GRAPHICAL METHOD BASED ON STOICHIOMETRIC DILUTION FOR THE CLASSIFICATION OF BINARY COMPLEXES WITH MOLE RATIO 1:1

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Summary—A simple approach to the treatment of spectrophotometric data obtained by stoichiometric dilution is presented for complexes of the type M_mB_n and $m/n = 1$. The values of m and n are obtained by matching the slope of a straight line with a mathematically predicted value. The method can be applied to moderately strong complexes, the concentration of which can be determined by absorbance measurements, and allows calculation of the formation constant as well.

Various methods have been reported for the characterization of metal complexes in solution.[1,2] In many cases the existence of several complex species must be assumed, and a large amount of very precise data must then be obtained, usually by potentiometric titration or polarography, and the data must be treated by computer for the values of the average ligand number, $\bar{n}$, and the formation constants to be obtained. The distribution of species under a given set of conditions may then be calculated. The study of such a system can be a very time-consuming task. The interested reader should refer to the work by Rossotti and Rossotti.[1] If, however, it is evident that only a single complex is formed, the study can be greatly simplified. Such chemical systems are commonly encountered in spectrophotometric analysis, in which conditions are adjusted to ensure the formation of a single complex. These systems are most conveniently studied by molecular absorption spectrophotometry.

Spectrophotometric methods for differentiating mononuclear and polynuclear complexes depend on the tendency of a complex to dissociate in solution at low concentrations. For a general equilibrium reaction

$$mM + nB \rightleftharpoons M_mB_n \qquad (1)$$

where M_mB_n is the only significant complex formed, three general approaches have been used. In the log ratio (or fixed logarithm) method,[3] the absorbance is measured as small amounts of one reactant are added to a large fixed amount of the other under such conditions that most of the reagent added remains uncomplexed. A second approach developed by Klausen and Langmyhr[4,5] depends on the presence of inflection points in a continuous variations plot to characterize complexes with mole ratio 1:1. A third and most useful approach depends on the degree of dissociation of the complex when the reactants are mixed in the stoichiometric ratio. Budĕšínský[6–10] has referred to this as the method of stoichiometric dilution, and it has been employed by several workers.[11,12] Recently several new mathematical treatments of data obtained by the method of stoichiometric dilution have been reported,[13,14] including one which can be applied to mixed-ligand complexes.[15] These methods allow the calculation of formation constants for moderately strong complexes, but are limited by the requirement that only a single complex must be formed. An alternative but similar approach, which might be called non-stoichiometric dilution, allows the calculation of formation constants of very weak complexes and can be used if more than one complex is formed.[16]

The present paper describes a new approach to the differentiation of mononuclear and polynuclear complexes with mole ratio 1:1, employing the method of stoichiometric dilution. It also yields the formation constants of moderately strong complexes.

THEORY

Consider the equilibrium reaction in equation (1) where M_mB_n is a coloured metal complex, the concentration of which can be measured spectrophotometrically, and for which the mole ratio has been previously determined by the usual methods and found equal to unity.[17–19] The degree of complex formation (α_c) is defined by

$$\alpha_c = \frac{m[M_mB_m]}{C_M} = \frac{A_{M_mB_m}}{A^{max}_{M_mB_m}} \qquad (2)$$

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where C_M = total metal ion concentration, $A_{M_mB_m}$ is the absorbance due to the complex M_mB_m, and $A^{max}_{M_mB_m}$ is the absorbance due to M_mB_m for 100% complexation of the metal ion. If M_mB_m is the only complex formed between M and B, the total absorbance $A = A_{M_mB_m} + A_B$, where A_B is the absorbance due to uncombined B. Correction for the absorbance due to uncomplexed reagent can be made by successive approximations, beginning with $A_{M_mB_m} \sim A$ in equation (2), and recalculating $A_{M_mB_m}$ after application of the relation $A_B = \epsilon_B(C_B - \alpha_c C_M)$, where ϵ_B is the molar absorptivity of B and C_B is the total concentration of B.

The fraction of uncomplexed metal ion, α_M, is defined by

$$\alpha_M = \frac{C'_M}{C_M} \tag{3}$$

where C'_M is the total concentration of uncomplexed metal ion. If M_mB_m is the only complex formed between M and B under the conditions of the experiment, then

$$C_M = m[M_mB_m] + C'_M \tag{4}$$

and dividing through by C_M yields

$$1 = \alpha_c + \alpha_M \tag{5}$$

The conditional formation constant for reaction (1) is

$$K' = \frac{[M_mB_m]}{(C'_M)^m(C'_B)^m} \tag{6}$$

where C'_B is the total concentration of uncomplexed B. If the experimental conditions are arranged so that $C_M = C_B$, then substitution of either α_M or α_c into equation (6), followed by a simple algebraic modification, will yield linear equations, which directly yield the value of m.

Substitution of α_c

Rearrangement of equation (2) gives

$$[M_mB_m] = C_M\alpha_c/m$$

Combination of equations (2) and (4) yields

$$C'_M = C_M(1 - \alpha_c)$$

Under the experimental constraints, $C'_M = C'_B$, and equation (6) becomes

$$K' = \frac{C_M\alpha_c}{mC_M^{2m}(1 - \alpha_c)^{2m}} \tag{7}$$

which on rearrangement and taking of logarithms gives

$$\log \alpha_c/(1 - \alpha_c)^{2m} = \log mK + (2m - 1)\log C_M \tag{8}$$

Thus a plot of $\log \alpha_c/(1 - \alpha_c)^{2m}$ *vs.* $\log C_M$ yields a straight line with slope $2m - 1$ and intercept $\log mK$. Selection of different values of m will yield straight lines with different slopes, but only the correct value of m will yield a slope which is equal to the value predicted by equation (8).

Substitution of α_M

The same results can be achieved by substituting appropriate expressions involving α_M into equation (6). Alternatively, substitution of equation (5) directly into (8) yields

$$\log(1 - \alpha_M)/\alpha_M^{2m} = \log mK + (2m - 1)\log C_M \tag{9}$$

Thus a plot of $\log(1 - \alpha_M)/\alpha_M^{2m}$ *vs.* $\log C_M$ will yield the same results as equation (8).

Calculation and precision of the formation constant

After the value of m has been determined, the value of the conditional formation constant can be obtained from the intercept according to equation (8), or by direct calculation from equation (7). The intercept method is likely to produce poor results because it involves a long extrapolation. Calculation of K' from equation (7) is preferable.

The relative error in the value of K' can be ascertained by differentiating equation (7) with respect to α_c, which yields

$$\frac{\delta K'}{\delta \alpha_c} = \frac{K'[1 + (2m - 1)\alpha_c]}{\alpha_c(1 - \alpha_c)}$$

Rearranging, converting to finite differences, and replacing $\Delta\alpha_c/\alpha_c$ with $\Delta A/A$, yields

$$\frac{\Delta K'}{K'} = \frac{\Delta A}{A}\left(1 + \frac{2m\alpha_c}{1 - \alpha_c}\right) \tag{10}$$

for a system in which the reagent blank is insignificant, that is, $A \sim A_{M_mB_m}$. The same expression was reported earlier for a similar approach[15] along with several curves to illustrate the relative error in K' for various values of m and α_c. Note that $\Delta K'/K'$ becomes very large as α_c approaches unity (complete complex formation), and approaches $\Delta A/A$ for small values of α_c.

RESULTS

Copper–orotic acid system

Earlier studies showed[13,16] that Cu(II) and orotic acid combine to form a 1:1 complex with $m = n = 1$, $\epsilon = 7.20 \times 10^3$ l.mole^{-1}.cm^{-1}, and $\log K' = 4.85$ in pH 5.01 acetate buffer. Table 1 shows calculation of the values of α_c from the equation

$$\alpha_c = A_{M_mB_m}/\epsilon' l C_M \tag{11}$$

where ϵ' is the apparent molar absorptivity calculated for $m = 1$ (this can be done without knowing the value of m, since C_M is used in the expression rather than the maximum concentration of the complex). Values of $\log \alpha_c/(1 - \alpha_c)^{2m}$ were calculated for m values from 1 to 4. Straight lines with correlation coefficients of 0.998 or better were obtained for each m

Table 1. Copper–orotic acid system*

C_{Cu}, μM	A	α_c	log $K'(m = 1)$
20	0.064	0.444	4.86
40	0.162	0.563	4.87
60	0.265	0.613	4.83
80	0.385	0.668	4.88
100	0.498	0.692	4.86
140	0.735	0.729	4.85
200	1.095	0.760	4.82

Assumed m	Predicted slope	Observed slope	Δslope	Δ^2slope
1	1	0.997 ± 0.02	−0.02	1.26
2	3	1.72 ± 0.04	−1.28	1.26
3	5	2.46 ± 0.05	−2.54	1.26
4	7	3.20 ± 0.07	−3.80	

*Data taken from reference 13.

value (Fig. 1), but only for $m = 1$ did the observed slope agree with the predicted slope. This was in agreement with previous results.

The formation constant calculated from equation (7) for the seven cases is log $K' = 4.85 \pm 0.02$, in excellent agreement with the previously reported result.[13]

Iron–Chrome Azurol S system

In a recent paper[15] we reported (in agreement with earlier studies[4,5,13]) that iron(III) combines with Chrome Azurol S (CAS) at pH 3.4 to form a single complex, with $m = n = 2$, a molar absorptivity at 570 nm equal to 4.60×10^4 l.mole^{-1}.cm^{-1}, and a formation constant log $K' = 17.88$ in 0.01M potassium chloride. Treatment of our previous results according to the present approach yields the results shown in Table 2. Values of α_c were calculated from equation (11), with $\epsilon' = 2.30 \times 10^{-4}$ l.mole^{-1}.cm^{-1}. The predicted and observed slopes agree for $m = 2$ (Fig. 2), corresponding to the composition $Fe_2(CAS)_2$. The average value of the formation constant calculated from equation (7) is log $K' = 17.89 \pm 0.05$, in agreement with 17.88 found in our previous study.[15]

Treatment of the data reported by Garcia *et al.*[13] yielded similar results. For $m = 1$, the predicted slope was 1 and the observed slope 1.67. For $m = 2$, the predicted slope was 3 and the observed slope 3.02, confirming the composition $Fe_2(CAS)_2$. The conditional formation constant, in this case at pH 3.40 and in 0.1M potassium chloride, was log $K' = 16.55$.

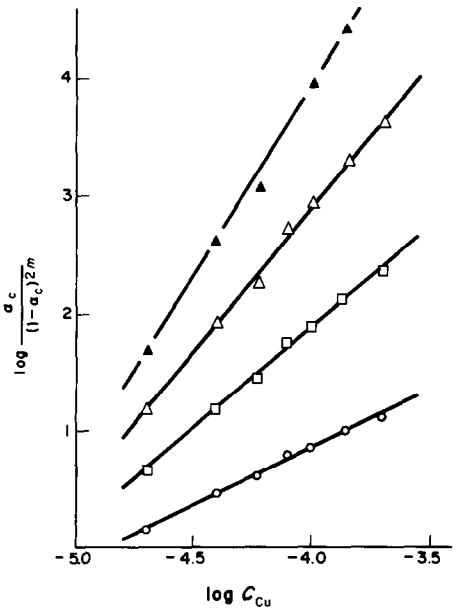

Fig. 1. Plots of log $\alpha_c/(1 - \alpha_c)^{2m}$ *vs.* log C_M for the copper–orotic acid system, assuming different values of m. ○, $m = 1$; □, 2; △, 3; ▲, 4.

DISCUSSION

The proposed method has been shown to yield the correct results for two well-studied systems. Its application is very straightforward, and although it is experimentally similar to previously described approaches,[7–15] its mathematical simplicity makes it particularly attractive. The success of this new approach depends on several factors as discussed below.

Since the degree of complexation is calculated from absorbance measurements, the apparent molar absorptivity of the complex must be obtained experimentally. Usually this means that excess of ligand must be added to a known concentration of metal ion to make complexation practically 100% complete. This raises the possibility that higher complexes will be formed, with a resultant error in the molar absorp-

Table 2. Iron–Chrome Azurol S system*

C_{Fe}, μM	A	α_c	log $K'(m = 2)$
4	0.065	0.707	17.87
6	0.107	0.775	17.85
8	0.152	0.826	17.94
10	0.194	0.843	17.84
12	0.240	0.870	17.95

Assumed m	Predicted slope	Observed slope	Δslope	Δ^2slope
1	1	1.65 ± 0.08	+0.65	0.54
2	3	3.11 ± 0.15	+0.11	0.54
3	5	4.57 ± 0.22	−0.43	0.54
4	7	6.03 ± 0.29	−0.97	0.54
5	9	7.49 ± 0.36	−1.51	

*Data taken from reference 15.

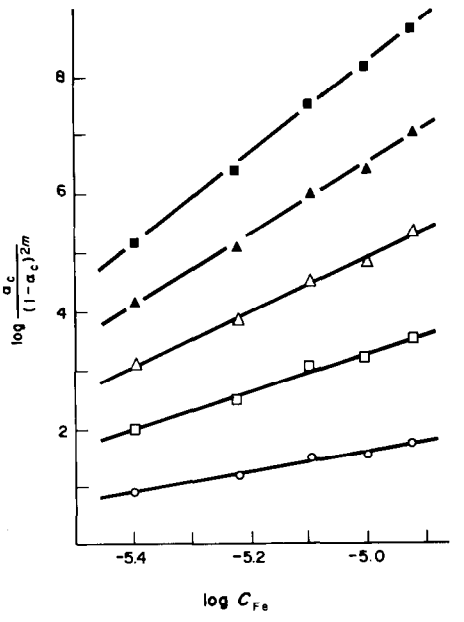

Fig. 2. Plots of log $\alpha_c/(1-\alpha_c)^{2m}$ *vs.* log C_M for the iron–Chrome Azurol S system, assuming different values of *m*. ○, *m* = 1; □, 2; △, 3; ▲, 4; ■, 5.

tivity. For this reason it is important to make a complete mole-ratio study in which the metal concentration is held constant and the concentration of ligand is increased. An absorbance *vs.* added ligand concentration graph giving two straight-line portions connected by a smooth curve is indicative that additional ligand molecules are not incorporated into the complex. The molar absorptivity determined in this experiment can be applied to solutions in which the reactants are mixed in the stoichiometric ratio. Such a study was performed for the iron–Chrome Azurol S system, and no evidence was found for formation of higher complexes.

Further evidence for the existence of only a single major complex in solution can be obtained from absorption spectra. If only one complex exists, the background-corrected spectrum obtained for the stoichiometric solution will be identical (except in amplitude) to the spectrum of the solution containing excess of ligand (provided the ligand itself does not absorb in the region examined).

The uncertainty in the formation constant for various values of $m + n$, A, and α_c, which was illustrated by graphs in the previous report,[15] also applies to this method. In general it is desirable to keep $\alpha_c < 0.5$ and $0.1 < A < 0.8$. Under these conditions the relative error in K' due to uncertainty in the absorbance measurement will be less than 10% for $m \leq 2$. The error becomes larger for $m > 2$, but such complexes are rarely encountered.

It is interesting to note that if the difference (Δ slope) between the observed and predicted slope is taken for each *m* value, and the difference between successive Δ slope values is then taken,

$$\Delta^2\text{slope} = (\Delta\text{slope})_{m+1} - (\Delta\text{slope})_m$$

the values of Δ^2slope are constant for a given system (Tables 1 and 2). A plot of the observed slope *vs.* assumed *m* value must therefore yield a straight line of the general form

$$y = ax + b \tag{12}$$

where y = observed slope and x = assumed *m* value. There can be only one correct pair of x and y values for a specific system, given by the equation for the predicted slope line

$$y = 2x - 1 \tag{13}$$

where in this case x = true *m* value and y is the correct predicted slope. It follows that the correct solution will be given by the intersection of the lines corresponding to equations (12) and (13). In Fig. 3 the observed slopes obtained for the two systems studied are plotted against the assumed *m* values. The point of intersection can be estimated from the graph, or calculated exactly by solving equations (12) and (13) for x. For the copper–orotic acid system equation (12) is $y = 0.741x + 0.237$, which yields $x = m = 0.98$. For the iron–Chrome Azurol S system, equation (12) is $y = 1.460x + 0.190$, which yields $x = m = 2.20$. Treatment of the data of Garcia *et al.*[13] gives $m = 1.94$.

Figure 3 suggests that as the true value of *m* increases, the line for the observed slope *vs.* assumed *m* and the predicted slope line will tend to merge, leading to larger uncertainties in the point of intersection. It follows that the best precision is obtained for small values of *m*.

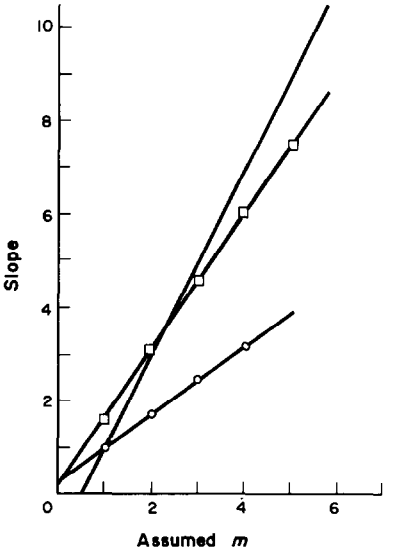

Fig. 3. Observed slope *vs.* assumed *m* for the copper–orotic acid (○) and iron–Chrome Azurol S (□) systems. Unbroken line is the predicted slope line.

The requirement that the complex must undergo significant dissociation limits the application of this method to complexes that are not extremely stable. Various allowable combinations of formation constants and molar absorptivities can be calculated from equations (6) and (11).

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A COMPARISON BETWEEN SECONDARY-ION MASS-SPECTROMETRY AND SPARK-SOURCE MASS-SPECTROMETRY FOR THE QUANTITATIVE ANALYSIS OF STEEL WIRE

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Summary—A comparison has been made between the results of the matrix-ion species ratio (MISR) method for quantification of secondary-ion mass-spectrometry data and spark-source mass-spectrometry analysis using photoplate detection for analysis of the steel basis of Al–Zn coated wire products. For SIMS quantification a suitable set of sensitivity factors, corrected for the actual surface sampling condition, was used. The results of both methods compare well. The SIMS results were, for most elements, within 25% of the concentration determined by SSMS. This could indicate that reasonably accurate results can be obtained by using the matrix-ion species ratio method for SIMS.

Secondary-ion mass-spectrometry (SIMS) is a powerful technique for the surface analysis of solid samples. The surface is bombarded with a beam of primary ions of several keV energy. As a result of the impact, surface particles are sputtered as atoms or ions. These ions are extracted from the target region and passed through a mass analyser, where they are separated according to their mass-to-charge ratio. These secondary ions are detected by suitable means.

The ability of SIMS to produce quantitative results is hampered by the large variations in sensitivity for different elements. Although the primary utility of SIMS is the possibility of performing both elemental distribution mapping of surfaces (in the ion microprobe or ion microscope version) and depth profile analysis, quantitative information is also often required.

The elemental sensitivities range over five orders of magnitude for various elements and moreover are sensitive to the matrix composition. Thus a set of sensitivity factors (SF) has to be determined for each element in each matrix. Smith and Christie[1] made a comparison of a theoretical model and SF determinations for quantification of SIMS data for glass and iron standard reference materials. Their experimental results based on use of SF were within a factor of 2 of expectation for 85% of the elements determined whereas for the theoretical approach, using the Anderson and Hinthorne model,[2] this level of agreement was achieved for only 55% of the elements.

More accurate quantitative results can be obtained, however, for SIMS data by using the matrix-ion species ratio (MISR) method described by Ganjei *et al.*[3] This method consists essentially of the use of a ratio of matrix-ion intensities (*e.g.*, M^+, MO^+, M_2^+, M_2O^+) to calibrate the elemental SF. Through admission of oxygen into the sample chamber during analysis of an external standard, a series of SF-values is generated as a function of the matrix-ion ratio. This ratio effectively indexes the sampling environment. The relative error of the MISR method, for different metallic matrices, is about 10–20%.

In this study, quantitative SIMS analysis using the MISR procedure is compared with results obtained with spark-source mass-spectrometry (SSMS). The latter technique is based on the formation of ions from a solid sample by a radiofrequency spark. The positive ions generated are accelerated and separated in a high-resolution double-focusing mass spectrometer. They are finally detected either simultaneously as a line spectrum on an ion-sensitive emulsion, or separately by a suitable dynode of an electron multiplier. SSMS has a distinct advantage for general analytical work on conducting solids, because it is possible to prepare two specimens of the sample for use as a pair of electrodes. This reduces the possibility of contamination. Some important advantages of SSMS in comparison with other methods are the possibility of detecting almost all elements simultaneously, the very low detection limits down to the ng/g level, an approximately equal sensitivity for all elements and the absence of matrix effects.[4]

EXPERIMENTAL

Samples

The samples investigated consisted of transverse cuts of the steel base of 55% Al–Zn coated steel wires (Bekaert N.V., Zwevegem, Belgium). As a standard sample, NBS SRM 467 low-alloy G steel was used, the composition of which closely corresponds to that of the steel wire.

For SIMS measurements the samples were mounted in the sample holder and embedded in a conducting resin.

They were subsequently wet-polished with 280 and 400 mesh abrasive paper and fine-polished with 25-μm, 15-μm, 3-μm and 1-μm metallurgical diamond paste used successively. They were presputtered during at least 15 min over a 1 × 1 mm area until stable ion signals were obtained. In this way any contamination due to the pretreatment was removed.

For SSMS analysis, the steel wire (diameter 3.4 mm) was metallographically ground and chemically polished in 20% HNO_3–10% HF solution until the surface coating was entirely removed. The resulting samples had a mirror-bright appearance and 10-mm long pieces were used as electrodes. They were presparked for at least 30 min, before SSMS analysis.

SIMS analysis

Measurements were performed with a CAMECA IMS-300 ion analyser, equipped with an electrostatic sector analyser for energy discrimination of the secondary ions. The apparatus was automated for spectral data acquisition and processing. The computer system used and program possibilities are described elsewhere.[5,6] The experimental SIMS measurements are summarized in Table 1. For quantitative analysis by the MISR method a series of SF is generated as a function of the reactive-gas adsorption on the NBS standard sample surface by progressively admitting oxygen into the sample chamber to increase the pressure from 2×10^{-7} to 7×10^{-5} mmHg. In this way a large range of secondary-ion yields is obtained, with only one external standard. Its homogeneity was investigated earlier.[7] The sampling constants provided a means for estimating the number of analyses needed for achieving a precision of 10%. They are listed in Table 2. Therefore at least five measurements were performed after each pressure increment, several minutes being allowed for stabilization between sputtering and adsorption. The actual surface sampling condition was thus measured by the ratio of two matrix ions which react differently upon adsorption of oxygen. The Fe_2^+/Fe^+ ratio was chosen in this work. The SF were calculated by using the relation:

$$SF_x = \frac{I_{x_i}}{I_{Fe_j}} \times \frac{C_{Fe}}{C_x} \times \frac{f_i}{f_j} \qquad (1)$$

where I_{x_i} and I_{Fe_j} denote the secondary-ion intensities of the ith isotope of the element in question and the jth isotope of the reference element Fe, while f_i and f_j symbolize the respective abundances of the chosen isotopes. Since the $^{56}Fe^+$ signal was too high to be measured ($> 3 \times 10^6$ cps) and since $^{57}Fe^+$ and $^{58}Fe^+$ were interfered with by $^{56}FeH^+$ and $^{58}Ni^+$ respectively, $^{54}Fe^+$ was chosen as the reference ion. The spectral interferences of $^{54}Fe^{2+}$ with $^{27}Al^+$ and of $^{56}Fe^{2+}$ with $^{28}Si^+$ were eliminated by applying high mass-resolution ($M/\Delta M = 3000$), while the interference of $^{54}FeH^+$ with $^{55}Mn^+$ could be sufficiently reduced by energy discrimination against secondary ions with kinetic energy less than 100 eV. For all other elements in the standard interference-free isotopes could be chosen, or the interference could be proved to be insignificant.

Table 1. Experimental SIMS conditions

Primary ion type	$^{40}Ar^+$
current density	~0.3 mA/cm²
energy	6 keV
source working pressure	1.5×10^{-4} mmHg
beam diameter	250 μm
Immersion lens aperture	55 μm
Magnetic field diaphragm	750 μm
Electrostatic analyzer energy window	4 eV
Working O_2-pressure in sample chamber	$\sim 2 \times 10^{-5}$ mmHg

Table 2. Number of analyses needed for achieving a 10% precision with a 0.05 probability of exceeding this error, for the NBS SRM 467 standard (area analysed = 0.02 mm²)

Element	Number of analyses
Al	2
Si	4
Ti	5
V	1
Cr	3
Mn	3
Co	2

The SF were fitted as a function of the matrix-ion ratio according to

$$SF_x = a(Fe_2^+/Fe^+)^b \qquad (2)$$

The sensitivity curves for V, Cr, Co and Mo are shown in Figs. 1 and 2 as an example. Table 3 lists the coefficients a and b and the correlation coefficient r for the sensitivity curves of the elements detected in the SRM.

The logarithm of SF at a given matrix-ion ratio is linearly related to the first ionization potential, as appears from Fig. 3. For the elements for which no SF can be determined because they are not detectable in the standard,

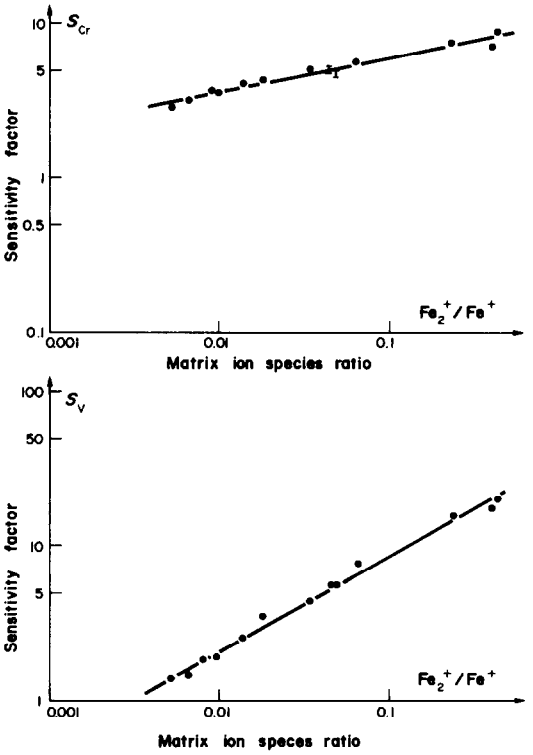

Fig. 1. Sensitivity factors for V and Cr as a function of the surface sampling condition (matrix-ion ratio Fe_2^+/Fe^+).

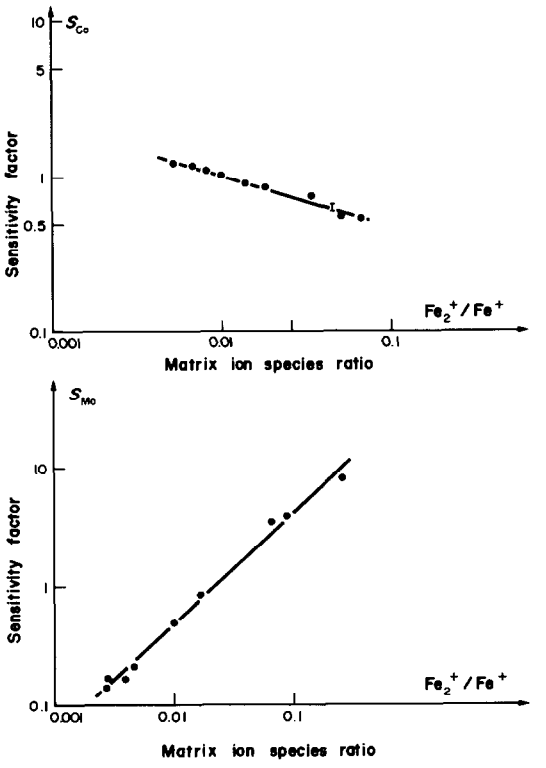

Fig. 2. Sensitivity factors for Co and Mo as a function of the surface sampling condition (matrix-ion ratio Fe_2^+/Fe^+).

namely Na, Mg, K, Ca, Zn, Ga and Sb, at least semiquantitative results can be obtained by using this relationship. From Fig. 3, the error expected from use of this procedure should be less than a factor of 2.

SSMS analysis

The instrument used was a Jeol JMS-01-BM-2 double-focusing mass spectrometer, incorporating a spherical electrical sector (*z*-focusing). Detection was done with Ilford Q2 ion-sensitive plates. The transmission profiles of the mass lines were stored on magnetic tape after automated densitometry, and the data reduction was performed on a PDP 11/45 computer, following the program developed by Pilate.[8] The SSMS spectrographic conditions are given in Table 4.

The quantitative analysis of the ion-sensitive plates in SSMS is very time-consuming because of the necessity of using several exposures to cover adequately the entire concentration range from the matrix level down to the ppm region.

The transmission profiles were integrated by using the Hull function,[9] and the plate "gamma" was determined by using the isotopic abundances of ^{54}Fe and ^{56}Fe and of ^{52}Cr and ^{53}Cr. The different exposures were correlated by using "calculated" exposures instead of the unreliable coulometer readings. ^{54}Fe was used as an internal standard. The interference of ^{54}Cr was insignificant (<0.01%). For the quantitative analysis only singly-charged ions were used. The following interference-free isotopes were selected: ^{23}Na, ^{24}Mg, ^{27}Al, ^{29}Si, ^{30}Si, ^{31}P, ^{32}S, ^{34}S, ^{35}Cl, ^{37}Cl, ^{39}K, ^{40}Ca, ^{50}Cr, ^{52}Cr, ^{53}Cr, ^{51}V, ^{55}Mn, ^{54}Fe, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{62}Ni, ^{63}Cu, ^{65}Cu, ^{64}Zn, ^{68}Zn, ^{69}Ga, ^{71}Ga, ^{75}As, ^{92}Mo, ^{98}Mo, ^{116}Sn, ^{118}Sn, ^{120}Sn, ^{121}Sb and ^{123}Sb.

Three individual measurements were performed on the sample. Also, in order to compensate for differences in sensitivity of the photoplates, 8 exposures of the sample were compared with 7 exposures of the NBS SRM 467 standard, collected on the same photoplate. For the certified elements the concentrations in the unknown sample could then be directly calculated.

For some uncertified elements an empirical approach was used for the calculation of the elemental sensitivities. For the analysis of steel, Van Hoye[10] found a good agreement between his experimental SF and those calculated by the method of Williardson and Socha,[11] using the relation

$$SF_{(x/y)} = \left[\frac{IP_x}{IP_y}\right]^{3.3} \qquad \text{for } IP_x < IP_z \tag{3}$$

The concentration of the elements Na, Mg, K, Ca and Ga could be calculated by using this method. For S, Cl, Zn and Sb, which cannot be determined by means of equation (3), the approach of Taylor and Gorton[12] was used [equation (4)]

$$SF_{(x/y)_z} = \frac{(IP_x)^2\,(BP_x)^{1/2} M_x}{(IP_y)^2\,(BP_y)^{1/2} M_y} \tag{4}$$

In equations (3) and (4) the indices x and y refer to the unknown element and the reference element respectively, z symbolizes the matrix, BP is the boiling point (K), *M* the mass, and IP the first ionization potential (eV).

Table 3. Coefficients *a* and *b* for the relation between sensitivity factors (SF) and matrix-ion species ratio [$SF = a(Fe_2^+/Fe^+)^b$] and the corresponding correlation coefficients *r* for NBS SRM 467

Element	*a*	*b*	*r*	Number of data points collected
Al	159	0.60	0.989	13
Si	0.71	0.052	0.707	7
P	0.034	0.148	0.800	7
V	35.4	0.617	0.995	13
Cr	9.54	0.214	0.976	13
Mn	2.11	0.073	0.943	12
Co	0.225	−0.329	0.989	10
Ni	7.5	0.458	0.955	8
As	0.0042	−0.446	0.989	8
Zr	102	1.01	0.990	13
Mo	37	0.94	0.994	9
Sn	5.16	0.624	0.974	10

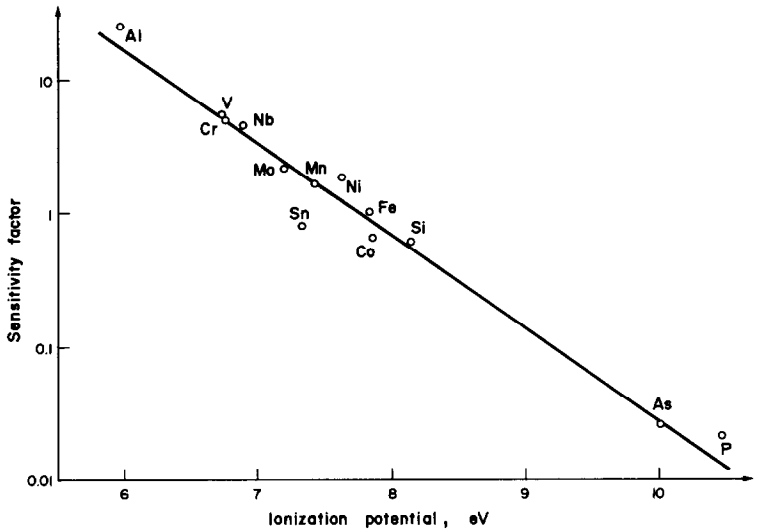

Fig. 3. Linear relationship between the logarithm of the sensitivity factor (SF) and the elemental first ionization potential (IP) for a matrix-ion ratio $Fe_2^+/Fe^+ = 0.047$.

RESULTS

Figure 4 shows the mass spectrum of the steel wire for the region m/e = 20–120. The elements C, Na, Mg, Al, Si, P, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Mo, Sn and Sb are detected (SIMS).

The resulting concentrations, determined by SIMS, are given in Table 5. For Cl, Ca, Zn, Zr and Sb only upper limits can be given, owing either to the absence of detectable ion signals (Cl, Zr) or to the presence of spectral interferences (Ca, Zn, Sb). About 20 analyses were performed for each element, except for Si and Co, for which up to 40 data points were collected, and for Ga, for which significant peaks were obtained in only two mass spectra. The precision is better than 20% for all elements, except for K and Ga, for which the errors amount to 40 and 50% respectively. Silicon was found to be quite heterogeneously distributed. Concentrations ranging from 0.1 to 0.3% were found, averaging around 0.21%.

Table 4. Spark-source mass-spectrographic conditions

Vacuum conditions	
Electrostatic analyser	$<10^{-8}$ mmHg
Magnetic analyser	$<10^{-8}$ mmHg
Source (when sparking)	$\sim 10^{-7}$ mmHg
Spectrometer parameters	
Spark voltage	60 kV
Repetition frequency	3 kHz
Pulse width	20 μsec
Accelerating voltage	29.0 kV
Main slit aperture	20 μm
α,β Slit openings	0.7 mm
Magnet current	4.75 A
Electrostatic analyser	2.9 kV
Exposure range	$0.3–1.5 \times 10^4$ pC
Detector	Ilford-Q_2 glass photographic plate

For the determination of the Al, Si, P, Cl, Ca, V, Cr, Mn, Co, Ni, As, Zr, Mo, Sn and Sb concentrations in the steel-wire sample several analyses were performed on different locations within the presputtered area. The Fe concentration was assumed to be 100%, as the total concentration of impurities as determined by SSMS is less than 1%.

The resulting concentrations, determined by SSMS, are also given in Table 5. The overall precision is 25%.

DISCUSSION

The overall agreement between the concentrations determined by SIMS and SSMS is good, as can be seen from Fig. 5. For 55% of the elements determined by using experimental SF, the difference is smaller than 25% relative error, and for 90% of these elements the error is better than 70%. Only for Sn is a large deviation obtained (factor 2.7); no explanation has yet been found to account for this discrepancy.

Where the concentrations are calculated by empirical methods for the compensation of differences in relative sensitivity, the agreement is less satisfactory, especially for K and Ga (factors 2.1 and 4.5 respectively). This could in principle be due to errors in the standardization of either the SIMS or SSMS. For Ga, heterogeneities and the lack of a sufficient number of data points could be responsible for the lack of accuracy from use of SIMS. For K the sensitivity factor was obtained by extrapolation of the log SF *vs.* IP graph. Taking into account the very low ionization potential of this element, the accuracy might be limited. It can be noted that the use of a similar MISR procedure for analysis of electric furnace dust also provided exceptionally low K concentrations, (low by a factor of 4).[13]

Also, SSMS results might lead to rather large inaccuracies, as Van Hoye[10] showed that differences in

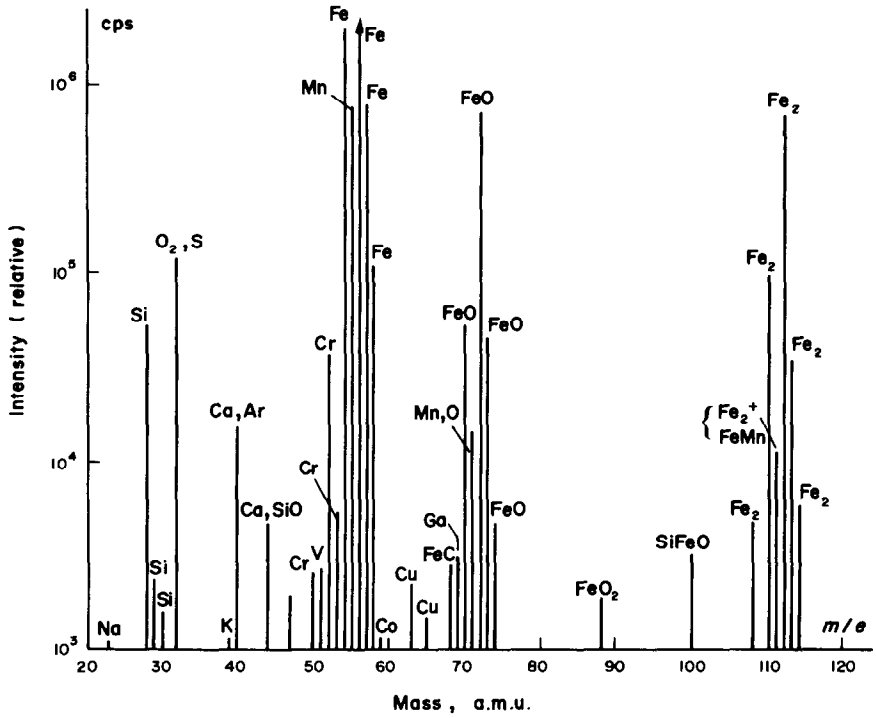

Fig. 4. Positive secondary-ion mass spectrum of the steel wire, for m/e = 20–120.

sparking conditions affect the sensitivities of most elements in steel samples and that the use of empirical formulas for the sensitivity factors might be doubtful.

Considering all this, it can be concluded that the matrix-ion species ratio method leads to reasonably accurate and precise results with SIMS, provided a standard sample with similar composition and adequate homogeneity is used.

Table 5. Quantitative results of SIMS and SSMS analysis of the steel basis of the coated wire (in weight percentages)

Element	Concentrations ± precision	
	SIMS	SSMS
Na*	0.000038 ± 0.000005	0.000035 ± 0.000011
Mg*	0.00044 ± 0.00004	0.00043 ± 0.00013
Al	0.00012 ± 0.00002	0.00016 ± 0.00005
Si	0.21 ± 0.04	0.22 ± 0.06
P	0.051 ± 0.009	0.057 ± 0.017
S*	n.m.	0.066 ± 0.020
Cl*	<0.1	0.0003 ± 0.0001
K*	0.000019 ± 0.000007	0.000086 ± 0.000015
Ca*	<0.0047	0.0021 ± 0.0006
Ti	n.m.	<0.0003
V	0.0027 ± 0.0004	0.0024 ± 0.0005
Cr	0.034 ± 0.006	0.031 ± 0.004
Mn	0.52 ± 0.09	0.42 ± 0.13
Co	0.0040 ± 0.0005	0.0060 ± 0.0018
Ni	0.0071 ± 0.0009	0.012 ± 0.004
Cu	0.050 ± 0.005	0.042 ± 0.012
Zn*	<0.013	0.0018 ± 0.0006
Ga*	0.0012 ± 0.0006	0.0025 ± 0.0008
As	0.016 ± 0.003	0.010 ± 0.003
Zr	<0.00017	<0.0002
Nb	n.m.	<0.00001
Mo	0.0031 ± 0.0003	0.0030 ± 0.0014
Sn	0.0011 ± 0.0002	0.0030 ± 0.0010
Sb*	<0.0057	0.0009 ± 0.0002

* Based on calculated SF.
n.m. = not measured.

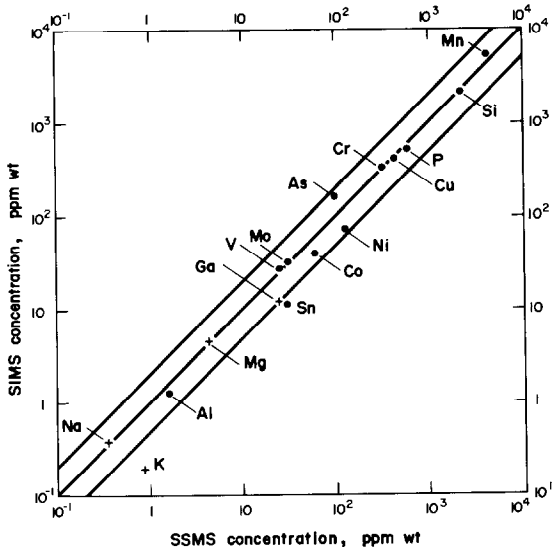

Fig. 5. Correspondence between elemental concentrations in the steel wire, determined by SIMS and SSMS (● symbolizes results obtained by use of experimental SF, + refers to results obtained by using estimated SF). The two limiting lines represent error factors of ±(×2).

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o-IODOSOBENZOATE AS AN OXIDIMETRIC TITRANT

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Summary—*o*-Iodosobenzoate has been used as a titrant at pH 7 with potassium iodide and starch as indicator, under Andrews' titration conditions with iodine monochloride as preoxidant, and in acid medium with potassium bromide and Methyl Red as indicator. Methods are described for the determination of tetrathionate (through disulphide cleavage by cysteine), hydrogen sulphite, thiosulphate, xanthates, cysteine and glutathione in certain combinations (involving masking of sulphite and thiols with acrylonitrile). Hexacyanoferrate(II) is determined in the presence of arsenic(III), antimony(III) and thallium(I). Sulphathiazole, sulphadiazine and sulphamerazine consume six equivalents of bromine per mole, but phthalation or diazotization of the aromatic amino group prevents disubstitution of bromine in the benzene ring. Chloramphenicol is determined by reduction of its aromatic nitro group to an amino group, followed by bromination. Rutin and vitamin C react with eight and two equivalents of bromine per mole respectively.

The aryl iodoso, iodoxy and related compounds constitute a group of considerable interest. They act as powerful oxidizing agents and are reduced practically irreversibly.[1–3] A number of iodoso compounds can be conveniently synthesized and it is an interesting question whether this series of oxidizing agents will be found of wide analytical value.

o-Iodosobenzoic acid was proposed by Hellerman *et al.*[4] for the specific determination of cysteine, glutathione and other protein thiols. The high results yielded by this method were thought to be due to the iodine liberated in the iodometric titration of the surplus reagent. Modified methods have been proposed that involve back-titration with ascorbic acid[5] or direct titration with *o*-iodosobenzoate[6] (with leuco-2,6-dichlorophenolindophenol and potassium iodide as indicator). Ascorbic acid has been determined in the presence of cysteine and hydrogen sulphite after masking of these two substances with acrylonitrile.[7] Xanthates in neutral solutions and thiols in acid medium have been determined, with starch and potassium iodide as indicator.[8] Chlorpromazine reacts with *o*-iodosobenzoic acid in acid medium, yielding a coloured substance of free radical nature.[9] *o*-Diacetoxyiodobenzoic acid behaves similarly to *o*-iodosobenzoic acid.[10,11]

The oxidation potential of *o*-iodosobenzoic acid has been found to be pH-dependent.[7] Thus, the reagent oxidizes iodide to iodine in neutral solution, and to iodine monochloride under Andrews' conditions, and bromide to bromine in acid medium. These conditions have been used in the present work for the determination of certain mixtures of organic and inorganic substances. On account of its ease of preparation,[12] excellent stability and applicability under various experimental conditions, *o*-iodosobenzoic acid can be recommended as an oxidimetric titrant for a variety of determinations.

EXPERIMENTAL

Reagents

*o-Iodosobenzoate, 0.02*M *solution.* Made by stirring 5.28 g of the free acid with 20 ml of 6% potassium hydroxide solution, diluting to 1 litre with demineralized water, and standardized iodometrically.[12] This solution loses about 1% titre on storage for 8 months. It can be diluted further as required.

*Iodine monochloride, 0.05*M *solution.* Prepared as described previously.[13]

Phosphate buffer, pH 7. Prepared by dissolving 117.7 g of K_2HPO_4 and 44.1 g of KH_2PO_4 in 1 litre of water.

Methyl Red. A 0.04% solution in ethanol.

Samples

Potassium ethyl and butyl xanthates[14] and sodium tetrathionate,[15] prepared and purified by known methods, were dissolved in water and the solutions were standardized, the xanthate solutions by mercurimetry[16] and the tetrathionate solution by periodate oxidation.[17]

Glutathione and cysteine were high-purity biochemicals, and their solutions were standardized by titration with chloramine-T[18] and by tetrathionate oxidation[13] respectively.

Solutions of high-purity salts of antimony(III), arsenic(III), thallium(I), hexacyanoferrate(II), thiosulphate and hydrogen sulphite were standardized by appropriate methods.[19]

All drug samples used were fresh.

Determination of tetrathionate

Mix 10 ml of solution containing up to 12 mg of tetrathionate with 5 ml of phosphate buffer and 10 ml of 0.02*M* cysteine and swirl the mixture for 5 min. Add 2 ml of acrylonitrile and let stand for 10 min. Add 10 ml of water, 100 mg of potassium iodide and 1 ml of 0.5% starch solution and titrate with 0.005*M* *o*-iodosobenzoate to appearance of a blue colour.

$$S_4O_6^{2-}\ (\text{mg}) = 224\ VM$$

where V is the volume (ml) of *o*-iodosobenzoate (molarity M) used.

Analysis of mixtures of xanthate or thiosulphate with hydrogen sulphite

To determine the xanthate or thiosulphate, mix 10 ml of the sample solution with 5 ml of phosphate buffer and 2 ml of acrylonitrile and shake the mixture for 5 min. Add 20 ml of water, 100 mg of potassium iodide and 1 ml of 0.5% starch solution and titrate with 0.01*M* *o*-iodosobenzoate to a blue colour.

$$S_2O_3^{2-} \text{ (mg)} = 224\ VM$$

$$\text{Alkyl xanthate (mg)} = 2\ VM\ w$$

where w is the formula weight of the alkyl xanthate ion. Mix another 10 ml of sample with 5 ml of phosphate buffer, 10 ml of water, 100 mg of potassium iodide and 1 ml of 0.5% starch solution and titrate with 0.01*M* *o*-iodosobenzoate to a blue colour. The amount of hydrogen sulphite is obtained by difference:

$$HSO_3^- \text{ (mg)} = 81\ \Delta VM$$

where ΔV is the difference between the two titration volumes.

The same set of methods can be used to analyse a mixture of a xanthate with cysteine or glutathione. Titration after reaction with acrylonitrile gives the amount of xanthate; the cysteine or glutathione is obtained by difference.

Cysteine or glutathione (mg) = $2\ f\Delta VM$ where f is the appropriate formula weight.

Analysis of mixtures of antimony(III) or thallium(I) with hexacyanoferrate(II)

Mix 10 (or 20) ml of sample with 25 ml of water, 5 ml of carbon tetrachloride and 5 ml of 0.05*M* iodine monochloride in a 250-ml iodine flask, shake the stoppered flask for 2 min, add 20 ml of 6*M* hydrochloric acid and titrate the liberated iodine with 0.02*M* *o*-iodosobenzoate to an Andrews end-point.

Determine the antimony(III) or thallium(I) alone by treating an equal aliquot of sample with 0.5 g of zinc sulphate and 25 ml of water, then applying the procedure above. Hexacyanoferrate(II) is obtained by difference.

$$\text{Sb(III) or Tl(I) (mg)} = aVM$$

where a = atomic weight

$$Fe(CN)_6^{4-} \text{ (mg)} = 424\ \Delta VM$$

Analysis of mixtures of arsenic(III), thallium(I) and hexacyanoferrate(II)

Mix an aliquot with 5 ml of phosphate buffer, 500 mg of potassium iodide and 1 ml of 0.5% starch solution, and titrate with 0.02*M* *o*-iodosobenzoate to obtain the amount of arsenic(III).

$$\text{As(III) (mg)} = 75\ VM$$

Take another aliquot to determine all three components by treating the mixture with iodine monochloride and titrating the liberated iodine to the Andrews end-point (V_1 ml). Treat a third aliquot with 500 mg of zinc sulphate, and then repeat the Andrews titration (V_2 ml)

$$Fe(CN)_6^{4-} \text{ (mg)} = 424\ (V_1-V_2)M$$

$$\text{Tl(I) (mg)} = 204\ (V_2-V)M$$

Analysis of chloramphenicol/sulphadiazine mixtures

Treat a known volume of drug suspension with 10 ml of 4*M* sulphuric acid and 50 ml of water, shaking well. Filter off insoluble matter on a Whatman No. 41 paper and wash it with water. Make up the filtrate and washings to known volume.

To determine the sulphadiazine, mix an aliquot with 200 mg of potassium bromide, 5 ml of 1*M* sulphuric acid and 2 or 3 drops of Methyl Red indicator and titrate with 0.02*M* *o*-iodosobenzoate till the red colour has almost faded, add 2 or 3 drops more indicator and titrate until the indicator is bleached.

$$\text{Sulphadiazine (mg)} = 83.3\ VM$$

Determine the sum of both components by treating another aliquot with two successive 0.25-g portions of zinc dust, heating on a water-bath for 5 min then cooling to room temperature, and titrating as before after adding 200 mg of potassium bromide and the indicator. Chloramphenicol is obtained by difference

$$\text{Chloramphenicol (mg)} = 161.5\ \Delta VM$$

Analysis of rutin/vitamin C mixtures

Stir a known weight of ground sample with 30 ml of water. Filter off insoluble matter on a Whatman No. 41 paper, wash with water, and make up the filtrate and washings to known volume. Treat the residue on the filter paper with two 25-ml portions of methanol and dilute the filtrate to a known volume with methanol.

Analyse an aliquot of the aqueous extract, which contains vitamin C (and any hydrogen sulphite added as preservative) by the method for xanthate.

$$\text{Vitamin C (mg)} = 176\ VM$$

Titrate a portion of the methanolic extract, which contains the rutin, by the method used for sulphadiazine.

$$\text{Rutin (mg)} = 160\ VM$$

Analysis of mixtures of phthalylsulphathiazole and chloramphenicol with sulphamerazine or sulphadiazine

Stir a known weight of finely ground sample with 25 ml of 1*M* potassium hydroxide, for 10 min, dilute to 100 ml with water and filter. Dilute the filtrate to a known volume. Analyse four equal aliquots as follows.

Aliquot 1. Add 5 ml of 2*M* sulphuric acid, 200 mg of potassium bromide, 20 ml of methanol, 2 or 3 drops of Methyl Red indicator and titrate with 0.02*M* *o*-iodosobenzoate to the sharp bleaching of the red colour (V_1 ml).

Aliquot 2. Acidify with 5 ml of 2*M* sulphuric acid; dissolve any precipitated material by adding 20 ml of methanol. Then add 5 ml of 2% sodium nitrite solution with shaking, and 2 min later 1 g of urea. Let stand for 5 min, add potassium bromide and indicator and titrate as for aliquot 1 (V_2 ml).

Aliquot 3. Add 20 ml of 2*M* sulphuric acid and heat on a water-bath for 20 min. Cool, add bromide *etc.* and titrate (V_3 ml).

Aliquot 4. Add 25 ml of 2*M* sulphuric acid and two 0.25-g portions of zinc dust. Heat on a steam-bath for 20 min, cool, add bromide *etc.* and titrate (V_4).

Sulphadiazine or sulphamerazine (mg) = $f(V_1-V_2)M/2$

(f = formula weight)

$$\text{Phthalylsulphathiazole (mg)} = 201.5\ (V_3-V_1)M$$

$$\text{Chloramphenicol (mg)} = 161.5\ (V_4-V_3)M$$

RESULTS AND DISCUSSION

Results obtained by these procedures are given in Tables 1–6 (CV = coefficient of variation). Results obtained by other methods are shown for comparison.

A number of oxidimetric methods have been proposed for determining tetrathionate but they lack specificity.[17,20] Cyanolysis and determination of the thiosulphate or thiocyanate produced has been used

Table 1. Determination of tetrathionate

	Found,* mg			
Taken, mg	Present method	CV, %	Periodate oxidation	CV, %
2.76	2.74	0.6	2.72	0.5
3.78	4.02	0.4	3.97	0.4
4.66	4.63	0.3	4.69	0.4
6.12	6.10	0.5	6.08	0.5
8.37	8.33	0.4	8.42	0.6
9.25	9.19	0.5	9.31	0.7

*Average of 10 replicates.

Table 2. Determination of sodium hydrogen sulphite

	Found,* mg (CV, %)	
Taken, mg	o-Iodosobenzoate	Iodine at pH 7
3.13	3.09 (0.6)	3.17 (0.8)
5.68	5.62 (0.5)	5.79 (0.9)
7.83	7.90 (0.6)	8.12 (0.9)
9.40	9.48 (0.8)	9.53 (1.5)
10.96	11.10 (0.8)	11.23 (1.2)
15.66	15.77 (1.1)	16.07 (1.8)

*Average of 6 replicates.

quite extensively.[21,22] In the present work, cysteine has been found to react with tetrathionate at pH 7:

$$S_4O_6^{2-} + 2CySH \rightarrow 2S_2O_3^{2-} + CySSCy + 2H^+$$

At least a twofold molar excess of cysteine should be added. The surplus cysteine is masked by reaction with acrylonitrile at the same pH, to produce *S*-2-cyanoethylcysteine which does not interfere in the titration of the thiosulphate. Sulphite, if also present with tetrathionate, is also masked by acrylonitrile.

$$CySH + CH_2{=}CHCN \rightarrow CySCH_2CH_2CN$$

$$HSO_3^- + CH_2{=}CHCN \rightarrow {}^-O_3SCH_2CH_2CN$$

$$SO_3^{2-} + CH_2{=}CHCH + H_2O \rightarrow {}^-O_3SCH_2CH_2CN + OH^-$$

Thiosulphate interferes, of course, but a mixture containing tetrathionate and thiosulphate can be analysed by titrating the thiosulphate alone with *o*-iodosobenzoate at pH 7 (using potassium iodide and starch), then the sum of the two compounds by using the method given for tetrathionate. The tetrathionate is obtained by difference. Xanthates are not masked by acrylonitrile, and their oxidation yields the dixanthates:

$$2ROC(=S)S^- + OI.C_6H_4.CO_2^- + H_2O \rightarrow (ROC(=S)S)_2 + I.C_6H_4.CO_2^- + 2OH^-$$

Methods have been recommended for sulphite determination that involve reaction with excess of iodine followed by titration of the surplus.[19] Direct titration with iodine gives erratic results, perhaps because acid is also produced in the reaction, since

Table 3. Determination of certain oxidizable substances

	Purity			
Substance	Present method, %[a]	CV, %	Comparison method, %[a]	CV, %
Ethyl xanthate	98.2	0.3	98.0[b]	0.3
Butyl xanthate	99.5	0.4	99.2[c]	0.4
Amyl xanthate	97.9	0.3	98.2[d]	0.5
Sodium thiosulphate	99.8	0.3	99.9[e]	0.3
Thallium(I) nitrate	99.7	0.4	99.9[f]	0.3
Arsenic(III) oxide	99.6	0.5	99.4[e]	0.3
Antimony(III) potassium tartrate	99.2	0.5	99.5[f]	0.4
Potassium hexacyanoferrate(II)	99.4	0.3	99.1[g]	0.5
Chloramphenicol	99.0	0.5	98.7[h]	0.6
Sulphadiazine	99.5	0.4	99.2[i]	0.3
Sulphamerazine	99.6	0.5	98.8[j]	0.5
Sulphanilamide	99.7	0.3	99.4[k]	0.4
Sulphathiazole	98.6	0.4	98.3[i]	0.3
Phthalylsulphathiazole	99.1	0.6	98.8[j]	0.5

[a] Average of 10 replicates.
[b] Mercurimetry.[16]
[c] Iodimetry.[28]
[d] Argentometry.[29]
[e] Iodimetry.[19]
[f] Iodate titration.[19]
[g] Cerimetry.[19]
[h] Reductimetry.[30]
[i] Alkalimetry.[31]
[j] Nitrite titration.[32]
[k] *N*-Bromosuccinimide titration.[23]

Table 4. Analysis of mixtures of certain reducing substances

Taken, *mg*			Found,* *mg*			
I	II	III	I	CV, %	II or III	CV, %
$NaHSO_3$	$Na_2S_2O_3$	*Ethyl xanthate*				
6.18	7.63	—	6.08	0.9	7.72	0.6
4.71	9.22	—	4.68	0.7	9.35	0.5
2.25	12.41	—	2.23	0.6	12.30	0.4
3.16	—	10.60	3.18	0.5	10.70	0.8
4.53	—	7.42	4.48	0.8	7.36	0.6
7.16	—	3.95	7.11	1.1	3.90	0.6
Butyl xanthate	*Cysteine*	*Glutathione*				
5.68	10.16	—	5.73	0.6	10.23	0.5
8.55	6.28	—	8.62	0.8	6.15	0.8
13.21	3.95	—	13.40	0.8	4.06	0.6
6.10	—	20.25	6.18	0.5	20.35	0.6
7.42	—	16.92	7.36	0.7	16.80	0.7
12.69	—	13.70	12.78	1.0	13.62	0.8
$Fe(CN)_6^{4-}$	Sb^{3+}	Tl^+				
7.86	10.84	—	7.80	0.7	10.76	0.6
13.21	7.31	—	13.32	0.8	7.21	0.8
19.05	4.62	—	18.89	1.0	4.58	0.6
17.12	—	5.68	17.30	1.1	5.74	0.7
12.56	—	8.31	12.49	0.8	8.40	0.7
6.99	—	10.12	6.89	0.6	10.01	0.8

*Average of 6 replicates.

Table 5. Analysis of mixtures of hexacyanoferrate(II), arsenic(III) and thallium(I)

Taken, *mg*			Found,* *mg* (CV, %)		
I	II	III	I	II	III
$Fe(CN)_6^{4-}$	As^{3+}	Tl^+			
6.12	2.35	12.52	6.19 (0.8)	2.39 (0.6)	12.46 (0.8)
10.25	5.22	8.37	10.13	5.06	8.45
14.67	7.31	6.83	14.79 (0.7)	7.42 (0.6)	6.80 (1.0)
18.06	8.49	5.50	18.26	8.56	5.42
22.11	4.86	5.92	21.96 (1.2)	4.72 (0.7)	6.00 (1.0)

*Average of 6 replicates.

Table 6. Analysis of drugs (averages of 6 replicates)

Drug	Compounds determined	Manufacturer's specification, *mg*	Found, *mg*	CV, %
Chromostat*	Rutin	50	48.6	1.2
	Vitamin C	150	146.2	0.6
Diastrep†	Sulphadiazine	100	98.5	0.8
	Chloramphenicol	125	121.8	1.1
Chlorosulf	Phthalylsulphathiazole	300	295.4	1.8
	Chloramphenicol	100	97.5	1.2
	Sulphamerazine	100	101.2	1.6
Enteromycetin Sulfa	Phthalylsulphathiazole	150	152.6	1.0
	Chloramphenicol	150	146.7	1.6
	Sulphadiazine	200	196.8	0.8

*Excipients adrenochrome monosemicarbazide (0.5 mg), menaphtheone sodium hydrogen sulphite (10 mg), calcium dihydrogen phosphate (132 mg), calciferol (500 IU).

†Excipient streptomycetin sulphate (125 mg).

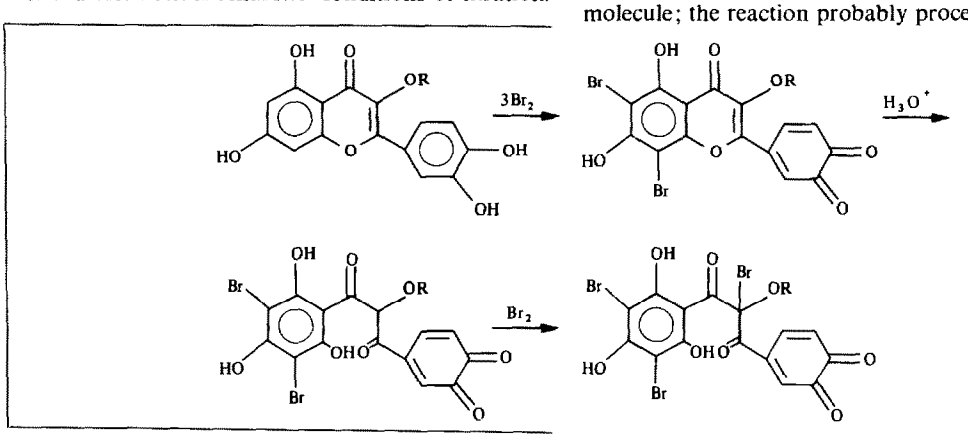
the *o*-iodosobenzoate titration, which does not yield any acid, gives accurate results.

$$SO_3^{2-} + I_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2I^-$$

$$SO_3^{2-} + OI.C_6H_4.CO_2^- \rightarrow SO_4^{2-} + I.C_6H_4.CO_2^-$$

This view is supported by the fact that when sulphite is titrated with iodine in pH-7 phosphate buffer, the results are precise (contrary to those yielded in non-buffered medium) and agree with those from *o*-iodosobenzoate titration (Table 2).

o-Iodosobenzoate oxidizes iodide to iodine monochloride under Andrews' conditions of titration.



Thallium(I), arsenic(III), antimony(III) and hexacyanoferrate(II) are also oxidized. If iodine monochloride is used as a preoxidant the Andrews end-point detection method can be used. Hexacyanoferrate(II) can be masked by precipitation with zinc, allowing analysis of mixtures of hexacyanoferrate(II) with other oxidizable ions.

Sulphonamides have been determined with reagents that produce bromine *in situ*,[23,24] but the stoichiometry changes with the reaction conditions and the substituents attached to the sulphonamide group.[25,26] Sulphadiazine, sulphamerazine and sulphathiazole have been examined in the present work and found to consume six atoms of bromine per molecule. The carbon atom β to a nitrogen atom in the substituent is active towards electrophilic bromination.[27]

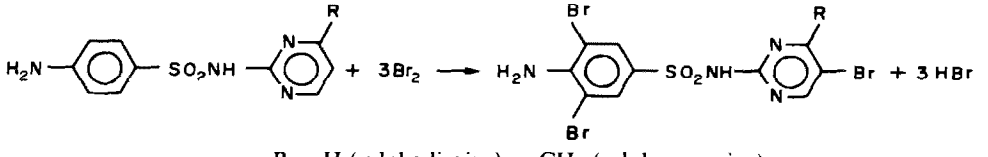
$$OI.C_6H_4.CO_2H + 2HBr \rightarrow Br_2 + I.C_6H_4.CO_2H + H_2O$$

Phthalation of the aromatic amino group prevents substitution at the two *ortho*-positions in the benzene ring, so phthalylsulphathiazole consumes only two atoms of bromine per molecule. The same effect results when the amino group is diazotized. Thus, diazotized sulphathiazole, sulphadiazine and sulphamerazine react with only two atoms of bromine per molecule. Hydrolysis of phthalylsulphathiazole produces sulphathiazole.

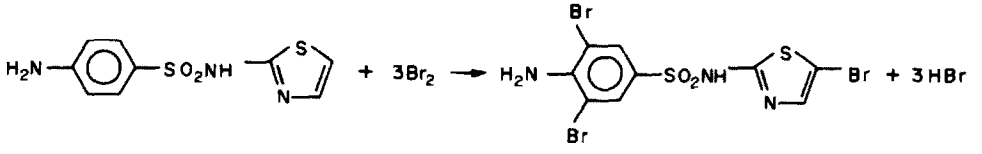
Chloramphenicol does not react with bromide and *o*-iodosobenzoate, but reduction produces an amino group which activates two *ortho*-positions for bromination. Rutin consumes eight atoms of bromine per molecule; the reaction probably proceeds as below:

(R = rotinose)

The methods for tetrathionate, thiosulphate, hydrogen sulphite, xanthates, cysteine and glutathione are unaffected by the presence of up to 20-fold molar excess of thiocyanate, chloride, bromide, acetate, oxalate, citrate, tartrate, formate and ammonium. Sulphide, dithionite and dithiocarbamates interfere severely. Titrations under Andrews' conditions are interfered with by hydrazine, hydroxylamine and similar reducing agents. The methods for the analysis of drugs tolerate glucose, starch, lactose, urea, histidine, glutamic acid, thiamine hydrochloride and iron(II) sulphate, but methionine, cystine, tyrosine, paracetamol, oxyphenbutazone, tryptophan, iodide, resorcinol and thiobarbituric acid interfere severely. Folic acid, phenacetin and acetylsalicylic acid also do not interfere, but if the drug solution is hydrolysed (as in the



R = H (sulphadiazine) or CH_3 (sulphamerazine)



analysis of Chlorosulf and Enteromycetin Sulfa), their interference is severe.

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SHORT COMMUNICATIONS

GRAVIMETRIC DETERMINATION OF RHENIUM AS 2,4,6-TRIPHENYLPYRYLIUM PERRHENATE

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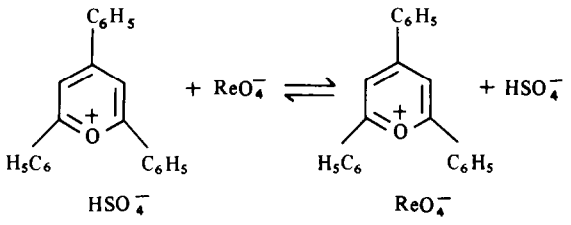
Summary—A gravimetric method for determination of rhenium is based on formation of sparingly soluble 2,4,6-triphenylpyrylium perrhenate. The conditional solubility product in 0.1*N* sulphuric acid medium was found to be $(3.72 \pm 0.08) \times 10^{-9}$. The interference of foreign anions (simple or complex) is eliminated by preliminary extraction of perrhenate with acetone from strongly alkaline medium. The method is applied to the determination of rhenium in perrhenates and rhenium alloys.

It is known that 2,4,6-triphenylpyrylium chloride $(C_6H_5)_3C_5H_2O^+$. Cl^- yields sparingly soluble salts with a number of complex anions.[1] This reaction has been used for gravimetric determination of boron, gold, platinum and perchlorate.[1,2] The performance characteristics of these methods show that 2,4,6-triphenylpyrylium chloride is equal or even superior to nitron and tetraphenylarsonium chloride for the purpose.

The present work deals with the gravimetric determination of perrhenate by precipitation with 2,4,6-triphenylpyrylium bisulphate, which has the advantages of being simpler to make and more selective than the chloride salt (in the presence of chloride various anionic chloro-complexes could be formed and interfere).

The precipitate is formed according to the reaction:



EXPERIMENTAL

Reagents

2,4,6-Triphenylpyrylium bisulphate was prepared according to Chadwick.[2] All other reagents were of Merck analytical grade.

Synthesis of 2,4,6-triphenylpyrylium perrhenate

Perrhenate was precipitated with a solution of 2,4,6-triphenylpyrylium bisulphate in sulphuric acid, to yield a lemon yellow substance sparingly soluble in water, m.p. 270–274°. Elemental analysis was in agreement with the composition $C_{23}H_{17}O_5Re$, m.w. 559.6, rhenium content 33.28%.

Determination of the conditional solubility product of 2,4,6-triphenylpyrylium perrhenate

A weighed amount of the salt was stirred continuously with 0.1*N* sulphuric acid at 20 ± 0.1° in a thermostat. Samples of the solution were taken with a submerged filter tube at fixed time intervals and the rhenium content determined spectrophotometrically with thiocyanate.[3]

RESULTS AND DISCUSSION

Solubility product

The solubility of 2,4,6-triphenylpyrylium perrhenate was determined in order to establish whether it would be suitable for the gravimetric determination of rhenium. The dependence of rhenium concentration on contact time with water is shown in Fig. 1, curve 1. The rhenium concentration continuously increases. Taking into consideration the chemical behaviour of the pyrylium cation it seems that the curve is in agreement with the effect of the hydrolysis processes which may occur (causing cleavage of the pyrylium ring). The same experiments were done with 0.1*N* sulphuric acid instead of water (curve 2) to prevent hydrolysis. It is seen that equilibrium was established within 15 min. The solubility began to increase after about 5 hr, but is constant for the duration of the gravimetric determination. The conditional solubility product for 0.1*N* sulphuric acid medium was found to be $(3.72 \pm 0.08) \times 10^{-9}$, very close to that for tetraphenylarsonium perrhenate, $(3.72 \pm 0.2) \times 10^{-9}$.[4]

Interferences

All complex anions forming insoluble triphenylpyrylium salts, such as $AuCl_4^-$, $PtCl_6^{2-}$, ClO_4^-, MnO_4^-, BF_4^-, CrO_4^{2-}, CNS^-, NO_3^-, MoO_4^{2-}, WO_4^{2-}, VO_3^- interfere. The interference of Mo, W, V, Cr (the elements which most often accompany rhenium) can be effectively eliminated by a preliminary extraction of perrhenate with acetone from 5*M* sodium hydrox-

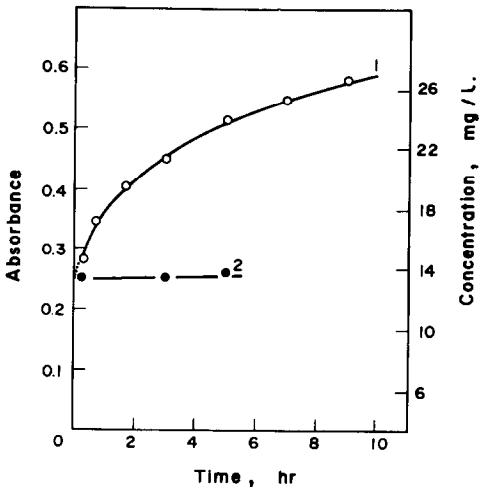


Fig. 1.

ide. The solubility of acetone in the aqueous phase is negligible and good separation of the phases is achieved. The presence of hydroxide precipitates does not interfere with the quantitative extraction of perrhenate. The organic layer is separated and the acetone evaporated by heating on a water-bath. The residue is dissolved in 30 ml of 0.1*N* sulphuric acid and perrhenate precipitated as in the normal procedure.

Procedure

The test solution, containing 20–50 mg of rhenium in 0.1*N* sulphuric acid is treated with a 50–60% excess of 1% solution of the reagent in 0.1*N* sulphuric acid, with stirring. The precipitate is allowed to stand for 30 min at room temperature, then for 1 hr in a refrigerator, and is finally filtered off on a porosity-4 fritted glass filter and washed first with cooled 0.1% solution of the precipitant and finally with small portions of ice-water. The final volume of filtrate should not exceed 50–60 ml.

Applications

The proposed method was applied to the analysis of potassium perrhenate (*p.a.*), ammonium perrhenate (technical grade) and rhenium–molybdenum alloy. The results were as follows.

1. Potassium perrhenate ($KReO_4$) *p.a.*
   Theoretical assay 64.36% Re
   Sample 50 mg
   Found, $\bar{x}_{Re} = 64.35\%$ $n = 14$, $P = 95\%$, $S = 0.25$,
   Re = 64.35 ± 0.15%, where $0.15 = t(n,P)S/\sqrt{n}$.

A parallel analysis was performed with tetraphenylarsonium chloride. The following results were obtained.

$\bar{x}_{Re} = 63.93\%$, $n = 10$, $P = 95\%$, $S = 0.20$,
Re = 63.94 ± 0.14%.

2. Ammonium perrhenate (NH_4ReO_4), technical grade
   Theoretical assay 69.42% Re
   Sample 50 mg
   Found, $\bar{x}_{Re} = 65.03\%$, $n = 4$, $P = 95\%$
   Comparative analyses gave $\bar{x}_{Re} = 64.93\%$, $n = 4$, $P = 95\%$
3. Rhenium–molybdenum alloy

With a model system it was shown that the proposed method can be applied to the analysis of rhenium–molybdenum alloys. Rhenium was separated from molybdenum by extraction with acetone. The model system contained about 10% Re and 90% Mo. With 9.94% Re present, the concentration found was 9.89% Re.

A standard rhenium–molybdenum alloy containing 46.7% Re was analysed. It was dissolved by anodic oxidation in 5*M* sodium hydroxide. The proposed method gave 47.0% Re.

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POTENTIOMETRIC AND VISUAL TITRATIONS WITH BROMAMINE-B

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Summary—Bromamine-B is proposed as an oxidimetric titrant for potentiometric and visual end-point titrations of arsenic(II), hexacyanoferrate(II), antimony(III), hydroquinone, semicarbazide hydrochloride, isonicotinic acid hydrazide, hydrazine sulphate, ascorbic acid, phenylhydrazine hydrochloride and metol. Quinoline Yellow, naphthidine, dimethylnaphthidinedisulphonic acid, *o*-dianisidine, diphenylbenzidine, Variamine Blue, α-naphthoflavone, Amaranth, Methyl Orange and Methyl Red are proposed as indicators in macro and micro titrations of the reductants with bromamine-B. The transition potentials of Quinoline Yellow, naphthidine, dimethylnaphthidinedisulphonic acid, and *o*-dianisidine in the titration of ascorbic acid are reported. Arsenic(III) and hexacyanoferrate(II) are suggested for the standardization of bromamine-B solutions.

This communication describes simple and accurate methods for standardization of bromamine-B (BAB) solution, which is proposed for the potentiometric and visual titration of macro and micro amounts of arsenic(III), hexacyanoferrate(II), antimony(III), hydroquinone (HQ), semicarbazide hydrochloride (SCH), isonicotinic acid hydrazide (INH), hydrazine sulphate (HS), ascorbic acid (AA), phenylhydrazine hydrochloride (PHH) and metol, with Quinoline Yellow (QY), naphthidine (N), dimethylnaphthidinedisulphonic acid (DMNS), *o*-dianisidine (ODA), diphenylbenzidine (DB), Variamine Blue (VB), α-naphthoflavone (NF), Amaranth (Am), Methyl Orange (MO) and Methyl Red (MR) as indicators.

EXPERIMENTAL

Indicator solutions

Solutions (0.1%) of QY, DMNS, VB, Am, MO and MR in water, N in glacial acetic acid, ODA in 0.04*N* sulphuric acid, DB in concentrated sulphuric acid and NF in ethanol were prepared.

Standardization of BAB solution with arsenic(III) and hexacyanoferrate(II)

A mixture of 20 ml of 0.05–0.01*N* arsenic(III), 5 ml of 10% potassium bromide solution and 0.5 ml of QY or NF or 0.2 ml of Am or MO was diluted to 40 ml with acid to give 1*M* sulphuric or hydrochloric acid or 2*M* phosphoric acid at the end-point, and titrated with 0.05–0.01*N* BAB solution to the disappearance of the colour or the appearance of a rust-brown, depending on the indicator.

A mixture of 20 ml of 0.05–0.01*N* hexacyanoferrate(II) and 0.1 ml of ODA was diluted to 40 ml with enough phosphoric acid to give a 0.5*M* acid concentration at the end-point, and titrated with 0.05–0.01*N* BAB solution to the appearance of a reddish brown colour.

Potentiometric titrations

Hexacyanoferrate(II), antimony(III), HQ, SCH or INH solution (20 ml, 0.05–0.01*N*) was diluted to 60 ml with enough acid to give 1*M* sulphuric or hydrochloric acid or 2.5*M* phosphoric acid concentration [only phosphoric acid medium for hexacyanoferrate(II)] at the end-point. The mixture was titrated potentiometrically with 0.05–0.01*N* BAB solution, a bright platinum gauze electrode being used. Potassium bromide solution (10%, 5 ml) was added before the titration of SCH and INH.

Visual end-point titrations

A mixture of 10–20 ml of 0.05–0.01*N* antimony(III), SCH, INH, HS, AA or PHH, 3–5 ml of 10% potassium bromide solution and 0.5 ml of QY (except for PHH) or 0.2 ml of Am, MO or MR, was diluted to 25–40 ml with enough acid to give 1*M* sulphuric or hydrochloric acid or 2*M* phosphoric acid at the end-point, and titrated with 0.05–0.01*N* BAB solution to the disappearance of the colour.

HQ was similarly titrated, with 0.1 ml of N, DMNS or ODA indicator: AA was titrated with 0.1 ml of N, DMNS or ODA or 0.5 ml of DB or VB as indicator, HS was titrated with 0.5 ml of NF as indicator, and metol was titrated only in 0.3*M* sulphuric acid medium, with 0.1 ml of ODA indicator.

RESULTS AND DISCUSSION

The transition potentials of QY, N, DMNS and ODA in the titration of AA with BAB vary over the ranges 843–867, 809–797, 744–715 and 766–754 mV respectively over the range of phosphoric acid concentration 2.0–3.5*M*.

BAB solution can be standardized with the primary standard arsenic(III), with QY, NF, Am and MO as indicators, or against hexacyanoferrate(II), with ODA indicator or a potentiometric end-point. QY, NF and ODA act as reversible indicators and Am and MO as irreversible indicators. The results indicate that these methods of standardization give satisfactory agreement but the arsenic(III) method is superior because it uses direct titration of a primary standard.

Potentiometric titrations

In the potentiometric titration of hexacyanoferrate(II), antimony(III), HQ, SCH and INH, the presence

of potassium bromide is desirable only for SCH and INH. Equilibration of the electrode potential takes about a minute. The potential jump at the end-point is 100 mV for hexacyanoferrate(II) in 1*M* phosphoric acid, 116 mV for antimony(III), 180 mV for HQ, 230 mV for SCH and 198 mV for INH in 1*M* hydrochloric acid medium, for addition of 0.1 ml of 0.01*N* BAB. Titrations of hexacyanoferrate(II) in 0.5–1.5*M* phosphoric acid, antimony(III) in 1.0–2.5*M* hydrochloric acid, HQ in 0.5–1.5*M* hydrochloric or sulphuric acid or 1.5–3.0*M* phosphoric acid, and SCH and INH in 0.5–1.5*M* hydrochloric or sulphuric acid or 1.5–2.5*M* phosphoric acid solution containing 0.6–2.5% potassium bromide, give accurate results (Table 1).

Visual end-point titrations

The indicators used in the visual end-point titrations depend on the nature of the indicator, the reductant and the titration medium. For example QY, NF, Am, and MO function in the titration of arsenic(III); QY, Am, and MO in the titration of antimony(III); N, DMNS and ODA in the titration of HQ; QY, Am, MO and MR in the titration of HS; QY, DB, N, DMNS, ODA, VB, Am, MO, and MR in the titration of AA; and Am, MO and MR in the titration of PHH in 0.5–1.5*M* sulphuric or hydrochloric acid [1–2.5*M* for antimony(III)] or 1.5–3*M* phosphoric acid (2–3.5*M* for HQ and HS) containing 0.6–2.5% potassium bromide. ODA gives sharp end-points in the titration of hexacyanoferrate(II) in 0.3–1.5*M* phosphoric acid and in the titration of metol in 0.3*M* sulphuric acid solution containing 0.6–3.7% potassium bromide. The total volume of the titration mixture at the end-point is about 60 ml in the titration of 0.05*N* reductant and 35 ml in the titration of 0.01*N* reductant.

QY, N, DMNS, ODA, DB, VB, and NF give sharp reversible end-points. QY gives a colour change from yellow to colourless and N, DMNS, ODA, DB, VB and NF from colourless to pinkish violet red, reddish brown, blue-violet, purple and rust-brown respectively at the equivalence point. The end-point colour is stable for 2–15 min. Am, MO and MR give sharp irreversible colour changes from red to colourless at the equivalence point.

Table 1. Potentiometric titrations with BAB

Reductant	Range studied, *mg*	Maximum error, %
$Fe(CN)_6^{4-}$	2.5–220	0.2
Sb(III)	7.5–128	0.3
HQ	5.5–150	0.4
SCH	5.3–53	0.1
INH	3.1–76	0.1

Table 2. Titration of arsenic(III), hexacyanoferrate(II), antimony(III), HQ, SCH, INH, HS, AA, PHH and metol with BAB

Reductant	Taken, *mg*	Mean found, *mg*	Relative std. devn., %
As(III)	68.8	68.9	0.1
	3.29	3.28	0.1
$Fe(CN)_6^{4-}$	221.7	222.0	0.3
	2.46	2.45	0.1
Sb(III)	128.2	128.5	0.2
	7.58	7.57	0.2
HQ	150.5	150.4	0.3
	5.54	5.54	0.1
SCH	52.8	52.8	0.2
	5.28	5.27	0.4
INH	76.2	76.2	0.3
	3.13	3.12	0.2
HS	68.3	68.3	0.2
	3.40	3.42	0.1
AA	178.6	178.5	0.2
	21.3	21.3	0.1
PHH	75.2	75.4	0.4
	3.79	3.84	0.1
Metol	164.6	164.5	0.8
	3.96	3.96	0.4

For all the reductants, 0.5–1.5 ml of 0.1% QY, DB, VB and NF, 0.1–0.5 ml of 0.1% N, DMNS and ODA and 0.2–0.8 ml of 0.1% Am, MO and MR indicators are recommended. Smaller amounts of indicator are suitable for titrations of 0.01*N* reductant. The average indicator correction is 0.03–0.05 ml of 0.01*N* BAB for the amounts of indicators recommended in the procedures. The sharpness of the end-point is in the order QY > ODA > N = DMNS > DB > VB > NF > Am > MO = MR.

A large deviation from the permissible ranges of acidity, indicator, and potassium bromide leads to sluggish, premature or overshot end-points.

The titration of AA and INH was used for the determination of AA in vitamin-C tablets and injections and INH in isoniazid tablets and syrup. A synthetic mixture containing 305 mg of starch, 304 mg of gelatin, 255 mg of talc, 285 mg of stearic acid, 270 mg of sodium alginate, 329 mg of citric acid, 242 mg of reserpine, 484 mg of pulvis acaciae and 400 mg of dextrose, sucrose or oxalic acid did not interfere in the determination of 75 mg of AA in 1*M* phosphoric acid or 50 mg of INH in 0.5*M* sulphuric acid. The results of the assay of tablets, injections and syrup compare favourably with those obtained by the standard methods.[1,2]

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DETECTION AND SEMIQUANTITATIVE DETERMINATION OF NICKEL WITH DIMETHYLGLYOXIME-LOADED FOAM

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Summary—Rapid, sensitive and selective detection and semiquantitative determination of nickel in aqueous solution can be obtained by using dimethylglyoxime loaded on polyurethane foam, either by batch or column extractions, the detection limits being 0.05 and 0.01 ppm respectively.

Polyurethane foam loaded with water-insoluble reagents which yield coloured reaction products seems very attractive for raising the sensitivity of spot-tests. This method has been tested for the detection of cobalt(II),[1] chromium(VI)[2] and iron(III)[3] with polyurethane foam loaded with Amberlite LA-1, 1,5-diphenylcarbazide and tricaprylamine, respectively. These tests were more sensitive than the normal spot-tests on a spot-plate,[4] on paper impregnated with reagent[4] or with the resin spot-test,[5] and had good selectivity.

They could also be used for semiquantitative determination.

In the present work polyurethane foam loaded with dimethylglyoxime was evaluated as a reagent for detection and semiquantitative determination of nickel.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade unless otherwise specified. Tricaprylamine (Alamine 336), pure grade, was kindly provided by General Mills, Kankakee, Illinois, U.S.A. An open-cell, polyether type polyurethane foam (bulk density 30 kg/m^3) was supplied by Greiner KG, Schaumstoffwerk-Kremsmunster, Austria. The foam material (cubes of 5 mm edge) was washed and dried as previously described.[1,2]

The dimethylglyoxime solution was prepared by dissolving about 0.2 g in 10 ml of Alamine 336. The reagent-foam was prepared by immobilizing the dimethylglyoxime–amine solution on the polyurethane foam by a procedure similar to that described previously.[6]

In the flow experiment, 0.5 g of the loaded foam was packed in a glass tube 5 mm in diameter and 10 cm long, by the vacuum method.[7]

RESULTS AND DISCUSSION

The colour reaction of dimethylglyoxime with nickel is well known. Experience with reagent-loaded polyurethane foam has shown that metal ions at very low concentration in aqueous solution can be collected on the reagent-foam simply by shaking, or by percolating the solution through a foam column at a reasonable flow-rate.

In preliminary experiments, an alcohol solution of dimethylglyoxime was tried for loading the foam, but the results were not satisfactory. A saturated solution of dimethylglyoxime in Alamine 336 proved suitable, however, the reagent not being leached from the foam on shaking vigorously with aqueous solution or on elution at relatively high flow-rates. Evidently, the amine serves a dual purpose, as a suitable solvent for the dimethylglyoxime and as a plasticizer for the foam material.

Shaking one cube (5 mm edge) of the foam for 1–2 min with 2 ml of aqueous nickel solution (pH 7–9), allowed detection of the nickel at relatively very low concentrations (0.05 ppm) by means of the red colour formed on the foam. The sensitivity was superior to that of the normal spot-test on a spot-plate (3.2 ppm) or impregnated paper (0.5 ppm).

It was found possible to detect 1 μg of nickel in the presence of 20 mg of Tl^+, In^{3+}, Hg(I), Cd^{2+}, Zn^{2+}, Ca^{2+}, Ba^{2+}, Rb^+, Mg^{2+}, NH_4^+, PO_4^{3-}, $S_2O_3^{2-}$, SO_4^{2-}, SCN^-, CH_3COO^-, CO_3^{2-}, HCO_3^-, Cl^-, Br^-, I^-, SO_3^{2-}, $B_4O_7^{2-}$, ascorbate or tartrate. Bi^{3+}, Au^{3+}, Pd^{2+} and Pt^{4+} interfere in the same way as in the usual spot-test.

In the presence of some other ions simple modifications of the aqueous solution are needed for the clear and sensitive detection of nickel (Table 1). Iron(II) must be oxidized to iron(III) before addition of fluoride.

Semiquantitative determination is possible by comparison of the colour of the foam cube with standards prepared with 0.05, 0.1, 1, 10 and 50 ppm nickel solutions under the same conditions.

A still lower concentration of nickel (0.01 ppm) can be detected by percolating 100 ml of the nickel solution through 0.5 g of reagent-foam (packed in a column) at a flow-rate of 3–5 ml min. The length of the coloured zone is proportional to the concentration of nickel, which can be estimated by using standards covering the concentration range 10–50 ng/ml.

Table 1. Effect of various ions on the detection of 1 μg of nickel(II) in 2 ml of aqueous solution

Foreign ion	Compound added	Colour of the foam*	Tolerance limit,† μg	Notes
Cu(II)	$CuCl_2$	White	$1:10^4$	Crystals of KI added, followed by $Na_2S_2O_3$ crystals.
Co(II)	$Co(NO_3)_2$	White	$1:10^2$	
Ce(IV)	$Ce(SO_4)_2$	White	$1:10^3$	
Fe(III)	$FeCl_3$	Yellow	$1:10^4$	One drop of saturated KF solution added
La(III)	$La(NO_3)_3$	White	$2:10^3$	
Mn(II)	$MnSO_4$	White	$1:10^4$	Oxidation with Br_2 water, excess of bromine eliminated by heating
Pb(II)	$Pb(NO_3)_2$	White	$1:10^2$	
Oxalate	$Na_2C_2O_4$	White	$1:10^4$	Crystals of $CaCl_2$ added
CrO_4^{2-}	K_2CrO_4	Pale yellow	$1:10^2$	
NO_2^-	$NaNO_2$	White	$2:10^3$	
VO_3^-	NH_4VO_3	Pale yellow	$1:10^2$	
MoO_4^{2-}	$(NH_4)_6Mo_7O_{24}$	White	$1:10^4$	Saturated solution of $H_2C_2O_4$ added, followed by a few crystals of $CaCl_2$; values obtained by comparison with a blank.
MnO_4^-	$KMnO_4$	White	$2:10^3$	Few drops of 1*M* H_2SO_4 and 1 ml of $Na_2C_2O_4$ added, then heating to *ca.* 80°; after cooling, nickel detected as usual.
Formate	HCOONa	White		Oxidation with Br_2 water, excess of bromine eliminated by heating

*The colour in the absence of nickel.
†The amount of the foreign ion below which 1 μg of nickel can easily be detected.

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EXTRACTIVE-SPECTROPHOTOMETRIC DETERMINATION OF MOLYBDENUM AS MIXED-LIGAND COMPLEXES WITH THIOCYANATE AND AMIDOPYRIDINES

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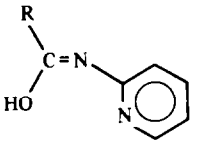
Summary—A fairly selective and sensitive method is described for the determination of microgram amounts of molybdenum(V) by means of its reaction with thiocyanate and the enolic form of various amidopyridines and extraction into benzene. The molar absorptivity of the complexes is in the range 1.5–1.9 × 10^4 l.mole^{-1}.cm^{-1} at λ_{max} 470 nm. The method is applicable in 1.5–7*M* hydrochloric acid or 1.2–6*M* sulphuric acid media. Cu^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{3+}, Al^{3+}, Cr^{3+}, Ti^{4+}, Zr^{4+}, V(V), Nb^{5+}, Ta^{5+}, W(VI) and U(VI) do not interfere.

The classical thiocyanate method is frequently used for the determination of molybdenum in spite of its drawbacks.[1] The spectrophotometric methods based on other reagents[2–12] also suffer from various difficulties, including interference from ions commonly associated with molybdenum, such as Cu^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, Bi^{3+}, Ti^{4+}, Zr^{4+}, Nb^{5+}, V(V), W(VI).

We now report on 2-benzamidopyridine and similar compounds as simple and selective reagents for molybdenum in presence of thiocyanate. The organic ligand is believed to behave as a singly charged bidentate chelating agent with the functional grouping shown below.



EXPERIMENTAL

Reagents

Standard molybdenum(VI) solution. Prepared by dissolving 2.04 g of ammonium heptamolybdate tetrahydrate in doubly distilled water and standardized gravimetrically.[13] The working solution was prepared by appropriate dilution.

Amidopyridines. Prepared as described in the literature[14] and used as 1% solutions in benzene.

Ascorbic acid and ammonium thiocyanate solutions. Freshly prepared 10 and 20% solutions respectively.

All solutions used were presaturated with benzene.

Procedure

An aliquot of solution containing 5–100 μg of Mo(VI), 5 ml each of ascorbic acid and ammonium thiocyanate solutions and 12 ml of 10*M* hydrochloric or sulphuric acid were transferred to a 100-ml separatory funnel, diluted to 25 ml with distilled water and shaken with 25 ml of benzene solution of the amidopyridine for 2 min. The organic layer was dried over anhydrous sodium sulphate (2 g) in a 50-ml beaker and its absorbance measured at λ_{max} against a suitable reference.

Analysis of steel

The sample was dissolved in *aqua regia* and the solution evaporated to dryness. The residue was heated with 3 ml of concentrated sulphuric acid till fuming. The digestion procedure was repeated 2 or 3 times. The dried mass was cooled and dissolved in 0.5*M* hydrochloric acid and diluted to known volume with distilled water. An aliquot was taken in a 100-ml separatory funnel and 0.2 g of sodium oxalate (in solution) was added to reduce any V(V). The molybdenum was then determined as described in the procedure above.

RESULTS AND DISCUSSION

Spectral characteristics

The absorption spectra of the metal chelates and 2-benzamidopyridine are shown in Fig. 1. The reagent has negligible absorption at the wavelength of maximum absorption. The metal chelate gives a sharp absorption peak with λ_{max} at 470 nm. Replacing the phenyl group by a 2-furyl group lowers the absorbance of the complex, but the introduction of substituents in the phenyl group only slightly affects the colour intensity (Table 1).

Choice of solvent and reducing agent

Benzene, toluene, chloroform, carbon tetrachloride, ether, ethyl acetate, acetophenone and amyl alcohol were tried as solvents. The complex was extractable into all these, but the molar absorptivity varied considerably. The position of λ_{max} shifts slightly towards shorter wavelengths as the dipole moment of the solvent increases (to 460 nm for alcohol). Benzene was chosen because of the high extractibility of the metal

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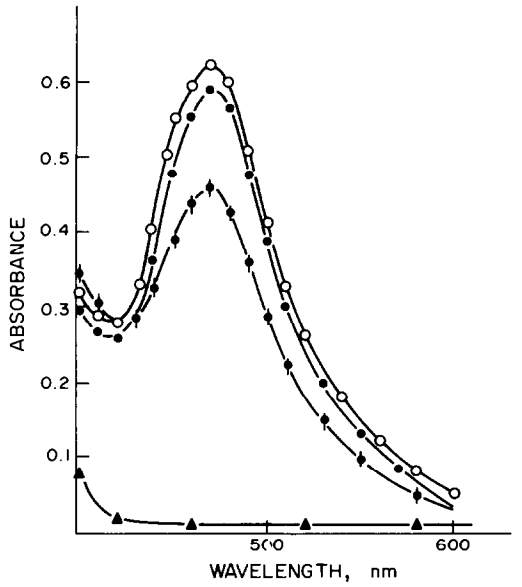

Fig. 1. Absorption spectra. [HCl] 5M; [amidopyridine] 0.05M; [SCN^-] 0.5M; [ascorbic acid] 0.1M. (⊙——⊙) 2-(*p*-toluamido)pyridine complex; (●——●) 2-benzamidopyridine complex; (◆——◆) 2-(*p*-chlorobenzamido)pyridine complex; (▲——▲) 0.05M 2-benzamidopyridine.

chelate into it. Other solvents were unsuitable owing to low absorbance, or slow phase separation or instability of the colour. Ascorbic acid and stannous chloride were tried as reducing agents, but ascorbic acid was chosen because it gave a high absorbance for the complex and the amount to be added was not critical (unlike stannous chloride). The extraction is complete even in the absence of ascorbic acid if the reaction mixture is shaken for at least 30 min.

Effect of variables

Systematic variation of the reagent concentrations indicated that at least 0.015M amidopyridine solution in benzene and 0.3M ammonium thiocyanate were necessary for complete extraction of the metal, but that up to 0.2M amidopyridine and 1.2M thiocyanate did not affect the spectral characteristics of the complex. However, thiocyanate concentrations $>1.2M$ decreased the extraction efficiency. The order of addition of reagents was not critical. A shaking time of 2 min was sufficient for the complete extraction reaction. Variation in temperature from 15° to 35° and in volume of aqueous phase from 15 to 60 ml did not affect the distribution coefficient. The absorbance was constant for at least 2 hr. Salting-out agents had no effect.

Table 1. Spectral data for the ternary complexes in benzene, at λ_{max} 470 nm

R*	[HCl] ([H_2SO_4]) range for extraction, M	ϵ, $l.mole^{-1}.cm^{-1}$
Phenyl	3.0–7.0 (2.0–6.0)	1.90×10^4
p-Tolyl	1.5–6.6 (1.2–6.0)	1.88×10^4
p-Chlorophenyl	2.5–6.0 (1.5–6.0)	1.92×10^4
2-Furyl	3.0–7.2 (2.0–6.0)	1.49×10^4

*In RC(OH)N Py.

The acidity of the solutions was adjusted with 10M hydrochloric or sulphuric acid, the optimum acidity ranges being 1.2–7.0M hydrochloric acid and 1.2–6.0M sulphuric acid, which gave the same values of λ_{max} and ϵ for the complex. Higher sulphuric acid concentrations decreased extraction of the metal because of increased miscibility of benzene with the aqueous phase. Hydrochloric acid was preferred because it gave better selectivity. Nitric acid and acetic acid are unsuitable for the extraction. The effect of hydrochloric acid is shown in Fig. 2 and a concen-

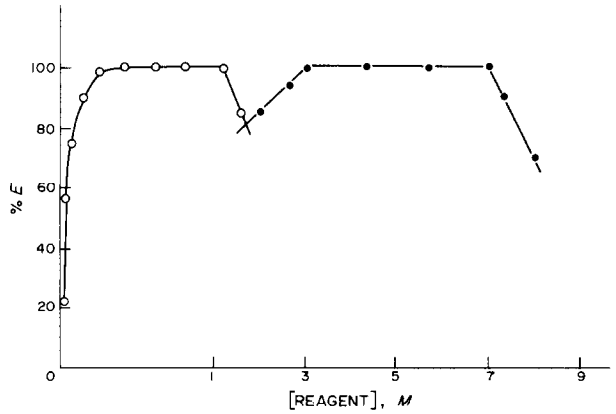

Fig. 2. Effect of thiocyanate and hydrochloric acid concentrations on the extraction. [Mo] $2.79 \times 10^{-5}M$; [KCl] 0.1M; [2-benzamido pyridine] 0.05M. (⊙——⊙) %E *vs.* [SCN^-] (in 5M HCl). (●——●) %E *vs.* [HCl] (in 0.5M SCN^-).

Table 2. Effect of diverse ions in determination of 2 ppm Mo(V) with thiocyanate and 2-benzamidopyridine

Ion added	Amount tolerated,* mg/ml	Ion added	Amount tolerated,* mg/ml
Fe^{2+}	4	Fe^{3+}	1
Co^{2+}	0.5	Al^{3+}	6
Ni^{2+}	6	Cr^{3+}	6
Cu^{2+}	0.5	Sb^{3+}	2
Zn^{2+}	4	Bi^{3+}	1
Cd^{2+}	4	Tl^{3+}	1.5
Hg^{2+}	2	La^{3+}	6
Pb^{2+}	1.5	Th^{4+}	2.5
Mn^{2+}	10	Ti^{4+}	0.4
Pd^{2+}	0.6	Zr^{4+}	2
W(VI)	2	V(V)	0.4
V(VI)	10	Nb^{5+}	0.1†
EDTA	8	Ta^{5+}	0.7
F^-	1	$S_2O_3^{2-}$	1
I^-	6	$C_2O_4^{2-}$	6
PO_4^{3-}	6	Citrate	8
AsO_4^{3-}	3	Tartrate	8

*Causing error <2%.
†In presence of sodium oxalate.

tration of 5*M* was chosen because it gave the highest rate of extraction.

Performance characteristics

The calibration graph is linear over a wide concentration range. The optimum range on the basis of a Ringbom plot[15] is 0.5–4.4 ppm Mo. The relative standard deviation (10 replicates, 2 ppm Mo) is 0.8%.

Composition of complex

This was determined by a curve-fitting method,[16] using plots of log *D* *vs.* log $[amide]_o$ or log $[SCN^-]$. The results obtained indicate the mole ratio to be 1:2:1 Mo:thiocyanate:aminopyridine, so the species extracted into benzene is probably $MoO(SCN)_2(RC_6N_2O)$ where RC_6N_2O is the deprotonated enolic form of the amidopyridine, co-ordinated to the metal through the oxygen atom of the amide group and the nitrogen atom of the pyridyl group.

Table 3. Determination of molybdenum in ore and steels

Sample	Mo certified, %	Mo found, %†	Relative standard deviation, %
Ore*	2.09	2.08	0.7
BCS 64	4.11	4.09	0.6
BCS 219/3	0.60	0.59_5	0.9
BCS 225/2	0.34	0.34_0	0.9
BCS 406/1	1.00	0.97§	—
BCS 219/4	0.58	0.60§	—
BCS 261/1	0.11	0.10_6§	—
BCS 432	0.039	0.043§	—

*Obtained from Indian Bureau of Mines, Nagpur, India.
†Average of six determinations.
§Single determination.

Table 4. Determination of molybdenum in various synthetic matrices

Composition	Mo found,* μg	Relative standard deviation, %
50 μg Mo + 10 mg Fe(III) + 10 mg Cr(III) + 5 mg W(VI) + 10 mg U(VI)	49.8	0.9
60 μg Mo + 5 mg V(V) + 2 mg Nb(V) + 2 mg Ti(IV) + 10 mg Zr(IV)	60.0	0.6
70 μg Mo + 6 mg V(V) + 10 mg W(VI) + 10 mg Cr(III) + 50 mg PO_4^{3-} + 50 mg PO_4^{3-}	69.8	1.0
40 μg Mo + 2 mg Co(II) + 5 mg Ni(II) + 5 mg Mn(II) + 5 mg Zn(II)	40.1	0.7

*Average of six determinations.

Effect of diverse ions

The effect of other ions in the determination of 2 ppm of Mo by the procedure given (hydrochloric acid) was examined. Chloride, bromide, nitrate, sulphate or alkali metal up to 2*M* and alkaline-earth elements up to 0.1*M* do not interfere. The tolerable amounts of other ions are tabulated in Table 2.

Applications

The validity of the method was tested with ores, alloy steels and synthetic solutions. The results are shown in Tables 3 and 4.

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ANALYTICAL DATA

RADIOCHEMICAL EXTRACTION INVESTIGATION OF THE ION-ASSOCIATION COMPLEX OF Sb(V) WITH THIAZOLYL BLUE IN STRONGLY ACIDIC SOLUTIONS

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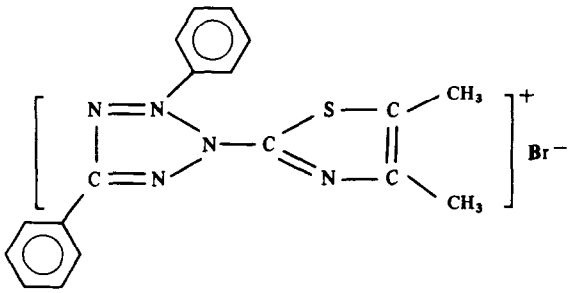
Summary—The interaction between Sb(V) and Thiazolyl Blue (MTT) in 6*M* HCl has been studied by a radiochemical extraction method. It has been proved that the ion-association complex $MTT^+ \cdot SbCl_6^-$ is extracted. The constants characterizing its extraction into $C_2H_4Cl_2$ and a 1:1 $C_2H_4Cl_2/CHCl_3$ mixture have been determined.

It has been established[1] that an extraction-photometric determination of antimony can be based on the formation of an ion-association complex between Sb(V) and MTT (I) in strongly acidic medium [MTT is used here as an abbreviation for Thiazolyl Blue, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-2H-tetrazolium bromide]. Because of the high absorbance of MTT in the organic solvent it was not possible to determine the extraction and association constants in



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the two-phase extraction system spectrophotometrically. In an earlier work[2] one of us used a radiochemical method to obtain these constants for a similar system. By extending this approach and using Sb tracers it proved possible to determine the constants characterizing the extraction equilibrium in the Sb(V)/HCl/MTT system.

EXPERIMENTAL

MTT (Koch-Light analytical grade) and $SbCl_5$ (Merck-Schuchardt) were used. The hydrochloric acid and all solvents were of reagent grade quality.

The tracers ^{125}Sb (Izotop, USSR) and ^{124}Sb (Institute of Nuclear Research, Poland) were obtained as Sb(III) in 4.1*M* and 0.1*M* hydrochloric acid, respectively. Sb(III) was oxidized to Sb(V) with sodium nitrite in hydrochloric acid medium, and the specific activity was adjusted to 7.4 kBq/ml (0.2 μCi/ml) by adding a solution of antimony(V) chloride. The concentration of antimony was determined iodometrically.

It was found earlier[1] that dichloroethane was the best organic solvent for the extraction of the Sb(V)/MTT complex. In the present study we used both dichloroethane and its 1:1 mixture with chloroform. The extraction procedure was as follows. Either $^{125}Sb(V)$ or $^{124}Sb(V)$ tracer in 6*M* hydrochloric acid, at the desired Sb concentration, was equilibrated with the organic solvent for 30 min, which was found to be sufficient for equilibrium to be reached. The phase volumes were 10 ml. The distribution ratios (organic/aqueous), *D*, were obtained from the count-rate ratios for aliquots of the corresponding phases.

The gamma activity was measured with the scintillation detector of an NP 424 nuclear counter (Hungary) or in the 3-in. well-type NaI(Tl) crystal of the 1185 Automatic Gamma Counting System (Nuclear Chicago).

RESULTS AND DISCUSSION

The values of *D* are plotted in Fig. 1 as a function of the equilibrium concentration of MTT in the

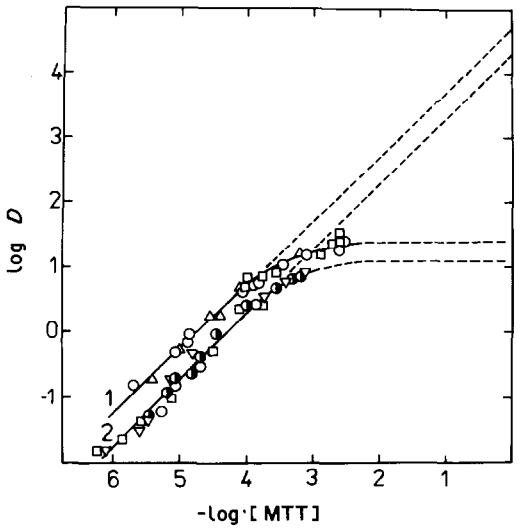

Fig. 1. Distribution ratio of Sb as a function of equilibrium MTT concentration in the aqueous phase. Curve 1: $C_2H_4Cl_2/CHCl_3$ (1:1); Sb(V) concentration: □ 3.0×10^{-5} ○ 5.0×10^{-5} △ 6.0×10^{-5} *M*. Curve 2: $C_2H_4Cl_2$; Sb(V) concentration: ▽ 1.34×10^{-5} □ 2.68×10^{-5} ○ 8.12×10^{-5} ◑ 13.56×10^{-5} *M*.

aqueous phase. The latter was calculated from D and the total Sb(V) and MTT concentrations. The left-hand parts of the plots have a slope of +1 and thus confirm the 1:1 ratio of Sb(V) and MTT in the complex.

The extraction equilibria in the Sb(V)/MTT system can be described by the extraction constant

$$K_{ex} = \frac{[MTT^+ \cdot SbCl_6^-]_{org}}{[MTT^+][SbCl_6^-]} \quad (1)$$

and the equation for the distribution ratio

$$D = \frac{K_D \beta [MTT^+]}{1 + \beta [MTT^+]} \quad (2)$$

where $K_D = [MTT^+ \cdot SbCl_6^-]_{org}/[MTT^+ \cdot SbCl_6^-]$ is the partition coefficient of the ion-association complex and $\beta = [MTT^+ \cdot SbCl_6^-]/[MTT^+][SbCl_6^-]$ is the association constant. The constants are related by

$$K_{ex} = K_D \beta \quad (3)$$

Table 1. Extraction parameters for the Sb(V)/MTT system

Organic phase	K_{ex}	K_D	β
$C_2H_4Cl_2/CHCl_3$ (1:1)	5.0×10^4	25.1	2.0×10^3
$C_2H_4Cl_2$	1.8×10^4	12.5	1.45×10^3

Extrapolation of the curves in Fig. 1 to high MTT concentrations yielded, according to equation (2), the values log $K_D = 1.40$ for the $C_2H_4Cl_2/CHCl_3$ mixture, and approximately 1.10 for $C_2H_4Cl_2$. Following the approach used in earlier papers[2,3] the curves in Fig. 1 were further analysed graphically to yield the constants K_{ex} and β. The constants obtained are summarized in Table 1.

From the constants obtained the log D *vs.* log $[MTT^+]$ curves were back-calculated from equation (2). These are shown as full lines in Fig. 1. The value of K_{ex} for the $C_2H_4Cl_2/CHCl_3$ mixture was also determined by the method of Likussar and Boltz[4] as $K_{ex} = 5.76 \times 10^4$, in good agreement with the description of the equilibria given in this work.

The results show that the Sb(V)/MTT^+ interaction in 6*M* hydrochloric acid is rather strong ($\beta \sim 10^3$.) with the equilibrium markedly shifted towards the formation of the ion-association complex.

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ANNOTATIONS

ON THE STORAGE OF THE SODIUM BOROHYDRIDE SOLUTION USED IN THE HYDRIDE-GENERATION ATOMIC-ABSORPTION TECHNIQUE

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Summary—A critical examination has been made of the stability of sodium borohydride solutions on storage at room temperature and at 4° in a refrigerator, by comparison of the reductive power for selenium(IV) in a hydride-generator, with that of a freshly prepared solution. No significant change in reactivity appeared to be caused by storage for a period of three weeks, and there seems no need to use freshly prepared solutions for analytical work.

During work with the hydride-generation atomic-absorption technique in this laboratory, it was found that the sensitivity for selenium changed somewhat with the age of the alkaline sodium borohydride solution used. This observation was not at first considered as being very remarkable, as many workers had reported that the solution was unstable on storage. The manufacturer of the hydride-generation assembly also recommended that the borohydride solution should be prepared daily.[1] However, it was discovered that some workers had found that the solution was stable for several weeks if certain precautions were taken, *e.g.*, for at least 8 weeks if the borohydride was dissolved in 2% sodium hydroxide solution and stored in a refrigerator,[2] or if the solution was filtered.[3] These claims did not help to resolve the problem, however, as both addition of sodium hydroxide and filtration are recommended in the Operator's Manual, yet it was still advised to prepare the solution daily. Because quantitative arguments for either statement could not be found in the literature, and it would be advantageous if the solution need not be prepared daily, it was decided to try to clarify the problem.

EXPERIMENTAL

Apparatus and operating conditions

A Perkin–Elmer model 300 atomic-absorption spectrometer was used under the standard conditions for selenium. Deuterium background correction was not used. The hydride-generation assembly was the pneumatically operated Perkin–Elmer MHS-10 system with a T-shaped quartz tube placed in the air–acetylene flame. Argon (99.99%) was used as purging gas. The system was operated according to the Operator's Manual.

Reagents

A 2% sodium hydroxide solution containing 3% sodium borohydride was made by dissolving 40.0 g of sodium hydroxide (Merck, *pro analysi*) in about 500 ml of water, cooling, then dissolving 60.0 g of sodium borohydride (Fluka, *purum*, >97%) in this solution, diluting to about 1800 ml, and filtering into a 2000-ml standard flask, making up to volume and mixing. This solution was divided into two parts, each of 1000 ml, which were stored in screw-cap polypropylene bottles. One solution was stored in the dark at ambient temperature and the other at 4° in a refrigerator. These solutions were used in measuring the signals from aliquots of 0.4*M* hydrochloric acid (Merck, *suprapur*) containing a constant amount of selenium(IV). The signals were compared with those obtained by use of a borohydride "reference" solution prepared just before use, by dissolving 2.00 g of sodium hydroxide and 3.00 g of sodium borohydride in water and making up to 100 ml after filtration. The sodium borohydride powder was delivered in a sealed can, which was furnished with a plastic cap for use after the can was opened the first time. Only one can was used during the experiment.

Procedure

Each borohydride solution was examined in turn, starting with the "reference" solution and ending with the solution that had been stored in the refrigerator, 100 ml being placed in the reductant reservoir. The 100 ml of the solution stored in the refrigerator were transferred to a vessel in the laboratory 1 hr before use, to allow the solution to reach ambient temperature. The 10 ml of 0.4*M* hydrochloric acid were transferred to the reaction vessel and 50 μl of 1-ppm standard Se(IV) solution were added by means of a Hamilton syringe. The reaction vessel was attached to the generation unit and the flame was ignited. After 30 sec the plunger was activated to allow the borohydride solution to be dispensed into the hydrochloric acid solution, and the signal was recorded on a strip-chart recorder operated at 5 mV sensitivity. This procedure was repeated five times for each of the three solutions on the day of the test.

RESULTS AND DISCUSSION

The means ($\bar{X}$) and estimates of the standard deviations (S) of the absorbance values for each of the two test solutions were calculated and compared with

those for the reference solution by means of the equation

$$t = \frac{\bar{X}_{ex} - \bar{X}_{ref}}{\sqrt{\frac{S^2_{ex} + S^2_{ref}}{n - 1}}}$$

where $n = 5$. If the calculated t-values exceeded the t-values from Student's table for 8 degrees of freedom ($2n - 2$) at 90% probability, the mean values were concluded to be significantly different.

Measurements were made on days 1, 3, 5, 9, 11, 15, 19 and 22 after preparation of the test solutions. From the t-test, none of the mean values for the test solution appeared to be different from the mean value for the reference solution obtained each day. The values for the test solutions for the first, third and fifth day appeared to be somewhat higher (more than 1S) than those for the reference solution, but the differences were not significant at the 90% level. This effect can probably be explained as due to the sodium borohydride powder adsorbing some moisture from the air when the can was opened, and hence losing some of its reactivity. This effect should be most pronounced in the first few days after opening of the can. After the fifth day the reactivity of the stored solutions very slowly fell below that of the reference solution, but the differences were never significant.

The reactivity of the solution which was stored in the cold was always slightly better than that of the solution stored at ambient temperature, except for the first three days of storage, but the difference was always very small and less than one standard deviation.

F-tests proved that there were never significant differences between the standard deviations of the five aliquots of the test solutions and the reference solution taken for determination in each set.

From this it might be reasonable to conclude that the sodium borohydride solution used for the hydride generation is stable for at least three weeks, probably longer. It seems there should be of no special need to prepare the solution daily. The main reason for this is probably that the loss of reactivity of the borohydride solution is not much larger than the loss of reactivity of the sodium borohydride powder caused by adsorption of moisture each time the can is opened.

If the solution is stored in the cold, it is important to allow the solution to reach ambient temperature before use, especially when peak heights are to be measured, because the peak heights should be dependent on the reactivity of the borohydride, but the peak areas will probably be much less so.

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EFFECT OF WASHING ON THE KINETIC STABILITY OF THE COPPER(II)/APCD/IBMK SYSTEM IN STRONGLY ACIDIC MEDIA

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Summary—The extraction behaviour of copper(II) with ammonium 1-pyrrolidinecarbodithioate in isobutyl methyl ketone has been investigated at various acidities of the aqueous phase. The chelate is produced even under strongly acidic conditions (0.01–6*M*), and if the organic phase is washed with water it remains stable for at least 1 hr. The extraction is quantitative over the entire range of acidity.

Low concentrations of copper are often determined by solvent extraction of a copper chelate and a number of chromogenic chelating agents have been proposed. One of the most widely used spectrophotometric reagents for copper(II) is sodium diethyldithiocarbamate (DDTC). However, the disadvantages of this reagent are its instability in acidic media and the narrow pH range for extraction.[1,2] On the other hand, ammonium 1-pyrrolidinecarbodithioate (APCD) is more stable, especially at low pH values, and so is often preferred to DDTC when extractions are performed from acidic solutions.[3,4] Although both chelating agents are decomposed at increased hydrogen-ion concentrations, their half-lives in acidic media are very different (30 sec for DDTC at pH 4 and 26 min for APCD at pH 2).[5] Several reports[4–10] have commented on the kinetic stability of metal–PCD chelates in isobutyl methyl ketone (IBMK). Dellien and Persson[10] reported that the stability in the organic phase increases in the order cadmium < lead < nickel < cobalt. They also observed that the decomposition of the complexing agent and the metal chelates is rapid in the two-phase system IBMK/water. The kinetic stability of the PCD extracts is evidently influenced by experimental conditions such as the contact area between the organic and aqueous phases.

The copper dithiocarbamate complexes exhibit good stability under acidic conditions,[4,6,11] but precise comparison of the results reported is difficult, owing to differences in experimental conditions. Although the experiments were performed on acidic solutions, data for very low pH values (below 0) were not reported. We have studied the change in the kinetic stability of the copper(II)–PCD chelate in IBMK with change in the acidity of the aqueous phase. We have also investigated the effect of washing the organic phase, with the object of improving the stability of the extracts.

EXPERIMENTAL

Reagents

All chemicals used were of reagent grade or better. Water was redistilled from an all-glass apparatus. A 0.1*M* stock solution of copper(II) was prepared by dissolving copper sulphate pentahydrate in water. A working solution was prepared daily by appropriate dilution of the stock with water.

A 0.1% APCD solution was prepared by dissolving 100 mg of ammonium 1-pyrrolidinecarbodithioate in 100 ml of water containing 0.1 ml of concentrated ammonia solution. It was filtered and stored in an amber glass bottle. The reagent was stable for at least 10 days. All solvents were used without further purification.

Procedure

Copper solution (5 ml containing 1 μmole of Cu) was transferred to a 100-ml separatory funnel and 10 ml of 0.1% APCD solution were added. The acidity was adjusted with very dilute hydrochloric acid and the aqueous phase was then diluted to 50 ml with water. The solution was mixed and mechanically shaken vigorously for 5 min with 25 ml of IBMK. The organic/aqueous phase volume ratio was kept constant throughout the experiments. In some experiments, after the aqueous phase had been withdrawn the organic phase was washed with 50 ml of water. In other experiments, the organic phase was washed with 50 ml of hydrochloric acid of the same acidity as the aqueous phase. The absorbance of the copper chelate in the IBMK phase was measured at 435 nm in 10-mm silica cells against a reagent blank prepared in the same manner.

To study the concentration of chelating agent needed for quantitative extraction, various amounts of 0.1% APCD solution were added to a solution containing 1.6×10^{-6} mole of copper. When absorbance measurements were made in 1.0*M* hydrochloric acid solution, the absorbances did not change when the mole ratio of reagent to copper was changed from 100:1 to 1000:1. While this means that the amount of chelating agent used is not critical, a variation in the amount of APCD will affect the degree of extraction of the copper chelate, because the decomposition of APCD is rapid at low pH and the degree of decomposition differs for different pH values. Hence at low pH a large excess of chelating agent is needed to allow for decomposition of the reagent. That is why a volume of 10 ml of 0.1% APCD solution was used in all our studies.

The effect of shaking time on the degree of extraction was examined. Although a 30-sec extraction time appeared to suffice for extraction from 1*M* hydrochloric acid it may not apply for other acidities, because the rate at which the metal chelate is formed and extracted decreases as the pH is reduced,[12] which is why a shaking time of 5 min was selected.

RESULTS AND DISCUSSION

The time stability of the extracts was studied for various acidities of the aqueous phase. The IBMK phase was separated rapidly from the aqueous phase without washing, after drying (filter paper) of the inside of the funnel stem, transferred to a glass bottle and stored. The rate of decomposition of the extract was found by taking samples at various time intervals and measuring the absorbance at 435 nm. The timing was started when the acidity was adjusted by addition of hydrochloric acid. It was found that there was an induction period (the length of which depended on the acidity) during which there was little or no decomposition, and this was followed by more or less rapid decomposition. Table 1 gives the time ($t_{\frac{1}{2}}$) taken for the absorbance of the extract to decrease to half the original value, but most of this time is the induction period. At an acidity of more than 7*M* the chelate readily decomposes during the extraction step. However, at less than 0.1*M* acidity the copper chelate is stable in IBMK and is formed quite readily in the acidity range 2–6*M*. The value of $t_{\frac{1}{2}}$ for 0.01 and 0.1*M* acidity was not determined, but the extracts remain stable for at least 4 hr. Surprisingly, most papers do not provide data on the extraction at high acidity; however, the extracts are fairly stable even in strongly acidic media.

Table 1. Half-life for Cu(II)–PCD chelate in IBMK at 25°

Acidity of aqueous phase, [HCl], *M*	$t_{1/2}$, *min*	t_{ind}, *min*
0.01	*	
0.1	*	
2.0	58 ± 5	
4.0	26 ± 4	~20
6.0	6 ± 1	
7.0	†	
8.0	§	

The separated organic phase, stored in a glass bottle, was permitted to stand without treatment.
*Stable for more than 4 hr.
†Decomposed during the 5-min shaking period.
§Decomposed during the 1-min shaking period.

Table 2 shows that the kinetic stability of the copper(II) chelate in IBMK depends on the treatment of the extract. At very low pH, if the extract is separated quickly from the aqueous phase and washed with water, there is no measurable decrease in absorbance in 1 hr, and the extraction is unaffected by the acidity (even > 1*M*). The decomposition of the extract is suppressed, because the free acid in the IBMK phase is removed by the wash water. On the other hand, if the IBMK phase is washed with acid of the same acidity as the aqueous phase, the absorbance decreases rapidly with time, showing that the decomposition is accelerated by increased acidity in the IBMK phase. These results suggest that copper(II) reacts practically quantitatively with APCD even in strongly acidic solution, and that the extract decomposes only slowly if the acid is removed from it.

The effect of washing on the efficiency of extraction was also studied for extraction from 4*M* hydrochloric acid. After the 5-min shaking period the IBMK phase was washed with an equal volume of water and the absorbance of the organic phase was measured. The washing was repeated four times, but the absorbance remained constant, showing that no stripping occurred.

Changing the mixing order of the reagents used had no effect on the efficiency of the extraction except when the acid was added to the APCD before the addition of the copper solution (Table 3).

Figure 1 shows the effect of acidity on the extraction. The IBMK phase was washed with 50 ml of water for 5 min and the measurements were then made directly. Extraction was quantitative in the acidity range 0.01–6*M*, but incomplete at acidities above 6*M*. There is thus no need to adjust the acidity of the aqueous phase if it is less than 6*M* hydrochloric acid. Munro[13] reported that the copper concentration in the organic phase increased with increasing sulphuric acid concentration in the aqueous phase before the extraction. Although precise comparison of the results reported[4,7] is difficult owing to differences in experimental conditions, the present results indicate that APCD is a superior chelating agent under

Table 2. Effect of acidity of washing on the time stability of Cu(II)–PCD chelate extracted from 4*M* HCl (Cu 2 × 10^{-5}*M*, temp. 25°)

	Absorbance				
Washing	20 min	40 min	60 min	2 hr	10 hr
50 ml of 4*M* HCl	0.305	0.140	0.050	0.011	0
50 ml of water	0.540	0.538	0.541	0.512	0.475

Table 3. Effect of order of mixing reagents on absorbances; extraction from 4*M* HCl

Order of mixing					
1	2	3	4	5	Absorbance
Water, 15 ml	Copper, 5 ml	APCD, 10 ml	10*M* HCl, 20 ml	IBMK, 25 ml	0.561
Water, 15 ml	Copper, 5 ml	10*M* HCl, 20 ml	APCD, 10 ml	IBMK, 25 ml	0.561
Water, 15 ml	APCD, 10 ml	10*M* HCl, 20 ml	Copper, 5 ml	IBMK, 25 ml	0.541

The separated organic phase was washed with 50 ml of water.

strongly acidic conditions, and that the copper(II)–PCD extract in IBMK has good stability.

In the method described here, if the extractions from strongly acidic solutions are carried out rapidly with an excess of APCD (to compensate for loss by decomposition) and the hydrochloric acid remaining in the separated IBMK phase is removed by washing, then it is possible to extract the complex regardless of the acidity of the aqueous phase, and the extract remains stable for 1 hr. The method is therefore recommended for the extraction of trace copper from strongly acidic media.

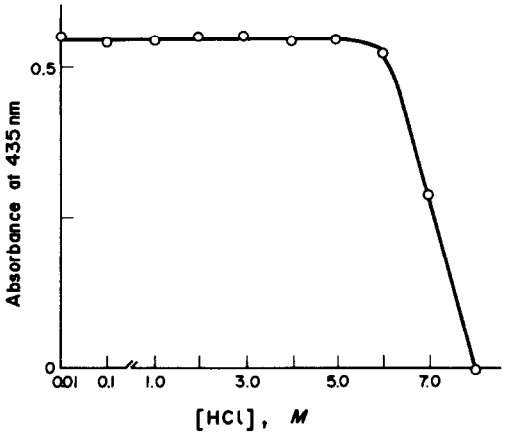

Fig. 1. Effect of acidity on the extraction of Cu(II) with APCD. Aqueous phase, 50 ml; organic phase, 25 ml; Cu(II), $2 \times 10^{-5}M$.

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LETTER TO THE EDITOR

SOME ANALYTICAL APPLICATIONS OF CHLORBROMAMINE-B

Sir,

Organic haloamines have received considerable attention as oxidimetric reagents,[1-3] the most recently reported being chlorbromamine-T.[4] The corresponding benzene derivative, chlorbromamine-B (N-chloro-N-bromobenzenesulphonamide, (CBB), also serves as an oxidimetric titrant in acetic acid medium for potentiometric determination of hydrazine, ascorbic acid, hydroquinone, semicarbazide, ferrocyanide, oxine, thallium(I) and antimony(III) and, by back-titration methods, of thiocyanate, thiosemicarbazide, isoniazid and indigo carmine.

The reagent can be prepared by Paterson's method,[5] by dissolving 18 g of benzenesulphonamide in 500 ml of water containing 4 g of sodium hydroxide, cooling to below 5°, adding 8.5 g of bromine dropwise with constant stirring, then when all the bromine has reacted, adding 4 g of sodium hydroxide dissolved in 20 ml of water, cooling to below 5° and passing chlorine through the solution (kept at below 5°), until it is neutral. The lemon yellow precipitate is filtered off, washed with water, dried over phosphorus pentoxide in the absence of light and stored in brown bottles. Yield ~ 100%; m.p. 83°.

CBB is only slightly soluble in water, but fairly soluble in glacial acetic acid (204 g/l. at 30°) and other common organic solvents. Solutions of CBB are light-sensitive and have to be kept in brown bottles. They should be standardized daily as for dichloramine-T.[6] The conditional standard redox potential in glacial acetic acid is +1.31 V at 30°.

Hydrazine sulphate, ascorbic acid, hydroquinone, semicarbazide hydrochloride, ferrocyanide, antimony(III), thiocyanate, thiosemicarbazide, isoniazid, indigo carmine, oxine and thallium(I) can all be titrated potentiometrically [with addition of potassium bromide for titration of semicarbazide, oxine, Sb(III) and Tl(I)], or by a back-titration procedure in which the excess of oxidant is determined iodometrically after 15 min reaction time. Steady potentials are attained almost instantaneously in all the potentiometric titrations. In the back-titration procedure indigo carmine is oxidized to isatin sulphonate. The maximum error was 1% for the potentiometric titrations and 0.8% for the back-titrations.

Assay of ascorbic acid and isoniazid in tablets and injections gave results in good agreement with those obtained by the British Pharmacopoeia[7] and United States Pharmacopoeia[8] methods, the maximum relative difference being about 2% and the average difference 0.6%. Gum acacia, magnesium stearate, citric acid, sodium chloride and reserpin (100 mg) did not interfere

Chlorbromamine-B, like the other organic haloamines, serves as a versatile redox titrant in non-aqueous media.

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7 April 1982

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DERIVATIVE HYDRODYNAMIC-MODULATION VOLTAMMETRY

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Summary—A technique based on derivative hydrodynamic-modulation voltammetry has been developed for improving the resolution in sensitive analyses of mixtures, at solid electrodes. By simple treatment of the hydrodynamic-modulation data the voltammetric current–potential waves can be transformed into peak-shaped curves, which are frequently more convenient for further data processing. To obtain such curves the rate of change in hydrodynamic-modulation current with potential is plotted against potential. Single and multiple peak systems are evaluated at the micromolar concentration level, by use of stopped-rotation and stopped-flow voltammetry. Dopamine, ascorbic acid, NADH, homovanillic acid and chlorpromazine were used as test compounds. The method is simple and suitable for on-line computerization.

Hydrodynamic-modulation voltammetry (HMV) has been shown to have advantages over other voltammetric techniques for obtaining current–voltage (i–E) data for low concentrations of electroactive species at solid electrodes.[1] For both reversible and irreversible electrode processes HMV produces sigmoidal curves, which give rather poor resolution (*cf.* d.c. and normal pulse polarography). For anodic oxidations at solid electrodes the resolution is even poorer because of the irreversible nature of most of these reactions, *i.e.*, the voltammetric waves are spread out over the potential range; for this reason a separation procedure (usually liquid chromatography) is frequently used before the voltammetric analysis. To improve the resolution of d.c. and normal pulse polarography (at the dropping mercury electrode) it is common practice to plot di/dE against E,[2,3] but this approach has not hitherto been applied to electroanalytical techniques at solid electrodes.

This paper describes the characteristics and applications of this approach, which will be called derivative hydrodynamic-modulation voltammetry (DHMV). It is based on plotting the increments in pulsed convection-current amplitude resulting from increasing the potential in equal steps, as a function of the potential. The resulting DHMV curve has a peak, in contrast to the sigmoidal shape of the conventional hydrodynamic-modulation voltamperogram. This approach is specially suited to HMV, because many of the modulation procedures used (*e.g.*, pulsed rotation[4] or pulsed stirring[5]) are based on pointwise recording of the voltamperogram (*i.e.*, making a 50- or 100-mV change in the applied potential and applying the convection pulse).

A summary of results obtained by such numerical manipulation of raw HMV data is presented in this paper. The DHMV response characteristics of some reversible and irreversible single-peak systems are evaluated, together with applications to batch and flow analyses of mixtures containing various oxidizable species at the micromolar level.

EXPERIMENTAL

Apparatus

The rotating disk electrode assembly, the electrochemical cell and the instrumentation (polarograph, recorder, *etc.*) used in the stopped-rotation (batch) experiments have been described in detail previously.[6] A 0.75-cm glassy carbon disk served as the working electrode.

Pulsed-flow experiments were done with a home-made wall-jet electrode, the details of which have been described elsewhere.[7] A 0.25-cm diameter glassy carbon disk served as the working electrode, with the solution inlet nozzle (0.34-mm diameter) 0.25 mm from the centre of the disk. All potentials are referred to an Ag/AgCl reference electrode. The sample solution was stored in a 250-ml Nalgene beaker, and flowed by gravity to the cell through 0.5-mm bore Teflon tubing.

Reagents

Chemicals and reagents used have been described in detail previously,[6] except as noted. Millimolar stock solutions of dopamine, homovanillic acid and reduced nicotinamide adenine dinucleotide (NADH) were prepared each day.

Procedures

Stopped-rotation experiments. Supporting electrolyte solution (200 ml) was added to the cell and pretreatment of the electrode begun. This consisted of applying potentials of +0.9 and −0.9 V alternately for 8 min, with 1 min wait at each potential. Following this, measurements were made on the blank and analyte solutions by making 25- or 50-mV changes in the applied potential and waiting about 30 sec before applying the rotation pulse. To shorten the overall time of the experiment, only the "off" to "on" part of the pulse was employed, thus eliminating the long decay time that characterizes the "on" to "off" part of the cycle.

Pulsed-flow experiments. After electrode-conditioning (similar to that for the batch analysis described above), current–potential data were obtained by making 50-mV

changes in the applied potential and applying the flow pulse at each potential. The flow was pulsed by raising and lowering the outlet level for the sample solution.

In experiments involving ascorbic acid oxygen was removed from the solution by bubbling nitrogen through it.

Data treatment

The strip-chart current amplitudes were read as described earlier.[6] The current difference Δi, at potential E_1 was subtracted from that at the next potential, E_2. Plots were made of $\Delta i_n - \Delta i_{(n-1)}$ *vs.* E_n.

RESULTS AND DISCUSSION

Single-peak systems

Figure 1 shows DHMV (a) and HMV (b) current–potential curves for the oxidation of 5μM dopamine by stopped-rotation voltammetry. The DHMV curves were obtained by use of potential increments of 25 and 50 mV. Some important characteristics should be noted with respect to Fig. 1. Because the rate of change of current with change in potential is at a maximum at around the half-wave potential, the DHMV peak potential and the HMV half-wave potential are similar, about +0.1 V. The resolution is improved by using the smaller potential increments; the data yield for ΔE = 25 mV a width of about 50 mV at half peak-height, while for ΔE = 50 mV the width is 70 mV. On the other hand, the larger ΔE value gives higher sensitivity and shorter recording time. As ΔE approaches the potential change responsible for the entire HMV wave, no further gain in the sensitivity will be obtained (*i.e.*, the peak current approaches a maximum) but the peak width continually broadens; it seems, therefore, that there should be an optimum ΔE value, which represents the best compromise between resolution, sensitivity and speed, and will differ from one system to another, depending on the reversibility of the reaction and the number of electrons involved (which controls the wave width), as well as on the presence of other species with adjacent redox potentials.

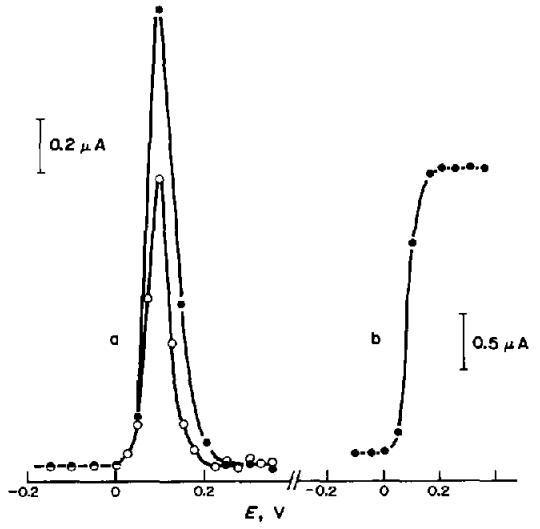

Fig. 1. Derivative (a) and conventional (b) hydrodynamic-modulation voltamperograms for the oxidation of 5μM dopamine in 0.1M phosphate buffer (pH 7.4). Stopped-rotation conditions, rotation on (1600 rpm) for 15 sec. ΔE 25 mV (○) and 50 mV (●).

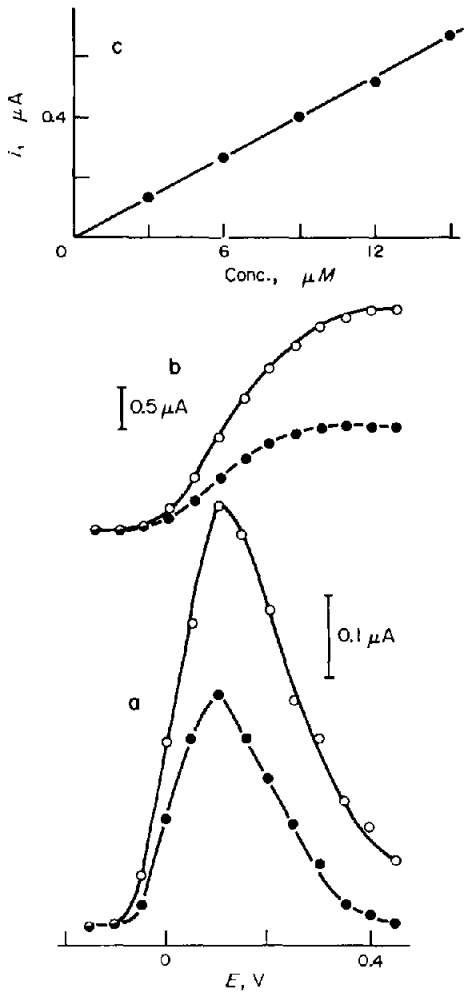

Fig. 2. Characteristic response for ascorbic acid. Derivative (a) and conventional (b) HMV response for 3 and 6μM ascorbic acid. Stopped-rotation conditions, as in Fig. 1; ΔE 50 mV; 0.1M phosphate buffer (pH 7.4).

Figure 2 shows the DHMV and HMV response for ascorbic acid at the micromolar concentration level. As ascorbic acid is oxidized irreversibly at carbon electrodes the hydrodynamic-modulation waves are spread out along the potential axis ($E_{3/4} - E_{1/4}$ about 150 mV), and thus the derivative peaks are wider than those for the reversible oxidation of dopamine (Fig. 1). The width at half peak-height for the two ascorbic acid derivative voltamperograms is about 0.25 V. The DHMV peak potential and the HMV half-wave potential for ascorbic acid are about +0.1 V. The two derivative curves shown in Fig. 2a are from a series of five covering the range 3–15μM ascorbic acid, which gave a linear calibration plot (peak current *vs.* concentration) with a slope of 44 nA.1.μmole^{-1}. The data of Fig. 2 indicate the feasibility of determining

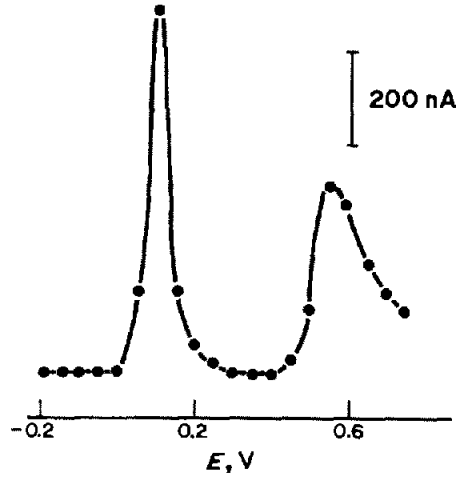

Fig. 3. Simultaneous measurement of 3μM dopamine and chlorpromazine. Conditions as in Fig. 2.

ascorbic acid at the micromolar level by this method, in contrast to differential pulse voltammetry (*e.g.*, ref. 6) which gives poorly-defined peaks for ascorbic acid because of the irreversibility.

Analysis of mixtures

Figure 3 shows a characteristic DHMV response for a mixture of dopamine and chlorpromazine (each at 3μM). The dopamine response is similar to that in Fig. 1. The chlorpromazine peak potential is +0.55 V, with a width of 165 mV at half peak-height, *i.e.*, the oxidation is more irreversible than that of dopamine.

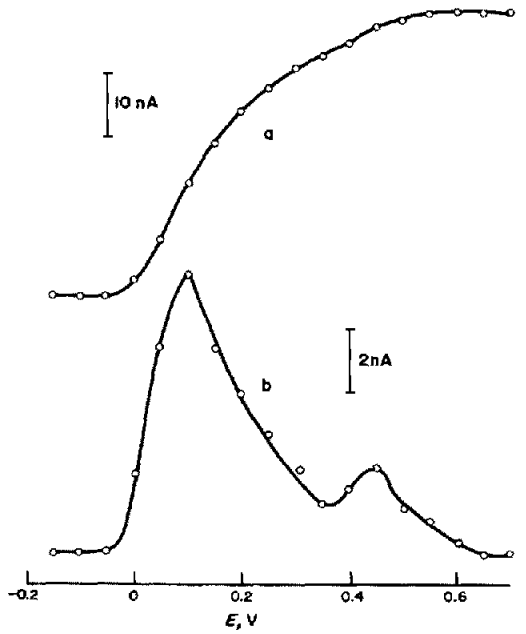

Fig. 4. Consecutive measurement of 2.5μM NADH and 5μM ascorbic acid in a flowing solution. Conventional (a) and derivative (b) pulsed-flow voltamperograms. Pulsed-flow conditions. 1.8 ml/min for 10 sec, 3.0 ml/min for 10 sec; 0.1M phosphate buffer (pH 7.4).

The decreased DHMV sensitivity associated with decreased reversibility is obvious, but nevertheless the 450-mV difference between the peak potentials of the two species provides good resolution (as might be expected since the HMV response gives well-separated waves anyway). It might be expected that 3 or 4 electroactive species could be measured over a 1-V working potential range, depending on the separation of their peak potentials and the nature of their redox reactions (*i.e.*, reversibility and number of electrons).

One of the most important applications of solid electrodes is as sensitive sensors for monitoring flowing streams, usually by constant-potential amperometry, resulting in high sensitivity and poor selectivity (unless a chromatographic separation is used). When the substances present must be identified it is necessary to obtain current–potential data for the flowing solutions. Flow-modulation techniques can provide reproducible voltamperograms for a low concentration of a single analyte at flow-through solid electrodes (*e.g.*, ref. 7). The improved selectivity of DHMV allows it to be applied to the analysis of mixtures of species with relatively close redox potentials, as shown in Fig. 4 for a mixture of 2.5μM NADH and 5μM ascorbic acid. Both species are irreversibly

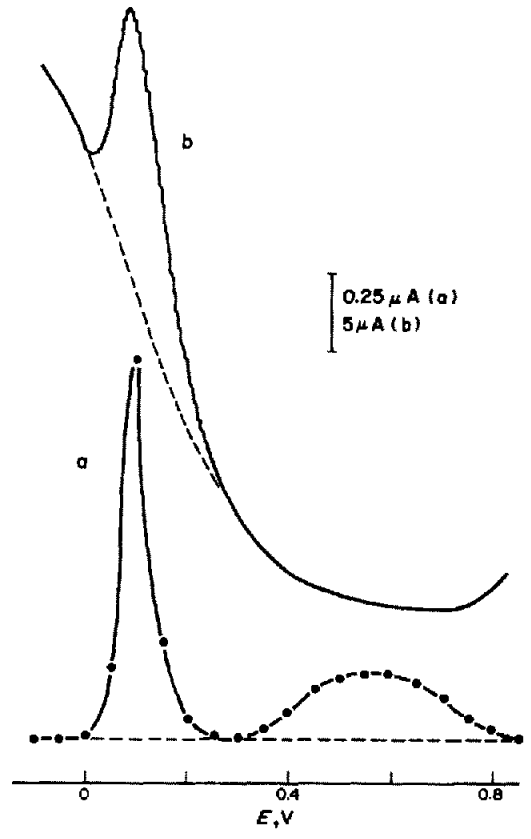

Fig. 5. Comparison of DHMV (a) and differential-pulse voltammetry (b) of 4μM dopamine and 3μM homovanillic acid in 0.1M phosphate buffer (pH 7.4). DHMV conditions as in Fig. 2. Differential-pulse conditions: amplitude 50 mV, scan-rate 5 mV/sec, rotation speed 1600 rpm. Dotted lines represent blanks.

oxidized and therefore give low sensitivity in conventional voltammetry. Because their redox potentials are relatively close the HMV voltamperogram gives strong overlap of the waves, making analysis of this mixture impossible by this method. Differential-pulse voltammetry also fails in this case. The derivative voltamperogram, however, shows two reasonably resolved peaks, about 350 mV apart and even for highly irreversible redox reactions and a peak separation of only 350 mV, DHMV provides useful qualitative and quantitative data at micromolar concentrations.

The advantages of DHMV over differential-pulse voltammetry in analysis of mixtures are clearly demonstrated in Fig. 5 [for batch analysis of a $4\mu M$ dopamine and $3\mu M$ homovanillic acid mixture by stopped-rotation modulation (a) and differential-pulse with continuous rotation (b)]. Both techniques give peaks (with similar peak potentials) for the oxidation of dopamine, but the differential-pulse peak coalesces with a high background current, due mainly to surface-controlled processes against which the technique is not effective. The DHMV dopamine peak does not suffer from this limitation, because the technique compensates for non-convective background currents. Because the oxidation of homovanillic acid is highly irreversible this reaction is not detected by the differential-pulse technique but does result in a broad (but well defined) DMHV peak. Good baseline resolution is obtained for the two DHMV peaks. Recording a differential-pulse voltamperogram takes about 4 min whereas the DHMV procedure (including data manipulation) takes about 25 min. However, at the micro- and submicromolar concentration levels, only DHMV offers the required sensitivity, the detection limits being similar to those for conventional HMV procedures, 10–50nM, depending on the species monitored and method used. The use of scanning potential-modulation procedures incorporated with on-line computerized differentiation would significantly shorten the observation time and make it more suitable for on-line work.

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VALIDATION OF THE SULPHUR CONCENTRATION OF SELECTED IRON-BASE NBS STANDARD REFERENCE MATERIALS BY ISOTOPE-DILUTION SPARK-SOURCE MASS-SPECTROMETRY

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Summary—An isotope-dilution spark-source mass-spectrometric procedure has been developed for the accurate determination of sulphur in iron-base alloys. Dissolution in a sealed tube is used to prevent volatilization losses and to effect isotope equilibration. Application of this technique to the re-analysis of existing NBS Standard Reference Materials yields results that are generally in good agreement with the certified values.

Sulphur is present in iron and steel as a result of impurities in the raw materials and fuels used in their production. Usually it is regarded as a detrimental element because of the potential brittleness that it imparts to the product. In special instances, however, sulphur is not removed and indeed may be intentionally added at controlled levels in order to produce a free-cutting material such as a screw stock. Whatever the kind of iron-base material produced, it is an accepted fact in the industry that its sulphur concentration must be closely controlled to achieve the desired mechanical properties.

The routine laboratory methods that are used to determine sulphur must not only be accurate but also inexpensive and rapid. The automated analytical instrumentation currently in use is capable of meeting these criteria provided an adequate range of accurate sulphur standards is available. Currently, a need exists for iron-base standards with sulphur concentrations at the 50 ppm level and above, but further needs will soon push the lower end of this range to about the 10 ppm level.

At the National Bureau of Standards (NBS), the philosophy for certification of Standard Reference Materials (SRMs) requires either that the analytical methods used are definitive, *i.e.*, free from systematic errors, or that at least two methods are used which are sufficiently different that the likelihood of their having the same kinds of bias is small. Stable isotope dilution mass spectrometry is widely used at the NBS as a definitive technique for trace element determination.[1–3] This paper describes an isotope-dilution spark-source mass-spectrometric procedure (ID–SSMS) that has been developed recently at the NBS for the accurate determination of sulphur in iron-base alloys. It was developed in response to two reports[4,5] which suggested that the certified values for sulphur in several of the currently available iron-base NBS SRMs might be high by as much as 20%. Analysis of these SRMs by this procedure has yielded results in close agreement with the certified values.

EXPERIMENTAL

Reagents

The high-purity nitric, hydrochloric and sulphuric acids used were prepared at the NBS by sub-boiling distillation.[6] Except where noted, all other chemicals were analytical-reagent grade.

Reducing solution. Hypophosphorous acid (60 ml, 50%), concentrated hydriodic acid (125 ml) and concentrated hydrochloric acid (205 ml) were mixed. This reducing mixture is the same as that recommended by Thode *et al.*[7] and subsequently used by Watanabe.[4] The high concentration of sulphur initially present in this mixture was removed by refluxing it for 1.5 hr with nitrogen continuously bubbling through it to sweep out any hydrogen sulphide produced.

Sulphur spike solution. Sulphur enriched to contain 98.5% ^{34}S was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee. It was dissolved by sealing it in a borosilicate tube along with 8 ml of NBS high-purity nitric acid and heating for 8 hr at 180°. The solution was transferred to a modified 200-ml standard flask and diluted with distilled water to obtain a working stock solution with a nominal sulphur concentration of 100 μg/g. The flask was modified by cutting off most of the neck and flaring the top to accept a standard-taper polyethylene stopper. This modification prevented loss of water by evaporation and thus kept the concentration of the spike solution from changing after calibration. The stopper was held firmly in place by covering it with a Teflon disc and clamping this to the neck by crimping an aluminium band round it. When not in use, this flask was kept in a closed box at 100% relative humidity.

Standard sulphur solutions. Two solutions containing known amounts of natural sulphur were prepared for calibration of the enriched ^{34}S spike solution. One was made from a weighed amount of recrystallized potassium sulphate and the other from NBS sub-boiling distilled sulphuric acid that was assayed coulometrically.

Apparatus

Dissolution. The sealed-tube dissolution procedure was similar to the one developed by Wichers *et al.*[8] The tubes used were constructed from borosilicate glass (2 mm wall thickness) and were about 25 cm long. The body of the tube was sealed at one end, had an outside diameter of 1.8 cm, was 15 cm long, and ended in a 10-cm stem having an outside diameter of 0.8 cm. The sample and reagents were added (in that order) and frozen in the tube by means of a slush of solid carbon dioxide and a 1:1 v/v mixture of chloroform and carbon tetrachloride. The tube was sealed (while still in the slush) by rotating it in an oxygen–gas flame until the open end had flowed together. Heating was continued until the internal pressure had blown the end into a smooth rounded shape. The tube was placed in a steel shell and about 50 g of solid carbon dioxide were added to provide a compensating external pressure. A copper gasket was used to make a tight seal between the shell and the screw cap. The tube was then heated in an oven at 180° for 8–16 hr (usually overnight for convenience).

Isolation of sulphur. The glass apparatus used for the separation of sulphur as hydrogen sulphide and its collection as silver sulphide consisted of a 100-ml round-bottomed flask fitted with a 15-cm straight water-cooled condenser, a 25-ml midget impinger, and a 15-ml centrifuge tube. Passing through the centre and sealed in a standard taper cap placed in the top of the condenser was a 6-mm outside diameter glass tube that extended to approximately 3 mm from the bottom of the flask. The end of this tube was drawn down to 1 mm bore to facilitate smooth bubble formation. The midget impinger was connected to a side-arm attached to the cap. All non-ground joints were butted and held together with Tygon tubing.

For initial purification of a large volume of reducing solution, a scaled-up version of this apparatus was used in which a 500-ml flask and a 30-cm condenser were employed.

Measurement of isotopic ratio. The $^{32}S/^{34}S$ ratio was measured with a JEOL model 01BM-2 spark-source mass-spectrometer equipped with electronic detection and an ion-multiplier. This instrument has magnetic switching between mass peaks and the magnetic field is controlled by a Hall-probe gaussmeter. To eliminate dispersion and discrimination of different masses by magnetic fields in the electrostatic and drift regions of the flight path, magnetic shielding was installed inside the vacuum envelope between the ion-source and the entrance to the magnet. A second modification involved splitting the ion-current monitor into upper and lower halves, each having its own vibrating-reed electrometer. This modification permits the monitoring and centring of the ion-beam in the z-axis and is necessary to achieve precise measurements. This centring is dependent on both the positioning of the sample electrodes in the z-axis and their positions relative to one another along the x-axis.

Procedure

The same analytical procedure was used for all samples analysed in this study in which the sealed-tube dissolution technique was employed. Generally, three samples and a blank were processed as a group. The order of addition of the reactants, the techniques used, and the precautions taken are given in some detail below.

Spike addition and sample dissolution. A weighed amount of sulphur spike solution with a ^{34}S content approximately equal to the amount of natural sulphur expected to be present in the sample was transferred to the tube. To do this the polyethylene stopper in the flask of spike solution was replaced by a similar stopper with a hole in its bottom. An 18-gauge platinum needle with a Kel-F hub was inserted through this hole, a 10-ml polyethylene syringe with the rubber tip of its plunger covered with a thin Teflon sheet was attached to the needle and a suitable volume of spike withdrawn. The syringe was disconnected and its end sealed with a Kel-F cap. Any static charge on the syringe was removed by wiping with a damp lintless cloth. The syringe and contents were weighed on a semi-micro balance to ± 0.02 mg. An appropriate volume of spike solution was then carefully delivered from the syringe into a vertically held dissolution tube and the syringe was again capped, wiped and weighed. The spike solution was washed down to the bottom of the tube with 1 ml of distilled water added slowly from a disposable Pasteur pipette while the tube was rotated. The tube was placed in the cooling slush to freeze the contents. A sample weighing between 0.5 and 1 g was added, followed by 8 ml of the purified nitric acid and 2 ml of the hydrochloric acid. Sufficient time was allowed for the nitric acid to freeze before the hydrochloric acid was added (this was done to prevent chemical reactions that could have led to the volatilization of sulphur before the tube was sealed). The tube was then sealed, placed in the steel shell, and heated *etc.*, as described under *Dissolution*, above. After cooling, the tube was cooled in the slush, and a small hole was blown in the tip by using an oxygen–gas torch. The neck of the tube was scored and opened and the contents were transferred to a 100-ml borosilicate glass beaker. A 0.5-ml portion of 0.02*M* sodium carbonate was added to both the sample and blank solutions to provide a counter-cation to prevent loss of sulphur by volatilization as sulphuric acid. The solutions were evaporated to dryness on a hot-plate under a borosilicate glass cover. This cover was 20 cm in diameter, with a 10-cm vertical wall which tapered to a cone of height 5 cm, with a glass tube sealed through its centre. The tube was sealed at its end but had four evenly spaced holes in it just above the end, and high-purity dry nitrogen was dispersed through these holes and down over the solution to hasten evaporation. The cover rested directly on the ceramic hot-plate and the nitrogen escaped through the gaps caused by the irregularity of the surfaces. Any condensate drained to the sides, where it evaporated, thus preventing any fall-back into the beakers. The high temperature and the nitrogen flow swept the vaporized acids efficiently from the system, and condensate occurred only at the start of the evaporation, if at all. This system was adopted for the express purpose of preventing contamination by sulphur compounds in the laboratory atmosphere; before its use the laboratory atmosphere contributed 70–90% of the total blank. After the initial evaporation, the sides of the beaker were washed down with hydrochloric acid (2, 1 and 1 ml volumes in succession, with evaporation to dryness after each addition), to ensure all the nitric acid was removed.

Reduction and separation of sulphur as hydrogen sulphide. First, 25 ml of the reducing mixture were placed in the round-bottomed flask of the reflux apparatus and heated to boiling. The mixture was refluxed for 45 min, with nitrogen bubbling through it at 20–25 ml/min. This second pretreatment of the reducing solution was used to ensure that all residual sulphur was removed and also to clean out the apparatus. After the solution had cooled, the spiked sample, dissolved in 5 ml of hydrochloric acid, was added to the flask and the mixture was again refluxed for 45 min. The hydrogen sulphide generated was passed first through the midget impinger containing 10 ml of distilled water to remove hydrochloric and hydriodic acids, and then into the centrifuge tube, containing 10 ml of 0.1*M* silver nitrate. The silver sulphide precipitate formed was centrifuged and washed three times with 5-ml portions of hot distilled water. Before the last rinse, 100-mesh gold powder (99.999% pure, 50 mg) was added and the mixture was co-dispersed before final centrifugation. The supernatant liquid was removed with a disposable Pasteur-type pipette and the residue was dried at 110° for 30 min. It was then transferred to a stainless-steel vial and the silver sulphide was homogenized with the gold powder by mixing for 3 min with a stainless-steel ball on a "Wig-L-Bug"

mixer. The mixture was then pressed between two flat, polished, tungsten carbide dies under a 2000-kg load. The resulting disc was cut in half with steel cutters, stacked, and re-pressed. Multiple halvings and pressings were used to homogenize the mixture further and to form it into two electrodes each having dimensions of 2 × 5 × 0.2 mm. The electrodes were mounted in the spark-source mass-spectrometer and the system evacuated to a pressure of less than 2×10^{-7} mmHg. A mass scan was made from $m/z = 240$ to $m/z = 10$, with the ion-multiplier electronic detection system. The maximum gain was used in order to survey each sample for any element present above the 0.1-ppm level. The altered isotopic ratio of the sulphur was then measured by use of magnetic peak switching under the control of a Hall probe. The ratio of $^{32}S/^{34}S$ was measured by means of the singly-charged sulphur ions [S^+] at nominal m/z ratio of 32 and 34. Three sets of measurements, each containing 25 ratios, were collected for every sample. Each set of 25 ratios required 25–30 min of measurement time. The ratio data were stored directly in a computer as they were collected and, after each set of measurements, the sulphur concentration was calculated by using the equation:

$$\text{sulphur (ppm)} = \frac{WK}{m} \frac{(A_{sp} - B_{sp}R)}{(BR - A)}$$

where R = measured $^{32}S/^{34}S$ ratio, A_{sp} and B_{sp} = abundances of ^{32}S, ^{34}S in the spike, A and B = abundances of natural ^{32}S and ^{34}S, W = sulphur spike added (μg), m = sample weight (g), and K = ratio of atomic weight of natural sulphur to atomic weight of the spike sulphur.

RESULTS AND DISCUSSION

By use of the sealed-tube dissolution and ID-SSMS procedure, sulphur was determined in a number of NBS iron-base SRMs. The results obtained and the IDMS results reported by Watanabe[4,5] are compared with the NBS certified values in Table 1. With the exception of SRM 365, the agreement of the certified values with those obtained in the present study is significantly better than their agreement with the values reported by Watanabe. In general, his results are considerably lower than the certified values.

In an attempt to explain the discrepancy between the two sets of IDMS results, the pertinent experimental parameters in both procedures were examined for potential systematic bias. In doing so, it was noted that in the dissolution techniques employed by Watanabe, the ^{34}S spike was always added *after* the sample dissolution. Hence, if sulphur were lost during the dissolution step before the addition of and equilibration with the spike, the resulting IDMS values would be low. To check this possibility, SRMs 362 and 364 were analysed by the open-beaker dissolution procedure employed by Watanabe[4] and compared with the sealed-tube dissolution results. These SRMs were selected for this test because the discrepancy between the two sets of IDMS results was largest for these samples. The results of this study, given in Table 2, clearly show that there is a significant loss of sulphur during sample dissolution for these particular low-alloy steels. This loss is almost certainly due to volatilization of sulphur during the dissolution. In addition, the reproducibility of the open-beaker results suggests that it is a particular form of sulphur present in these

Table 2. Intercomparison of dissolution techniques

SRM	Sulphur, μg/g	
	Open beaker*	Sealed tube
362	318; 328; 320	360; 364; 357
364	225; 225; 227	247; 251; 251

* ^{34}S spike added after dissolution

Table 1. Comparison of certified and IDMS values for sulphur in selected NBS SRMs

SRM	NBS certified value*, μg/g	Average IDMS values, μg/g	
		Present work (sealed tube)	Watanabe (open beaker)
32e, Ni–Cr steel	210 ± 10†	204 ± 4§	171.5‡; 181‖
33d, Ni steel	100 ± 10	95 ± 2	86.1‡
72f, Cr–Mo steel	240 ± 10	222 ± 6	196.9‡
361, low-alloy steel	150 ± 10	142 ± 3	131¶
362, low-alloy steel	380 ± 10	360 ± 6	301¶
363, low-alloy steel	68 ± 2	69 ± 2	63¶
364, low-alloy steel	250 ± 3	250 ± 6	213¶
365, electrolytic iron	56 ± 1	55 ± 2	55¶

*Values stated on most recently revised certificates. For SRMs 361–365, these are somewhat different from the values given on the original certificates issued in 1971 and 1972.

†The indicated uncertainties are based on current judgment and represents an evaluation of the combined effects of imprecision, possible systematic errors among methods, and material variability.

§95% confidence level for minimum of three analyses.

‡Ref. 4.

‖Ref. 5.

¶Ref. 9.

Table 3. Analysis of other NBS iron-base alloys

SRM	Certified value, %	Sulphur found, μg/g
C1288, high-alloy steel	0.010 ± 0.001*	115 ± 5†
342a, nodular cast iron	0.006	23 ± 2

*Estimated uncertainty.
†95% confidence level for a minimum of three analyses.

alloy steels that is lost. Although no open-beaker tests were done in which the spike was added to these samples before dissolution, the fact that the ^{34}S is added in the relatively non-volatile sulphate form suggests that these results would also have been low.

The sulphur results obtained for two other NBS iron-base SRMs by the sealed-tube ID-SSMS procedure are summarized in Table 3. The agreement between the certified value for sulphur in SRM C1288 and the value found by the proposed procedure is considered acceptable. However, the value found for SRM 342a is significantly lower than the certified value. Since this SRM was certified more than a decade ago, by use of procedures acceptable at the time by industry, this discrepancy is perhaps understandable. Because the present study indicates that the sulphur value certified for SRM 342a is too high, it would be advisable for the procedures used in the initial certification to be examined for possible bias.

Through correspondence, provision of additional samples, and by the use of the sealed-tube dissolution technique, Watanabe has confirmed that his earlier results were low.[10] His latest thermal IDMS results for the NBS SRMs listed in Table 1 are almost identical with the NBS ID-SSMS results. Two reasons have been identified for the initial low values. The principal one was the volatilization of some sulphur from the sample before equilibration with the spike sulphur. A secondary reason, proposed by Watanabe, was that additional losses could have occurred when he rinsed the chip samples before analysis. However, the SRMs issued by NBS are certified for use in the "as received" condition unless explicitly specified to the contrary on the analysis certificate. The cleaning of chip steel samples before analysis has never been recommended.

The key step in the proposed procedure for obtaining accurate sulphur values is the dissolution of the iron samples in a sealed tube. This dissolution technique enables the sulphur in the sample to equilibrate completely with the spike sulphur without possibility of loss of either by volatilization. A critical operation after the dissolution is the complete removal of nitric acid. Even traces of nitric acid will prevent complete reduction of sulphate to sulphide with the acid reducing mixture used. In experiments done to evaluate the sulphur blank, 10 μl of nitric acid proved sufficient to prevent this reduction. Following reduction, the collection of the evolved hydrogen sulphide in silver nitrate solution was preferred to the cadmium acetate solution used by Watanabe[4] because it is more efficient and also eliminates the need for subsequent conversion of cadmium sulphide into silver sulphide.

The reduction procedure used for isolating sulphur from iron samples is relatively specific and is applicable to sulphur levels of a few micrograms. Complete mass scans of the gold–silver sulphide mixtures showed major lines for only sulphur, silver and gold. Apart from a small amount of phosphorus, estimated to be less than 1% of the sulphur recovered, and trace levels of iodine and chlorine, no other elements were detected in significant quantities. The last three elements apparently originate as volatile components of the reducing solution that are not completely removed by the water scrubber.

Although no experiments were performed to measure the efficiency of the recovery of sulphur, the absolute sulphur signal obtained by processing 5 μg of ^{34}S spike indicated a high overall yield. The combination of good recovery for this amount of sulphur and the high sensitivity of the spark-source mass-spectrometer indicated a theoretical detection limit well below 0.1 μg of sulphur. However, as is the case for most common elements at levels below 1 μg, it is the blank that is the limiting factor. The total sulphur blank for all steps in the present procedure averaged 0.5 ± 0.1 μg. About 0.2 μg originated from the gold powder used.

The sealed-tube dissolution ID-SSMS procedure described in this paper is illustrative of the analytical techniques required to meet the growing need for more accurately characterized standards. The availability of steel standards with accurately determined sulphur content is especially important since almost all sulphur determinations in industry today are performed with automated analysers. These analysers clearly have the desired speed, precision, and sensitivity to meet existing needs. Their accuracy, however, is highly dependent on the quality and variety of standards available for calibration. The application of this technique for the accurate measurement of the sulphur concentration of NBS SRMs 361, 362, 363, 364 and 365 is especially important since this series of standards should be extremely useful for calibrating automated sulphur analysers to be used for accurate determination of the sulphur content of low-alloy and carbon steels. Similarly, the technique should be equally valuable in validating the sulphur concentration of SRMs 1261, 1262, 1263, 1264 and 1265, which are currently used for calibrating optical emission spectrometers.

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METAL COMPLEXES OF CYCLIC TETRA-AZATETRA-ACETIC ACIDS

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Summary—The cyclic tetra-aza complexones cDOTA ([12]ane N_4·4ac), cTRITA ([13]ane N_4·4ac) and cTETA ([14]ane N_4·4ac) have been synthesized and characterized by elemental analysis, titration, melting-point determination and NMR (and infrared) spectroscopy. The ionization constants and the stability constants of the MH_2L, MHL and ML complexes formed with alkali, alkaline-earth and some transition metals were determined at 25.0 ± 0.1° and ionic strength 0.10*M* [KNO_3 and $(CH_3)_4NNO_3$]. It was confirmed that cDOTA forms the most stable Ca^{2+} and Sr^{2+} complexes but the reported inversion of the order of stability of the complexes of these two ions with cTRITA was not confirmed. Also, the values of the stability constants determined in this work differ substantially from those previously reported for ML species. cDOTA is an interesting alternative to classical non-cyclic complexones for the complexometric determination of Ca^{2+} and Mg^{2+} but neither this ligand nor the other two offer advantages over EDTA or DCTA for the complexometric titration of transition metals.

Cyclic polyoxa and polyaza ligands which form stable complexes with a variety of metal ions are of current interest either because of their usefulness in new processes of organic synthesis or as models for the study of certain biological reactions or systems.

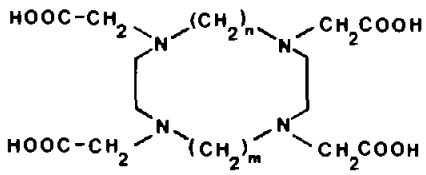
The effects of cavity size and of conformation on the spectroscopic and magnetic properties of the metal complexes, on the kinetics of complex formation and on the thermodynamic stability of the species formed have also been studied by many inorganic chemists but, so far, few analytical chemists have been concerned with such ligands as possible reagents for complexometric titrations.

Indeed, the macrocycles that have been studied are not suitable for this purpose, but derivatives of them might be of interest. For example, the polyaminocarboxylic acids derived from cyclic polyaza ligands should be relatively soluble in water, and should form quite stable complexes with metal ions.

Very few such derivatives have been reported in the literature; references have been found to a dipropionic acid derivative of a diazatetraoxa macrocycle,[1] to the tetra-acetic acid derivatives of three tetra-aza macrocycles[2] and to the tetra-acetic acid derivative of a dimethyltetra-aza macrocycle.[3]

In the first two cases, the authors were indeed concerned with the determination of stability constants of alkaline-earth and transition metal complexes; the third case, however, is a preparative and spectroscopic study of the cobalt, nickel, copper and zinc species formed with the particular ligand chosen.

The dipropionic acid derivative mentioned above[1] forms only very weak complexes and is not of special analytical interest; in contrast, the tetra-acetic acid derivatives of tetra-aza macrocycles synthesized by Stetter and Frank[2] form very stable complexes and one of them (**a**) was reported to form the most stable Ca^{2+} complex in aqueous solution known to date. For a second ligand of this group (**b**) the stability constant of the strontium complex was reported[2] to be higher than that of the calcium complex by a factor of almost 10^4, which would be a quite exceptional result for classical non-cyclic complexones, but possible, at least in principle, for derivatives of macrocycles with the correct cavity size. Stetter and Frank reported later[2a] that they were unable to confirm their unusual initial results. On the other hand, the stability constants of the complexes formed with the transition-metal ions are high but not exceptional.



a $m = 2$, $n = 2$:
1,4,7,10-tetra-azacyclododecane-*N*,*N*′,*N*″,*N*‴-tetra-acetic acid ([12]ane N_4·4ac) or cDOTA

b $m = 2$, $n = 3$:
1,4,7,10-tetra-azacyclotridecane-*N*,*N*′,*N*″,*N*‴-tetra-acetic acid ([13]ane N_4·4ac) or cTRITA

c $m = 3$, $n = 3$:
1,4,8,11-tetra-azacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetra-acetic acid ([14]ane N_4·4ac) or cTETA

These reports led us to undertake the synthesis of the ligands to allow more detailed study of the species formed in aqueous solution, the thermodynamic data for the reactions with metal ions and their suitability as reagents for complexometric titration. In the present paper we report the synthesis and analysis of the ligands, and give values for the stability constants of the MH_2L, MHL and ML species formed with various metal ions. Our values differ, sometimes quite considerably, from those reported by Stetter and Frank;[2] in particular, no inversion of the normal

order of stability of the calcium and strontium complexes of **b** was found.

The trend for the three cyclic complexones is the same as for the non-cyclic classical ones.

EXPERIMENTAL

Synthesis and characterization of the ligands

The cyclic tetra-azatetra-acetic acids were prepared by reaction of the corresponding cyclic amines with chloroacetic acid in aqueous alkaline solution. The cyclic tetramines [12]ane N_4 and [13]ane N_4 were synthesized as described in the literature;[4,5] [14]ane N_4 was obtained from a commercial source (Strem Chemicals). The method of preparation of the cyclic tetramines involved the condensation of a linear tosylated amine with a tosylated diol at 100–120°, in dry dimethylformamide as solvent. Tosylation of the amines and of the diols was done as described in the literature,[6,7] but the yield from the diols, particularly from 1,3-propanediol, was rather low (40–60%).

Hydrolysis of the tosyl groups in the cyclic tetramines was better achieved by refluxing with a mixture of glacial acetic acid with 48% hydrobromic acid (9:16 v/v) for 48–60 hr.

The condensation of the amines with chloroacetic acid offered no difficulties but the pH had to be kept below 10 and the temperature between 40 and 60°. The reaction mixtures were acidified to pH 2 with hydrochloric acid, and the products (cDOTA and cTETA) crystallized as small bright colourless needles on standing overnight in the refrigerator; the crystallization of cTRITA required the previous separation of the potassium chloride present.

All products were recrystallized from water. Both cDOTA and cTRITA crystallized with two moles of potassium chloride per mole of product and recrystallization did not change their composition. cTETA was obtained free from potassium chloride.

All intermediate and final products were characterized by elemental analysis, melting points, infrared and/or NMR spectra, and by the titration curves in the case of the tetra-acetic acids.

cDOTA (with 2 KCl): m.p. 298° (dec.); m.w. (titration) 553.5. Found: C 34.7%, H 5.4%, N 10.3%; required: C 34.70%, H 5.06%, N 10.12%. Proton NMR spectrum (solvent D_2O, reference DTSS, pD = 4.50), Fig. 1(*a*): δ 3.683 (8 H, acetate groups), δ 3.281 (16 αCH_2 ring protons).

cTRITA (with 2KCl): m.p. 242° (dec.); m.w. (titration) 567.6. Found: C 36.1%, H 5.6%, N 9.9%; required: C 35.97%, H 5.33%, N 9.87%. Proton NMR spectrum (solvent D_2O, reference DTSS, pD = 3.05), Fig. 1(*b*): δ 3.71, 3.67 (2 singlets, 8 H, acetate groups), δ 3.33 (multiplet, 16 αCH_2 ring protons), δ 2.05 (quintuplet, 2 βCH_2 ring protons).

cTETA: m.p. 313° (dec.); m.w. (titration) 432.5. Found C 49.7%, H 7.3%, N 12.9%; required C 50.00%, H 7.40%, N 13.00%. Proton NMR spectrum (solvent D_2O, reference DTSS, pD = 3.40), Fig. 1(*c*): δ 3.475 (8 H, acetate groups a), δ 3.210 (singlet, 8 αCH_2 ring protons b), δ 3.142 (triplet, 8 αCH_2 ring protons c), δ 1.924 (quintuplet, 4 βCH_2 ring protons d).

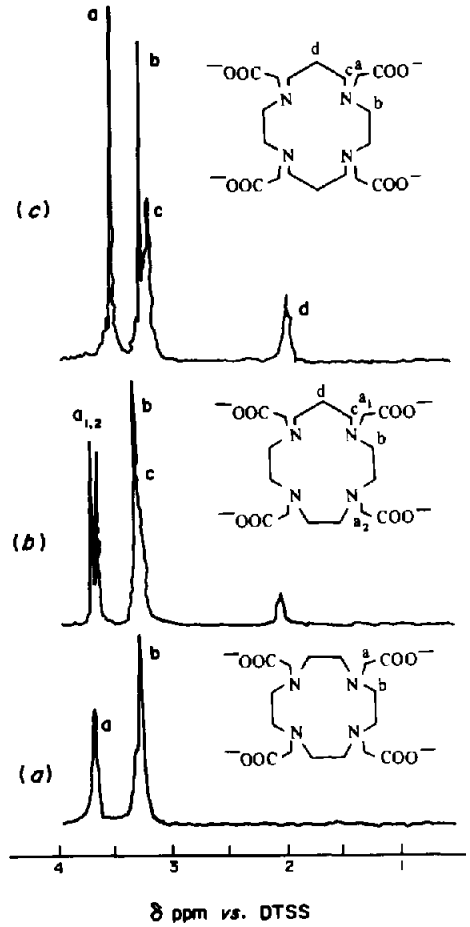


Fig. 1. Proton NMR spectra of (*a*) cDOTA at pD 4.5; (*b*) cTRITA at pD 3.05; (*c*) cTETA at pD 3.40.

Reagents

Metal salts. Metal nitrates of analytical reagent grade were used and solutions were prepared in demineralized water and standardized by EDTA titration or gravimetry (Be).

The ionic strength was adjusted with solutions of potassium or tetramethylammonium nitrate (prepared from tetramethylammonium hydroxide and nitric acid and recrystallized twice from 80% ethanol).

Carbonate-free potassium and tetramethylammonium hydroxides. Carbonate-free solutions of these titrants were prepared according to Schwarzenbach and Biederman,[8] under purified nitrogen. The solutions (*ca.* 0.050*M*) were standardized by titration with 0.01*M* hydrochloric acid. Carbonate was tested for regularly[9] and the solutions were discarded when the concentration reached 0.5% of the hydroxide concentration.

Potentiometric titrations

The experimental set-up has been described previously;[10] a Radiometer pHM 4 measuring instrument was used together with a Radiometer G 202 B glass electrode and a K 401 saturated calomel reference. The temperature was controlled at 25.0 ± 0.1° by circulating water through the jacketed titration cell. The ionic strength was kept to 0.10*M* by use of potassium nitrate or tetramethylammonium nitrate as background salts; the ionic product of water in these media was taken as 1.68×10^{-14}.[10]

The glass electrode was calibrated in terms of $[H^+]$ by titrating solutions of hydrochloric acid and potassium hydroxide of known concentrations and correlating the mV readings with calculated values of $[H^+]$. The $[H^+]$-dependent junction potentials were found to be negligible (from Gran plots[9]) and the correlation between measured e.m.f. and calculated $[H^+]$ was strictly represented by $E = E^{0\prime} + Q \log [H^+]$ for both the acid and alkaline zones, with slightly different values of $E^{0\prime}$. In each zone the relevant value of $E^{0\prime}$ was used; in intermediate pH ranges (4.5–8.5) an average $E^{0\prime}$ value was adopted. With this procedure experimental and calculated titration curves were completely superimposable.

To obtain the titration curves of the complexones, 50.0 ml of approximately $10^{-3}M$ solutions of the ligands, 5.0 ml of demineralized water and 5.0 ml of 1.200M potassium nitrate or tetramethylammonium nitrate were titrated with 0.050M carbonate-free potassium hydroxide or tetramethylammonium hydroxide. To obtain the titration curves of the complexones in the presence of metal ions, 5.0 ml of 1.00×10^{-2} or $2.00 \times 10^{-2}M$ solutions of the metal salts were added instead of the demineralized water.

Other measurements

NMR spectra were recorded with a 100-MHz Jeol JNM 100 PTF spectrometer coupled to a Jeol 980 A computer. When deuterated chloroform or acetone was used as solvent, tetramethylsilane (TMS) was the reference compound; when deuterium oxide was the solvent, the reference compound was the sodium salt of 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid (DTSS).

Melting points were determined with a Reichert-Thermovar instrument provided with a microscope, and are uncorrected.

Elemental analyses were done with a Perkin-Elmer 240 Elemental Analyzer.

Calculation of the stability constants

The stability constants of the various species formed were obtained from the experimental data with the aid of the program MINIQUAD[11,12] and an IBM 360 computer. The program uses a non-linear least-squares method to optimize data and requires previous knowledge of approximate values for the constants.

Suitable approximate values for the constants were obtained by use of simpler programs based on methods of calculation previously described in other publications from our laboratory.[10] The values were improved by comparison of the experimental titration curves of the complexones in the presence of the various metal ions with calculated titration curves for values of stability constants close to the estimated values, until a satisfactory superimposition of the curves was achieved. This was done on a 2200WANG computer coupled to a 2212 plotter. The β values for the best superimposition were then refined by MINIQUAD. In the present work, the values selected were those for which the calculated relative standard deviation was less than 10%, the "fitting index" R was less than 0.003 and the confidence level was higher than 95% for 6 degrees of freedom, following the procedure of other authors.[13,14]

The standard deviations quoted refer to calculation from data obtained in one experiment; however, the logarithmic values obtained from a series of titrations performed on different occasions were differed by not more than ± 0.05 from those presented, even in the most unfavourable cases.

RESULTS AND DISCUSSION

Titration curves for the three complexones studied, alone and in the presence of several metal ions, are shown in Figs 2, 3 and 4.

As can be seen, all the ligands are tetrabasic, with two pKs of the order of 3–4 and two of the order of 10–11. The values refined by MINIQUAD are presented in Table 1 together with those determined by Stetter and Frank[2] at 20.0° and $\mu = 0.1$ (potassium chloride) and by Desreux *et al.*[15] at 25.0° and $\mu = 1.0$ (sodium chloride). Our values were determined at $25.0 \pm 0.1°$ and $\mu = 0.10$ (tetramethylammonium nitrate or potassium nitrate); both sets of results are presented to allow comparisons.

Agreement between the several sets of values is fair, taking into account the different experimental conditions in which they were determined. The major differences are found in the values of pK_4 for cDOTA and cTETA and of pK_3 for cTETA.

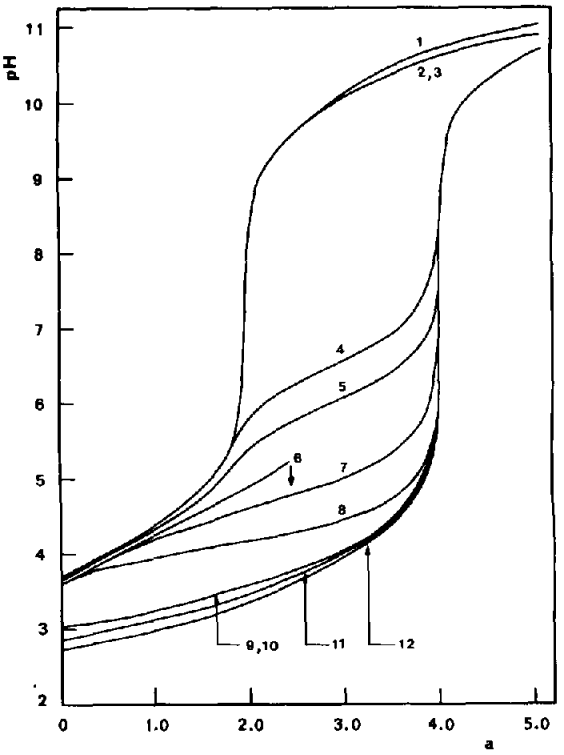


Fig. 2. Titration curves for cDOTA alone and in the presence of metal ions in 1:1 ratio. $T = 25.0 \pm 0.1°C$. $\mu = 0.1M$ [$(CH_3)_4NNO_3$]. 1—cDOTA alone, $10^{-3}M$, and with 2,3—Na^+ or Li^+; 4—Mg^{2+}; 5—Ba^{2+}; 6—Be^{2+}; 7—Sr^{2+}; 8—Ca^{2+}; 9, 10—Co^{2+} or Ni^{2+}; 11—Zn^{2+}; 12—Cu^{2+}.

The discrepancy in the case of cDOTA may be explained by the fact that we used non-complexing tetramethylammonium nitrate medium and Desreux *et al.* used 1M sodium chloride. Although they made a correction for the formation of a sodium complex this may have been insufficient. In our determinations we obtained a value for the stability constant of this sodium complex that was higher than their guessed value, and if our value is adopted, the constants of Desreux *et al.* come close to ours. The values are already close for 0.1M potassium nitrate medium (but potassium also forms a complex with cDOTA).

The discrepancy in the case of cTETA may also be explained in the same way, but now the correction made by Desreux *et al.* may be too high. We found for the sodium complex of this ligand log $K = 0.4 \pm 0.1$ (with a 100:1 molar ratio of sodium to ligand) and if this value is used instead of log $K = 1.64$, again closer agreement between their value and ours is obtained.

It seems, from the available data, that the first two protons ionize from two *trans* acetic acid moieties and the last two from protonated *trans* nitrogen atoms, but hydrogen-bonding to pairs of nitrogen atoms is not excluded.[15,16]

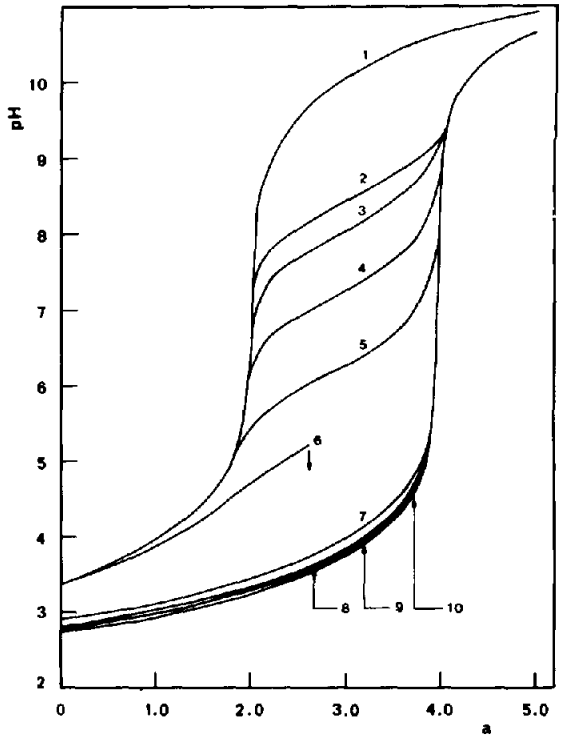


Fig. 3. Titration curves for cTRITA alone and in the presence of metal ions in 1:1 ratio. $T = 25.0 \pm 0.1$°C. $\mu = 0.1M$ (KNO_3). 1—cTRITA alone, $10^{-3}M$, and with 2—Mg^{2+}; 3—Ba^{2+}; 4—Sr^{2+}; 5—Ca^{2+}; 6 Be^{2+}; 7—Zn^{2+}; 8—Co^{2+}; 9—Ni^{2+}; 10—Cu^{2+}.

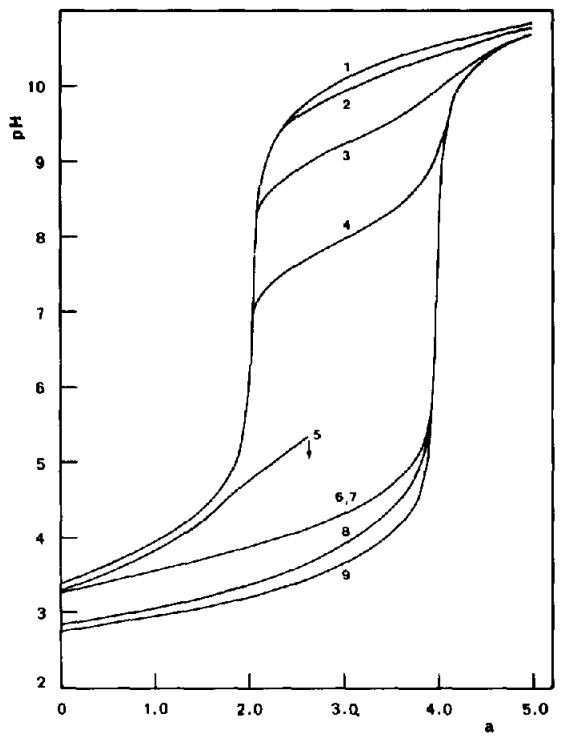


Fig. 4. Titration curves for cTETA alone and in the presence of metal ions in 1:1 ratio. $T = 25.0 \pm 0.1$ C. $\mu = 0.1M$ (KNO_3). 1—cTETA alone, $10^{-3}M$, and with 2—Ba^{2+}; 3—Sr^{2+}; 4—Ca^{2+}; 5—Be^{2+}; 6,7—Co^{2+} or Zn^{2+}; 8—Ni^{2+}; 9—Cu^{2+}.

Regarding the formation of the metal complexes, two observations immediately arise from Figs 2–4. First, the stabilities of the complexes of the alkaline-earth metals decrease as the size of the cavity of the ligands increases. Secondly, there is no inversion of the order of stability of the calcium and strontium complexes of cTRITA, contrary to the original report by Stetter and Frank,[2] and log K_{CaL} > log K_{SrL} for

Table 1. Ionization constants of cyclic tetra-azatetra-acetic acids

Constant	[12]ane N_4·4ac (cDOTA)		[13]ane N_4·4ac (cTRITA)		[14]ane N_4·4ac (cTETA)	
pK_1	4.130 ± 0.003	(*a*)				
	4.36 ± 0.01	(*b*)	3.323 ± 0.004	(*b*)	3.347 ± 0.004	(*b*)
	4.41	(*c*)	3.28	(*c*)	3.46	
	4.18 ± 0.03	(*d*)	—		3.38 ± 0.04	(*d*)
pK_2	4.548 ± 0.003	(*a*)				
	4.37 ± 0.01	(*b*)	4.157 ± 0.006	(*b*)	4.091 ± 0.004	(*b*)
	4.54	(*c*)	4.59	(*c*)	4.31	(*c*)
	4.24 ± 0.02	(*d*)	—		4.05 ± 0.02	(*d*)
pK_3	9.680 ± 0.001	(*a*)				
	9.750 ± 0.002	(*b*)	9.734 ± 0.002	(*b*)	10.136 ± 0.002	(*b*)
	9.73	(*c*)	9.18	(*c*)	9.75	(*c*)
	9.23 ± 0.02	(*d*)	—		10.18 ± 0.02	(*d*)
pK_4	12.09 ± 0.04	(*a*)				
	11.22 ± 0.03	(*b*)	11.35 ± 0.05	(*b*)	10.682 ± 0.005	(*b*)
	11.36	(*c*)	11.22	(*c*)	11.07	(*c*)
	11.08 ± 0.07	(*d*)	—		11.56 ± 0.02	(*d*)

(*a*) Present work: $T = 25.0 \pm 0.1$°C; $\mu = 0.10M$ [$(CH_3)_4NNO_3$].
(*b*) Present work: $T = 25.0 \pm 0.1$°C; $\mu = 0.10M$ (KNO_3).
(*c*) Stetter and Frank:[2] $T = 20$°C, $\mu = 0.1M$ (KCl).
(*d*) Desreux *et al.*:[15] $T = 25.0$°C, $\mu = 1.0M$ (NaCl), corrected.

Table 2. Stability constants (log K) of metal complexes of cDOTA, cTRITA and cTETA

Metal ions	Species	cDOTA		cTRITA		cTETA	
		(*a*)	(*c*)	(*b*)	(*c*)	(*b*)	(*c*)
Li^+	ML	4.32 ± 0.03					
Na^+	ML	4.38 ± 0.02	2.52 ± 0.07(*d*)			0.4 ± 0.1	1.64 ± 0.02(*d*)
K^+	ML	1.64 ± 0.05				negligible	
Be^{2+}	MH_2L	2.26 ± 0.03		2.41 ± 0.05		2.47 ± 0.05	
	MHL	7.68 ± 0.04		7.58 ± 0.03		7.82 ± 0.05	
	ML	13.64 ± 0.01		13.36 ± 0.01		13.38 ± 0.03	
Mg^{2+}	MHL	3.917 ± 0.005		2.781 ± 0.005		1.743 ± 0.005	
	ML	11.915 ± 0.005	11.03	7.620 ± 0.004	6.36	1.967 ± 0.001	3.02
Ca^{2+}	MH_2L	3.11 ± 0.06					
	MHL	8.68 ± 0.04		5.451 ± 0.005		5.09 ± 0.05	
	ML	17.226 ± 0.005	15.85	12.085 ± 0.003	8.06 10.4(*e*)	8.322 ± 0.004	9.48
Sr^{2+}	MH_2L	2.28 ± 0.03					
	MHL	7.80 ± 0.05		3.688 ± 0.005		3.987 ± 0.005	
	ML	15.22 ± 0.02	12.80	9.995 ± 0.002	11.70 8.5(*e*)	5.728 ± 0.005	6.15
Ba^{2+}	MHL	6.415 ± 0.005		3.641 ± 0.005		2.519 ± 0.005	
	ML	12.873 ± 0.002	11.3(*e*)	8.342 ± 0.002	7.24	3.854 ± 0.004	4.32
Co^{2+}	MH_2L	6.05 ± 0.03		6.17 ± 0.02		2.63 ± 0.07	
	MHL	12.08 ± 0.02		12.73 ± 0.01		9.949 ± 0.005	
	ML	20.17 ± 0.02	18.42	20.10 ± 0.01	14.98	16.557 ± 0.004	15.00
Cu^{2+}	MH_2L	8.316 ± 0.008		7.21 ± 0.04		7.36 ± 0.03	
	MH	14.416 ± 0.007		14.03 ± 0.04		14.60 ± 0.02	
	ML	22.21 ± 0.01	19.06	21.53 ± 0.04	17.29	21.60 ± 0.03	18.60
Ni^{2+}	MH_2L	6.49 ± 0.03		7.175 ± 0.006		6.52 ± 0.03	
	MH	11.45 ± 0.09		13.639 ± 0.006		13.35 ± 0.02	
	ML	20.03 ± 0.03	17.25	20.821 ± 0.006	15.75	19.91 ± 0.03	15.26
Zn^{2+}	MH_2L	7.01 ± 0.01		5.56 ± 0.01			
	MH	13.145 ± 0.006		12.138 ± 0.007		9.84 ± 0.03	
	ML	21.049 ± 0.009	18.90	19.42 ± 0.02	14.42	16.27 ± 0.01	15.81

(*a*) Present work: $T = 25.0 \pm 0.1$ °C; $\mu = 0.10M$ [$(CH_3)_4NNO_3$].
(*b*) Present work: $T = 25.0 \pm 0.1$°C; $\mu = 0.10M$ (KNO_3).
(*c*) Stetter and Frank:[2] $T = 20$°C, $\mu = 0.1M$ (KCl).
(*d*) Desreux *et al.*:[15] $T = 25.0$°C; $\mu = 1.0M$ (NaCl).
(*e*) Stetter and Frank.[2a]

Table 3. Comparison of stability constants (log K) for some alkali, alkaline-earth and transition-metal complexes of cDOTA, ethylenediamine-*N*,*N*'-diacetic acid (EDDA), ethylenediaminetetra-acetic acid (EDTA) and *trans*-1,2-diaminecyclohexanetetra-acetic acid (DCTA) $T = 25.0°C$, $\mu = 0.1$ (potassium nitrate or tetramethylammonium nitrate)

Metal ion	cDOTA	EDDA	EDTA	DCTA
Li^+	4.32	..	2.85*	4.13
Na^+	4.38	—	1.79*	2.70
Be^{2+}	13.64	—		
Mg^{2+}	11.92	3.95	8.83	11.07
Ca^{2+}	17.23	—	10.61	13.15
Sr^{2+}	15.22	—	8.68	10.58
Ba^{2+}	12.87	—	7.80	8.6
Co^{2+}	20.17	11.25	16.26	19.58
Ni^{2+}	20.03	13.65	18.52	20.2
Cu^{2+}	22.21	16.2	18.70	21.92
Zn^{2+}	21.05	11.22	16.44	19.35
pK_1	4.13	6.53	2.0	2.4
pK_2	4.55	9.59	2.61	3.5
pK_3	9.68		6.11	6.12
pK_4	12.09		10.17	12.3

*$\mu = 0.3M$ CsCl.

these cyclic complexones, as with the classical non-cyclic ones, and as later found by Stetter and Frank.[2a]

For the transition metals the situation is not so clearly defined; from the titration curves the only conclusion which can be reached is that the cobalt and zinc complexes of cTETA are markedly less stable than those of nickel and copper, in contrast with what happens with the other two ligands.

Values for the stability constants of the various species identified in 1:1 mixtures of the metal ions and of the ligands* are given in Table 2, along with the corresponding values for some ML species.[2,15] These values confirm the qualitative observations made above but differ quite considerably from those reported by Stetter and Frank,[2] which are generally lower than ours by a factor of 10^2–10^3.

Since these authors did not consider MH_2L and MHL species, their values might be expected to be higher rather than lower, so we see no obvious reason for the difference. It should, however, be stressed that our values were found to be reproducible from titration to titration, and to vary little with differences in the nature of the ionic medium. To obtain such reproducibility required great care, particularly with the transition metals which gave rather slow reaction. Usually, the mixtures of metal and ligand were left to equilibrate for 2 hr before the titration, and in the case of Ni^{2+} and cDOTA it took 24 hr to reach stable pH values. After each addition of titrant, the reading was not taken until the pH was stable, and this took 1–3 min, sometimes more, particularly in the case of nickel and cobalt complexes. With cTRITA, the reactions were generally faster.

For the alkali-metal complexes of cDOTA and for the alkaline-earth metal complexes of all ligands the stability follows the order of the ionic potential, except for magnesium and beryllium, which behave "abnormally", as usual, because of their small radii.

Beryllium forms quite stable complexes with the three complexones and, at first sight, this seems of interest for the development of complexometric methods for this metal, but the side-reaction of beryllium with hydroxide prevents this (see below).

For the transition metals, the Irving–Williams order of stability is observed; it is interesting to note, however, that the difference in stability between the complexes of the same metals with cDOTA, cTRITA and even cTETA (in the cases of nickel and copper) is rather small, unlike the behaviour with the alkaline-earth metals and also with the complexes of the transition metals with the parent cyclic amines (judging from the few values available for copper[17]) and with the corresponding non-cyclic amines.

This seems to imply that for the alkaline-earth metals the size of the internal cavity of the ligand and its conformation are critical and that all nitrogen atoms may be involved in bonding to the metal, as postulated for the complexes of rare-earths with cDOTA.[18]

In contrast, the transition metals do not seem to coordinate to all the nitrogen atoms of the ligands. This conclusion was also reached by Häflinger and Kaden on the basis of a spectral study of the complexes formed by cobalt, nickel, copper and zinc with meso-5,12-dimethyl-1,4,8,11-tetra-azacyclotetradecane-*N*,*N*',*N*'',*N*'''-tetra-acetic acid, which is similar to cTETA. A comparison with the values of stability constants for the complexes of a few classical non-

*2:1 ratios were also used but the partial formation constants of the 2:1 complexes were too low to be calculated with precision in these conditions; however, in the case of Ni^{2+}–cTETA we obtained log $\beta_{M_2L} = 23.01 \pm 0.05$.

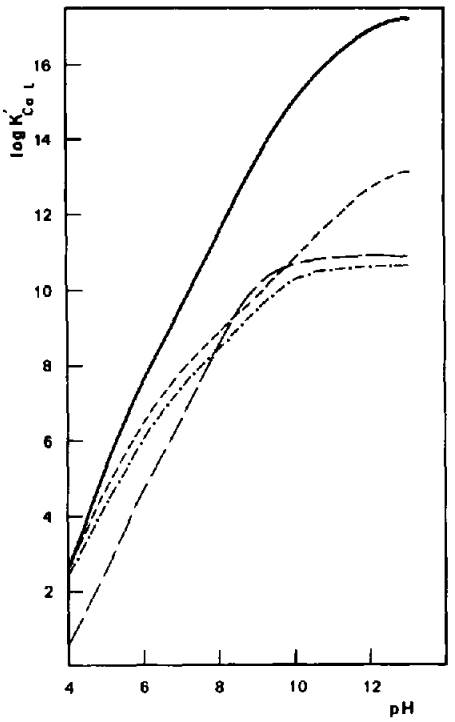


Fig. 5. Variation of the conditional stability constants (log K'_{CaL}) of the calcium complexes of cDOTA (——), *trans*-DCTA (---), EGTA (--) and EDTA (—·—) with pH.

cyclic complexones (Table 3) provides some support for this hypothesis.

Although these complexones are not directly comparable with those studied in the present work a few conclusions can be extracted from the values presented in this table.

1. Co-ordination by one strongly basic nitrogen atom (pK ~ 10), one nitrogen atom of intermediate basicity (pK ~ 6) and two carboxylate groups, as in ethylenediaminediacetic acid (EDDA), gives complexes with stability constants lower than those determined for the corresponding cDOTA complexes by a factor of about 10^6–10^{10}, but that are not too different from those determined for the cDOTA MHL complexes.

2. Co-ordination by a ligand such as EDTA, *i.e.*, EDDA plus two carboxylate groups, gives transition-metal complexes with stability constants lower by a factor of up to 10^4 than those determined for the cyclic complexones.

3. When the basicity of one of the nitrogen atoms is increased as in DCTA (pK_4 ~ 12.3), the complexes of the transition metals compare in stability with those of the cyclic complexones but the complexes of the alkaline-earth metals have stability constants that are 4–6 orders of magnitude below the values found for those of the strongest cyclic complexone, cDOTA.

Hence, it seems that for the transition metals the MHL complexes of the cyclic complexones may be like those of EDDA *i.e.*, co-ordinated to two nitrogen atoms and to two carboxylate groups. Their ML complexes require more ligand atoms to be co-ordinated and it is tempting to suggest that the two remaining carboxylate groups may be involved. In any case it is unlikely that the four nitrogen atoms can participate since then much higher stability constants would result (the log K values for the copper complexes of the cyclic amines are[17] about 25–29). The fact that the less stable complexes of the cyclic complexones form in preference to the more stable ones involving the four nitrogen atoms, as in the parent amines, may be the result of the combination of various factors (conformational, thermodynamic and kinetic) but at the present stage of our work it is not possible to advance a concrete soundly based explanation.

Our final comments refer to the possible use of the cyclic complexones, particularly cDOTA, as reagents for complexometric titrations. In Figs. 5 and 6 the logarithms of the conditional constants of the calcium and magnesium complexes of cDOTA, EDTA, EGTA and DCTA are plotted *vs.* pH. These show that cDOTA is indeed the most powerful ligand for both metal ions and that the selectivity ratio log K'_{CaL}/log K'_{MgL} is as good for cDOTA as for EGTA, at pH > 10 (and is better still for cTETA). These ligands could then be interesting alternatives for titrations of these ions.

For beryllium, the conditional constants of the complexes are too low (log K'_{BeL} < 3) even at the most favourable pH values, so none of the new ligands is suitable for beryllium determination.

For the transition metals, the cyclic complexones have no advantages over the classical non-cyclic ones such as EDTA or DCTA.

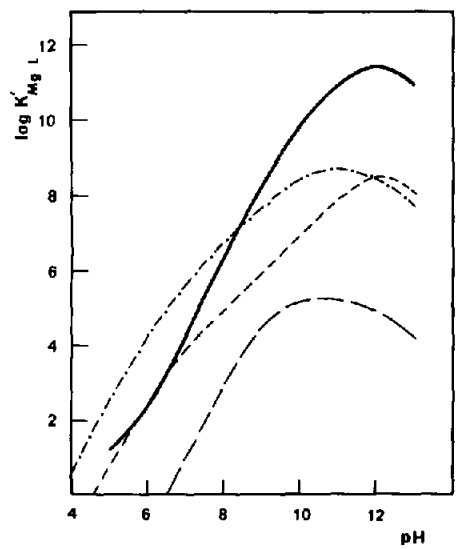


Fig. 6. Variation of the conditional stability constants (log K'_{MgL}) of the magnesium complexes of cDOTA (——), *trans*-DCTA (---), EGTA (--) and EDTA (—·—) with pH.

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SELENIUM IN ENVIRONMENTAL WATERS: DETERMINATION, SPECIATION AND CONCENTRATION LEVELS

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Summary—This article reviews the different methods used for the determination of selenium species in all types of environmental waters. Basic difficulties are discussed and the efficiency of the methods is explained in view of the sub-μg/l. concentration levels. Special attention is paid to preconcentration steps. Published data on speciation and concentration levels in various water samples are critically reviewed.

As the toxicological and physiological importance of selenium has become more and more evident[1–12] during the past twenty years, there has been an increasing interest in this essential element.

Deficiency of selenium results in selenium-responsive diseases in various animal species. Selenium also prevents several types of chemically induced cancer in animals.[4,5] Although its role in human health is still not fully understood and remains controversial, Shamberger[6–9] has pointed out that human cancer death rates are lower in countries where more selenium occurs in the environment and that human mortality from heart disease is also lower in the high-selenium areas. Since the initial observations that selenite prevents the injurious effects of cadmium on the testes[10] and of mercury(II) on the kidney and intestine,[11] there has been much work and speculation on the mechanisms of these antagonistic interactions, as summarized by Magos and Webb.[12]

As the difference between the essential and the toxic concentration level is rather narrow, precise knowledge of the selenium content in the environment and food products is mandatory. Hofsommer and Bielig[13] give a summary of methods for the determination of selenium in foodstuffs, the source that contributes most to the intake of selenium. Drinking water contributes only 1–6% to selenium uptake, according to Commins,[14] for typical concentrations and a water consumption of 3 l./day. Underwood,[15] however, calculated that the human intake of selenium ranges from 22 to 220 μg/day, with a mean value of 60 μg/day, in which case a daily intake of 2 litres of drinking water containing the EPA upper limit of selenium (10 μg/l.) would be responsible for a significant fraction of the total intake. Valentine *et al.*[16] screened a population in New Mexico, exposed to selenium from well waters having a selenium content ranging from 10 to as much as 3900 μg/l. Presumably these waters were contaminated by a uranium-mill tailing pond nearby. These various data stress the importance of determining selenium concentrations in all kinds of environmental waters, since these are frequently used as a source for drinking water.

All the publications discussed deal with aqueous solutions, although not all specifically refer to environmental waters. The different procedures for selenium determination in environmental water samples are critically reviewed below, with special attention to determination at the sub-μg/l. level.

Since there is much confusion about the definition of the terms "detection limit" and "sensitivity", users of the data should always check the definition applied. The preconcentration steps, mostly involving co-precipitation or complexation, are examined for interferences and basic difficulties. Since selenium is present at such a very low level, sampling and storing need special care.

Knowledge of the chemical speciation of trace metals in natural waters is essential to an understanding of the toxicity and bio-availability of these elements, so special attention is also paid to the content of the different forms of selenium in water samples. Selenium concentrations in the different types of water in various countries are compiled at the end of this article.

SAMPLING AND STORAGE

Progress in handling natural waters and analysing them for ultratrace amounts of metals has mainly been achieved by laboratory work under relatively favourable conditions. Unfortunately, samples from the sea or rivers often become heavily contaminated during sampling; this is a crucial step, the importance of which has not yet been fully realized by all investigators. Consequently many published data, some of them varying by several orders of magnitude, are associated with gross and mainly positive errors. Mart[17,18] has discussed some general and specific

contamination factors affecting the accuracy of heavy-metal analysis at the trace level, with particular reference to the determination of zinc, cadmium, lead, bismuth and copper. The pretreatment and cleaning procedures for sampling bottles and other laboratory ware, the sampling method, the filtration of the samples and their long-term storage can be adapted and taken into account in the determination of other trace elements, such as selenium. Numerous reports concerning adsorption effects in bottles have been published[19–28] but most of them result from experiments with trace elements in doubly distilled water. In analysis for selenium, not only adsorption losses but also volatility risks have to be taken into account. Shendrikar and West[29] investigated rates of selenium losses from aqueous solutions stored at different acidities (pH 7, pH 3.8 and in 0.5% v/v nitric acid) in various materials. The losses by adsorption from a 1-mg/l. selenium solution in Pyrex beakers in 15 days were 4, 1.5 and 1%, respectively. In flint-glass beakers, losses of about 5, 2.5 and 1.5%, respectively, occurred. Losses in polyethylene beakers were not greater than 8, 3 and 2%, respectively. These observations are useful but inadequate with respect to the preservation of environmental samples, for the following reasons.

(*a*) The selenium concentration of 1 mg/l. used was much higher than that found in most environmental samples, where concentrations lie at the μg/l. level.

(*b*) Only one type of water (probably distilled water) was used, whereas both pure synthetic solutions and contaminated natural waters should preferably be tested.

(*c*) The selenium oxidation was presumably Se(IV) and both Se(VI) and Se(IV) should be investigated, because both can be important in the natural environment.

(*d*) A test period of 15 days is inadequate for a major control study, which can require no less than three months from the time of preparation of samples to confirmatory analyses, packaging, shipping and final analyses by participating laboratories.

Cheam and Agemian[30] studied the stability of inorganic Se(IV) and Se(VI) species at levels of 1 and 10 μg/l. under various conditions of pH, type of water and type of container. Use of polyethylene containers and adjustment to pH 1.5 will provide optimum conditions of preservation for both distilled and natural water samples up to 125 days. Algal growth is detrimental to solution stability at natural pH values of pH 5.4–7.2, but adjustment to pH 1.5 with sulphuric acid successfully prevents this effect. Container size and temperature are also discussed. Storage of samples at 4° gives satisfactory stability but is less practicable. However, sulphuric acid interferes with some analytical methods, and if natural waters are to be tested it may be preferable to use unacidified natural water stored in polyethylene bottles at 4°. It was noted that Se(VI) is generally more stable than Se(IV) in aqueous solutions. An interlaboratory quality control study[31] used the preferred conditions of acidification of 0.02% v/v sulphuric acid and storage at room temperature in polyethylene bottles. Excellent recoveries over the selenium concentration range 0–1000 μg/l. confirmed the effectiveness of the proposed preservation method. Cutter[32] stated that acidification can be used for the preservation of water samples, but that it should not be excessive because the speciation can be changed. A sample spiked with Se(VI) and stored in 4*M* hydrochloric acid for 7 days showed 60% conversion into Se(IV). Sample storage in 1*M* hydrochloric acid preserves the Se(VI) as well as the Se(IV). Freezing the samples avoids the introduction of contaminants and, in addition, the frozen samples can be analysed for volatile compounds—the methyl species, even in airtight containers, are completely lost from liquid samples within a day.

Massée *et al.*[33] studied sorption losses for selenium, arsenic, cadmium, silver and zinc at the $10^{-7}M$ level from distilled water and artificial sea-water, during storage in containers made of borosilicate glass, high-pressure polyethylene or Teflon. The effects of pH and storage time were studied, and special attention was paid to the effect of the ratio of inner container surface area to sample volume. For Se(IV) at the 8-μg/l. level, losses were insignificant in all three container materials, irrespective of the water matrix composition. This is probably because the selenium is present as oxy-acids, which are partly dissociated, and the anions do not adhere to the container walls. Schutz and Turekian[34] observed no losses of selenium onto borosilicate glass from sea-water. Glickstein[35] used radioisotope tracers (Na_2SeO_3) to study the volatilization and surface adsorption losses of nanogram quantities of selenium in sea-water. For concentrations below ~0.02 μg/l., serious losses were observed at pH 8.1 after 40 hr. Thompson *et al.*[36] spiked acidified river water with selenium at the 50-μg/l. level. They observed losses starting after 16 days of storage at 20°. Robberecht and Van Grieken,[37] using different tracer experiments, showed that losses of both Se(VI) and Se(IV) from simulated natural waters onto Pyrex and polyethylene containers were negligible, even after prolonged storage. Elemental selenium, however, appeared to be rapidly lost on both materials. Measures and Burton[38] compared selenium values resulting from sea-water samples analysed at sea with those obtained in the laboratory. They found that samples acidified to pH 2 with hydrochloric acid and stored either in glass or linear polyethylene containers, showed no significant change in concentration of either oxidation state over a period of 4.5 months. This is in good agreement with earlier results.[30,38–40]

Reamer *et al.*[41] studied selenium adsorption in several hydride generation systems and construction materials by use of ^{75}Se as radiotracer (100 μg/l.). Polypropylene, two types of Teflon, and both silaned and unsilaned glass were evaluated. Glass and polypropylene cause the highest adsorption losses (23–32%) and silaned glass the lowest (2–8%). The adsorption decreased as a function of the number of

reductions done and adsorbed selenide was leached from the material only by 2*N* nitric acid.

The various literature data indicate that adsorption losses of selenium will depend on various factors such as element concentration, chemical form, container material, contact time, pH, salinity, suspended matter and micro-organisms. Reduction of contact time and acidification with a strong acid will generally prevent or minimize the losses. Acidification, however, can change the initial composition of the aqueous sample, but storage of the unacidified water at 4° in pre-cleaned polyethylene bottles can prevent this problem. For the study of volatile organoselenium compounds, freezing the samples is the best method of storage.

DETERMINATION PROCEDURES

Spectrophotometric analysis

This was probably the most cited technique in the past, but it was not always sensitive enough for determination of selenium in trace amounts. An excellent review of methods and reagents is given by Shendrikar.[42]

Spectrophotometry. Osburn *et al.*[43] have described a simple method for the determination of 0.1-mg/l. Se(IV) in aqueous solutions, involving oxidation of hydroxylamine hydrochloride to nitrous acid by selenious acid followed by diazotation of sulphanilamide by the resulting nitrite and subsequent coupling of the diazonium salt with *N*-(1-naphthyl)ethylenediamine dihydrochloride. Interferences from $Cu(II)$ can be removed by ion-exchange. Any Se(VI) can be reduced to Se(IV) by heating the sample with 4*M* hydrochloric acid.

Campbell and Yahaya[44] determined microgram amounts of Se(IV) by measuring the decrease in absorbance of dithizone in carbon tetrachloride at 620 nm. Of several metals tested, only copper (at the 10-μg level) and iron (at the 100-μg level) interfered. High concentrations of nitric or perchloric acid caused low results.

Pal and Das[45] determined Se(IV) in the range 0.05–100 mg/l. by measuring the yellow acetothioacetanilide complex at 400 nm. Silver, platinum, palladium and gold interfered.

Spectrophotometric methods based on catalytic reactions are generally much more sensitive than those based on stoichiometric reactions.[46] Most catalytic methods involve oxidation–reduction reactions in which the catalyst, usually a multivalent ion, changes its oxidation state.[47] Feigl and West[48] proposed a very sensitive qualitative test for selenium, based on the catalytic reduction of Methylene Blue by sodium sulphide. The reaction was later developed for the determination of low-levels of selenium.[49–52]

A similar catalytic method[50] was modified by Tzeng and Zeitlin[53] for determination of selenium as selenite in sea-water, after preconcentration by adsorption on a colloid which was then floated. They stated that their method is temperature-sensitive and that this can contribute some uncertainties to the results.

Kawashima and Tanaka[54] proposed determination of Se(IV) based on its catalytic effect on the reduction of 1,4,6,11-tetra-azonaphthalene by hypophosphorous acid and attempts have been made[55] to use this procedure for Se(IV) in sea-water. When arylhydrazines are oxidized, then coupled with aromatic compounds, they produce intensely coloured azo dyes.[56] Kawashima *et al.*[57] found that, in the presence of chlorate, Se(IV) catalyses the oxidative coupling reaction of *p*-hydrazinobenzenesulphonic acid with 1-naphthylamine to produce an intensely coloured compound, and based on it a new catalytic method for small amounts of Se(IV). Kawashima *et al.*[58] replaced 1-naphthylamine by *m*-phenylenediamine. The sensitivity and reproducibility were improved by extracting the product into organic solvents. Under optimum conditions 0.12–0.5 μg of Se(IV) in 10 ml of extract could be determined. This corresponds to a working range of 8–33 μg/l. in water. A method based on the catalytic effect of selenium on the reduction of picrate by sodium sulphide is described by Diamandis and Hadjiioannou.[59] Selenium (3–30 μg) may be determined in 15 ml of solution with an average error of about 4%. The reaction can be followed spectrophotometrically, or by potentiometric monitoring with a picrate-selective electrode. The oxidation state of selenium is not mentioned, but the method is probably only sensitive to Se(IV).

Fluorimetry. Haste[60] and Haste and Gillis[61] proposed 3,3′-diaminobenzidine (DAB) as a sensitive fluorimetric reagent for selenium, since the piazselenol formed with Se(IV) shows a strong fluorescence. It has been used for fluorimetric determinations in many materials. In water analysis, the DAB reaction has been the most popular for determination of selenium. The method is sensitive but is lengthy and pH-dependent, and the reagent solutions are generally unstable and must be prepared daily. Strong oxidizing or reducing agents must be absent, so the addition of masking reagents[62] or EDTA[62–64] is necessary. Rossum and Villarruz[63] proposed two methods for the determination of selenium in water samples. In their distillation method, they claimed to separate selenium quantitatively from other elements by distillation of the tetrabromide. In the second method they first oxidized all selenium compounds to selenate with acid permanganate, then reduced selenate to selenite with warm 4*M* hydrochloric acid. The addition of EDTA eliminated negative interference from iron(III). Engberg[65] used both methods for determination of selenium in ground and river water. Chau and Riley[66] used co-precipitation with iron(III) hydroxide at pH 4–6 to concentrate Se(IV) from sea-water. Selenium was separated from the iron and other cations by ion-exchange and determined fluorimetrically with DAB. Selenium(VI) could be determined similarly after reduction to Se(IV) by boiling with sulphur di-

oxide, but the method was insufficiently sensitive for sea-water analysis. Morette and Divin[67] used the DAB method for the determination of Se(IV) and Se(VI) in ground waters in France. Selenate was reduced to selenite by boiling with hydrobromic acid and determined by difference. More recently, Desai and Paul[68] reported on rapid and accurate methods for simultaneous determination of Se(IV) and Se(VI). Selenium(IV) is determined by spectrophotometry after sequential complexation by diethyldithiocarbamate (DDTC) and solvent extraction by chloroform in the presence of perchlorate ions. Selenium(VI), which remains in the aqueous phase, is reduced to Se(IV) by 25% hydrobromic acid and determined either spectrophotometrically with DDTC or fluorimetrically after reaction with DAB.

2,3-Diaminonaphthalene (DAN) was first introduced as a fluorimetric reagent for Se(IV) by Parker and Harvey.[69] The fluorescence intensity and extractability into organic solvents from acidic media are superior to those obtained with DAB. Rankin[64] claimed to convert inorganic selenium into Se(VI) by oxidation with hydrogen peroxide. Hydrochloric acid was then used for the reduction to Se(IV) for reaction with DAN. Nitrite, which interferes, can be removed by oxidation to nitrate, and interfering metals masked by complexation. Verlinden and Deelstra[73] applied this technique to drinking water and found a recovery of only 48% for added selenium.

Raihle[70] established a technique for the determination of elemental selenium and Se(IV). Elemental selenium is oxidized to selenious acid by a bromine–bromide redox buffer, before addition of DAN; Se(VI) is not reduced to Se(IV) under these conditions. Hiraki *et al.*[71,72] and Sugimura *et al.*[39,74,75] used this technique to study selenium speciation in sea-water. These authors preconcentrated Se(IV) either by adsorption of the DDTC complex on a macroreticular resin[39,74] or by co-precipitation on ferric hydroxide. To remove Fe(III) before the fluorimetry, they first used ion-exchange separation[71] but more recently have used extraction with a capric acid–chloroform solution.[72] The Se(VI) in the filtrate is reduced to elemental selenium by boiling with hydrazine sulphate, then concentrated by co-precipitation on sodium tellurite.[39,72,74] The precipitate is redissolved and the DAN method is applied, with a recovery of around 95%. Organic selenium compounds are separated from the sea-water by adsorption on Amberlite XAD-2 resin.[75] Up to 45% of the selenium is present in organic form. Another DAN fluorimetric procedure has been described by Nazarenko and Kislova[76,77] for the specific determination of selenites, selenates and organoselenium compounds in water with the aid of various selective reducing and oxidizing reagents (Table 1). The limit of detection is 0.01 μg/l. The relative standard deviation is 23% for 0.05–1.0 μg/l. of selenium and 5% for 50–500 μg/l. No organoselenium compounds were found in well water or tap water. This is probably due to the fact that heating the acid solution hydrolyses the organoselenium species, which therefore appear as selenite.

Table 1 summarizes the commonly used preconcentration techniques for the fluorimetric determination (with DAN) of various selenium species in all types of water.

Turbidimetry and nephelometry. Some older publications deal with the turbidimetric or nephelometric de-

Table 1. Determination of various selenium species in natural water samples by fluorimetry of the DAN–selenite complex

Preconcentration and sample treatment method	Sample volume, *l.*	Species	Detection limit, μg/*l.*	Ref.
H_2O_2 oxidation, HCl reduction	0.05	Se (total)	0.2	64
HBr/Br_2 oxidation	0.01	Se(0) + Se(IV)	—	70
Co-precipitation with $Fe(OH)_3$, redissolution, removal of Fe(III)		Se(IV)	—	71
Co-precipitation with $Fe(OH)_3$, redissolution, capric acid $CHCl_3$ extraction, removal of Fe(III)	2–3	Se(IV)	—	72
Hydrazine sulphate reduction, co-precipitation with Te, redissolution, HCl reduction	2–5	Se(VI), Se(total)	—	39, 72, 74
Complexation with DDTC	5	Se(IV)	—	39, 74
Adsorption on XAD-2 resin, elution (with CH_3OH, or NH_4OH)	—	organic Se (neutral, basic or acidic)	—	75
HNO_3 heating	0.05–0.1	Se(IV)	0.01	76, 77
$HClO_4/HNO_3$ heating	0.05–0.1	Se(IV), organic Se	0.01	76, 77
$HClO_4/HNO_3$ heating, HCl reduction	0.05–0.1	Se(total)	0.01	76, 77

termination of selenium in water.[78–80] Fogg and Wilkinson[78] described a turbidimetric procedure for use after reduction of Se(VI) to Se(IV) by distillation with hydrobromic acid and bromine, and subsequent precipitation as elemental selenium with ascorbic acid. Destruction of organic material by heating with nitric acid–perchloric acid mixture is necessary for effluent analysis. Sherrat and Conchie[79] isolated selenium from interfering substances by ion-exchange, reduced Se(VI) to Se(IV) in 6*N* hydrochloric acid, then reduced Se(IV) to elemental selenium by ascorbic acid or hydrazine sulphate.

Atomic-absorption spectrometric methods

The use of atomic-absorption spectrometry (AAS) has increased during the last two decades to cover a large fraction of selenium determinations. Very recently, Verlinden *et al.*[81] have comprehensively reviewed AAS determination of selenium in various materials, including water.

Flame AAS (FAAS). This technique in its conventional form is not sensitive enough for direct determination of selenium in environmental water; indeed, even with optimized experimental conditions, the detection limits are at best[81] around 100 μg/l.

With the "tantalum sampling boat" technique it is possible[82] to reach a limit of detection of 10 μg/l. Use of the Delves cup or an electrically heated platinum loop cannot significantly enhance the sensitivity for selenium.[83]

Various chemical preconcentration methods have been proposed, mostly involving extraction with DDTC or ammonium pyrrolidinedithiocarbamate (APDC) into methyl isobutyl ketone (MIBK), but none has been used for selenium determination in environmental water by conventional FAAS. Only Guegueniat *et al.*[85] used a double preconcentration on manganese dioxide for trace element determination in coastal waters by FAAS; it was possible to detect 0.03 μg/l. of soluble selenium in 30-l. samples.

Electrochemical preconcentration of Se(IV) by reduction and deposition of elemental selenium on a platinum spiral was used by Lund and Bye[86] for air–acetylene FAAS. The electrolysis is done in the presence of hydrazine dihydrochloride to prevent the generation of chlorine, which would oxidize Se(IV) to the non-reducible Se(VI). A detection limit of 5 μg/l. and an electrolysis efficiency of 10% are obtained for a 25-ml sample and a 5-min electrolysis time. Recently Holen *et al.*[87] reached a detection limit of 0.5 μg/l. by electrothermal heating of the filament before its introduction into an argon–hydrogen (entrained air) flame.

In recent years, there has been a growing interest in the technique of hydride generation for the determination of selenium and a few other elements in various materials.[81] The volatile selenium hydride is formed by reduction of Se(IV) with reducing agents such as Zn–$SnCl_2$–KI, $TiCl_3$–Mg or in particular, $NaBH_4$. The hydride is flushed from the solution with argon, helium or nitrogen as carrier gas. The hydride evolved is either conveyed directly into the atomization system as it is generated, or is stored in a rubber balloon, a collapsible plastic bag or a pressurized chamber before transfer to the atomizer, or it is collected in a liquid-nitrogen trap and flushed into the flame as the cold trap is warmed. Hydride generation FAAS techniques for selenium are more sensitive by three orders of magnitude than nebulization FAAS, as the detection limit can be around 0.2 μg/l.[82] An additional advantage is that selenium is separated from the matrix before atomization, thus avoiding the interferences that occur when the conventional techniques are used.

The literature on hydride-generation FAAS for environmental water analysis is summarized in Table 2.

It appears that with regard to sensitivity, the sample pretreatment step is of paramount importance. For most uncontaminated environmental waters, only the last three methods listed in Table 2 will be suitable.

Electrothermal AAS (ETAAS). The flameless technique, ETAAS, in which a small sample aliquot is electrothermally atomized in a graphite furnace, is especially suitable for direct analysis of samples as it offers a high sensitivity (50 pg of Se is the detection limit), but it is not simple, nor free from interferences or volatility losses. Treatment of the graphite furnace, or addition of various metal ions, such as nickel,[101–107] cobalt,[106,108] molybdenum,[100] lanthanum,[103] copper,[109–111] mercury,[112] chromium,[113] proved to diminish the chemical interferences in water analysis, allows use of higher ashing temperatures without losses and results in a significantly enhanced sensitivity. Vickrey and Buren[103] reported that added metal solutions counteract signal depression by interfering elements not only because they reduce the volatility of selenium, but also because they modify the graphite furnace surface, leading to more efficient atom formation. Surface treatment can replace the matrix modifier—use of metal-coated or pyrolytic graphite-coated cuvettes[103] or graphite tubes lined with tantalum foil[114] yields optimal results.

Various detection limits have been reported for determination of selenium in water samples by ETAAS without preconcentration. Table 3 summarizes some published results. Pretreatment of the water samples and some slight modifications can give enhanced sensitivity. Henn[100] developed a method for the determination of selenium (1–50 μg/l.) in waters and industrial effluents which involved mineralization with hydrogen peroxide, drying, redissolution in hydrochloric acid, preliminary purification on cation-exchange resins, and ETAAS after addition of molybdenum salts (100 mg/l.) to increase the sensitivity, raise the ashing temperature and decrease the interferences. The same technique was used by Martin *et al.*[115] for determination of selenium in fresh water, waste, sediments and muds after digestion with nitric acid–hydrogen peroxide mixture and addition of nickel

Table 2. Determination of selenium in environmental waters by hydride-generation FAAS

Pretreatment	Reducing agent	Flame type	Detection limit, μg/l	Ref.
None	$NaBH_4$	Ar/H_2	50	88
None	$NaBH_4$	N_2/H_2	5	89
HCl, H_2SO_4	$KI/SnCl_2/Zn$	Ar/H_2	2	90
Digestion with acid $KMnO_4$ solutions, reduction by HCl	$KI/SnCl_2/Zn$	N_2/H_2	2	91
None	$TiCl_3/Mg$ or $NaBH_4$*	Air/C_2H_2	1.7	92
H_2SO_4, HNO_3, $K_2S_2O_8$	$NaBH_4$	Ar/H_2	1	93
Acid digestion	$NaBH_4$	N_2/H_2	0.6	94
None	$NaBH_4$*	Ar/H_2	0.15–0.25	95
$K_2S_2O_8$/HCl	$KI/SnCl_2/Al$	Ar/H_2	0.1	96
HCl heating (1 hr)	$NaBH_4$	Tube furnace	0.02	97
Adsorption of hydrides on $HgCl_2$- or $AgNO_3$-impregnated filters, re-extraction with HNO_3, $HClO_4$	$NaBH_4$ or $KI/SnCl_2/Zn$	Ar/H_2	0.02	98
Co-precipitation with $Fe(OH)_3$, flotation with air bubbles, scavenging, redissolution in HCl	$NaBH_4$	N_2/H_2	0.02	99

*The hydrides were collected in a collapsible bag. In all other cases there was direct injection.

nitrate to prevent losses during ashing. They obtained a detection limit of 0.02 μg/l. Use of the standard-additions method was mandatory. They were not able to analyse sea-water or brines since the suppressive effect of chloride on the sensitivity was not sufficiently decreased by the additive.

Chemical preconcentration has often been combined with ETAAS. Kamada *et al.*[109,110] proposed selective extraction of Se(IV) and differential determination of Se(IV) and Se(VI) by using DDTC, APDC and dithizone in organic solvents, combined with ETAAS. The sensitivity was 0.4 μg/l. for 1% absorption, and the relative standard deviation was 3% at 0.8 μg/l. Recently Kamada and Yamamoto[110] showed that copper(II) was useful in diminishing the volatility of selenium in ETAAS, but the detection limit was only slightly improved to 0.3 μg/l. Ohta and Suzuki[111] also tried to determine selenium in various water samples in the same way, but found no species present above the 0.4 μg/l. level.

Subramanian and Meranger[116] selected optimum conditions for the quantitative extraction of selenium, arsenic and antimony from aqueous solution with APDC into MIBK, and subsequent determination by ETAAS. Nitrite (75 μg/l.) completely suppressed the extraction of all three elements. The detection limit of 0.3 μg/l. for selenium, and the fact that low levels of nitrite suppressed the signal suggests that this technique is unlikely to be useful for the analysis of polluted waters. Nève *et al.*[104] recently proposed a simple evaporation step for water samples, with nitric acid added to minimize loss of selenium. The Se(IV) is selectively reacted with 4-chloro-1,2-diaminobenzene and the reaction product is detected by ETAAS after addition of nickel as matrix modifier. Total selenium is determined similarly after hydrogen peroxide digestion. The limit of detection is 20 ng/l. There are some questions concerning the speciation, since in this procedure for selenite, heating in the presence of concentrated nitric acid may result in partial hydrolysis of any organoselenium compounds and oxidation of selenide and elemental selenium to Se(IV).

Hydride generation in combination with ETAAS is probably the most studied method for determination of selenium in environmental water, yet it is plagued by numerous interferences.[96,117–133] Meyer *et al.*[131] found that these depend very strongly on the concentration of hydrochloric acid in the sample solution. Vijan and Leung[132] utilized the capability of hydrochloric acid to form chloro-complexes with common interfering heavy metals, such as copper and nickel, to eliminate practically all their suppressive effect. Bujmovic[120] found that organic interferences could be removed by irradiation with ultraviolet light.

The applications of hydride-generation ETAAS to water analysis are included in Table 3. Many authors lead the evolved selenium hydride directly into a carbon furnace but some authors[118,125,134] prefer a low-cost quartz tube. Others[117,118,135,136] collect the hydride, stripped from the solution, in a liquid-nitrogen trap. This tube is warmed to cause instantaneous transfer of the hydride into the atomizer. Because of the lesser dilution, better sensitivity can be obtained.

Various automated hydride-generation ETAAS

Table 3. Determination of selenium in environmental waters by ETAAS

Type of water	Pretreatment	Detection limit, μg/l.	Ref.
	(a) Direct analysis		
—	none	36	137
Waste	none (tantalum-foil lined tube)	8	114
Waste	none	3.1	112
Power-plant effluent	none	0.25	138
	(b) Digestion only		
Sewage	predigestion	10	139
Industrial effluent	predigestion + addition of Mo salts	1	100
Estuarine/fresh	addition of 0.2% Ni(II)	0.5	102
Waste/fresh	predigestion + addition of Ni(II)	0.02	115
	(c) Chemical preconcentration		
Waste/river/sea	APDC or DDTC/CCl_4 extraction	0.4	109
	DDTC/CCl_4 extraction + addition of Cu(II)	0.4	101
Waste/river/sea	APDC/MIBK extraction + addition of Cu(II)	0.3	110
Waste/river	APDC/MIBK extraction	0.3	116
Drinking/source/rain	evaporation piazselenol–toluene extraction + addition of Ni(II)	0.02	104
	(d) Hydride generation		
	$NaBH_4$ reduction	4	127
	$HCl/K_2S_2O_8$ digestion + $NaBH_4$ reduction	1	133
	H_2SO_4/HNO_3 digestion + $NaBH_4$ reduction	0.1	96
	$NaBH_4$ reduction	0.2	128
	$NaBH_4$ reduction	0.12	125, 134
Estuarine/drinking	$NaBH_4$ reduction	0.1	31, 129
Lake	$NaBH_4$ reduction	0.05	122, 123
—	$NaBH_4$ reduction	0.02	121
Sea/river	$NaBH_4$ reduction cold trap concentration	0.005	135
Sea/river	cold trap concentration GC/AAS detection for $(CH_3)_2$ Se and $(CH_3)_2Se_2$	0.0007	135

techniques have been described[96,121,133] allowing 30–70 water analyses per hour. In the procedure of Pyen and Fishman,[133] organic selenium-containing compounds are first decomposed by $HCl–K_2S_2O_8$ digestion. The Se(VI) produced, along with any inorganic selenium, is reduced to Se(IV) with stannous chloride and potassium iodide, and then to selenide with sodium borohydride. The hydrogen selenide is stripped from the solution with nitrogen, and then decomposed in a tube furnace at 800° placed in the optical path of the ETAAS unit. Goulden and Brooksbank[96] used digestion with either HNO_3/H_2SO_4 or acid persulphate to break down organoselenium compounds, and found hydride-generation ETAAS, with a 0.01-μg/l. detection limit, at least two orders of magnitude more sensitive than hydride-generation FAAS.

Cutter[135] proposed a complex method for determination of selenite, selenate, dimethyl selenide and dimethyl diselenide in natural waters. Volatile methyl species of selenium are removed from the sample by a stream of helium and inorganic forms of selenium are selectively reduced to the hydride with sodium borohydride and stripped. Both the organic selenides and the H_2Se are trapped in a liquid-nitrogen trap. The methyl species are separated by gas–liquid chromatography and measured in a quartz-tube furnace. No free methyl selenides were detected in lake, rain and sea-water samples, probably because they are present at levels below the detection limit (0.7 ng/l.) or because they have disappeared during sample storage. For Se(IV) and Se(VI), the detection limit is 5 ng/l. and the time of analysis is 15 and 30 min per sample, respectively. It was not possible to analyse chlorinated drinking water since chlorine interferes with the reduction step.

Atomic-fluorescence spectrometry

The method of determination of selenium, arsenic, antimony, and tellurium by sodium borohydride reduction with subsequent atomic-fluorescence determination of the evolved hydride was studied by Thompson.[140] The hydrides were passed directly into an argon–hydrogen (entrained air) flame. The atomic fluorescence was excited by use of modulated microwave sources and detected by a dispersive measuring system. The detection limit for selenium in 15 ml of sample was 0.06 μg/l. Nakahara *et al.*[141] used a non-dispersive system to compare the zinc and sodium borohydride reduction systems. The best attainable detection limits for selenium were 0.43 ng and 0.2 ng, respectively. With the proposed method, 1-μg/l. levels of selenium can be determined accurately. The presence of several elements, including other hydride-forming elements, in 1000-fold ratio to selenium caused a negative interference, but tellurium gave a positive interference. The method was applied to the determination of selenium in waste waters in the range 0.9–26 μg/l.

Emission spectroscopy

The use of the inductively coupled plasma (ICP) as an excitation source in optical atomic emission spectroscopy (AES) for single and multielement inorganic trace analysis of pollutants is becoming widespread. The recent availability of commercial instruments has stimulated use for the determination of metals and metalloids in diverse environmental materials ranging from airborne particulates and fly-ash to industrial effluents and sewage waters. The capabilities and limitations of ICP–AES in water pollution analysis have been reviewed by Barnes.[142] Winge *et al.*[143] evaluated the accuracy, precision and detection limits for ICP–AES water analyses. Pneumatic nebulization gives a detection limit of 30 40 μg/l. Only with ultrasonic nebulization is it possible to reach a limit of detection of 1 μg/l., which is well below the EPA-recommended levels for public water supplies and irrigation water, 10 and 20 μg/l. respectively.[144,145]

Thompson *et al.*[146] developed a method for simultaneous determination of selenium, antimony, bismuth, arsenic and tellurium by generation of their gaseous hydrides and introduction of these hydrides into an ICP-source. A detection limit of 0.8 μg/l. was obtained for selenium in aqueous solutions. The presence of certain metal ions in solution resulted in low recoveries of some of the elements, especially selenium and tellurium, but this could be overcome by co-precipitation separation on lanthanum hydroxide. With 200 ml of river water it was possible to reach a detection limit of 0.05 μg/l.[147] Goulden *et al.*[148] concentrated 100 ml of water to 10 ml and were able to detect selenium at the 0.03 μg/l. level. Koyama *et al.*[149] used ICP–AES for determination of organic selenium compounds after destruction with concentrated nitric acid. The method allowed determination at the 10–100 mg/l. selenium level. The method is thus of little use, since organoselenium species occur in natural waters only in the ng/l. range.

Fricke *et al.*[150] coupled a semi-automated hydride-generation device to a microwave-induced Ar/H_2 plasma (MIP) and used a chromatographic column to separate species causing spectral interference. In this way they separated the hydrides of selenium, arsenic, tin, germanium and antimony and, with a 20-ml sample, obtained a limit of detection of 1.25 μg/l.

Belcher *et al.*[151,152] have proposed methods for the determination of selenium, based on molecular emission cavity analysis (MECA). Kouimtzis *et al.*[153] elaborated this technique for the determination of the element in water samples. Selenium is first co-precipitated with ferric hydroxide. After dissolution of the precipitate in hydrochloric acid, Se(IV) is reduced by hydroxylamine or sulphur dioxide; the elemental selenium is filtered off and determined by MECA. For a 250-ml sample the detection limit is 0.2 μg/l. It should be emphasized that the selenium must be in the selenite form before co-precipitation. If the concentration of selenium in the samples is higher than 5 μg/l. there is no need for co-precipitation. It was suggested that organic selenium compounds, if present, may first be decomposed by digestion with permanganate in hot acid solution, but Pyen and Fishman[132] found this treatment to be insufficient to destroy all organic compounds.

X-ray emission analysis

As with most other techniques, it is impossible to determine selenium directly by X-ray emission methods at the concentration levels in natural waters: indeed the detection limits are around 200 μg/l. for elements such as selenium[154] with secondary target-energy-dispersive X-ray fluorescence analysis or X-ray energy spectrometry (XES). A simple evaporation enrichment step is not very helpful: even in optimized conditions, spotting on a cellulose filter paper and drying results in a detection limit of 30 μg/l. for low-salinity samples by XES,[155] and spotting a droplet of water onto a Mylar foil and analysis by the highly sensitive proton-induced X-ray emission analysis (PIXE) yields detection limits of around 60 μg/l. for selenium.[156] More favourable sensitivities are obtained when a large volume of water, *e.g.*, 200 ml, is freeze-dried after addition of graphite and the pelletized residue is analyzed by a versatile XES-procedure in which the matrix corrections are based on the scatter peaks in the spectrum, but the detection limit of 1 μg/l. is still well above natural concentrations.[157] Greathouse and Craun[158] evaporated 30 ml of water and applied PIXE to reach a detection limit of 1 μg/l. In a screening analysis of 3834 household tap-water samples, only 10% showed selenium levels above this limit. An additional common drawback to all evaporation procedures is the volatility of selenium compounds.

Clearly, there is a need for chemical preconcentra-

tion techniques to boost the sensitivity of X-ray emission analysis sufficiently for environmental water applications, and over 100 articles have been published on ion-exchange, co-precipitation, solvent extraction, immobilization after chelation, or electrodeposition. Most lead to thin homogeneous targets which are ideal for accurate and sensitive X-ray emission analysis. In view of the multielement potential of X-ray emission methods, most of these papers deal with non-selective enrichment. By using multielement extraction with APDC into chloroform and evaporation onto filter paper, Marcie[159] was able to determine selenium down to 10 μg/l. by wavelength-dispersive X-ray fluorescence.

By co-precipitation on polyvinylpyrrolidine thionalide[160] or DDTC[161] a detection limit of a few μg/l. can be obtained. By using chelating 2,2'-diaminodiethylamine cellulose filters, Smits and Van Grieken[162,163] could collect both Se(IV) and Se(VI) from water at pH 3–6, and reach a detection limit of 0.05 μg/l. for XES-analysis, but ionic strengths above 0.001 appeared to interfere with the collection.

Specifically aiming for selenium determination in water, Pradzynski *et al.*[164] used co-precipitation with APDC in the presence of iron(III), followed by XES to determine 0.6–50 μg/l. of selenium in fresh waters, even when appreciable concentrations of transition metals were present. They stated that the relative speed and economy made the method suitable for application in environmental monitoring.

Robberecht and Van Grieken[37] reported that selenate and selenite in various environmental waters can be determined by XES after preconcentration of elemental selenium on activated carbon. Selenite is reduced to elemental selenium with ascorbic acid. Selenate plus selenite is determined after refluxing the samples with thiourea in sulphuric acid and then adsorbing the elemental form on activated carbon. Selenate is determined by difference. The limit of detection is 50 ng/l. for selenite and 60 ng/l. for total selenium. The coefficient of variation is approximately 10% for both species at the 0.5–1 μg/l. Se level. Humic material, common abundant ions and oxidizing substances do not interfere. The applicability of this method to environmental water has been clearly demonstrated.[165]

Neutron-activation analysis (NAA)

Because of its high sensitivity (10^{-8}–10^{-9} g of Se) this technique is widely used for determination of selenium, especially in biological material, but also in environmental waters. Thermal neutrons are most often used for the activation because radioactive selenium isotopes are formed in (n,γ) reactions. Only the ^{77m}Se and ^{75}Se radioisotopes are currently utilized in activation analysis. ^{77m}Se, providing the highest sensitivity thanks to a very short half-life (17.5 sec), is used only in instrumental neutron-activation analysis (INAA). ^{75}Se is used more often because its long half-life (120.4 days) allows chemical separation, but long activation times are required. The sample material is usually irradiated in a nuclear reactor with a flux of 10^{13}–10^{15} n.cm^{-2}.sec^{-1} for 7–32 days (for ^{75}Se) or several seconds (for ^{77m}Se). The activity of the irradiated samples is measured with a high-resolution Ge(Li) γ-ray detector coupled to a multichannel analyser, or sometimes with an NaI(Tl) detector.

The most important step in the NAA of water is the enrichment of selenium. Three ways of preconcentrating the element from the water have been described, namely selective extraction, non-selective extraction and freeze-drying. The irradiation can also be followed by radiochemical separation. As can be seen in Table 4, only five papers deal with irradiation of whole water samples without any pretreatment, and physical preconcentration by evaporation or freeze-drying usually does not give sufficient sensitivity for analysis of unpolluted water.

Kharkar *et al.*[177] and Schutz and Turekian[178] applied a very elaborate multielement chemical post-irradiation separation scheme for the analysis of ocean and river water in which ^{75}Se was purified by precipitation as SeS_2, distillation of Se with HBr–HCl and final precipitation with sodium bisulphite. Hashimoto and Winchester[179] performed post-irradiation separation by distillation and precipitation with hydrazine for rain and snow analysis. Jørstad and Selbu[180] used freeze-drying and irradiation, then concentrated ^{75}Se by controlled-potential electrolysis, and obtained a 0.68-μg/l. detection limit for sea-water.

Massée *et al.*[181] determined selenium in fresh water and sea-water by reduction of selenite to elemental selenium with ascorbic acid and adsorption of selenium onto activated carbon. They also determined Se(VI) after reduction to Se(IV) by refluxing with concentrated hydrochloric acid. This allowed determination of Se(IV) and Se(VI) in 40 samples daily, with a detection limit of 10 ng/l. Very recently, Orvini *et al.*[182] presented an extended speciation scheme for the determination of selenium in polluted river waters. They used filtration, charcoal adsorption, selective reduction by ascorbic acid followed by charcoal adsorption and collection on anion-exchange resin for determination of suspended and colloidal compounds, selenite and selenate, respectively. They found an important fraction of the selenium to be present in the colloidal fraction. This was probably due to the fact that at pH 6.5 organoselenium compounds and some selenite adsorb on the charcoal and are included in this fraction. Lieser *et al.*[183,184] also employed carbon adsorption either alone or in the presence of DDTC or dithizone as chelating agents, for the partial recovery of selenium from sea-water for subsequent NAA determination.

Spark-source mass-spectrometry (SSMS)

Although it has several suitable stable isotopes, selenium is not an ideal element for SSMS because of its volatility in a high-temperature spark, which results in a low relative sensitivity coefficient[185] and

Table 4. Determination of selenium in water by non-destructive neutron-activation analysis without chemical preconcentration

Experimental conditions	Type of water	Detection limit, μg/l.	Ref.
10 ml enclosed in quartz 2.6×10^{12} n.cm^{-2}.sec^{-1} (14 hr) 30 days decay time	Subsurface waters	0.7	166–168
5 ml enclosed in quartz 1.4×10^{13} n.cm^{-2}.sec^{-1} (3 days) 17 days decay/60 min counting	River water	0.34	169
250 ml in specially designed quartz bottles 10^{13} n.cm^{-2}.sec^{-1} (20 hr)	Sewage	—	170
5 l., evaporation 10^{14} n.cm^{-2}.sec^{-1}	Rain water	-	171
Progressive evaporation 3×10^{16} n.cm^{-2}.sec^{-1}	Rain water	—	172
250 ml, freeze-drying, pressing into pellets 1.6×10^{12} n.cm^{-2}.sec^{-1} (32 hr) 20 days decay time	Rain water	3.0	173
100 ml, freeze-drying 5×10^{13} n.cm^{-2}.sec^{-1} (4 hr)	River water	—	174
200 ml, freeze-drying 10^{14} n.cm^{-2}.sec^{-1} (1 day) 1 day decay time	River water	0.1	175
1 l., freeze-drying in the presence of 25 mg of ultra carbon, 2×10^{14} n.cm^{-2}.sec^{-1} (35 hr) 4 weeks decay time	River water	0.07	176

poor precision and accuracy. Hamilton and Minski[185] freeze-dried 1 litre of water in the presence of pure carbon powder and pressed the residue into electrodes, using bismuth as an internal standard. A limit of detection of 0.05 μg/l. was obtained for selenium. Similarly Wahlgren *et al.*[187] freeze-dried 1-litre samples, ashed the residue under ozone at low temperature, spiked with yttrium as an internal standard and slurried with graphite and compressed into electrodes. They reported a detection limit of 0.1 μg/l.

Gas-liquid chromatography (GLC)

Determination of selenium by GLC is based almost exclusively on the detection of the amount of volatile piazselenol formed in the reaction of Se(IV) with an appropriate reagent. Species other than Se(IV) must first be converted into the quadrivalent form by reduction or oxidation. Piazselenols can readily be extracted from water into organic solvents for direct injection into a gas chromatograph. Piazselenols are usually detected with an electron-capture detector, which gives the highest sensitivity and selectivity for such compounds. Table 5 lists applications of GLC to environmental water, and the various reagents that have been proposed.

Nakashima and Tôei[188] proposed a GLC procedure for selenium involving electron-capture detection of 5-chloropiazselenol and 4,5-dichloropiazselenol. They reported the latter as being three times more sensitive than the former, owing to the additional electronegative group. The minimum amount of selenium detectable was about 0.04 μg as the 5-chloropiazselenol. By extracting the 4,6-dibromopiazselenol into 1 ml of toluene from 500 ml of water sample, Shimoishi and Tôei[196] were able to determine Se(IV) and total selenium in natural waters down to 0.002 μg/l. Young and Christian[194] confirmed the work of Nakashima and Tôei[188] and found the 4,5-dichloropiazselenol to give the same sensitivity as the selenium–DAN complex. However, they used DAN because of impurities encountered in commercial chlorophenylenediamine products. Measures and Burton[195] studied the speciation of dissolved selenium in estuarine waters by developing the procedures described by Shimoishi[193] and Gosink and Reynolds.[192] Total dissolved selenium was determined after photo-oxidation by ultraviolet radiation at a well-defined pH. This led to a reproducible proportionation of species between selenite and selenate. Afterwards the determination procedures were opti-

Table 5. GLC determination of various selenium species in natural waters

Pretreatment and compound extracted	Sample volume, *l.*	Species	Detection limit, *μg/l.*	Ref.
4,5-Dichloropiazselenol	—	Se(IV)	—	188
2,3-Diaminopiazselenol	0.1	Se(IV)	2	189
Reduction + 2,3-diaminopiazselenol	0.1	Se(total)	2	189
5-Nitropiazselenol	—	Se(IV)	0.1	190
Reduction + 5-nitropiazselenol	—	Se(total)	0.1	190
5-Chloro-piazselenol	0.1	Se(IV)	0.05	191
Reduction + 5-chloropiazselenol	0.1	Se(total)	0.05	191
Reduction + 4-nitropiazselenol	0.5	Se(total)	0.04	192
4-Nitropiazselenol	0.1	Se(IV)	0.02	193
Reduction + 4-nitropiazselenol	0.1	Se(total)	0.02	193
2,3-Diaminopiazselenol	0.005	Se(IV)	0.01	194
4-Nitropiazselenol	0.1	Se(IV)	0.01	195
Photo-oxidation + 4-nitropiazselenol	0.1	Se(total)	0.01	195
4,6-Dibromopiazselenol	0.5	Se(IV)	0.002	196
Reduction + 4,6-dichloropiazselenol	0.5	Se(total)	0.002	196
4,6-Dibromopiazselenol	0.5	Se(IV)	0.002	40
Bromine oxidation + 4,6-dibromopiazselenol	0.5	Se(−II, 0, IV)	0.002	40
Br_2/Br- buffer + 4,6-dibromopiazselenol	0.5	Se(−II, 0, IV, VI)	0.002	40
5-Nitropiazselenol	0.1	Se(IV)	0.0008	38, 197, 198
Photo-oxidation + 5-nitropiazselenol	0.1	Se(total)	0.0008	38, 197, 198

mized so that as little as 0.8 ng/l. of selenite in sea-water could be determined.[38] Organic selenium compounds were not tested, and the calibration curve was found to be linear only over the range 0.8–240 ng/l. The method was applied for routine determination of both Se(IV) and total dissolved selenium in sea-water.[197,198] Uchida *et al.*[40] determined various selenium species in river water and sea-water by electron-capture detection GLC after reaction with 1,2-diamino-3,5-dibromobenzene. This reagent reacts with Se(IV) to form 4,6-dibromopiazselenol which is extracted into toluene. After Se(−II) and Se(0) have been oxidized by bromine and Se(VI) has been reduced by a bromine–bromide solution to Se(IV) state, total selenium is determined by the same method. The limit of detection is 0.002 μg/l.

Talmi and Andren[189] coupled the gas-chromatographic separation to a microwave-emission spectrometric detection system for the determination of selenium in various environmental samples. By reaction with 5-nitro-*o*-phenylenediame and extraction of the piazselenol into toluene, they were able to determine selenium in water at the 0.1 μg/l. level.

HPLC

The potential of high-pressure liquid chromatography (HPLC) as a method in inorganic trace analysis has been reviewed by several authors.[199–201] Several piazselenols and selenium diethyldithiocarbamates can be separated from the reagents by reversed-phase chromatography and determined by ultraviolet detection in the nano- and picogram range. The separation of selenium from copper, nickel and lead as diethyldithiocarbamates makes possible the simultaneous determination of these elements. Schwedt and Schwarz[201] tested the method for the determination of selenium in drinking, river and waste water. They reported a limit of detection of 0.7, 0.3 and 5.0 μg/l. for the piazselenol, 5-chloropiazselenol and diethyldithiocarbamate, respectively.

Electrochemical methods

In acidic medium selenite can be reduced at a mercury electrode in two discrete steps. The reaction $Se^{4+} + Hg + 4e \rightarrow HgSe$ is irreversible. Selenium can be accumulated at an electrode surface in this way. At −0.45 V the enriched selenide layer can be stripped cathodically by the reversible process $HgSe + 2H^+ + 2e \rightleftharpoons Hg + H_2Se$. Both reactions can be used in voltammetry. Direct-current polarography of Se(IV) in acidic medium gives rise to two reduction waves at the dropping mercury electrode, but, in practice, this technique is too insensitive and it suffers from severe interference problems, especially from elements that form insoluble selenides. Alternating-current polarography, differential-pulse polarography and cathodic-stripping voltammetry have been used increasingly successfully for trace selenium determinations in recent years. Some of the relevant publications will be discussed here, although not all were specifically intended for environmental waters.

Shafiqul Alam *et al.*[202] determined Se(IV) in hydrochloric and perchloric acid solutions by a.c. polarography at a dropping mercury electrode, with detection limits around 10 μg/l. The use of a hanging mercury drop electrode and a 2–12 min selenium accumulation before cathodic stripping yielded detection limits of 0.1–2 μg/l. The most serious interference, from lead(II), can be prevented by the addition of EDTA.

Howard *et al.*[203] determined Se(IV) by differential-pulse polarography of the 4-chloro-*o*-phenylenediamine piazselenol, which gives a reduction peak at 0.11 V *vs.* SCE at pH 2.5 in formate buffer. The detection limit was 0.4 μg/l. Interferences from chromium(VI), copper(II), molybdenum(VI), nickel(II), tin(II), tellurium(IV) and vanadium(V) were overcome by treatment of the sample with a Chelex-100 resin column. The method appeared to be applicable to the analysis of fresh water and of saline solutions such as estuarine water and sea-water.

A cathodic-stripping voltammetric procedure for the determination of selenium in the range 0.2–20 μg/l. was described more than ten years ago by Henze *et al.*[204] The electrolytic preconcentration on the hanging Hg droplet was effected at −0.4 V *vs.* SCE from a supporting electrolyte containing ammonium sulphate in the presence of EDTA and copper chloride. The procedure was substantially free from interferences. Arlt and Naumann[205] added copper(II) (1 mg/l.) and stripped the intermetallic compound Cu_2Se or Cu_6Se cathodically from a hanging mercury drop electrode, with the specific aim of determining selenium in drinking water. Only sulphide at concentrations above 20 μg/l. appeared to interfere. Krapivkina *et al.*[206] studied the voltammetric behaviour of selenium at a graphite electrode and recommended cathodic or anodic stripping of intermetallic copper–selenium compounds, to obtain a detection limit of 0.06 μg/l.

Recently Henze[207] discussed the determination of selenium, arsenic and tellurium at sub-μg/l. levels. After electrolytic depositions of the elements as intermetallic compounds after addition of copper(II), the determination is done by differential-pulse cathodic-stripping voltammetry. At a selenium concentration of 1 μg/l., the standard deviation is only 2.3%. The simultaneous determination of selenium and tellurium or of selenium and arsenic is possible but inorganic compounds that may be present in natural waters, can, in practice, cause depression or even complete suppression of the peaks.

Deldime and Hartman[208] discussed the electrochemical characteristics of the differential pulse- and cathodic-stripping polarography of selenium. The addition of copper and of 2*N* hydrochloric acid as supporting electrolyte can decrease the interfering effect of certain trace metals. Denis *et al.*[209] used reduction of selenium to selenide by sodium borohydride, evolution of hydrogen selenide and its trapping in an alkaline cell, as a preseparation step before differential-pulse cathodic-stripping voltammetry. The detection limit was 1 μg/l. with a precision of approximately 5% at concentrations above 4 μg/l. Metal interferences can be removed by batch extraction with immobilized 8-hydroxyquinoline.

Nguyen *et al.*[210] used differential-pulse anodic-stripping voltammetry for the simultaneous determination of copper, lead, cadmium and zinc, and differential-pulse cathodic-stripping voltammetry at a hanging mercury drop electrode for selenium determination, specifically in rain water and snow. With special care to purify and pretreat sampling containers and laboratory ware, they were able to determine selenium down to 10 ng/l. in a 15-ml sample.

Alternative electrochemical techniques, such as amperometric titration on a rotating platinum electrode[211] and potentiometric titration with a fluoride-selective electrode[212] yield detection limits of only around 1 mg/l., and are thus definitely not sensitive enough for direct application to environmental waters.

CHEMICAL SPECIATION OF SELENIUM IN NATURAL WATERS

Knowledge of the speciation of environmentally and biomedically relevant elements, *i.e.*, their distribution in different chemical forms, is important because bio-availability and toxicity both depend critically on the chemical form. The measurement of the total concentration of a trace element provides little information about the bio-availability since different forms have different assimilability.[213] Generally, the free (hydrated) metal ion is the form most toxic to aquatic life. Strongly complexed metal, or metal associated with colloidal particles, is much less toxic.[213–219] For speciation determination at ultratrace concentration levels, very sensitive analysis is required and the whole analytical procedure must be designed to keep blanks and contamination to an absolute minimum. Also, any preliminary separation step and the analytical measurement must wherever possible avoid altering the equilibria between the various chemical species in the sample. However, in a dynamic system such as a natural water, some disruption of the chemical equilibria is inevitable, although it can be minimized by use of rapid separation procedures.[220]

The protein-bound form of selenium seems to be the most available form for animal and man, so the study of organoselenium compounds in water is very important. Nhimi and Laham[221] studied the relative toxicity of organic and inorganic selenium to newly-hatched zebra fish (*Branchydanio rerio*) and found that selenium dioxide was the most toxic of the compounds tested. Palmer and Olson[222] stated that there are only small differences in toxicity between selenate and selenite when they are added to the drinking water of rats. Earlier, Schroeder and Mitchener[223] reported severe toxicity at the 2-mg/l. level for selenite but not for selenate.

Selenium, like sulphur, exists in several oxidation states, *viz.* −2, 0, +4 and +6. The chemical diversity of these oxidation states is a major factor affecting the behaviour of the element in the environment. Gissel-Nielsen[224] presented some possible cycles for selenium in its different forms. Some of the properties are summarized here from more comprehensive presentations.[225,226] Hydrogen selenide is readily oxidized to non-toxic elemental red selenium, which is insol-

uble in aqueous systems and is generally resistant to oxidation and reduction. In water it will be adsorbed on particulate material present. Methylated selenium (dimethyl selenide or dimethyl diselenide) can be produced in water by microbial or fungal activity.[227–230] Since these compounds are poorly soluble in water, they will be lost from solution.[135,231,232] Ridley *et al.*[232] discussed the formation of dimethyl selenide by biomethylation reactions. They found the products to be volatile and slowly oxidized by molecular oxygen to stable, water-soluble species. Their presence in natural waters has yet to be demonstrated, although methods for their detection have been developed.[135,232]

Selenium(IV) exists as the weak selenious acid, H_2SeO_3, and as a number of inorganic selenites. Most selenites are less soluble than the corresponding selenates. In aqueous solution (pH range 3.5–9.0), dissolved selenites exist predominantly as the hydrogen selenite ion. Selenites have high affinity for iron, aluminium and manganese hydroxides.[233,234] Under acidic conditions, selenites are rapidly reduced to elemental selenium by mild reductants such as ascorbic acid or sulphur dioxide. Alkaline and oxidizing conditions favour the formation and stabilization of selenate. Most selenates are very soluble and do not form stable complexes. The conversion of selenate into the less soluble selenite or elemental selenium is a slow process, which is not appreciably enhanced by acid environments. In natural waters selenium exists in two common oxidation states, Se(VI) and Se(IV). Sillén[235] has suggested that most of the selenium in sea-water should be present in the thermodynamically stable sexivalent state, but in view of the oxidation–reduction potential, Chau and Riley[66] concluded that the quadrivalent state was more probable. The geochemical behaviour of selenium in aqueous systems has been mapped in pH–*E*h diagrams, such as calculated[236] by Delahay *et al.*, Tischendorf and Ungethum, and Dyachkova and Khodakovsky. Such diagrams reveal that over wide ranges of *E*h and pH, selenium is stable as H_2SeO_3, $HSeO_3^-$ and SeO_3^{2-}, all quadrivalent forms. On the other hand, the field of predominance of SeO_4^{2-} is restricted to extremely oxidizing conditions, so the existence of selenates is rather limited. If selenates exist, it could only be in hot arid climates, where waters normally have a strongly alkaline character.[236] However, selenates may have a metastable existence outside their *E*h–pH equilibrium range.[94] There are contradictory observations concerning the ratio of Se(IV) to Se(VI) in different types of waters.

Literature information on the speciation of selenium in environmental water is very confusing. The findings of Measures and Burton[195,237] indicate that Se(IV) is present in some rivers as a minor fraction of the total dissolved selenium (less than 10%). Hiraki *et al.*[238] report that Se(IV) accounts for 2–16% of the total selenium in samples from two rivers in east central Japan, whereas it comprises 75% of the dissolved selenium in the Kuji river.[239] Several investigators have found that Se(IV) forms a substantial fraction of the dissolved selenium content in some oceanic waters, particularly deep waters. Selenium(IV) was found to be uniformly distributed with depth, but Se(VI) increased to about three times the surface value. The ratio of Se(IV) to Se(VI) ranged from 1–4 for surface samples, to 0.7–1.5 for deep samples. Recently Measures and Burton[198] found that the ratio of selenite to selenate in ocean water increased from 0.1 in the surface layer to a maximum of 0.6 at 1300 m, the deep-water value being 0.55. In ocean water the similarity of depth profiles of selenium species to those of dissolved silicate and phosphate indicates that the distribution of the element is probably mainly controlled by biological processes[197] and that the oxidation phenomena are restricted to the surface-layers.[198] Shimoishi *et al.*[193,196] found the percentage of selenite in sea-water and river water to vary from 35 to 70%. Sinemus *et al.*[97] studied the influence of the oxidation state on the determination of selenium in lake water and found that the element was present only as Se(VI). Uchida *et al.*[40] determined various selenium species in river water and sea-water and concluded that the amount of Se(−II, 0) in river water was larger than that in sea-water, whereas Se(IV) in sea-water was more prevalent than in river water. Total selenium values for river water were found to vary widely, whereas those for sea-water were fairly constant, with Se(VI) dominating. Cutter's findings[135] were similar. For lake water and rain water the selenite concentration was about ten times that of selenate. The reverse was true for sea-water, where the selenate content was at least fifteen times that of selenite. Selenite concentration seemed to increase with the depth of sampling. Massée *et al.*[181] proved that selenite was the predominant species in various environmental waters, and the sexivalent form contributed about 60% of the total selenium only in drinking water. In waste water, 30–100% of the selenium was found to be in the quadrivalent form.[109] Robberecht and Van Grieken[165] stated that the type of water is important for the ratio of selenate to selenite. They found that for the quite polluted Scheldt river, its estuary and the North Sea, most of the selenium was present as selenite. In drinking water, swimming pool water, geothermal water and some waste waters, however, the element was predominantly present as selenate.

The redox state of chemical elements is controlled not only by thermodynamic equilibria but also by some other processes such as biochemical reactions, chemical treatment, surface interaction and adsorption. For this reason other characteristics of water samples are important.

Another reason for discrepancies between the various investigations lies in artefacts arising during the analysis, every step of which must be chosen very carefully. Acidification probably causes desorption and hydrolysis of adsorbed organoselenium com-

Table 6. Reduction of various selenium species

Modification	Remarks	Ref.
(a) Se(VI) to Se(IV)		
6*N* HCl		16, 79, 91, 118, 132, 153
5*N* HCl		111, 240
4*N* HCl		39, 43, 63, 65, 71, 74, 76, 77, 97, 109, 110, 116, 116, 135, 241
Concentrated HCl		64, 76, 77, 91, 125, 134, 148, 179, 181, 190, 191, 233,
SO_2		66
HBr		67, 68, 242
HBr/Br_2	redox buffer	40, 243
KBr		147
H_2O_2		104, 244, 245
HNO_3/H_2SO_4		189
HCl/HNO_3	50% conversion	246
KI/HCl		94, 109
$KI/SnCl_2/HCl$		96
(b) Se(VI) to Se(0)		
$H_2SO_4/TiCl_3$	boiling	193, 196
hydrazine sulphate		72, 73
NH_2OH	(+HCl)	153
thiourea/H_2SO_4	90% conversion	37, 165, 247
sodium hypophosphite		76
(c) Se(IV) to Se(0)		
$H_2SO_4/TiCl_3$	boiling	193, 196
hydrazine sulphate		39, 72, 248
hydrazine		179
NH_2OH	(+HCl)	153
thiourea/H_2SO_4		37, 165, 247
ascorbic acid	various concentrations	37, 78, 79, 165, 181, 182, 247
SO_2	no Se(VI) reduction	153, 178, 249
(d) Se(IV) to Se(−II)		
$Zn/SnCl_2/KI$		90, 91, 95, 98, 117, 141, 250, 251
$NaBH_4$	several procedures	88–90, 92, 93, 95, 97–99, 117, 118, 122, 125–135, 141, 240, 250, 252–254

pounds from colloidal matter. In this way the easily adsorbed selenite can return to the soluble fraction. Heating in the presence of nitric acid causes partial or total oxidation of selenides and elemental selenium.[76,77,104] Sugimura *et al.*[73,74] collected selenite as the DDTC complex on Amberlite XAD-2 resin. Since this resin adsorbs all organic compounds, these authors recently changed their procedure so that all organoselenium compounds are adsorbed before the selenite complex is formed.[75] In this way, they found that up to 45% of the selenium in sea-water was present in an organic form.

In many of the methods of selenium determination, the element must be present as Se(IV), and many ways of conversion into this form have been proposed. Cutter[135] discussed several methods for reduction of Se(IV), and found heating the solution in a boiling water-bath with 4*N* hydrochloric acid the only suitable method. He found that acid concentration is important, and also that the boiling time must be controlled to avoid reduction to the metallic state. Thus, uncontrolled conversion steps may explain some of the literature discrepancies concerning selenium speciation in water. Various published reduction and oxidation methods used in selenium analysis are summarized in Tables 6 and 7. Reported selenium concentration levels in different water samples can only be compared after critical examination of the pretreatment steps. The speciation of selenium in water samples can only be elucidated if other characteristics of the water are taken into account and the different separation methods and conversion steps are shown not to disturb the speciation equilibrium.

CONCENTRATION LEVELS

Summaries of the literature values for the concentrations and major chemical forms of selenium in various types of waters are listed in Tables 8–12.

It is remarkable that most of the selenium in drinking water is present as Se(VI), probably because of chlorination in the preparation of potable water. For all types of water very little information is available concerning the organoselenium compounds.

Apparently, waters rarely contain selenium at levels

Table 7. Oxidation of various selenium species

Modification	Remarks	Ref.
(a) Se(IV) to Se(VI)		
Cl_2		86
(b) Se(−II) and Se(0) to Se(IV)		
Br_2/HBr	redox buffer	40, 70, 193, 196, 243
HNO_3		104, 191, 255
(c) Organoselenium compounds		
HNO_3/H_2SO_4		96
$KMnO_4$ (acidic medium)		91, 153
	partly to Se(VI)	133
	completely to Se(VI)	63, 65
H_2O_2	to Se(VI)	64
$HClO_4/HNO_3$		76, 77, 104
(d) Photo-oxidation		
ultraviolet radiation	reproducible disproportion	
(special buffer)	Se(IV)/Se(total):	
	0.74	195, 237
	0.86	38, 197, 198

above a few μg/l. Only older publications report higher values. However, drainage water from seleniferous soils can contain high amounts of selenium. Although the WHO limit for selenium, like the EPA-proposed maximum contaminant level, is 10 μg/l.[144] and the USSR limit is 1 μg/l. as SeO_3^{2-},[262] most evidence indicates that there is greater overall potential for selenium deficiency than for toxicity at current levels of selenium intake.[261] The maximum no-observed-adverse-health-effect level for selenium in water is at least 100 μg/l. and may be as high as 500 μg/l. A concentration of 20 μg/l. just barely provides a minimum nutritional amount of selenium with a consumption of 2 l./day. The criteria for protection of fresh-water aquatic life, 9.7 μg/l. as a 24-hr average and a concentration never above 22 μg/l., and those for the protection of salt-water aquatic life,[275] 4.4 μg/l. as a 24-hr average and a concentration never above 10 μg/l., are seldom exceeded.

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Table 8. Concentrations of various selenium species (μg/l.) in drinking waters

Sampling place	Total dissolved Se	Se(IV)	Se(VI)	Ref.
Australia	<1	—	—	256
Belgium (Antwerp)	0.32	0.07	0.25	165
Belgium	<0.05–0.66	<0.04–0.085	0.06–0.64	247
Belgium (Brussels)	0.34–0.375	0.02	—	101
Belgium	0.13–0.14	0.05	—	101
England	<1	—	—	257
England	1.1–3.3	—	—	186
France	<2–10	—	—	189
Germany (Darmstadt)	0.12	<0.04	0.087	165
Germany (Darmstadt)	1.1	—	—	175
Germany	<0.12–3	—	—	134
(Stuttgart—tap water)	1.6	—	—	258
(Stuttgart—mineral water)	5.3	—	—	258
Israel (Jerusalem)	0.44	0.06	0.38	165
	26–1800	—	—	16
New Mexico	5	—	—	115
Sweden (Stockholm)	0.061	—	—	259
The Netherlands	0.16	0.10	0.06	181
USA (New York)	<0.2	—	—	64
USA	<0.32	—	—	201
USA	3.5	—	—	158
USA	<1–2	—	—	260
USSR (Moscow)	0.125	0.05	0.075	76, 77
US/EPA—upper limit	10	—	—	144, 261
USSR—upper limit	1	—	—	262
FRG—upper limit	8	—	—	263

Table 9. Concentrations of various selenium species (μg/l.) in sea-water

Sampling place	Total dissolved Se	Se(IV)	Se(VI)	Organo-Se	Ref.
Antarctic Sea	0.052	—	—	—	178
Canada (harbour water)	1.12	—	—	—	129
	1.24	—	—	—	31
Caribbean Sea	0.11	—	—	—	178
English Channel	—	0.34	—	—	66
English Channel	0.03	—	—	—	85
England	—	<0.1	—	—	140
Irish Sea	—	0.50			66
Israel (Dead Sea)	0.80	0.70	0.10	—	165
Japan (seashore)	0.033–0.047	0.012–0.032	—	—	196
Japan (seashore)	0.04–0.08	0.04–0.08	<0.02	—	193
Japan (seashore)	—	0.023	—	—	99
Japan (coastal water)	0.04–0.11	—	—	—	39, 74
Japan (off-shore, upper layer)	—	0.026	0.103	—	71, 72
(off-shore, 25-m depth)	—	0.055	0.096	—	71, 72
Japan (seashore)	0.05–0.07	0.006–0.031	0.031–0.051	—	40
Japan (seashore)	0.110	—	—	—	178
Japan (seashore)	<0.3	—	—	—	110
Japan (seashore)	—	0.40	—	—	53
Japan (seashore)	4–6	—	—	—	264
North East Atl. Ocean	0.025–0.138	0.002–0.055	—	—	197
North East Atl. Ocean	0.088				178
North East Atl. Ocean	0.122–0.162	0.04–0.049	—	—	38
North West Pacific Ocean					
—upper layers	0.06–0.12	0.04–0.08	0.01–0.06	—	39, 74
upper layers	0.07–0.08	—	—	0.019–0.036	75
—deeper layers	0.06–0.20	0.06–0.09	0.06–0.09		73, 74
North Sea					
Belgian shore	0.13–0.27	<0.04–0.14	<0.05–0.23	—	165
(10 km off-shore)	0.11	0.07	<0.05		165
North Sea	0.045	—	—	—	183
North Sea	0.078	—	—	—	184
North Sea	4	—	—	—	265
The Netherlands					
(North Sea)	0.07	—	—	—	266
(North Sea)	0.052–0.12	—	—	—	178
(North Sea shore)	0.12	—	—	—	266
(North Sea)	0.13	—	—	—	181
(North Sea)	3.3–4.4	—	—	—	267
(Wadden Sea)	0.24	—	—	—	266
USA (California)					
—upper layers	—	<0.005	—	—	135
—120-m depth		0.021	—	—	135
—250-m depth	—	0.033	—	—	135
—960-m depth	—	0.056	—	—	135
—1310-m depth	—	0.070	—	—	135
(California)	—	<0.005	0.058–0.080	—	135
(San Diego)	—	0.004–0.063	0.04–0.12	—	198
(San Diego—upper layer	0.045	—	—		198
(San Diego—4000-m depth)	0.178	—	—	—	198

Table 10. Concentrations of various selenium species (μg/l.) in river waters

Sampling place	Total dissolved Se	Se(IV)	Se(VI)	Organo-Se	Ref.
Belgium					
(Scheldt)	0.23–1.78	0.13–1.45	<0.05–0.03	—	165
(Scheldt estuary)	0.66	0.60	0.06	—	165
('unpolluted' rivers)	<0.05–0.58	<0.04–0.20	<0.05–0.38	—	165
Brazil (Amazon)	0.21	—	—	—	177
Canada (Baltimore)	0.33	—	—	—	174
England	0.2–0.9	—	—	—	147
(Southampton)	0.5–15	—	—	—	186
estuary	0.085–0.39	<0.002–0.031	—	—	237
estuary	0.33	0.018	—	—	195
(Southampton)	1.4–4.1	—	—	—	192
(Southampton)	4–19	—	—	—	192
(Thames)	0.13–0.41	—	—	—	176
France (Rhône)	0.15	—	—	—	177
	<2–10		—	—	189
Germany					
(Rhine)	0.14	—	—	—	266
(Rhine)	0.17	0.065	0.10	—	165
(Main)	0.54	—	—	—	175
	0.8	—		—	201
(Donau)	2.3–2.6	—	—	—	242
(Rhine)	2–5	—	—	—	242
Italy					
	<0.02–0.17	—	—	—	233
	<0.7–1.1	—	—	—	166–168
(Ticino river)	29.1–32.7	7.5	3.2–5.8	7.8–9.2	182
(Navigliaccio River)	104.4	55.8	40.6	8.0	182
Japan					
(Asahi)	0.023	0.008–0.012	—	—	196
	0.016–0.23	0.002–0.016	0.005–0.202	—	40
	–	0.006–0.017	0.084–0.171	—	71, 72
	0.04	—	—	—	71, 72
	2	—	—		110
Mexico					
(Guanajuato river)	200	—	—	—	268
Norway	<0.34	—	—	—	169
Panama	0.325	—	—	—	177
The Netherlands	0.20	—	—	—	181
(Western Scheldt)	1.85	—	—	—	266
USA					
(Ohio)	—	<0.01	—	—	194
(Mississippi)	0.114	—	—	—	177
(Michigan)	0.8–10	—	—	—	187
(Nebraska)	<1–20	—	—	—	65
(Colorado River)	30	—	—	—	268

Table 11. Concentrations of various selenium species (μg/l.) in ground water, lake water, swimming pool water, rain water and snow

Sampling place	Total dissolved Se	Se(IV)	Se(VI)	Ref.
Argentina (ground water)	48–67	—	—	269
Asia (thermal waters)	6	—	—	270
Australia				
(ground water)	0.008–0.33	—	—	233
(lake water)	0.2	—	—	233
Belgium				
Antwerp (rain water)	0.25	0.05	0.20	165
Brussels (rain water)	0.91	—	—	138
Ghent (rain water)	<3.0	—	—	173
(ground water)	<0.06–1.33	<0.04–0.10	0.10–1.20	247
Antwerp (snow)	0.29	0.22	0.07	165
Brussels (ground water)	0.125–0.175	0.025	—	104
Antwerp (swimming pool)	0.2–0.33	<0.04	0.17–0.30	247
Canada				
(Lake Michigan)	0.083	—	—	271
(lake water)	<0.1	—	—	272
Denmark				
(rain water)	0.11–0.39	—	—	171
England				
(rain water)	0.21	—	—	179
France				
Pyrenees (ground water)	2.36	<0.04	2.32	165
(ground water)	<5–75	—		67
(well water)	200	—	—	273
Germany				
(lake water)	<0.5	—	—	126
(rain water)	2.5	—	—	242
Greenland				
(ice sheet)	0.008–0.025	—	—	274
Israel				
(ground water)	0.9–27	<0.04	<0.05–26	165
Italy				
(thermal water)	<0.002–0.11	—	—	233
(geothermal water)	<0.002–0.02	—		233
(ground water)	<0.002–1.94	—	—	233
(Lago Maggiore)	0.4	—	—	175
Sweden				
(well water)	0.11 0.15	—	—	259
The Netherlands				
(Yssel lake)	1.85	—	—	266
USA				
California (lake water)	—	0.018	<0.005	135
California (rain water)	—	0.052	—	135
Nebraska (ground water)	<1–480	—	—	65
USSR (well water)	0.095–0.950	0.09–0.85	0.001–0.100	76, 77
West Pacific (rain water)	<0.001	—	—	206

Table 12. Concentrations of various selenium species (μg/l.) in sewage waters

Sampling place	Total dissolved Se	Se(IV)	Se(VI)	Ref.
Germany	0.8–2.1	—	—	201
	1.5	—	—	170
Japan (Osaka)	0.9 26	—	—	141
—	14–38	—	—	109, 110
—	280	—	—	110
—	—	480–700	—	112
USA				
(power plant effluent)	0.4–9.3	—	—	138
—	<5–75	—	—	64
	10–280	—	—	139
USSR	0.182–0.268	0.071–0.115	0.111 0.153	76, 77

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AN IMPROVED SENSITIVE ASSAY FOR POLONIUM-210 BY USE OF A BACKGROUND-REJECTING EXTRACTIVE LIQUID-SCINTILLATION METHOD*

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Summary—A procedure is described for the determination of polonium-210 in various types of materials, including ores, mill tailings, and environmental samples, by a combined solvent-extraction liquid-scintillation spectrometry method. Concentration of polonium-210 and separation from interfering elements (such as iron) are accomplished by extraction from a 7*M* phosphoric acid–0.01*M* hydrochloric acid solution with 0.20*M* trioctylphosphine oxide solution (together with a scintillator) in toluene. The polonium-210 is determined by counting the 5.3-MeV alpha-radiation with a photon/electron-rejecting alpha liquid-scintillation spectrometer. Extraction coefficients of over 1000 for polonium ensure quantitative recovery, and no other alpha-emitters in the decay chains of uranium-238, uranium-235 and thorium-232 are extracted. The results for several samples show the relative standard deviation to be ~1.2%. A lower limit of detection of 0.0038 pCi is proposed, based on a counting time of 1000 min and an easily obtainable background of 0.01 cpm for the alpha peak.

Polonium-210, the daughter of naturally occurring uranium-238, is a common alpha-emitting radionuclide found in biological and environmental materials. It is rated as a Class 1 radionuclide (very high radiotoxicity),[1] yet few methods for determining polonium-210 levels in environmental samples have been developed. Most such methods involve lengthy chemical separations, deposition onto silver disks, and counting on a surface barrier detector. They also depend on the use of polonium-208 and polonium-209 tracers to determine polonium-210 recovery and counting efficiency.[2,3]

Early methods for determination of polonium-210 used stannous chloride to co-precipitate polonium with tellurium or selenium, followed by deposition of the polonium on silver disks from ~0.5*M* hydrochloric acid, and counting with a gas-flow proportional counter.[4,5] Later methods[2] used pyrosulphate fusion, along with solvent extraction with trilaurylamine chloride for polonium separation, followed by deposition onto silver disks and alpha-counting on a surface barrier detector. Polonium-208 or polonium-209 tracers were used to determine recovery and counting efficiency; the precision and accuracy were reported to be within the statistical accuracy of the counting. Recent methods reported by Chou *et al.*[6] and Godoy *et al.*[3] are very similar to past procedures.

This paper describes use of a new technique, developed at Oak Ridge National Laboratory (ORNL),[7] that combines solvent extraction and alpha liquid-scintillation spectrometry[8] and is of wide applicability to alpha-assay problems. Pulse-shape discrimination electronics are used to reject beta and gamma pulses[9] and thus lower the background count to acceptable levels.

The use of liquid-scintillation methods for alpha counting has been known for some time,[10-13] and more recently the ability to obtain a useful degree of alpha-energy resolution has been demonstrated.[14,15] However, the new technique of combining solvent extraction of a nuclide into a solvent containing a scintillator and counting on a high-resolution spectrometer (in conjunction with pulse-shape discrimination electronics to reject beta and gamma pulses) is competitive with, and in some cases more desirable than, other methods. At ORNL, this concept, called photon/electron-rejecting alpha liquid-scintillation (PERALS) spectrometry, has been used extensively.[7,8,16,22] The present method for separation of polonium-210 and its assay by PERALS spectrometry was developed and used for a study of polonium distribution in uranium milling streams.[23]

EXPERIMENTAL

Reagents

The scintillator, [2-(4-biphenylyl)-6-phenylbenzoxazole] (PBBO), and the extractant, trioctylphosphine oxide (TOPO), were obtained from Eastman Organic Chemicals. Eastman scintillation-grade naphthalene and high-purity distilled-in-glass toluene from Burdick and Jackson Laboratory, Inc. were used. All other chemicals used were reagent grade.

The extractive solution was composed of 77 g of TOPO, 180 g of naphthalene, and 4.0 g of PBBO dissolved in and diluted to 1 litre with toluene.

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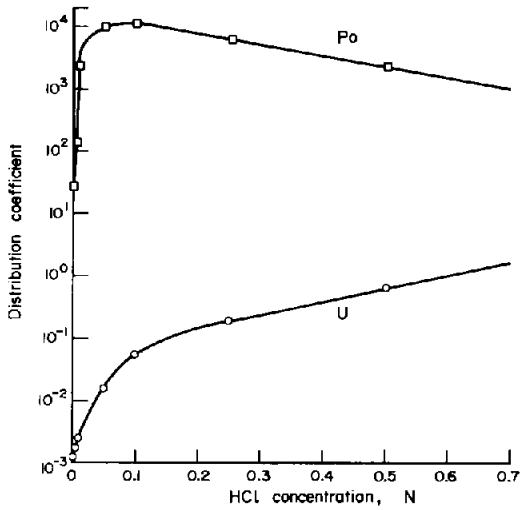

Fig. 1. Polonium and uranium extraction by 0.20*M* trioctylphosphine oxide in toluene as a function of hydrochloric acid concentration.

Equipment

The PERALS spectrometer and pulse-shape discrimination (PSD) equipment previously developed at ORNL were used to count the 5.3-MeV alpha radiation of polonium-210. The PSD equipment was an improved version (developed by D. G. Prater of ORNL Instruments and Controls Division) of that previously[9] described. The detector and associated electronics have also already been described.[24] A Teflon-lined pressure vessel was used for dissolving solid samples. A mechanical wrist-action shaker was used for equilibration in the polonium extractions.

Procedure for solids

Place approximately 0.5 g of the sample (accurately weighed) in a Teflon-lined pressure vessel, and add 2 ml each of concentrated hydrochloric, nitric, and hydrofluoric acids. Seal the vessel, and heat it in an oven at 140° for ~2 hr. Remove the vessel from the oven, let it cool for ~1 hr, and transfer the dissolved sample to a 250-ml Teflon beaker with ~5 ml of water. Add 5 ml of concentrated phosphoric acid. Using a hot-plate with a heat control, evaporate the solution to ~5 ml, rinse down the beaker with ~5 ml of water, and again evaporate to ~5 ml. The acid remaining in the sample should now mainly be phosphoric acid. Retention of hydrochloric acid will increase the extraction of uranium (see Fig. 1), but the uranium can be removed by a scrub (see below). Transfer the sample to a 30-ml separatory funnel with ~5 ml of water and add 1 ml of 0.1*M* hydrochloric acid. The solution should now be ~7.0*M* phosphoric acid–0.01*M* hydrochloric acid. Add by pipette a known volume (usually in the range 1.2–1.5 ml) of extractant solution and shake the funnel on a wrist-action shaker for at least 30 min; run off the aqueous phase and scrub the organic phase with ~5 ml of 7.4*M* phosphoric acid–0.001*M* hydrochloric acid to remove any co-extracted uranium. After the phases have separated, pipette 1.0 ml of the organic phase into a 10 × 75 mm culture tube. Deoxygenate the solution by bubbling argon, methane, propane or acetylene (presaturated with toluene) through it for ~2 min, using a disposable transfer pipette as a sparging lance. (Deoxygenation is necessary if the beta and gamma pulses are to be discriminated from alpha pulses by the electronics.) Seal the sample with a cork coated with a room-temperature vulcanizing silicone sealer, and count the polonium-210 with a beta/gamma-rejecting PERALS spectrometer. Samples are generally stable for 48 hr when sealed as described. Good pulse-shape characteristics can be restored by repeating the sparging.

Procedure for aqueous samples

Combine a measured quantity of the aqueous sample with 5 ml of concentrated phosphoric acid and 2 ml of concentrated hydrochloric acid in a 250-ml Teflon beaker. Apply the procedure given above for solid samples, starting with the evaporation step. Some aqueous samples may require or permit modified procedures; for example, analysis of a sample of ~6*M* commercial (wet-process) phosphoric acid needs only the addition of 0.1*M* hydrochloric acid and extraction *etc.*, before the scintillation counting.

RESULTS AND DISCUSSION

Sample preparation

Dissolution. It was found necessary to use mixed acids in a pressure vessel for the dissolution of solid samples. Some forms of polonium (usually the bivalent state)[2] have been reported to be volatile, causing some concern over the possibility of losing polonium in the dissolution or subsequent evaporation; however, recovery tests with known amounts of polonium showed no loss in the procedures described here. This is in agreement with earlier work by Scott and Stannard.[25]

Extraction. TOPO is an excellent extractant for polonium from hydrochloric acid media.[26] Figure 1 shows the distribution coefficients for polonium and uranium from 7.4*M* phosphoric acid, as a function of hydrochloric acid concentration. Essentially 100% of the polonium is extracted over a very wide hydrochloric acid concentration range. Although phosphoric acid has little effect on polonium extraction, the extraction of uranium and iron is suppressed by increasing phosphoric acid concentration.[26] At 0.01*M* hydrochloric acid and 7.4*M* phosphoric acid, the separation factor for polonium from uranium is about 10^6. Suppression of iron extraction by complexing with phosphate is necessary because small amounts of Fe(III) in the extractive scintillator can quench the light emission to the extent that counting of the polonium is impossible. At higher hydrochloric acid concentrations some uranium is extracted along with the polonium but can be easily removed by scrubbing as described in the procedure. Figure 2 shows the spectra obtained with and without the scrubbing step. These spectra show no evidence for the extraction of any other alpha-emitting nuclides.

The rate of extraction was found to be low, so adequate time must be allowed to ensure complete extraction. Equilibration for 1–2 min gave extraction coefficients in the range 100–200 for 1*M* hydrochloric acid–7.4*M* phosphoric acid medium. After 20–30 min equilibration, however, extraction coefficients up to 900–1000 were obtained for this system. For ~0.005–0.1*M* hydrochloric acid–7.4*M* phosphoric acid, the extraction coefficients were always $\gg 1000$ (approaching 10^4) after the same length of time. Equilibration times in this work were, therefore, always greater than 20 min to ensure quantitative polonium extraction.

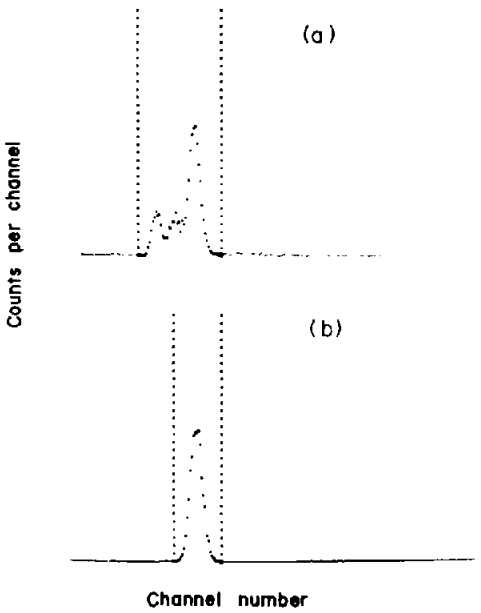

Fig. 2. Energy spectra of uranium and polonium extracted together and of polonium after scrubbing with 0.001 *M* hydrochloric acid–7.4 *M* phosphoric acid. (a) ^{238,234}U and ^{210}Po spectra when extracted together by 0.20 *M* TOPO extractive scintillator; (b) ^{210}Po spectrum after scrubbing the organic with equal volume of 0.001 *M* HCl–7.4 *M* H_3PO_4.

Counting. Figure 3 compares alpha spectra obtained with and without pulse-shape discrimination (PSD). The high-energy alpha-radiation (~8 MeV) is from polonium-214. White and Ross[26] found that bismuth is extracted by TOPO from hydrochloric acid solutions. Our work has confirmed that bismuth is extracted from the hydrochloric acid–phosphoric acid medium. However, the beta and gamma radiation from bismuth-214 and bismuth-210 is effectively rejected by the PSD electronics, leaving only the two alpha-radiation peaks for polonium-210 and polonium-214. Since the alpha energies of the two polonium isotopes are widely separated, integrating the polonium-210 peak gives an accurate determination of the counts from this isotope. No error occurs in the polonium-210 count because of production of this isotope from the decay of extracted bismuth-210 if the sample is counted within 8 hr after extraction ($t_{1/2}$ is 5 days for ^{210}Bi and 138 days for ^{210}Po).

To evaluate the accuracy and reproducibility of the method, eight portions of New Brunswick Laboratory (NBL) Standard No. 104 were assayed. This counting standard was prepared by blending a weighed quantity of pitchblende ore (NBL-analysed sample No. 6-A) with a pulverized silica sand. The uranium content, as reported from the NBL chemical analysis of the final product, is 0.0103 ± 0.004% U at the 95% confidence level. The radium to uranium ratio is 3.49×10^{-7}, in secular equilibrium with the uranium.

If the polonium-210 in the NBL standard is assumed to be in equilibrium (a reasonable assumption because of the method of sample preparation), its activity may be calculated from the uranium (or the radium) concentration. The resulting value is 76.32 dpm (polonium-210) per gram of sample. Table 1 gives results obtained for this standard by the present

Table 1. Counting results from samples of New Brunswick Laboratory (NBL) standard No. 104 (0.0103 ± 0.004% U)

Sample weight, *g*	Total counts*	Observed ^{210}Po activity, *dpm/g*
0.6104	3085	75.8
0.4863	2412	74.4
0.7230	3616	75.0
0.3865	1940	75.3
0.8210	4094	74.8
0.2946	1451	73.9
0.5002	2531	75.9
0.1965	984	75.1

*The NBL sample, after dissolution, was extracted into 1.5 ml of extractive scintillator, and 1.0 ml was then pipetted for counting. Counting time for each sample was 100 min.

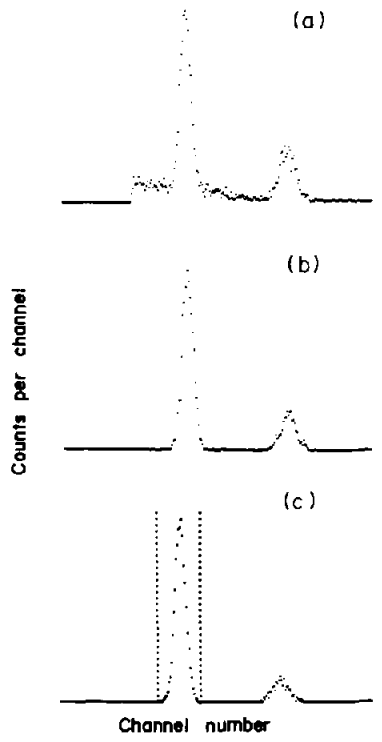

Fig. 3. Examples of polonium energy spectra. The low energy cut-off in (*a*) is caused by a low-level discriminator for the purpose of improving operation of the pulse-shape discriminator. The polonium-214 peak on the right decreases as time elapses between successive counts, because the polonium-214 ($t_{1/2}$ = 164 μsec) arises from the beta-gamma decay of the bismuth-214 ($t_{1/2}$ = 20 min) extracted. (a) ^{210,214}Po energy spectra with beta-gamma background; (b) ^{210,214}Po energy spectra with background rejected by pulse-shape discrimination; (c) ^{210,214}Po energy spectra showing integration of the ^{210}Po peak.

method. The relative standard deviation is 0.9%, and the average value is 75.0 dpm (polonium-210) per gram (>98% recovery of the calculated available polonium-210). Additional assays with another NBL standard gave similar results; eight analyses gave a relative standard deviation of 1.3% and >99% recovery of calculated available polonium-210.

The overall reproducibility of the method for uranium ore samples was examined by analysing six portions of well-mixed Kerr-McGee Ambrosia Lake ore, ranging in weight from 0.2 to 0.89 g. The calculated dpm of polonium-210 per gram showed a relative standard deviation of 1.2%.

The lower limit of detection by this counting method has been estimated previously[2] on the basis of a 1000-min counting time and a background of 0.01 cpm for the alpha peak. Backgrounds as low as this were not always obtained in this work, but were not greater than 0.03 cpm.

CONCLUSIONS

The TOPO/PERALS spectrometry method is effective in analysis for polonium-210 in ores, mill tailings, and other materials, and provides an excellent quantitative assay. Sample preparation is less complicated and requires fewer steps than previous methods, with no need to use tracers for calculating recovery and counting efficiency. Although the method was developed for assay of samples from uranium process streams, it could also be effectively used for low-level environmental samples because of the high extraction coefficients, low background, and high counting efficiency.

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ORIGIN OF DOUBLE-PEAK SIGNALS FOR TRACE LEAD, BISMUTH, SILVER AND ZINC IN A MICROAMOUNT OF STEEL IN ATOMIC-ABSORPTION SPECTROMETRY WITH DIRECT ELECTROTHERMAL ATOMIZATION OF A SOLID SAMPLE IN A GRAPHITE-CUP CUVETTE

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Summary—Atomic-absorption signals of trace lead, bismuth, silver and zinc in steel, obtained by directly atomizing one sample particle, were found to consist of a small first peak and a large second peak. It was concluded that the first peak was caused by the analyte element existing around the grain-boundaries of the steel and near the sample surface and the second by the fraction of the analyte element existing within the crystal grains of the steel.

When a solid sample is directly atomized in atomic-absorption spectrometry, there should be no contamination and/or loss of the elements sought, and the analytical procedure should be shorter because no decomposition of the sample and preconcentration of the elements of interest will be required. Various direct methods of atomization[1–3] of solid samples have been reported, the electrothermal graphite atomizer[4–8] and induction-heated graphite atomizer[9–11] being used. When an electrothermal graphite-furnace atomizer was used, the atomic-absorption signals for trace lead in iron,[4] low-alloy steel,[4,7] stainless steel,[5,6] copper,[4] copper-base alloy[4] and nickel-base alloy[7] usually appeared as double peaks. Therefore, in this case peak-area measurements[4–7] give better accuracy and precision than peak-height measurements. However, the cause of the double peaks has not been described. We have studied this problem by direct atomization of solid samples with an electrothermal graphite-cup cuvette. We conclude that the first peak comes from lead on the sample surface and at the grain boundaries, and the second from lead within the grains. The atomization mechanism for trace bismuth, silver and zinc in iron and low-alloy steel has also been examined.

EXPERIMENTAL

Apparatus

A Hitachi 170-50 atomic-absorption spectrometer equipped with a Hitachi GA-2 electrothermal graphite-cup cuvette atomizer, a Hitachi 056 chart recorder, a Tokyo Kagaku RD-202 digital integrator and a Shimadzu LM-20 microbalance (standard deviation 5 μg) was used, with hollow-cathode lamps as light sources and deuterium-lamp correction of background absorption. Table 1 gives the parameters used for the lamps.

Samples

The samples[4,8] used were standard low-alloy steel, carbon steel and iron samples. Certified values and analytical values obtained in our institute by flame atomic-absorption spectrometry are shown in Table 2.

Procedure

A micro sample was prepared by carefully cutting a solid sample with a pair of nippers. The micro samples were irregular in shape. A single particle of sample was weighed on the microbalance, then put into a graphite-cup cuvette atomizer. The cuvette was sheathed by argon at 2.0 l./min flow-rate. It was heated directly to atomization temperature without any preheating (*i.e.*, no drying or ashing stage). The shapes and areas of the atomic-absorption signals were recorded.

RESULTS

Double peaks of the lead signal

The lead was uniformly distributed as very small particles in the steel.[4] The shapes of the absorption signals of lead at various atomization temperatures are shown in Fig. 1. They usually consisted of a small first peak and a large second peak, at atomization temperatures from 1350° to 2080°. The appearance behaviour of peaks was examined by use of various atomization procedures. When atomization was interrupted at the end of the appearance of the first peak (1st atomization), only the signal corresponding to the second peak was obtained when the atomization was resumed (2nd atomization) as shown in Fig. 2(a). When atomization was interrupted during the appearance of the second peak (2nd atomization), however, double peaks were obtained on resumption of atomization (3rd atomization) as shown in Fig. 2(b).

On the alternate repetition of the 1st and 2nd atomizations as in Fig. 2(b), the first peak and second peak (partially) were alternately obtained, as shown in Fig. 3. It was assumed from these results that lead on the sample surface or in the grain boundary region was easily atomized, and that at the same time lead inside the grains began to move to the sample surface. Therefore the following experiments were performed.

Table 1. Operating conditions of hollow-cathode lamps

Element	Type	Wavelength, *nm*	Lamp current, *mA*
Ag	Hitachi HLA-3	328.1	2.5
Bi	Westinghouse WL22932A	223.1	5.0
Pb	Hitachi HLA-4S	283.3	3.5
Zn	Hitachi HLA-3	213.8	5.0
D_2	Hitachi HLA-3	—	20

Table 2. Samples used

Element	Sample	Type	Certified value, %	Value by flame AAS, %
Ag	NBS 1165	Ingot iron	(0.0002_5)	—
Bi	JSS 370-1	Carbon steel	—	0.0012
Pb	JSS 159-3	Low-alloy steel	0.001	0.0012
Pb	JSS 160-3	Low-alloy steel	0.002	0.0021
Pb	JSS 161-3	Low-alloy steel	0.003	0.0028
Zn	JSS 370-1	Carbon steel	—	0.0007

Micro samples were annealed in quartz boats for 23.5 hr *in vacuo* (1.3×10^{-5} Pa) at 100°, 300° or 600°, and the absorption signals for lead were recorded. As shown in Fig. 4, the higher the annealing temperature, the smaller the first peak. No first peak was detected for the sample annealed at 600°. However, when a micro sample that had been annealed for 23.5 hr *in vacuo* (1.3×10^{-5} Pa) at 800°, was cut into a few particles and each particle was directly atomized, a very small first peak was obtained.

Atomic-absorption signals of bismuth and silver

The atomic-absorption signals for bismuth and silver in carbon steel and iron are shown in Figs. 5 and 6. These also had double peaks at low atomization temperature (<1610°). At an atomization temperature of 1610°, the first peaks began to overlap with the second peaks, giving peaks with a shoulder, and these absorption signals had a single peak at higher atomization temperature (1830–2080°).

Similarly to lead in steel, when the atomization was stopped at the end of the appearance of the first peak of bismuth [1st atomization in Fig. 7(a)], the absorption signal in the next atomization [2nd atomization in Fig. 7(a)] had only a single peak (corresponding to the second peak). Moreover, when the atomization was stopped during appearance of the second peak [1st atomization in Fig. 7(b)], the absorption signal in the next atomization [2nd atomization in Fig. 7(b)] showed double peaks. For micro samples annealed for 23.5 hr *in vacuo* (1.3×10^{-5} Pa), one at 100° and one at 600°, the absorption signals for bismuth appeared as shown in Fig. 8. The first peak disappears if the sample is annealed at 600°. When individual

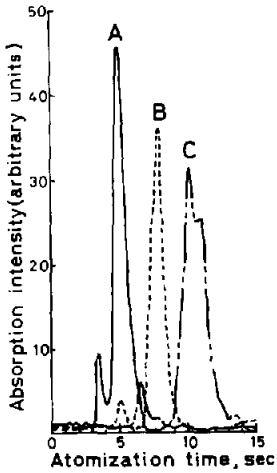

Fig. 1. Profiles of atomic-absorption signal for lead in steel at various atomization temperatures. Atomization temperature: A 1830°, B 1610°, C 1450°. Sample: JSS 160-3. Sample weight: A 70, B 60, C 78 μg.

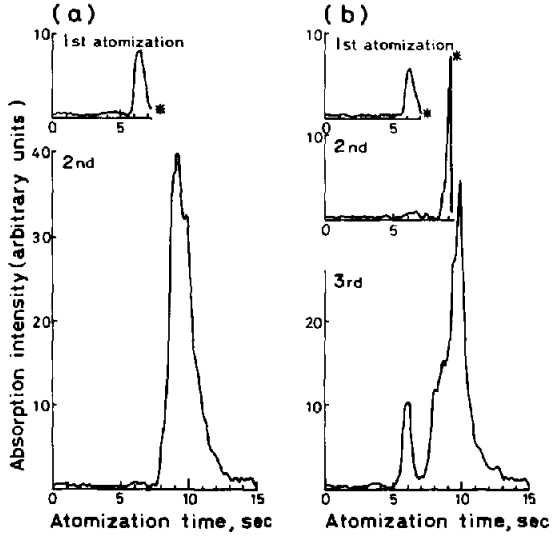

Fig. 2. Profiles of atomic-absorption signal for lead in steel by interrupted atomization. Atomization temperature: 1450°. Sample: JSS 160-3. Sample weight: (a) 120, (b) 215 μg. *Point at which atomization was stopped.

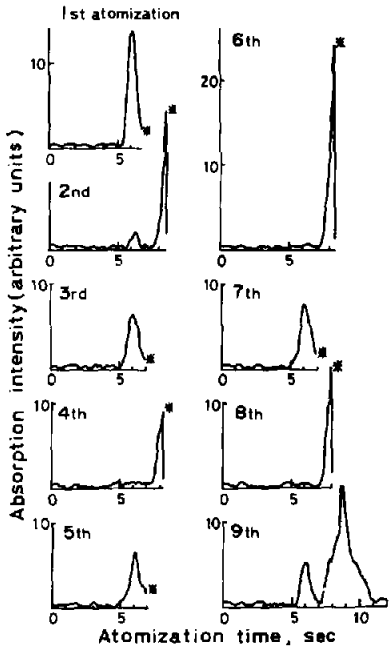

Fig. 3. Profiles of atomic-absorption signal for lead in steel by repeating interrupted atomization. Atomization temperature: 1450°. Sample: JSS 160-3, 305 μg. *Point at which atomization was stopped.

pieces cut off from a sample annealed for 23.5 hr at 1.3×10^{-5} Pa and 800° were directly atomized, the bismuth absorption signal had only a single peak (corresponding to the second peak). These phenomena were similar to those for lead in steel, and similar behaviour was observed for silver in iron.

Atomic-absorption signal of zinc

Atomic-absorption signals for zinc in carbon steel at various atomization temperatures are shown in Fig. 9. They usually consisted of a small first peak and a large second peak for atomization at 1300–2080°. The signals for micro samples annealed for 23.5 hr at

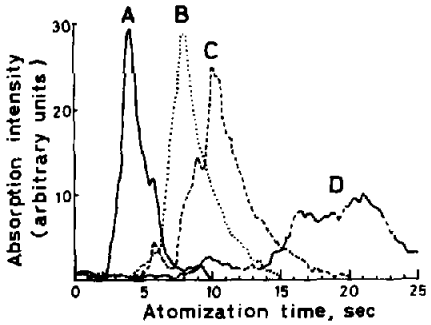

Fig. 5. Profiles of atomic-absorption signal for bismuth in steel at various atomization temperatures. Atomization temperature: A 1830°, B 1610°, C 1450°, D 1300°. Sample: JSS 370-1. Sample weight: A 387, B 500, C 457, D 367 μg.

1.3×10^{-5} Pa and 100, 300 and 600° are shown in Fig. 10. The first peak was removed by annealing at 600°. When the atomization was stopped at the end of the appearance of the first peak for zinc (1st atomization), only the absorption signal corresponding to the second peak appeared in the next atomization (2nd atomization), as shown in Fig. 11. These phenomena were similar to those for lead in steel. However, when the atomization was stopped during appearance of the second peak for zinc (2nd atomization in Fig. 11), the absorption signal in the next atomization (3rd atomization in Fig. 11) usually gave only a single peak corresponding to the second peak for zinc. Atomization of individual pieces cut off from a sample annealed for 23.5 hr at 1.3×10^{-5} Pa and 800° generally gave double peaks which were similar to those for zinc in an unannealed sample. This phenomenon was different from the behaviour of lead in steel.

DISCUSSION

Mechanism of atomization of lead

The appearance behaviour of the absorption signals can be accounted for by the following metallurgical

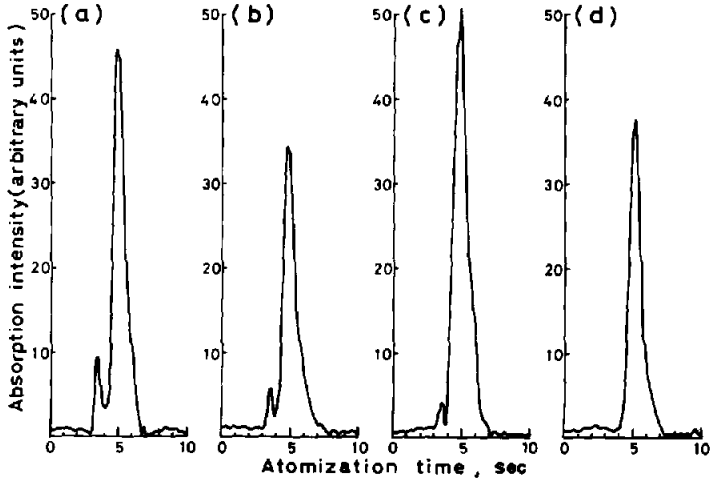

Fig. 4. Profiles of atomic-absorption signal for lead in steel after annealing for 23.5 hr at 1.3×10^{-5} Pa. Annealing conditions: (a) no annealing, (b) 100°, (c) 300°, (d) 600°. Sample: JSS 160-3. Sample weight: (a) 70, (b) 67, (c) 90, (d) 50 μg. Atomization temperature: 1830°.

considerations. The steel samples used are polycrystalline, and the phase-diagram of the lead–iron system does not show a solid solution at any concentration range. Therefore trace lead may exist as particles at the grain boundaries and in the grains. A model of a polycrystalline sample of lead–iron alloy is illustrated in Fig. 12(a). In the atomization procedure, lead near the surface of the sample and at the grain boundaries

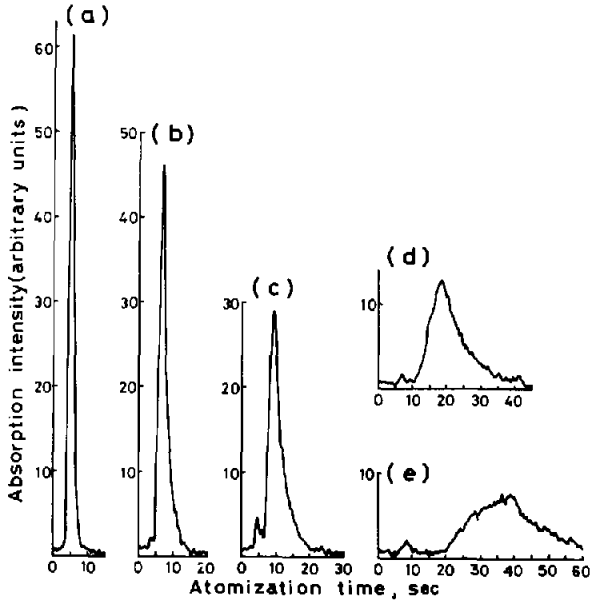

Fig. 6. Profiles of atomic-absorption signal for silver in ingot iron at various atomization temperatures. Atomization temperature: (a) 1830°, (b) 1610°, (c) 1450°, (d) 1300°, (e) 1200°. Sample: NBS 1165. Sample weight: (a) 178, (b) 185, (c) 190, (d) 198, (e) 238 μg.

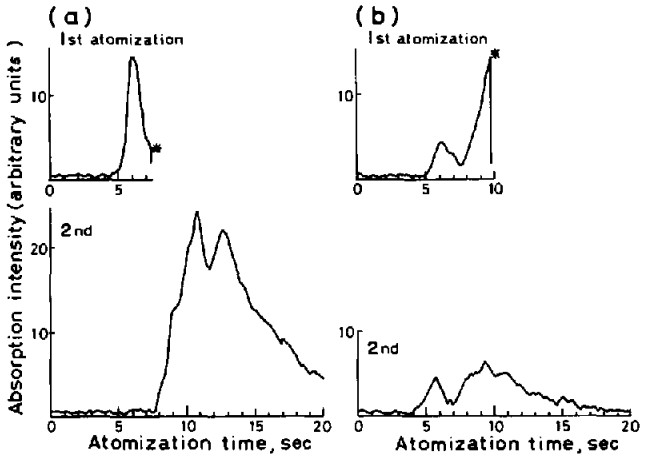

Fig. 7. Profiles of atomic-absorption signal for bismuth in steel by interrupted atomization. Atomization temperature: 1450°. Sample: JSS 370-1. Sample weight: (a) 945, (b) 455 μg, *Point at which atomization was stopped.

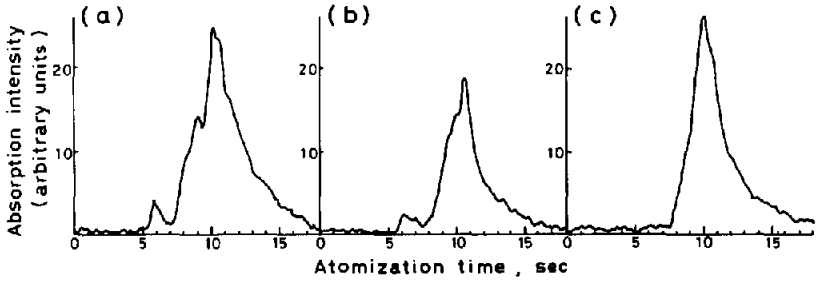

Fig. 8. Profiles of atomic-absorption signal for bismuth in steel after annealing for 23.5 hr at 1.3×10^{-5} Pa. Annealing conditions: (a) no annealing, (b) 100°, (c) 600°, Sample: JSS 370-1. Sample weight; (a) 457, (b) 463, (c) 458 μg. Atomization temperature: 1450°.

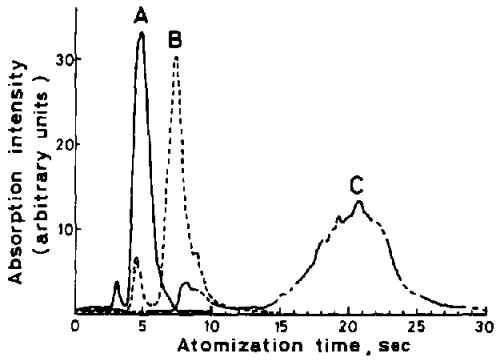

Fig. 9. Profiles of atomic-absorption signal for zinc in steel at various atomization temperatures. Atomization temperature; A 1830°, B 1610°, C 1300°. Sample: JSS 370-1. Sample weight: A 82, B 80, C 100 μg.

rapidly evaporates from the sample: at the same time grain growth occurs and then lead in each grain is evaporated, as shown in (a)–(c) in Fig. 12. The surface and boundary lead (which we will call lead A) gives the first peak and the lead inside the grains (lead B) gives the second peak in the double-peak signal for lead. As indicated in Figs. 2 and 3, lead A had evaporated out of the sample by the end of appearance of the first peak [Fig. 12(a)→(b)], and then lead B gave a single peak corresponding to the second peak [Fig. 12(b)→(c)]. When the atomization was stopped during the appearance of the second peak, lead B (which migrated onto the cut surface of the sample and into the grain boundaries by diffusion during the atomization) remained there on cooling of the sample. When this sample was atomized again, the lead remaining on the sample surface and at the grain boundaries

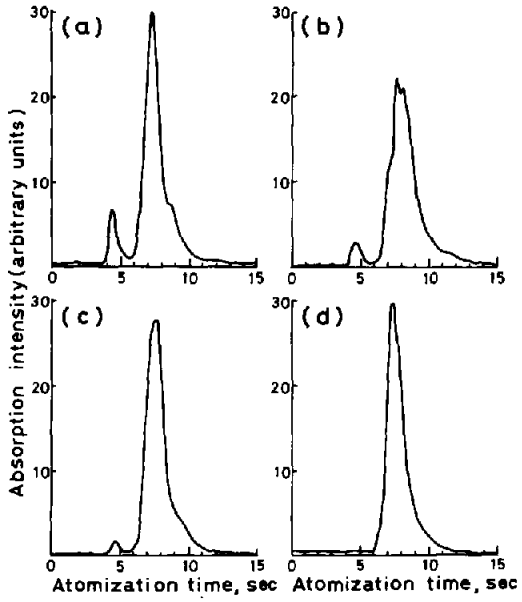

Fig. 10. Profiles of atomic-absorption signal for zinc in steel after annealing for 23.5 hr at 1.3×10^{-5} Pa. Annealing conditions: (a) no annealing, (b) 100°, (c) 300°, (d) 600°. Sample: JSS 370-1, Sample weight: (a), (b), (c) 80, (d) 93 μg. Atomization temperature: 1610°.

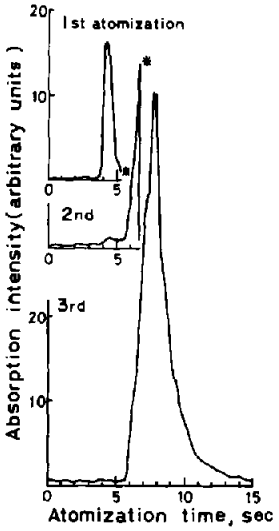

Fig. 11. Profiles of atomic-absorption signal for zinc in steel by interrupted atomization. Atomization temperature: 1610°. Sample: JSS 370-1, 272 μg, *Point at which atomization was stopped.

gave the first peak and the lead still left inside the grains gave the second peak. Thus a double-peak absorption signal again appeared. It is shown in Fig. 4 that lead A evaporates more rapidly than lead B out of the sample. Lead B was not affected by annealing at below 600–800°. Because the lead B precipitates as very small particles in the grain, its diffusion temperature is higher. Therefore the area of the second peaks obtained before and after annealing does not differ (for similar sample weight). The grain size of the steel increased during the annealing. When the sample from which lead A had been removed by annealing *in vacuo* at 800° was cut up, some of lead B was exposed at and near the cutting surface. Therefore the appearance of a very small first peak after annealing and cutting was due to this lead.

As lead is a typical segregation element in steel, any segregation may affect the accuracy of lead determination by direct atomization. When the direct-atomization absorption signal consisted of a large first peak and a small second peak for lead, the lead values found were higher than those obtained by flame atomic-absorption spectrometry. For example, when the lead in two pure irons was determined, we obtained values of 0.0014 and 0.0033% by direct atomization (means of 5 determinations), but below 0.0001 and 0.0011% (means of duplicates) by flame atomic-absorption. For another pure iron, when the first peak was small and the second large, (as in Fig. 1), the values (0.0033 and 0.0034%) obtained by direct atomization agreed reasonably with that obtained by flame atomic-absorption (0.0029%), but whenever the area of the first peak was about equal to or bigger than that of the second peak, the values (0.0062, 0.0086 and 0.0049%) found by direct atomization never agreed with that from flame atomic-absorption (0.0029%). This may be explained as follows: the *sensitivity* of the

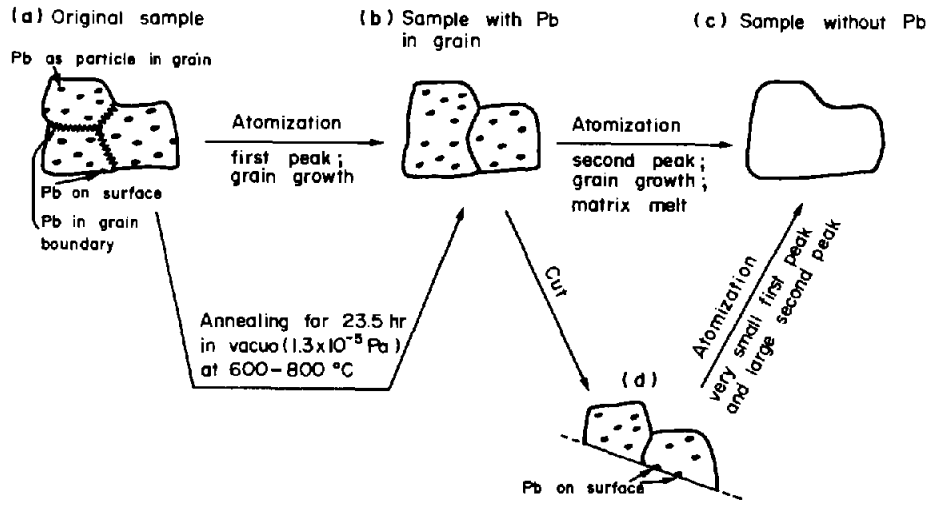

Fig. 12. Model of atomization process of lead in steel.

first peak was larger than that of the second, because lead vaporized from the sample at low atomization temperature had longer retention time (first peak) in the cuvette than the lead vaporized at high atomization temperature (second peak). Bäckman and Karlsson[5] have reported that the absorption signal area for lead in thin-flake samples (large surface area) was 20% larger than that for ball-type samples (small surface area). This may also indicate that the absorption signal of the first peak will generally have a sensitivity different from that of the second peak. Thus segregation of lead in micro samples may be detected from the shape of the double-peak signal for lead. In our experiments, the area of the first peak lead signal was usually 2–8% of the total area of the double-peak signal.

Mechanism of atomization of bismuth and silver

Neither bismuth nor silver gives a solid solution phase on the iron side of the phase system. Moreover the boiling points and vapour pressures of bismuth and silver are similar to those of lead. Therefore the various phenomena of the absorption signals for bismuth or silver were similar to those for lead and can be explained in the same way. The only differences from the behaviour of lead were the overlap of the two peaks for bismuth or silver at high atomization temperature, and the complete elimination of the first peak of bismuth or silver for individual pieces cut off from the samples annealed at 800°. The first difference might be due to the diffusion rate of bismuth or silver in the sample at the high atomization temperature being larger than that for lead, and the second might be due to differences in sensitivity.

Mechanism of atomization of zinc

Zinc does give solid solution on the iron side of the phase system, so trace zinc exists in the grain boundary and almost homogeneously in the grains. A model of a polycrystalline sample of zinc–iron alloy is illustrated in Fig. 13. In the atomization, zinc on the cut surface and in the grain boundaries (zinc A) is evap-

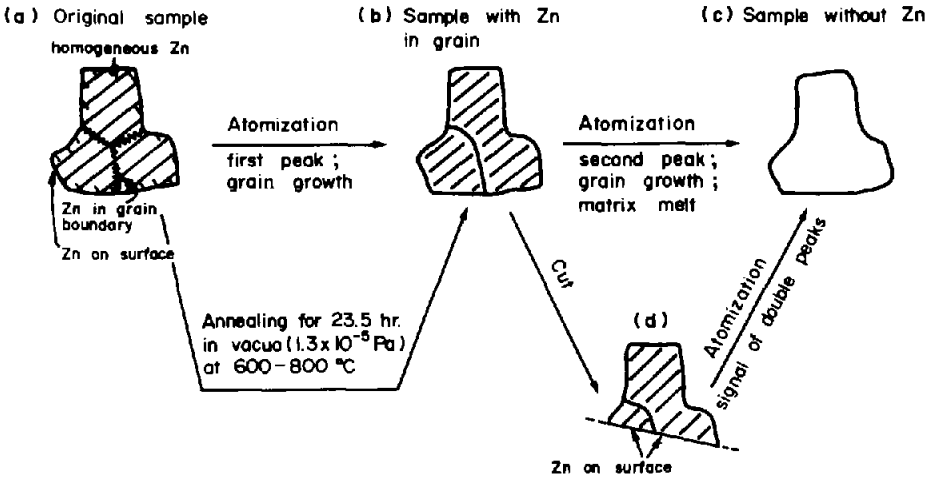

Fig. 13. Model of atomization process of zinc in steel.

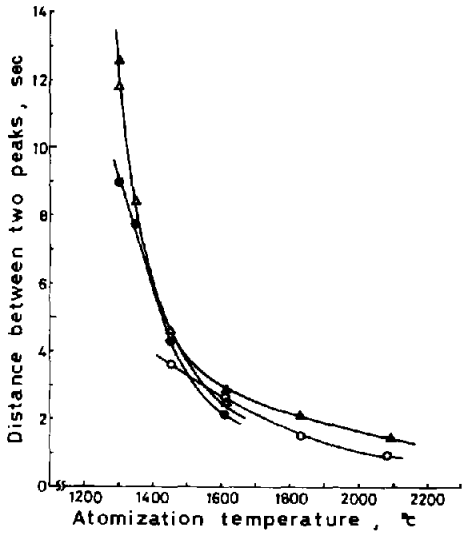

Fig. 14. Effect of atomization temperature on the distance between the first and second peaks. ○ Pb (JSS 160-3, 60–93 μg), ● Bi (JSS 370-1, 367–500 μg), △ Ag (NBS 1165, 178–238 μg), ▲ Zn (JSS 370-1, 80–100 μg).

orated first, and the zinc within the grains (zinc B) is evaporated subsequently. If it is assumed that zinc A gives a small first peak and zinc B a large second peak in the double peaks, various phenomena in the absorption signal from zinc in steel can be explained. When only zinc A has been evaporated, because atomization has been stopped at the end of the first peak, zinc B left in the grains will give only a single peak (corresponding to the second peak) on subsequent atomization, as seen in Fig. 11. Atomization of zinc A, diffusion of zinc B and grain growth occur simultaneously. These phenomena are similar to those for lead. However, when the atomization is stopped during the appearance of the second peak, zinc B, which has arrived at the cut surface of the sample and at the grain boundaries by diffusion will not be left there during cooling of the sample, because of the high vapour pressure of zinc. Therefore, when this sample is atomized again, only zinc B left inside the grains of the sample will be atomized, and will usually give a single peak corresponding to the second peak in the double peaks. It is shown in Fig. 10 that zinc A evaporates more rapidly than zinc B, because of the higher volatilization of zinc near the sample surface. The area of the second peak obtained before and after annealing was found to be approximately equal for samples of the same weight. When the sample that had lost its zinc A by annealing was cut up, some of zinc B in the grains was exposed at the cut surface, and became zinc A. Therefore the double peaks for zinc after annealing and cutting had a shape similar to that for zinc before annealing.

CONCLUSION

As trace lead, bismuth and silver do not give solid solutions with large amounts of iron, these elements mainly exist as particles in the grains, whereas trace zinc, which does give a solid solution, is almost homogeneously distributed in the grains. Moreover, these elements will be exposed at the grain boundaries and on the sample surface when the sample is cut. When such samples are directly atomized, these fractions in the grain boundaries and near the surface give a small absorption signal, which is followed by a large absorption signal from the fraction within the grains. Thus direct atomization of these elements in steels gives double peaks in the absorption signals. In practical analysis, however, it is desirable to obtain a single peak. As shown in Fig. 14, the distance between the two peaks becomes shorter as the atomization temperature is increased. It is therefore recommended that the sample should be rapidly heated to above 2500° and then held at the appropriate temperature for the ideal absorption signal; this temperature will depend on the rate of diffusion of the trace element in solid and molten iron, retention time of the atoms, vapour pressure, carbide formation and so one. Even so, it seems to be difficult to obtain an "ideal" single peak for some elements, such as lead and zinc.

Lead in copper, copper alloys and silver gives double atomization peaks. Lead is practically insoluble in these metals and is more volatile than they are. The behaviour of lead in copper and in silver is similar to that in iron.

On the other hand, lead in Sn, Sb, Bi, Zn, Cd, Te, In and Tl gives a single atomization peak. These elements have a lower melting point or higher vapour pressure than lead, and lead dissolves in them to various extent.

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THE EFFECT OF INCOMPLETE SPIKE REACTION ON THE DETERMINATION OF TRACE METALS IN A COMPLEXING MATRIX BY STANDARD ADDITIONS

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Summary—In the determination of trace metals, a preconcentration step is frequently necessary. A simple model is developed to demonstrate the magnitude of the error incurred in the application of the standard-additions method if full equilibration of the spike with a sample matrix that contains complexing species is not reached before the preconcentration is completed.

The standard-additions method (SAM) is nowadays routinely used and reference to it can be found in many textbooks on instrumental analysis, e.g., that by Skoog and West.[1] SAM is especially valuable in trace and subtrace metal determinations in conjunction with such techniques as atomic-absorption and atomic-emission spectroscopy (AAS and AES) and anodic-stripping voltammetry (ASV). The method, which is intended to compensate for any effect of the sample matrix on the response characteristics, is based on the assumption that the analyte spike added in increasing amounts to aliquots of the sample behaves identically to the sample analyte throughout the analytical procedure and is affected to the same extent by any chemical interference.[2] The blank value is determined in the absence of the analyte element in a closely matched matrix, and subtracted to yield the analytical result.

Much use has been made of SAM in the determination of trace metals in natural waters. Natural waters contain a multitude of ligands that are both natural and anthropogic in origin. Consequently, the soluble metals (*i.e.*, ions) are highly speciated in forms that include the free (hydrated) metal ion, metal complexes of various labilities, and colloidally adsorbed metal.[3,4] The determination of metals in natural waters by AAS or AES very often includes a preconcentration step to "enhance" the metal concentration. In sea-water analysis, preconcentration also permits isolation of the metals from the salt matrix. Solvent extraction and ion-exchange are the most frequently used means of preconcentration.[5–8]

In such complicated systems as natural waters, the analyte spikes may not be completely equilibrated with the sample before the preconcentration step, and, as pointed out by others,[5,9,10] the application of SAM in these circumstances can lead to an erroneous estimate of the *total* soluble analyte concentration. To date, however, the magnitude of the potential error has not been demonstrated. The purpose of this study is to show the relationship between the analyte concentration recovered (*i.e.*, the analytical result) and the degree of reaction of the spike with the matrix components. A simple model is used which takes into account the fraction of reacted spike plus original analyte transferred to the preconcentration phase.

Procedure

In the model, the sample solution is assigned a total analyte metal (M_a) concentration of 1.00 ng/ml with respect to a particular metal, which is assumed to be distributed between hydrated (*i.e.*, ionic) and complexed forms. Four aliquots of the sample solution are taken and after being spiked with known amounts of the analyte metal (M_s), contain final spike concentrations of 0.00, 1.00, 2.00 and 3.00 ng/ml. The total analyte (M_a plus M_s) is then subjected to a preconcentration procedure. It is between the time of spike addition and completion of the preconcentration step that M_s can react with matrix components. In the model, we are concerned particularly with the degree of reaction of the spike with a ligand, L, during this time interval. In a real sample, several different ligands can be present that form metal complexes of various labilities but in order to keep the model simple only a single ligand, L, is considered. For the time interval, the following degrees of spike reaction (DSR) with L are assigned: 0, 25, 50, 75 and 100%. For DSR = 0, none of M_s has yet reacted with L; for DSR = 100%, M_s has come into complete equilibrium with L (whether the equilibrium constant be high or low) and is no longer distinguishable from M_a. The following equilibrium (charges omitted) depicts this situation:

$$(M_a + M_s) + L \overset{K_1}{\rightleftharpoons} (M_aL + M_sL) \qquad (1)$$

For intermediate values of DSR, only a portion of M_s has participated in the reaction with L. Thus, for DSR = 50%, half of the M_s ions have "seen" a ligand

Table 1. Calculation of combined spike and analyte recovery for DSR = 50%, F_t = 0.50; original analyte concentration 1.00 ng/ml

Spike added, *ng/ml*	Unreacted spike recovered, *ng/ml*	Reacted spike recovered, *ng/ml*	Analyte recovered, *ng/ml*	Combined spike and analyte recovered, *ng/ml*
0	0	0	1.00 × 0.50	0.50
1.00	0.50	0.50 × 0.50	0.50	1.25
2.00	1.00	1.00 × 0.50	0.50	2.00
3.00	1.50	1.50 × 0.50	0.50	2.75

and are either bound to L (and so are indistinguishable from M_aL) or have dissociated from M_sL (so are indistinguishable from M_a). The remaining half have not been complexed to L at all and in this sense are distinguishable from M_a ions.

In the calculation of the combined amount of added spike and original analyte that is recovered in the preconcentration step, the portion of unreacted spike is differentiated from the portion of reacted spike (and from the original analyte); it is assumed that the separation method is efficient enough to achieve quantitative separation of unreacted spike. In contrast, for the portion of reacted spike and for the original analyte, the degree of separation is unknown, because it will depend on the stability constants, concentrations and rates of reaction of the species involved, as well as the temperature, pH and other reaction conditions. Therefore, in the model the total amount of added spike and original analyte that is separated at each value of DSR is calculated for a range of fractions (F_t) of *reacted* spike plus original analyte transferred in the separation step. Nine values of F_t have been chosen (from 0.001 to 0.999) so nine standard-addition plots can be constructed for each value of DSR. Table 1 shows the calculation for the construction of the line represented by DSR = 50% and F_t = 0.50. A plot of combined recovery (last column) against spike concentration added (first column) gives an SAM line that yields 0.67 ng/ml as the original concentration analyte (1.00 ng/ml actually present) when extrapolated to the x-axis. For simplicity, the instrument response is assumed to be a linear function of concentration and is plotted as combined spike and analyte concentration found and the degree of preconcentration is not included in the calculation, since its use would simply involve multiplication and eventually division by the same number (the preconcentration factor).

Table 2. Recovery (ng/ml) of original analyte (1.00 ng/ml) as a function of DSR and F_t

F_t	DSR, % 0	25	50	75	100
0.001	0.001	0.001	0.002	0.004	1.00
0.0099	0.0099	0.013	0.020	0.038	1.00
0.091	0.091	0.12	0.17	0.28	1.00
0.24	0.24	0.30	0.39	0.56	1.00
0.50	0.50	0.57	0.67	0.80	1.00
0.76	0.76	0.80	0.86	0.93	1.00
0.91	0.91	0.93	0.95	0.98	1.00
0.990	0.990	0.992	0.994	1.00	1.00
0.999	0.999	0.999	0.999	1.00	1.00

Table 2 records the analytical estimates of the original analyte concentration for various combinations of DSR and F_t. Each value is derived from calculations as shown in Table 1 and the corresponding SAM plot. It should be noted that the range of F_t values corresponds to distribution ratios ranging from 10^{-3} to 10^3 for a solvent-extraction process involving equal phase volumes; strictly speaking, however, F_t is the fraction of reacted spike and original analyte transferred to the second phase in *any* two-phase separation procedure, whether or not equilibrium exists.

DISCUSSION

Complexing species in natural waters range from simple unidentate ligands such as chloride and hydroxide to amino-acids and high molecular-weight polyfunctional molecules such as polypeptides and humic materials. Trace metals exist in these media as hydrated ions, and as labile, moderately labile, and non-labile complexes and as colloidally-bound metal (inert). Useful operational definitions of lability have been proposed in terms of the time-frame of the experimental technique and evidence shows that the proportion of metal in each form is greatly dependent on the nature of the metal.[3,4,10]

A quantity often sought in the analysis of natural waters is the *total* soluble concentration of particular trace metals. Frequently, the determination is made by graphite-furnace AAS in conjunction with SAM and a preconcentration step. The relevant equilibria can be depicted simply as

$$M_a + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M_aL \quad (K_L) \qquad (2)$$

$$M_s + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M_sL \quad (K_L) \qquad (3)$$

$$M_a + R \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} M_aR \quad (K_R) \qquad (4)$$

$$M_s + R \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} M_sR \quad (K_R) \qquad (5)$$

where L denotes a natural ligand, R the separation agent (*e.g.*, a solvent-extraction reagent or an ion-

exchange resin). K_L and K_R are the relevant equilibrium constants and k_1 *etc.* represent the appropriate rate constants.

A source of concern in these determinations is the problem of whether M_s has reached complete equilibration with the complexing matrix components (L) before completion of the preconcentration. The degree of spike reaction with L will decide the accuracy of the analysis. In Table 2, the recovery of analyte metal, present originally at 1.00 ng/ml, is shown for various degrees of spike reaction with L. At each value of DSR, the recovery has been calculated as a function of the fraction of reacted spike plus original analyte transferred to the organic phase or resin (see *Procedure*). The effect of DSR is illustrated by the data for $F_t = 0.50$ and the corresponding SAM plots (Fig. 1). Note that quantitative recovery of the analyte is possible only if the spike has completely equilibrated with L (DSR = 100%). If the spike has not reacted at all with L (DSR = 0%), only 50% of the original analyte is recovered (*i.e.*, 0.5 ng/ml). Also of interest is the fact that although the accuracy is diminished as DSR is decreased, the sensitivity is increased. This arises because the calculation is made on the basis that *unreacted* spike is quantitatively transferred and that the parameter F_t applies only to *reacted* spike and original analyte. At DSR = 0% the basis of the calculation is certainly valid since this situation merely corresponds to the case in which the rate constant k_1 is so small that in the time interval between spiking and the completion of preconcentration (usually only some minutes), no M_s has entered reaction with L. At the other extreme (DSR = 100%), k_1 is large enough to allow complete equilibration in the experimental time interval and no unreacted spike exists.

Several other features in Table 2 are noteworthy. (*i*) If DSR = 100%, the analytical recovery of analyte metal will be complete, regardless of the value of F_t

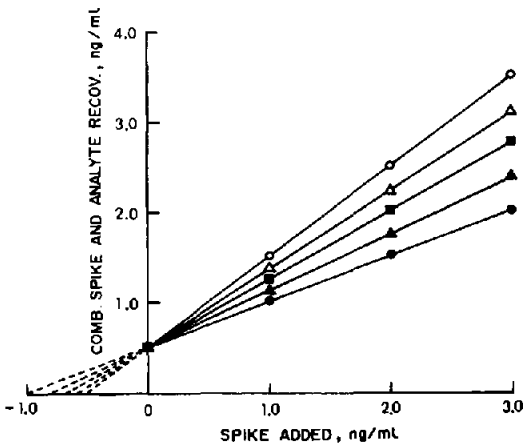

Fig. 1. Effect of DSR on analytical recovery of original metal analyte present (1.00 ng/ml), at $F_t = 0.50$. DSR = 0% (—○—); 25% (—△—); 50% (—■—); 75% (—▲—); 100% (—●—).

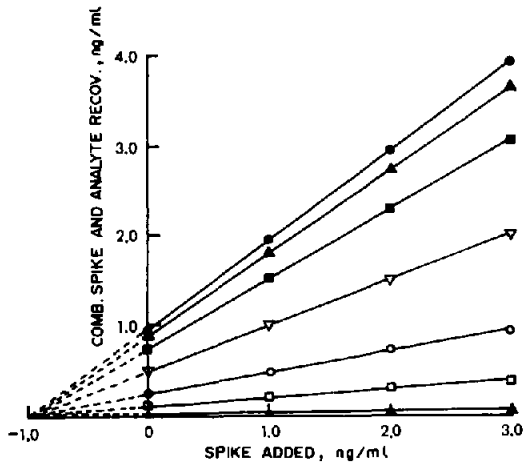

Fig. 2. Effect of F_t on analytical recovery of original metal analyte (1.00 ng/ml), at DSR = 100%. F_t = 0.99 (—●—); 0.91 (—▲—); 0.76 (—■—); 0.50 (—▽—); 0.24 (—○—); 0.091 (—□—); 0.0099 (—△—).

(last column and Fig. 2). The effect of F_t on sensitivity, however, is great and for the examples illustrated here, values of $F_t < 0.50$ are not practical. For higher concentrations of analyte and spike, lower F_t values could be tolerated. (*ii*) No matter what the value of DSR, complete recovery of the analyte metal is possible if F_t is sufficiently large (last two rows, where $F_t = 0.99$ and 0.999 corresponds to solvent-extraction distribution ratios of 10^2 and 10^3 for the matrix containing L).

It is assumed, of course, that the dissociation kinetics for M_sL (M_sL) and the formation kinetics for M_sR (M_sR) are favourable for the time interval involved. In fact, if the preconcentration reaction is sufficiently powerful to remove the analyte element quantitatively from its matrix, the application of SAM, which is a method for calibration of a chemical procedure and not merely of an instrument, is not necessary, because chemical interference from matrix components will not occur. A simple calibration graph prepared with use of the appropriate solvent would suffice. In this regard, it is interesting to observe that in a study of the preconcentration of trace elements from sea-water with silica-immobilized 8-hydroxyquinoline,[7] calibration was done by addition of a standard spike to the solution obtained *after* elution of the analyte element from the column. (*iii*) For the examples shown, the application of SAM is most practical for $F_t = 0.50$, 0.76 and 0.91. It should be stressed that quantitative separation of the spike and analyte element ($F_t \geq 0.99$) is not a prerequisite for a good analytical result. What *is* a prerequisite is knowledge that the spike has fully equilibrated with the matrix components. Very infrequently do investigators possess this knowledge. Indeed, complete recovery of the spike is not sufficient evidence that the analytical result is to be trusted, as such a recovery could simply be the result of unfavourable kinetics (*i.e.*, DSR = 0%), with no recovery of the analyte.

In summary, suitable combinations of thermodynamic and kinetic factors are required for SAM to yield reliable results. Two combinations are: (*i*) K_R sufficiently greater than K_L for F_t, if not in the range 0.99–0.999, to be large enough to be practical, and k_1, k_{-1} and k_2 ($\gg k_{-2}$) sufficiently large to allow complete spike equilibration and rapid formation of M_uR (M_sR); (*ii*) K_R large and $\gg K_L$, to give complete separation of the spike and analyte element ($F_t = 0.99$–0.999); k_1 can vary from a small value (DSR = 0%) to a high value (DSR = 100%) but M_uL (M_sL) should be labile (k_{-1} large) and M_uR (M_sR) should form quickly.

In the analysis of natural waters, investigators do not have enough knowledge of all relevant thermodynamic and kinetic parameters to be certain that complete spike equilibrium has occurred, and of the extent of separation of the analyte metal. Under these conditions, it would seem prudent to use powerful preconcentration separation methods—for example, solvent extraction systems that employ strong chelating agents which give complexes having good solubility in organic solvents, or strong chelating ion-exchange media, even more powerful than Chelex-100. Such media require considerably more development. One advantage of solvent extraction methods is the solubility of organic colloids, onto which metal ions are adsorbed.[9] If quantitative separation of spike and analyte cannot be ensured, then complete equilibration of the spike should be promoted, *e.g.*, by heating the spiked aliquot and allowing a reasonable amount of standing time before the preconcentration step. For storage of water samples, many investigators acidify the sample with nitric acid to pH 1–2. This practice is effective in minimizing adsorption of trace elements on the container walls. It would also appear to be an appropriate way to promote equilibration of the spike, in that organometallic complexes should be effectively dissociated at low pH. Thus, on adjustment of the pH for preconcentration, M_s and M_u should be indistinguishable, even if complexes reform. Finally, more drastic measures such as destruction of matrix components (*e.g.*, by ultraviolet irradiation) can be used when deemed necessary.

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DETERMINATION OF COPPER, CADMIUM, LEAD AND BISMUTH IN PHOSPHORIC ACID SOLUTIONS BY ATOMIC-ABSORPTION SPECTROMETRY AFTER EXTRACTION WITH DIETHYLAMMONIUM DIETHYLDITHIOCARBAMATE AND BUTYL ACETATE

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Summary—The possibility of applying diethylammonium diethyldithiocarbamate and butyl acetate for the extraction of copper, cadmium, lead and bismuth from molar solutions of orthophosphoric acid, and from solutions containing up to 55% pyrophosphoric acid and 20% tripolyphosphoric acid has been investigated. Some characteristics of the various diethylammonium diethyldithiocarbamate–butyl acetate–phosphoric acid systems are described. Suitable conditions have been found for flame atomic-absorption spectrometric determination of the ions extracted. This extraction/AAS approach has been used to determine copper, cadmium, lead and bismuth in some iron-, aluminium- and tin-containing samples dissolved in concentrated orthophosphoric acid. The same approach is used for determining 10^{-5}–10^{-6}% copper, cadmium and lead in orthophosphoric acid and in alkali-metal mono and dihydrogen phosphates.

Orthophosphoric acid and condensed phosphoric acid (CPA) are effective solvents for many inorganic substances, especially those containing iron, aluminium, chromium and silicon as matrix elements.[1–7] However, they find "somewhat limited use in analytical chemistry",[1] since the formation of various phosphate complexes and insoluble phosphates interferes in many of the subsequent steps of the analysis. In order to determine some major and especially some minor elements in phosphate media, efforts have been made to suppress the influence of the phosphate ions either by dilution[5] or by introduction of other mineral acids.[4]

In the present work an attempt is made to combine the efficiency of phosphoric acid as a solvent with a suitable method for separation of the determined elements both from the interfering phosphate ions and from the matrix element(s). Extraction of the determined elements directly from the phosphoric acid solution with diethylammonium diethyldithiocarbamate (DADDTC) and butyl acetate (BA) is found to be effective, and the procedure is applied to determination of Cu, Cd, Pb and Bi in some iron-, aluminium- and tin-containing samples.

DADDTC is a suitable reagent for extracting metal ions from highly acidic solutions.[8–13] It has the advantage of being poorly soluble in water and is therefore introduced into the extraction system as a solution in the organic solvent used. DADDTC is in this way somewhat protected against decomposition in the acidic aqueous phase. The extraction of metal ions from hydrochloric, sulphuric or nitric acid media with DADDTC in chloroform or tetrachloromethane has been studied[11,12] and the first of these solvents is widely used. In our work, flame-AAS determination of the extracted metals was envisaged and butyl acetate was therefore chosen as the solvent.

Extraction of dithiocarbamate complexes from orthophosphoric acid media has not been widely investigated.[14,15] In a previous work,[16] we showed that when phosphoric acid is heated, considerable amounts of pyrophosphoric, tripolyphosphoric and tetrapolyphosphoric acids are formed. The formation and extraction of diethyldithiocarbamate complexes in such media has not hitherto been studied. Possible interactions between the organic and aqueous phases have to be taken into account. Low stability has been reported[17–19] for some dithiocarbamate complexes extracted with basic organic solvents. Butyl acetate is also able to solvate the hydroxonium ion and thus to affect the stability and distribution of the extracted chelates.

EXPERIMENTAL

Reagents

Diethylammonium diethyldithiocarbamate. Solutions (0.01%, 0.1% and 1.0%) in n-butyl acetate or chloroform were prepared.

Cu(II), Cd(II), Pb(II) and Bi(III). Working solutions (10 μg/ml and 50 μg/ml) were prepared from Merck standard solutions.

Saturated aqueous solution of sodium bicarbonate. All

solutions were prepared with demineralized and doubly-distilled water and were tested for Cu, Cd, Pb and Bi.

The iron powder, potassium alum and stannous chloride (all Merck products) contained less than 10^{-6}% Cu, Cd, Pb or Bi.

Procedure

Fresh DADDTC solutions were prepared every third day. Preliminary experiments showed that on longer storage the concentration of the reagent decreased sufficiently to invalidate the results. The decrease is practically the same in both organic solvents tested.

The organic and aqueous phases were shaken mechanically in 100-ml reagent flasks and then transferred to separatory funnels. The aqueous phase was discarded and the organic phase filtered (medium-pore paper) into small glass test-tubes provided with stoppers. The filtration was found not to affect the results.

The distribution of the chelating agent was checked by determining the DDTC concentration in the organic phase: after the aqueous phase had been discarded, a saturated aqueous sodium bicarbonate solution containing an excess of Cu(II) was added to the separatory funnel and the concentration of the $Cu(DDTC)_2$ formed in the organic phase was determined spectrophotometrically.[20]

The metal ions were extracted with 5-ml portions of DADDTC solution in butyl acetate from 50 ml of aqueous solution containing different concentrations of phosphoric acid or mixtures of the various phosphoric acids. The composition of the mixtures was as follows: (*a*) 90% H_3PO_4 + 10% $H_4P_2O_7$; (*b*) 30% H_3PO_4 + 55% $H_4P_2O_7$ + 15% $H_5P_3O_{10}$; (*c*) 25% H_3PO_4 + 35% $H_4P_2O_7$ + 20% $H_5P_3O_{10}$ + 20% $H_6P_4O_{13}$; these solutions were prepared from 4 *M* orthophosphoric acid by heating. The procedure and the method of checking the acid contents are described in a previous paper.[16]

The concentration of the metal ions in the organic phase was determined by AAS (Pye Unicam SP 90, three-slot burner and air/propane flame) at the following wavelengths: Cu 324.8 nm, Cd 228.8 nm, Pb 217.0 nm and Bi 223.1 nm, with background correction. The calibration graphs were prepared by extraction of the metal ions from 15 ml of saturated aqueous sodium bicarbonate solution with 10 ml of 1% DADDTC solution in butyl acetate, over the concentration interval 0.1–1.0 μg/ml. Triple extraction was used to prove the quantitative recovery of Cu, Cd, Pb and Bi.

RESULTS AND DISCUSSION

Some characteristics of the extraction system

The extraction of a metal ion from acidic aqueous solutions with DADDTC is a complex and, in general, a non-equilibrium process. It may be represented as follows:

$$\begin{array}{ccccc} (\text{DADDTC})_0 & & (\text{HDDTC})_0 & & [\text{M(DDTC)}_n]_0 \\ \downarrow & & \rightleftarrows & & \\ (\text{DADDTC})_{aq} & \xrightarrow{H^+} & (\text{HDDTC})_{aq} & \rightleftharpoons & [\text{M(DDTC)}_n]_{aq} \\ & & \downarrow & & \\ & & (C_2H_5)_2NH_2^+ + CS_2 & & \end{array}$$

The following facts deserve special attention. The DADDTC is introduced into the extraction system as a solution in the organic solvent and decomposes rapidly (within a few seconds) into diethyldithiocarbamic acid (HDDTC) and diethylamine when the phases are shaken together. Thus, HDDTC is the chelating agent under these conditions.[21] At pH <4 over 99% of the HDDTC should be in the organic phase.[22] In acidic aqueous solutions HDDTC decomposes rapidly into carbon disulphide and protonated diethylamine. The apparent rate constant is pH-dependent at pH > 2 but constant at pH < 2.[23,24] A half-life of about 7 sec has been calculated for decomposition of HDDC in mineral acid solutions at 20°.[21] The diethyldithiocarbamate complexes are formed in the aqueous phase.

The properties of the organic solvent must also be taken into account, since (*a*) the solvent chosen determines the distribution of HDDTC and $M(DDTC)_n$ between the phases; (*b*) butyl acetate and water are partly mutually soluble; (*c*) being a basic solvent, butyl acetate solvates hydroxonium ions and in this way extracts mineral acids from the aqueous phase. These interactions may cause decomposition of the reagent and the complexes in the organic phase, or at least may change those characteristics of the two phases which determine the distribution of the species between the phases.

Because of the complex character of this extraction system, in this work only some overall effects due to the interactions mentioned were examined. The results were used to optimize the extraction conditions for Cu, Cd, Pb and Bi.

As a first step, the distribution of HDDTC was checked. Some information can be obtained from the fact that decomposition of HDDTC in the aqueous phase causes gradual decrease in its concentration in the organic phase when the phases are contacted. In the case of the 1*M* $HCl/CHCl_3$–HDDTC and 1*M* HCl/CCl_4–HDDTC systems it has been shown[21] that the ratio of the time needed for a 50% decrease of the HDDTC concentration in the organic phase ($\tau_{50\%}$) to the half-life of HDDTC in the aqueous phase ($\tau_{\frac{1}{2}}$) is approximately equal to the distribution constant. We studied this relation for the systems 4*M* H_3PO_4/BA–HDDTC, 4*M* $H_3PO_4/CHCl_3$–HDDTC and 1*M* $HCl/CHCl_3$–HDDTC, where BA stands for butyl acetate. The results were expressed as described by Bode and Neumann[21] (Fig. 1). The following values were found for the ratio if $\tau_{\frac{1}{2}}$ is taken as 7 sec: for 4*M* H_3PO_4/BA–HDDTC 34, for 4*M* $H_3PO_4/CHCl_3$–HDDTC and 1*M* $HCl/CHCl_3$–HDDTC 2570 (*cf.*[21] 2700 for 1*M* $HCl/CHCl_3$–HDDTC). No influence of the nature of the mineral acid was noted in the case of chloroform as solvent, but in the case of BA it was found that $\tau_{50\%}$ depends on the acid used, the order being $H_3PO_4 > H_2SO_4 > HCl$. The extractability of these acids with basic organic solvents increases in the same order.[25,26]

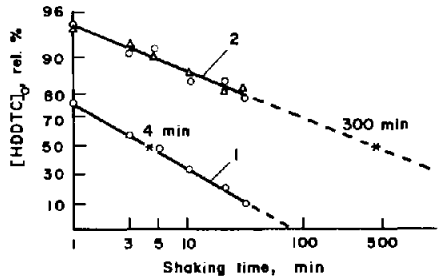

Fig. 1. The HDDTC concentration in the organic phase in relation to the shaking time; 1 butyl acetate; 2—chloroform: ○—4*M* H_3PO_4; △—1*M* HCl; $V_{org} = V_{aq} = 20$ ml.

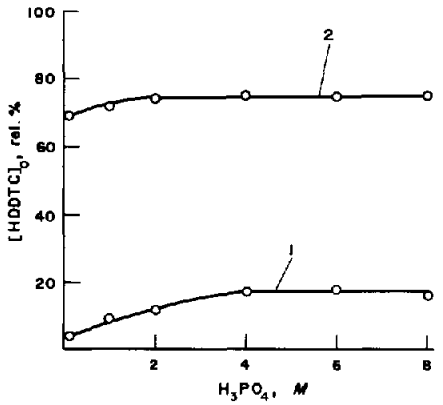

Fig. 2. The HDDTC concentration in the organic phase in relation to the H_3PO_4 concentration in the aqueous phase; $V_{org} = 5$ ml; $V_{aq} = 50$ ml; shaking time 10 min; 1—butyl acetate; 2—chloroform.

The influence of the phosphoric acid concentration (Fig. 2) and of the degree of condensation of the acid (Table 1) on the HDDTC distribution was also investigated. The acidity was always in the range where the HDDTC decomposition rate can be considered constant. Thus changes in the HDDTC concentration in the organic phase as a function of type of acid used could be considered as due solely to variations in the HDDTC distribution. As can be seen, the HDDTC concentration in the organic phase increases with increasing phosphoric acid concentration (up to 4*M*) in the aqueous phase and also with the degree of the phosphoric acid condensation. No significant difference between the two organic solvents was noted. In practice this means that the poor extraction of metal ions from phosphoric acid solutions may be due not only to the formation of phosphate complexes but also to the lower HDDTC concentrations in the aqueous phase.

We assume that there is a slight salting-out effect when the concentration of the highly hydrated phosphoric acids increases.

Stability of the extracted complexes with time

Irreproducible AAS signals were obtained for Cu, Cd, Pb and Bi extracted from solutions with acidity higher than 1*M* phosphoric acid. The values varied with the time needed for the phases to separate and with the interval of time between the extraction and the AAS determination. Rapid discard of the aqueous phase did not lead to significantly better results. Gradual decomposition of the extracted DDTC complexes caused by acidic aqueous phase finely dispersed in the organic phase was assumed to be the possible reason. Washing the organic phase with water, or better with saturated sodium bicarbonate solution, was found to be very effective for preventing this. Washing with bicarbonate solution does not appreciably change the extraction recovery of the metal ions.

When treated in this manner and stored in the cold and the dark, the extracts were stable for at least 20 days.

The shaking time

The extraction does not reach equilibrium, because the HDDTC decomposes rapidly in the aqueous phase. Thus the shaking time is critical in obtaining quantitative results. The relation between the recovery and the shaking time for Cu, Cd, Pb and Bi was investigated. It was found that shaking for no longer than 5–7 min is optimal for the simultaneous extraction of the four elements from 4*M* phosphoric acid with 1% DADDTC solution in butyl acetate. Longer shaking decreases the recovery of Cd and Pb.

Extraction of Cu, Cd, Pb and Bi from phosphoric acid

The extraction of each element was studied at different phosphoric acid concentrations and in the presence of polyphosphoric acids. Three DADDTC concentrations were used (Table 2). Cu, Cd, Pb and Bi are extracted simultaneously with 1% DADDTC in BA from solutions with concentrations of up to 4*M*

Table 1. Concentration of HDDTC in the organic phase in relation to the degree of condensation of H_3PO_4 in the aqueous phase; $V_{org} = 5$ ml, $V_{aq} = 50$ ml; shaking time 10 min

Composition of the aqueous phase	$(HDDTC)_0$, rel. %	
	chloroform	butyl acetate
4*M* H_3PO_4	75	18
90% H_3PO_4 + 10% $H_4P_2O_7$*	87	41
30% H_3PO_4 + 55% $H_4P_2O_7$ + 15% $H_5P_3O_{10}$*	90	57

*The overall acid concentration (expressed as H_3PO_4) in these solutions is 4*M*.

Table 2. Extraction of Cu, Cd, Pb and Bi from phosphoric acid solution with DADDTC and BA; V_{org} = 5 ml, V_{aq} = 50 ml, shaking time 5–7 min

Composition of the aqueous phase	Copper*, R% (DADDTC %)†			Bismuth*, R% (DADDTC %)†			Lead*, R% (DADDTC %)†			Cadmium*, R% (DADDTC %)†		
	0.01	0.1	1.0	0.01	0.1	1.0	0.01	0.1	1.0	0.01	0.1	1.0
0.05M H_3PO_4	98	100	99	98	97	99	70	98	98	25	39	100
0.1M H_3PO_4	100	98	97	98	100	98	58	99	100	15	29	100
1.0M H_3PO_4	100	100	97	100	100	100	58	99	98	1	5	100
2.0M H_3PO_4	98	99	99	98	97	99	38	88	100	0	0	98
4.0M H_3PO_4	99	100	100	99	98	97	15	40	98	0	0	98
6.0M H_3PO_4	100	98	97	98	96	98	3	15	87	0	0	78
8.0M H_3PO_4	99	97	99	98	97	98	0	3	55	0	0	48
H_3PO_4 + $H_4P_2O_7$	96	98	100	99	98	97	0	5	98	0	0	95
H_3PO_4 + $H_4P_2O_7$ + $H_5P_3O_{10}$	70	88	98	98	97	98	0	0	95	0	0	97
4M KH_2PO_4	99	100	98	99	97	97	90	99	100	95	100	99

*Concentration of the metal ion in the aqueous phase, 0.2 μg/ml.
†Concentration of DADDTC in the organic phase.

H_3PO_4, 50% $H_4P_2O_7$, 20% $H_5P_3O_{10}$ and 4M alkali-metal mono or dihydrogen phosphate. BA is not a suitable solvent when the aqueous phase contains tetrapolyphosphoric acid, because a stable emulsion is formed.

Extraction of Cu, Cd, Pb and Bi from phosphoric acid solutions containing Fe, Al and Sn as matrix elements

Potassium alum, stannous chloride and iron powder were used as matrices for testing the extraction of Cu, Cd, Pb and Bi from a complex metal-ion–phosphate matrix. Model solutions were prepared by dissolving the matrix substance in concentrated phosphoric acid with heating. Cu, Cd, Pb and Bi, 10 μg each, were added prior to heating. No influence of the matrix on the extraction or the AAS determination was found in the presence of up to 300 mg of Al or Sn. The extraction of Cd and Pb was completely suppressed in the presence of 2 mg of iron, but masking of iron with 50% ammonium fluoride solution was found to be effective in this case. A 2–4% ammonium fluoride solution was found to be effective for preliminary stripping of the organic phase to remove the matrix element from the aqueous phase finely dispersed in the organic phase (prior to the stripping with bicarbonate). Without this preliminary stripping there will be considerable extraction of iron, accompanied by interference in the AAS determinations,[27] and in the case of aluminium and tin as matrix elements there will be extensive hydrolysis accompanied by losses of the determine elements. It was found experimentally that stripping with ammonium fluoride did not adversely affect the extraction.

Applications

The general scheme is as follows. The sample is dissolved in concentrated phosphoric acid or in a mixture of concentrated phosphoric acid and 1–1.5 ml of concentrated nitric, sulphuric and hydrochloric acid in fused silica beakers. The solution thus obtained is diluted to 50 ml (final phosphoric acid concentration ~4M) with demineralized and doubly-distilled water. Cu, Cd, Pb and Bi are extracted from the total volume or from a suitable fraction of it, depending on the expected Cu, Cd, Pb and Bi content. If a fraction is used, enough concentrated phosphoric acid is added to give a final concentration of 4M, and the resulting solution is diluted to 50 ml. The Cu, Cd, Pb and Bi are then extracted by shaking

Table 3. Determination of Cu, Cd, Pb and Bi in samples containing matrix elements Fe, Al, Sn*

Sample	Copper			Lead			Bismuth		
	X, %	CV, %	Relative error, %	X, %	CV, %	Relative error, %	X, %	CV, %	Relative error, %
Fe, metal†	5.0×10^{-5}	10		—	—	—	—	—	—
Cr–Ni steel‡§	7.7×10^{-2}	5	3	1.4×10^{-4}	14	8	2.5×10^{-4}	8	4
Al, metal†	1.0×10^{-3}	10	—	1.7×10^{-3}	12				
γ-Al_2O_3†	—	—	—	5.6×10^{-3}	16	—	—	—	—
Sn, metal§	1.3×10^{-2}	2	2	1.7×10^{-1}	5	3	2.3×10^{-2}	6	4

*Results of 8 parallel analyses.
†Determined by the standard addition method.
‡Cr 11.20% and Ni 19.47%.
§Certified standard.

Table 4. Determinaton of Cu, Cd and Pb in orthophosphoric acid and alkali metal phosphates with 1% DADDTC in BA, V_{org} = 5 ml, V_{aq} = 50 ml, shaking time 5 min

Sample	H_3PO_4 (n = 9)			KH_2PO_4 (n = 10)			Na_2HPO_4, (n = 9)		
Element	X, %	S	CV, %	X, %	S	CV, %	X, %	S	CV, %
Copper	5.3×10^{-6}	0.5×10^{-6}	9		—		7.4×10^{-6}	1.3×10^{-6}	18
Lead	7.8×10^{-6}	0.6×10^{-6}	8	5.2×10^{-4}	0.2×10^{-4}	4	2.2×10^{-5}	0.1×10^{-5}	5
Cadmium	3.9×10^{-6}	0.7×10^{-6}	18	1.2×10^{-5}	0.1×10^{-5}	8	3.7×10^{-5}	0.2×10^{-5}	5

n = number of parallel analyses; CV = coefficient of variation.

for 5–7 min with 5 ml of 1% DADDTC solution in BA. The aqueous phase is discarded and the organic phase stripped by shaking for 10–20 sec with 5 ml of 2–4% ammonium fluoride solution. The aqueous phase is discarded and the organic phase again stripped, by shaking for 15–30 sec with 10 ml of saturated sodium bicarbonate solution. The aqueous phase is discarded and the organic phase filtered through a medium paper into a suitable test-tube. A blank containing all the reagents is prepared and extracted and stripped in the same way as the samples. Cu, Cd, Pb and Bi are determined in the samples and blanks by AAS. The calibration graphs covering the range 0.1–2.0 μg/ml are prepared by simultaneous extraction of the four elements from 50 ml of 0.1*M* phosphoric acid with 10 ml of 1% DADDTC solution in BA, and AAS analysis of the extract.

By this procedure the Cu, Cd, Pb and Bi content was determined in iron metal, in Cr–Ni high-alloy steel, in aluminium metal, in γ-alumina, in tin metal, in orthophosphoric acid and in alkali-metal mono and dihydrogen phosphates. The results are presented in Tables 3 and 4. Some specific details follow.

Iron metal. A 0.1–1.0 g sample is dissolved in 15 ml of concentrated phosphoric acid and a few drops of concentrated nitric acid. The time needed for complete dissolution depends on the grain size of the sample.

Cr–Ni steel. A 0.1–1.0 g sample is dissolved in a mixture of 15 ml of concentrated phosphoric acid, 1.5 ml of concentrated nitric acid, and 1.0 ml each of concentrated sulphuric and hydrochloric acids with heating at 270–280°. About 2 hr are needed for complete dissolution. Ten ml of 50% ammonium fluoride solution are needed to mask the iron in a 1.0-g sample.

Aluminium metal. Samples of 0.1–1.0 g are dissolved as for iron metal. When the vigorous reaction is over, the samples are heated for about 2–3 hr on a sand-bath.

γ-Alumina. Fifteen ml of concentrated phosphoric acid are heated at 270–280° on a sand-bath for 40 min so that polyphosphoric acids are formed. Then a sample of 0.1–2.0 g of γ-alumina is carefully added and heating continued until dissolution is complete. Before the dissolution is over, 10–15 ml of doubly-distilled water and 0.1–0.2 ml of concentrated sulphuric acid are *cautiously* added to prevent condensation of the phosphoric acid to too high a degree.

Tin metal. A sample of 0.1–1.0 g is dissolved as for Cr–Ni steel; 20–40 min are needed for complete dissolution.

Phosphoric acid and alkali-metal phosphates. A sample of 10–15 ml of concentrated phosphoric acid or 5–10 g of alkali-metal phosphate is diluted (or dissolved) with doubly-distilled water to give a total volume of 50 ml. Extraction is done as described above. Only the stripping with bicarbonate is required.

The results presented in this work show that DADDTC and BA may be successfully used to extract Cu, Cd, Pb and Bi from concentrated solutions of phosphoric acid even when the aqueous solution contains considerable amounts of pyrophosphoric and/or tripolyphosphoric acid. Rapid discard of the aqueous phase and stripping of the organic phase are required in order to obtain results with good reproducibility. We assume that this conclusion will also apply to systems employing other basic organic solvents used in AAS. We assume also that the procedure may be successfully applied when CPA is used as the solvent. In that case, phosphoric acid solutions with a composition near to that employed here can be prepared by partial hydrolysis of the CPA (when the sample has dissolved) with sulphuric acid and water.

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SHORT COMMUNICATIONS

ORGANOSULPHUR BEHAVIOUR IN A GC/MS MEMBRANE INTERFACE

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Summary—The behaviour of 0.1–1.0 ng amounts of S as dimethyldisulphide, n-butanethiol and di-isopropylsulphide in a membrane gas-chromatograph/mass-spectrometer interface has been studied. Results are presented for an all-Teflon system which incorporated a membrane housing constructed from either glass or polymer.

The identification of organosulphur compounds is important in environmental, industrial, clinical and biochemical analysis. Various methods have been used to study such species present in a wide spectrum of samples, including atmospheric pollutants, petroleum spills, marine sediments, pesticide residues, flavour volatiles, mouth air and natural gas. Much of this work has been done by gas chromatography with flame photometric detection (FPD), where both loss due to adsorption and catalytic structural changes caused by components of the analytical system have been a source of concern for some time.[1] It is of note that similar difficulties have been encountered in the HPLC of metal diethyldithiocarbamates.[2,3] Interfacing transfer lines and devices that are constructed from metal and glass to connect the gas chromatograph to the mass spectrometer are expected to exhibit analogous behaviour. Recently we presented the design of a relatively inert (Teflon) connection, with a membrane separator, for the packed-column GC/MS analysis of organosulphur compounds of low molecular weight.[1] The present paper describes an appraisal of losses due to adsorption of these compounds on glass, based on comparison of the performance of a similar device constructed from glass.

EXPERIMENTAL

Equipment

The all-polymer interface consisting of transfer lines with four-port valve system, membrane housing and re-entrant tube between a Varian 2740 gas chromatograph and a modified AEI MS-902 mass spectrometer was as described previously. A glass separator similar in design to the polymer device, and fitted with a glass frit for membrane support, was used interchangeably with the polymer separator. A flame photometric detector (FPD) was used.

Procedure

The performance of the Teflon separator was assessed at various nitrogen flow-rates and interface temperatures with sequential injections of 0.1-μg amounts of standard compounds, once without connection of the mass spectrometer, and the second with the MS operating in the single-ion monitoring (SIM) mode at the m/z value of a common fragment (CH_3S^+). The yields were evaluated by peak-area subtraction of the second FPD signal from the first. The amount entering the ion source at this relatively high concentration was confirmed by a separate calibration check. By a similar procedure used at 60° and a flow-rate of 15 ml/min the test compounds (0.1, 0.5 and 1.0 ng as S) were introduced into the all-polymer system and the interface incorporating the glass separator.

RESULTS AND DISCUSSION

The yields for the 0.1-μg amounts of the standard organosulphur compounds with the all-Teflon system exhibit a decreasing trend with increase of separator temperature and diminish markedly with higher flow-rates (Table 1). These results are consistent with the expected behaviour of the silicone membrane with respect to compound solubility and diffusion, although the highest value (70%) does not match the yield of over 90% reported by other workers.[4]

The yields for dimethyldisulphide, n-butanethiol and di-isopropylsulphide obtained with the Teflon separator, together with the relative SIM signals and amount lost in the glass device, are shown in Table 2. The mass-spectral signals appear to reflect the amounts transferred by the membrane, although at the 0.1-ng level the signals were barely discernible, a reasonable result in view of the fact that the limit-of-detection of the mass spectrometer system used was approximately 0.05 ng. A complete absence of any

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Table 1. Yield (%) when using an all-Teflon interface with separator temperature and carrier gas flow-rate*

Temperature, °C	50	60	60	60	80	100	120
N_2 flow-rate, *ml/min*	30	15	30	60	30	30	30
Methanethiol	34.3	47.7	37.0	19.8	21.8	18.4	17.3
Diethylsulphide	39.4	60.5	39.3	29.1	31.8	32.1	43.4
n-Butanethiol	38.1	65.8	36.9	34.1	37.2	35.4	34.1
Dimethyldisulphide	47.1	69.4	45.1	34.8	40.2	41.4	37.9

*For 0.10 μg of each compound as S.

Table 2. Performance of Teflon and glass separators in all-polymer interface (60°C, N_2 flow-rate 15 ml/min)

	Teflon			Glass*
Compound (SIM *m/z* value)	Amount injected, *ng*	Apparent yield, %	Relative SIM signal	Amount lost, *ng*
Dimethyldisulphide	0.10	40.0	0.2	0.04
(94)	0.50	46.9	0.6	0.22
	1.0	47.9	1.8	0.37
n-Butanethiol	0.10	36.4	0.4	0.07
(90)	0.50	53.2	1.5	0.11
	1.0	63.1	3.5	0.25
Di-isopropylsulphide	0.10	43.0	—	0.04
(43)	0.50	45.0	0.5	0.14
	1.0	52.0	1.2	0.30

*No SIM signal detected.

SIM signal was observed for the experiments conducted with the glass separator. Accordingly, we attribute the loss to surface adsorption both before and after the membrane in the analytical train. The relatively large loss of n-butanethiol at the 0.10-ng level is not surprising in the light of well-known tendency of thiols to adsorb on surfaces. Silanization of the glass separator by conventional methods improved the situation marginally but did not produce the results given by the all-polymer system.

This preliminary work confirms that adsorption losses of organosulphur species at low concentration levels are to be expected in glass interfaces and that the polymer system can be used successfully, even if only at moderate temperatures.

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DETERMINATION OF TIN IN POLY(VINYL CHLORIDE) BY ATOMIC-ABSORPTION SPECTROSCOPY

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Summary—A simple procedure is described for the determination of tin in PVC by atomic-absorption spectroscopy with an air–hydrogen flame, after wet digestion of the sample with sulphuric acid and hydrogen peroxide.

Organotin compounds have been used as stabilizers in poly(vinyl chloride) for several years and are generally present to the extent of 1–2%. A significant amount of work has been done on the analysis of these stabilizers but mostly in relation to qualitative aspects[1,2] or to the determination of specific organotin compounds.[3,4] On the other hand, not much work has been published on the determination of total tin in poly(vinyl chloride).

Bergnes *et al.*[5] determined tin in PVC by titration with EDTA after dissolution in hot tetrahydrofuran and dilution with ethanol to precipitate the PVC, which was filtered off. Olivier[6] adopted a similar approach for extracting the tin into an aqueous phase but then back-extracted it into methyl isobutyl ketone (MIBK) from the aqueous solution at pH 4–5 containing ammonium pyrrolidine dithiocarbamate. The MIBK extract was then aspirated into an air–acetylene flame. Fassy and Lalet[7] determined tin in PVC at a level of 300 μg/g by ashing the sample, converting tin into its acetate and nebulizing the solution into an air–acetylene flame for atomic-absorption measurement at 224.6 nm.

In attempts to determine tin in PVC samples in this laboratory none of the above-mentioned methods gave adequately precise results. Therefore a comparatively simple procedure based on decomposition with sulphuric acid and hydrogen peroxide[8] and aspiration of the aqueous solution into an air–hydrogen flame to measure the tin absorption, has been adopted. The method is easy, rapid and sufficiently precise.

EXPERIMENTAL

Instrument

A Perkin–Elmer 305 atomic-absorption spectrophotometer was used with a 10-cm single-slot burner and readout on a chart recorder. Tin absorption was measured at 224.6 nm; an air–hydrogen flame was used.

Procedure

Weigh out 0.1 g of PVC sample and transfer it to a micro Kjeldahl tube. Add 1 ml of concentrated sulphuric acid and char by gentle heating. Complete the decomposition by heating for about 10 min with slow dropwise addition of 2 ml of 50% hydrogen peroxide. Boil the contents for another 5 min to remove all the hydrogen peroxide and water. The liquor should be colourless. Let it cool and transfer it to a 25-ml standard flask. Rinse the Kjeldahl tube and make up to volume with water. Determine the atomic absorbance as for the standards (see below).

Calibration

Prepare a 500-ppm solution of tin by dissolving 0.250 g of pure tin metal in 25 ml of concentrated hydrochloric acid and diluting accurately to 500 ml with water. Transfer an aliquot containing 0.5–2.5 mg of tin into a 100-ml standard flask followed by 3 ml of concentrated sulphuric acid and 8 ml of hydrogen peroxide. Shake to mix and make up to the mark with water. Prepare a blank by taking the same volumes of sulphuric acid and hydrogen peroxide. Nebulize the calibration solutions and samples into a fuel-rich air–hydrogen flame and measure the tin absorbance at 224.6 nm.

RESULTS AND DISCUSSION

The effects of acids and the blank

It has been shown[9] that the presence of concentrated hydrochloric acid, even up to 20% v/v, does not effect the atomic-absorption determination of tin, so the presence of less than 0.3% v/v hydrochloric acid in the calibration solutions but none in the samples was considered not to present any difficulties. Sulphuric acid does cause a slight depression in the tin absorption, however,[9] so equal amounts of this acid should be present in both calibration and sample solutions.

Sodium stannate is sometimes used as stabilizer to prevent the slow decomposition of hydrogen peroxide. Though with analytical-reagent grade hydrogen peroxide the amounts of sodium and additional tin were so minute that in practice the blank (containing a volume of hydrogen peroxide equivalent to that used for the sample) did not give any noticeable absorbance, and was unnecessary, in cases where the hydrogen peroxide does contain considerable amounts of sodium stannate, the preparation of a blank would be essential.

Table 1. Determination of tin by various methods in sample A containing butyltin maleate as additive

Determination	H_2SO_4/H_2O_2 decomposition followed by AAS, Sn, %	Extraction of Sn by precipitation followed by	
		Complexometry of ethanolic solution Sn,%	AAS of aqueous solution Sn,%
1	0.147	0.141	0.110
2	0.155	0.112	0.095
3	0.154	0.092	0.065
4	0.153	0.130	0.087
5	0.156	0.082	0.105
S.D.	0.003	0.024	0.017

Table 2. Determination of tin in PVC samples

PVC Sample	Additive	No. of determinations	Sn,%	
			Stated	Found
A	Dioctylthiotin	3	0.152	0.146
B	Dibitylthiotin	3	0.152	0.144
C	Butyltin maleate	5	0.159	0.153

Other methods

The methods mentioned in the introduction were all tried, but none gave acceptable results, which led to the need for a new method.

A dry-ashing procedure was tried in which up to 1 g of sample was ignited at 800° in a silica crucible, but nothing was left after half an hour. In another attempt 0.5 g of sample was heated with 2 g of magnesium nitrate at 800° for half an hour. The residue was dissolved by adding hot hydrochloric acid but no trace of tin could be detected in the solution.

Extraction of tin by dissolving the PVC in an organic solvent and reprecipitating only the polymer by addition of water or ethanol was also tried. The ethanol extract was titrated with 0.001*M* EDTA, with Catechol Violet as indicator, as described by Burgnes *et al.*[5] and also the aqueous extract was aspirated into an air–hydrogen flame, but the results obtained in both cases, and given in Table 2, were poor and imprecise, which is most probably due to incomplete extraction of the tin or its adsorption on the precipitated polymer.

Another approach suggested by Olivier[6] for the determination of trace metals in polymers is based on the direct aspiration of a 2% solution of the polymer in an organic solvent into the flame. This method was also tried but in this case rapid blockage of the burner assembly was indicated by a sharp decrease in the absorption with time. A comparison of results and standard deviations obtained by the different methods is shown in Table 1.

Analysis of PVC samples

By courtesy of Messrs. Albright and Wilson three samples of PVC, each containing a different additive at around the 1–2% level, were made available: 100 mg of sample could be easily and completely decomposed with 1 ml of concentrated sulphuric acid and 2 ml of 50% hydrogen peroxide. The whole decomposition procedure took 15 min at the most.

The air–hydrogen flame was found adequately sensitive for the amounts of tin present in the samples and a linear calibration graph for tin in the range 5–20 μg/ml could easily be obtained by use of a slightly fuel-rich flame.[10] The results obtained for the analysis of PVC samples containing various organotin additives are given in Table 2. A relative standard deviation of 2% was obtained for five successive determinations of butyltin maleate in PVC.

In conclusion, with the present method tin can be determined with more satisfactory results and with very little effort, compared to other methods given in the literature.

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RADIOCHEMICAL DETERMINATION OF COBALT-60 IN ENVIRONMENTAL SAMPLES*

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Summary—A procedure for the radiochemical determination of ^{60}Co in low-activity samples of sediment and biological material is described. Cobalt recovery is high and decontamination from tervalent lanthanides and naturally-occurring radionuclides is complete. Cobalt is precipitated with 1-nitroso-2-naphthol, decontaminated from iron by precipitation of the iron as ferric phosphate, extracted into methyl isobutyl ketone, and finally precipitated as cobalt mercury(II) thiocyanate for yield determination and beta-counting.

Decreasing concentrations of radiocobalt (principally ^{60}Co) in the environment during the past twenty years have made its accurate measurement in natural materials increasingly difficult. Reliance on NaI(Tl) gamma-ray spectrometry, a technique that worked well when activities of radiocobalt were much higher than those of naturally-occurring gamma-emitters in the environment, is no longer of sufficient accuracy now that the isotopes of cobalt are often masked by the spectrum of natural radioactivity. Nor does the refinement of Ge(Li) detectors solve this problem: while affording much better resolution than NaI(Tl) detectors, the efficiencies of Ge(Li) for the high-energy gamma-ray transitions of ^{60}Co (1.17 and 1.33 MeV, respectively) are low and make its use for quantification of trace amounts of this radionuclide questionable. The solution lies in the radiochemical separation of cobalt from interfering stable and radioactive elements, thus allowing the ^{60}Co to be determined by low-level beta-counting techniques.

Previously published radiochemical separation procedures for cobalt radioisotopes have emphasized purification from highly radioactive fission-product or neutron-activation product matrices.[1-4] In these instances, neither high chemical yield nor separation from natural radioactivities is important. The same cannot be said, however, for the determination of low levels of radiocobalt in environmental samples.

This paper describes a simple, effective method of extracting cobalt from sediment and biological matrices in a form suitable for the ^{60}Co beta-emissions to be counted. The advantages of the separation scheme described are (1) cobalt is isolated from a wide range of elements existing in natural samples, by precipitation with 1-nitroso-2-naphthol; (2) the difficult isolation of cobalt from iron is accomplished by precipitation of ferric phosphate; (3) it is the first procedure to confirm decontamination from residual fallout and naturally-occurring radionuclides present in environmental materials today.

It should be mentioned that nuclear power plants produce more ^{57}Co and ^{58}Co than ^{60}Co. In the radiocobalt analysis of samples collected from such locales, the purified cobalt fraction should be counted on a gamma-ray spectrometer to determine the isotopic composition of the sample.

EXPERIMENTAL

Reagents

Use reagent grade chemicals unless otherwise specified, and use doubly distilled water in the preparation of all solutions and in steps requiring water washes.

Ammonium mercury(II) thiocyanate. Dissolve 316.8 g of mercury(II) thiocyanate, in 250 ml of 1*M* ammonium chloride.

Cobalt carrier and yield monitor (Co 10 mg/ml). Dissolve 4.04 g of cobalt chloride hexahydrate in water and dilute to volume in a 100-ml standard flask. The solution can be standardized by precipitating the cobalt in a 1-ml aliquot with sodium hydroxide, centrifuging the precipitate and then washing it with water, transferring it with ethanol to a 2.5-cm stainless-steel planchet and drying it under a heat lamp. The weight of cobalt hydroxide obtained is reproducible.

1-Nitroso-2-naphthol solution. Dissolve 10 g of 1-nitroso-2-naphthol in 100 ml of glacial acetic acid.

Trisodium phosphate solution. Dissolve 23.05 g of trisodium phosphate (12-hydrate), in 1 litre of water.

Procedures

Sediment dissolution. Weigh 10 g of sediment into a 250-ml plastic beaker, add 1 ml of cobalt yield monitor, 50 ml of 8*M* nitric acid, 50 ml of concentrated hydrofluoric

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acid and evaporate to dryness under a heat lamp. Add about 100 ml of 6*M* hydrochloric acid and evaporate to dryness again. Transfer the residue to a glass beaker with 6*M* hydrochloric acid; add 100 ml of 6*M* hydrochloric acid to the plastic beaker along with 1 g of boric acid, warm under a heat lamp until the boric acid is dissolved, and then transfer to the glass beaker. Evaporate on a hot-plate to 50 ml and then filter through S&S white-band filter paper. Put the filter back into the glass beaker, add 10 ml of concentrated nitric acid and 10 ml of concentrated perchloric acid, boil till fumes of perchloric acid appear and filter through a glass fibre filter, combining this filtrate with the previous one. Retain the filtrate for cobalt determination.

Total dissolution is preferred to leaching, because Duursma *et al.*[5] have shown that the longer clay sediments are in contact with a solution containing radioactive cobalt, the greater is the fraction that is not extracted by weak leaching agents. Moreover, 6*M* hydrochloric acid has been found to remove only 75–85% of the ^{55}Fe and 65% of the stable iron from Columbia River sediments,[6] and cobalt would be expected to behave similarly.

Dissolution of biological material. Place a known weight of dried biological sample in a beaker, add 1 ml of cobalt yield monitor, slowly bring the temperature up to 450° in a muffle furnace, and ash the sample for 24 hr or until a light grey ash forms. Cool the sample, dissolve the residue in 100 ml of 6*M* hydrochloric acid, then reduce the volume to 50 ml on a hot-plate. Filter through S&S white-band filter paper, and continue as described above for sediments, from the same stage in the procedure.

Cobalt determination. Dilute the filtrate from the dissolution procedure until its acid concentration is 1*M*. Add approximately 2 g of hydroxylamine hydrochloride. Bring almost to the boil, add 7 ml of the 10% 1-nitroso-2-naphthol solution and boil for 2 min. Filter off on S&S white-band filter paper, wash several times with water and discard the filtrate. Place the filter and precipitate in a 250-ml beaker, add 10 ml each of concentrated nitric and perchloric acids and heat to fumes of perchloric acid to destroy the precipitate. Transfer to a 50-ml centrifuge tube, dilute to 20 ml and precipitate cobaltous hydroxide with sodium hydroxide. Centrifuge, discard the supernatant solution and wash the precipitate with water. Dissolve the precipitate in a minimum of concentrated hydrochloric acid with heating in a water-bath (80°). Dilute to about 20 ml with water. For each 20 mg of iron present, add 5 ml of 0.1*M* trisodium phosphate. Adjust to approximately pH 5–6 with ammonia solution, then to pH 3–3.5 by adding 1 ml of glacial acetic acid. Heat in a boiling water-bath to coagulate the precipitate of ferric phosphate. Filter off on S&S white-band paper, collecting the filtrate in a clean 50-ml centrifuge tube, and wash the precipitate three times with hot, dilute (2.5% v/v) acetic acid. Discard the precipitate. Precipitate cobaltous hydroxide from the combined filtrate and washings with sodium hydroxide, centrifuge and wash the precipitate with water. Dissolve the precipitate in a minimum of concentrated hydrochloric acid (2 or 3 drops) with heating in a hot water-bath, dilute to 10 ml with water and add 2 ml of concentrated ammonia solution. Filter into a 125-ml separatory funnel. Discard the filter. Add 2 ml of glacial acetic acid, 20 ml of 25% ammonium thiocyanate solution and 20 ml of methyl isobutyl ketone (MIBK) to the separatory funnel and shake it for 5 min. Allow the phases to separate and discard the aqueous phase. Add 20 ml of 25% ammonium thiocyanate solution and shake for 1 min. After phase separation, discard the aqueous phase. Repeat this washing step. Strip the cobalt by shaking with two 5-ml portions of water for 30 sec each time. Combine these two aqueous extracts in a clean 50-ml glass centrifuge tube. Add 3 drops of concentrated hydrochloric acid. Heat nearly to boiling and add 2 ml of 1*M* ammonium mercuric thiocyanate. Cool in an ice-bath and stir vigorously with a glass rod until precipitation of the blue cobalt mercuric thiocyanate is complete. Centrifuge, and discard the supernatant solution. Wash the precipitate with cold water, then with cold ethanol, discarding the washings. Add 1 ml of ethanol, shake to obtain a suspension of the precipitate and transfer this with a Pasteur pipette to a weighed 2.5-cm stainless-steel planchet. Rinse the centrifuge tube with 250 μl of ethanol and add this immediately to the planchet to ensure an even coating with the precipitate. Dry under a heat lamp and weigh as $Co[Hg(SCN)_4]$ (11.98% cobalt). Count on a low-background beta-counter, making appropriate corrections for chemical yield, self-absorption and efficiency.

RESULTS AND DISCUSSION

A radiochemical procedure for cobalt can be in error if it fails to separate the cobalt from major stable element interferences or if it is not sufficiently specific and contaminating radionuclides are isolated along with the cobalt. Part of the procedure described here has been shown to decontaminate cobalt from reactor fission products and activation products in samples cooled for only 5–24 hr,[1] but no consideration was given to separation of cobalt from environmental samples or its isolation from natural radionuclides. Present-day environmental materials, such as Columbia River sediments, are much more likely to be contaminated by transuranic radionuclides (Pu and Am), and long-lived fission and neutron-activation products such as ^{137}Cs, ^{55}Fe and tervalent lanthanides, especially radionuclides of Eu. This is evident in the work of Robertson *et al.*[7] and from the radionuclides certified by the National Bureau of Standards (NBS) for certain of their standard reference materials discussed below.

As shown in Table 1, the extraction by MIBK is completely selective for separation from tervalent ^{152}Eu; the slightly higher count-rate for ^{152}Eu in the aqueous phase after the MIBK extraction is due to the slight decrease in volume of the aqueous phase. The efficiency of decontamination from naturally-occurring radionuclides was confirmed by extracting cobalt from deep-sea sediments which measurements of ^{239,240}Pu activity had previously shown to be below the depth of man-made radioactivity. Replicate analyses produced count-rates indistinguishable from the background. The completeness of decontamination of cobalt from naturally-occurring radionuclides is further supported by the fact that our results on the Columbia River sediment standard reference material supplied by the NBS (Table 2) are in excellent agreement with the certified value. This material also contains substantial amounts of ^{228}Th, ^{230}Th and ^{232}Th in radioactive equilibrium with their daughter products, as well as ^{137}Cs, ^{152}Eu and ^{55}Fe. A 10-g sample was used for the sediment analysis because the amount available is often limited, but the procedure should be applicable to samples of up to 50 g.

The procedure is applicable to the analysis of both sediments and biological materials (Table 2), attesting

Table 1. Decontamination of Co from ^{152}Eu by MIBK extraction

Sample	^{152}Eu activity of aqueous fraction, *cpm*		
	Before Co extraction	After Co extraction	^{152}Eu remaining in aqueous fraction, %
1	877 ± 11	904 ± 11	103 ± 2%
2	889 ± 11	897 ± 11	101 ± 2%

Table 2. Cobalt-60 determinations in reference material

Sample	Measured activity, *dpm/g*	Accepted activity, *dpm/g*
Columbia River Sediment*		
	0.28 ± 0.03†	0.28 ± 0.01
	0.27 ± 0.02†	0.28 ± 0.01
Vegetable Meal Standard§		
	3.68 ± 0.05†	3.65
Lake Sediment‡		
	32.9 ± 0.7	33.1 ± 0.1
	33.1 ± 0.8	33.1 ± 0.1

*Standard Reference Material 4350B from National Bureau of Standards.
†Standard deviation calculated from sample and background count-rates and are 1σ confidence intervals.
§EMRM-VM-1 from Environmental Measurements Laboratory BERLI Intercalibration Study[8] (no error terms reported).
‡Standard Reference Material from National Bureau of Standards (reference number not yet assigned).
Errors about the measured values are calculated from the propagated sample and background count rates, and are 1σ confidence intervals.

to its utility in the analysis of environmental materials. When the procedure was traced with ^{60}Co, 92 ± 2% of the cobalt was found to be carried through the extraction with MIBK, and the recovery of $Co[Hg(SCN)_4]$ was 95 ± 5%, giving an overall chemical recovery of 86 ± 5%. With care and familiarity, the entire procedure should easily provide cobalt recoveries of at least 75%.

Some notes from our testing of this procedure may be helpful in circumventing problems that occur if this analysis is attempted in series with other radiochemical separations. First, cobalt is not collected effectively by either calcium oxalate or calcium sulphate from dilute acid solutions. Secondly, a substantial but variable proportion of the cobalt is co-precipitated when hydrous ferric oxide is precipitated with ammonia as suggested by Marsh and Maeck.[1] To separate cobalt from iron we used Young and Hall's[9] procedure precipitating ferric phosphate, which we found co-precipitated only 3% of the cobalt.

Finally, the precipitate of $Co[Hg(SCN)_4]$ will not form easily unless the volume is kept low and 3–4 mg of cobalt are present in the sample. If a plastic centrifuge tube is used for this step, a substantial amount of the precipitate adheres to the walls and cannot be recovered. In our experiments, 55 ± 7% of the cobalt was recovered in plastic tubes and 95 ± 5% in glass tubes.

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ANNOTATIONS

ON THE ACID DECOMPOSITION OF HUMAN BLOOD AND PLASMA FOR THE DETERMINATION OF SELENIUM

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Summary—A systematic investigation has been made of various methods for decomposition of blood and plasma for determination of selenium, and various sources of error have been identified. The method recommended is graded destruction by heating with nitric acid and perchloric acid, to a final temperature of 210°.

Because of the increasing interest in selenium as an essential trace element for human physiology, classical methods for its determination have given way to more sensitive techniques such as fluorimetry[1] and hydride-generation atomic-absorption spectrometry[2] which are capable of determining ng amounts of the element. Most methods require dissolution of the sample, and in the case of the two techniques just mentioned, selenium must be present as selenium(IV). As with any analytical procedure, the sample preparation must not introduce appreciable errors. With selenium, two opposing phenomena are involved. Many selenium compounds are volatile and can be lost during an ill-controlled destruction procedure. It seems less well recognized that very acid-resistant organoselenium compounds such as selenomethionine, selenocysteine and the trimethylselenonium ion are often not fully decomposed, hence causing low results. Loss of volatile molecules such as H_2Se, Se_2Cl_2, $SeCl_4$, $SeOCl_2$ and $SeO_2.2HCl$ can be avoided[3] by inhibiting their formation in the first place and by keeping the temperature and the rate of decomposition under control. Moreover, decomposition in a closed vessel prevents loss of volatile products at elevated temperatures. Selenomethionine, a normal metabolite in plants, is easily incorporated into mammalian proteins.[4] Selenocysteine is synthesized from H_2Se and selenite[5] and has been proved to be the active centre of the enzyme glutathione-peroxidase, which represents about 10% of the selenium in human blood.[6] Evidence was presented by Burck[4] suggesting that trimethylselenonium ions are normal excretory products in human urine. It is therefore necessary to focus attention on problems associated with the decomposition of such compounds. In this paper the wet acid digestion of biological materials, especially of human blood and plasma, will be discussed and the accuracy of selenium determinations in these matrices by hydride-generation AAS (HGAAS) established.

HISTORICAL OUTLINE

Of the methods available, digestion with acid in an open system is undoubtedly the most popular, because of its large oxidative capacity and ability to give stringent control of each digestion stage. When a sufficiently high oxidation potential is maintained throughout, temperatures up to 200–250° can be applied without significant loss of selenite or selenate.[7]

Wet digestion with nitric acid under elevated pressure, in a closed PTFE vessel, has been applied less frequently. Its correct use prevents loss of volatile compounds, but the capacity is small (approximately 0.5 g of dry organic material for a 125-ml vessel), and its use is time-consuming. Table 1 surveys procedures (published in 1959–1980) for destruction of biological materials before determination of selenium. Columns B, C and D reflect the evolution that took place.

A predigestion period was included and the oxidative power increased stepwise by adding the acids consecutively instead of all at once. This served two major purposes: to prevent formation of volatile selenium compounds whilst giving drastic but controlled conditions for the breakdown of resistant organoselenium compounds. The rate and extent of destruction are mainly governed by the rate of temperature increase and the highest temperature reached. Appreciable uncertainty as to completion of the digestion arises from the fact that the final temperature is often described only qualitatively, *e.g.*, "the temperature at which copious white fumes are evolved...", or "the temperature at which the liquid briskly boils...". This should (but may not) be 200–210° when nitric/per-

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Table 1. Survey of literature data on the destruction of biological materials, for determination of selenium A: destruction reagents; B: (a); oxidizing acids added as a mixture, (b): stepwise oxidation; C: predigestion at $T° < 100°C$; D: end-point of the digestion, with time spent in the final stage; E: matrix; F: year of publication and bibliographical reference; f: fumes

A	B	C	D	E	F
HNO_3 $HClO_4$	a	–	⩾210°C (–)	cocoa	1950 (8)
	b	+	180°C (–)	plants, blood	1963 (9)
	b	+	$HClO_4$ f(15 min)	plants, blood	1966 (1)
	b	+	$HClO_4$ f(15 min)	plants, tissues	1969 (10)
	a	–	$HClO_4$ f(–)	food	1974 (11)
	b	+	$HClO_4$ f(15 min)	plants	1974 (12)
	b	+	$HClO_4$ f(30 min)	plants	1975 (13)
	a	+	180°C (–)	liver	1976 (14)
	a	+	125°C (12 hr)	plants, meat	1976–77 (15, 16)
	b	–	$HClO_4$ f(–)	food	1976 (17)
	a	–	210°C (15 min)	plants, blood	1977 (18)
HNO_3–H_2SO_4 HNO_3 $HClO_4$	a	–	?	cocoa	1959 (8)
$HClO_4$–H_2O_2	a	–	⩾210°C (–)	feed	1975 (19)
HNO_3–$HClO_4$–H_2O_2	b	+	$HClO_4$ f(⩾ 10 min)	plants	1969 (20)
HNO_3–$HClO_4$–$HClO_3$	a	–	240°C	food	1980 (21)
HNO_3–$HClO_4$–H_2SO_4–H_2O_2)/(NH_2OH)/ $(NH_4)_2C_2O_4$	a	–	?	cocoa	1959 (8)
	a	–	$HClO_4$ f(–)	urine	1963 (22)
	a	–	⩾290°C	plants	1968 (23)
	b	–	?	fish	1975 (24)
	a	–	⩾290°C	food	1975 (25)
	b	(+)	SO_3 f(–)	food	1974–80 (17, 26–29)
	b	(–)	SO_3 f(–)	urine	1977 (30)
	a	–	⩾290°C	fish	1978 (31)
	b	+	SO_3 f(–)	food	1978 (32)

chloric acid mixtures are used, and around 250–300° when sulphuric acid is present.

The AOAC gradually refined the methods it recommended. In 1968 Hoffman *et al.*[23] proposed the addition of nitric, perchloric and sulphuric acids to the dry sample. Watkinson's method[1] (the basis for most of the digestion procedures developed over the past 20 years) was modified by Olson[10] who introduced a predigestion stage involving solubilization of the organic matter and mild oxidation of easily hydrolysed compounds at low temperature (20–80°). The initial reaction is vigorous, with foaming and evolution of dense brown nitrogen dioxide fumes. When this reaction has subsided slight heating results in complete solubilization of all suspended matter and the real destruction can commence. As the temperature is increased, nitric acid condenses in the lower neck of the digestion flask.[23] Perchloric acid is added and heating continued until all organic materials are mineralized (near the boiling point of perchloric acid). A new reaction is seen to ensue, from the evolution of residual nitric acid; the digest rapidly turns from pale green to colourless and clear. Dense white vapours of perchloric acid are evolved, and the temperature rises to approximately 200°.[20]

Hall and Gupta[20] used a procedure in which the rate of destruction was not closely monitored, and attributed loss of selenium (up to 60%) to use of high temperatures, prolonged digestion, or both. However, it seems more logical to attribute these losses to charring during the second stage of the decomposition. Charring occurs when the acid-to-sample ratio is too low or water is withdrawn from the sample too suddenly. It yields carbon particles with reducing properties, thus converting inorganic selenium into the gaseous hydride.[1,8,32] It is often seen when sulphuric acid is added to the digest too early,[8,12,27] or the temperature is increased too fast, so that the nitric acid is driven off before solubilization is complete. It follows that predigestion should proceed smoothly if the temperature is slowly increased[13,27] with sufficient nitric acid present.[9,27] At the end of the predigestion the volume may be reduced as this shortens the total digestion time.[9] Losses due to charring are largely determined by the nature of the sample.[27] Olson demonstrated that the degree of decomposition is mainly dependent upon the length of the perchloric acid stage. His paper dealt directly with the problem of very resistant selenium compounds. He initially modified Watkinson's method by increasing the heating time to 15 min beyond the appearance of perchloric acid fumes[10] and later extended this to 30

min, to ensure the complete mineralization of $(CH_3)_3Se^+$.[13]

Both nitric and perchloric acid interfere severely in the fluorimetric determination of selenium[12,13] and to a lesser extent in the HGAAS determination.[2] Therefore the procedure finally adopted by the AOAC includes the use of a perchloric/sulphuric acid mixture at a late stage to enable nitric acid to be boiled off.[27] In a later modification sulphuric acid was added to the digest after all the organic material had already been destroyed, so that the incidence of charring could be reduced.[32] Repeated heating and cooling of the digest after the addition of small amounts of water, hydrogen peroxide, ammonium oxalate or hydroxylamine hydrochloride can be used to drive off residual traces of nitric acid.[10,17,23,26,27,30,32] During digestion with perchloric acid, selenite is easily oxidized to selenate.[1,9,26] Reduction back to selenium(IV) can be achieved in sulphuric acid medium by addition of hydrogen peroxide and heating.[9] When a nitric/perchloric acid digestion is used the reduction can be done with 4*M* hydrochloric acid.[1]

EXPERIMENTAL

Digestion apparatus

Twenty destructions were conducted concurrently, by using a temperature- and time-controlled heated aluminium block in which Pyrex tubes (diameter 42 mm, height 30 cm, usable volume 250 ml) were inserted to a depth of 6 cm. The tubes were surrounded by a shield to protect them against temperature fluctuations caused by air draughts. The system is commercially available (Tecator Digestor 20). Glass air-condensers were made and fitted to the tubes; to ensure effective reflux of acid vapours they needed to be at least 30 cm long. Selenium(IV) was reduced to selenite by heating with 4*M* hydrochlorie acid for 5 min at 100°.

For comparison some destructions were done in pressure vessels (Perkin-Elmer, Autoklav III; maximum temperature 160°, maximum pressure 145 atm) in which a 125-ml PTFE vessel fitted with a magnetic rod and a PTFE lid is accommodated by a steel mantle heated by an electrical hot plate (IKA-Combimag RCT).

Reagents and glassware

Reagents were of analytical grade. Quartz and polypropylene 25-ml standard flasks were used for aqueous standards and samples. Acid-soaked and rinsed polyethylene flasks were used for storage of digested materials at −18°.

Biological materials

Freeze-dried standard (reference) materials were stored in a desiccator and before use were dried as recommended by the manufacturer.

Blood was obtained by venepuncture with evacuated tubes (Vacutainer, Becton-Dickinson), with Li-heparin as anticoagulant. Blood was aspirated into the air-free tube, thus minimizing the risk of contamination. Plasma samples were obtained by centrifuging blood at 1100 *g* for 10 min. The supernatant plasma was transferred to polystyrene disposable tubes by means of a polyethylene syringe. Transfer of red blood cells was carefully avoided; haemolysed plasma samples were discarded. Pipetting was done with Eppendorf micropipettes provided with an n-heptane-treated polyethylene tip.

Assessment of selenium concentration

Selenium concentrations were determined by HGAAS, using optimized parameters as published elsewhere.[2] The generation medium was 0.5*M* sulphuric acid. A Perkin–Elmer 372 atomic-absorption spectrometer and MHS-1 hydride generation system were used. The method of standard additions was used throughout.

Destruction procedures

Two open wet-digestion procedures were tested; they differed in the final temperature achieved.

Procedure I. On the basis of some literature data[10,15,16,18] the following procedure was established. Accurately weigh dry material or pipette blood (or plasma) into a digestion tube. Add a few glass beads to prevent bumping. Add 20 ml of concentrated nitric acid for 2–5 g of organic material (50 ml for samples weighing between 10 and 15 g). As pointed out by Ihnat and Miller,[32] the sample size is mainly determined by the water and fat, starch or sugar content. In general, 2 ml of blood or plasma are digested.

Fit the air-condensers and place the tubes in the aluminium block. Increase the temperature to 75° during approximately 15 min. Let the mixture stand overnight at 75°, and another 6 hr at 125°. Remove the condensers and reduce the volume to 3–5 ml by heating at 125°. Add 2.0 ml of concentrated perchloric acid (72%). Keep the block temperature at 125° until the liquid volume is approximately 2 ml. Allow to cool. Add 1 ml of concentrated hydrochloric acid and heat for 5 min in a boiling water-bath. The solution should generally be clear and citron-yellow. Transfer quantitatively to a 25-ml standard flask and dilute to volume. When only 1-ml blood (or plasma) samples are available, solution should not be made up to more than 10.0 ml if the blood selenium concentration is expected to be only moderate.

A total digestion run (18 samples and 2 blanks) takes between 22 and 30 hr.

Procedure II. As will be shown, the most reliable destruction of blood was achieved with the following procedure, which combines several aspects of procedures described by others.[8–10,12–14,18,20]

The predigestion is performed as in procedure I. When the volume has been reduced to 3–5 ml at 125°, 5.0 ml of perchloric acid (72%) are added, the condensers are replaced and the temperature of the heating block is gradually increased to 210°. This usually takes 30 min. The digest is held at this temperature for 30 min. Initially brown nitrogen dioxide vapours appear in the condensers, their colour gradually becoming lighter. Nitric acid is driven off, suddenly yielding a clear and colourless solution, and the perchloric acid refluxes in the upper part of the condensers. The condensers are then removed and the digest is kept at 210°, without vigorous boiling, until the volume is reduced to about 2 ml. This is completed in less than half an hour. Selenate is then reduced to selenite as in procedure I. The digest is then transferred to a 25.0-ml standard flask and diluted to volume. The whole procedure takes between 15 and 23 hr.

In both procedures the predigestion period may be shortened to as little as 4 hr without appreciable loss of selenium.

Destruction in a Teflon bomb

A maximum of 0.5 g of dry material or 2 ml of blood (or plasma) is introduced into the vessel and 4.0 or 5.0 ml of concentrated nitric acid (according to the nature and amount of the sample) are added. A Teflon-coated magnetic-stirrer rod is introduced. The vessel is closed and placed in the steel mantle, then heated for 60 min, with magnetic stirring, the temperature at the Teflon wall being maintained at 120°. The entire bomb is then allowed to cool to room temperature, before the lid is removed. Residual

nitrogen dioxide vapours are driven off by magnetic stirring for 10 min. The contents are transferred to a 25-ml standard flask and diluted to volume. The Teflon vessel is then boiled in nitric acid and rinsed with water, to remove any stains left after the decomposition (tests of the nitric acid after this procedure have not shown any residual selenium). A blank is then prepared by applying the full treatment to the same volume of nitric acid as used for the decomposition, in the same Teflon vessel.

RESULTS AND DISCUSSION

The accuracy of a procedure refers to the overall error, and is largely determined by the systematic error or bias of the method.[34] In HGAAS, this bias, which is not due to random error of measurement, may arise in the sample preparation as well as in the final measurement. Aqueous standards that have been subjected to a given digestion procedure yield data on the recovery of selenium from the digestion matrix but the results may not reflect the complex situation which arises when selenium is present in an organic matrix.

As demonstrated by Nève *et al.*[26] and by Doll and Armbrecht[34] chemical constituents remaining after a nitric acid digestion of an organic sample such as serum may cause severe loss of both native and added selenium. Binding effects of the matrix on selenium and interference effects of the matrix on the method are generally evaluated by analysing a standard material similar in composition to test material.[34]

Table 2 gives the results obtained for several standard materials. Unfortunately the freeze-dried animal blood standard, IAEA A-2, cannot be considered a true standard reference material (SRM), as demonstrated by the large confidence interval of the recommended value for the selenium concentration. It is therefore a less suitable material for discriminating between procedures I and II; both seemed to recover all the native selenium. Appreciable losses were observed when blood was mineralized in a pressure bomb. The pressure often became too high, causing the safety valve to open, and volatile selenium compounds may thus have been lost. Such losses seemed to be more of a problem than incomplete destruction did. However, incomplete destruction (at 125°) seems to be responsible for the low concentration of selenium found in NBS bovine liver.

As no reliable SRM was available, the results for a large number of different blood samples treated by procedures I and II were compared. As it was impractical to withdraw sufficiently large blood samples from each donor for repeated analysis by both procedures, samples were analysed once by each procedure.

As the degree of biological variation is the same for both sets of results, the difference in variance between the two groups of results reflects the difference in repeatability of the destruction methods. Results for selenium in blood (170 donors) and plasma (17 donors) are given in Table 3. The mean selenium

Table 2. Analysis of standard (reference) materials. (n: number of analyses; $\bar{x}$: average Se concentration; s: standard deviation; $s_{\bar{x}}$: standard error of the mean; s_r: relative standard deviation)

Standard (reference) material and method	n	$\bar{x} \pm s$, $\mu g/g$	s_r, %	$s_{\bar{x}}$, $\mu g/g$	95% Confid. interv., $\mu g/g$	Recommended conc., $\mu g/g$ (95% conf. interv.)	Recovery, %
Bovine liver NBS 1577							
Bomb	7	$1.0_7 \pm 0.0_7$	6.5	0.02_6	1.0_1–1.1_4	1.1(1.0–1.2)	97
open, proc. I	3	$0.9_6 \pm 0.0_7$	6.5	0.04_6	0.7_9–1.1_3	1.1(1.0–1.2)	87
open, proc. II	3	$1.1_5 \pm 0.0_9$	7.8	0.05_2	0.9_3–1.3_7	1.1(1.0–1.2)	105
Animal blood, IAEA A-2							
Bomb	3	$0.36_3 \pm 0.02_2$	6.1	0.01_3	0.30_7–0.41_9	0.59(0.45–0.73)	61.5
open, proc. I	3	$0.59_0 \pm 0.01_7$	3.4	0.01_0	0.54_7–0.63_3	0.59(0.45–0.73)	100.0
open, proc. II	3	$0.64_1 \pm 0.00_9$	1.4	0.00_5	0.61_9 0.66_3	0.59(0.45–0.73)	108.5
Copepod (fish) IAEA MA-A-1							
open, proc. II	5	$2.8_3 \pm 0.1_9$	6.7	0.08_5	2.5_9–3.0_7	3.1(2.6–3.6)	91.3
Seaplant, IAEA SP-M-1							
open, proc. II	3	$0.18_0 \pm 0.01_1$	6.1	0.00_6	0.15_4 0.20_6	0.18(0.16–0.20)	100.0

Table 3. Comparison of the average Se concentration in human blood and plasma, after application of a low temperature (I) and a high temperature (II) wet digestion procedure. Symbols are the same as in Table 2

Statistical parameter	Procedure I		Procedure II	
	Whole blood	Plasma	Whole blood	Plasma
$\bar{x} \pm s$, *ng/ml*	103 ± 15	97 ± 12	125 ± 18	101 ± 12
n	170	17	170	17
s_x, *ng/ml*	1.15	3	1.38	3
s_r, %	14.6	12.4	14.4	11.9

levels in plasma did not differ significantly (two-sided t-test, $P > 0.05$), but procedure II gave an average blood-selenium concentration about 20% higher than that obtained procedure I. This difference was highly significant ($t = 12.26$, paired observations with unequal variances,[35] $P < 0.000025$, one-sided test). The variances were slightly different, but only at the 2.5% significance level; hence the repeatability of both procedures was considered very similar. All destructions were performed on fresh samples.

In recovery experiments, several selenium species were added to pooled blood samples, at the beginning of the digestion. Aqueous standards were treated in the same way. Average recoveries are given in Table 4.

Selenite was recovered quantitatively from either water or blood by the low-temperature procedure, but only 65% of the selenomethionine-selenium. When this selenomethionine digest was subjected to a second destruction in an pressure bomb, the recovery rose to 83%, indicating incomplete destruction at 125°. The high-temperature procedure gave practically complete recovery of selenite from water and blood, and of selenomethionine from water, but only 85% recovery of selenomethionine from blood. Selenocystine was recovered to a varying extent by procedure I. It is clear that blood and plasma give matrix effects. Possibly acid-resistant organic selenium compounds prevail in the erythrocytes of whole blood, which might explain the different behaviour of blood and plasma toward acid mineralization. The need for drastic decomposition was stressed by Nève *et al.*,[26] who attributed low recoveries in decompositions with nitric acid at 140–150° to matrix effects.

Recently Robberecht *et al.*[36] studied possible losses of biologically incorporated selenium during destruction by both procedures described here. Radioactive ^{75}Se was incorporated physiologically into various organs of rats. The ratio of the radioactivity before and after destruction was measured. In both cases this ratio was almost 1 for the various organs, whereas for blood 95% of the selenium was recovered.[36]

These results support our finding that the low recoveries for blood-selenium by procedure I are a consequence of incomplete decomposition and that this loss of Se(IV) is considerably more serious than volatilization losses.

Further studies were undertaken to evaluate the accuracy and the overall reproducibility of the hydride generation technique for the determination of selenium. Binding and other matrix effects could account for part of the method bias. Youden[37] has described a method to distinguish "constant bias" from "constant % bias". Increasing sizes of sample are analysed and the expected and measured concentrations of the analyte are compared. If the measurements are unbiased, the slope of the regression line should be unity. When the determinand concentration is unknown, as in our case, the quantity recovered may be expected to be directly proportional to the sample size, in the absence of bias. Selenium concentrations were therefore determined in one 0.5-ml, two 1.0-ml, two 2.0-ml, one 3.0-ml, one 5.0-ml and one 6.0-ml portions of

Table 4. Concentration of native Se in whole blood, and recovery of added Se. Symbols as in Table 2

Sample (vol.)	Native Se		Se added			
	$\bar{x} \pm s$, μg/ml	(n)	Se species + quantity of Se *ng*	Destruction procedure	Recovery, %	(n)
blank			H_2SeO_3, 300	I	106 ± 7	(4)
blood (3 ml)	0.130 ± 0.007	(4)	H_2SeO_3, 300	I	106 ± 13	(4)
blank	—		Se-methionine, 300	I	65 ± 8.5	(3)
blank	—		Se-methionine, 300	I + bomb	83 ± 4	(3)
blood	0.130 ± 0.007	(4)	Se-methionine, 300	I	64 ± 9	(4)
blank	—		Se-cystine, 300	I	87 ± 11	(3)
blood (3 ml)	0.130 ± 0.007	(4)	Se-cystine, 300	I	93 ± 14	(4)
blank	—		Se-methionine, 400	II	94 ± 0.5	(3)
blood (2 ml)	0.135 ± 0.014	(9)	Se-methionine, 400	II	85 ± 15	(5)
blank	—		H_2SeO_3, 200	II	104 ± 5	(2)

Table 5. Assessment of systematic errors due to effects of the matrix after digestion of whole blood

Se measured		Amounts of Se expected				
		With bias	Without bias ($y_{i0} = x$), with x:			
Vol., *ml*	y_i, Se found, ng	$y_b = 8.6 \pm 163.3x$	87 ng/0.5 ml	322 ng/2 ml	1004 ng/6 ml	202 ng/ml
0.5	87	90	87	81	84	101
1.0	154	172	174	161	167	202
1.0	184	172	174	161	167	202
2.0	349	335	348	322	335	404
2.0	294	335	348	322	335	404
3.0	566	499	522	483	502	606
5.0	778	825	870	805	837	1010
6.0	1004	988	1044	966	1004	1212
	$s_y^2 = \sum_{i=1}^{8} (y_i - y_{ib})^2 = 9308$					
	$s_y = \sum_{i=1}^{6} (y_i - y_{i0})^2 =$	15417	11189	9921	116637	
	Bias	−	−	−	+	

pooled blood samples, by destruction procedure I. The regression data, with and without bias, were calculated and are given in Table 5, the reference values for the "unbiased" selenium concentrations being taken successively as the concentrations found in the 0.5, 2.0 and 6.0 ml samples.

According to Youden, for 8 experimental points, the sum of the squares of the "biased" deviations must be less than 55.6% of the corresponding "unbiased" sum to indicate significant ($P < 0.05$) deviation of the experimental data from the unbiased line.[37]

From the figures in Table 5 it appears that there is no bias due to matrix effects that cannot be compensated for by the method of standard additions, whether a 0.5-ml or a 6.0-ml sample is used for estimation of the selenium concentration. The last column gives the results obtained when 202 ng/ml (the concentration found by procedure II) is taken as the "true" concentration, and these show a constant % bias from the unbiased line. This is likely to indicate that procedure I has a constant efficiency, lower than that of procedure II, for recovery of selenium as Se(IV) from blood. Efficient compensation of matrix effects by the standard-additions method was also shown by determining selenium in 10 blood and 3 plasma samples by both HGAAS and fluorimetry.[38] Each sample was decomposed by procedure II, as nitrous compounds that persist after procedure I severely interfere with the fluorimetric determination.[38] The results all belonged to the same distribution of values, as demonstrated by a paired *t*-test[35] ($P > 0.05$).

The precision and overall reproducibility of the combined destruction–HGAAS technique have been established in several ways. The liberation of selenium as H_2Se from a digest and the measurement of its absorbance at 196.0 nm are fairly precise,[39] the relative standard deviation (s_r) being below 2%, and hardly influenced by the nature of the sample. However, the condition of the atomization tube plays a major role, as discussed elsewhere.[40] The repeatability of the destruction procedures was established by analysis of pooled human blood. It was poor for destruction in the bomb ($s_r = 20\%$, $n = 8$), whereas s_r values between 8 and 11% are common for both wet procedures. Plasma may be mineralized somewhat more precisely ($s_r = 5\%$, $n = 8$). Blood samples digested in several batches did not exhibit significantly different results.

A modest interlaboratory study was undertaken to evaluate the overall reproducibility of the HGAAS technique. Blood and plasma from 3 male and 3 female donors was pooled and freeze-dried for 18 hr at 0.02 mbar pressure. The moisture content of the fresh blood was 77% w/w, and of plasma 91%. Samples were distributed to two other laboratories for determination of selenium by HGAAS.

Laboratory 3 used a destruction procedure very similar to our procedure II. Investigators in laboratory 2 used mineralization in stream of oxygen. Table 6 shows the results. From these data it is seen that differences encountered when procedures I and II are applied to fresh blood (Table 3), disappear when the procedures are applied to freeze-dried blood. When the water content was taken into account the results corresponded to those obtained for fresh blood by procedure II. It seems unlikely that the different behaviour of fresh and freeze-dried blood is merely due to chance. The plasma samples gave excellent overall reproducibility.

Our own results for blood selenium did not differ statistically from those found in laboratory 3. For procedure I the results from the three laboratories did not differ significantly, but procedure II produced a

Table 6. Evaluation of the overall reproducibility of HGAAS for the determination of Se in pooled, freeze-dried whole blood and plasma. Explanation of symbols, see Table 2

Statistical parameter	Se concentration in freeze-dried whole blood, μg/g			
	This work			
	Proc.II	Proc.I	Lab. 2	Lab. 3
$\bar{x} \pm s$	$0.52_5 \pm 0.02_1$	$0.51_9 \pm 0.02_0$	$0.48 \pm 0.02_5$	$0.53 \pm 0.03_3$
n	3	3	5	8
$s_{\bar{x}}$	0.012	0.012	0.011	0.012
s_r, %	4	3.9	5.2	6.2
range of x_i	$0.51_0 - 0.54_9$	$0.50_8 - 0.54_2$	—	$0.48 - 0.58$
$ts_{\bar{x}}$	0.052	0.052	0.031	0.028
$P_{0.95}$	0.47_3–0.57_7	0.46_7–0.57_1	0.44_9–0.51_1	0.50_2–0.55_8
	Se concentration in freeze-dried plasma, μg/g			
$\bar{x} \pm s$	$1.01_8 \pm 0.07_8$	$1.02 \pm 0.05_3$	$0.98_5 \pm ?$	
n	3	5	4	
s_x	0.045	0.024	—	
s_r, %	7.7	5.2	—	
range of x_i	0.94_4–1.01_1	—	0.92_6–1.08_5	
$ts_{\bar{x}}$	0.194	0.067	—	
$P_{0.95}$	0.82_4–1.21_2	0.95_0–1.08_7	—	

value different from that obtained in laboratory 2 (at the 5% significance level, but not at the 2.5% level). It is concluded that the overall reproducibility is very satisfactory for analysis of freeze-dried blood and plasma.

The overall reproducibility of HGAAS after mineralization of fresh blood by procedure II was tested on 40 different blood samples. After venepuncture the blood samples were divided into two parts, one of which was analysed as soon as possible, and the other frozen and transported in dry ice to laboratory 3, where it was analysed. We found an average selenium concentration of 121 ng/ml in whole blood, with a standard deviation of 13 ng/ml. The corresponding values from laboratory 3 were 121 and 19 ng/ml. Although the individual results diverged in a few instances, the overall agreement (based on pairing of observations with unequal variances[35]) was excellent ($t = 0$). Our own results gave a slightly better standard error of the mean (2.1 ng/ml, against 3 ng/ml from laboratory 3).

CONCLUSION

It has been shown that blood and plasma (especially blood) require harsb conditions for the liberation of their selenium content. Graded severity of destruction with nitric–perchloric acid mixture, with a final stage at 210°, is recommended. The accuracy and reproducibility of this method (combined with determination by hydride-generation atomic-absorption spectrometry) are satisfactory. Digestion at a lower temperature may lead to erroneous results, especially in the case of whole blood. It is emphasized that the stability of some organic selenium compounds may present a greater threat to the accuracy than does their volatility.

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THE ELECTROLYTIC TEST FOR DETECTION OF ARSENIC ALONE AND IN THE PRESENCE OF OTHER METALS

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Summary—A historical account is given of the development of the electrolytic generation of arsine in the Marsh test, showing how it was early realized that a step in an analytical procedure could be advantageously replaced by a more efficient one. It also shows that well-designed apparatus never really becomes outmoded.

The detection of toxins in the dead, especially in criminal cases, posed analytical problems in the 19th century. The same prohlems arose in the detection of toxins in adulterated food. In the preface to his book *On Poisons in Relation to Medical Jurisprudence*,[1] Taylor stated "There are many poisons which cannot at present be detected by chemical analysis, there are numerous circumstances which occur to prevent their detection in the food, the vomited matters, or the contents of the viscera in the dead." The Marsh test (1836), the Berzelius adaptation of this (1837) and the Reinsch test (1838) were available, but they only detected arsenic and antimony, and were quantitative only if applied with the utmost care. Their reliability often depended on the skill of the operator. Thus, according to Henry's diary for 1837,[2] Faraday insisted on the necessity for much practice before expressing an opinion about suspected arsenic poisoning, thought the Marsh test needed a skilled operator, and that, without proper precautions, the whole of the metal might escape without being caught. Henry also noted that Daniell considered the Marsh test a good one, but that arsenic-free tin was difficult to obtain, and a reagent blank was therefore necessary. Long before these tests were developed, however, Cruikshank, by observing the ease with which copper was deposited electrolytically, was the first to suggest electricity as a possible analytical tool for the detection of metals. By 1812, Fischer had detected arsenic electrolytically by the "galvoplastic" method, wherein the substance was precipitated on the cathode of the cell.[3] Fischer also first recognized the displacement of one metal from solution by another in what is now called the electrochemical series.[4] In 1840, Cozzi detected metals in animal fluids. In 1850 de Claubeny detected poisonous metals by electrolytic deposition on platinum, followed by dissolution and analysis by the standard methods. He considered his method totally reliable, especially for the detection of copper in bread. In 1857, Otto's *Lehrbuch de Chemie* gave a delicate test for manganese and lead by electrolysis and in the same year Despretz described the electrochemical decomposition of certain salts. Copper and lead acetates gave lead dioxide at the anode and copper at the cathode. Manganese gave no deposit on the cathode, but a black dioxide film on the anode. Potassium antimonyl tartrate gave a crystalline deposit of antimony at the cathode and a yellowish-red coating, supposed to be anhydrous antimonic acid, at the anode. Bismuth nitrate gave a reddish-brown deposit on the anode. Despretz thought his antimony, lead and manganese results were new, and that the separation of copper from lead was virtually complete.[5] In 1861, in a paper on the application of electrolysis to the detection of the poisonous metals in the presence of organic matter,[6] Bloxam stated "Every analyst is only too well aware of the difficulties which beset the detection of the poisonous metals in mixtures containing organic matters, such as the contents of the stomach, the solids and fluids of the body, and the articles of food." This may have been inspired by Todd's letter to "The Times" in 1859, following the conviction of Dr. Smethurst for murdering his mistress, Isabella Bankes, which stated: "I trust this very important case will not be lost on toxicologists ... and that it will induce analytical chemists to review carefully all the processes hitherto in use for the purpose of detecting mineral and other poisons with a view to clear up every possible source of fallacy."[7]

In his revision of Bowman's *Practical Handbook of Medical Chemistry* (4th Ed., 1862), Bloxam described his electrolytic test for arsenic,[8] listing the objections to the Marsh test, such as the presence of arsenic in commercial sulphuric acid, and of arsenic and antimony in zinc, the frothing of the reaction mixture despite the addition of alcohol, and the impossibility of further analysis of the mixture because of the large excess of zinc sulphate. He then stated: "The detection of the poisonous metals by the decomposing action of the galvanic current is, I think, free from these objections, and so minute quantities of the poisonous metals may be detected by this method, that it

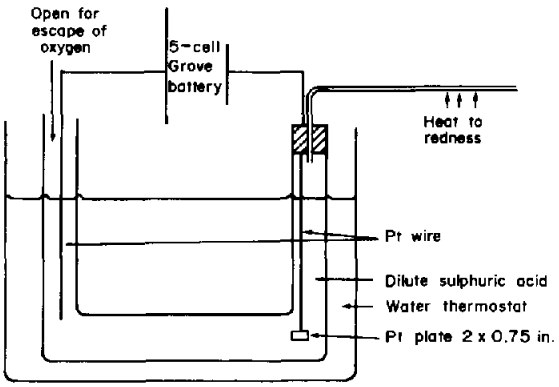

Fig. 1.

may safely be relied upon in most cases of chemico-legal investigations."[6] Bloxam's first apparatus for the analysis is shown in Fig. 1.

First the U-tube was charged with 1 fl. oz of sulphuric acid (1 + 4) and the emission tube was heated to redness for 15 min before introduction of the specimen, to verify that no arsenic was present in the acid. Then portions of arsenious acid solution containing 0.076, 0.0076 and 0.00076 grn of arsenic were successively introduced. In all cases good mirrors were obtained. Bloxam then pulped 1 oz of lean meat, $1\frac{1}{2}$ oz of milk and $\frac{1}{2}$ oz of white of egg in a mortar, mixed all this with 5 fl. oz of hydrochloric acid (1 + 4), digested the mixture on the water-bath for 15 min, filtered, and evaporated the filtrate to $3\frac{1}{4}$ fl. oz of dark brown viscid liquid. To this he added 0.1 grn of arsenious oxide and placed a quarter of the whole in the decomposition tube. He obtained an arsenic mirror for this and smaller quantities of arsenious oxide, down to 0.01 grn.[6]

One problem Bloxam had to overcome was that the arsenious sulphide often present in such mixtures was insoluble in hydrochloric acid, and he wondered whether heating with potassium chlorate and hydrochloric acid would lead to a positive result. He showed that it was necessary to reduce the resulting arsenic(V) back to arsenic(III) with sulphurous acid if a mirror was to be obtained from small quantities (0.05 grn). A mixture similar to that just mentioned gave a good arsenic mirror. He also showed that arsenic could be detected in beer (added to the mixture). He was still not satisfied, however, since not all the arsenic was detected. Using two cells, separated with a parchment membrane, to prevent the chlorine evolved at the anode from passing to the cathode and converting the liberated arsine into arsenic(III) chloride, he was able to show that this was the source of the discrepancy, and with this apparatus he could detect 0.0001 grn of arsenious oxide in a mixture of foods.[6] His apparatus at this stage was as shown in Fig. 2.

The advantages of this method were the ability to detect arsenic by use of platinum, a metal not contaminated with it, the same sulphuric acid could be used throughout and tested for any length of time before the introduction of the specimen, the experiment could be interrupted at any time by breaking the circuit, and both the clearest and the foulest mixtures could be analysed equally well, leaving a residue that could be further analysed for other metals. However, Bloxam concludes: "On considering the detection of the other poisonous metals in this way, it is obvious that lead must be altogether excepted on account of its insoluble sulphate. Silver must also be omitted, where hydrochloric acid is the solvent; and baryta, of course, would not be expected to answer. The remaining important poisonous metals, antimony, copper, mercury, bismuth and zinc, were therefore tried, bismuth being included on account of the medicinal use of its compounds."[6]

Bloxam was very interested in the determination of antimony at the cathode. To the food mixture used for the detection of arsenic he added 0.01 grn of tartar emetic (*i.e.*, 0.036 grn of Sb) and analysed as for arsenic. The antimony deposited on the cathode was dissolved in ammonium polysulphide solution and the solution was evaporated on a watch-glass till an orange stain (indicating antimony) appeared. On the strength of his results Bloxam compiled a scheme of analysis for the poisonous metals antimony, mercury, copper and bismuth but forgot to state specifically that arsenic was also detectable. If mercury interfered with the arsenic determination, the liquid from the decomposition cell or a fresh portion of the original hydrochloric acid solution should be distilled to separate mercury from arsenic. Later, Bloxam showed how the interference of mercury could be avoided and how the error caused by the evolution of stibine might be obviated.[9] He also improved his apparatus yet again by using broad strips of platinum foil instead of wire for the electrodes, and adding the sample to the cathode compartment through a thistle funnel.

To prevent the evolution of stibine, he boiled the sample with hydrochloric acid and potassium chlorate, evaporated the solution to low bulk, saturated it with hydrogen sulphide and placed it in the decompo-

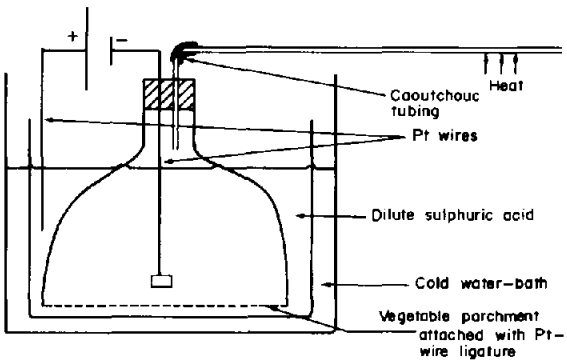

Fig. 2.

sition cell; deposits of arsenic and arsenious sulphide were formed in the heated tube. The dark precipitate collected after the experiment was identified as Sb_2S_3. Bloxam showed that, with 1 grn of mercuric chloride and 0.01 grn of arsenious oxide (or 0.25 and 0.0025 grn respectively) mixed with white of egg, bread, milk and beer, there was no difficulty in detecting the arsenic. The addition of hydrogen sulphide to the electrolytic cell yielded a crust of arsenic, whereas without it only mercury was deposited on the cathode. Similar results were obtained with remains which had putrified for about a year. Bloxam was particularly concerned about the purity of his reagents (with good reason) since he was unable to obtain hydrochloric acid pure enough for large quantities to be used without contributing a detectable blank. The sulphuric acid available was also impure, and he later published a paper on the production of pure sulphuric acid for the purpose.[10] He also feared that the potassium chlorate and hydrogen sulphide used were impure. He recommended the reaction:

$$Sb_2S_3 + 6HCl = 2SbCl_3 + 3H_2S$$

for the production of the hydrogen sulphide, but did not suggest how to obtain pure potassium chlorate.

Underlying the tests for the poisonous metals there clearly run organic (medical) and inorganic (mineral) criteria of applicability. Not only the poisonous metals, however, were to come under investigation by electrolytic methods. As the science of electrochemistry developed, its range was extended to estimation of many more substances. This progress is well outlined by Smith.[11]

Bloxam's work in this direction was seminal. His papers of 1861 and 1862 were the efforts of an analyst attempting to achieve workable detection techniques for arsenic, originally in connection with death by arsenic poisoning. He also saw the use to which his test might be put in inorganic mineral analysis.

The medical analyst, seeing (as Bloxam saw) difficulties in the application of the test to large quantities of viscera, might well have been deterred from pursuing its application. For example, the Joint Committee of the Society of Public Analysts and the Society of Chemical Industry was still recommending the standard Marsh–Berzelius test for arsenic in 1909.[12] By this time, the hydrochloric and sulphuric acids were pure enough for the purpose, if subjected to special further purification. The zinc used was also specially purified, the standard arsenic mirrors were made with 4, 6, 8 and 10 μg of arsenious acid, and the test still involved visual comparison.[12]

In inorganic analysis, however, continued interest in the electrolytic arsenic test was predictable, and Thorpe[13] and Hefti[14] used it in mineral analysis.[13] The typical electrolytic apparatus used for this purpose in the present century,[13] apart from the use of a mercury cathode, is very reminiscent of Bloxam's 1862 apparatus.

The early researches of Bloxam into arsenic detection are thus seen to be both an important link in electrolytical analysis and illustrative of the zeal of an analyst to apply new methods to the problems of toxicology, as well as providing a period piece of instructive electrochemistry.

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SYSTEME D'ANALYSE ET D'ACQUISITION DE DONNEES CINETIQUES PILOTE PAR MICROPROCESSEUR

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Résumé—Cet article décrit un système interactif et économique d'analyse et d'acquisition de données cinétiques basé sur un microprocesseur 6809. La détermination de constantes de vitesse du second ordre nécessite le calcul préliminaire d'un paramètre du système chimique étudié. La valeur de ce paramètre, calculée et affichée par notre appareillage, permet le contrôle de la validité des conditions expérimentales. Les données cinétiques retenues sont finalement enregistrées sur cartouche magnétique en vue d'un traitement ultérieur sur mini-ordinateur.

On peut accéder à des constantes de vitesse élevées du second ordre par une méthode stationnaire en diminuant la concentration des réactifs et en ralentissant ainsi la vitesse de réaction. Dans ces conditions (TFCR EXSEL: Très Faible Concentration en Réactif Excès de Sel),[1] pour les constantes de vitesse les plus élevées accessibles (10^8 l.mole^{-1}.sec^{-1}), le temps de demi-réaction est de l'ordre de la seconde. Cependant plus la concentration des réactifs est faible plus la mesure sélective précise du courant faradique devient difficile. Par la mise au point d'un potentiostat à réaction positive et à circuit détecteur de courant à haute sensibilité, nous avons réussi à détecter spécifiquement le courant faradique parmi tous les courants parasites présents.[2]

Or le dépouillement manuel de la courbe cinétique traduisant la vitesse de disparition d'une espèce électroactive par l'enregistrement en fonction du temps de son courant limite de diffusion (Fig. 1) est une opération longue et peu précise. Le résultat obtenu peut en plus être fonction des choix de l'opérateur. En outre il est très difficile d'estimer rapidement *a priori* si les conditions expérimentales (état du solvant, agitation) sont adéquates pour obtenir des mesures valables.

Pour dépasser ces limitations nous avons conçu un système à microprocesseur (Fig. 2) adapté à nos conditions expérimentales et permettant:

—un contrôle rapide de la validité de ces conditions expérimentales;
—une amélioration de la précision des mesures par la mise en oeuvre d'un ensemble matériel et logiciel adapté à nos besoins;
—une amélioration de la reproductibilité des résultats par un traitement informatique ultérieur des données cinétiques enregistrées sur cartouche magnétique.

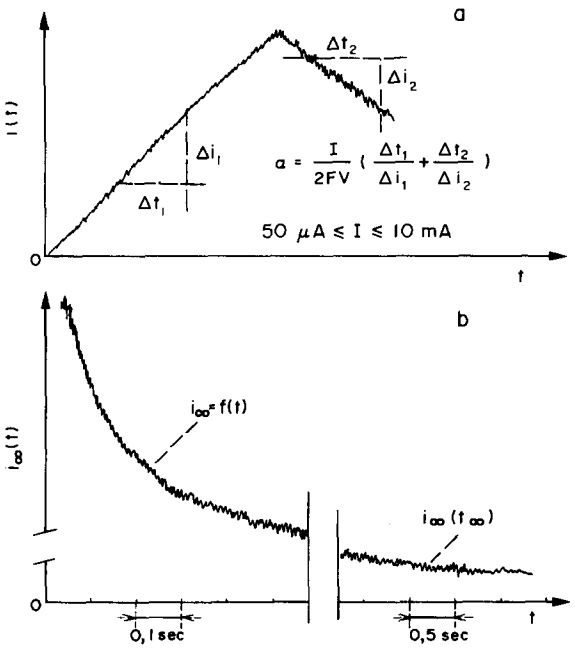

Fig. 1(*a*) Electrolyse et détermination de α: F = 1 Faraday (96500 C); V = volume de la cellule; I = courant d'électrolyse. (*b*) Courbe cinétique.

Contraintes expérimentales

La mesure précise du courant faradique $i(t)$ (Fig. 1) auquel correspond une variation globale de tension de quelques mV doit répondre à différentes exigences:

—pour ne pas diminuer la précision de l'enregistrement de $i_\infty(t)$, il faut que le traitement analogique introduise une erreur de l'ordre de 0,1% maximum, ce qui correspond à une résolution de 10 bits;
—vu le mode de calcul de la constante de vitesse k (à partir de la pente p de $\log([i_\infty(t)]/[i_\infty(t) - i_\infty(t_\infty)])$, il faut que $i_\infty(t) - i_\infty(t_\infty)$ soit connu avec la meilleure précision possible;
—à cause du bruit (dû en partie à l'agitation) superposé à $i_\infty(t_\infty)$, il est nécessaire d'accroître la réso-

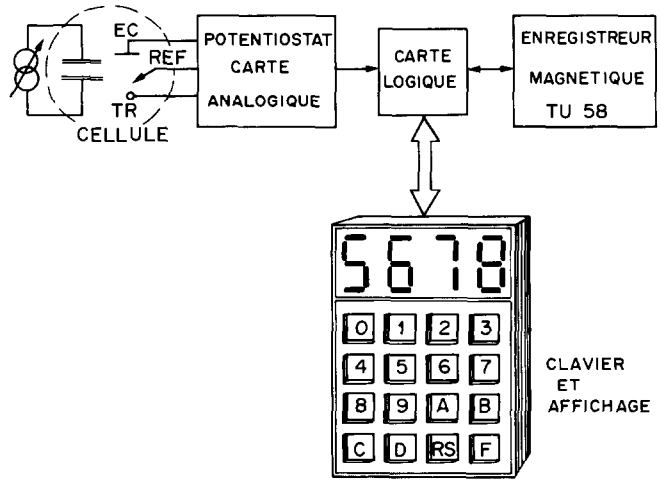

Fig. 2. Schéma descriptif de l'appareillage.

lution de cette mesure en vue d'un moyennage ou lissage ultérieur.

PARTIE EXPERIMENTALE

Description des circuits analogiques

Pour réduire au minimum les erreurs (statique, dynamique et de réjection) nous avons choisi (Fig. 3):

—un amplificateur d'instrumentation type AD522[3] stable et précis à faible niveau, peu bruyant entre 0 et 10 Hz, avec une erreur de non-linéarité de gain très nettement inférieure à 0,1% sur toutes les gammes (G = 1, 2, 5, 10, 20 et 50 dans notre cas) et avec un RRMC élevé indispensable à la réjection de la tension parasite de 50 Hz superposée au signal et provenant de la haute impédance de l'électrode de référence;

—un convertisseur tension–fréquence type AD537K[4] associé à un compteur programmable; il intègre le signal pendant le temps de conversion choisi et possède ainsi, pour des temps de conversion multiples entiers de la période du secteur, une réjection théoriquement infinie pour le 50 Hz et ses harmoniques. En fait, pour une fréquence du secteur variant entre 49,5 et 50,5 Hz, la réjection reste[5] au minimum de 37 db qui ajoutés aux 75 db de l'amplificateur d'instrumentation (gain = 1) donne au minimum 112 db de réjection du secteur;

—un photocoupleur qui transmet le signal de sortie du convertisseur tension-fréquence vers l'unité logique qui de ce fait peut être éloignée du dispositif expérimental sans risques de perturbation. De plus ce dispositif assure de la façon la plus efficace possible la séparation entre la masse analogique et la masse numérique.

Microprocesseur et circuits périphériques

L'acquisition des données en provenance du convertisseur tension–fréquence, leur prétraitement (calcul de α), leur enregistrement sur cartouche magnétique et la gestion de l'ensemble du système sont confiés à un microprocesseur MC6809.[6] A ce microprocesseur nous avons associé un certain nombre de circuits (Fig. 4) dont les caractéristiques et la fonction sont décrites ci-après:

—un circuit "mémoire" EPROM effaçable par U.V., de capacité 2K × 8 bits, type 2716[7] contenant l'ensemble logiciel;

—4 circuits "mémoire" RAM statiques type 2114[8] de capacité globale 2K × 8 bits, 512 octets permettant le stockage temporaire des données cinétiques, 512 autres octets étant réservés à la gestion de l'enregistrement magnétique;

—un PTM 6840[9,10] dont les 3 compteurs sont utilisés de la manière suivante:

(*a*) le compteur 1 compte les impulsions en provenance du convertisseur tension–fréquence après synchronisation sur l'horloge du microprocesseur à l'aide d'une bascule type D,

(*b*) le compteur 2 programmé en mode monostable établit les temps de porte pour le compteur 1 et, par conséquent, la résolution de la mesure,

(*c*) le compteur 3 détermine la cadence d'échantillonnage et déclenche une interruption de programme (ligne $\overline{\text{FIRQ}}$) pour chaque point acquis;

—un UART 6850[10] auquel sont associés un générateur de bauds et des circuits convertisseurs de niveau (standard RS232C); ces circuits assurent une liaison type série avec l'enregistreur magnétique à cartouche TU 58;[11] seize vitesses de transmission standard sont sélectionnables au moyen de commutateurs DIL (50 à 19200 bauds), différents formats pouvant être choisis par programme; on peut donc facilement envisager l'utilisation d'autres types de périphériques (ordinateur hôte, disquette, etc...);

—un PIA 6821[10] dont un port est utilisé pour l'interfaçage du clavier; une pression sur l'une quelconque des touches du clavier déclenche une interruption de programme (ligne $\overline{\text{IRQ}}$) dont la priorité est inférieure à celle générée par le PTM: une perturbation accidentelle de la mesure est ainsi exclue.

Clavier et affichage (Fig. 5)

Afin de permettre le contrôle interactif du déroulement de l'expérience, nous avons adjoint au système un clavier et un afficheur gérés tous les deux par le microprocesseur. Ceux-ci ont été placés dans un boitier séparé style calculatrice de poche (Fig. 2) pour des raisons ergonomiques.

Clavier. Il s'agit d'un clavier matriciel à 16 touches dont les fonctions sont définies par programme. Ce clavier est encodé par un circuit intégré CMOS encodeur de clavier type 74C922.[12]

Afficheur. L'afficheur comporte 4 indicateurs "7 segments + point" à diodes électro-luminescentes pilotés par un circuit intégré CMOS type 74C911.[12]

Logiciel

L'ensemble logiciel de 2K octets comporte un moniteur permettant (Fig. 6):

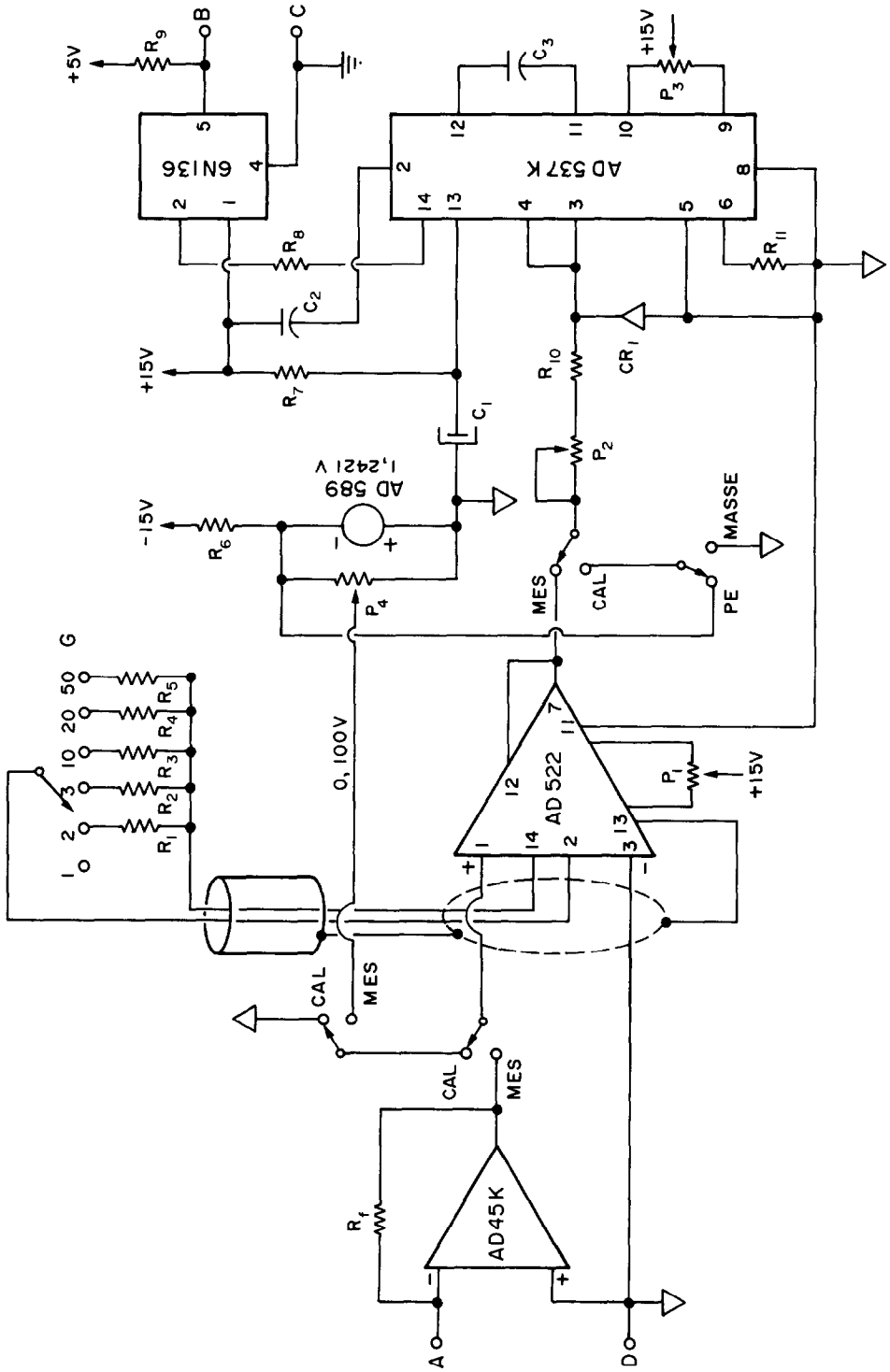

Fig. 3. Carte analogique $R_1 = 200000\ \Omega$; $R_2 = 50000{,}0\ \Omega$; $R_3 = 22222{,}2\ \Omega$; $R_4 = 10526{,}3\ \Omega$; $R_5 = 4081{,}63\ \Omega$; $R_6 = 2{,}7$ kΩ; $R_7 = 100\ \Omega$; $R_8 = 820\ \Omega$; $R_9 = 2{,}4$ kΩ; $R_{10} = 820\ \Omega$; $R_{11} = 8{,}3$ kΩ; $P_1 = 10$ kΩ (20 tours); $P_2 = 200\ \Omega$; $P_3 = 20$ kΩ; $P_4 = 10$ kΩ; $C_1 = 2\ \mu$F; $C_2 = 10$ nF; $C_3 = 680$ pF (NPO céramique); $CR_1 = HP\ 5082 - 2811$ Diode Schottky.

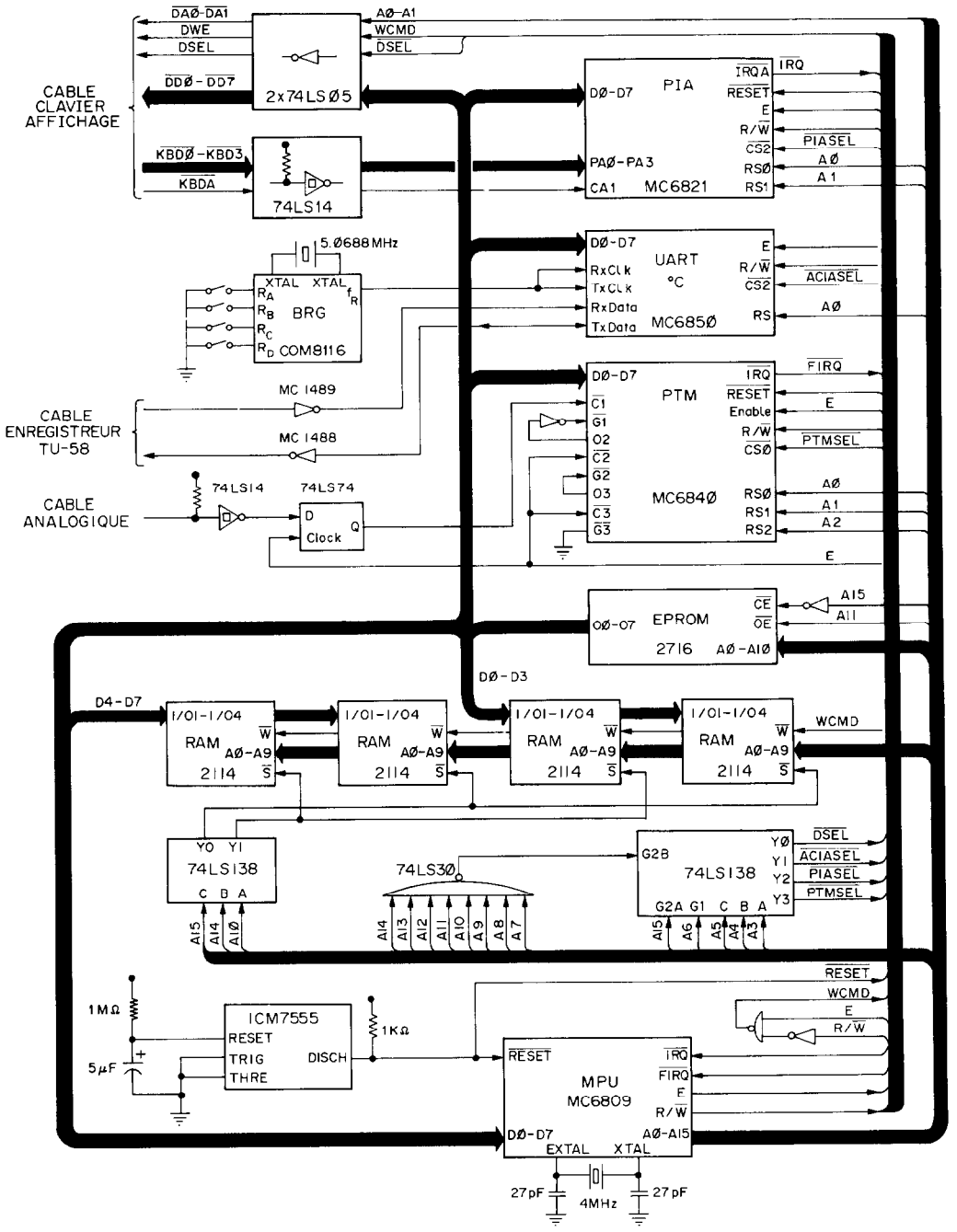

Fig. 4. Carte logique.

—d'initialiser et de tester le système à sa mise sous tension;

—de gérer les différents périphériques;

—d'interpréter les différentes commandes (acquisition, calcul de α, enregistrement).

Programme moniteur et de gestion des périphériques (Fig. 6). Les différents programmes associés aux périphériques (clavier, afficheur, cartouches) permettent à l'opérateur d'une manière interactive.

—d'entrer par le clavier en mode interruptible les commandes suivantes:

(*a*) C0: calcul de α par la méthode des pentes,
(*b*) C1: acquisition d'un signal,
(*c*) C2: enregistrement des données,

—de contrôler par affichage (résultats, messages, erreurs) le bon déroulement de la commande en exécution;

—d'enregistrer en fin de cinétique les valeurs de $i_\infty(t)$, $i_\infty(t_\infty)$, ainsi qu'un certain nombre de paramètres (gains,

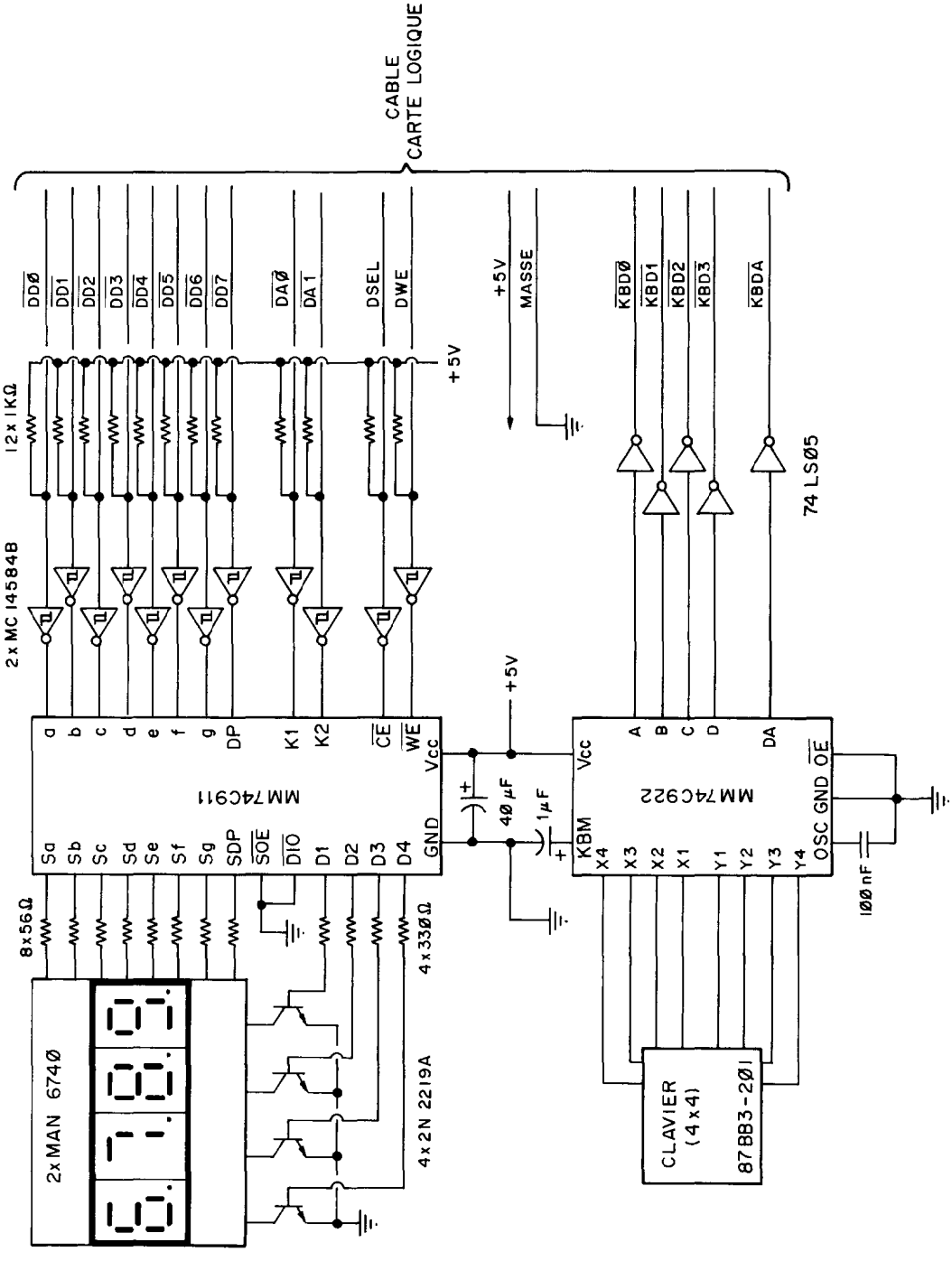

Fig. 5. Clavier et affichage.

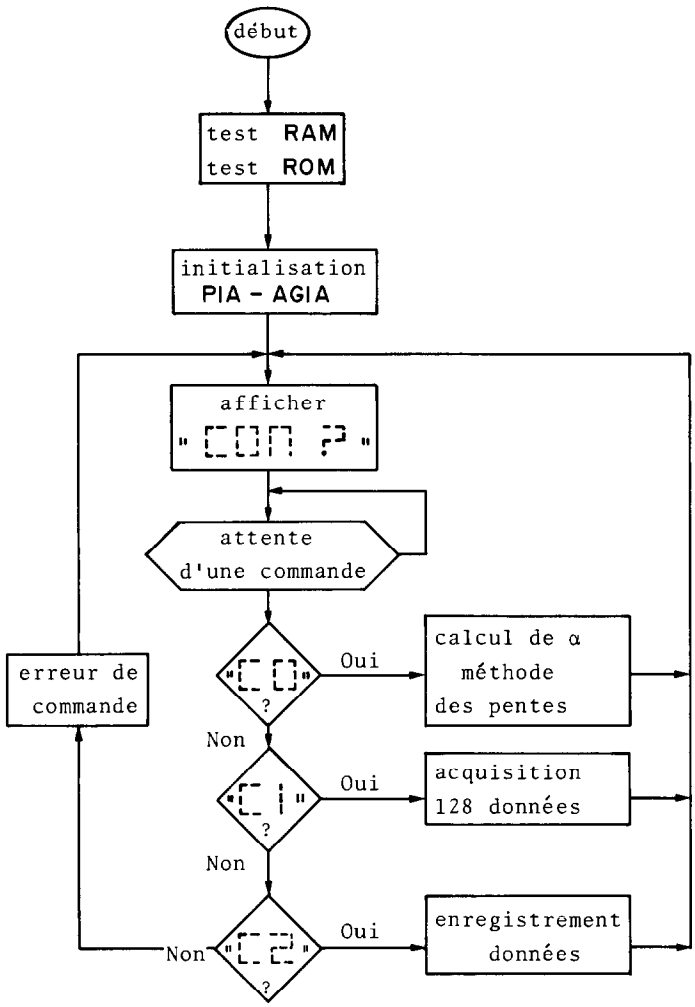

Fig. 6. Organigramme général.

pas d'échantillonage) sur cartouche magnétique dans un fichier de 512 octets.

La structure de fichier choisie est compatible avec le format de fichier du système d'exploitation RT 11[13] en vue d'un traitement plus élaboré sur ordinateur PDP 11/10.

Module d'acquisition. Ce module gère l'acquisition, soit du signal cinétique $i_\infty(t)$ (128 points de 16 bits), soit de $i_\infty(t)$ et $i_\infty(t_\infty)$ (64 points supplémentaires de 16 bits).

Le programme demande à l'opérateur d'entrer un pas d'échantilonnage de 0,1 sec pour les cinétiques rapides ou 0,5 sec pour les cinétiques plus lentes auquel correspond respectivement un temps de porte de 20 ou 40 msec; les registres du PTM sont alors initialisés en fonction de ce choix. Par pression sur la touche F du clavier l'opérateur déclenche le processus d'acquisition.

Module de prétraitement des données (Fig. 7). Ce module, appelé par la commande C0, détermine la valeur de la constante α qui, d'une part, permet de contrôler la validité des conditions expérimentales avant la cinétique et, d'autre part, entre dans le calcul de la constante de vitesse.[14]

L'opérateur, après avoir entré à l'aide du clavier les valeurs du gain, du pas d'échantillonnage et du courant d'électrolyse, commande l'acquisition de deux signaux linéaires successifs (128 points par signal) dont le module calcule les pentes, ces dernières permettant la détermination de α (Fig. 1a). Cette valeur est calculée en virgule fixe sur 32 bits puis affichée en vue d'interprétation.

PERFORMANCES DU SYSTEME

A partir des spécifications garanties par le constructeur et pour un temps de porte de 20 msec, l'erreur de mesure maximale calculée est de 0,12% à 25° et de l'ordre de 0,25% pour une variation de température de 30°. Des mesures effectuées sur des périodes de 10 hr et pour des variations de température de l'ordre de 15° montrent que l'erreur globale reste inférieure à 0,1% même pour des courants faradiques très faibles (quelques mV à l'entrée de l'amplificateur d'instrumentation).

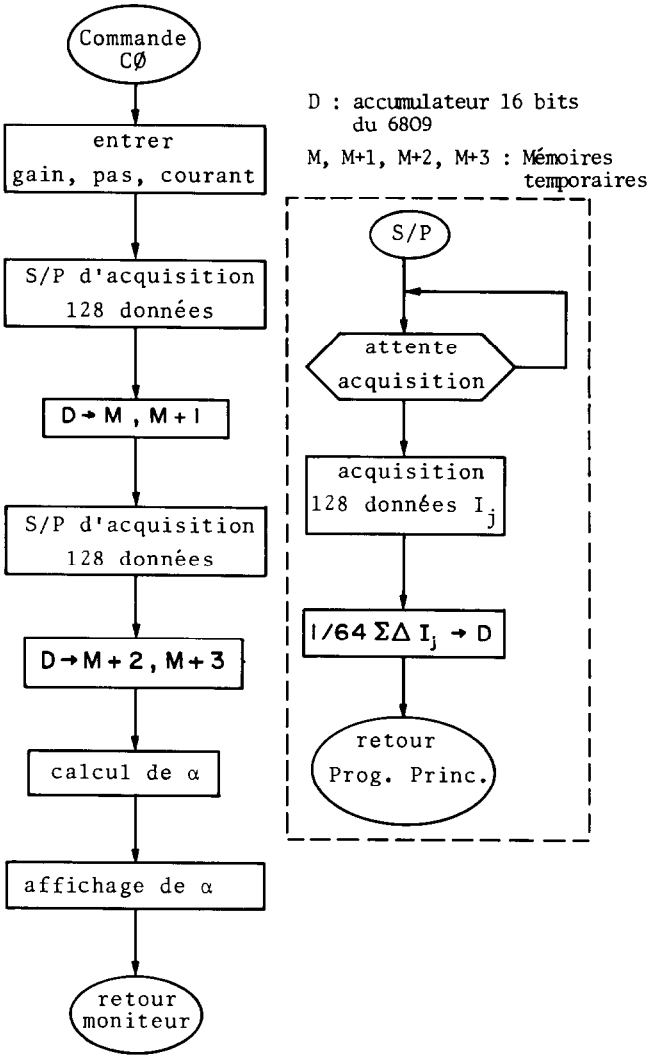

Fig. 7. Organigramme du calcul de α (Commande C0).

CONCLUSION

La structure modulaire du système (matériel et logiciel) et sa très grande souplesse d'emploi devraient, moyennant quelques adaptations (conversion de niveau, programme), permettre son application à d'autres techniques cinétiques et spectroscopiques (écoulement bloqué, spectroscopie de masse, UV, IR, *etc.*).

La liaison série RS 232C en sortie permet d'envisager d'autres dispositifs de stockage temporaire (disques souples) ou de traitement sur le site par liaison à un ordinateur hôte.

Remerciement—Nous tenons à exprimer notre vive gratitude au Professeur J. E. Dubois pour l'intérêt qu'il a porté à ce travail et pour l'aide précieuse qu'il nous a apportée.

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Summary—This paper describes a low-cost 6809 microprocessor-based system designed for the acquisition, analysis, preprocessing and recording of electrochemical kinetic data. Determination of second-order rate constants involves a preliminary calculation of a characteristic parameter of the electrochemical system investigated, the value of which, computed and displayed by our apparatus, allows checking of the correctness of the experimental conditions. At the end of the experiment the data are recorded on a magnetic tape cartridge and can be transferred from the tape to a minicomputer for further mathematical processing.

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A DUMMY CELL FOR DIFFERENTIAL-PULSE POLAROGRAPHIC ANALYSERS

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Summary—A non-linear network is proposed for simulating the response of electrochemical cells in differential-pulse polarographic (DPP) analysis. The response of a DPP analyser connected to the dummy cell is a bell-shaped current peak (I_p) located at any desired point along the potential-scan range. Approximate model calculations of the expected I_p as a function of the dummy-cell parameters are in good agreement with measured data. It is suggested that the dummy cell could be useful in the analytical laboratory and during studies for improving DPP analysers.

Differential pulse polarography (DPP) has been shown to have great potentialities as an analytical tool for the determination of trace heavy metals[1,2] and other compounds.[3] In this voltammetric method, the electrochemical cell is subjected to a pulse-type potential-scan which produces large transient currents, thereby increasing the sensitivity of analysis. The signal-to-noise ratio is further improved by applying a differential mode of operation in which the background current preceding the potential-pulse period is subtracted from the pulse current.[4]

A typical DPP polarogram for a single oxidation–reduction reaction is a bell-shaped current peak, located at a characteristic potential E_p which is a function of $E_{\frac{1}{2}}$ of the reaction and other parameters of the experimental conditions and electrochemical reactions involved.[5] This characteristic response is not reproduced when a classical dummy cell (composed of a linear RC network[6]) is substituted for the electrochemical cell. The classical dummy cell being a linear network, its response is independent of the scan potential. Consequently, the response obtained with such a dummy cell is constant, except for the transient currents at the beginning and end of the scan. Such a cell therefore cannot be used as a means of testing the operation of polarographic analysers in the DPP mode.

The purpose of this study was to investigate the possibility of devising an electrical dummy cell that would reproduce the normal DPP response of an electrochemical cell. It was deemed necessary for the response of the polarographic analyser, when loaded by the dummy cell, to be a current peak located at any desired point on the potential-sweep range. To meet these goals, the dummy cell must reproduce the behaviour of the real electrochemical cell in at least two respects: (*a*) it should simulate the response of the cell to a pulsed potential-excitation, and (*b*), its response must be dependent on the scan potential in such a way that the maximum response (vis-à-vis DPP) is obtained at a preselected potential. The first requirement could be met by including a capacitor in the dummy cell to simulate the transient diffusion-dependent current produced in response to the pulse excitation. The second requirement calls for the inclusion of a non-linear response with respect to the voltage applied across the dummy cell. This could be accomplished with a diode network that would switch from a state of cut-off to a state of conductance at a given potential.

THE DUMMY CELL

The design of the proposed dummy cell follows the concept outlined above. It was found that the design could be simplified by allowing a connection to the electronics earth (ground) besides the normal connection to the terminals for the working, reference and auxiliary electrodes. The consequence of using this approach is that the response of the dummy cell will depend not only on the potential difference between the working electrode (WE) and reference electrode (REF) but also on the potential of these electrodes with respect to the ground potential of the system. Since these voltages are dependent on the electronic design of each particular polarographic analyser, it is not possible to offer a universal dummy cell that will be suitable for operation with any polarograph. However, once the concept of operation of the proposed dummy cell is comprehended, it is relatively simple to modify the design of the cell so that it can be used in conjunction with polarographic analysers of different designs. The particular dummy-cell design given here is directly compatible with potentiostats in which the WE is at ground, (or virtual ground) potential.[4,7–9] The polarographic analyser used in this study is of this design. It includes a three-electrode potentiostat, an amplifier, and analogue gates to generate the exci-

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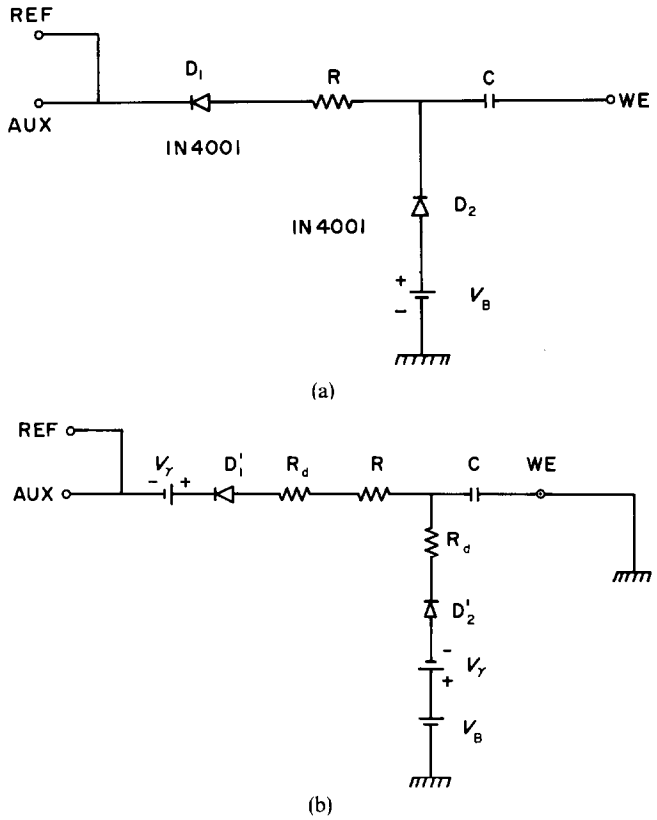

Fig. 1. Proposed dummy cell (*a*) and equivalent linear circuit used in model calculations (*b*) (for meaning of V_γ, see Appendix).

tation potentials and process the current response in differential mode. The design is similar to the one described by Vassos[4] except that the potential scan is not a linear ramp on which the pulses are superimposed, but rather a staircase waveform as described earlier.[5,8,10]

The dummy cell (Fig. 1a) comprises a resistor (R, resistance R), a capacitor (C, capacitance C), two diodes (D_1, D_2), and a bucking-off voltage source that could be a battery, a floating power supply or a grounded power supply (V_B). The diodes form a switch that blocks the passage of the excitation pulses to the RC network (and to the WE) when V_B is negative with respect to V_{REF} (referred to ground and the WE). Current pulses will be fed to the WE when V_B is sufficiently positive with respect to the REF electrode for D_1 to be conducting. Thus with ideal diodes conduction should occur only at the voltage of V_B, but because of the exponential nature of real diode conduction curves at emf below 0.6 V, the conduction window is broadened, giving the peak shape displayed by a DPP polarogram.

No attempt has been made to conduct an accurate mathematical analysis of the response of the proposed dummy cell to the pulsed-potential excitation used. Such an analysis, which must take into account the non-linearity and non-ideality of the practical diodes used, could be done by standard numerical-analysis methods. Such a treatment was deemed superfluous, in view of the fact that the response could be easily measured, but because in practical applications it would be desirable to have a rough estimate of the peak current (I_p), and peak-current potential (E_p), we present in the Appendix an approximate derivation of these parameters. In most cases, however, determination of the components of the dummy cell by trial and error will probably be preferred.

EXPERIMENTAL

Instruments and cells

The dummy cell was tested in conjunction with a Ben-Gurion University Model E1204 polarographic analyser[8,10] to which a Rekondenki Model BW-11 x–y plotter was connected. A standard power supply was used as the buck-off voltage source (V_B). The dummy cell was constructed from standard electronic components.

DPP procedure

All measurements were made with $E_{pulse} = 50$ mV, $E_{step} = 10$ mV, $t = 640$ msec.[8,10] The potential scan was started at $V_{REF} = 1400$ mV and terminated at $V_{REF} = 200$ mV. V_B was set to 1500 mV.

RESULTS AND DISCUSSION

The response of the polarographic analyser, when loaded by the proposed dummy cell and operated in the DPP mode, was a bell-shaped current peak with an amplitude dependent on the series resistance

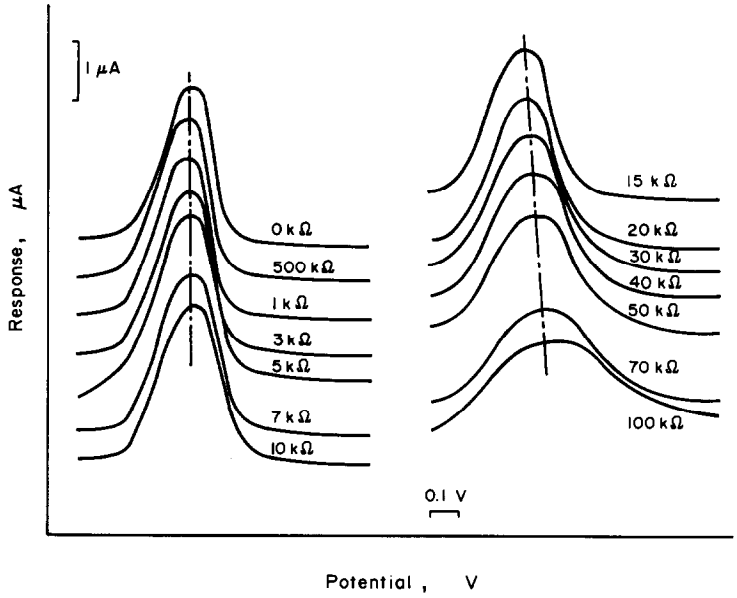

Fig. 2. DPP current response obtained with proposed dummy cell, for various resistors, R; V_B = 1500 mV. Peaks are located at about V_{REF} = 800 mV.

(Fig. 2). When V_B (Fig. 1) was 1.5 V, the current peak was obtained at E_p = 0.8 V as expected (see Appendix). The normalized peak current (Fig. 3) was found to be dependent only on τ and E_{pulse}, as predicted from the derivation given in the Appendix. However, the functional relationship between I_P/C and τ_R (= CR) did not follow exactly the predicted values obtained from the approximate analysis given in the Appendix. This is attributed to the fact that the approximate derivation does not allow for variations of R_d along the V–I curve for the diodes. Nonetheless, the values predicted by the linear model were close to the measured ones when R_d is between 6 and 10 kΩ, which was the actual range of R_d.

Besides determining the current peak-height, the charging time-constant also had a marked effect on the shape of the I_P curve (Fig. 2). The width at half-height ($\Delta E_{\frac{1}{2}}$) increased as a function of τ_R (Fig. 4), ranging from about 200 mV for $\tau_R = 0$ to about 550 mV for τ_R = 250 msec. It should be noted, however, that long time-constants are inconsistent with the approximate analysis given in the Appendix, which assumes that the response for each pulse is independent of that for the preceding pulse. By the

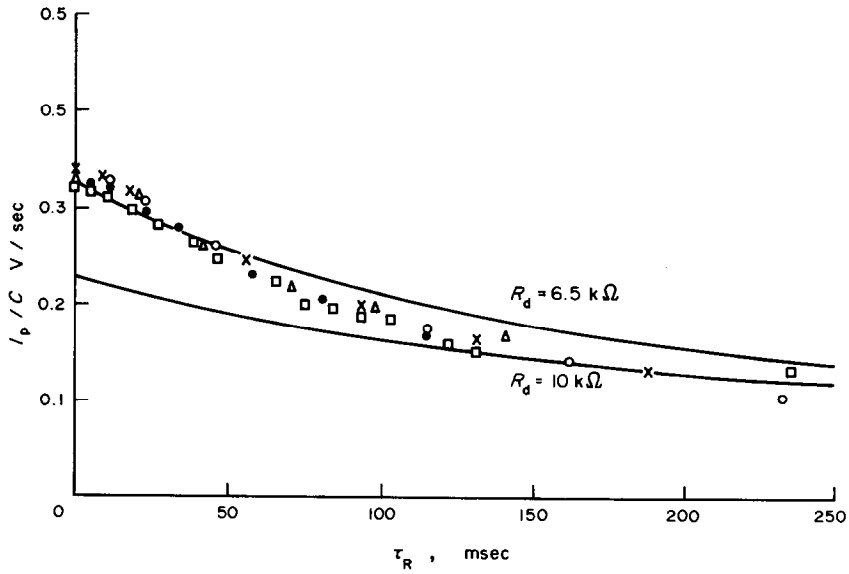

Fig. 3. Calculated and measured peak response (I_P/C) as a function of charging time-constant, $\tau_R = RC$. Solid line: model calculation; □ 9.4 μF; ○ 23.2 μF; ● 11.6 μF; △ 14.1 μF; × 18.8 μF.

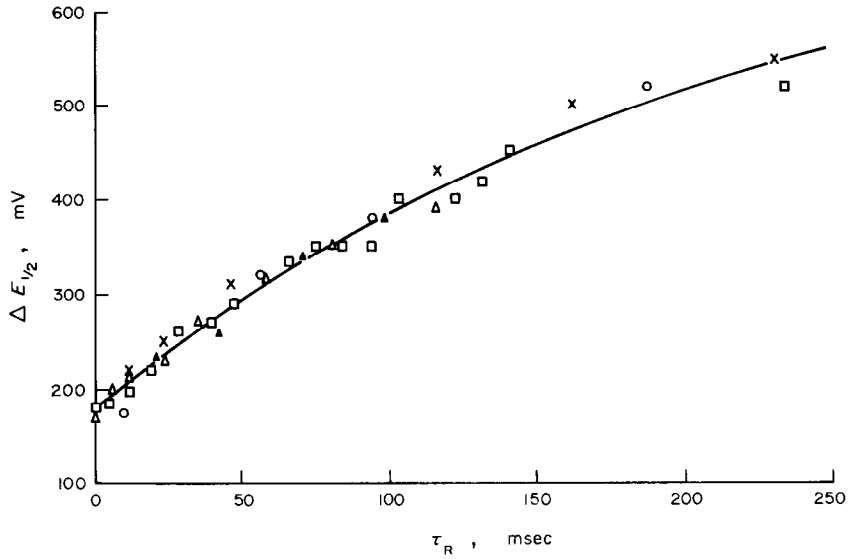

Fig. 4. Current-peak width at half-height ($\Delta E_{\frac{1}{2}}$), as a function of charging time-constant $\tau_R = RC$. □ 9.4 μF; △ 11.6 μF; ○ 18.8 μF; × 23.2 μF. Solid line is a least-squares fit of the data to an exponential curve.

least-squares fitting technique, the $\Delta E_{\frac{1}{2}}$ *vs.* τ_R function was approximated by an exponential function of the form:

$$\Delta E_{\frac{1}{2}} = V_{off} + V_{max}[1 - \exp(a\tau)] \qquad (1)$$

The best fit (Fig. 4) was obtained for $V_{off} = 178.9$ mV; $V_{max} = 557.1$ mV; $a = -4.608$ sec^{-1}.

This relationship is presumably a result of the exponential nature of the characteristic *V–I* curves of the diodes.[11,12] No attempt was made to derive an analytical expression for $\Delta E_{\frac{1}{2}}$.

A general comparison cannot be made between the DPP response obtained with an electrochemical cell and the one obtained with the proposed dummy cell, because the electrochemical response is a function of the particular experimental conditions used.[1,13] Comparison between the I_P obtained with the dummy cell and the I_P range found in an ASV analysis conducted with the same analyser and a glassy-carbon electrode,[10] showed that an I_P range of a few μA corresponds to a heavy metal concentration range of few ng/ml for a 30-sec deposition time. The $\Delta E_{\frac{1}{2}}$ previously obtained with an electrochemical cell[10] was about 70 mV, whereas the range 200–500 mV was obtained in this study. However, these comparisons are superficial since the DPP response is extremely sensitive to the particular experimental conditions used.

A closer examination of the processes which determine the response of the electrochemical cell and the dummy cell reveals that the mathematical relationships are of different nature. The time-dependence of the diffusion-controlled current in response to a potential step is proportional to $1/\sqrt{t}$,[14,15] whereas the charging current of the dummy-cell capacitor is proportional to $\exp(-t/\tau)$ (see Appendix). The I_P responses could be made equal for any given experimental conditions, but the equality may break down if any of the experimental parameters, say E_{step}, E_{pulse} or the excitation-pulse forms, is changed.

Notwithstanding the different processes which control the response of the real cell and the proposed dummy cell, the latter can be extremely useful in particular experimental situations. A typical problem which arises during a set of DPP measurements is to locate the source of trouble when malfunction occurs. An important step in a systematic procedure for pinpointing the reason for an analytical difficulty is to determine whether the source of the trouble is the analyser or the electrochemical cell. This could be easily accomplished by using the proposed dummy cell for testing the analyser response independently of the electrochemical cell. Since the analyser response when connected to a dummy cell is predetermined, any deviation from normal operation, such as a change in amplifier gain, incorrect scan-potential or waveform, or a malfunction of the differential processor, will cause a change in the standard response. The proposed dummy cell can also be applied in instrumentation studies aimed at improving the sensitivity or noise-rejection capability of DPP analysers.[8,16] In such studies it is imperative to obtain a reproducible response during many measurements that may extend over a long period of time. Under these conditions, the real electrochemical cell may prove to be inconvenient, owing to problems associated with the long-term stability and reliability of reference and working electrodes. Hence, in such situations, the application of the proposed dummy cell could be beneficial. However, since the response of the proposed dummy cell is only a first approximation to that of the electrochemical cell, care should be taken to examine the

extent to which the dummy cell is capable of simulating the real cell in any given experimental conditions.

APPENDIX

Approximate analysis of the dummy cell

The analysis is done on an equivalent circuit (Fig. 1b) which is based on a linear, piecewise approximation of the dummy cell network.[13,14] The peak current will be obtained when the two diodes just about reach the edge of conductance; *i.e.*, when

$$V_{REF} \cong V_B - 2V_\gamma \qquad (A.1)$$

where V_γ is the break-point of the *V–I* characteristic curves for the diodes.

The *V–I* characteristic curves of the IN4001 diodes used here show a voltage break at approximately 350 mV and a dynamic resistance range of 5–10 kΩ. It should be noted that this break-point at low current levels is at a much lower voltage than the 0.6 V break-point usually assumed for silicon diodes. This fact is well known[11,12] and is attributed to different conduction mechanisms in the silicon *p–n* junction.

The *V–I* characteristic curve of a diode and resistor in series, which represents the case in hand, seems to justify the linear piecewise equivalent circuit used in the approximate analysis.

The potential pulse, superimposed on this V_{REF} (equation A.1) will drive D_1 into conduction and will charge C through R. This charging current is amplified by the input circuit of the polarograph and processed by the analyser to produce the output response. It is assumed that the base-line current, which is subtracted from the pulse response, is zero, since the diodes were at the edge of conductance until the pulse appeared.

Since *C*, which simulates the diffusion process, is large, the voltage fluctuation across it will be relatively small, *i.e.*, it is assumed that C does not charge appreciably during the pulse period. Hence, D_2 will remain at the edge of conduction during the pulse period. An approximate value for I_P—or more accurately, the upper limit of I_P—can thus be derived by calculating the charging current of C through $(R + R_d)$ assuming that the current through D_2 is zero, and that the response to the preceding pulse excitation has already subsided.

The charging current will thus be:

$$I_C = \left(\frac{E_{step} + E_{pulse}}{R + R_d}\right)\exp\left(-\frac{t}{\tau}\right) \qquad (A.2)$$

where E_{step} = step height, E_{pulse} = pulse height, $\tau = (R + R_d)C$.

The response output of the polarograph is obtained by integrating the input current over one cycle of the power-line frequency:

$$I_C = \frac{E_{step} + E_{pulse}}{R + R_d}\int_{t_1}^{t_1+\Delta}\exp\left(-\frac{t}{\tau}\right)dt \qquad (A.3)$$

where t_1 = initial integration delay (20 msec), $\Delta = 1/f$ = period of power-line frequency (50 Hz in our case). Hence:

$$\frac{I_P}{C} = (E_{pulse} + E_{step})\left[\exp\left(-\frac{t_1}{\tau}\right) - \exp\left(-\frac{t_1+\Delta}{\tau}\right)\right] \qquad (A.4)$$

which predicts that the normalized peak current (with respect to *C*) will be dependent only on E_{pulse} and τ, given that t_1 and τ are fixed for a given polarographic analyser.[8]

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FLOW-INJECTION ANALYSIS OF OXIDIZABLE SPECIES WITH REVERSE-PULSE AMPEROMETRIC DETECTION

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Summary—The technique of reverse-pulse amperometry is applied for the detection of oxidizable organic species at a solid-electrode flow detector. Exploiting the reverse-pulse amperometric waveform gives better sensitivity than d.c. amperometric detection. Species (*e.g.*, phenols) giving responses that are poorly separated from background are easily monitored. Reducible species can be monitored without deaeration of the solution. The effects of flow-rate, waiting time between pulses, precision, and linearity of response are reported. At a flow-rate of 1.0 ml/min injection rates of 180 samples per hour and detection limits of a few tenths of a nanogram are obtainable.

Continuous analysis in flowing streams with solid-electrode flow detectors has gained increased attention in recent years.[1,2] Most solid-electrode detectors utilized in liquid chromatography (LC) or flow-injection analysis (FIA) employ d.c. amperometric detection. Recently, several pulsed-potential waveforms have been applied to LC and FIA detection, mainly in conjunction with cathodic reactions at the dropping mercury electrode. These include differential-pulse detection,[3] square-wave detection,[4] and reverse-pulse amperometric detection of amalgam-soluble metals.[5] Applications of reverse-pulse amperometry (RPA) to the flow analysis of oxidizable organic species at a solid-electrode detector have not yet been described.

This study characterizes the analytical utility of RPA in a flow-cell with a carbon electrode, as applied to redox reactions in which the (oxidizable) reactant and the (reducible) product coexist in solution. This approach is a variant of normal pulse voltammetry; it is based on the application of an unsymmetrical square-wave potential with a long application of a positive initial potential E_i, followed by a short pulse at a more negative final potential E_f. Oxidizable species are measured by monitoring the reduction (at the end of the more negative pulse) of the oxidation product from the initial potential (in the plateau region):

$$A \xrightarrow{E_i} B \xrightarrow{E_f} A$$

The current sampled at this point is proportional to the concentration of the species in the flowing stream. The generation/detection capability of RPA yields, at a single electrode, advantages similar to those reported recently for dual-electrode detectors.[6,7] Different aspects of electrochemical detection can be improved with the RPA detection mode: sensitivity, detection of species with high redox potentials, and detection of reducible species without interference from dissolved oxygen. The equipment is simple (a modern polarographic analyser with the normal pulse mode and a potential-hold capability) and available in most laboratories.

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EXPERIMENTAL

Apparatus

The electrochemical "wall-jet" detector has been described in detail previously.[8] The working electrode was a planar glassy-carbon disk (0.25 cm diameter) with a solution stream directed onto it from a solution inlet nozzle (0.34 mm diameter). The distance between the nozzle tip and the surface of the glassy carbon was 0.025 cm. An Ag/AgCl reference electrode was placed in the cell downstream from the working electrode.

The carrier and sample solutions were stored in two Nalgene beakers, with similar hydraulic heads to provide equal flow-rates. These reservoirs were connected, through two Teflon tubes (1.0 mm bore, 0.05 mm wall), to a three-way Teflon stopcock located 12 cm from the detector. The stopcock and the cell were connected by 1.0-mm bore Teflon tubing. All measurements were made with a Princeton Applied Research Model 174 Polarographic Analyzer. Detection peaks were recorded on a Houston Omniscribe strip-chart recorder.

Reagents

The chemicals and reagents used have been described in detail previously,[9] except as noted. Stock solutions of NADH, chlorpromazine, phenol and benzoquinone were prepared each day. Aliquots of the stock solution were added to the phosphate buffer supporting electrolyte to give the desired concentration.

Procedure

The potential limits (E_i and E_f) for the RPA were set with the PULSE mode of the polarographic analyser, in a similar way to that described by Maizota and Johnson.[5] E_i and E_f were chosen to be in the limiting current regions for the forward and reverse reactions, respectively. The flow-

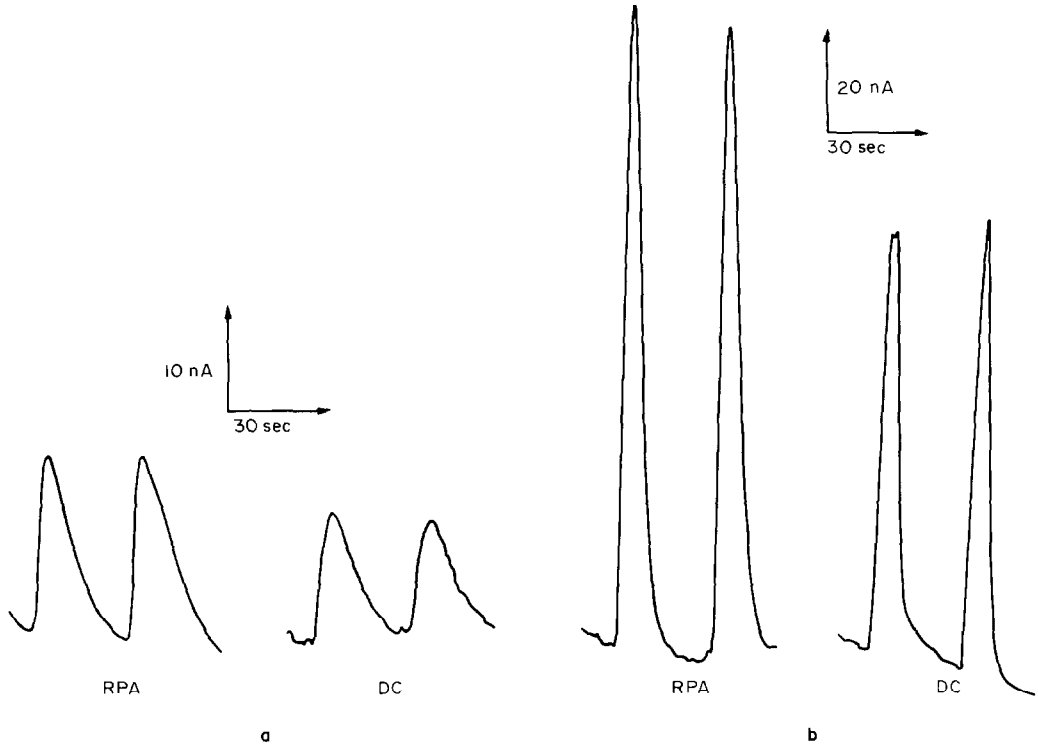

Fig. 1. Typical peaks for detection of 10μM ferrocyanide (a) and NADH (b) by FIA and RPA and d.c. detection modes. Conditions: flow-rate, 1.0 ml/min; Sample flow, 5 sec; carrier flow (0.1M phosphate buffer), 25 sec; sample size, 83 μl; pulse repetition time, 0.5 sec; low-pass filter, 1 sec; potentials, (a) RPA with $E_i = +1.0$ V, $E_f = -0.3$ V; d.c. at +1.0 V; (b) RPA, $E_i = +0.9$ V, $E_f = +0.2$ V; d.c. at +0.9 V.

injection measurements were performed with the three-way valve by alternating periodically, for fixed times, between the carrier solution (the supporting electrolyte) and the sample solution. Details are given in the following section.

RESULTS AND DISCUSSION

Figure 1 compares RPA detection peaks with those obtained in conventional d.c. amperometric detection for injections of 10μM solutions of ferrocyanide (a) and NADH (b). The advantage of the RPA detection mode is evidenced by its higher (by ~50%) peak currents. A surprising aspect of the RPA detection is that compounds (*e.g.*, NADH, ascorbic acid) which appear chemically irreversible with "slower" potential-scan techniques (such as cyclic voltammetry), have a significant RPA response because of the different time scale. (Initially, we intended to exploit the expected discrimination against compounds with irreversible redox reactions for improving the selectivity in flow-analysis of mixtures containing "reversible" and "irreversible" compounds.) A similar observation was reported recently for d.c. measurement at a dual-electrode detector with a short spacer between the upstream and downstream electrodes.[7]

For comparison of the results obtained by RPA with those for d.c. detection, the relative signal, expressed as RPA-peak current/d.c. peak current (i_{RPA}/i_{dc}) may be used. The value of i_{dc} is given by the equation for the limiting current at the "wall-jet" detector [ref. 10 equation (10)], and the RPA response can be described on the basis of the mixed hydrodynamic–Cottrell behaviour of pulse voltammetry at convective solid electrodes.[11] At low convection rates and short pulse-widths the RPA current will obey the Cottrell equation (and will be independent of convection transport); at higher convection rates, the convection transport will control the current. This behaviour is indicated by the data of Fig. 2a. The relative insensitivity of the RPA response to the flow-rate (below 1.0 ml/min) would be advantageous for making measurements in flowing systems with poorly controlled flow-rates. The mixed hydrodynamic–Cottrell RPA response assumes that the oxidation product is stable and is not adsorbed on the electrode. Therefore, RPA may be used as a tool for confirming the identity of an analyte which gives a product (at E_i) that is subject to chemical reaction or adsorption at the electrode (with the i_{RPA}/i_{dc} value as the criterion in a similar way to the collection efficiency in ring–disk or other dual electrode experiments). Table 1 gives the i_{RPA}/i_{dc} values for several compounds. Though for most compounds a 50% increase in sensitivity is observed ($i_{RPA}/i_{dc} \sim 1.5$), for chlorpromazine (a compound known to interact with a carbon surface[12]) there is a 20% decrease ($i_{RPA}/i_{dc} = 0.79$).

The effect of the waiting time between pulses (pulse repetition time) on the RPA response is shown in

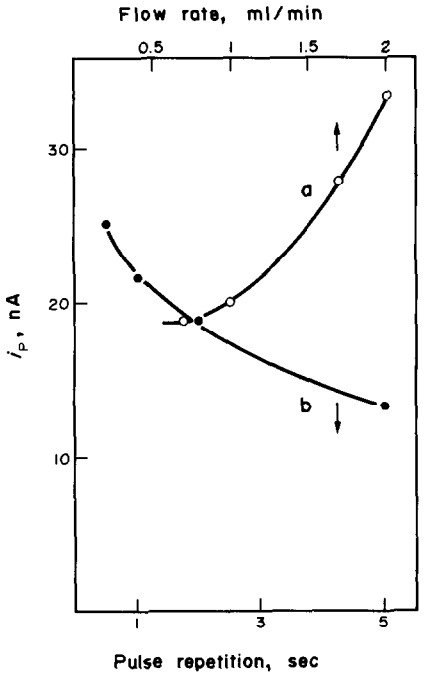

Fig. 2. Dependence of the FIA/RPA peak currents on the volume flow-rate (a) and the waiting time between pulses (b). Conditions: (a) 10μM ferrocyanide; sample flow, 5 sec; carrier flow (0.1M phosphate buffer) 25 sec; $E_i = +1.0$ V; $E_f = 0.3$ V; low-pass filter, 1 sec; (b) 5 μM benzoquinone; sample flow, 5 sec; carrier flow (0.1M phosphate buffer) 20 sec; flow-rate, 1.0 ml/min; $E_i = -0.8$ V; $E_f = +0.6$ V; low-pass filter, 1 sec.

Fig. 2b. The four repetition times available with the PAR 174 analyser were examined. As the waiting time increases, from 0.5 to 5 sec, the FIA/RPA peak current decreases by more than 50%. This is because as the waiting time increases, the frequency of sampling the current decreases, *i.e.*, fewer points are sampled during the passage of the short peak-shaped sample-plug profile through the detector (a peak-shaped response was obtained at the different times employed). Different effects on the peak current are expected if larger volumes are injected or if the product formed at E_i is not stable. From the data found, a repetition time of 0.5 sec was selected.

The main application of RPA detection is not to measurement of easily-oxidized analytes. Amperometric d.c. detection of compounds which react at high potentials (> 1.0 V), where background processes (water or mobile-phase oxidation) occur, suffers from detector drift, increased background and noise levels, and the need for high current gain.[6,7,13] These compounds may be better detected by using the generation/detection capability of RPA, *i.e.*, by detecting the oxidation product at lower potentials. A reverse-pulse procedure was exploited in a similar way to deal with the interfering hydrogen-evolution reaction, in batch analysis at the dropping mercury electrode.[14] Among the important compounds that are oxidized at potentials near that for solvent decomposition is phenol, the oxidation of which reaches a current plateau at potentials higher than +1.2 V (*vs.* Ag/AgCl electrode).[15] Problems associated with d.c. amperometric detection of phenolic compounds have been reported.[15,16] Figure 3a illustrates the RPA detection peaks for successive injections of 2.6μM phenol in phosphate buffer solution (corresponding to 20 ng in the injection volume used). The sampling rate is 180/hr. Well-defined and reproducible peaks are observed. A detection limit near 50nM (0.4 ng) is expected (signal/noise = 2).

Two separate experiments were performed to estimate the precision of the results. For a series of 10 repeated injections of a 25μM ferrocyanide solution, the average value of the peak current was 0.364 μA (with a range of 0.354–0.370 μA), and the relative

Table 1. Values of i_{RPA}/i_{dc} for several compounds*

Compound	E_i, V	E_f, V	i_{RPA}/i_{dc}
Ferrocyanide	+0.9	0.0	1.55
Chlorpromazine	+0.9	0.0	0.79
NADH	+0.9	+0.2	1.46
Phenol	+1.4	−0.1	1.54
Benzoquinone	−0.8	+0.6	1.41

*Concentration, 10 μM. FIA conditions and instrumental parameters as for Fig. 1. The d.c. measurements were made at potential E_i.

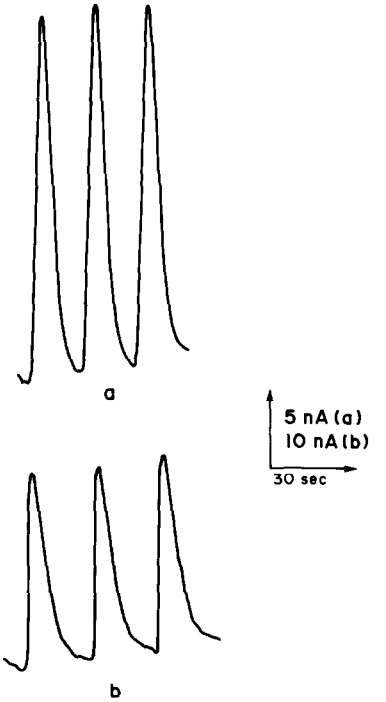

Fig. 3. Detection peaks in flow-injection analysis of a 2.6μM phenol solution (a) and a 5μM benzoquinone solution (b). Conditions: flow-rate, 1.0 ml/min; repetition time, 0.5 sec; low-pass filter, 1 sec; sample flow, 5 sec; carrier flow (0.1M phosphate buffer), 15 sec (a); 20 sec (b); potentials, $E_i = +1.4$ V, $E_f = +0.6$ V (a).

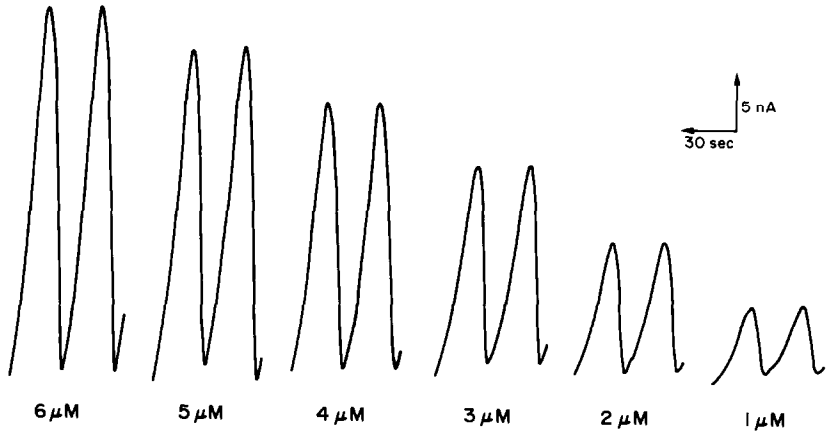

Fig. 4. Reverse-pulse amperometric detection for injections of 1, 2, 3, 4, 5 and 6 μM chlorpromazine solutions. Flow conditions, repetition time and filter, as for Fig. 1. $E_i = +0.9$ V, $E_f = 0.0$ V.

standard deviation was 1.3% (conditions as in Fig. 1a, except that the carrier flowed for 10 sec). A series of 8 repeated injections of an 11μM phenol solution yielded an average value for the peak current of 139 nA (range 134–142 nA), and a relative standard deviation of 1.0% (conditions as in Fig. 3a, except that the carrier flowed for 25 sec).

Another benefit of RPA detection is that low concentrations of reducible species can be monitored without interference from dissolved oxygen. The classical d.c. amperometric detection of reducible species usually requires the removal of oxygen from the test solution. MacCrehan and Durst[6] employed serial dual-electrode detection for eliminating oxygen interferences. In RPA detection, a similar advantage is obtained with a single electrode. Species are reduced at E_i (where reduction of oxygen occurs), and the species generated are oxidized and measured at E_f (which is insufficient to oxidize the hydrogen peroxide produced in the reduction of oxygen). This advantage of RPA detection was demonstrated[5] for detection of amalgam-forming metal ions by use of a dropping mercury electrode. We have exploited this capability for detecting reducible organic species at a solid electrode. Typical peaks for 5μM benzoquinone obtained by FIA and RPA are shown in Fig. 3b. A defined anodic response is obtainable through the oxidation, at $E_f = +0.6$ V, of the hydroquinone generated (by reduction) at $E_i = -0.8$ V.

To demonstrate the utility of RPA for measurements of low concentrations of organic compounds, FIA current–time data were recorded (Fig. 4) for chlorpromazine in the 1–6μM concentration range (corresponding to 29–174 ng for the 83-μl injection). Well-defined peaks and low noise level are observed. The estimated detection limit (signal/noise = 2) is 57 nM, which corresponds to 1.6 ng for the sample volume injected. The data of Fig. 4 yielded a linear calibration plot, the slope of which corresponds to a sensitivity of 5.9 nA.l.μmole^{-1} (correlation coefficient 0.995, intercept −0.2 nA). The tailing of the peak and the longer (25 sec) wash time needed in the chlorpromazine experiment may indicate that for compounds like chlorpromazine,[12] that interact with the carbon surface the RPA involves a slow "stripping" of the analyte from the surface.

In view of the results presented here, RPA flow-detection of electroactive species may be considered as a rival approach to d.c. amperometric detection. The low detection limit is a result of combining a sensitive detection mode with an effective "wall-jet" detector. The improved sensitivity, and detection of compounds with extreme redox potentials or of reducible species without the need for deaeration, indicate great promise and applicability for flow-through detectors.

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DETERMINATION OF tert-BUTYLHYDROQUINONE IN EDIBLE OILS BY DIFFERENTIAL-PULSE POLAROGRAPHY

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Summary—A differential-pulse polarographic method for the determination of tert-butylhydroquinone (TBHQ) in vegetable oils has been developed and tested with a variety of oil samples. No prior extraction or other separation of TBHQ from the sample matrix is required. An oil sample is dissolved in a 1:5 v/v toluene/ethanol mixed solvent containing an acetate buffer, and polarograms are obtained directly for this solution. Recovery studies indicate that this method is suitable for routine determination of TBHQ at the concentrations normally used in edible oils.

Various phenolic antioxidants are commonly added to cooking oils and other food products to increase their stability. Among the most commonly used of these antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG). One of the newest food-grade antioxidants to be approved for use in certain classes of food is tert-butylhydroquinone (TBHQ). Regulations issued by the U.S. Food and Drug Administration permit the use of TBHQ by itself, or combinations of TBHQ with BHA and/or BHT, in food products at a maximum antioxidant concentration of 0.02% w/w of the fat or oil in the food.[1] TBHQ can also be used in animal and poultry fats at a maximum concentration of 0.01%.[1]

TBHQ has been determined at low concentrations in fats, oils and other food products by means of a colour reaction with dimethylamine.[2] A gas chromatographic method for TBHQ has been described in another publication.[3] These procedures necessarily require prior extraction of the TBHQ from the food product.

Most antioxidants can be anodically oxidized at suitable electrodes in appropriate solvent systems. The electrochemical behaviour of a number of phenolic antioxidants has been reported,[4–7] with correlation of polarographic half-wave potentials with structure and/or antioxidation efficiency.

The anodic oxidation reactions of 2-substituted hydroquinones in aqueous perchloric acid solutions have been studied by cyclic voltammetry and controlled-potential coulometry.[8] With a methyl group in the 2-position, a simple 2-electron oxidation of the hydroquinone to the quinone occurs. A similar 2-electron oxidation of TBHQ to the corresponding quinone (TBQ) would be expected under normal voltammetric conditions:

$$TBHQ \rightarrow TBQ + 2H^+ + 2e^-$$

The polarographic behaviour of a series of alkylated hydroquinones, including TBHQ, was studied by Ryba *et al.*[7] They determined the half-wave potentials ($E_{1/2}$) for a number of these compounds in 50% v/v aqueous ethanol buffered with acetate. The $E_{1/2}$ values for anodic oxidation of TBHQ exhibited a pH-dependence consistent with a simple 2-electron oxidation reaction. The $E_{1/2}$ value for TBHQ at 25° was 0.067 V *vs.* SCE in a supporting electrolyte consisting of 0.10*M* sodium acetate/0.10*M* acetic acid in 50% v/v aqueous ethanol.

Luckadoo[9] developed a method for determination of TBHQ in safflower oil by d.c. polarography. This procedure involved extraction of the oil sample with methanol and polarographic determination of TBHQ after addition of a small volume of aqueous acetate buffer. The average recovery for TBHQ was 98%.

The previously cited methods for determination of TBHQ all involve a separation step prior to analysis. McBride and Evans[10] reported a method for rapid voltammetric determination of tocopherols, BHA and PG in oils and fats that does not require a prior separation. This linear-sweep voltammetric procedure allows determination of BHA and PG at concentrations above 0.001%. The differential-pulse polarographic method reported here also allows the direct determination of TBHQ in common types of vegetable oil without prior separation.

EXPERIMENTAL

Reagents

Antioxidants. The following food-grade antioxidants were obtained from Eastman Chemical Products. Inc.: Tenox® TBHQ, Tenox® 22 (20% BHA, 6% TBHQ, 4%

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anhydrous citric acid, 70% propylene glycol), Tenox® 26 (10% BHA, 10% BHT, 6% anhydrous citric acid, 28% corn oil, 6% TBHQ, 28% glyceryl mono-oleate, 12% propylene glycol), Tenox® 20 (20% TBHQ, 10% anhydrous citric acid, 70% propylene glycol) and Tenox® 20A (20% TBHQ, 3% anhydrous citric acid, 32% glyceryl mono-oleate, 15% propylene glycol, 30% corn oil).

Cooking oils. Several brands of cooking oil containing no preservatives were purchased in local supermarkets: A (pure soybean oil), B (partially hydrogenated soybean oil), C (partially hydrogenated soybean oil with polyglycerides), D (partially hydrogenated soybean oil with polyglycerides), E (partially hydrogenated soybean oil, polyglycerides, artificial colour and flavouring), F (sunflower and soybean oils), G (sunflower oil), H (safflower oil), I (corn oil with isopropyl citrate), J (peanut oil).

An additional oil which contained TBHQ as a preservative was also purchased locally: K (sunflower oil with TBHQ and citric acid).

Solvents and other reagents. Absolute ethanol (U.S.P.) was obtained from U.S. Industrial Chemicals Co. All other chemicals used were reagent grade.

Apparatus

All polarograms were obtained with a Princeton Applied Research (PAR) Model 174A Polarographic Analyzer equipped with a PAR 174/70 drop-timer. The current–potential curves were recorded on an MFE Model 815 x–y recorder.

A three-electrode cell system including a Metrohm EA874 cell top and Metrohm EA875-20 cell bottom was used for all experiments. A saturated calomel electrode (SCE) with a porous Vycor® junction and a separate salt bridge with porous Vycor® junction was used as the reference electrode. The auxiliary electrode was a platinum foil of about 8 cm^2 area. The dropping mercury electrode (DME) had a flow-rate of 1.71 ± 0.03 mg/sec. This flow-rate was checked weekly.

TBHQ standard solutions. A stock solution of TBHQ was prepared by weighing 0.1662 g into a 10-ml standard flask, and dissolving and diluting it to volume with absolute ethanol. A $1.00 \times 10^{-3}M$ TBHQ standard solution was prepared by 100-fold dilution of the stock solution with absolute ethanol. Fresh solutions were prepared daily.

Preparation of TBHQ solutions in oils. The following approximate amounts of TBHQ were weighed accurately into 150-ml beakers: 0.005, 0.01 and 0.02 g. The selected oil was added carefully to bring the individual weights to about 100 g and the mixtures were weighed accurately. The samples were dissolved in the oil by stirring for about 3 hr at room temperature, with an argon atmosphere maintained over the sample. The oil samples were subsequently stored in polyethylene bottles.

Solutions of TBHQ in one of the oils were also prepared by using the Tenox® 20, 20A, 26 and 22 antioxidant preparations in such quantities as to give final antioxidant concentrations that corresponded to the levels allowed by U.S. FDA regulations.

Procedures

For investigation of the polarographic behaviour of solutions of TBHQ in various solvent/supporting electrolyte systems, all working solutions were prepared from stock solutions of the supporting electrolyte by adding increments of the TBHQ standard solutions. The solvent systems used were 50% ethanol, absolute ethanol and 1:5 v/v toluene/absolute ethanol. Various acetic acid (HOAc)/sodium acetate (NaOAc) buffer systems were used as the supporting electrolytes.

Differential-pulse polarography. Differential-pulse polarograms were obtained with solutions that had been purged with argon (saturated with the appropriate solvent) for at least 15 min immediately before the initial scan. The solutions were blanketed with argon during each scan and purged briefly with argon after each increment of TBHQ standard was added, before the next scan. The scans were all made at 2 mV/sec scan-rate, 25 mV modulation amplitude and 0.50 sec drop-time.

Procedure for determination of TBHQ in oil samples. A 1.00-ml aliquot of the oil sample was weighed accurately into the cell bottom. A 25.00-ml aliquot of buffer solution ($0.050M$ NaOAc/$1.0 \times 10^{-4}M$ HOAc in 1:5 toluene/absolute ethanol) was added, and argon was bubbled through the mixture for about 15 min to dissolve the sample and purge the solution. A differential-pulse polarogram was obtained over the range from −0.30 to 0.10 V *vs.* SCE, with the instrumental parameters already listed. Four standard additions of $1.00 \times 10^{-3}M$ TBHQ were made, each followed by a differential pulse polarographic scan. The peak currents were measured by the three base-line methods discussed in the following section.

RESULTS, DISCUSSION AND CONCLUSIONS

Initial investigation of the polarographic behaviour of TBHQ

A well-defined anodic differential-pulse polarogram was obtained for TBHQ with a TBHQ supporting electrolyte of $0.10M$ NaOAc/$0.10M$ HOAc in 50% v/v aqueous ethanol. The peak potential (E_p) was 0.065 V *vs.* SCE, which is very close to the $E_{1/2}$ value reported by Ryba *et al.*[7] for the same medium. Plots of peak current *vs.* TBHQ concentration were found to be linear in the range from 1.00×10^{-6} to $8.00 \times 10^{-5}M$.

The electrochemical behaviour of TBHQ was next studied with a supporting electrolyte of $0.10M$ NaOAc/$0.10M$ HOAc in absolute ethanol. The differential-pulse polarograms showed very well-defined peaks at TBHQ concentrations down to $1.0 \times 10^{-6}M$. The peak potential in this solvent/supporting electrolyte system shifted to −0.035 V *vs.* SCE. Plots of peak current *vs.* TBHQ concentration were linear over the same concentration range as for the aqueous ethanol system, but the sensitivity was much greater, the slope of the least-squares line being about 58% greater for the pure solvent than for the mixed solvent system. There was also less background noise.

Obviously, TBHQ could be determined in a number of food products by this method if a suitable extraction procedure were used to separate the TBHQ from the sample matrix. However, it was desired to develop a method for direct determination of TBHQ in cooking oils, without a separation step. Since the cooking oils were essentially insoluble in ethanol, a series of polarographic experiments was performed with toluene/absolute ethanol mixed solvent systems. Higher proportions of toluene adversely affected the anodic oxidation of TBHQ at the DME; however, it was found that 1:5 v/v toluene/ethanol would dissolve an adequate amount of common cooking oils and that the polarographic behaviour of TBHQ was quite satisfactory in this solvent system, though the peak current was smaller and the sensitivity somewhat lower than for the absolute ethanol system.

Differential-pulse polarograms were obtained for a solution containing 1.00 ml of a soybean oil (C) dissolved in 25.0 ml of 0.10*M* NaOAc/0.10*M* HOAc in 1:5 v/v toluene/ethanol. Standard additions of TBHQ were made to the solution and differential-pulse polarograms recorded for the concentration range 1.00–8.00 × 10^{-5}*M*. This procedure was repeated using another soybean oil (B) and a safflower oil (H). The peak potential (E_p) of TBHQ was about +0.010 V *vs.* SCE in these solutions, but there was an interfering substance (or substances) present in these oils which had a peak potential between 0.028 and 0.042 V *vs.* SCE. All the vegetable oils used in this study exhibited an oxidation peak in the range 0.02–0.05 V *vs.* SCE. By decreasing the concentration of HOAc in the supporting electrolyte, it was possible to shift the anodic differential-pulse polarographic peak of TBHQ to potentials sufficiently negative for the other peak not to interfere to so great an extent.

Differential-pulse polarograms were obtained for solutions containing 1.00 ml of a sunflower oil sample (K) dissolved in 25.0 ml of various acetate buffers in 1:5 v/v toluene/ethanol. (Oil K contained TBHQ as a preservative.) The peak potential of the TBHQ was found to be about −0.05 V *vs.* SCE with a 0.10*M* NaOAc/1.0 × 10^{-4}*M* HOAc supporting electrolyte or about −0.04 V with a 0.050*M* NaOAc/1.0 × 10^{-4}*M* HOAc supporting electrolyte. The peak at about 0.03 V *vs.* SCE was not shifted by the change in pH and was only a minor source of interference in the less acidic buffer systems. The supporting electrolyte used in all subsequent polarographic work was the 0.050*M* NaOAc/1.00 × 10^{-4}*M* HOAc buffer, because it was difficult to dissolve enough NaOAc in the solvent system to give 0.10*M* solutions.

Methods of quantitation

Because all the oils used in this study contained a substance (or substances) which gave an anodic peak at between 0.02 and 0.05 V *vs.* SCE, the TBHQ peak was always superimposed on the rising portion of this peak. This made it necessary to devise empirical methods for estimating the base-line current in order to measure the peak current for TBHQ. Obviously, a blank can be used to determine a base-line if a sample of the oil being analysed, and not containing TBHQ, is available for reference. This was the case for the recovery studies reported here, but would not be the case for most practical oil analyses. Therefore in addition to use of a blank for base-line determination, two empirical base-line methods were devised and tested. Figure 1 illustrates the three methods used for base-line determination and subsequent measurement of peak currents in the recovery studies: curve 1 illustrates the "blank" method, curve 2 the "tangent" method and curve 3 the "extrapolation" method.

The TBHQ in each oil sample was determined by the standard-additions method.

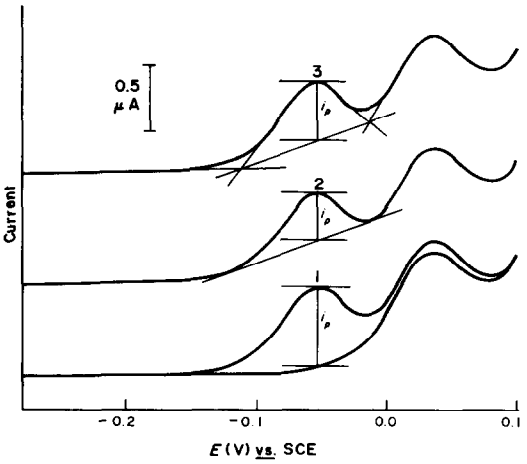

Fig. 1. Three methods for estimation of base-line current and measurement of peak current of TBHQ in cooking oils: 1, "blank" base-line method; 2, "tangent" base-line method; 3, "extrapolation" base-line method.

Recovery studies

Recovery studies were performed on ten brands of vegetable oil, which were obtained locally. None of these oils contained TBHQ. Solutions containing about 0.005, 0.01 and 0.02% TBHQ in each oil were prepared accurately. The TBHQ in each sample was determined as already described. In most cases, peak height was measured by use of all three base-line methods. Ten replicate determinations were done on the three samples of oil D, and triplicate determinations on the other oil samples. The results are reported in Table 1.

The "tangent" method invariably gave low results, as would be expected, especially for the samples containing 0.005 or 0.01% TBHQ. The recoveries by the "blank" method were generally satisfactory, but in some cases (oils E, A and G) the results were excessively high. Under normal circumstances, this method could not be used for "real" samples because samples of the "pure" oil would not be available for reference.

The "extrapolation" method gave good recoveries for all three concentrations of TBHQ in most of the oils used in this study. The only really poor results obtained with this method were for TBHQ at the 0.005% level in the sunflower oil (G) and the corn oil (I). Therefore, this should be the method of choice.

The best indication of the inherent precision of the method is given by the results obtained for oil D. The relative standard deviation for the "extrapolation" method is below 1% at all three concentrations in this case. The recoveries are also quite satisfactory.

It should be noted that the peak potentials for TBHQ are somewhat dependent on which oil is used. In addition, the peaks are shifted to more positive potentials at higher concentrations of TBHQ. This shift is due to the hydrogen ion that is produced in the electrode reaction and the relatively low buffer capacity of the supporting electrolyte. The lower

Table 1. TBHQ recovery from oil samples*

Cooking oil	E_p, V vs. SCE	TBHQ added, %	No. of replicates	Mean recovery, % (±σ) "Blank" base-line method	"Tangent" base-line method	"Extrapolation" base-line method
A (soybean oil)	−0.060	0.00530	3	105 (±3)	79 (±4)	100.3 (±0.9)
		0.0097	3	104 (±2)	81 (±1)	95 (±1)
		0.0195	3	112 (±2)	98.2 (±0.6)	103 (±1)
B (soybean oil)	−0.055	0.00550	3	105 (±2)	88 (±2)	98 (±2)
		0.0100	3	100.0 (±0.8)	81.5 (±0.3)	97.6 (±0.8)
		0.0199	3	—	96.6 (±0.5)	104 (±3)
C (soybean oil)	−0.055	0.00569	3	98.7 (±0.4)	70.0 (±0.6)	91.8 (±0.0)
		0.0108	3	101 (±1)	75 (±2)	96.8 (±0.5)
		0.0214	3	—	94.1 (±0.3)	97.1 (±0.6)
D (soybean oil)	−0.055	0.00532	10	101 (±2)	77.2 (±0.8)	95.1 (±0.9)
		0.0106	10	101 (±1)	85.9 (±0.6)	98.8 (±0.5)
		0.0200	10	101 (±2)	99.5 (±0.6)	99.4 (±0.7)
E (soybean oil)	−0.060	0.00538	3	116 (±3)	74 (±2)	98.0 (±0.6)
		0.0110	3	106 (±3)	82 (±7)	99 (±1)
		0.0193	3	111 (±3)	93 (±3)	102.4 (±0.6)
F (sunflower, soybean oil)	−0.050	0.00561	3	102 (±4)	66 (±3)	93 (±2)
		0.0118	3	99 (±3)	76 (±2)	93 (±2)
		0.0198	3	108 (±3)	90 (±5)	101 (±2)
G (sunflower oil)	−0.040	0.00511	3	94.5 (±0.9)	51 (±4)	72 (±3)
		0.0103	3	100 (±5)	80 (±1)	96 (±2)
		0.0206	3	109 (±3)	93.1 (±0.6)	96.8 (±0.8)
H (safflower oil)	−0.050	0.00529	3	91.5 (±0.3)	69 (±1)	92 (±1)
		0.0100	3	98 (±3)	86 (±1)	99.7 (±0.8)
		0.0213	3	95 (±2)	90.9 (±0.8)	99.5 (±0.0)
I (corn oil)	−0.092	0.00526	3	94.7 (±0.3)	61.3 (±0.2)	76 (±8)
		0.0100	3	101 (±4)	75 (±6)	99 (±3)
		0.0200	3	96.0 (±0.5)	88.8 (±0.4)	99.0 (±0.7)
J (peanut oil)	−0.055	0.00596	3	96.1 (±0.3)	63.8 (±0.7)	91.1 (±0.3)
		0.0104	3	100 (±1)	75.9 (±0.5)	95 (±1)
		0.0188	3	104 (±1)	85 (±1)	99 (±2)

*A more complete description of the oils is given in the reagents section.

instantaneous pH at the electrode surface in these cases causes a slight positive shift in the potential at which TBHQ may be oxidized. The peak potentials (E_p) reported in Table 1 are for oils which contain about 0.005% TBHQ. The uncertainty is about ±5 mV in each case. With the exception of the corn oil (I), all the peak potentials are within about 10 mV of −0.050 V *vs.* SCE.

The TBHQ peaks obtained for the corn oil (I) were extremely broad and poorly resolved, but the results obtained at the two higher concentrations of TBHQ were quite satisfactory. The peaks obtained for TBHQ in the other nine oil samples were all well resolved. Figure 2 shows typical differential-pulse polarograms obtained for TBHQ in one of them (oil D). The steep base-line due to the interfering peak at about 0.036 V *vs.* SCE made it necessary to use either the "blank" or the "extrapolation" method for the oil samples containing 0.005% TBHQ in order to achieve adequate recoveries.

Recovery studies were also performed on several commercial antioxidant preparations marketed by Eastman Chemical Products, Inc. for direct addition to cooking oils and other food products, *viz.* Tenox® 22, 26, 20 and 20A (the compositions of which were given in the reagents section). Appropriate amounts were added to oil D and the TBHQ determined as in the other recovery studies. The polarograms were of the same form as those obtained by direct addition of TBHQ to the oil (see Fig. 2). Five replicate determinations were done on each sample. The results are

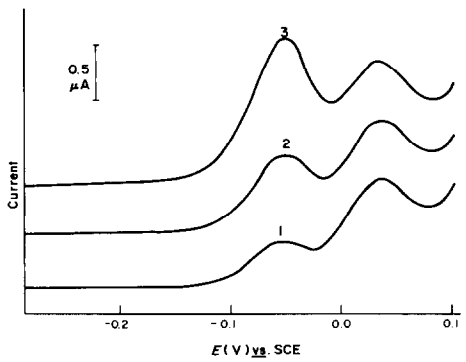

Fig. 2. Differential-pulse polarograms of TBHQ recovery studies in soybean oil D: 1, 0.00532% TBHQ added; 2, 0.0106% TBHQ added; 3, 0.0200% TBHQ added.

Table 2. TBHQ recovery from soybean oil D plus antioxidant preparations

Antioxidant preparation	TBHQ added, %	No. of replicates	Mean recovery, % ($\pm\sigma$) "Blank" base-line method	"Tangent" base-line method	"Extrapolation" base-line method
Tenox® 22	0.00456	5	99.9 (±0.5)	76.0 (±0.3)	96.6 (±0.5)
Tenox® 26	0.00465	5	99.9 (±0.7)	75.7 (±0.6)	93 (±2)
Tenox® 20	0.0200	5	101 (±2)	94.5 (±0.7)	100.1 (±0.6)
Tenox® 20A	0.0200	5	103 (±2)	93 (±1)	99 (±1)

reported in Table 2. The recoveries obtained by either the "blank" or the "extrapolation" method were satisfactory. Obviously, none of the other components of the preparations interfered. Both BHA and BHT can be electrochemically oxidized but only at electrode potentials more positive than the working range of a DME.

One sunflower oil sample (K) which contained TBHQ as a preservative was obtained locally. The TBHQ was determined in this sample by the procedure given. The amount of TBHQ found in this sample by the "extrapolation" method was 0.0088%. Since U.S. FDA regulations allow adding TBHQ to vegetable oils in amounts not greater than 0.02%, this result would seem to be reasonable.

The differential pulse polarographic method described in this paper should be useful as an alternative method for determination of TBHQ in cooking oil samples. The accuracy and precision should be adequate for most routine analyses of this type of sample. One of the principle advantages of this technique is that no prior separation of TBHQ is required: the oil sample (1 ml) is weighed directly into a polarographic cell, a known volume of solvent/supporting electrolyte mixture is added and a polarogram is run. Quantitation is straightforward by the "extrapolation" method in conjunction with standard additions.

Acknowledgement—The TBHQ and the Tenox® antioxidant preparations were provided by the Health and Nutrition Division of Eastman Chemical Products, Inc., Kingsport, Tennessee 37662.

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POTENTIOMETRIC DETERMINATION OF CERTAIN α-AMINOHYDROXY COMPOUNDS BY USING AN AMMONIA GAS-SENSING ELECTRODE

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Summary—A simple method is described for the determination of certain α-aminohydroxy compounds, based on the potentiometric measurement of ammonia released after oxidation with an excess of periodate. Ammonia is measured directly in the reaction mixture, with an ammonia gas-sensing electrode. Ethanolamine, diethanolamine, triethanolamine, serine, threonine, and glucosamine (0.3–6 μmole) can be determined with average errors of about 1–2%.

The ammonia gas-sensing electrode has been used extensively for the direct potentiometric determination of ammonia in a variety of matrices of clinical or environmental importance.[1] It has also been used for the construction of bioselective sensors, such as enzyme electrodes, by taking advantage of the fact that ammonia is a product of biodegradation of many nitrogen-containing organic compounds.[2] Ammonia is also obtained from the chemical degradation (*e.g.*, hydrolysis) of many organic compounds, thus making possible their potentiometric determination.

In a previous paper we described a simple method for assaying nicotinamide in multivitamin preparations by potentiometric measurement of ammonia released after alkaline hydrolysis of the sample.[3] In this paper we describe a simple procedure for the determination of various α-aminohydroxy compounds, based on the determination of ammonia released after oxidation with an excess of periodate.

It is known that periodate cleaves the carbon–carbon bond of α-aminohydroxy compounds smoothly.[4] The general reaction scheme for the three α-amino alcohols, ethanolamine, diethanolamine and triethanolamine, is

$$(HOCH_2CH_2)_nNH_{3-n} + nIO_4^- \rightarrow 2nHCHO + NH_3 + nIO_3^- \quad (n = 1\text{–}3).$$

The optimum conditions (pH, temperature and reaction period) for completion of these reactions have been reported[5] and various analytical schemes have been developed. Titrimetric methods are based on the iodometric determination of either the excess of periodate or the iodate produced, after masking of the excess of periodate with molybdate.[6] Other schemes are based on the spectrophotometric measurement of the formaldehyde produced.[7] These methods offer high accuracy and sensitivity but they lack selectivity when other compounds reacting with periodate, such as vicinal glycols, polyhydroxy compounds and carbohydrates are present. The titrimetric determination of the ammonia released is not subject to these interferences but it involves tedious and time-consuming separation steps (*e.g.*, by microdiffusion[8]).

With the ammonia gas-sensing electrode, ammonia can be determined directly in the reaction mixture. No separation steps are necessary and the sensitivity of the analysis is greatly improved. To evaluate the method, it was applied to the determination of three ethanolamines, the α-hydroxyamino-acids serine and threonine, and the amino-sugar glucosamine. Microamounts of these compounds in the range 0.3–6 μmole were determined with average errors of about 1–2%.

EXPERIMENTAL

Apparatus

An Orion Model 95-10 ammonia gas-sensing electrode was used, and the potential measurements were made with an Orion Model 801 digital pH/mV meter. All measurements were made at 25°C ± 0.1° in a 10-ml cell equipped with a magnetic stirrer.

When not in use, the electrode was kept in 0.05*M* ammonium chloride.[9]

Reagents

Analytical-grade materials and demineralized, distilled water were used throughout.

*Sodium metaperiodate, 0.100*M. Dissolve 21.4 g of reagent in water and dilute to 1 litre. Store in an amber bottle.

*Potassium carbonate, 1.00*M *solution.*

*Sodium bicarbonate, 0.20*M *solution.*

*Potassium hydroxide, 2.00*M *solution.*

α-Aminohydroxy compounds. Prepare 0.0200*M* stock solutions of serine, threonine and glucosamine hydrochloride by dissolving the appropriate amount of each compound in water. Prepare 0.0200*M* stock solutions of ethanolamines by diluting 1*M* solutions, which have been titrated potentiometrically with standard 1.000*M* hydrochloric acid solution. Prepare fresh standard solutions, $1.00 \times 10^{-4}M$, $2.50 \times 10^{-4}M$, $7.50 \times 10^{-4}M$ and $2.00 \times 10^{-3}M$, as needed, by appropriate dilution of the stock solutions.

Procedures

Monoethanolamine, diethanolamine, serine and threonine. Pipette 4.00 ml of a 1:1 mixture of 0.100*M* sodium metaperiodate and 1.00*M* potassium carbonate (this mixture is stable for at least one day at room temperature) and 3.00 ml of the standard or unknown α-aminohydroxy compound solution into the measurement cell. Start the stirrer and read the potential when it has stabilized to within ±0.1 mV (in about 3–4 min). Find the unknown concentration from a calibration graph of potential *vs.* log [α-aminohydroxy compound].

Triethanolamine. Incubate the reaction mixture (prepared as before) in a 10-ml vial fitted with a well-fitting stopper, for 60 min, in a water-bath at 60°, cool to room temperature, transfer the contents of the vial into the measurement cell and measure as for monoethanolamine *etc.*

Glucosamine. Pipette 3.00 ml of a 2:1 mixture of 0.100*M* sodium metaperiodate and 0.20*M* sodium bicarbobonate (this mixture is stable for at least 15 min at room temperature, but then disodium paraperiodate, $Na_2H_3IO_6$, starts to precipitate) and 3.00 ml of the standard or unknown glucosamine solution into a 10-ml vial with a well-fitting stopper. Shake the vial and immerse it in a water-bath at 60° for 60 min. Cool the vial to room temperature, pipette 1.00 ml of 2.00*M* potassium hydroxide into it, mix, and transfer the contents into the measurement cell. Measure as above.

RESULTS AND DISCUSSION

Completion of the reactions

Figure 1 shows recordings of the potential of the ammonia gas-sensing electrode during the course of the reaction of α-aminohydroxy compounds with periodate in alkaline (carbonate) solution. Since gas-sensing electrodes are slow-response concentration transducers, for comparison of speed of response an increase of ammonia concentration by addition of ammonium chloride has been included. The latter was added in an amount equivalent to that of the α-aminohydroxy compound.

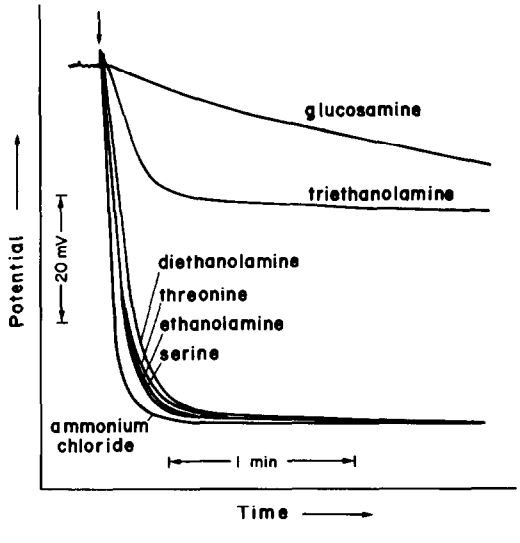

Fig. 1. Records of the potential of the ammonia gas-sensing electrode during the reaction of periodate with α-aminohydroxy compounds. In a mixture of 4.00 ml of composite 0.0500*M* periodate–0.500*M* carbonate solution and 3.00 ml of $6.67 \times 10^{-5}M$ ammonium chloride, 100 μl of 0.0200*M* solution of the relevant α-aminohydroxy compound are injected. Ammonium chloride is also injected for comparison. ↓: α-aminohydroxy compound (or ammonium chloride) solution injection. Concentrations at the start of the reaction: $NaIO_4$, 0.0282*M*; K_2CO_3, 0.282*M*; NH_4Cl, $2.82 \times 10^{-5}M$ (added in all cases to keep the initial potential stable); α-aminohydroxy compound (or NH_4Cl), $2.82 \times 10^{-4}M$. Temperature: 25°C.

It has been reported that the ammonia electrode responds to lower organic amines.[1] It can be seen from Fig. 1 that there is no direct response of the electrode to α-aminohydroxy compounds. This is probably because the hydroxyl groups make these compounds more hydrophilic and therefore less able to diffuse through the hydrophobic membrane of the ammonia gas-sensing electrode.

Ethanolamine, diethanolamine, serine and threonine react quantitatively and almost instantaneously with periodate, and weakly alkaline (bicarbonate) media have been recommended[4,6] for making the reaction quantitative. However, the reactions also proceed quickly in more alkaline (carbonate) solutions, and then practically all the ammonia released is in molecular form and thus is measured quantitatively with the ammonia gas-sensing electrode.

On the other hand, triethanolamine and glucosamine react very slowly and incompletely. In fact, under all conditions tested (*e.g.*, bicarbonate solutions, phosphate buffers of pH 7–9, carbonate and borate buffers of pH 9–10, use of Mn^{2+} as catalyst plus nitrilotriacetic acid as activator,[10] at temperatures from 25° to 75°) the ammonia released never exceeded 90% of the theoretical amount. These findings contradict those of other workers[6] who claim complete oxidation, at least for triethanolamine. It is possible that the reaction is stoichiometric with regard to the periodate consumed or iodate produced per molecule of triethanolamine but that some of the ammonia condenses with the formaldehyde formed as one of the reaction products. Attempts to release ammonia quantitatively by addition of glycine as a scavenger for formaldehyde failed, because glycine is oxidized by periodate at elevated temperature and releases large amounts of ammonia. Plots of the percentage of ammonia released from triethanolamine and glucosamine *vs.* reaction period, under a variety of conditions, are shown in Fig. 2. The ammonia released from triethanolamine and glucosamine was 80 and 88% of the theoretical amount, respectively, when the reaction with periodate took place in alkaline (carbonate) and weakly alkaline (bicarbonate) solutions respectively, at 60° for 60 min.

Analytical results

The potential of the ammonia gas-sensing electrode was found to be linearly dependent on the logarithm of the concentration of the α-aminohydroxy compound, according to the general equation.

$$E = \text{constant} - S\log[\alpha\text{-aminohydroxy compound}]$$

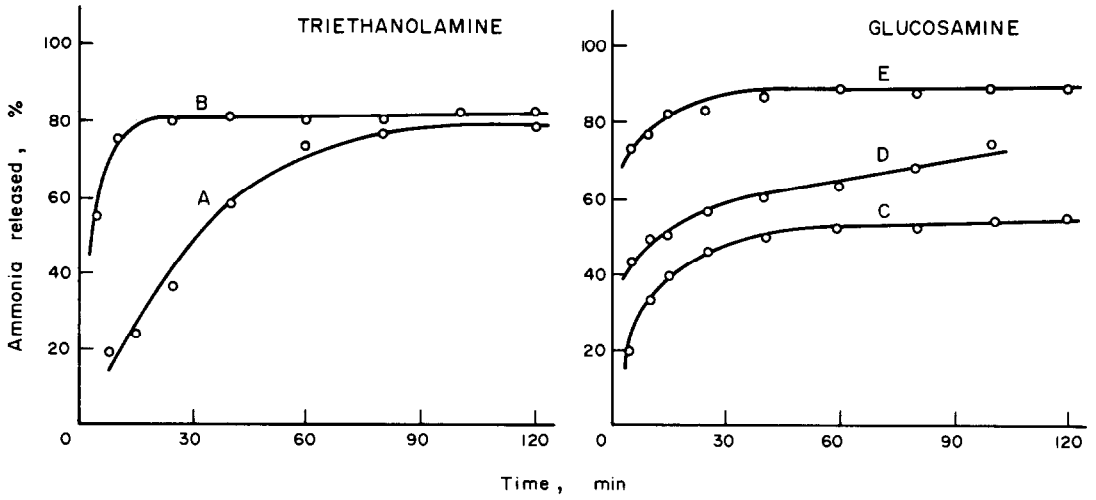

Fig. 2. Rate of release of ammonia for triethanolamine and glucosamine. Initial concentrations and temperatures: A–$NaIO_4$, 0.0286M; K_2CO_3 0.286M; triethanolamine, 3.21 × $10^{-4}M$; 25°C. B—as in A but at 60°C. C—$NaIO_4$, 0.0286M; K_2CO_3, 0.286M; glucosamine, 3.21 × $10^{-4}M$; 25°C. D—same as C but at 60°C. E—$NaIO_4$, 0.0333M; $NaHCO_3$, 0.0333M; glucosamine, 3.75 × $10^{-4}M$; 60°C.

for the concentration range 1 × 10^{-4}–2 × $10^{-3}M$. The working range can be extended to higher concentrations provided that enough periodate is added to oxidize the compound to be determined plus any other oxidizable compound. Working curves for ethanolamine, diethanolamine and α-hydroxyamino-acids, obtained by plotting potential *vs.* log[α-aminohydroxy compound], practically coincide with a working curve obtained with standard solutions of ammonium chloride. Therefore, for these compounds the

Table 1. Results for the potentiometric determination of α-aminohydroxy compounds

	Concentration, $10^{-4}M$			
Compound	Taken	Found	Error, %	Regression equation
Ethanolamine	1.00	1.02	+2.0	$E = -156.6 - 54.3 \log C$,
	2.50	2.43	−2.8	$r = -0.9993$
	7.50	7.32	−2.4	
	20.0	20.4	+2.0	
			Av. 2.3	
Diethanolamine	1.00	0.99	−1.0	$E = -167.8 - 55.9 \log C$,
	2.50	2.39	−4.4	$r = -0.9996$
	7.50	7.56	+0.8	
	20.0	20.2	+1.0	
			Av. 1.8	
Triethanolamine	1.00	1.04	+4.0	$E = -161.9 - 57.8 \log C$,
	2.50	2.52	+0.8	$r = -0.9994$*
	7.50	7.41	−1.2	
	20.0	20.4	+2.0	
			Av. 2.0	
Serine	1.00	1.02	+2.0	$E = -172.0 - 57.8 \log C$,
	2.50	2.42	−3.2	$r = -0.99995$
	7.50	7.62	+1.6	
	20.0	20.2	+1.0	
			Av. 2.0	
Threonine	1.00	1.00	—	$E = -176.0 - 58.2 \log C$,
	2.50	2.54	+1.6	$r = -0.99994$
	7.50	7.55	+0.7	
	20.0	19.9	−0.5	
			Av. 0.7	
Glucosamine	1.00	1.02	+2.0	$E = -155.3 - 53.4 \log C$,
	2.50	2.43	−2.8	$r = -0.9992$
	7.50	7.65	+2.0	
	20.0	20.2	+1.0	
			Av. 2.0	

*The lower concentration standard deviates from linearity and has not been included in the calculation of the regression equation.

Table 2. Effect of various compounds on the determination of α-amino-hydroxy compounds

Compound ($5.00 \times 10^{-4}M$)	Interferent	[Interferent]/[Compound]	Error, %
Ethanolamine	Propylene glycol	30/1	+0.4
		100/1	−0.4
		300/1*	−12.0
	Glucose	3/1	−4.5
		10/1	−5.5
		30/1	−10.4
		100/1*	−43.0
	Formaldehyde	30/1	−2.8
		100/1	−7.4
		300/1	−33.5
Diethanolamine	Glycerol	30/1	−0.8
		100/1	−2.0
		300/1†	−69.6
	Formaldehyde	30/1	−1.6
		100/1	−5.9
		300/1*	−36.9
Triethanolamine	Glycerol	10/1	−1.6
		30/1	−3.2
		100/1	−13.0
		300/1	−14.7
Glucosamine	Glucose	3/1	−3.8
		10/1	−9.8
		30/1	−32.4
		100/1	−46.2

*At these interference levels it took about 10 min to obtain a steady potential indication.

†At this interference level the potential was not stabilized even after 10 min, drifting continuously toward smaller values (smaller error).

working curve can be based on standard ammonium chloride solutions. The working curves for triethanolamine and glucosamine were shifted slightly toward more positive potentials because of the incomplete release of ammonia.

Table 1 gives typical analytical results for the determination of six α-aminohydroxy compounds, together with the regression equations of the corresponding working curves. The relative standard deviation for $5 \times 10^{-4}M$ ethanolamine was 2.0% ($n = 12$).

Interferences

Positive analytical errors are to be expected in the presence of free ammonia or ammonium salts, or compounds releasing ammonia under the conditions of the measurement, such as amides or nitriles. Such interferences can be overcome by running blanks from which periodate is omitted.

Negative analytical errors are to be expected in the presence of compounds that react with ammonia, such as aldehydes. Negative analytical errors are also to be expected in the presence of compounds that consume periodate, such as vicinal glycols, polyhydroxy compounds and carbohydrates. Such interferences can be minimized by using as much periodate as possible. Again the problem is only partially solved because aldehydes released from the cleavage of these compounds may react with some of the ammonia. The interference, expressed in terms of analytical error, caused by such compounds in the analysis of various α-aminohydroxy compounds at various concentration ratios is shown in Table 2.

It can be seen from Table 2 that α-aminohydroxy compounds which are oxidized at 25° by periodate in alkaline (carbonate) solution can be determined even in the presence of a hundredfold excess of propylene glycol and glycerol, because these interferents react very slowly with periodate in alkaline solutions. On the other hand, glucose reacts quickly with periodate, reducing its oxidative capacity. Formaldehyde masks ammonia only when it is present in large excess. Determination of triethanolamine and glucosamine results in more intense interference because of the higher temperature used for their determinations.

At high interferent concentrations, it was noticed that the ammonia was released only slowly for the easily oxidized compounds, probably owing to the reduced availability of periodate because of the equilibrium existing between the free anion and its complex compound with the polyhydroxy compound, favoured by the alkaline pH.[4]

CONCLUSIONS

The proposed method for the determination of α-aminohydroxy compounds is simple, fast, sensitive and selective enough for many possible applications. The accuracy attained is similar to that of other direct potentiometric measurements. This method comple-

ments other methods for the determination of α-aminohydroxy compounds and broadens the spectrum of possible applications of the ammonia gas-sensing electrode. A possible use for the method would be the determination of total serine and threonine in the hydrolysis products of peptides and proteins, and of ethanolamines in petroleum plant effluents and cutting fluids. The possibility of the determination of glucosamine in serum glycoproteins is under consideration.

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SPECTROPHOTOMETRIC DETERMINATION OF SOME PHENOLS WITH SODIUM METAPERIODATE AND AMINOPHENOLS

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Summary—New spectrophotometric methods have been developed for the assay of some phenols by use of two pairs of reagents, *m*-aminophenol and periodate (for catechol, catecholamines, eugenol and guaiacol) and metol and periodate (for pyrogallol, gallic acid, propyl gallate, phloroglucinol and butylated hydroxyanisole). The methods are simple, sensitive, reproducible, accurate within ±1.0%, and applicable to the assay of antioxidants (gallic acid, propyl gallate and butylated hydroxyanisole) in oils and fats, catecholamines in dosage forms, and eugenol in clove oil.

It has long been known that phenols are readily oxidized. The products are mostly complicated mixtures of dimeric, polymeric, and quinonoid compounds.[1–3] Several spectrophotometric and colorimetric methods for assay of phenols are cited in the literature.[4–23] Recently kinetic determination of phenols by a fixed-time method using sodium metaperiodate as oxidant has been reported.[24] We have now developed simple, rapid, sensitive and accurate spectrophotometric determinations of some phenols at the microgram level, with sodium metaperiodate in the presence of aminophenol. They are based on our observations that catechol (reagent: $NaIO_4$ and aniline or *m*-aminophenol), catecholamines, eugenol and guaiacol (reagent: $NaIO_4$ and *m*-aminophenol), pyrogallol, gallic acid and propyl gallate (reagent: $NaIO_4$ and metol or *p*-aminophenol) and phloroglucinol and butylated hydroxyanisole (reagent: $NaIO_4$ and metol) develop characteristic colours (λ_{max} shown in Table 1) and that the intensity of colour is directly proportional to the amount of phenol. These methods have wide applicability in purity assays of antioxidants (gallic acid, propyl gallate and butylated hydroxyanisole) and sympathomimetics (catecholamines), and also for estimation of these antioxidants in oils and fats, catecholamines in dosage forms and eugenol in clove oil.

Table 1. Experimental conditions for determination of phenols

Phenol	pH	Aminophenol soln., *ml*	$NaIO_4$ soln., *ml*	Time for maximum colour development, *min*	Stability of colour, *min*	λ_{max}, *nm*
Catechol	2.0*(2.0–3.2)†	A:1.0*(0.7–3.0)	2.0*(1.5–3.0)	immediate	10	510–520
	3.1 (2.5–3.5)	B:2.0 (1.5–3.0)	2.0 (1.0–2.5)	30	45	510–520
	3.1 (1.5–3.5)	C:0.5 (0.5–1.0)	1.0 (0.5–2.0)	immediate	8	510–520
Guaiacol	3.1 (2.0–3.5)	A:1.0 (0.7–3.0)	2.0 (1.5–3.0)	20	50	490–520
Eugenol	3.1 (2.0–3.5)	A:1.0 (0.7–3.0)	2.0 (1.5–3.0)	20	50	490–520
Adrenaline	3.1 (2.0–3.5)	A:1.0 (0.7–3.0)	0.5 (0.5–2.0)	50	150	480–490
Noradrenaline	3.1 (2.0–3.5)	A:1.0 (0.7–3.0)	0.5 (0.5–2.0)	50	150	480–490
Isoprenaline	3.1 (2.0–3.5)	A:1.0 (0.7–3.0)	0.5 (0.5–2.0)	25	45	480
Methyldopa	3.1 (2.0–3.5)	A:1.0 (0.7–3.0)	0.5 (0.5–2.0)	40	60	480–490
Dopamine	3.1 (2.0–3.5)	A:1.0 (0.7–3.0)	0.5 (0.5–2.0)	10	120	450–480
Pyrogallol	3.1 (2.0–3.5)	D:2.0 (1.0–2.5)	4.0 (2.5–4.0)	immediate	30	550, 580, 620*
	3.1 (2.0–3.5)	B:1.0 (0.5–2.0)	2.0 (2.0–3.0)	immediate	30	510–550
Gallic acid	2.5 (2.0–3.1)	D:2.0 (1.5–2.5)	1.5 (1.0–2.0)	immediate	10	580
Propyl gallate	2.5 (2.0–3.1)	B:1.5 (1.0–2.0)	2.0 (1.0–3.0)	immediate	10	560
	2.5 (2.0–3.1)	D:2.0 (1.5–2.5)	1.5 (1.0–2.0)	10	40	580
Butylated hydroxyanisole	2.5 (2.0–3.1)	D:3.0 (2.0–3.0)	1.5 (1.5–2.0)	110	240	500–520
Phloroglucinol	3.1 (2.0–3.5)	D:3.0 (1.0–2.5)	4.0 (2.5–4.5)	5	10	520

A: *m*-Aminophenol; B: *p*-aminophenol; C: aniline; D: metol (*p*-*N*-methylaminophenol sulphate).
*Used in suggested procedure.
†Range for maximum absorbance and stability.

EXPERIMENTAL

Reagents

All solutions were prepared in doubly distilled water. The solutions (0.2%) of aminophenols or aniline in pH 3.0 buffer were always freshly prepared. The sodium metaperiodate solution was 0.01*M*. Buffer solutions of glycine and hydrochloric acid (pH 1.0–2.1) and of potassium hydrogen phthalate and hydrochloric acid (pH 2.2–3.7) were prepared according to Lurie.[25]

All the phenols used were of commercially available general-reagent or C.P. grade. Their stock solutions were prepared in distilled water, the compounds insoluble in water being dissolved initially in the minimum volume of alcohol or pH 3.0 buffer solution. Working solutions were prepared by appropriate dilution of the stock solutions. All other reagents were of analytical grade.

General procedures

To a 25 ml standard flask, add, in the following order, 15 ml of buffer solution, aminophenol solution (*m*-, *p*- or *p*-*N*-methyl), sodium metaperiodate solution and 1–4 ml of the phenol solution, and dilute to the mark. Measure the absorbance at λ_{max} after maximum colour development, against a reagent blank prepared under similar conditions. Prepare a calibration graph in the same way. The buffer pH, the volume of perrodate solution, and the other experimental conditions needed are shown in Table 1.

Procedure for propyl gallate or gallic acid in oils and fats

Dissolve 10 g of oil or fat in 50 ml of carbon tetrachloride and extract with four 20-ml portions of 50% aqueous alcohol. Neutralize the combined alcoholic extracts with calcium carbonate (1 g) then dilute with water to 100 ml in a standard flask and filter through a dry paper. Use the filtrate for propyl gallate or gallic acid determination by the procedure above.

For the determination of butylated hydroxyanisole in oils or simultaneous determination of butylated hydroxyanisole and propyl gallate if both are present, separate the antioxidant(s) by the procedure suggested by Schwien and Conroy,[26] and apply the general procedure.

Procedures for dosage forms of catecholamines

Use pH 3.0 buffer to bring the catecholamine present in the dosage form (tablet, powder or injection) into the working range (100 μg/ml) and apply the general procedure.

Procedure for adrenaline in biological fluids

Take a 1-ml sample of urine or blood and precipitate the protein with trichloroacetic acid (0.5 ml of 5% solution). To the clear centrifugate, add a few drops of sodium hydroxide solution to adjust the pH to 3.0. Then apply the general procedure to determine the adrenaline.

Procedure for eugenol in clove oil

Dissolve the clove oil in methanol and dilute to bring the eugenol concentration into the working range (100 μg/ml), and apply the general procedure.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of the reaction products show characteristic maxima (shown in Table 1), whereas the reagent mixtures used have practically no (or only low) absorption in these regions (Figs. 1–3). The time taken for maximum colour development and the stability period in each case are shown in

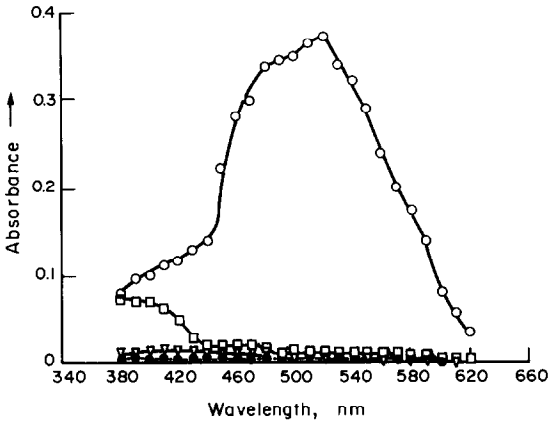

Fig. 1. Absorption spectrum of catechol–periodate–*m*-aminophenol system ○——○ catechol–IO_4^-–mAP *vs.* reagent blank; □——□ IO_4^-–catechol *vs.* IO_4^-; ◇——◇ IO_4^-–mAP *vs.* IO_4^-; ●——● mAP–catechol *vs.* mAP. $[IO_4^-] = 8.0 \times 10^{-4}M$; [mAP] = $7.3 \times 10^{-4}M$; [catechol] = $9.1 \times 10^{-5}M$.

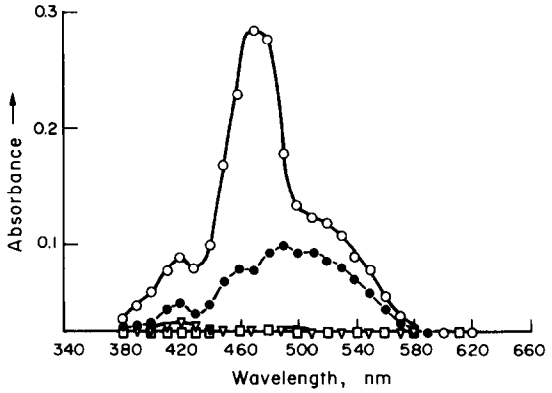

Fig. 2. Absorption spectrum of adrenaline–periodate–*m*-aminophenol system. ○——○ Adrenaline–IO_4^-–mAP *vs.* reagent blank; ◇——◇ IO_4^-–mAP *vs.* IO_4^-; ●——● IO_4^-–adrenaline *vs.* IO_4^-; □——□ mAP–adrenaline *vs.* mAP, $[IO_4^-] = 2.0 \times 10^{-4}M$; [mAP] = $7.3 \times 10^{-4}M$; [adrenaline] = $4.4 \times 10^{-5}M$.

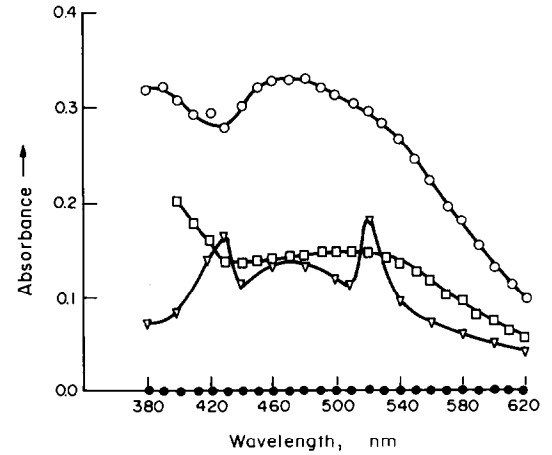

Fig. 3. Absorption spectrum of butylated hydroxyanisole–periodate–metol system. ○——○ BHA–IO_4^-–metol *vs.* water; □——□ BHA–periodate–metol *vs.* reagent blank; ◇——◇ IO_4^-–metol *vs.* IO_4^-; ●——● IO_4^-–BHA *vs.* IO_4^-. $[IO_4^-] = 6.0 \times 10^{-4}M$; [metol] = $7.0 \times 10^{-4}M$; [BHA] = $1.7 \times 10^{-4}M$.

Table 2. Optical characteristics, precision and accuracy

Phenol	Reagent	Beer's law limits, $\mu g/25$ ml	Molar absorptivity at λ_{max}, $l.mole^{-1}.cm^{-1}$	Phenol, mg		Relative standard deviation, %
				Taken	Found*	
Catechol	m-AP-IO_4^-	25–200	4.4×10^3	0.200	0.199	0.8
Guaiacol	m-AP-IO_4^-	50–250	2.8×10^3	0.200	0.198	0.1
Eugenol	m-AP-IO_4^-	50–200	3.5×10^3	0.200	0.199	0.6
Adrenaline	m-AP-IO_4^-	40–200	6.4×10^3	0.200	0.199	0.3
Noradrenaline	m-AP-IO_4^-	40–160	4.9×10^3	0.160	0.159	0.9
Isoprenaline	m-AP-IO_4^-	20–85	4.6×10^3	0.080	0.079	0.1
Methyldopa	m-AP-IO_4^-	50–200	6.2×10^3	0.200	0.198	0.4
Dopamine	m-AP-IO_4^-	75–200	2.7×10^3	0.150	0.149	0.6
Pyrogallol	Metol-IO_4^-	100–600	1.9×10^3	0.500	0.496	0.7
Gallic acid	Metol-IO_4^-	50–350	5.0×10^3	0.300	0.299	0.4
Propylgallate	Metol-IO_4^-	50–400	5.8×10^3	0.300	0.300	0.2
Butylated hydroxyanisole	Metol-IO_4^-	250–1000	8.8×10^2	0.800	0.800	0.3
Phloroglucinol	Metol-IO_4^-	20–150	7.6×10^3	0.150	0.148	1.0

*Mean of three readings.

Table 1. The studies show that *m*-aminophenol/periodate is the best reagent for catechol, catechol monoalkyl ethers and catecholamines, and metol/periodate for pyrogallol, gallic acid, propyl gallate, phloroglucinol and butylated hydroxyanisole.

Effect of pH and reagent concentration

The optimum conditions are presented in Table 1. Higher pH should not be used, as the phenols themselves then develop colours with periodate.

Effect of order of addition

The order of addition of the reactants has no influence on the colour development if mixing is done immediately, but any delay in adding the aminophenol to the phenol–periodate mixture or adding the phenol to the aminophenol–periodate mixture (exception: catechol) causes a considerable decrease in absorbance.

Table 3. Recovery of added antioxidants from edible oils and fats

Sample	Antioxidant added, mg	Recovery, %	
		Proposed method	Literature method
Coconut oil	PG, 10	96.4	95.7[29]
Groundnut oil	PG, 10	97.1	96.6
Sunflower oil	PG, 10	98.3	97.8
Cottonseed oil (hydrogenated)	PG, 10	95.6	95.9
Groundnut oil	GA, 10	95.5	96.1[28]
Sunflower oil	BHA, 10	96.1	96.5[29]
Sunflower oil	PG, 5*	97.2	97.5
	BHA, 5*	95.8	96.3

*Separated initially by the Schwien and Conroy method.
PG, propyl gallate; GA, gallic acid; BHA, butylated hydroxyanisole.

Effect of solvent

Investigation of other polar solvents (60% v/v solution in water) such as acetonitrile, methanol and tert.-butyl alcohol showed that aqueous medium is the best for maximum colour development. Extraction of the coloured products into organic solvents gives a spectral shift and reduces the colour intensity.

Analytical data

The Beer's law ranges and molar absorptivities are given in Table 2. The precision and accuracy were found by analysis of 10 separate samples containing known amounts of the phenol tested, and the results are summarized in Table 2. The recovery of propyl gallate, gallic acid and butylated hydroxyanisole from various oils and fats by the proposed and literature methods are shown in Table 3, and results for catecholamines in dosage forms and for eugenol in clove oil are shown in Table 4.

Interferences

Phenol and its *o*-, *m*- or *p*-monosubstituted derivatives (other than catechol and its monoalkyl ethers) do not respond to the procedure, except for *o*-aminophenol which itself produces a colour with periodate and so interferes.

In the determination of propyl gallate (250 μg), the following amounts of other substances do not interfere: antioxidants—butylated hydroxyanisole (250 μg), butylated hydroxytoluene (500 μg); phenols—phenol (750 μg), hydroquinone (500 μg), resorcinol (500 μg); other constituents in foods—ascorbic acid (1 mg), citric acid (5 mg), benzoic acid (10 mg), tryptophan (1 mg), cysteine (1 mg), glucose (10 mg), sucrose (10 mg) and sodium chloride (10 mg).

In the determination of adrenaline in dosage forms, the excipients usually present, *viz.* chlorbutol, chloro-

Table 4. Assay of catecholamines in dosage forms

Sample	Nominal amount, *mg*	Found, *mg*	
		Literature method[19]	Proposed method
Injections, *mg/ml*			
1. A 10 mg; CB 40 mg; CC 10 mg; SMB 10 mg; SC 80 mg; 10 ml	10	9.88	9.94
2. A 10 mg; CC 20 mg; SMB 10 mg/20 ml	10	9.75	9.81
3. AT: 1 mg; SMB: 1 mg/ml	1	0.97	0.99
4. NABT: 4 mg/2 ml	4	3.89	3.93
5. DMH: 200 mg/5 ml	200	197.9	197.9
Tablets			
6. IPS: 20 mg; T: 0.033/	20	19.6	19.7
7. MD: 250 mg; T:	250	246.9	247.2
8. Eugenol in clove oil		3.15%[27]	3.2%

Each value represents the mean of two readings.
A, Adrenaline; CB, chlorbutol; CC, chlorocresol; SMB, sodium metabisulphate; SC, sodium chloride; AT, adrenaline tartrate; NABT, noradrenaline bitartrate; DMH, dopamine hydrochloride; IPS, Isoprenaline sulphate; T, tartrazine; MD, methyldopa.

cresol, sodium metabisulphite, sodium chloride and tartrazine, do not interfere.

The methods proposed are very sensitive and have reasonable accuracy. If interfering substances are present in real samples, suitable separation techniques must necessarily be applied before the individual determinations are done.

Mechanism

The colour developed may be due to oxidative coupling of the phenol and aminophenol, as reported earlier for monohydric phenols, or to charge-transfer complex-formation involving electron transfer between the aminophenol (or its intermediate oxidation product) and the phenol (or its oxidation product).

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MEASURING AND MAXIMIZING PRECISION IN ANALYSES BASED ON USE OF CALIBRATION GRAPHS

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Summary—Confidence-band statistics are used as a basis for measuring and improving precision in chemical analysis. Currently used calibration procedures often result in loss of precision and are therefore replaced by weighted least-squares and multiple-curve calibration techniques. Measures of precision such as correlation coefficient, relative standard deviation, and detection limit are replaced by more meaningful parameters based on confidence-band statistics. These new techniques are incorporated into a general scheme for developing methods for routine analysis.

To yield accurate data, an analytical method must meet four basic conditions. First it must be inherently precise; without precision, accuracy is impossible, unless a large number of replicate analyses are done. Secondly, it must be free from systematic error, or at least free from unpredictable error. These two conditions are fundamental, and together define the upper limit of performance. During routine analysis based on use of calibration graphs, the actual performance will be significantly worse unless the following additional conditions are met: the calibration procedure itself must not excessively degrade the precision, and repetition of the calibration at intervals of time should give the same results (within the precision of the method) as the original calibration (*i.e.*, the calibration should be stable as a function of time).

During the development of an analytical method, the analyst will first measure performance without using a calibration graph. Thus precision will be measured by repetitive analysis of standards at a few different concentrations, the detection limit will be measured by repetitive analysis of low-concentration standards, and accuracy will be measured by repetitive analysis of standard materials. The next developmental step will be choice of calibration standards, calibration range and a method for calculating or plotting the calibration graph. The analyst should then measure the precision of the data obtained by use of the calibration graph. In practice, this step is often omitted, because of the extra work involved, lack of awareness of its effects on data quality, and lack of suitable measurement techniques.

Here we present general procedures for measuring and maximizing precision in calibration-graph analysis. Both these aspects of precision are important. Generally used measures of precision are inadequate for calibration-based data, and the calibration graph itself may be the major source of error in an analytical method (this is particularly likely at low concentrations). These procedures are based on the use of confidence bands or confidence-interval statistics to measure precision and to select calibration conditions yielding optimum precision.[1,2] These statistical techniques are not widely known. Their major advantage is that data quality can be measured in terms of all the factors affecting precision, thus providing meaningful data-quality parameters and facilitating selection of conditions which minimize the loss in precision inherent in the calibration process.

PRECISION OF CURRENTLY USED CALIBRATION PROCEDURES

The most precise calibration procedure is the two-standard method. Standards closely bracketing the sample concentration are selected, and the high standard, the sample, and the low standard are analysed in sequence. The sample concentration is calculated by linear interpolation between the two standards. Although very precise, this method is usually impractical for routine analysis because it requires at least one standard measurement for each sample analysed.

For routine analysis most analysts use calibration graphs, the precision of which is considerably lower than that of the two-standard method, for three reasons.

(*a*) Calibration data often do not fit the chosen mathematical model. Calibration graphs are almost always computed by using a least-squares best-fit procedure with an *n*th-order polynomial. Although a first-order equation is appropriate for many analyses (*e.g.*, absorption measurements at low absorbances), for many others the calibration graph is non-linear, and usually convex. For convenience a second- or

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higher-order model is used to calculate the curve, but it may not fit the data. In fact, the curve is often first-order at low concentrations, approximately second-order at high concentrations, but neither of these over the entire range.

(*b*) The data show non-constant variance. The least-squares best-fit procedure minimizes the sum of squares of residuals about the calibration curve. Even if the data fit the mathematical model, the least-squares procedure will yield precise results only if the data show constant variance.[2] With calibration over moderate-to-wide calibration ranges the assumption of constant variance is almost always false. For example, many atomic- and molecular-absorption procedures have constant relative standard deviations (RSDs) over absorbance ranges of about 0.1–0.7, and if the relative variance is constant, the absolute variance must increase with concentration.

A common result of incorrectly assuming constant variance is gross error at low concentrations. The analytical method usually has the largest *absolute* errors at high concentrations. The least-squares procedure minimizes these errors, often rotating the line so that it does not pass through the origin, thus causing large *relative* errors at low concentrations.

(*c*) The precision varies with position on the calibration graph. A given calibration graph shows maximum absolute precision at a concentration equal to the weighted mean concentration of the standards. Variance is greater at both higher and lower concentrations. It increases rapidly with concentration at concentrations above that of the highest standard, particularly with curves of second- and higher-order equations.

The two-standard method does not have any of these disadvantages and hence yields excellent precision. Over narrow ranges calibration graphs are linear, and the data show approximately constant variance. Each measurement is near the mean concentration of the standards.

Manual graph-plotting can also yield good precision because the analyst is not bound by a mathematical model, and non-constant variance can be intuitively compensated for. This approach, however, is time-consuming for routine analysis, and data quality will be very dependent on the skill of the analyst, and on the number of calibration points he is willing to generate.

In general, the wider the calibration range, the worse the precision obtained with conventionally computed calibration graphs. Although a wide range offers direct analysis of samples with widely differing concentrations, the precision is poorer than in analysis over a more limited range. This trade-off between data quality and cost can be minimized by using the improved computational procedures described here.

Confidence-band statistics

A confidence interval is a band either side of a measurement, which will include the "true" value of the measurement with a given significance level α. In calibration-graph analyses, confidence bands around both the sample signal and the calibration graph can be calculated. These are then combined to yield a confidence band around the predicted concentration. The combined band estimates the precision of sample analysis in terms of *all* the factors which affect precision, provided standards are subjected to the same treatment as the samples (Fig. 1). First a calibration graph, say $Y = b_0 + b_1X$ is obtained by a least-squares procedure, then a confidence band around it is computed. The area enclosed by the band will include the line for the "true" equation at, say, a 90% significance level. Confidence bands around the regression equation are usually shaped like a double concave lens, wider at high concentrations because variance increases with increasing concentration. Next, a confidence band around the sample signal Y_0 is computed. This also broadens with increasing concentration, with a resulting increase in the *absolute* precision. These bands are combined as shown in Fig. 1 to produce a confidence band around the predicted sample concentration X_0. Procedures for calculating confidence bands are given in previous papers,[1,2] and

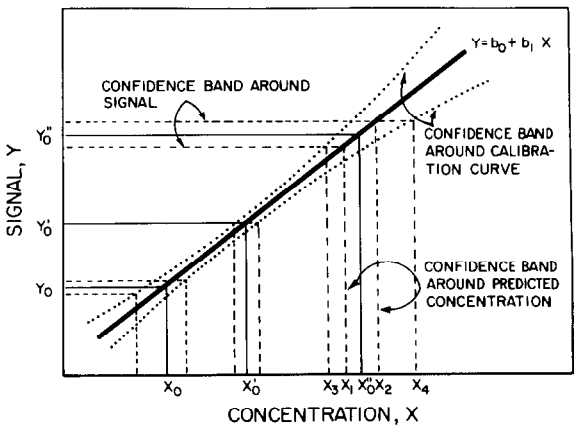

Fig. 1. First-order calibration graph showing confidence bands around the line and around predicted sample concentrations. The latter bands include variation due to (*a*) both sample signal and the curve (band around X_0), (*b*) the curve only (band around X'_0), and (*c*) the sample only (band around X''_0).

the reader is referred to these for the mathematical treatment. Qualitatively, the precision of a given calibration-graph analysis can be improved by (*a*) using an inherently more precise physicochemical measurement method, (*b*) using a more appropriate mathematical model, (*c*) selecting standards such that their weighted mean concentration is roughly equal to the sample concentration, (*d*) making more replicate sample measurements, and (*e*) increasing the number of standard measurements. (This assumes that the calibration curve is "valid" during all measurements, *i.e.*, there is no drift).

Procedures for minimizing loss of precision during calibration

To minimize the loss of precision during calibration, the range covered can be limited, but this may limit the usefulness of the method. Some loss could be avoided by improving the computational procedure by correcting the weakness of the conventional least-squares procedure. Error due to data not fitting the mathematical model could be minimized by finding a more correct model, ideally a single mathematical function to fit the data. This is difficult with non-linear graphs because the data do not usually fit a simple second-order model, and confidence-bands widen as the number of terms in the equation increases.

A simple, semi-empirical approach is to use a multiple-curve procedure.[1] Several calibration curves are computed from various combinations of contiguous standard measurements. The curve giving the narrowest confidence band around each predicted sample concentration is the most appropriate for that particular sample and is used to calculate the sample concentration. For example, if six standard concentrations are used, a low-concentration sample is often best analysed with a calibration graph based on the four standards of lowest concentration rather than all six standards. This approach may intuitively seem incorrect, since we are choosing not to use all the available data, but inclusion of measurements based on the two highest standards may result in a very poor fit to the mathematical model, outweighing the benefits of the additional information.

The effects of non-constant variance can be compensated for by weighting each standard measurement according to the variance at that concentration and then using the least-squares procedure.[2] Without weighting, the least-squares procedure is heavily influenced by variation in measurements of high-concentration standards, which have the worst (absolute) precision. The weighting factors are inversely proportional to variance at each concentration, and a knowledge of the ratio of variances at these concentrations is required for the computation. Use of a weighted least-squares procedure also allows self-consistent rejection of outlying standard measurements and outlying (replicate) sample measurements.

Suggested data-quality parameters

Since confidence-band statistics measure data quality in terms of all the factors affecting precision, they can be used to develop a set of very useful data-quality parameters. In this section, we present these parameters.

(*a*) *Calibration-curve quality.* This is usually measured with parameters such as length of linear range, correlation coefficient, and standard error of estimate. Despite the usefulness of these, their fundamental defects include not indicating whether the chosen mathematical model is adequate and not measuring precision in terms directly meaningful to the data user.

A long linear calibration range is desirable because the linear segment of the curve usually has the maximum sensitivity and because it allows use of a first-order regression equation over a wide range. However, information on length of linear range is of very limited value to the analyst because it cannot be converted into a quantitative statement of precision. The coefficient of correlation, r, between signal and standard concentration measures both precision and the adequacy of the mathematical model. It has no units and has a maximum value of 1.0. Although in analytical chemistry calibration graphs typically show r values of >0.98, this parameter is of limited value for the following reasons. (*i*) r values cannot be meaningfully compared if they are obtained from curves based on different standards (since r is a function of calibration range, it is easier to get a high r value with well-spaced standards than with standards within a narrow concentration range). (*ii*) Curve shape can change without necessarily affecting r. Anscombe[3] shows four 11-point curves for a first-order equation, each with the same r value. One curve shows points spread around a straight line; another set of data is parabolic, misfitted with a straight line; another perfectly fits a straight line but with one outlier; and the fourth shows 11 replicate measurements at the same level. (*iii*) r values do not yield quantitative comparisons of data quality, even if the same standards are used. Although a curve with an r of 0.99 is usually "better" than a curve with an r of 0.98, this is not a quantitative statement. It does not state, for example, that increasing r from 0.98 to 0.99 may improve precision by say 20% at a given concentration. (*iv*) The r value does not indicate whether the chosen mathematical model (*e.g.*, a first- or second-order equation) adequately fits the data.

The standard error of estimate (SEE) of a calibration curve is the SD of the residuals about the curve. It is not affected by the calibration range, and it has the same advantages as a correlation coefficient. However, SEE values are affected by choice of units.

To achieve a more meaningful approach to calibration-curve quality, we use the following two-step procedure. A mathematical model is chosen and the data are tested for lack of fit. If there is significant lack of fit, another model is chosen and tested. When an

appropriate mathematical model is found, calibration-curve quality is measured in terms of confidence-band widths.

The adequacy of the mathematical model can be tested in several ways. If replicate standard measurements are available, an F-test for lack of fit can be used to compare the variation between replicate standard measurements with the variation between residuals about the calibration curve. If variation about the curve is significantly greater than variation between replicate standards, the curve is inappropriate, and a better mathematical model is sought. Otherwise, the model is considered to fit the data adequately. If replicate measurements are not available, the extra-sum-of-squares test can be used to test the effect of adding an extra term to the equation (usually the square of the concentration). If the extra-sum-of-squares due to inclusion of the extra term is significant, then the extra term should be included in the equation. This latter test is inferior to the lack of fit test because it tests only the effect of adding an extra term to the equation, and does not consider the adequacy of either equation.

If a multiple-curve technique[1] is used, an appropriate regression equation for all standard measurements is not always needed, although it can be used for rejecting outlying standard measurements. First- and second-order equations would certainly be appropriate for various segments of the calibration range.

Calibration-curve quality can be measured directly in concentration terms by using confidence-band statistics. A confidence band around the calibration curve is calculated and then used to calculate the confidence band around each predicted sample concentration. Figure 1 illustrates the procedure for predicting sample concentration X'_0 from signal Y'_0 by using $Y = b_0 + b_1x$. The sample signal is assumed to be accurately measured, so as to isolate the effects of calibration-curve quality. A confidence band is calculated around the curve. Signal Y'_0, projected onto the line and bands, intersects the confidence bands, yielding the lower and upper bounds for concentration X'_0. The width of the confidence band about X'_0 will be different at each concentration. For quality-control purposes, confidence bands at a few key concentrations should be calculated for each set of calibration data and checked against a chosen quality standard.

(*b*) *Sample-data quality*. Precision of sample analysis is usually measured in terms of RSD at various sample concentrations. The RSDs are obtained by repetitively analysing samples or standards and then calculating the mean and SD. In practice, RSD values overestimate the precision obtainable from a routine method, for two reasons. The RSD data are often based on analysis of replicate fractions from sample extracts or digests rather than of independent, replicate samples. Such data estimate the precision of the measurement step rather than of the complete analysis. (*ii*) Even when independent replicate samples are analysed, the same calibration graph is usually used, thus ignoring the effects of calibration-curve quality and overestimating the precision of the method. In Fig. 1, signal Y''_0 and a confidence band around the signal are projected onto the calibration line $Y = b_0 + b_1X$ and its confidence band. The narrow band $X_2 - X_1$ around the predicted concentration is obtained when the band around the line is ignored. Use of the band yields the correct confidence band, $X_4 - X_3$.

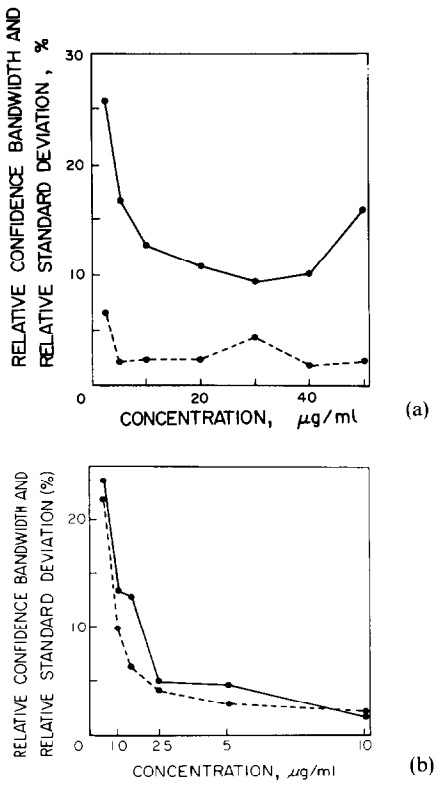

Fig. 2. Comparison of relative standard deviation and relative confidence-band widths for (*a*) determination of chloride and (*b*) determination of lead. (——) Relative confidence-band width = (upper limit—lower limit) × 100/predicted concentration; (– – –) relative standard deviation.

The net effect is that typical RSD data may overestimate precision by a factor of up to about 3. For example, Fig. 2 shows RSDs obtained by replicate sample analysis with a single calibration graph and relative confidence-band widths calculated by assuming triplicate sample measurements for the determination of chloride in water by an AutoAnalyzer procedure[4] and of lead by conventional atomic-absorption spectrometry. Over the range studied the chloride procedure yielded lower RSD values than the lead procedure, but the confidence-band widths were wider for the chloride procedure. This was because the chloride measurements did not closely fit the chosen second-order model, whereas the lead measurements more closely fitted their model.

Thus an analyst would incorrectly conclude from RSD data that the chloride procedure was more precise than the lead procedure. To avoid this disadvantage, we recommend determining confidence-band widths for measuring the precision of a given calibration-graph procedure, for intermethod comparisons, and for monitoring precision on a day-to-day basis.

(*c*) *Minimum reportable concentration.* The lower limit of the concentration range can be defined in several ways (see for example, the third paper in the series by Wilson[5–8]). It is typically some multiple of the detection limit, itself often defined as the concentration at which the RSD is 50%. For a useful discussion of detection limits, see Liteanu and Rîcă.[9] In this paper, we define the lower limit as the lowest concentration at which the analyte can be shown to be present (with a given confidence level). Then samples with predicted concentrations below this threshold level will be reported as containing less than the minimum reportable concentration. For calibration-graph analyses, the detection limit, which is really a measure of the signal:noise ratio for the method, is only one factor (though the major one) affecting whether an analyte at low levels can be shown to be present. Once again calibration parameters are important. For example, if a measurement technique yields a detection limit of 1 arbitrary unit (based on repetitive analysis without calibration), an analyte at a concentration of 2 units is more likely to be detected by use of a 0–3 unit calibration range than a 0–100 range.

Confidence-band statistics provide a useful measurement of the minimum reportable concentration. It is the concentration at which the confidence band around the predicted sample concentration just includes zero as a lower bound (with a given significance level). For example, consider a sample with a predicted concentration of 2 and a symmetrical 90% confidence band of ± 2. There is a $90 + 10/2 = 95\%$ probability that the sample has a concentration above a lower bound of zero. Thus the minimum reportable concentration is 2 at the 95% level of significance.

Choice of calibration conditions has an important effect on the minimum reportable concentration, but not on detection limits as usually defined. To illustrate this, we made a number of replicate measurements of iron in water by conventional atomic-absorption spectrometry. These yielded a detection limit, without a calibration graph, (defined as the concentration at which the RSD is 50%), of 0.015 μg/ml. The minimum reportable concentration was then calculated for four different calibration graphs, each calculated from various combinations of randomly selected data points but always using a total of 18 measurements. As expected, minimum reportable concentrations increased with increasing dynamic range (Table 1), and were always higher than the detection limit.

Note that the minimum reportable concentrations varied by a factor of 12, depending on the calibration range.

The minimum reportable concentration can be routinely calculated from calibration-graph data and will yield more realistic estimates of the concentration at which the analyte can be detected with given calibration conditions on a given day.

(*d*) *Maximum reportable concentration.* In many analytical procedures the sensitivity decreases with increasing concentration, the signal asymptotically approaching a value which is constant irrespective of further increase in concentration. The resulting nonlinearity, increased variance and loss of sensitivity at high concentrations result in less precise data than those obtained at lower concentrations. The analyst should select a cut-off or maximum reportable concentration, above which samples should be diluted and re-analysed, or analysed with use of a different calibration graph. This cut-off level is usually selected arbitrarily (*e.g.*, at the concentration of the highest standard used or at the concentration at which the deviation from linearity exceeds, say, 5%).

The maximum reportable concentration should be selected with care. If it is too low, many samples may require re-analysis. If it is too high, the precision for the more concentrated samples will be poor.

Wilson[7] suggests several criteria for selecting the upper limit of the concentration range for analyses not based on calibration graphs. For calibration-based methods, the maximum reportable concentration can be defined as the maximum concentration at which the method yields adequate precision. This can be readily determined by using confidence band statistics.

To illustrate this approach, we determined iron in water by conventional atomic-absorption spectro-

Table 1. Effect of choice of calibration conditions on the minimum reportable concentration for the determination of iron by atomic-absorption spectrometry

Range, μg/ml	Number of standards	Number of replicates per standard	Number of sample measurements	Minimum reportable concentration,* μg/ml
0.05–0.100	2	6	6	0.026_4
0.05–0.5	5	3	3	0.028_7
0.05–1.5	7	2	4	0.033_5
0.05–5.0	9	2	1	0.33

*Compare with detection limit, 0.015 μg/ml.

Table 2. Determination of maximum reportable concentration

Standard concentrations, μg/ml	Absorbance	Single-curve calibration* relative confidence-band width,† %	Multiple-curve calibration	
			Calibration range, μg/ml	Relative confidence-band width,† %
0.05	0.004	40	0.05–1.5	33
0.10	0.008	26	0.05–1.5	19
0.25	0.022	13	0.05–1.5	6
0.50	0.045	9	0.1–1.5	5
1.00	0.093	8	0.1–1.5	4
1.5	0.142	7	0.1–20.0	6
2.5	0.222	7	0.1–20.0	6
5.0	0.430	5	0.1–20.0	4
10.0	0.750	6	0.1–20.0	5
15.0	0.961	11	0.1–20.0	8
18.0	1.054	>60	15.0–40.0	11
20.0	1.086	>60	15.0–40.0	12
22.0	1.145	>60	15.0–40.0	16
25.0	1.191	>60	15.0–40.0	25
40.0	1.268	>60	25.0–100.0	38
50.0	1.300	>60	0.05–100.0	>60
75.0	1.360	>60	40.0–100.0	>60
100.0	1.405	>60	40.0–100.0	>60

*A weighted least-squares technique was used; calibration range 0.05–18 μg/ml.
†Relative confidence-band width, %, = (upper band − lower band) × 100/2 × predicted value.

metry over the very wide dynamic range of 0.05–100 μg/ml (Table 2 and Fig. 3). Inspection of the plot shows that a first-order equation should fit the data up to a concentration of about 5 μg/ml, with a second-order equation appropriate at higher concentrations. Confidence bands around the predicted concentrations were calculated from the weighted least-squares curves of best fit. Relative confidence-band widths were obtained by two computational procedures: a single second-order curve was fitted to data over the iron-concentration range 0.05–18 μg/ml, and multiple first- and second-order curves were fitted to segments of the data. As expected, relative confidence-band widths were high at low and high concentrations, and the multiple-curve techniques yielded narrower bands than the single-curve technique (Table 2).

With 20% RSD taken as an adequate confidence-band width, the maximum analysis concentration was about 15 μg/ml with the single-curve procedure and about 22 μg/ml with the multiple-curve procedure.

Note that the multiple-curve procedure always includes a calibration standard of higher concentration than the sample. This is contrary to much present practice, but it suggests that an imprecise measurement of a higher concentration standard is better than no measurement at all.

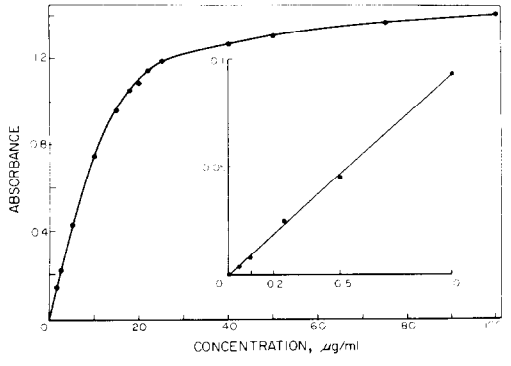

Fig. 3. Calibration data for the determination of iron by atomic-absorption spectrometry.

METHOD DEVELOPMENT WITH CONFIDENCE-BAND STATISTICS

Analytical method development involves a number of steps, such as choice of sample preparation and measurement techniques, studies of systematic error, precision and potential interferents. The confidence-band statistics and precision parameters described above can make a useful contribution. To illustrate this, we present below diagramatically (and in Fig. 4) a general procedure for developing a calibration-graph analytical method. Steps benefiting from confidence-band statistics are discussed in some detail. Others are included for completeness and are only briefly discussed. We use, as an example, data for the determination of iron in potable water.

1. *Select sample preparation and measurement techniques*

The inherent precision and accuracy of these techniques will be the major factor in determining overall data quality. They should be carefully chosen on the basis of published or otherwise known performance data, plus practical factors such as degree of technical difficulty, availability of instrumentation and trained

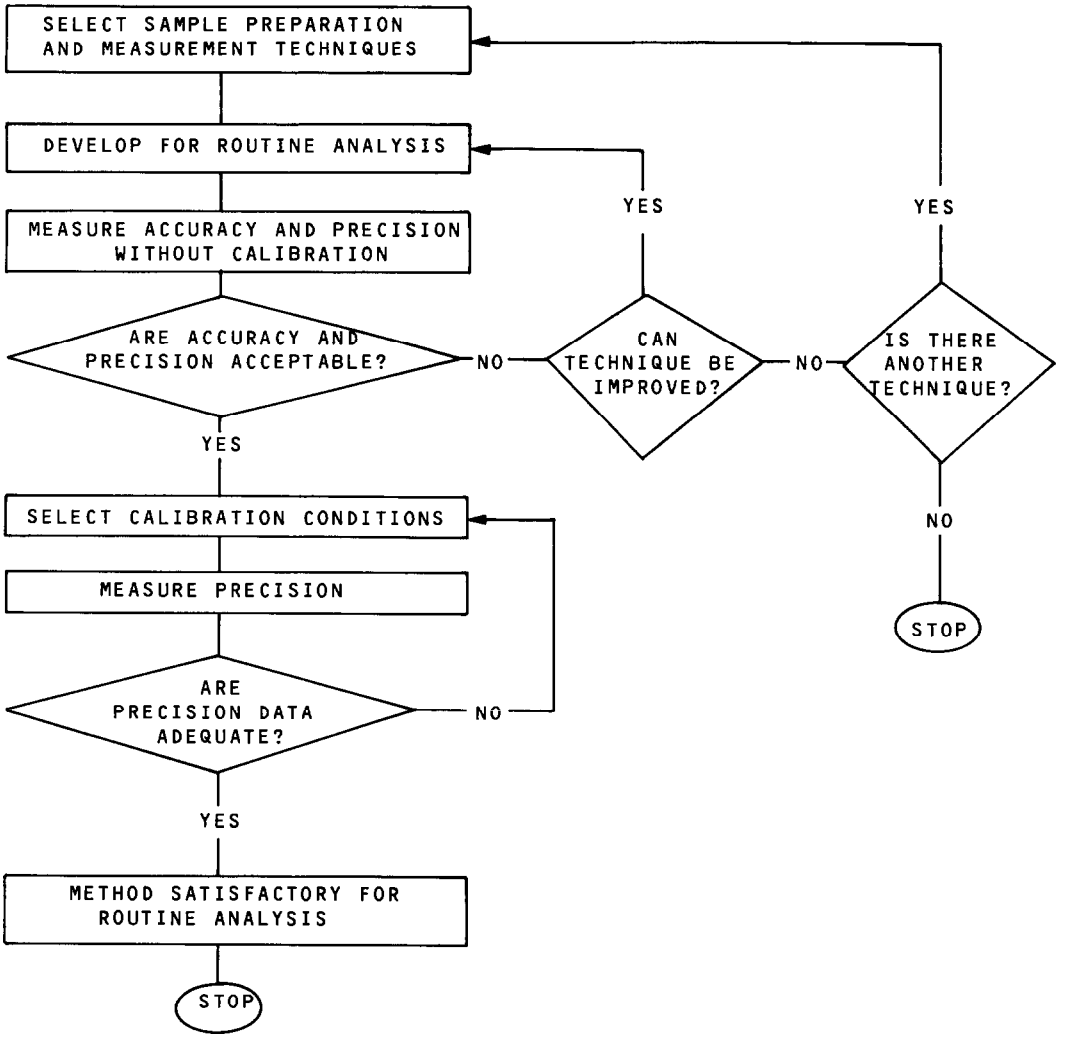

Fig. 4. Method-development procedure for calibration-based analyses.

staff, and cost. In our example, we chose an atomic-absorption procedure using an air–acetylene flame.

2. *Develop method for routine analysis*

The proposed procedure should be carefully "debugged", with error-prone steps (such as critical pH-adjustments) eliminated, simplified or automated.

3. *Measure accuracy and precision without calibration*

These two quality-parameters define the upper limit of performance. The proposed method should be studied in considerable detail. Wilson[5–8] gives excellent guidelines for this step. The studies should also generate sufficient data for calculation of standard deviations at the concentration of each proposed calibration standard (this is required for calculating a weighted least-squares curve of best fit[2]). The stability (freedom from drift) of the proposed method should also be determined. If unstable, it may be improved, for example, by improving temperature control or the instrumentation. Alternatively, drift can be compensated for by frequent calibration. If overall performance is still inadequate, the analyst can consider improving the technique or changing to an alternative technique.

4. *Select calibration conditions*

These can be chosen to meet analytical requirements. Thus, if accuracy at low concentrations is required, the analyst should choose low concentration standards or the multiple-curve technique. If there is a key concentration, such as an action level, the analyst can choose standards with a weighted mean concentration equal to the key value. If a wide determination range is required, the concentration of the highest standard can be at the top of the linear range (or higher with the multiple-curve technique).

In our example we chose an iron-concentration range of 0–2.0 μg/ml, the upper end being the maximum iron level found in at least 99% of samples analysed. The standards ranged from 0.50 to 2.5 μg/ml, with the graph linear over the entire range. The

Table 3. Summary of calibration data for the determination of iron by atomic-absorption spectrometry

Concn., *μg/ml*	SD, *arbitrary units*	Signal, *arbitrary units*	Predicted signal, *arbitrary units*	Difference in signal, *arbitrary units*
0.05	1.8	10	9.6	0.4
0.05		13		3.4
0.10	2.2	23	30	−7
0.10		32		
0.25	3.3	85	91	−6
0.25		92		1
0.50	8.3	200	194	6
0.50		202		8
1.00	11.1	416	398	18
1.00		416		18
2.50	15.2	991	1012	−21
2.50		1014		2

Calibration data: the curve is first-order, by lack-of-fit test, with no outliers, the equation being signal = 10.8 + 409.2 × concentration; correlation coefficient $r = 0.99887$.

weighted mean concentration of the standards was 0.3 μg/ml, the maximum allowable level of iron in potable water in New York State.

5. Measure precision of the proposed method

This should include measurement of relative confidence-band widths at several concentrations and of the maximum and minimum reportable concentrations. In our example, a FORTRAN program REGRES (available from the authors) was used to calculate calibration-graph statistics (Table 3). The program calculated a first-order line of best fit and then tested it for lack of fit (no significant lack of fit was found). It also printed out the calibration equation, the correlation coefficient and detailed calibration data (Table 3).

6. Are precision data adequate?

Precision at several key concentrations was measured by analysing appropriate standards. Data for standards containing iron at the 0.1, 0.3 and 1.0 μg/ml levels are listed in Table 4. The predicted concentrations and relative confidence-band widths for both the multiple-curve and the single-curve procedures and the calibration range for the continguous standards chosen by the multiple-curve technique are listed.

For this analysis, use of the multiple-curve technique did not greatly improve precision (there would have been a much larger improvement if the graph had been non-linear). At the key 0.3 μg/ml level, the multiple-curve technique yielded a relative confidence-band width of 13.9% A sample with a predicted concentration of 0.3 × (100 − 13.9/100) = 0.26 μg/ml or less, does not give a signal exceeding that of the standard at the 95% level of significance (assuming no systematic error).

Thus, the confidence-band width at the key 0.3 μg/ml level should be quite adequate for this application. If it were not, the analyst could consider replicate sample analysis, analysing more standards, or changing the method. For example, triplicate sample analysis would reduce the band width to 9.5% ($13.9/\sqrt{3}$) and raise the cut-off value from 0.26 to 0.27 μg/ml.

7. Method satisfactory for routine analysis

If the precision is adequate, the analyst can, with a reasonable degree of confidence, introduce the method into routine use. It is necessary to document the method, add quality-control procedures and continually monitor analytical performance to ensure that the method continues to work satisfactorily. Large changes in confidence-band widths and errors in control-standard concentrations indicate problems, such as poor instrument response and inaccurate or

Table 4. Control standard data

Mean signal		Predicted concn., *μg/ml*		Confidence-band width, %		Calibration range, *μg/ml*
		Multiple-curve	Conventional	Multiple-curve	Conventional	
31.5		0.104	0.104	31	31	0.05–2.5
114	(single analysis)	0.308	0.305	13.9	18.8	0.25–1.0
114	(triplicate analysis)	0.308	0.305	9.6	12.3	0.25–1.0
399		0.983	1.002	13.2	15.0	0.5–2.5

unstable standards. Other sources of error, such as changes in sample matrix, changes in sampling techniques, and unstable samples, may require more detective work to identify.

DISCUSSION

Confidence-band statistics provide a useful tool for studying and monitoring analytical methods and often give considerable insight into method performance. For example, the fact that confidence bands are usually much wider than RSDs often explains the lower-than-expected precision found in interlaboratory comparisons. Also, minimum reportable concentrations are usually greater by a factor of 3–10 than detection limits reported in the literature and are a much more realistic estimate of the levels at which the analyte can be shown to be present.

In our view, confidence-band data-quality parameters should always be used in calibration-based routine analysis instead of such parameters as RSD, detection limit and correlation coefficient, which are poor measures of the data quality to be expected from a routine procedure. In some cases confidence-band statistics yield information not readily obtainable with other techniques. For example, suppose measurements are to be made over a concentration range of 1–100. If the graph is linear up to a concentration of 120, 120 would be a better highest standard than 100. However, if the curve is non-linear above a concentration of 100, the 120 standard might degrade the fit to the mathematical model sufficiently to negate the benefits of the higher-level measurement. The decision can be based on confidence-band data. As another example, an analyst in our laboratory was determining nitrate in drinking water and used two calibration graphs covering different but overlapping ranges. The results were interpreted by use of both curves. For sample concentrations in the overlapping range, the analyst assumed that the data from the more sensitive calibration graph would be the most precise, and reported the results obtained from this graph. Comparison of confidence-band data showed that this assumption was incorrect.

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SPECTROPHOTOMETRIC DETERMINATION OF METHYLPHENOBARBITONE BY USE OF ORTHOGONAL POLYNOMIALS

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Summary—A direct spectrophotometric method for the determination of methylphenobarbitone in presence of degradation products is presented. The method is based on use of the combined polynomial method. The coefficient of the combined polynomial P_w, calculated over the wavelength range 228–272 nm at 4-nm intervals, is unaffected by the presence of any degradation products and is linearly related to the methylphenobarbitone concentration with a relative standard deviation of 0.4%. The mean recovery is 99.1 ± 0.8%. The method can also be used for assessing the stability of methylphenobarbitone.

Methylphenobarbitone, a member of the barbiturate group, can be determined by several methods including potentiometric titration,[1,2] photometric titration in non-aqueous solvents,[3] other acid–base titrations,[4] complexometric,[5,6] argentimetric,[7] mercurimetric,[8] amperometric,[9–11] coulometric[12] and conductometric titration methods.[13] Colorimetric,[14,15] spectrophotometric[16–18] and spectrofluorimetric[19,20] methods have also been reported. An official method for methylphenobarbitone and phenobarbitone[21] is based on titration with silver nitrate in sodium carbonate solution.[7,22,23]

The present work is concerned with applying the combined polynomial method[24] (a modification of Glenn's method[25] of orthogonal functions) to the determination of methylphenobarbitone in presence of its degradation products. The method has been successfully applied to the determination of thiamine hydrochloride in the presence of its degradation products.[26]

Being an *N*-substituted barbiturate, methylphenobarbitone is unstable in alkaline solutions. Stainier *et al.*[27] reported that the time taken for disappearance of the absorption peak in 1*M* sodium hydroxide is 1 hr. The spectrum of the product from alkaline degradation of methylphenobarbitone has an inflection at about 246 nm, which interferes in determination of the parent compound by means of its absorbance maximum at 246 nm (Fig. 1). The degradation products spectrum has an inflection at about 246 nm.

APPLICATION OF THE COMBINED POLYNOMIAL METHOD

According to the general rules,[25,28–31] the quadratic, P_2, and cubic, P_3, polynomials have been chosen for construction of the required combined polynomial, P_w. The quadratic polynomial makes the major contribution to the absorption curve of methylphenobarbitone and therefore its coefficient p_2 should give a precise estimate of the concentration. Accordingly, p_2 and p_3 (the polynomial coefficients) were calculated from the absorption spectra of methylphenobarbitone (0.002% solution) and its degradation products (equivalent in concentration to 0.002% methylphenobarbitone) in pH-10 buffer, by using 12-point orthogonal polynomials at 2- and 4-nm intervals. The signs and values of the integers a and b were varied until $p_w(Z)$ was negligible in comparison with $\alpha_w c_X$ (see previous work[26] for details). This was achieved by calculating the ratio $p_w(Z)/p(X)$, which gives the relative error in the determination of methylphenobarbitone (X), caused by the presence of the degradation products (Z). It was found that for $p_w = 4p_2 - 3p_3$ calculated over the wavelength range 228–272 nm at 4-nm intervals, with 12-point orthogonal polynomials, $p_w(Z) = +0.0149 \times 10^{-3}$ and $p_w(X) = -15.95 \times 10^{-3}$, so the expected error, $100\, p_w(Z)/p_w(X) = -0.09\%$. Accordingly, the p_w-convoluted curves for the analyte (X) and the irrelevant absorption were plotted (Fig. 1). The finally chosen set of wavelengths (for the integers $a = 4$, $b = -3$) was found to occur on a slope in the p_w-convoluted curve of methylphenobarbitone. This means that the calculated p_w will be affected by any shifts in the spectrophotometer wavelength scale which may occur during measurement. For that reason, an experiment was designed to estimate the

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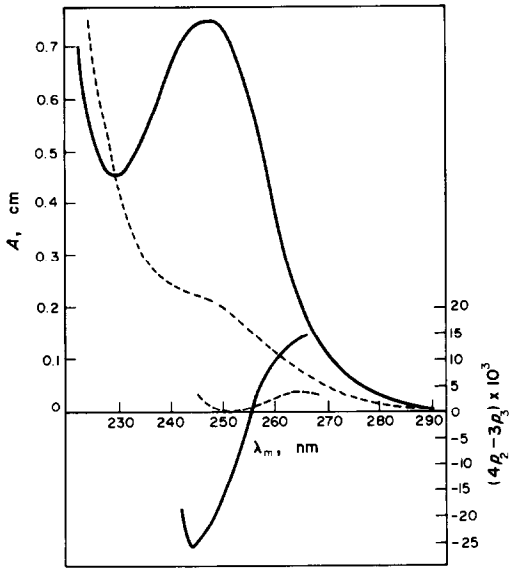

Fig. 1. Absorption curves of 0.022% methylphenobarbitone (——) and its degradation products (---) in pH-10 buffer, and the corresponding ($4p_2$-$3p_3$) convoluted curves derived therefrom, by use of 12-point orthogonal polynomials (4-nm intervals).

effect of a $\pm$0.5-nm shift in the wavelength scale, on the calculated p_w for methylphenobarbitone. A methylphenobarbitone solution buffered at pH 10 was measured at three sets of wavelengths centred at 249.5, 250 and 250.5 mm, with measurements at 4-nm intervals. Then $p_w = (4p_2 - 3p_3)$ was calculated for each set of 12 absorbances. The results are shown in Table 1. To minimize such effects, a standard and a sample should be measured side by side in a constant-temperature laboratory.

Once the wavelength range and intervals, and the values of a and b have been chosen, the combined polynomial can be constructed as shown in Table 2. The value of p_w can be obtained directly by dividing $\Sigma A_i P_{wi}$ by D, where A_i is the absorbance at the ith wavelength, and P_{wi} and D are defined in Table 2.

Linearity of P_w with respect to concentration

Seven solutions of methylphenobarbitone covering a concentration range of 0.5–3.5 mg per 100 ml of buffer solution (pH 10) were prepared by accurate dilution of a stock solution of methylphenobarbitone. The absorbance (in 1-cm cells) of each solution was then measured at 4-nm intervals from 228 to 272 nm inclusive, against the buffer solution. The coefficient p_w of the combined polynomial, P_w, was then calculated for each set of twelve absorbances. Graphs of $p_w = (4p_2 - 3p_3)$ and A_{max} against concentration were plotted and showed reasonable linearity (correlation coefficients 0.9999 and 0.9997 respectively). Linear equations were calculated by regression analysis; they were

$$10^3 p_w = 0.0273 - 7.2902c$$

$$A_{248} = -0.006 + 0.3473c$$

where c is the concentration in mg per 100 ml.

Reproducibility of p_w

Separate determinations of p_w were made for different concentrations of methylphenobarbitone in pH-10 buffer solution, and p_w (for a 1% solution in a 1-cm cell) was calculated for each solution. The results had a relative standard deviation of 0.4%, indicating reasonable reproducibility.

EXPERIMENTAL

Determination of methylphenobarbitone by the combined polynomial method

Weigh accurately about 40.0 mg of methylphenobarbitone and transfer it to a 100-ml standard flask with 10 ml of ethanol. Warm to dissolve and make up to volume with pH-10 buffer solution. Pipette 5 ml into a 100-ml standard flask and dilute to volume with pH-10 buffer solution. Measure the absorbance of this solution at 4-nm intervals over the wavelength range 228–272 nm, against a corresponding blank.

Prepare and treat a standard reference solution similarly.

Preparation of alkali-induced degradation products of methylphenobarbitone

Weigh accurately about 40.0 mg of methylphenobarbitone, transfer it to a 100-ml standard flask with 10 ml of 1*M* sodium hydroxide and leave to stand for 2 hr. Add 10 ml of 1*M* hydrochloric acid, then dilute to volume with pH-10 buffer. Pipette 5 ml of this solution into a 100-ml standard flask and dilute to volume with pH-10 buffer. Measure the absorbance of this solution at 4-nm intervals over the wavelength range 228–272 nm against a blank.

Table 1. Sensitivity of $p_w = (4p_2 - 3p_2)$ to $\pm$0.5-nm shift in the wavelength scale

	(λ_m° − 0.5 nm) 249.5 nm	λ_m° 250 nm	(λ_m° + 0.5 nm) 250.5 nm
Wavelength range,* *nm*	227.5–271.5	228–272	228.5–272.5
$p_w \times 10^3$	−17.16	−15.95	−14.61
Deviation,† %	+7.6	0	−8.4

*Measurements at 4-nm intervals.
†Relative to p_w at λ_m°.

Table 2. Computation of the combined polynomial P_w and the corresponding divisor for the determination of methylphenobarbitone in the presence of degradation products

$$P_{wi} = \frac{ap_{ji}N_k/F + bp_{ki}N_j/F}{N_jN_k/F}$$

$a = 4$ $\quad j = 2$ $\quad N_2 = \Sigma P_2^2 = 12{,}012$

$b = -3$ $\quad k = 3$ $\quad N_3 = \Sigma P_3^2 = 5148$

F = factor to reduce to lowest integers (= 5148)

$$D = N_2N_3/F = 12{,}012$$

$$\sum_{i=0}^{11} P_{wi}A_i/D = (4p_2 - 3p_3)$$

i	P_2	aP_2	$\frac{aP_2N_3}{F}$	P_3	bP_3	$\frac{bP_3N_2}{F}$	P_{wi} $(aP_2N_3 + bP_3N_2)/F$
0	+55	+220	+220	+33	−99	−231	− 11
1	+25	+100	+100	− 3	+ 9	+ 21	+121
2	+ 1	+ 4	+ 4	−21	+63	+147	+151
3	−17	− 68	− 68	−25	+75	+175	+107
4	−29	−116	−116	−19	+57	+133	+ 17
5	−35	−140	−140	− 7	+21	+ 49	+ 91
6	−35	−140	−140	+ 7	−21	− 49	−189
7	−29	−116	−116	+19	−57	−133	−249
8	−17	− 68	− 68	+25	−75	−175	−243
9	+ 1	+ 4	+ 4	+21	−63	−147	−143
10	+25	+100	+100	+ 3	− 9	− 21	+ 79
11	+55	+220	+220	−33	+99	+231	+451

RESULTS AND DISCUSSION

Six mixtures of methylphenobarbitone and its degradation products, in pH-10 buffer, were prepared, and assayed by the combined polynomial method. The results obtained are shown in Table 3. The mean recovery was found to be 99.1 ± 0.8%

Errors in the method can be attributed to (*i*) wavelength setting errors, (*ii*) non-zero coefficients that may have been contributed by the added degradation products to the assay coefficient, (*iii*), overall shifts in the wavelength calibration, which could affect coefficients sited on slopes in the corresponding p_w-convoluted curve (Fig. 1).

Several trials have been made of the application of the single polynomial method for the determination of methylphenobarbitone in presence of its degradation products, by use of P_2 from the twelve-point orthogonal polynomials for measurements at 2- and 4-nm intervals, but without success. For example, the mean recovery was found to be 95.5 ± 1.5% from use of p_2 calculated over the same set of wavelengths (228–272 nm at 4-nm intervals, Table 4). The poor results obtained by using p_2 alone were due to the contribution to p_2 from the degradation products, which was cancelled by the method using $4p_2 - 3p_3$.

For comparison, recoveries were calculated from the maximum absorbances and were unacceptably high (Table 3). The error in each result decreased with increase in the concentration of methylphenobarbitone relative to the concentration of the degradation products (the latter being kept constant in the mixtures).

The modified Vierordt method[32] was applied to the determination of methylphenobarbitone (λ_1 = 228 nm, λ_2 = 248 nm) in the same six mixtures. The mean recovery was found to be 102.6 ± 1.4%.

However, the presence of a linear irrelevant absorption, such as may originate from differences between batches of the samples and the "reference" standard of methylphenobarbitone, will certainly lead to erroneous results in the modified Vierordt method. On the other hand the results obtained by using the combined polynomial method will not be affected by interferences contributing to coefficients other than those involved in p_w. In other words, the calculation of $(4p_2 - 3p_3)$ will correct for constant, linear, quartic, quintic, *etc.* components of an irrelevant absorption. In support of this argument, Table 3 shows a systematic error in the recovery experiments when the modified Vierordt method was used, the recovery decreasing systematically from 104.8 to 100.9%. This systematic error could originate by chance from contamination of either the reference solutions of methylphenobarbitone or the prepared mixtures. However, this systematic error does not appear in the results obtained by using p_w. Moreover, it also does not appear in the results obtained by using p_2, although the latter gives poor results. This example illustrates the superiority of the combined polynomial method to the modified Vierordt method.

Table 3. Spectrophotometric determination of methylphenobarbitone in presence of the degradation products, by different methods

Expt.	Added,* mg	Recovery, %			
		A_{248}	p_2	p_w $(4p_2 - 3p_3)$	Modified Vierordt
1	1.0	120.9	92.7	97.7	104.8
2	1.5	114.6	95.2	99.7	103.9
3	2.0	111.0	95.8	99.7	102.6
4	2.5	108.9	96.1	98.6	102.1
5	3.0	107.4	96.7	99.3	101.6
6	3.5	106.1	96.7	99.6	100.9
Mean		112.5	95.5	99.1	102.6
s.d.		5.6	1.5	0.8	1.5

*Each contains degradation product corresponding to 0.50 mg methylphenobarbitone.

Degradation of methylphenobarbitone

The recovery experiments reported in Table 3 are not enough to prove the specificity of the method. There may be different intermediate products which, of course, will not be revealed by these recovery experiments. A 0.05% solution of methylphenobarbitone in 0.1*M* sodium hydroxide was therefore prepared and kept at room temperature in the dark, and 5-ml portions of this solution were diluted to 100 ml with pH-10 buffer solution at zero time and then at hourly intervals. The absorbances were measured at 4-nm intervals in the range 228–272 nm. The 0.1*M* sodium hydroxide medium was used to accelerate the rate of decomposition of methylphenobarbitone. The coefficient p_w was calculated from each set of twelve absorbances and the concentration of methylphenobarbitone was computed. The plot of log c% against time (data in Table 4) gave a straight line with a slope of $-0.02031\ hr^{-1}$ and a correlation coefficient of 0.9976. It may therefore be concluded that the proposed method based on calculating $(4p_2 - 3p_3)$ is specific for the intact molecule, and not affected by the degradation products.

To test the specificity of the official method of analysis for the intact molecule of methylphenobarbitone, a 2% solution of the drug in 0.1*M* sodium hydroxide was assayed at hourly intervals by the argentimetric method after neutralization with a precalculated volume of 0.1*M* nitric acid and addition of 25 ml of a 4% solution of anhydrous sodium carbonate. The volume of silver nitrate solution consumed did not vary with time and so did not reveal the degradation of methylphenobarbitone. This indicates that the official method of analysis is not specific for the intact molecule of methylphenobarbitone, which is shown by the polynomial method to undergo cleavage in 0.1*M* sodium hydroxide medium. In this respect, the proposed method is considered superior to the official method in determining methylphenobarbitone in presence of degradation products.

Table 4. Decrease of concentration of methylphenobarbitone with time, calculated from $(4p_2 - 3p_3)$

Time, hr	Conc., %
0	100.0
1	95.1
2	89.9
3	86.4
4	82.7
5	79.3

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SHORT COMMUNICATIONS

N-PHENYL-*N'*-ACETYLTHIOUREA—A SPECIFIC REAGENT FOR PHOTOMETRIC DETERMINATION OF RUTHENIUM

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Summary—*N*-Phenyl-*N'*-acetylthiourea is recommended as a specific chelating agent for the spectrophotometric determination of ruthenium. The blue complex formed in 5–8*M* hydrochloroic acid is not extractable into common organic solvents but is soluble in 30% ethanol solution. The colour is stable for up to 48 hr and Beer's law is obeyed at 650 nm over the metal concentration range 2–18 μg/ml. The molar absorptivity is 4.7×10^3 l.mole^{-1}.cm^{-1}.

Beamish and Van Loon[1] have critically reviewed the use of organic thio-ligands in the photometric determination of ruthenium, but these reagents generally lack selectivity. Many of the more recent reagents are also deficient in this respect,[2–8] but *o*-hydroxythiobenzhydrazide[9] and *N,N*-diphenylthiourea[10] have good selectivity. We now introduce *N*-phenyl-*N'*-acetylthiourea (PhAT) as a chelating agent that is specific for the spectrophotometric determination of ruthenium in the sense that it does not give a colour reaction with any other metal ion in acid medium.

EXPERIMENTAL

Reagents

Ruthenium(III) chloride monohydrate was dissolved in dilute hydrochloric acid, and the solution was standardized by hydrolytic precipitation and diluted as required. Solutions of other ions were prepared by dissolving the corresponding salts in distilled water or dilute hydrochloric acid. All reagents used were of analytical or general reagent quality.

Preparation and properties of N-*phenyl*-N'-*acetylthiourea*

The reagent was prepared by the general procedure described by Charonaut and le Perdriel.[11] Ammonium thiocyanate (0.1 mole) was dissolved in acetone and 0.1 mole of acetyl chloride was added slowly with constant shaking. The mixture was refluxed for 15 min on a hot water-bath, cooled and filtered. A solution of 0.1 mole of aniline in acetone was added slowly to the filtrate with constant shaking. The mixture was refluxed for 10 min on a hot water-bath, then most of the acetone was removed by distillation. The residue was added slowly to crushed ice with constant stirring. The crude solid reagent was filtered off and recrystallized from aqueous ethanol. The pure compound melts at 174°. It is appreciably soluble in water and highly soluble in ethanol and most of the common organic solvents. It is stable towards heat, light, air, acids and alkalis, but attacked by strong oxidants. A 0.01*M* ethanolic solution of PhAT was used throughout.

Procedure

A measured volume of ruthenium(III) chloride solution was placed in a 25-ml Erlenmeyer flask and its acidity adjusted to between 5 and 8*M* hydrochloric acid. Then 3–4 ml of 0.01*M* ethanolic PhAT solution were added and the mixture was stirred well and warmed for 10 min on a hot water-bath, then cooled. The contents of the flask were transferred quantitatively to a 25-ml standard flask and made up with concentrated hydrochloric acid and ethanol so that the final solution was about 6*M* in the acid and contained about 30% ethanol. The absorbance was measured at 650 nm against a reagent blank prepared under identical conditions.

RESULTS AND DISCUSSION

Spectral characteristics of the complex

The complex exhibits maximum absorption at 620 nm and the reagent has no absorption at wavelengths longer than 550 nm when measured against distilled water. The absorbance is constant for up to 48 hr. The ruthenium concentration range for conformity to Beer's law at 650 nm is 2–18 μg/ml, and the optimal range[12] is 4–15 μg/ml. The molar absorptivity at 650 nm is 4.7×10^3 l.mole^{-1}.cm^{-1}.

The optimal final acidity is 5–8*M* hydrochloric acid. The complex is appreciably soluble in water, highly soluble in ethanol and incompletely extracted by the common organic solvents. The minimal molar-ratio of reagent to ruthenium for complete colour development is 16. Both ruthenium(III) and ruthenium(IV) give the same colour reaction with PhAT, so presumably the reagent reduces Ru(IV).

Table 1. Effect of diverse ions in the spectrophotometric determination of 190 μg of ruthenium

Foreign ion	Amount added,* mg	Foreign ion	Amount added,* mg
Fe^{3+}	1.5	Cd^{2+}	1.5
Co^{2+}	1.5	Hg^{2+}	1.5
Ni^{2+}	1.5	Pb^{2+}	2.0
Cr^{3+}	2.0	UO_2^{2+}	5.0
Mn^{2+}	2.0	Ti^{4+}	2.0
W(VI)	2.5	Th^{4+}	5.0
Mo(VI)	1.0	Au^{3+}	2.0
Cu^{2+}	2.0	Rh^{3+}	1.8
Zn^{2+}	2.0	Pd^{2+}	2.0
Ga^{3+}	1.5	Os(VIII)†	2.0
In^{3+}	1.5	Ir^{3+}	2.0
Tl^{3+}	2.5	Pt^{4+}	2.0

*Causes an error less than ±2%.
†Os (VIII) is reduced to Os (VI) in alcoholic medium.

The coefficient of variation of the measurements is 0.5%.

Effect of other ions

The organic reagent produced no colour with any of the ions tested. Hence, no masking agent was required in the determination of ruthenium. The tolerance limits for the ions tested are shown in Table 1. Ruthenium can be determined with PhAT in the presence of appreciable amounts of foreign ions commonly associated with it.

Specific and sensitive methods for the spectrophotometric determination of ruthenium are of importance because the metal occurs only as a minor constituent in its sources. Though thiourea and substituted thioureas offer sensitive colour reactions with reuthenium, the advantage of *N*-phenyl-*N'*-acetylthiourea lies in its selectivity and the simplicity of the procedure.

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DETERMINATION OF THORIUM AND SCANDIUM BY INDIRECT CHRONOPOTENTIOMETRIC STRIPPING ANALYSIS

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Summary—A highly sensitive chronopotentiometric determination of thorium or of scandium is described. It is based on the substitution of thorium or scandium for lead (or zinc) in the lead–EDTA complex, and measurement of the liberated lead by deposition on a mercury film electrode, followed by a chemical oxidation stripping process during which the potential of the mercury electrode is monitored. Thorium has been determined at concentrations in the range 10^{-5}–$10^{-7}M$ by use of deposition times from 1 min to 1 hr.

Potentiometric stripping analysis was introduced by Jagner and Graneli.[1] The method, which is related to anodic stripping voltammetry, is based on two steps: in the first the metal ions in solution are concentrated by electrolysis onto a mercury film electrode from a solution containing also mercury(II). At the completion of this step the potentiostat is disconnected from the electrochemical cell. In the second step the potential of the electrode is recorded as a function of time as the metal in the mercury film is oxidized, either by the excess of mercury(II) or by the dissolved oxygen in the solution, and thus stripped back into the solution.

This approach has been used[2–4] for the determination of ions forming amalgams and being reversibly reduced and oxidized at the mercury electrode, including copper, zinc, lead, cadmium, bismuth and thallium. We have extended the study to cover the determination of metals which do not form amalgams, such as thorium, scandium and the rare earths, by making use of a substitution reaction between the EDTA complex of lead, or of zinc, and those metals having EDTA complexes with formation constants at least four orders of magnitude greater than those for lead and zinc:

$$PbY^{2-}(ZnY^{2-}) + Me^{2+} \rightarrow MeY^{2-} + Pb^{2+}(Zn^{2+})$$

The relevant log K values are: PbY 18, ZnY 16.5, ThY 23.2, ScY 23.1, InY 25. Free lead and zinc can readily be determined by potentiometric stripping analysis. Note that the thermodynamic formation constants can be used, as the side-reaction coefficient for protonation of the EDTA will affect all the constants equally, at a given pH.

EXPERIMENTAL

Reagents

All solutions ($10^{-3}M$) of the metals were prepared from their *p.a.* chlorides or nitrates dissolved in doubly distilled water. Hydrochloric acid ($0.5M$ and $0.1M$) and acetate buffer solutions ($0.1M$, various pH values) were prepared from reagent-grade chemicals diluted with doubly-distilled water.

Apparatus

Potentiometric stripping measurements were made with an REC 80 Servograph with the REA 120 scanning unit and TTA 80 titration assembly, and pH values were measured on a model PHM 64 meter, all from Radiometer, Copenhagen. Micropipettes with capacities from 5 to 500 μl (Bie & Bentsen, Denmark) were used for measuring out the metal solutions.

Electrodes

Radiometer electrodes were used: a P 1312 platinum counter-electrode, a K 4040 saturated calomel electrode and an F 3500 glassy-carbon electrode. The surface of the glassy-carbon electrode was polished for 1–2 min with 3-μm diamond paste, then rinsed with acetone. When not in use, the electrode was stored in ethanol.

The following procedure is recommended for precoating the surface of the glassy-carbon electrode: immerse the electrode in $0.1M$ hydrochloric acid containing 25 mg of mercury(II) per litre and plate the mercury at -0.5 V *vs.* SCE for 2 min, then strip it off again. Repeat this cycle, plating at -0.7 V, then -0.8 V and finally at -0.9 V. Wash the electrode with distilled water and use for the measurements. This preparation procedure should be repeated every two days.

Procedure

To 10 ml of $5 \times 10^{-3}M$ lead–EDTA solution add 5 ml of pH-3.17 buffer and then the sample containing thorium or scandium: for the calibration take from 0.1 to 0.9 ml of $5 \times 10^{-3}M$ thorium or scandium solution. Boil for 5 min under an infrared lamp, then cool to room temperature and add air-saturated buffer solution to make up to 25 ml. There is then enough dissolved oxygen to oxidize the metal back into solution quantitatively in the stripping process. Transfer the solution to the cell and electrolyse for 12 min at -0.8 V *vs.* SCE (or at -1.3 V for zinc), then disconnect the potentiostat and record the potential as a function of time (a suitable chart speed is 1 mm/sec). The time taken to complete the stripping process is proportional to the initial concentration of thorium (or of scandium): completion is indicated by the potential moving rapidly to less negative

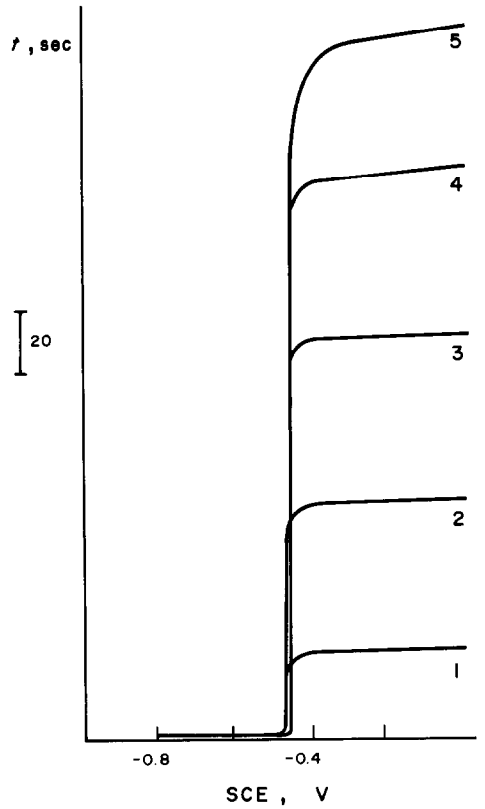

Fig. 1. Set of stripping chronopotentiometric curves calibration graph for thorium or scandium in acetate buffer at pH 3. Electrolysis for 1 min at −0.8 V. Curves 1–5: 2, 6, 10, 14, 18 × $10^{-5}M$ Th or Sc.

values. Figure 1 shows a typical set of curves obtained with the lead–EDTA complex. Similar results are obtained when the zinc–EDTA complex is used.

RESULTS AND DISCUSSION

Thorium and scandium can displace lead or zinc from their respective EDTA complexes in weakly acidic solution, but to speed up the reaction it is preferable to heat the solution to boiling for a few minutes. The best results are obtained by working in the pH range 2.7–4.4. At higher pH the products of hydrolysis of the thorium aquo-ions are adsorbed on to the mercury electrode, and at lower pH the substitution reaction is too slow.

The determination of thorium suffers interferences from traces of complexones such as EDTA, DCTA *etc.*, and from sulphate, which precipitates lead and complexes thorium. Other cations with log K_{MY} values close to 23 [in the range 20–29, including Sn(II), Cr(III), Sc, In and Zr] interfere as they react similarly to thorium with the lead and zinc complexes, though the rates of reaction are sometimes different.

The determination of scandium, which has a log K_{MY} value of 23.1, is similar to that of thorium, and so are the interferences. The optimum pH range is 2.6–3.0, limited by slowness of reaction and by hydrolysis at low and high pH values respectively, as with thorium. The variation of stripping time with pH in the initial chemical steps is shown in Fig. 1.

The limit of detection depends on the electrolysis time, but the determination of thorium or of scandium is feasible in the concentration range from $10^{-5}M$ (1 min electrolysis) to $10^{-7}M$ (1 hr electrolysis) when dissolved aerial oxygen is used as the oxidizing agent. The concentration of thorium or scandium can be found from a calibration graph, but is better obtained by using the standard-addition method as it is difficult to keep a constant ionic strength in all samples and standards, and this factor does have a significant effect on the measured stripping times. Table 1 includes some results for determinations of thorium in the presence of a number of other metals. Thorium, scandium and indium would be measured as their sum.

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Table 1. Determination of thorium in the presence of other metals (10 replicates)

Metals added, *mg*		Thorium added, *μg*	Thorium found, *μg*	$\bar{x}$, *μg*	Std. devn., *μg*	Electrolysis time, *min*
Al, Mn, Ca, Mg	20	2.3	2.1	2.08	0.11	6
Al, Mn, Ca, Mg	100	0.23	0.19	0.19	0.02	36
Cd, Zn, Ni, La, Ce	100	2.3	2.55	2.55	0.30	6
Cd, Zn, Ni, La, Ce	100	0.23	0.24	0.24_5	0.01	36

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COMPLEXOMETRIC DETERMINATION OF MERCURY(II) BY USE OF 4-AMINO-5-MERCAPTO-3-PROPYL-1,2,4-TRIAZOLE AS REPLACING REAGENT

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Summary—A simple, rapid, and selective complexometric method is proposed for the determination of mercury(II). Mercury(II) is first complexed with excess of EDTA and the surplus EDTA is back-titrated (pH 5–6) with zinc sulphate solution, with Xylenol Orange as indicator. 4-Amino-5-mercapto-3-propyl-1,2,4-triazole is then added to displace EDTA from the Hg–EDTA complex and the released EDTA is titrated with zinc sulphate solution. Reproducible and accurate results are obtained in the range 1–40 mg of mercury, with a relative error of approximately 0.4%.

Mercury(II) is normally not determined by direct EDTA titration, particularly in presence of other ions.[1] Usually the mercury(II) and other metal ions are first complexed with EDTA and the Hg–EDTA complex is then selectively decomposed by replacing reagents[1–3] and the EDTA released is titrated. However, many of these methods suffer severe interference from copper(II), some require heating for demasking the Hg–EDTA complex, and others require cooling to avoid the copper interference. A satisfactory method for use in presence of copper was described by Ueno, however.[4] The method now reported uses 4-amino-5-mercapto-3-propyl-1,2,4-triazole (HPAMT) as a selective demasking agent for the Hg–EDTA complex at pH 5–6. Copper(II) does not interfere and the decomposition takes less than 5 min even at room temperature.

EXPERIMENTAL

Reagents

HPAMT. Synthesized as reported in the literature[5] (found: C 38.2%, H 6.2%, N 35.6%, S 20.3%; $C_5H_{10}N_4S$ requires C 37.95%, H 6.37%, N 35.41%, S 20.26%; m.p. 104°), and used as a 0.025*M* solution in acetone.

Mercuric chloride solution. Prepared from analytical-grade material and standardized by the ethylenediamine method.[6]

Zinc sulphate solution. Standardized by the quinaldinate method.

EDTA solution, 0.025M.

Xylenol Orange indicator solution, 0.5%.

Procedure

An acidic solution of the sample, containing 1–40 mg of Hg(II), was mixed with excess (about 5 ml) of EDTA and adjusted to pH 5–6 with hexamine. The excess of EDTA was back-titrated with zinc sulphate solution (Xylenol Orange as indicator). At least 100% excess of HPAMT (calculated on the basis of a 2:1 reaction ratio of HPAMT to Hg) was added and the mixture allowed to stand for 5 min. The EDTA released was then titrated with zinc solution. The second titration gave the amount of Hg(II) present.

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RESULTS AND DISCUSSION

The fact that HPAMT displaces EDTA quantitatively (at pH 5–6) from the Hg–EDTA complex indicates that the $Hg(HPAMT)_2$ complex is more stable than the Hg–EDTA complex. The release is quantitative at room temperature. Further, unlike many sulphur ligands, HPAMT forms a highly soluble $Hg(HPAMT)_2$ complex, and the absence of a precipitate in the reaction mixture assists accurate determination of the end-point. At least 100% excess of the reagent is required for complete release of the EDTA, but a larger excess has no adverse effect. Zinc sulphate was found to be ideal as the titrant as it gives a sharp end-point and does not react with HPAMT. Equally good results are obtainable with lead nitrate as titrant, in the absence of a large amount of chloride or sulphate in solution.

Table 1. Determination of 24.89 mg of mercury in a mixture of metal ions

Metal ions	Quantity added, *mg*	Mercury found, *mg*
Pb(II) + Cd(II)	200 each	24.7_8
Cd(II) + Zn(II)	200 each	24.8_9
Cu(II) + Co(II)	70 + 100	24.9_9
Cu(II) + Pb(II) + Cd(II)	70 + 200 + 200	24.8_9

Mercury in the range 5–40 mg was determined with a relative error not exceeding 0.4% and an overall standard deviation of 0.07 mg. The effect of diverse metal ions on the determination of 25 mg of Hg^{2+} was studied and the tolerance limits found were 200 mg of Cd^{2+}, Pb^{2+} or Au^{3+}, 100 mg of Co^{2+}, 70 mg of Cu^{2+}, 50 mg of Ni^{2+} and 25 mg of Mn^{2+}, either singly or in combination. It is important to note that Cu^{2+} can be tolerated in up to threefold ratio without masking or cooling to below room temperature.

Some results for synthetic mixtures are given in Table 1. However, no satisfactory results were obtained in the presence of Bi^{3+}, As(III), Sb(III), Al^{3+}, Fe^{2+} and Fe^{3+}, as the end-point in these cases was not sharp.

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DETERMINATION OF PHOSPHORUS AND TUNGSTEN IN HETEROPOLY ACIDS BY EDTA-TITRATION

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Summary—Previous methods for the titrimetric determination of phosphorus and tungsten with EDTA are found to be unsatisfactory for heteropoly acids. Successful modifications of the procedures are described.

The application of heteropoly acids and their salts in catalytic processes has received increased attention in recent years.[1,2] Tungsto- and molybdophosphates decompose in alkaline media to form simple tungstate or molybdate ions and hydrogen phosphate,[1,3] which finds use in a popular method[4] for the titrimetric determination of phosphorus after precipitation as ammonium 12-molybdophosphate.[3]

$$[PMo_{12}O_{40}]^{3-} + 23OH^- \rightarrow 12MoO_4^{2-} + HPO_4^{2-} + 11H_2O \quad (1)$$

$$[P_2W_{18}O_{62}]^{6-} + 34OH^- \rightarrow 18WO_4^{2-} + 2HPO_4^{2-} + 16H_2O \quad (2)$$

However, calcination of the hydrated 12-tungstophosphoric acid at high temperatures results in decomposition of the heteropoly structure with some loss of the matrix oxide by sublimation.[2] The stoichiometry given above no longer applies to the catalyst thus obtained and it is necessary to analyse separately for both phosphorus and tungsten.

EDTA methods have been reported for the determination of phosphorus in uranium ores, concentrates and liquors, by precipitation as ammonium molybdophosphate and titration with EDTA.[5] Phosphates, metaphosphates, pyrophosphates, and tripolyphosphates have also been determined by precipitation as magnesium ammonium phosphate followed by EDTA titration.[6] Further discussion of EDTA techniques for the determination of phosphorus can be found in the literature.[4,7] The determination of tungsten by precipitation as lead tungstate and subsequent EDTA titration was suggested some years ago[8] and has been applied to tungsten–iron and tungsten–molybdenum alloys[9] and to tungsten oxides.[10]

Though these methods have been satisfactorily used for analysing a variety of relatively simple compounds of phosphorus and tungsten, attempts to apply them to tungstophosphates have produced less than satisfactory results. We report here some modifications of earlier procedures that make them satisfactory for analysis of 12-tungstophosphoric acid and dimeric 9-tungstophosphoric acid by EDTA titration.

EXPERIMENTAL

Samples

Analytical-reagent grade 12-tungstophosphoric acid, authentic simple tungstates and phosphates were obtained from BDH Chemicals. The 12-acid was shown to be the 24-hydrate by thermogravimetry.[2] The dimeric 9-tungstophosphoric acid was prepared from the A-form of the ammonium salt.[11]

Reagents

Stock solutions of 0.01*M* magnesium chloride and 1*M* ammonia/1*M* ammonium chloride buffer. Eriochrome Black T, 1% solution in 10% ammonia solution,[12] prepared fresh every month. Stock solutions of 0.02*M* lead nitrate and of acetate buffer[10] (50 g of sodium acetate and 12 ml of glacial acetic acid diluted to 1000 ml). Aqueous 1% Xylenol Orange solution.

Procedures

Phosphorus. Add 5 ml of 1*M* sodium hydroxide to an aliquot of heteropoly acid solution (containing ~50 μmole of phosphorus) and dissolve the resultant precipitate by gentle boiling. Neutralize with 5 ml of 1*M* hydrochloric acid, then add 20 ml of ammonia/ammonium chloride buffer and 10 ml of 0.01*M* magnesium chloride. Titrate the surplus magnesium with 0.01*M* EDTA (Eriochrome Black T indicator) after leaving sealed overnight.

Tungsten. To an aliquot of heteropoly acid solution (*ca.* 100 μmole of tungsten), add 1.6 ml of 1*M* sodium hydroxide and 20 ml of water. Boil gently to dissolve the sample and dilute with water to about 450 ml. Quickly add 20 ml of acetate buffer to make the pH 5.0, and then 10 ml of 0.02*M* lead nitrate. Boil for 15 min, cool, and filter off the white precipitate. Titrate the surplus lead in the filtrate with 0.01*M* EDTA (Xylenol Orange indicator). Correct the tungsten value for phosphate as discussed in the text.

RESULTS AND DISCUSSION

Table 1 shows the results of alkalimetric titrations of tungstophosphoric acids and their calcined

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Table 1. Alkalimetric analysis of tungstophosphoric acids and their calcined products*

Sample	Phosphorus, *mmole*		Recovery, %
	Taken	Found	
$H_3PW_{12}O_{42}$			
Uncalcined 24-hydrate	0.112_2	0.111_4	99.3
Calcined† at 400° (anhydrous)	0.110_2	0.109_5	99.4
Calcined† at 525° (decomposed)	0.125_8	0.121_8	96.8
$H_6P_2W_{18}O_{62}$			
Uncalcined 35-hydrate	0.100_8	0.101_8	100.7
Calcined† at 400° (anhydrous)	0.113_6	0.110_4	97.2

*Literature procedure[3] mentioned in the text.
†In helium, for 2 hr.

products, in which a known amount of heteropoly acid (*ca.* 100 μmole of phosphorus) was dissolved in 20 ml of 0.25*M* sodium hydroxide and the excess of alkali was titrated with 0.25*M* hydrochloric acid (phenolphthalein as indicator).[3] The uncalcined 12-tungstophosphoric acid was the 24-hydrate, calcination of which at 400° gave the anhydrous acid and at 525° resulted in decomposition. This thermal behaviour was confirmed by thermogravimetric analysis and powder X-ray diffraction.[2] The calcined products and the dimeric 9-acid required complete hydrolysis by gentle boiling in alkali before the titration. This simple technique seemed suitable for routine analysis of the stoichiometric heteropoly acid.

The EDTA determination of phosphorus[6] is based on the complete precipitation of phosphate as ammonium magnesium phosphate ($NH_4MgPO_4 \cdot 6H_2O$) with a known (and excessive) amount of magnesium chloride at pH 10.5 in ammonia/ammonium chloride medium. Fine crystals are deposited on the walls of the container within 5–10 min and titration after 30 min gives fairly good results for simple phosphates (Table 2), but not for the heteropoly acids. The modified procedure described above gives results in good agreement with the expected values for the uncalcined tungstophosphoric acids (Table 2). Evidence for some loss of phosphorus on calcination appears in these results though it is not shown by those obtained by alkalimetry (Table 1).

The modifications are simple, but essential both for complete decomposition of the heteropoly acid and quantitative precipitation of $NH_4MgPO_4 \cdot 6H_2O$.

Tungsten(VI) forms a white precipitate of lead tungstate with lead ions.[13] Titration[10] of the remaining lead with EDTA at pH 5 gives excellent results for a simple tungstate and an isopolytungstate (Table 3). The complete decomposition of the isopoly salt into simple tungstate by gentle boiling in alkaline medium is again important. However, the values obtained for tungsten in heteropoly acids were about 15% too high. The discrepancy was suspected to arise from the precipitation of lead phosphate, which is only slightly soluble in water,[14] along with the lead tungstate. Precipitates were obtained from both Na_2HPO_4 and Na_3PO_4 with lead in acetate buffer. EDTA titration of the excess of lead in the filtrate confirmed the precipitation of $Pb_3(PO_4)_2$. Since 1 mmole of phosphate will react with 1.5 mmole of lead (which in turn will

Table 2. EDTA determination of phosphorus

Sample	Phosphorus, *mmole*		Recovery, %	
	Taken	Found	This work	Independent analysis
Authentic phosphates*				
Na_2HPO_4	0.0491	0.0470	0.95_7	0.99_3†
$(NH_4)_2HPO_2$	0.0560	0.0555	0.99_1	1.00_3†
$Na_3PO_4 \cdot 12H_2O$	0.0527	0.0560	1.06_2	0.97_7†
Heteropoly acids§				
$H_3PW_{12}O_{40} \cdot 24H_2O$	0.0453	0.0460	1.01_5	1.00_4‡
Calcined at 400°	0.0571‖	0.0555	0.97_2	—
Calcined at 525°	0.0610‖	0.0540	0.88_5	—
$H_6P_2W_{18}O_{62} \cdot 35H_2O$	0.0421	0.0420	0.99_8	1.02_4‡

*Literature procedure[4] in the text.
†Laboratory A.
‡Laboratory B.
§Modified procedure (this work).
‖Based on anhydrous $H_3PW_{12}O_{40}$.

Table 3. EDTA determination of tungsten

Sample	Tungsten, *mmole*			Recovery, %	
	Taken	Found*	Corrected†	This work	Independent analysis
Simple and isopoly tungstate§					
$Na_2WO_4 \cdot 2H_2O$	0.1052	0.1070	—	101.7	100.5‡
$(NH_4)_{10}W_{12}O_{41} \cdot 5H_2O$	0.1218	0.1221	—	100.2	107.9‡
Heteropoly acids‖					
$H_3PW_{12}O_{40} \cdot 24H_2O$	0.1025	0.1170	0.1041	101.6	101.2¶
$H_6P_2W_{18}O_{62} \cdot 35H_2O$	0.1051	0.1236	0.1060	100.8	99.1¶

*Calculated as W, but including P for heteropoly species.
†Corrected for P by deduction of 1.5 mmole of W per mmole of P.
‡Laboratory A.
§Literature procedure.[10]
‖Modified procedure.
¶Laboratory B.

be equivalent to 1.5 mmole of tungsten), for each mmole of phosphorus present 1.5 mmole of tungsten should be deducted from the apparent total amount found. The lead phosphate was observed to react slowly with EDTA in the titration if it was not removed by filtration, but the modified procedure gives satisfactory results for the total amount of tungsten and phosphorus (Table 3).

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ELUTION AND SPECTROPHOTOMETRIC DETERMINATION OF GOLD AFTER ITS SEPARATION FROM NON-VOLATILE PLATINUM METALS BY COLUMN EXTRACTION CHROMATOGRAPHY

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Summary—Mixtures of gold(III) and iridium(IV) were separated by column extraction chromatography on silica treated with a tri-n-octylamine (TOA) salt. A mixture 2.25*M* in hydrochloric acid and 5*M* in nitric acid was used for elution of iridium. Gold was eluted together with the TOA salt by acetone, and after evaporation of the acetone, the TOA chloroaurate was dissolved in chloroform, converted into TOA bromoaurate and determined spectrophotometrically at 395 nm ($\epsilon = 3.4 \times 10^3$ l.mole^{-1}.cm^{-1}). Beer's law was obeyed over the concentration range 5–67 ppm of gold. The method was found suitable for determination of gold after its separation from other metals by extraction chromatography on supports treated with liquid anion-exchangers.

The determination of gold after its separation from other metals by chromatography on solid anion-exchange resins requires combustion of the resin, owing to irreversible retention of this metal.[1,2] A simple and rapid method of analysis for non-volatile platinum metals, based on their column chromatographic separation on silica treated with a tri-n-octylamine (TOA) salt and elution with mixtures of hydrochloric and nitric acid, has been described,[3] but it was found that gold(III) was very strongly retained and could not be eluted even by a mixture of concentrated hydrochloric and nitric acids. Preliminary experiments in our laboratory indicated that although the gold could be eluted from the column together with the TOA salt and remaining aqueous solution with acetone, and subsequently determined spectrophotometrically with Rodamine B,[4] this procedure was inconvenient since the presence of TOA interfered, and mineralization of the amine salt with a mixture of concentrated nitric acid, sulphuric acid and hydrogen peroxide required refluxing at a temperature of 220–230° for 4 hr.[5]

Many spectrophotometric methods for gold are based on ion-pair formation between halide complexes of gold and basic dyes.[6,7] A selective and precise extraction–spectrophotometric method utilizing the bromoaurate complex with tri-n-octylphosphine oxide was proposed by Holbrook and Rein.[8] Since gold had been reported to form coloured spots on paper treated with tri-n-octylamine hydrobromide,[9,10] it was thought that formation of an ion-pair composed of bromoaurate and tri-n-octylammonium ions could be utilized for extraction–spectrophotometric determination of gold after elution of chloroaurate from TOA-coated silica with acetone.

EXPERIMENTAL

Reagents

Pure tri-n-octylamine (Fluka) was further purified by vacuum distillation, the fraction boiling at 190–200°/5–10 mmHg being collected. Reagent grade chloroform was also distilled before use.

Standard gold solution was prepared by dissolving 46.5 mg of $HAuCl_4.4H_2O$ (pure, POCh, Poland) in 1*M* hydrochloric acid.

Iridium(IV) solution was prepared in the manner described previously.[3]

All other reagents were of analytical grade.

Procedure

Glass tubes (10 × 300 mm) were used, packed with purified silica gel coated with TOA salt. The sorbent (10 g) was slurried with 50 ml of the first eluent (2.25*M* HCl + 5*M* HNO_3), poured into the column and covered with a cellulose filter paper. The column length was 190 mm, the volume of organic phase 1.4 ml and the hold-up volume 8 ml. The volume of organic phase was determined by eluting the TOA salt together with the remaining aqueous phase from the column with 35 ml of acetone; the solvent was then evaporated and the volume of TOA was measured in a narrow calibrated test-tube.

After the column had been washed with 8 ml of the first eluent, 2 or 3 ml of a synthetic mixture containing 176 μg of iridium and 890 or 445 μg of gold was introduced into it. Elution under hydrostatic pressure (head 250 mm) was used for the separation. Iridium was eluted with 40 ml of the first eluent (and was found in a 15-ml fraction of the eluate). The column was then washed with 12 ml of 3*M* hydrochloric acid and gold was eluted together with the organic stationary phase and aqueous solution with 35 ml of acetone. The acetone was then evaporated on a water-bath and the bright yellow residue (TOA chloroaurate and excess of TOA salt) with remaining aqueous solution was treated with 40 ml of chloroform and transferred into a separatory funnel, which was then thoroughly shaken. When the phases had separated, the organic (lower) phase was run into a 50-ml standard flask and the aqueous phase

was washed with 5 ml of chloroform, the washings also being added to the flask. The combined extracts were diluted to the mark with chloroform and mixed. Then 5 ml of the chloroform phase were pipetted into a small separatory funnel and 5 ml of 1.8*M* aqueous hydrobromic acid (containing 4×10^{-4}% bromine) and 10 drops of concentrated phosphoric acid were added. The mixture was then shaken for 1 min, and after separation of the phases, the lower (organic) phase was dried by addition of a small amount of anhydrous sodium sulphate. The absorbance of the organic phase was then measured in a 1-cm cell at 395 nm against a reagent blank similarly prepared (the concentration of TOA salt in the reagent blank was 0.06*M*). A calibration graph was prepared with standard TOA bromoaurate solutions made as follows. A 0.12*M* solution of TOA in chloroform was shaken with an equal volume of the first eluent (2.25*M* HCl + 5*M* HNO_3), and after separation of the phases, the lower phase was filtered through a cellulose filter. More dilute solutions of the TOA salt were obtained by dilution of this 0.12*M* TOA salt solution with chloroform. A 5-ml volume of this organic solution was then introduced into a small separatory funnel and 5 ml of 1.8*M* hydrobromic acid (containing 4×10^{-4}% bromine) 10 drops of concentrated phosphoric acid and 0.1–1 ml of standard gold solution were added. The mixture was shaken for 1 min and then treated as described for samples.

The procedure for column preparation and chromatographic separation has already been described.[3]

RESULTS AND DISCUSSION

Since gold(III) can be very easily separated from other noble metals by column extraction chromatography, owing to the very strong retention of gold in the system TOA salt–(HCl + HNO_3), a simple method for elution of gold and its determination was sought. Attention was paid to the spectrophotometric method based on the bromoaurate complex with tri-n-octylphosphine oxide, which was found to be very selective for gold, only iridium(IV) and tin(II) interfering (at low concentrations).[8] Since it was found that gold and the tri-n-octylamine salt can be easily eluted from the column with acetone, and bromoaurate should form a coloured ion-pair with the tri-n-octylammonium cation, we decided to modify the method proposed by Holbrook and Rein[8] for the determination of gold in the eluate from the column. It was also supposed that chloroaurate tri-n-octylammonium ion-association complex should be convertible into the bromoaurate complex by treatment with a sufficient excess of hydrobromic acid owing to the higher stability of the bromide complexes of gold[11] and the higher affinity of bromide for alkylammonium cations.[12,13]

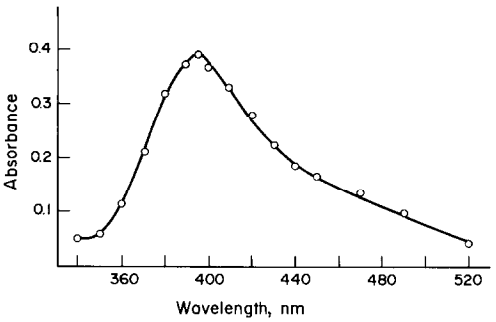

Fig. 1. Spectrum of bromoaurate–TOA complex in chloroform, measured *vs.* reagent blank. TOA concentration 0.06*M*; gold concentration 22.5 ppm.

Preliminary experiments indicated that gold(III) is not extracted by chloroform from hydrobromic acid media, but the organic phase becomes yellow after addition of a small amount of tri-n-octylamine. In further experiments, a small amount of bromine was added to the hydrobromic acid solutions to prevent the reduction of gold, and phosphoric acid was added to the aqueous solution (before the extraction) as masking agent for iron(III) if traces of this metal were present in the aqueous solution.

The absorption spectrum of the TOA–bromoaurate complex in chloroform (Fig. 1) is analogous to the spectrum of the TOPO-bromoaurate complex reported by Holbrook and Rein,[8] but the molar absorptivity is a bit lower (3.4×10^3 l.mole^{-1}.cm^{-1} at 395 nm). The absorbance of the organic phase is stable for 20 min and then slowly decreases.

The absorbance depends somewhat on the TOA salt concentration in the range 0.03–0.12*M* (see Table 1); since 40 ml of chloroform were used for the dissolution of the TOA–chloroaurate complex and the excess of TOA salt after their elution from the column, and the concentration of TOA salt was then 0.06*M*, a tri-n-octylamine hydrobromide solution of this concentration in chloroform was used as the blank for gold determination. It should be remarked that the absorbance of the organic phase was the

Table 1. Influence of tri-n-octylamine salt concentration in chloroform on absorbance of the bromide complex of gold (18 ppm); the organic phase was shaken with 1.8*M* hydrobromic acid containing 4×10^{-4}% bromine

	Absorbance at 395 nm			
	0.03*M* TOA	0.06*M* TOA	0.09*M* TOA	0.12*M* TOA
TOA blank *vs.* $CHCl_3$	0.100	0.108	0.120	0.136
Sample *vs.* $CHCl_3$	0.390	0.390	0.400	0.420
Sample *vs.* TOA blank	0.285	0.280	0.284	0.287

Table 2. The determination of gold after the separation of synthetic mixtures of iridium (176 μg) and gold by column extraction chromatography

Au taken, μg	Au found and standard deviation, μg	Error, %
890	880 ± 12	−1.1
445	432 ± 5	−2.9

* Mean value from 6 successive determinations.

same whether the gold was extracted from hydrobromic acid medium or the TOA–chloroaurate complex in chloroform was shaken with 1.8*M* hydrobromic acid. Beer's law is obeyed over the gold concentration range 5–67 ppm.

The results of gold determination after its separation from iridium(IV) by column extraction chromatography on silica treated with TOA salt and eluted with mixture of hydrochloric acid and nitric acid (2.25*M* HCl + 5*M* HNO_3) are given in Table 2.

In our opinion, the proposed method can be utilized not only for the determination of non-volatile noble metals after their separation by column extraction chromatography, but also for determination of gold in other materials containing larger amounts of this metal (owing to the relatively low sensitivity of the method) in the absence of metals which form coloured anionic bromide complexes extractable by TOA. Furthermore, extraction chromatography with the system TOA HCl–HNO_3 is more convenient than use of anion-exchange resins for the separation and determination of non-volatile noble metals, owing to the easier elution of platinum(IV) and iridium(IV) and the simple and rapid method for determination of gold (which is very strongly retained in both anion-exchange chromatographic systems).

It should be noted that gold(III) and the TOA salt can probably be eluted from the column with methyl isobutyl ketone, like gold bound with tributyl phosphate,[14] and subsequently determined in the organic solution by AAS, in a modification of the method described by Groenewald.[15]

The sensitivity of the method described here could be increased by using a smaller volume of chloroform (*e.g.*, 10 ml) for the extraction, but a blank made in the same way would have to be used as reference solution because of the effect of the higher TOA hydrobromide concentration.

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ANALYSIS OF SOME SYNTHETIC CO-POLYMERS FROM THEIR TITRATION CURVES IN NON-AQUEOUS MEDIA

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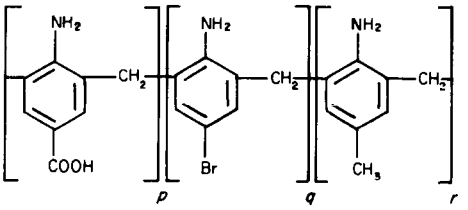
Summary—Electrometric titration in non-aqueous solvents is used for the analysis of some synthetic co-polymers with acidic and basic functional groups. The composition of the co-polymers can be deduced from the determination of these groups. The enhanced acidic/basic character of some of the functional groups in the co-polymer chain is interpreted in terms of intramolecular hydrogen bonding, and the inductive effect of the substituents. The nature of the titration curves is explained in terms of ion-association and homoconjugation.

In recent years, electrometric titration in non-aqueous solvents has been widely used for determination of organic acids and bases, alone or in mixtures.[1,2] However, there is little reference in the literature to use of these techniques for analysis of synthetic polymers. Preliminary investigations in this laboratory on some phenolic co-polymers revealed that electrometric titration in non-aqueous solvents may provide an elegant method for analysis of the acidic/basic functional groups, composition, and structural features of the co-polymers.[3–5] These observations encouraged us to extend this approach to the study of some basic co-polymers having various types of functional groups. For the present study, three substituted aromatic amines, *p*-bromoaniline (PBrA), *p*-toluidine (PT) and *p*-aminobenzoic acid (PAB), were co-polymerized with stoichiometric quantities of formaldehyde in the presence of acid catalyst to obtain the random co-polymer (**I**). In order to obtain co-polymers of various compositions, the feed was altered appropriately. These co-polymers are interesting in view of the fact that each monomeric unit in the chain has an $-NH_2$ group, and in addition some of the units have –COOH and Br as substituents. The composition of the co-polymers can be deduced from the determination of the $-NH_2$, –COOH, and Br groups present in the monomeric units. An attempt has been made in this paper to correlate characteristic features of the titration curves of the co-polymers with intramolecular hydrogen bonding, homoconjugation, and ion-association in a medium of low dielectric constant.

EXPERIMENTAL

Random co-polymer (**I**) was prepared by refluxing stoichiometric quantities of PBrA, PT and PAB and HCHO in the presence of 2 ml of concentrated hydrochloric acid as catalyst, for $2\frac{1}{2}$ hr at 130°.



I

The reaction mixture was poured into ice-cold water to precipitate the co-polymer. The product was washed several times with cold distilled water to free it from catalyst.

Three samples of the random co-polymer (**I**) were prepared by using the following feed compositions.

Sample 1: 0.1*M* PBrA + 0.1*M* PT + 0.8*M* PAB + 1.0*M* HCHO
Sample 2: 0.2*M* PBrA + 0.2*M* PT + 0.6*M* PAB + 1.0*M* HCHO
Sample 3: 0.33*M* PBrA + 0.33*M* PT + 0.33*M* PAB + 1.0*M* HCHO

The yield was 95–98% for sample 1 and 75–80% for samples 2 and 3. The halogen content of the samples was determined by treating the co-polymer with sodium metal[6] and filtering off the resultant sodium bromide.

A Radiometer pH-meter (pH M 26 C) with glass (G 202 B) and calomel (K 401) electrodes was used for pH titrations, and a Leeds and Northrup conductance bridge (4959) for conductometric titrations. The details of the titration have been described elsewhere.[3] For acidic functional groups, pyridine was used as the titration medium and sodium methoxide and tetramethylammonium hydroxide (TMAH) in methanol were used as titrants. Basic functional groups were titrated with perchloric acid in glacial acetic acid medium. The co-polymer concentration used was about 1.5 g/l. The titration curves were reproducible within ±2–3%.

RESULTS AND DISCUSSION

Figure 1 shows the potentiometric and conductometric titration curves of sample 1 in pyridine and

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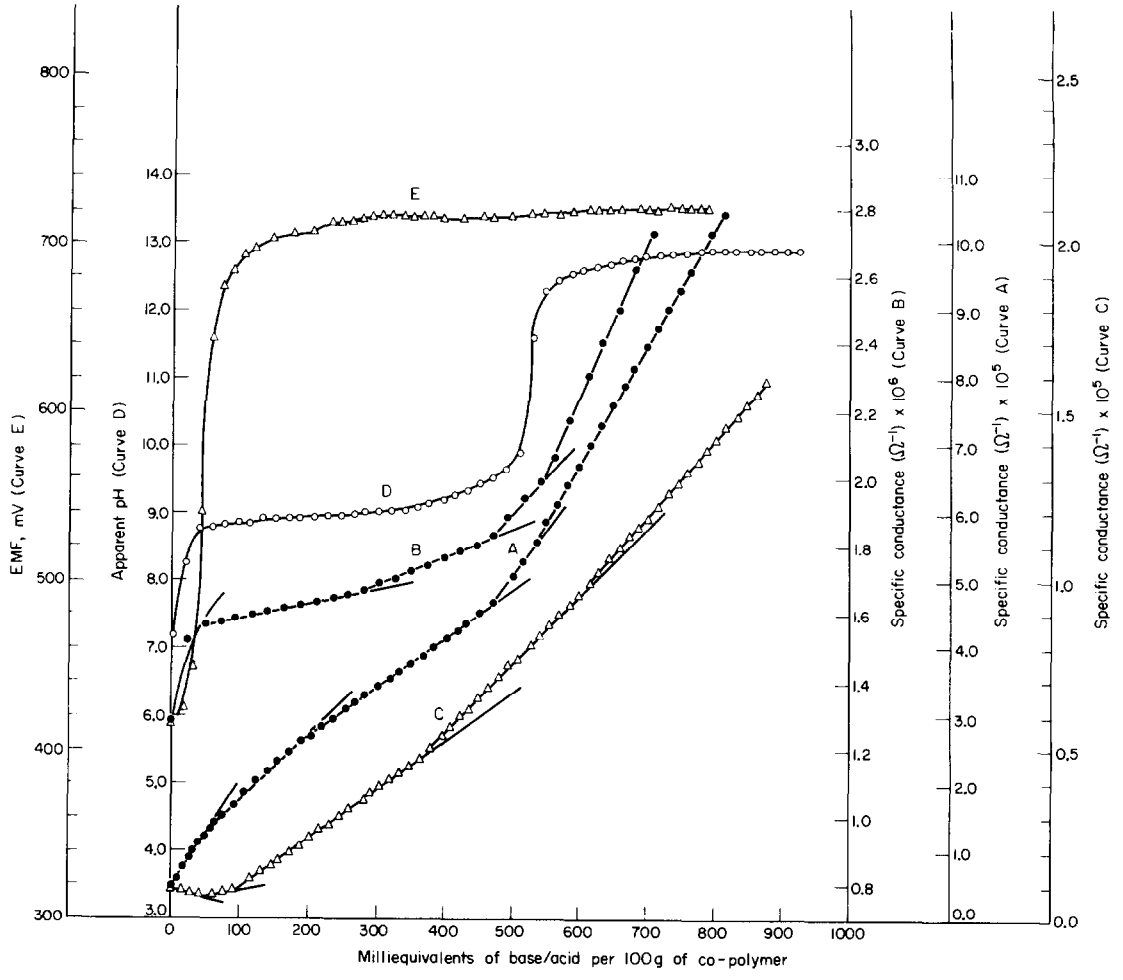

Fig. 1. Titration curves for sample 1. Conductometric curves: (A) in pyridine with TMAH, (B) in pyridine with sodium methoxide, (C) in glacial acetic acid with perchloric acid. Potentiometric curves: (D) in pyridine with sodium methoxide, (E) in glacial acetic acid with perchloric acid.

glacial acetic acid media. Those for samples 2 and 3 were similar to those for sample 1. The conductometric curves for titration with sodium methoxide (*cf.* curve B) showed a distinct final break, which coincided with the very sharp inflection on the potentiometric curve (*cf.* curve D). The titration with TMAH (*cf.* curve A) also had a final break which tallied (within experimental error) with the breaks on the other titration curves. This break obviously indicates the total amount of acidic functional groups (*e.g.*, –COOH) portion titrated. Similarly, the final break in the conductometric titration curves in glacial acetic acid medium (*cf.* curve C) indicates the complete neutralization of $-NH_2$ groups. The percentage of halogen found will give the relative proportion of PBrA units ($-C_6H_2 \cdot NH_2 \cdot CH_2 \cdot Br-$) in the co-polymer chains. The composition of the co-polymer can thus be deduced in terms of the three monomeric units PBrA ($-C_6H_2 \cdot NH_2 \cdot CH_2 \cdot Br-$), PT ($-C_6H_2 \cdot NH_2 \cdot CH_2 \cdot CH_3-$), and PAB ($-C_6H_2 \cdot NH_2 \cdot CH_2 \cdot COOH-$). Table 1 gives the results for the three samples.

The presence of additional breaks in the conductometric curves, before that for the complete neutralization of the functional groups, obviously indicates that some of the COOH and NH_2 groups in the co-polymer chain have stronger acidic (or basic) character compared to others in the same molecule. The hyperacidity of some of the OH-groups in phenolic polymers has been interpreted by several authors in terms of intramolecular hydrogen-bond formation between neighbouring OH-groups.[3–5,7] The presence of intramolecular hydrogen-bonding in such compounds has also been shown from infrared[8] and conformational studies.[9] The infrared spectra of all the co-polymers studied indicated absorptions in the ranges 3450–3600 and 3070–3350 cm^{-1}, showing the probable presence of O–H—O and N–H—N intramolecular hydrogen-bonding.

The basicity of NH_2-groups in the co-polymer chain is expected to be influenced by the inductive effect of the *para*-substituents present in the monomeric units. For instance, the conductometric curve for the co-polymers in glacial acetic acid medium (*cf.* curve C)

Table 1.

Sample	Bromine, %	Co-polymer composition*			Total COOH-groups found, *meq/100 g*	Total NH_2-groups, *meq/100 g*	
		PBrA	PAB	PT		Calc.†	Obs.
1	3.96	0.09	0.82	0.09	550	668	620
2	11.46	0.26	0.63	0.11	415	645	650
3	15.42	0.36	0.47	0.17	320	638	630

*Expressed as mole ratios.
†On the basis of halogen and COOH-group estimations.

showed first a fall in conductivity and then a region of constant conductance. There was invariably a sharp inflection in the potentiometric curve (*cf.* curve E), coinciding with the fall or constant stage of conductance. Incidentally, this portion of the titration curve also almost coincides with the relative proportion of PT in the co-polymer chain. This behaviour is expected in view of the fact that the pK values of the three monomeric units are in the order PT (pK = 5.07) > PBrA (pK = 3.97) > PAB (pK = 2.32).

This obviously shows that the inductive effect of the CH_3-group makes the $-NH_2$-groups of *p*-toluidine (PT) stronger than the NH_2-groups in the other two monomeric units. This is well reflected in the titration curves.

The difference in the nature of the conductometric curves with sodium methoxide and TMAH as titrants (*cf.* curves B and A) can be attributed to ion-association. It is expected that the smaller titrant cation (Na^+) will associate with the acid anion more strongly than the larger one [$(CH_3)_4N^+$] in a medium of low dielectric constant. The indication (on the conductometric curve) is probably due to the formation and different degrees of dissociation of acid–anion, or base–cation complexes in a medium of low dielectric constant. Such homoconjugation has been referred to by Kolthoff and co-workers in the case of weak acids in non-aqueous solvents.[10–12]

Another important aspect of this study is the general ability to account for the actual composition of the co-polymers on the basis of reactivity of the monomeric units used in the co-polymerization reaction. The rate of addition of monomeric units in the co-polymer chain will depend on factors such as (*i*) resonance stabilization of monomeric units, (*ii*) polarization of monomers, owing to the electron-attracting or electron-donating nature of the substituents, and (*iii*) the directive influence of the substituents. On the basis of these factors, the reactivity of the three monomeric units should be in the order PAB > PBrA > PT. This trend is in fact reflected in the actual composition of the products (Table 1).

In conclusion, it can be said that electrometric titration techniques in non-aqueous media may provide an elegant and simple method for estimating acidic and basic functional groups, and deducing the composition of polymers. Structural features, such as intramolecular hydrogen-bonding in polymers are well reflected in the titration curves.

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ANALYTICAL DATA

STRUCTURAL INVESTIGATION OF A NEW ORGANIC ANTISEPTIC: TAUROLIDINE

A SPECTROSCOPIC STUDY OF ITS STABILITY AND EQUILIBRIA IN VARIOUS SOLVENTS

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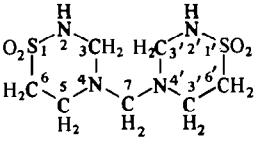
Summary—^1H and ^{13}C NMR investigations of aqueous solutions of Taurolidine and Tauroflex have been made to determine their stability. This study revealed two successive equilibria, leading particularly to Taurultam-methylol (in 15% proportion) from a 0.5% Taurolidine solution. The methylol derivative is supposed to be the component active against bacteria and their endotoxins.

Taurolidine (1) is the international common name of a new chemotherapeutic molecule with bactericidal and anti-endotoxin properties:[1–4] bis-(1,1-dioxoperhydro-1,2,4-thiadiazinyl-4)methane.



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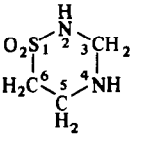


(2)

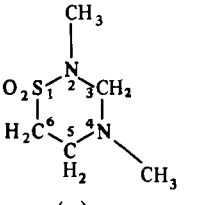
Its activity and that of Taurultam (2) [1,1-dioxoperhydro-1,2,4-thiadiazine] (DPT) should be due to the liberation of active methylol groups in aqueous solution and *in vivo*. Specialized forms (Tauroflex) are administered locally but mainly intraperitoneally and intravenously in digestive surgery.

The aim of this study was to contribute to the investigation of the different chemical species found in Taurolidine solutions, in order to better define its pharmacodynamic model. This paper describes ^1H and ^{13}C NMR, and infrared studies of Taurultam, Taurolidine solutions and Tauroflex.

EXPERIMENTAL

Reagents

Taurinamide, Taurolidine, Taurultam, Tauroflex [2% aqueous solution of Taurolidine, containing 5% poly(vinylpyrrolidone-17)] and 2,4-dimethyltaurultam (3), called compound 2012 in some publications, were a gift from Geistlich Sons Ltd., Wolhusen, Switzerland. All other reagents were obtained from commercial sources (DMSO-d_6, D_2O, TMS and $KHSO_3$ from Merck).



(3)

Apparatus

^1H and ^{13}C NMR studies were performed on Jeol C 60 HL and on Bruker WP 80 spectrometers respectively. All the chemical shifts were referred to TMS.

Infrared spectra were recorded with a Nicolet 7199 IR spectrometer. The software developed for the Nicolet 1180 computer was used for data-treatment, and particularly the subtraction of solvent spectra.

Polarographic measurements were obtained with a Tacussel PRG 5 polarograph, with 0.6*M* lithium hydroxide as supporting electrolyte.

RESULTS AND DISCUSSION

NMR study

The ^1H NMR study of a DMSO-d_6 solution of Taurolidine, illustrated in Fig. 1a, reveals all its protons and confirms its stability, mentioned in a recent publication.[5] Before investigation of the aqueous Tauroflex solution, we recorded the ^1H NMR spectra for Tauroflex solutions in mixtures of DMSO-d_6 and D_2O and of D_2O and H_2O. We used the integrated signal for the methylene bridge between the thiadiazine rings as an indication of the degree of hydrolysis; the integrated intensity was measured with an average error of 2%, taking into account the potential

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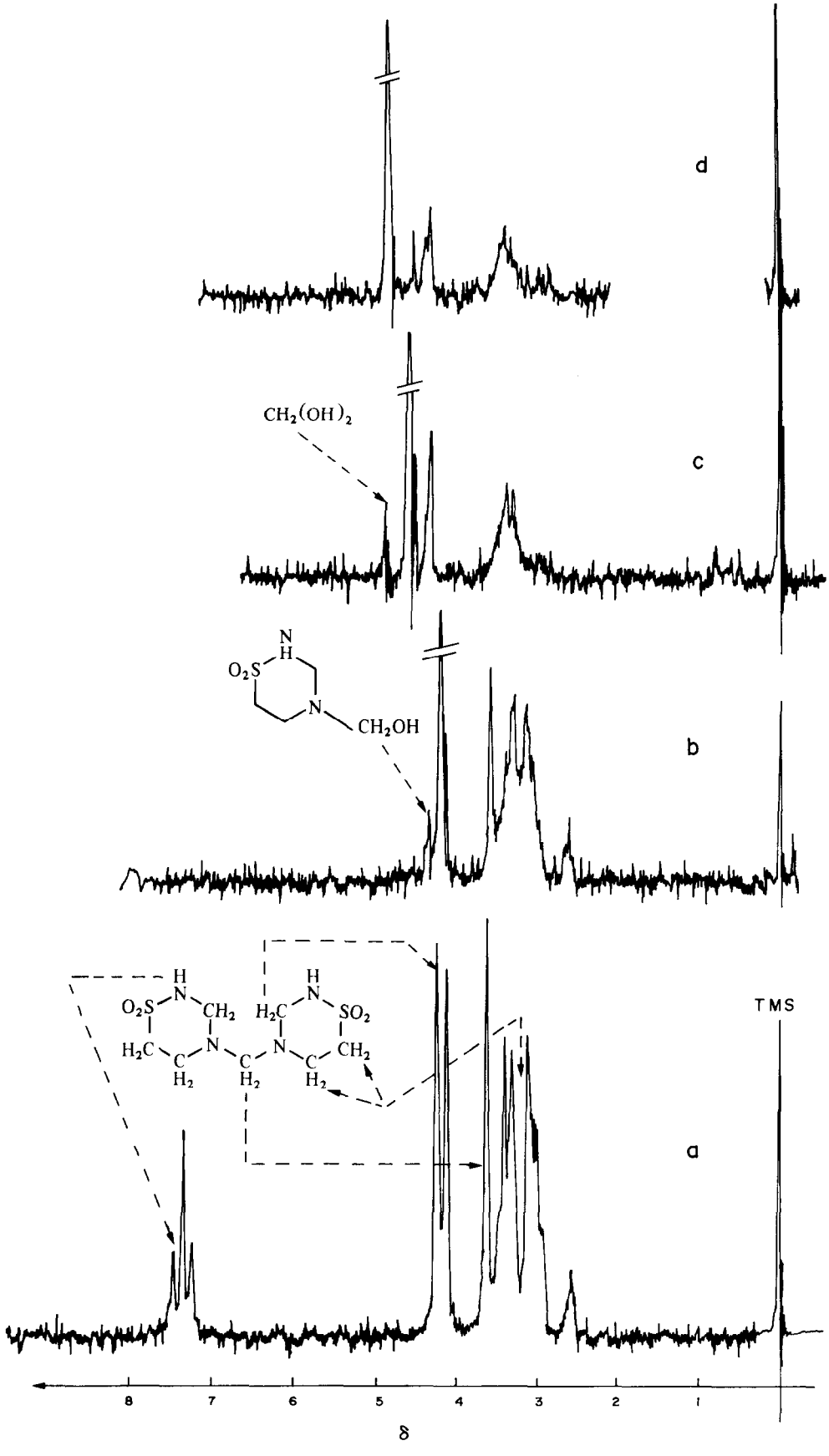


Fig. 1. ^{1}H NMR Spectra of Taurolidine. (a) DMSO-d_6; (b) binary, D_2O (17%)–DMSO-d_6; (c) D_2O, 70°C; (d) D_2O, 25°C.

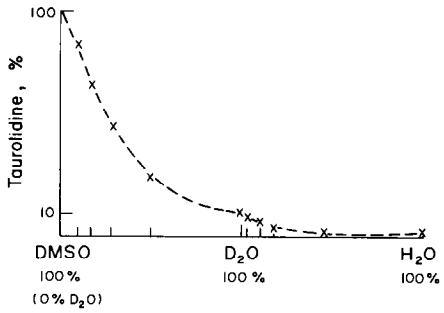
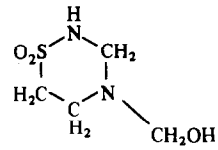


Fig. 2. Hydrolysis of Taurolidine.

interference from the adjacent high-field protons on C_5 and C_6. In pure D_2O at equilibrium there is no more than 10% of Taurolidine, and (by extrapolation) in pure H_2O no more than 2%: Fig. 2, based on ten observations for various proportions of each solvent, shows the extent of hydrolysis.

From the solution in 17:83 v/v D_2O–DMSO-d_6 mixture a signal at 4.45 ppm (Fig. 1b) was obtained which could be due to the methylene protons of an alcohol group, indicating the possible presence of Taurultam-methylol (4).



(4)

Moreover, Fig. 1c shows a signal at 4.80 ppm arising[6] from $CH_2(OH)_2$. This assignment is made on the basis of the thorough study by Maslovich *et al.*[6] of

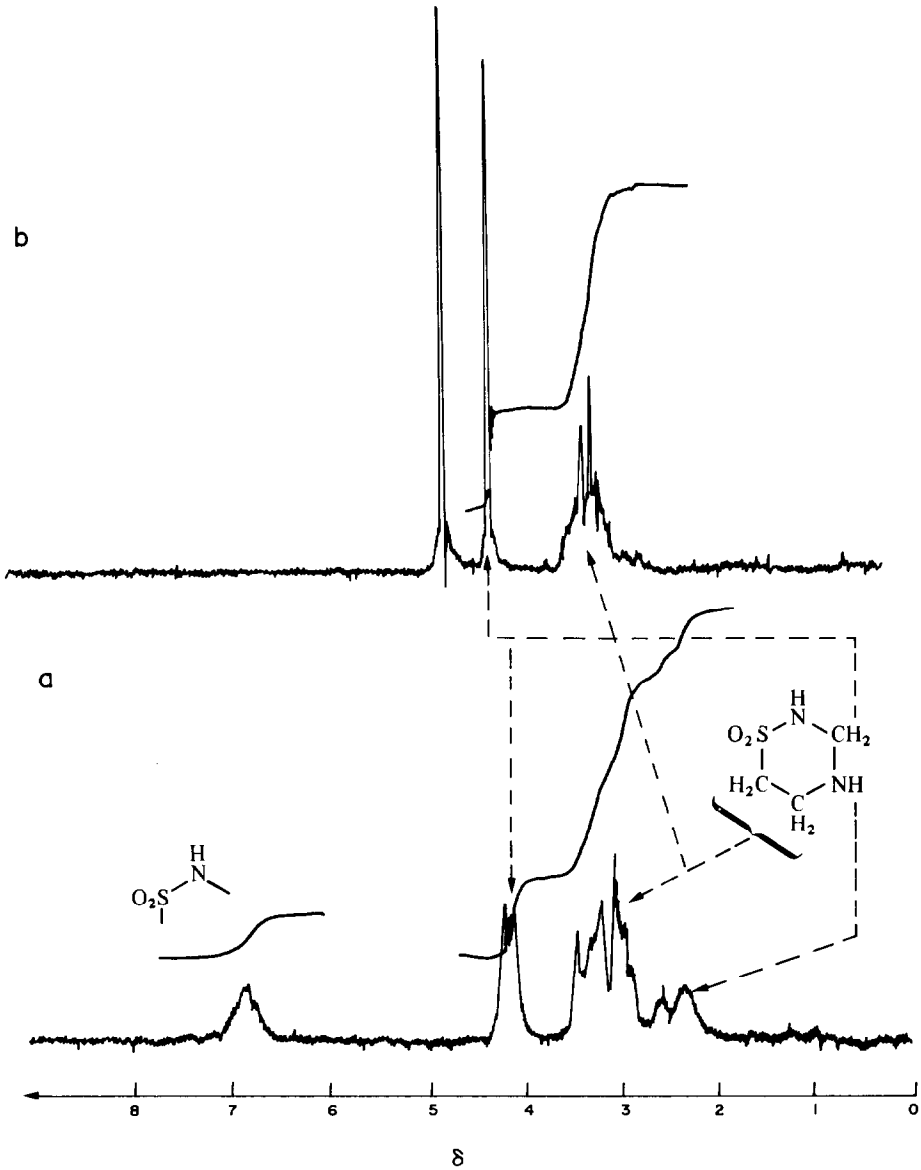


Fig. 3. ^{1}HNMR Spectra of Taurultam in (a) DMSO-d_6; (b) D_2O.

Table 1. Assignments in Tauroflex ^{13}C NMR spectrum

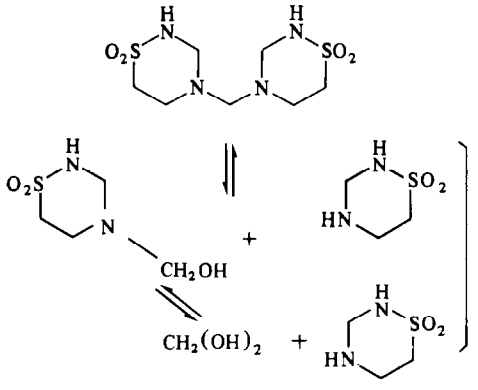
δ (*vs.* TMS), *ppm*	Assignment
19 large	P.V.P.
31 large	P.V.P.
36 large	P.V.P.
40.94	C_5 and $C_{5'}$
42 large	P.V.P.
44.64	C_3
47 large	P.V.P.
51.59	C_6
78.25	CH_2OH
122.44	CH_2OH
167.29	HCHO
177 large	P.V.P.

*P.V.P. = poly(vinylpyrrolidone).

the ^1H and ^{13}C NMR of formaldehyde in aqueous solutions. These two observations allow us to suppose that hydrolysis of Taurolidine proceeds in two steps, each of them leading to Taurultam.

A previous study of this compound (Fig. 3) had shown that chemical shifts relative to the protons on C_5 and C_6 are similar for Taurultam and Taurolidine, but that the methylene protons on C_3 resonate slightly more upfield for Taurultam than for Taurolidine. This difference causes dissymmetry in the signal observed at 4.3 ppm in the NMR spectra of aqueous solutions of Taurolidine (Fig. 1d).

The stability of the spectra with respect to time permits us to affirm the stability of Taurultam in all the solutions concerned. The different species at equilibrium in 0.5% solutions in D_2O could be determined and are shown below.



In order to confirm these proportions, particularly that of $CH_2(OH)_2$, a polarographic study was made of Taurolidine solutions with different additions: first $KHSO_3$ to detect which portion of the polarographic wave was due to formaldehyde, then known amounts of formaldehyde solution. The result confirms the low amounts previously observed.[11]

To support our hypothesis about the presence of Taurultam-methylol in aqueous solutions of Taurolidine, we recorded the ^{13}C NMR spectra of Tauroflex. The signals were assigned (Table 1) after subtraction of the poly(vinylpyrrolidone) spectrum and preliminary study of compound 2012 for which the ^{13}C NMR chemical shifts are 35.34 (C on N_4), 40.38 (C_5), 44.76 (C_3), 52.25 (C_6) and 74.13 ppm/TMS (C on N_2).

The signal at 78.25 ppm has been assigned to a methylol group on N_4 by means of Roberts's correlation relation[7] applied to C on N_4 of compound 2012 (calculated value 78.64 ppm). This relation is very useful in structural analysis; it gives the ^{13}C NMR chemical shift of a methylol derivative from its methyl analogue:

$$\delta\,(CH_2OH) = \delta\,(CH_3) + 43.3\ \text{ppm}$$

This result confirms the ^1H NMR investigation.

For the signal at 122.44 ppm, the application of the same relation to C on N_2 of compound 2012 gives 117.43 ppm. It is hence possible that a second substitution occurs on N_2 of Taurolidine.

Infrared study

The infrared spectrum of a drop of Tauroflex between two AgBr plates is reproduced in Fig. 4, after subtraction of the absorption bands due to water. To assign the observed bands correctly, it was necessary to record the spectra of Taurinamide, Taurultam and Taurolidine powders in KBr pellets.[8]

The proposed assignments were made by means of the group frequency concept (especially with the aid of the tables of Tipson and Parker[9]) and are given in Table 2. It must be said that in the literature only one reference[10] deals with an infrared and Raman study of Taurine.

In Table 2, the characteristic wavenumbers of thiadiazine rings can be recognized, for instance the symmetrical valence vibration of the sulphur–oxygen bond, showing two components (1180 and 1160 cm^{-1}), which indicates that at least two kinds of compound with thiadiazine rings are present. It may be noted that a polarographic study of Tauroflex indicated the presence of a very low proportion of formaldehyde, which was confirmed by Knight *et al.*[11] by GLC. They found there was less than 0.004% free formaldehyde and that poly(vinylpyrrolidone) stabilizes aqueous solutions of Taurolidine by reducing the formaldehyde content.

These results indicate that infrared spectroscopy and especially NMR spectroscopy are suitable for studying the stability of Taurolidine in various solvents. These non-destructive methods provide a very good example of application of analysis to the solution of a difficult structural problem.

CONCLUSION

This investigation on aqueous solutions allows identification of the different species in equilibrium

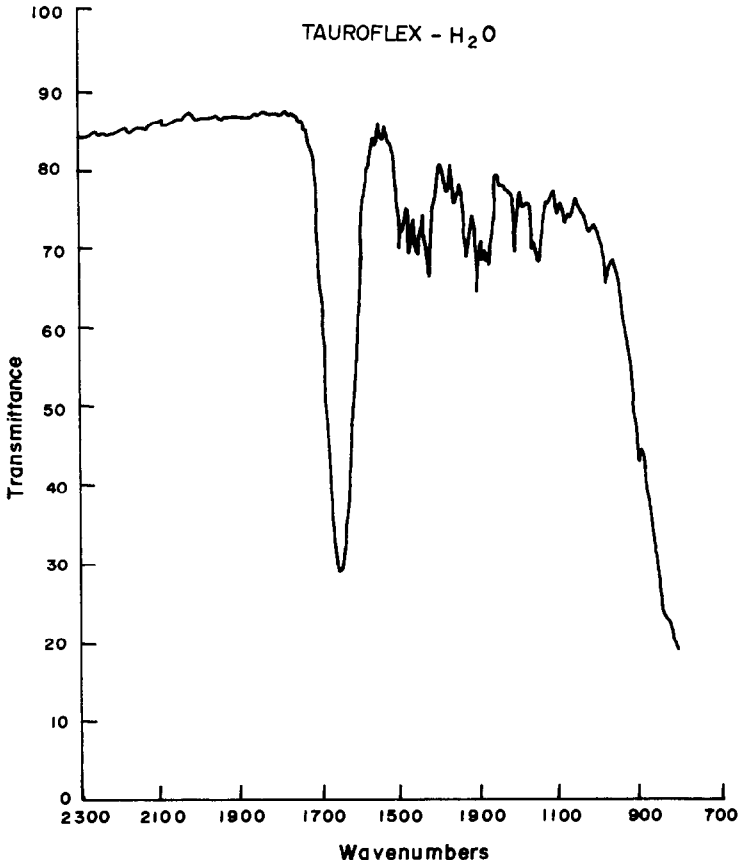


Fig. 4. Infrared spectrum of Tauroflex after subtraction of water bands.

Table 2. Assignments in Tauroflex infrared spectrum

Wavenumbers, cm^{-1}	Assignment	Wavenumbers, cm^{-1}	Assignment
1500	δ CH_2	1200	
1490		1180	ν s SO_2
1460		1160	
1445		1145	δ NH
1420		1095	ν CN + ν CC
1375	$\omega + \delta$ CH_2	1080	ν CN
1365	τ CH_2	1070	
1320		1020	
1300	ν a SO_2 + τ CH_2	980	ρ CH_2
1280		900	
1275			

and more accurately confirms the presence of methylol derivatives which are the basis of the pharmacologists' hypothesis about the antibacterial activity of Taurolin. A further publication will deal with the analytical aspects, including determination of the metabolites, an important step in better defining the biological applications of this new organic antiseptic.

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ANNOTATIONS

PLUTONIUM COULOMETRY

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Summary—The assumptions made in choosing the conditions for coulometric determination of plutonium are critically examined, and the chemical features making the determination possible are elucidated.

The efforts expended on the controlled-potential coulometric determination of plutonium have made it a method of choice for plutonium analyses. Factors such as electrode cleanliness and room temperature have all been considered in detail, and the success of this method has led to the belief that it is both well-understood and non-empirical.[1] The purpose of this note is to suggest that this confidence is premature, and that something remains to be studied before the method can be described in this way.

A popular adaptation of the controlled-potential technique begins with a solution in which the plutonium is apparently all in the tervalent state. By this it is implied that at the start of the electrolysis the oxidation number (N) of the plutonium is as close to 3.00 as possible, or that the initial value of N (which is close to 3.00) is accurately enough known. A current is then caused to flow through the working electrode, oxidizing the plutonium from the tervalent to the quadrivalent state:

$$Pu^{3+} = Pu^{4+} + e^- \quad (1)$$

The potential to be applied to the working electrode (*vs.* the reference electrode) is calculated by means of the Nernst equation:

$$E = E^0 + 0.05916 \log \frac{[Pu^{4+}]}{[Pu^{3+}]} \quad (2)$$

with the ratio $[Pu^{4+}]/[Pu^{3+}]$ typically set at some high value such as 999.[2] When the current has dropped to the "background" level, the number of coulombs passed through the cell is translated into a measure of the plutonium in the titration vessel. Sometimes "correction factors" to allow for incomplete electrolysis are found by manipulating equation (2).[1] In any case, it is tacitly assumed that the value of N is close to 4.00 at the end of the determination.

Although never explicitly stated, an assumption underlying the coulometric determination is that it is possible, by experimental manipulations, to produce an integral change in the plutonium oxidation number; in the present case, this change is ± 1 depending upon the direction of equation (1). Curiously, no-one seems to have calculated the plutonium oxidation numbers at the beginning and end of typical determinations to test this assumption. As the oxidation number of the plutonium in solution at equilibrium is easily calculated from the values of the solution acidity and potential, it is informative to examine these numbers.[3,4] For example, at a potential of 1.137 V (recommended by Shults[2] for the coulometric determination), plutonium *at equilibrium* in 1 *M* perchloric acid should have an oxidation number of about 5.998, and consist almost entirely of plutonium(VI). However, plutonium(VI) is not reported as present in the electrolysed solutions; what is reported is a solution containing more than 99% plutonium(IV). It follows that by the end of the electrolysis (taken as corresponding to the current falling to the background level) the solution has not come to thermodynamic equilibrium.

Equilibrium is established in the plutonium system in acid medium by the generation of tervalent, quinquevalent, and sexivalent plutonium,[5]

$$2Pu^{4+} + 2H_2O = Pu^{3+} + PuO_2^+ + 4H^+ \quad (3)$$

$$Pu^{4+} + PuO_2^+ = Pu^{3+} + PuO_2^{2+} \quad (4)$$

and the first step is rate-determining. The drift toward equilibrium establishes two reversible couples in the solution: Pu^{4+}/Pu^{3+} and PuO_2^{2+}/PuO_2^+. If the Nernst equation is to be applicable to the first of these couples under the conditions of the determination, then it should be applicable to the second couple under the same conditions. But the influence

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of the second couple on the potential of the electrode or upon the determination has always been ignored, and there is no evidence that application of the Nernst equation to the PuO_2^{2+}/PuO_2^{+} couple leads to the very same potential obtained by application of this equation to the Pu^{4+}/Pu^{3+} couple.

When a reaction between an oxidizing agent and a reducing agent [equation (4)] takes place at the surface of a conductor, the potential of the conductor lies between the equilibrium potential of the system which is oxidized and that of the system which is reduced.[6] In other words, the potential impressed at the electrode in the plutonium system amounts to a "mixed potential" maintained at the electrode surface by mixing unknown contributions from both reversible couples in the plutonium system. This "mixed potential" bears no simple relationship (such as that given by the Nernst equation) to the concentrations or activities of the reacting species.[7] Thus, mathematical analyses based upon applications of the Nernst equation to electrodes in non-equilibrium systems cannot be considered complete, for such potentials have an inherent tendency to drift.[8]

As the Nernst equation applies only when the net current through an electrode is zero and the electrode is at equilibrium,[9] conventional descriptions of the coulometric method solely in terms of equilibrium concepts are not satisfactory. It is therefore appropriate to reconsider the determination with a view to (1) exploiting the departure from equilibrium and (2) adapting the system to give near equilibrium conditions.

(1) A significant improvement in the performance of the controlled-potential method is realized if the analysis time is curtailed,[1] but it does not seem to be appreciated that this better performance is a logical consequence of operating under conditions far removed from equilibrium. The disproportionation reaction is not fast; if it were fast, the analysis as we know it today would not be possible. Because reaction (3) is slow, rapid electrolysis results in a much higher exchange-current density for the Pu^{4+}/Pu^{3+} couple than for the PuO_2^{2+}/PuO_2^{+} couple. In this non-equilibrium circumstance, the Nernst equation is a reasonable *approximation* to the true state of affairs.[9] Rapid electrolysis also minimizes the amount of Pu^{3+} and PuO_2^{+} internally generated by disproportionation. These latter species begin to appear as soon as substantial proportions of plutonium(IV) are generated by electrolysis;[5] both are electroactive and therefore additional sources of error which can be minimized by rapid electrolysis.

(2) A second improvement involves adding complexing agents to the plutonium solution, such as sulphuric or nitric acid, or phosphate.[10] Such reagents stabilize plutonium(IV) by forming complexes with it that are considerably more stable than those formed with other plutonium species. (That is, the α-coefficient for the side reactions of tetravalent plutonium is increased more than the α-coefficients for the other plutonium oxidation states.[11]) The effect of this is to decrease the formal (conditional) potential of the Pu(IV)/Pu(III) couple and increase that of the Pu(VI)/Pu(IV) and Pu(V)/Pu(IV) couples. Thus the solution at the end of the electrolysis will contain a very high proportion of Pu(IV), close to the proportion that would exist if the Pu^{4+}/Pu^{3+} equilibrium were the only one possible. The oxidation number of the plutonium will therefore be close to 4.00 at this time. Because the solution composition is effectively restricted to only these two lower oxidation states during the determination, the problem of mixed potentials never arises.[9] Likewise, the tendency to internal generation of other electroactive species is minimized, as can be verified by computation of the maximum (equilibrium) values of these species for selected values of N between 3.00 and 4.00.[12] These are good reasons for rapid electrolysis in sulphuric acid medium being preferred for the coulometric determination.

A discussion of non-equilibrium effects on plutonium determinations can be found elsewhere.[13]

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IODOMETRIC STANDARDIZATION AND BIAMPEROMETRIC DETERMINATION OF IRIDIUM(IV)

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Summary—Ir(IV) in the concentration range 0.4–0.8 g/l. can be easily determined iodometrically with 0.01*M* thiosulphate (starch as indicator). The relative standard deviation is 0.2–0.3%. When other coloured ions are present, biamperometric detection is recommended.

At present, solutions of a number of the platinum metals, including iridium, are standardized by methods, mostly gravimetric,[1] which are slow and tedious. Hexachloroiridate(IV) reacts quantitatively with iodide, resulting in an equivalent amount of iodine, according to the equation:

$$2IrCl_6^{2-} + 2I^- \rightleftharpoons 2IrCl_6^{3-} + I_2$$

The liberated iodine can be titrated with thiosulphate. This method, with starch as indicator,[2] has not found wide use because of difficulty with the end-point colour change from blue (starch–iodine) to pale yellowish-green ($IrCl_6^{3-}$). To overcome this difficulty, Woo and Yost[3] added a small amount of benzene to extract the iodine and titrated until the upper layer turned colourless. Other workers, however, do not agree that this modification furnishes correct results.[4,5] A potentiometric titration, with a platinum indicator electrode, was reported by Pshenitsyn and Ginzburg to give good results.[6] The aim of the present work was to re-examine the method.

EXPERIMENTAL

Reagents

A stock iridium(IV) solution (1.6 g/l.) was prepared by dissolving pure $(NH_4)_2IrCl_6$ (Johnson and Matthey) in 0.5*M* hydrochloric acid, and standardized gravimetrically with formic acid.[7] Sodium thiosulphate solution was prepared with freshly distilled water and standardized with sodium iodate. It was diluted immediately before use, to give a 0.01*M* working solution.

Procedures

Visual end-point. To 10.00 ml of a solution containing between 8 and 16 mg of iridium(IV) add 0.5 g of potassium iodide, whilst stirring. Then add 10 ml of distilled water and titrate with 0.01*M* thiosulphate added dropwise from a semimicro burette. When the colour changes from brown to yellow, add 2 drops of freshly prepared 1% aqueous starch solution and continue titration until the colour clearly changes from blue to pale yellowish-green.

Biamperometric detection. To 5.00 ml of solution containing between 1.5 and 7.5 mg of iridium(IV), add 0.5 g of potassium iodide and dilute to 30 ml with distilled water. Insert two platinum foils (0.2 cm^2 in area) and apply a potential difference of 30 mV between them. With continuous stirring, add fresh 0.01*M* thiosulphate from a semimicroburette until the current reaches zero ("dead-stop" end-point).

RESULTS AND DISCUSSION

Interferences

Iron(III) interferes but can be easily masked with fluoride; Ru(IV) interferes by reaction with the iodide; Os(IV), Os(III) and Ru(III) do not react with iodide and can be present when the biamperometric detection is used, but interfere in the visual end-point method because they are coloured. Ru(IV), Ru(III), Os(IV) and Os(III) can be previously eliminated by perchloric acid oxidation.[8]

Au(III) reacts with iodide but can be previously extracted as its chloro-complex into diethyl ether.[9] Pd(II) interferes owing to the reaction of thiosulphate with its iodo-complex, but can be eliminated by precipitation with dimethylglyoxime in acidic solution.[10]

Coloured ions such as Rh(III), Co(II) and Ni(II) do not interfere in the biamperometric determination, but can interfere in the visual method. The common ions sulphate, phosphate, perchlorate and nitrate do not interfere.

Visual end-point

This procedure was tested with 30 samples at the 0.8-g/l. Ir(IV) level and 30 samples at the 0.4-g/l. Ir(IV) level. The relative standard deviations were 0.17 and 0.26%, respectively.

The results were compared with those obtained by the formic acid gravimetric method[7] applied to three 100-ml samples, which gave relative standard deviations of 0.2 and 0.25% for the 0.8 and 0.4-g/l. solutions, respectively.

Biamperometric end-point

Thirty solutions containing 1.5 mg of Ir(IV) each and another 30 containing 7.5 mg of Ir(IV) each were titrated. Besides Ir(IV) each sample contained 1.0 g of each of Co(II), Ni(II), Rh(III), Ru(III) and Os(IV), 20 mg of Fe(III), 10 mg of Au(III) and 20 mg of Ru(IV).

The Fe(III) was masked with 1.0 ml of 1.0*M* sodium fluoride and Au(III) and Ru(IV) were eliminated beforehand as already mentioned. The relative standard deviations were 0.35 and 0.23%, respectively.

CONCLUSIONS

No difficulty was experienced in the visual end-point method, which can easily be used for the standardization of Ir(IV) solutions, and is rapid and accurate. It is much easier than the gravimetric method and does not need more than a few ml of solution. To avoid interference by coloured ions it is advisable to use biamperometric detection, which also shows good accuracy, is rapid and easy to perform, and can be used for routine determination of Ir(IV).

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ENVIRONMENTAL SPECIMEN BANKING: A CHALLENGE IN TRACE ANALYSIS

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Summary—The need for environmental specimen collection and storage ("specimen banking") is demonstrated. A survey of the banking concept, storage techniques and storage conditions for 14 selected human and environmental materials in the Federal Republic of Germany is given. This is followed by a description of the banking and analytical facilities of the Specimen Bank in Jülich, and of the sampling and sample preparation performed for marine macroalgae and several terrestrial specimens (carp, mussels, rain worms, beetles and poplar leaves), the rather new approach to the production and extensive application of appropriate control materials, and the methodology used for the determination of toxic metals, metalloids (Hg, Pb, Cd, Ni and As), anabolic steroids and polyaromatic hydrocarbons. Finally the results of the first analytical series and of particular stability studies as a part of long-term investigations are presented and future work is outlined.

Monitoring programmes on the world-wide spread of anthropogenic chemicals in the whole environment, including man himself, are increasingly recognized as of paramount importance as a basis for governmental actions to protect man, animals and plants against harmful organic and inorganic compounds.

At present, however, the number of chemicals and their metabolites entering the environment is of the order of 60000 and growing each year, so a systematic and all-encompassing determination of their ecotoxic behaviour and of discernible effects is practically impossible. Thus, it is no surprise that analytical progress and new toxicological findings are rather frequently concerned with the separation, identification and determination of those organic or inorganic compounds which are recognized to be particularly hazardous. Such observations immediately pose the question of whether these compounds have been spread quite recently, longer ago, or continuously over a certain time span, into the whole environment or just a part of it. A reliable answer could be obtained by retrospective analyses of stored materials.

This question, however, may only be answered successfully if we have available relevant specimens from the past, which have been carefully selected from important regions or environmental compartments analytically characterized at the time of sampling, and stored without deterioration. The information obtained from analysis of such "fossilized" materials would surely facilitate the making of decisions if, for instance, governmental and/or legal regulations should become necessary, since the data could form the basis for prognosis of at least the near future. Thus, from the point of view of environmental surveillance and protection it is highly desirable to investigate the feasibility of a meaningful long-term storage of carefully selected human and environmental materials. Possibilities for the realization of such storage in appropriate repositories ("specimen banks") have been amply discussed by experts from several countries within the last decade.[1,2] In the United States of America, in France and Japan as well as in the Federal Republic of Germany, such plans have recently been realized. After a preliminary period of informative meetings, the U.S. and German governments decided to face this challenge jointly through feasibility studies on national specimen banks.

In both countries, during the introductory phase, analytical investigations together with a few storage experiments were performed.[3–5] Subsequently, the world's first pilot specimen banks became operational. The bank in the United States, operated by the National Bureau of Standards (NBS), under the project leadership of the Environmental Protection Agency (EPA), was inaugurated in November 1979.[6] The German programme, designed and co-ordinated by the Umweltbundesamt (UBA) Berlin, differs from the U.S. approach. It is organized as a co-operative task shared by eleven independent research groups.[7] A central storage bank has been established in Jülich for the long-term storage of all specimens selected, together with organizational and analytical facilities. The institute at Jülich and the other groups also contribute to the project by sampling and/or analysis. The research groups in Ahrensburg, Berlin, Kiel and Münster are operating satellite (*i.e.*, comparative storage) banks for a few selected materials (see Table 1), and a data bank is located in Münster. The biggest satellite bank in Münster started operation in mid-1980, and the central bank in Jülich was inaugurated in May 1981. However, storage of a few materials had already begun early in 1980 and storage techniques had been studied before the bank building was finished.

Table 1. Specimens selected for storage and analytical investigations during the project "pilot specimen bank"

Human Materials
Whole blood*
Adipose (fatty) tissue*
Liver*
Terrestrial Ecosystems and Food Chain
Soil (Parabraunerde; US Soil Tax: Hapludalf)
Sewage sludge (from Hamburg)†
Lumbricus rubellus (rain-worm)
Carabus auratus (beetle)
Lolium multiflorum (grass)§
Wheat§
Populus nigra italica, leaves (lombardy poplar)
Cow milk‡
Aquatic Environment
Cyprinus carpio (carp)
Dreissena polymorpha (mussel)
Marine Environment
Fucus vesiculosus (brown algae)

*Parallel (comparative) storage at −80° in the satellite bank in Münster.
†Parallel (comparative) storage at −80° in the satellite bank in Ahrensburg.
§Parallel storage in the satellite bank in Berlin.
‡Parallel storage in the satellite bank in Kiel.

This paper intends first to explain the philosophy of the pilot phase in the Federal Republic of Germany. This is followed by a description of the central bank and the associated analytical facilities in Jülich, and a summary of experimental work performed so far in our institute on sampling, sample preparation, portioning and packaging of a few materials, as well as the production of control materials. Moreover, examples (illustrated by results) are given of the efforts to provide very precise analytical determinations, together with preliminary results of stability investigations.

Philosophy of the pilot specimen bank programme

An environmental specimen bank should provide, at acceptable cost, facilities for the long-term storage ($\geqslant$50 years) of carefully selected human and environmental materials. Such a bank should make possible a meaningful retrospective analysis for numerous organic and inorganic compounds regarded to be of toxicological and/or environmental significance. Further, the bank should also provide homogeneous and well characterized materials for the continuous improvement and checking of analytical methods used in environmental surveillance.[8,9]

The German approach now being realized has three main items.

I. Selection of human and environmental materials which can be regarded mainly as accumulators or indicators for organic and/or inorganic pollutants. These materials should be representative of man and the most important environmental fields (terrestrial, aquatic and marine). In selection of these materials, done during the planning phase, it is considered that they should be quite different chemically and structurally. This also allows the evaluation and testing of comprehensive analytical methods as a sound basis for further real-time analysis (see Table 1).

Since comparative and long-term analytical studies have to be performed during the pilot phase, the stored materials should be either homogeneous materials or else be homogenized as far as possible to achieve uniformity. Examples of homogenization procedures will be given below.

II. Selection of optimal storage conditions within the general banking concept, *i.e.*, a comprehensive approach ranging from storage facilities to containers and vessels. For the human materials and sewage sludge it was decided to use and compare two temperature levels, −80° with compressor cooling, performed at the satellite banks in Ahrensburg and Münster, and $\leqslant -145°$with liquid-nitrogen vapour cooling, performed at the central bank at Jülich.

It was also decided to use for the storage vessels two different materials resistant to very low temperatures: glass bottles of different sizes (from 22 up to 500 ml) made from Duran glass with plastic screw-caps sealed with either aluminium or indium foil (for samples to be analysed for organic pollutants), and polypropylene bottles with screw-caps made of the same material (for samples to be analysed for toxic metals). The 22-ml bottles are used for sample volumes from 5 to 15 ml, and samples smaller than 2 ml are capsuled in 3-ml minivials which are in turn inserted into 22-ml bottles.

The programme should further also contribute to a possible improvement of storage vessels with the goal of finding, if feasible, a standardized material useful for both organics and metals, and additionally to develop economical and completely sealed vessels for long-term storage.

III. Repeated analysis of the compounds selected (see Table 2) over about 2–3 years to investigate whether during this time span and under the given conditions any loss, contamination, metabolization or transformation occurs. This aim first of all required a decision on the number of total samples and of the sample portions needed for each analytical series as well as on the amount needed for each individual sample. This individual amount can be different for different materials and depends on the concentration level of the compound to be determined, the method of determination, and the homogeneity of the material.

These conditions were evaluated during the preliminary analytical investigations performed in the participating laboratories. The analytical task requires extremely precise and reliable analytical methods. This was and still is one of the crucial points of the whole banking programme, since the duration of the project, owing to the considerable cost, is short compared with the calculated total storage time of $\geqslant$50 years for samples in a specimen bank. Doubtless studies of this kind will have to be continued within

Table 2. Pollutants to be determined in the materials selected for the pilot specimen bank

Toxic Metals and Organometallic Compounds
Mandatory substances: Hg, methyl-Hg, Cd and Pb
Further desirable metals and metalloids: As, Ni, Cr, Cu, Se, *etc.*
Organic Pollutants
Halogenated hydrocarbons and polychlorinated biphenyls (PCB)
Polycyclic aromatic hydrocarbons (PAH)
Aromatic amines
Phenolic compounds
Hormones and anabolic steroids
Pesticides and insecticides
Other Organic Compounds
(regarded as most suitable as model compounds for stability investigations)
Ascorbic acid
Unsaturated fatty acids

future national specimen bank programmes. The problems with the methods and the performance attained are discussed in some detail in what follows, with respect to the role of our institute within the project.

Banking and analytical facilities

The Institute of Applied Physical Chemistry of KFA Jülich contributes to the pilot environmental bank programme the facilities of the central bank, the storage and general transport organization (logistics), sampling, sample preparation and various analytical facilities. The last are located in either newly constructed or well equipped laboratories with modern instrumentation.

The central bank building[7] was constructed in 1980–1981 and has a total space of 230 m^3. The storage and working part, accessible by a dust-sluice, on average provides a dust level that is less than 10% of that of a regular laboratory, by introduction of prefiltered air, according to VDI 2038, class 5.[10] Additionally, in each of the storage compartments and laboratories laminar air-flow hoods (US Fed. Stand. class 100, *i.e.*, VDI 2083, class 3) have been installed. Four storage compartments offer considerable storage volumes: 20.4 m^3 at liquid-nitrogen vapour temperatures, ranging from $\leqslant -145°$ at the top to $-190°$ at the bottom of the 18 storage containers in three of the compartments and 1.7 m^3 at $-80°$ and 1 m^3 at temperatures from $+4°$ to $-10°$ in the fourth. Containers of the first type are loaded with about 5000 samples of all the materials shown in Table 1. The last two types are used for comparative storage, intermediate storage and storage of control materials. There are also two laboratories for the determination of mercury, arsenic and PAH, and a smaller room for sample preparation with an atmosphere on average approaching clean-room (VDI 2083, class 3) conditions. The complete floor plan of the bank building is depicted in Fig. 1.

For specimen sampling and transport two vans and occasionally the institute's mobile laboratory[11] are used. The latter is equipped with a class 100 laminar air-flow hood. The vans carry samples at liquid-nitrogen vapour temperature to the satellite banks and the co-operating laboratories. Further analytical facilities for organic and inorganic compounds, also equipped with clean working places, are located inside the main institute building as well as in the radiochemical part situated in another complex of the Chemistry Department of KFA. A schematic flow chart for the interaction of all these sections is given in Fig. 2.

EXPERIMENTAL

Sampling and sample preparation

The institute performed the sampling, homogenization, portioning and packaging for the marine macroalgae (*Fucus vesiculosus*) and contributed to the homogenization, portioning and packaging of the carp (*Cyprinus carpio*), mussels (*Dreissena polymorpha*) rain-worms (*Lumbricus*

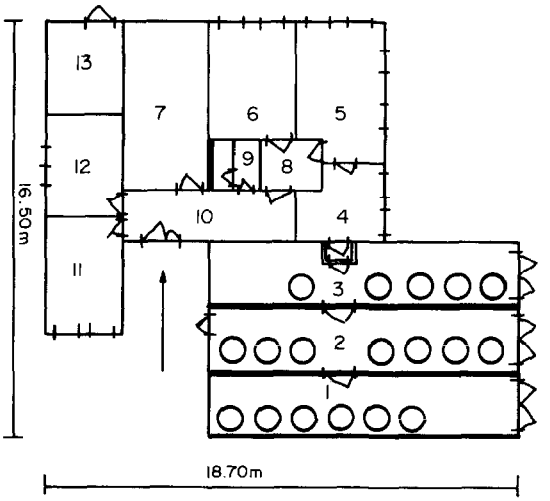

Fig. 1. Floor plan of the pilot specimen bank building. 1–3, Compartments for liquid-nitrogen vapour-phase storage (compartment 1 contains six 1.4-m^3 containers, compartments 2 and 3 contain twelve 1.0-m^3 containers); 4, sample preparation room; 5 and 6, analytical laboratories; 7, compartment for compressor cooling; 8, dust sluice; 9, lavatory; 10, vestibule with control panels for all technical functions; 11 and 12, offices (logistics); 13, technical maintenance room.

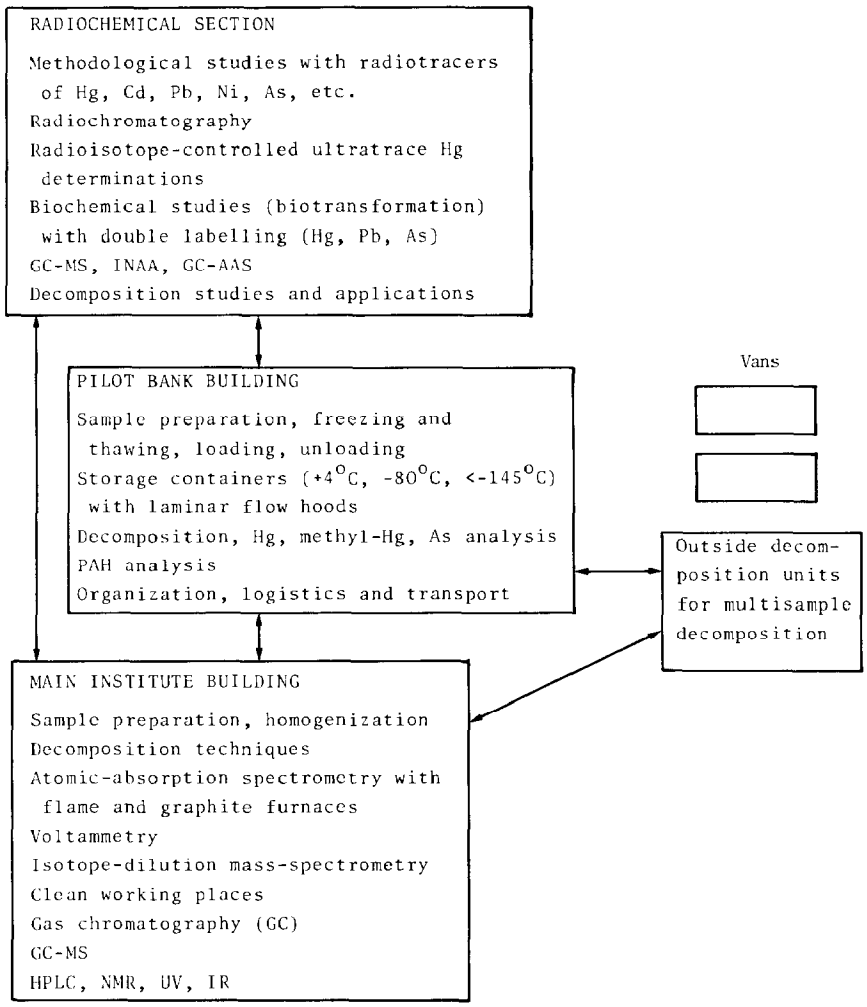

Fig. 2. Flow-chart of the pilot specimen bank and the connected analytical facilities.

rubellus), beetles (*Carabus auratus*) and Lombardy poplar (*Populus nigra italica*) under as clean conditions as possible.

Fucus vesiculosus was sampled in March 1981 at Strande, on the Baltic coast close to Kiel, with the aid of the mobile laboratory and a van.[12] The algae were first collected by hand from the shallow water (precleaned polyethylene gloves being worn), and were placed either in stainless-steel containers filled with sea-water (for the subsequent determination of organic compounds) or in polyolefin containers (for metals). Adhering material (small worms, crabs, beetles, *etc.*) was then separated by shaking and by use of stainless-steel, nickel or plastic forceps and spatulas. The samples were brought by van from the beach to the mobile laboratory, situated at a nearby field laboratory of the Institute of Marine Research, Kiel. Here, the samples were dealt with in the laminar-flow hood, being rinsed three times with clear sea-water freshly taken from the sampling site. Adhering water was removed by centrifugation with a stainless-steel salad centrifuge. The material to be reserved for organic analysis was passed three times through a mincer (stainless steel and aluminium alloy), carefully mixed and put (in ~50-g lots) into carefully precleaned 100-ml Schott Duran GL 45 bottles, weighed, sealed with aluminium foil, and immediately deep-frozen in liquid-nitrogen vapour containers.

The portions for metal determinations were first passed twice through the mincer after the preparation of material for organic analyses, then deep-frozen (with liquid nitrogen) in appropriate portions and milled in zirconium dioxide ball mills which before use were carefully cleaned with warm dilute nitric acid and checked for contamination. The whole was then thawed, carefully mixed and placed (in 1- or 2-g portions) in the minivials, which were then enclosed in the 22-ml bottles and immediately deep-frozen, as described above, in a liquid-nitrogen vapour atmosphere.

These tasks could be performed very rapidly. Thus, the time span from homogenization to packaging was only a few hours if amounts up to 5 kg had to be prepared, there was sufficient manpower to proceed without delay, and the sampling staff were experienced. Analytically significant deterioration in that time is not to be expected, even if rather sensitive organic compounds have to be investigated. Also, the unavoidable contamination from the mincer is minimized under the conditions chosen, as could be proved by comparison of, for example, nickel values for directly taken and processed materials [which yielded identical values within the analytical error limits (2*s*)].

Fillet of carp (*Cyprinus carpio*) for subsequent determination of metals and arsenic was freshly dissected at a clean-bench with special quartz knives, already approved for trace metal determinations in marine biota,[12] and 1–2 g portions were transferred into polyethylene minivials, weighed, and immediately deep-frozen as described for *Fucus vesiculosus*. This was done in co-operation with the

Table 3. Control materials made from surplus banking or other appropriate materials

Material	Characterized or to be characterized for	Portion (dry weight), g	Total amount produced, g
Pig liver I	Pb, Cd, Hg, As	2	180
Fresh whole blood I	Hg, Pb, Cd	5 (wet weight)	500
Fish fillet I (low Hg content)	Hg, As, Cd	5	720
Brown algae I	Pb, Cd, Cu, Ni, Zn, Cr, Hg	5	130
Brown algae II	Pb, Cd, Hg, As	5	1600
Soil ("Parabraunerde") I	Pb, Cd, Hg, As	10	2100
Soil ("Parabraunerde") II	Pb, Cd, Hg, As, Ni	5	1050
Grass II (*Lolium multiflorum*)	Pb, Cd, Hg, As, Ni	3.5	84
Mussels II (*Dreissena polymorpha*)	Pb, Cd, Hg, Cu, Cr, Ni	5	55
Poplar leaves I	Pb, Cd, Hg, Cu, Ni, Cr, As	5	265
Poplar leaves II	Pb, Cd, Hg, Ni, As	5	445

Remarks. Series I made from appropriate materials, series II made from surplus bank materials. Analytical methods used for the characterization: flame, graphite-furnace, cold-vapour/hydride AAS, DPASV and IDMS (Pb and Cd). Whole blood stored at $-80°C$ or $\leqslant -145°$

participating group from Ulm University (Prof. Ballschmiter and co-workers).

In another collaboration with the group from Saarbrücken University (Prof. Müller and co-workers) the homogenization, weighing and packaging of deep-frozen terrestrial and aquatic indicators or accumulators was performed. For that purpose the clean-room of the bank building was used and the species *Dreissena polymorpha* (mussel), *Lumbricus rubellus* (rain-worm), *Carabus auratus* (beetle) and leaves from *Populus nigra italica* Lombardy poplar) were processed in a manner similar to that described for *Fucus vesiculosus*. The samples were stored immediately after deep-freezing.

Production of control materials

Besides the current use of appropriate Standard Reference Materials from NBS (Bovine Liver, Orchard Leaves, Tomato Leaves, Spinach) and of materials from other sources (IAEA, BCR and EURATOM Ispra)[14] recently lyophilysed whole-blood control materials with assigned values have been made available and used successfully for determinations of cadmium, lead and mercury.[15,16] Since these materials could only be used for general calibration purposes, other materials which were completely identical with the specimen bank specimens were needed. Such materials have been produced from surplus banking materials under carefully controlled conditions. The results of characterization determinations, performed during 1981, whenever possible by physically different methods, show, at least for lead, cadmium and nickel, that the values are in excellent agreement with those found for the corresponding fresh materials. Some results for mercury, however, at the low ng/g level seem to indicate that at present a certain contamination from the laboratory environment cannot be completely ruled out. This is currently under thorough investigation. These materials (see Table 3) were freshly homogenized as described for the materials for the bank, freeze-dried, finely ground in agate or zirconium dioxide ball mills, sieved (200 μm sieves) if necessary, and subsequently bottled and stored at $-80°$ or $+4°$. The total amounts and the number of portions prepared, except when there was not enough available, were sufficient for the continuous quality-control and data evaluation during the pilot phase of the project, as described in the section on inorganic analysis.

General analytical aspects

From the philosophy of the programme it is obvious that for the pilot phase the most important challenges are the detection limits and the precision, which at present are more important than extreme accuracy. Good detection limits are a prerequisite for the scrupulous control of contamination and for reliable analysis at the low ng/g level. Good precision is essential if meaningful information is to be obtained about the effects of long-term storage of the samples.

Inorganic analysis

Since our institute is the only one participating that performs characterization determinations and long-term analytical studies for the relevant metals, metalloids and organometallic compounds in all the stored materials, these will be discussed in some detail. For most of the materials and elements the first or even the second analytical series has now been finished. Suitable routine methods have been selected and tested for the mandatory elements lead, cadmium and mercury, and in part also for nickel, manganese and arsenic, together with independent methods for checking the accuracy. The main aim was to attain an average day-to-day precision, for at least the mandatory elements, of $\leqslant 5\%$ (with a final goal of $\leqslant 3\%$), which corresponds to a confidence interval of less than 10% at $p = 0.05$. Otherwise no sound conclusions can be drawn concerning the homogeneity of samples, sources of contamination, and possible losses or decomposition of, for example, organometallic compounds.

Reagents, contamination precautions. Usually, all reagents have to be chosen so that blanks are as low as possible. This is achieved by the use of "ultrapure" reagents (*e.g.*, Merck Suprapur®) and, if necessary, by special purification procedures such as sub-boiling distillation of acids, which is of particular importance in determination of mercury (which has to be done in laboratories which are apart from any dealing with electrochemistry).

All tubes, dishes *etc.*, as well as the storage vessels for banking and control materials, have to be carefully cleaned either with warm dilute acids or, which from our experience is more promising, in a way which simulates the subsequent analytical step. An example is the cleaning of Teflon digestion vessels for decomposition under pressure, which can be done by running a "blank" decomposition with materials having very low trace-element levels

Table 4. Results of the first analytical series (performed from July to November 1981) for Pb, Cd, As and Hg in materials of the pilot specimen bank: the data for Pb, Cd and Hg are obtained from an average of 6 subsamples; analysis on different days with at least 2 × 6 determinations (firings) from each subsample

Material	Lead		Cadmium		Arsenic		Mercury	
	μg/g	C.V., %	μg/g	C.V., %	μg/g	C.V., %	ng/g	C.V., %
Whole blood	0.075	10	0.0007	7	0.001	—	1.60*	3
Adipose tissue	<0.005	—	≤0.010	—	n.d.	—	0.8	—
Liver	1.12	2.9	1.50	5	0.008	10	62*	3.5
Soil	14.5	1.7	0.170	2	4.40	2.3	53	3.3
Sewage sludge	25.0	2.0	0.830	4	0.48	6.3	470	2.6
Carabus auratus† (beetle)	0.860	1.3	0.83	1	0.23	1.7	65	4.1
Lolium multiflorum (grass)	0.250	6.0	0.02	3.2	0.05	1.8	4.3	2
Populus nigra italica (Lombardy poplar, leaves)	6.25	2.9	0.99	2.3	0.170	6.5	19	4
Cow milk§	<0.005	—	<0.005	—	n.d.	—	≤0.01	—
Cyprinus carpio (carp)	<0.005	—	<0.001	—	0.02	4.3	71*	8
Dreissena polymorpha (mussels)	0.33	13	0.470	6.7	1.20	4.7	12.2	7
Fucus vesiculosus (brown algae)	0.720*	5.6	0.260	4*	8.80	10	11†	20
Average C.V., % (blood not incl.)		4.7		3.5		5.3		4.2 (*Fucus* not incl.)

*In these materials and for these elements a second series has already been performed and a mean value found which agrees with the first series within the indicated C.V.

†From the data obtained it appears that *carabus auratus* is very well homogenized, at least for Pb, Cd and As.

‡Three series have already been analysed for Hg in *fucus vesiculosus* and the conclusion is that this material is not homogeneous for Hg. Thus, no sound conclusions on the behaviour of Hg in this material can be expected.

§Owing to the fact that levels of Pb, Cd and Hg in cow milk are extremely low, other elements for long-term control are sought. Mn and Cu would be better suited for that purpose but this has still to be investigated.

("cleaning decomposition") as described elsewhere.[14,17,18] The laboratory ware cleaned in this manner has to be stored in air-tight vessels, in sealed plastic bags or at a clean-bench if not to be used immediately. Additionally, all vessels used for analyte processing and storage have to be checked for contamination before use, and discarded or cleaned again if any blank above the acceptable level is detected. In wet digestions for subsequent cold-vapour (mercury) or solvent extraction–GFAAS analysis, blanks were determined for each individual digestion vessel before and after sample digestion and determination.[19]

The methods selected had usually already been published and thoroughly checked[20–22] and were then modified for the matrices in question.

Mercury was determined by two different versions of the cold-vapour method: one using instrumentation constructed in our own laboratory partly from commercially available parts, and the other using a commercial instrument (Perkin–Elmer MHS-20 coupled with a Perkin–Elmer 280 AAS). The first approach is based on a previously published principle[23] using gold wool to preconcentrate the mercury but has been considerably improved to attain extremely low instrumental and analytical blanks with an absolute detection limit of ≤0.005 ng and a determination limit of ≤0.02 ng.[24] The determination follows a carefully optimized wet digestion[25] continuously monitored with ^{203}Hg. This method enables us to reach the required day-to-day precision of less then 5%, even at the low ng/g level, provided the material analysed is homogeneous. For repeated determinations on a whole-blood control material (see Table 3) over several months a coefficient of variation of 4.2% (Hg level 2.4 ng/ml) could be obtained. Further data for banking materials are summarized in Table 4 and show that the first goal can be reached for mercury as long as the materials are homogeneous.

The commercial system is useful for higher concentrations and also to check the accuracy of the home-made system. Sample preparation in this case is performed by pressurized decomposition, with improved multisample decomposition systems and optimized (*i.e.*, prolonged) temperature programmes making possible nearly quantitative decomposition. The results obtained by the two methods agree within the precision limits (≤5%) for all the materials investigated.

Lead and cadmium were routinely determined by graphite furnace or flame AAS, usually after a pressure decomposition step.[18] The instruments used were the Perkin–Elmer 410 and 4000 systems coupled with either the HGA 400 or 500 graphite furnaces, and a Perkin–Elmer 5000 with computerized flame control. To improve precision, accuracy and partly also detection power, for lead and sometimes also for cadmium, the L'vov platform technique[26,27] was used. Routinely attainable absolute detection limits in aqueous solutions were ≤0.2 pg for cadmium and about 2 pg for lead. Results were evaluated by comparison against scrupulously analysed control materials (see Table 3) thus avoiding the possibly erroneous and time-consuming evaluation by standard addition or by matrix-matched calibration graphs, and certainly improving the precision and accuracy as well. The results shown in Table 4 indicate a rather satisfactory day-to-day precision, which is mainly below 5% variation. Lead frequently poses problems in GFAAS, owing to poor sensitivity and matrix interferences. From our experience, however, it appears that these problems may be less severe with the L'vov platform, though they cannot be completely overcome.

Voltammetry theoretically offers a better limit of detection than AAS for cadmium and lead,[28–30] but in practice, the difficulty of achieving complete digestion, and the mag-

nitude of the blanks (of the order of 0.1–5 ng for a digestion vessel) frequently offset this advantage, at least for cadmium. Despite these limitations, voltammetry constitutes an inexpensive and elegant physically-independent reference method, particularly useful for the characterization of control materials and for accuracy checks during methodological investigations.[14]

For voltammetric determinations the robust and versatile PAR 174 A instrument has been used successfully in conjunction with either the hanging mercury drop (HMDE) or the rotating mercury-film electrode (RMFE) on a glassy carbon support.[14,16,17,20,22,28–30]

Another analytical approach for extremely precise and accurate lead and cadmium determinations is isotope-dilution mass-spectrometry, IDMS.[14,20,31,32] In our laboratory IDMS is performed with a modified Varian-MAT CH-5 mass spectrometer coupled with an HP 9825 A dedicated computer for spectra evaluation. The mass-spectrometric determination is performed after low-temperature ashing in a microwave-excited oxygen plasma and allows reliable cadmium and lead determinations at the 15 and 50 ng/g levels respectively.[33] The method has become indispensable for the characterization of control and banking materials. Since the method also allows very precise thallium determinations, this element was also determined in some materials.

Total arsenic has been determined in nearly all the materials to assist in their characterization. The samples were opened out by digestion with a mixture of nitric, perchloric and sulphuric acids, or by digestion under pressure, or by decomposition under pressure followed by dry-ashing with magnesium nitrate as ashing aid. These approaches are necessary to ensure complete decomposition of the very resistant high molecular-weight organo-arsenic compounds which appear in various aquatic and marine species.[34,35] The determination was performed with the MHS-20 PE 280 combination already mentioned, providing absolute detection limits of about 0.4–0.7 ng, depending on the blank level.

Nickel can be determined with good sensitivity at the low pg level with modern GFAAS instruments using graphite tubes coated with pyrolytic carbon[36,37] but also by d.c. or differential pulse voltammetry (relative detection limit about 1 pg/ml) following preconcentration by accumulation of an adsorption layer of nickel dimethylglyoximate at the HMDE.[38] Because of the toxicological and occupational health-hazard significance of nickel and its compounds, thorough characterization measurements of this element in the banking materials are in the preparatory stage; approximate levels have already been determined and range from less than 2 ng/ml in whole blood[39] to around 1–2 μg/g for algae and mussels and 100 μg/g (dry weight) for sewage sludge. Characterization measurements for other metals, such as chromium, copper and manganese are just being started.

Organic analysis (anabolic steroids, polyaromatic hydrocarbons)

From the variety of organic pollutants to be monitored in the pilot phase (*cf.*, Table 2) it is evident that such detailed analyses can only accomplished by the combined efforts of different research groups, each selected for its expertise in the identification and precise determination of a certain pollutant or pollutant group. Since a full discussion of all these activities is outside the scope of this paper, only those compounds will be discussed in detail that are actually determined in our institute:[40] steroid hormones, including synthetic analogues with a comparable chemical structure (stilbestrols), and polyaromatic hydroxy groups.

Steroids. These are determined by methods employing high-pressure liquid chromatography (HPLC), gas chromatography (GC) and glass-capillary gas chromatography/mass spectrometry (GC/MS), developed with the aim of meeting the requirements of the programme. Much effort has been spent on bringing the analytical procedure to the highest degree of reliability, accuracy and precision.[41] Moreover, by introducing selective and sensitive detection systems (multiple ion detection, voltammetric detection)[42] it has proved possible to optimize the methods for determinations in the upper pg-range with a coefficient of variation of about 10%. Details of these developments are summarized in this section.

By making maximum use of the separation efficiency offered by the chromatographic techniques, it has become possible to simplify the clean-up procedure remarkably, thus minimizing the risk of contamination, sample loss and decomposition. The following summary emphasizes crucial points of various clean-up procedures rather than repeating known techniques for steroid analysis.

For *clean-up* steroid hormones, stilbestrols and their metabolites are removed from the different matrices (liver, fat, urine) by a single-step extraction with appropriate solvents such as dichloromethane, dichloromethane/propan-2-ol, diethyl ether and methanol/water. Since these solvents are used in relatively large amounts (up to 200 ml) they should be checked for their suitability before use as they are normally contaminated with a variety of interfering compounds and require repeated distillation for purification. Phenolic compounds and fatty acids are removed from the crude extracts by extraction with 2*M* sodium hydroxide and treated separately. Without further purification, the organic extracts are then dried, concentrated and used for preparation of derivatives. For drying, however, the usual inorganic desiccants, such as anhydrous sodium sulphate or magnesium sulphate are quite unsuitable on account of their high levels of interferents. Instead, filtration through a precleaned filter paper is employed to remove adhering water droplets.[43]

For analysis by HPLC the clean-up procedure is different and even simpler.[44,45] The crude organic extracts or untreated liquid samples (urine) are preconcentrated directly on a short HPLC column which also separates steroids from interfering compounds. By a column-switching technique the fractions of interest are then transferred to the analytical column, where the necessary separations are accomplished.

For steroid-conjugates, which are normally to be expected in urine samples, a similar column-switching technique is applied, resulting in a very pure conjugate fraction which in particular is free from cleavage inhibitors. Thus, hydrolysis with β-glucuronidase may be achieved in 20 min for phenols and in 2–3 hr for aliphatic hydroxy groups, compared to at least 24 hr in previously described procedures.[46]

Derivative formation is required for GC and GC/MS determinations, since it markedly improves the resolving power of the gas chromatographic columns and the sensitivity of the detection systems. In general, the TMS-ethers or similar silyl derivatives are preferable to the TFA or HFBA derivatives which are only suitable for phenolic compounds, numerous by-products being formed from aliphatic hydrocarbons.[46]

Various *instrumental aspects* require attention. Routine analyses are usually done by chromatography (liquid or gas) with conventional detectors, and capillary GC/MS is mainly used for the elucidation of unknown structures or for identification in cases of uncertainty. However, optimal performance of all methods may only be achieved if a number of prerequisites are carefully checked:

inertness of chromatographic columns
temperature stability (for GC)
solvent stability (for HPLC)
long-term instrument stability
reproducibility in terms of retention indices or other independent parameters

For gas chromatography these requirements may conveniently be tested with long-chain fatty acids in the form of their trimethylsilyl esters or with other compounds of similar polarity and structure.[47] If need be, columns may be de-activated by treatment with Dexil 300 or Carbowax 20 M. Particular attention has to be paid to the GC/MS interface, which may be similarly tested and de-activated.[48,49] In HPLC, additional problems arise mainly from unsuitable phases (size and coating), unsuitable transfer lines and bad detector design (especially for voltammetric detectors).[50] A very important aspect in this context is the purity of the eluents used. For ultratrace analysis in the upper pg-range, even the quality of quartz-distilled water is unsatisfactory, and additional cleaning procedures are mandatory.[51]

Finally, proper choice of the stationary liquid phase is of great importance. For GC and GC/MS determinations slightly polar liquid phases such as SE 30, SE 52, SE 54, OV 1, OV 101 and DB 5 are generally used. In HPLC cyano- and phenyl-bonded columns are recommended for the androgens, while for the separation of phenols and the preconcentration of conjugates C-2, C-3 and C-8 bonded phases are more effective, generally in conjunction with gradient elution with acetonitrile/water mixtures.

Polyaromatic hydrocarbons. The programme for monitoring the stability of polyaromatic hydrocarbons in stored environmental materials started in spring 1981. For preliminary experiments the methodology of Grimmer *et al.*[52] was followed, but on the basis of our own experience we decided to use glass capillary columns (30 m with Sil 5 coating) to improve the resolution, especially for critical compounds. These experiments have now been successfully concluded and it is intended to simplify the clean-up procedure to reduce the risk of contamination and sample loss.

PRELIMINARY RESULTS AND DISCUSSION

Metals

The results presented in Table 4 for the first series of determinations of total metals show that in general the first goal, to attain on average a coefficient of variation (C.V.) of around 5%, could be reached provided the concentrations were well above the determination limits and the materials were homogeneous enough. The rather new approach to determining trace-metal contents in a particular material by using a scrupulously analysed control material of exactly the same origin appears to be a significant step forward. That is clearly demonstrated for the determination of lead and cadmium in soil and in poplar leaves where such control materials have been used for the first time. It seems now, after thorough investigation, that the more widespread use of appropriate and carefully analysed control materials will render possible faster, cheaper and much more reliable monitoring and surveillance programmes in the future.

As expected, the homogeneity also had a great influence on the results. This can be seen for the relatively homogeneous materials *carabus auratus*, soil, poplar leaves and human liver. Materials such as whole blood and milk, which would seem to be already homogeneous as received, did, however, pose problems due to extremely low trace-metal levels (milk) or to matrix difficulties (blood). Blood for the pilot programme stemmed from a rather large amount of outdated Red Cross blood reserves. The first application of our precision procedures for the determination of lead and cadmium in whole blood[53,54] initially led to unsatisfactory results for lead, but more recent work with some fresh-blood samples has proved much more successful. This has persuaded us to prepare an appropriate material from other Red Cross samples, and this is now being characterized by different methods, including Zeeman-compensation AAS measurements, which we hope will yield a significant improvement in precision and accuracy. The results in Table 4 also indicate that the homogenization step has to be improved for some materials, particularly the marine and aquatic specimens, which certainly require further improvements in contamination-free field homogenization at low temperatures and under ultraclean conditions. Since this will also be of paramount importance for the homogenization of sufficient amounts of sample for long-term investigations and for reliable real-time monitoring, this aspect will have to be carefully studied during the second phase of the pilot specimen bank programme, which will also include approaches to the determination of organometallic compounds, mainly of methylmercury, at trace and ultrace levels, based on earlier investigations.[21,25]

Organic compounds

Studies on the determination and long-term stability of steroids in stored samples have been made over the last 5 years.[56–60] The first investigations were dedicated to different freezing techniques such as deep-freezing and freeze-drying.

In principle, the latter method seems to be advantageous, because samples can be stored in normal glass containers and kept at room temperature. Moreover, transportation, shipment and distribution are simplified. However, results from a couple of experiments indicate that the freeze-drying process itself needs further intensive basic research since the chromatographic "fingerprint" of numerous reference steroids is totally changed during this pretreatment process. On the other hand it may be predicted that by application of strictly controlled low-temperature and vacuum conditions, this method can be satisfactorily developed so that even compounds as unstable as steroids will not change their chemical structure.

Repeated determinations on stored human urine samples, kept at $-80°$ for 2 years in carefully cleaned glass containers, showed nearly unchanged "fingerprints" in the steroid portions of all chromatograms obtained by different methods (HPLC, GC, GC/MS). This was the case for a variety of synthetic anabolic steroids, their metabolites and numerous other compounds, not all of which have been identified. In a couple of samples, however, the concentration of cholesterol showed a significant decrease (as much as 70% being lost). The reason for this unexpected degradation is absolutely unknown, but may be the differ-

ence in enzyme concentrations in the urine samples investigated. Hence future programmes should record the chromatographic fingerprints of all compounds extracted in the clean-up procedure regardless of their chemical structure or significance as pollutants. If deterioration with storage time or temperature becomes evident, structure elucidations may have to be performed, which may then serve as supporting aids for future storage, monitoring and determination programmes.

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SAMPLE CONTAMINATION AS A SOURCE OF ERROR IN TRACE-ELEMENT ANALYSIS OF BIOLOGICAL SAMPLES

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Summary—Trace-element levels estimated by different investigators are often disparate. It is becoming increasingly evident that sample contamination may explain some of the discrepancies. A method has been developed for the direct estimation of potential errors. This shows that extraneous additions occurring during sample collection and preparation may give rise to grossly misleading results on subsequent analysis.

For a long time it was thought that no great problems were associated with sample collection and manipulation in trace-element determinations. Thus, most researchers concentrated on developing suitable analytical procedures and on improving their sensitivity and specificity. However, as soon as the results of a number of investigators were reported, it became apparent that widely divergent values had been obtained.

The origin of these discrepancies has frequently been discussed. Many factors such as age, sex, pregnancy, dietary habits, environmental conditions and occupational exposure have been documented and shown to influence the metabolism of certain trace elements in healthy subjects.[1–7] However, the advent of biological reference materials left no doubt that some of the inconsistencies could be attributed to analytical inaccuracies.[8–14] This is best illustrated by the discordant values for chromium (<0.005–3.5 μg/g) obtained for Standard Reference Material (SRM) 1577, National Bureau of Standards (NBS) Bovine Liver.[14] Furthermore, evidence has accumulated that there is a further source of error, namely unsuspected contamination of samples during collection or manipulation. In this respect, the observations of Cotzias and his associates[15,16] are of paramount importance. In 1961, these investigators published a mean serum manganese level of 2.50 ng/ml which, 5 years later they admitted to be unreliable, as a detailed examination revealed that there had been a systematic contamination of the samples with exogenous metal in their first study. Shortly after, Davies *et al.*[17] showed that routine plasma zinc estimations were of no value unless a few simple but stringent technical rules were observed. They emphasized that zinc is a ubiquitous contaminant of glass, lead and rubber piping, and of water and many chemicals (even of the highest analytical grade). During the early stages of their study, these authors performed over 100 zinc estimations on blood samples collected with ordinary sterile glass syringes into "chemically clean" glass bottles with metal caps. Furthermore, a number of these specimens were allowed to stand for several hours. The results were considerably different from those obtained during the later stages of their study on samples collected and processed with due observance of the essential safeguards against contamination. These and similar observations gave impetus to closer examination of the problem during the subsequent decade.

To what extent sample contamination may invalidate the results of trace-element analyses is not easily established. Different, usually indirect, approaches have been proposed. Using neutron-activation analysis, we have developed a direct method of estimating the resulting errors. The procedure is described in detail elsewhere,[18,19] and so only a brief account of the experimental details is given here. The present paper is a summary of our results and a survey of the observations of other investigators.

EXPERIMENTAL

Principle

Using neutron-activated apparatus, we reproduced *in vitro* several routine sample-collecting and handling procedures, starting from the hypothesis that under the experimental conditions used, transfer from the instruments to the samples would be reflected by radioactivity in the samples. Figure 1, which depicts the superimposed gamma spectra of an irradiated rubber stopper of a Venoject® tube

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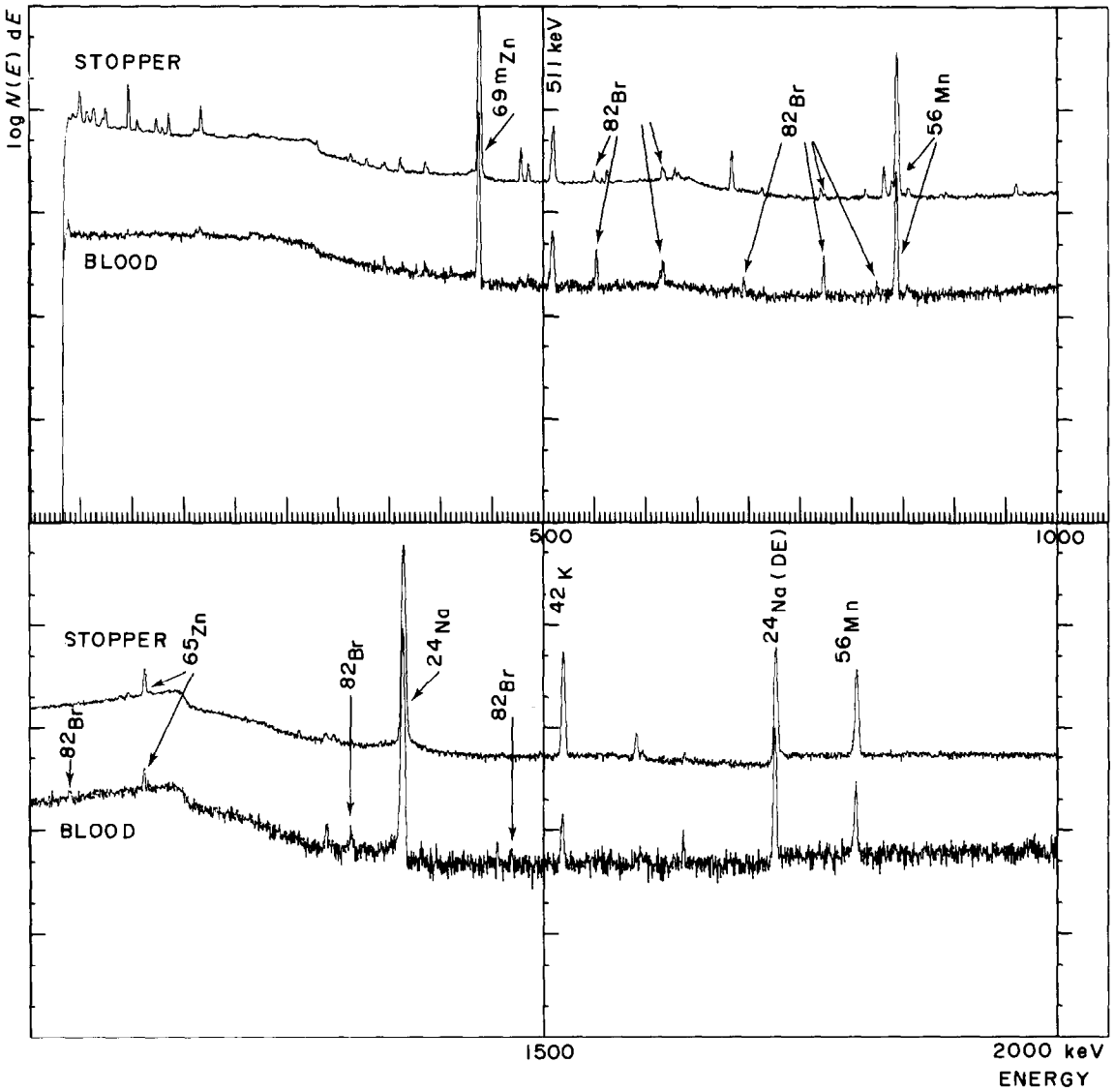

Fig. 1. Ge(Li) spectrum (waiting time 2 hr 55 min, measurement time 1 hr) showing the extraneous additions to a blood sample after contact (1 hr 30 min) with an irradiated (6 hr 25 min at 1.8×10^{12} n.cm^{-2}.sec^{-1}) rubber stopper of a Venoject® tube. The spectrum of the stopper is also shown.

(plain, silicone-coated, Terumo) and a blood sample that came into contact with it, proves that this actually occurs. It visualizes what has been observed by several workers, namely that ordinary rubber stoppers cause a marked zinc contamination.[20–27]

It is evident that the activities of the different photopeaks in such a contaminated sample may be converted into numerical values in exactly the same way as in routine neutron-activation analysis. By using various irradiation conditions and post-irradiation measurements, we succeeded in determining the extraneous additions of several elements, both qualitatively and quantitatively.

Scope

We investigated the potential additions in a number of circumstances: in blood samples collected with steel needles (sterile, disposable needle, 19Gl½, Terumo) or with polypropylene over-the-needle catheters (Intranule®, 110 16, Vygon), in blood and serum samples collected or stored in various containers (Vacutainer®, royal blue stopper, silicone-coated, brand tube with minimal trace element content, Becton-Dickinson; polyethylene containers, Kartell SpA), and in needle aspiration (Menghini needle, K. Storz, K. G.) or surgical wedge biopsies of the liver (Swann-Morton surgical blade, W. R. Swann & Co). In all these cases, the usual sampling techniques were reproduced as closely as possible in the laboratory. In order to assess additions from a steel needle or from a polypropylene catheter during venepuncture, a plastic tube was filled with normal human blood, kept at 37° and punctured with an irradiated needle or catheter. Additions from a royal blue stopper were estimated by introducing 10 ml of normal human blood into an opened Vacutainer®, which was then gently inverted 2 or 3 times every 5 min for 30 or 120 min after having been resealed with an irradiated stopper. Finally, to study additions in liver biopsies, we used the normal organ of a victim of a sudden accidental death. With irradiated Menghini needles, specimens of about 4–6 mg, comparable to small percutaneous biopsies, were obtained. Each series consisted of approximately 100 biopsies. With neutron-activated surgical blades, biopsies of about 500–750 mg were cut. In this case, each series corre-

Table 1. Trace element additions in blood samples collected with disposable steel needles, and literature values for intrinsic concentrations in plasma or serum

Needle No.	20-ml sample	Concentration of metal ion, *ng/ml*											
		Sc	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ag	Sn	Sb	Au
							Additions						
1	1			0.19				0.28					
	2			0.10				0.20					
	3			0.051				0.36					
	4			0.057				0.34					
2	1			0.22				0.59					
	2			0.013				0.092					
	3			0.012				0.33					
	4			0.017				0.13					
3	1			0.077				0.38					
	2			0.030				0.33					
	3			0.013				<0.23					
	4			0.039				0.22					
4	1			0.45				0.82					
	2			0.13				0.30					
	3			0.017				0.26					
	4			0.030				0.25					
5	1	$<2.3 \times 10^{-3}$	90		230	0.92	75		<0.32	21.2×10^{-3}	<1.4	$<38 \times 10^{-3}$	$<4.2 \times 10^{-3}$
	2	$<0.52 \times 10^{-3}$	13		32	0.22	10		<0.76	$<5.5 \times 10^{-3}$	<0.68	$<8.8 \times 10^{-3}$	$<2.0 \times 10^{-3}$
	3	$<0.50 \times 10^{-3}$	10		23	0.15	8.5		0.055	$<5.2 \times 10^{-3}$	<0.64	$<8.2 \times 10^{-3}$	$<2.5 \times 10^{-3}$
	4	$<0.54 \times 10^{-3}$	16		33	0.18	13		0.43	4.8×10^{-3}	<0.35	$<9.5 \times 10^{-3}$	$<3.2 \times 10^{-3}$
References							Intrinsic concentrations						
16				0.587									
17									950				
31								1120			37.2		
32		19.5×10^{-3}	1.4			0.36			905	195×10^{-3}		113×10^{-3}	
33				0.54									
34					1450	0.46		1050	1010			5200×10^{-3}	540×10^{-3}
35				0.63									
36			0.45			0.30				2100×10^{-3}		520×10^{-3}	48×10^{-3}
37		150×10^{-3}	9.3		1950	1.85		1040	1160	6200×10^{-3}		2500×10^{-3}	
38			0.14										
39			0.7										
40							1.8						
41							2.6						
42						0.074							
43				0.57				1070	940				
44					1630				1130				
45			0.160			0.108							
46		160×10^{-3}			1210	0.52		1010	950	900×10^{-3}		750×10^{-3}	9×10^{-3}

Table 2. Manganese and copper additions in blood samples collected with polypropylene catheters

Catheter No.	20-ml sample	Metal ion addition, *ng/ml*	
		Mn	Cu
1	1	$<8.8 \times 10^{-3}$	
	2	$<4.3 \times 10^{-3}$	
	3	$<3.1 \times 10^{-3}$	
	4	$<2.6 \times 10^{-3}$	
2	1	25×10^{-3}	
	2	8.0×10^{-3}	
	3	$<2.7 \times 10^{-3}$	
	4	$<2.5 \times 10^{-3}$	
3	1	18×10^{-3}	0.12
	2	5.6×10^{-3}	0.10
	3	$<2.4 \times 10^{-3}$	0.085
	4	$<2.8 \times 10^{-3}$	0.16

sponded to about 10 biopsies. The intervals between the end of the irradiation, the moment of the experiment and the subsequent measurement were fixed according to the half-life of the radioisotope selected for the investigations.

Reactor irradiations

Parts of our study (estimation of manganese, copper, and in some cases zinc transfer) were performed with devices irradiated for about 6 hr at fluxes of 1.0–1.8 × 10^{12} $n.cm^{-2}.sec^{-1}$, and other parts (estimation of scandium, chromium, nickel, iron, cobalt, zinc, silver, tin, antimony and gold transfer) with devices irradiated for 5 days at a flux of approximately 10^{14} $n.cm^{-2}.sec^{-1}$. Polypropylene catheters, rubber stoppers and polyethylene containers do not resist irradiation at high neutron-fluxes, so for these we determined only manganese, copper and sometimes zinc transfer. During some of the 6-hr irradiations, standards were co-irradiated. In most cases however, flux monitors or comparators were preferred.[18,19] For details, see the publications of De Corte *et al.*[28,29]

Radioactive measurements and computation

Our experiments were performed over a period of more than 10 years so different measuring systems have been used. The specifications of those used in the initial phase of our investigations can be found in previous papers.[18,19] For the last few years we have measured our gamma spectra with a coaxial 70-cm^3 Ge(Li) detector (Philips) (energy resolution 2.0 keV for the 1332-keV photopeak of ^{60}Co; relative detection efficiency 15.6%), coupled to a 4000-channel analyser (Didac, Intertechnique). At first data reduction was performed with a PDP9, but is now done with a PDP11/45 computer (Digital Equipment Corporation) by means of a program developed by Op de Beeck and Hoste.[30]

RESULTS

Table 1 lists the transfer of scandium, chromium, manganese, iron, cobalt, nickel, copper, zinc, silver, tin, antimony and gold observed in blood samples collected with disposable steel needles. Their significance may be assessed by comparing the observed figures with the reportedly normal intrinsic concentrations in plasma or serum given in the lower half of the table. In some cases, a definitive conclusion is hard to draw because of the continuing uncertainty surrounding the plasma or serum levels of some elements in healthy adults, as pointed out in a previous publication.[47] The most important contaminations were invariably found in the first 20-ml sample. Obviously, the transfer of copper and zinc is largely negligible. The iron contamination amounts to about 15% in the first and to about 2% in subsequent 20-ml samples*. The manganese contamination in the first 20-ml sample varies from about 13% (needle No. 3) to 77% (needle No. 4) and in the third or fourth 20-ml sample from about 2% (needle No. 2, third 20-ml sample) to 10% (needle No. 1, fourth 20-ml sample). The transfers of cobalt, and more particularly of chromium and nickel, are even more important as they may equal or even exceed the intrinsic levels of these elements in human serum. The additions of scandium, silver, tin, antimony and gold are difficult to interpret for reasons already mentioned and also because we only succeeded in determining upper limits in several instances.

To avoid these serious artefacts it was decided to take blood samples with a polypropylene catheter. Table 2 summarizes the results for manganese and copper. They are expressed in the same units as in Table 1 so that they may easily be compared with the normal levels given in that table. An analysis of the data shows that errors are significantly reduced. The manganese addition of 0.025 ng/ml observed in the first sample collected with catheter No. 2 corresponds to an error of about 4%.

Figure 2 depicts the gamma spectrum of a blood sample after contact with an irradiated stopper of the Vacutainer® introduced by Becton-Dickinson for trace element studies. Table 3 summarizes the manganese, copper and zinc results. Compared with the natural levels in serum (Table 1) the amounts of copper and zinc transferred are insignificant (1–3% for copper and 1–2% for zinc). On the other hand, the amounts of manganese are important (errors of 8–51% in samples that remained in contact with the stopper for 30 min and of 11–24% in those that remained in contact for 120 min). We have no data on the potential extra additions from the tube itself. Figure 3 shows the upper right part (500–1000 keV) of Fig. 2 in more detail. It shows that whereas sodium, manganese, copper and zinc are readily taken up by the sample, gallium, lanthanum, europium and dysprosium are not (or only to a much lesser extent). We have not examined this problem further. A similar phenomenon was observed with the rubber stopper of a Venoject® tube, as shown in Fig. 1. The photopeaks in the stopper that are not reflected in the sample are from gallium, barium, lanthanum, europium, dysprosium and tungsten radioisotopes.

Table 4 enumerates the transfer of manganese and copper to serum samples stored in polyethylene containers. Though the absolute amounts of both el-

*The percentages given are based on an approximate mean "normal" level when the literature reports a range of "normal" levels.

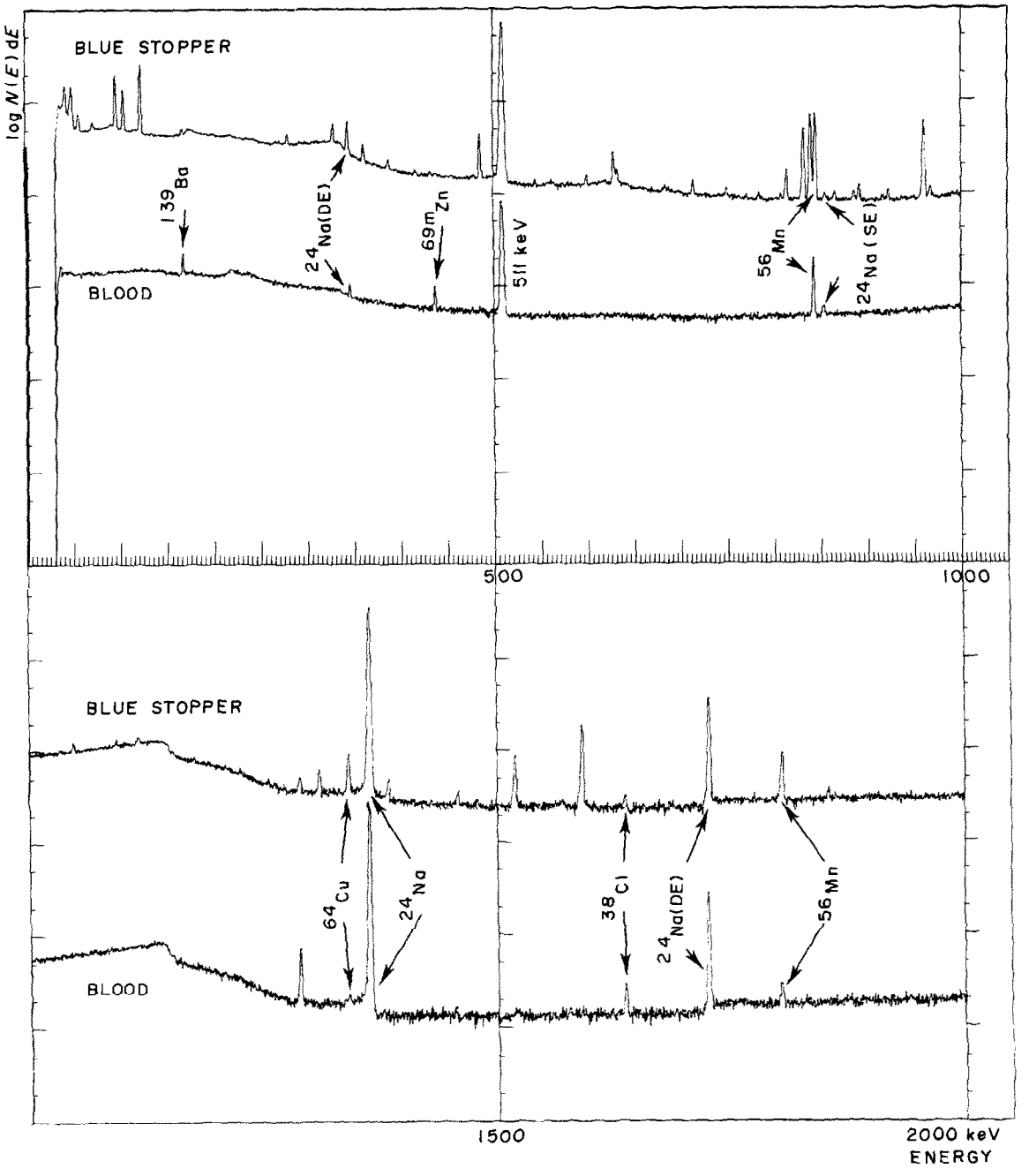

Fig. 2. Ge(Li) spectrum (waiting time 50 min, measurement time 2 hr 15 min) showing the extraneous additions to a blood sample after contact (30 min) with an irradiated (6 hr 10 min at 1.8×10^{12} n.cm^{-2}.sec^{-1}) royal blue stopper of the Vacutainer® recommended by its manufacturer for trace element studies. The spectrum of the stopper is also shown.

ements are roughly of the same order of magnitude (mean values, non-cleaned containers—manganese 0.57 ng/ml, copper 0.96 ng/ml; rinsed containers—manganese 0.084 ng/ml, copper 0.27 ng/ml), their significance is widely different. Indeed, when compared to the normal mean plasma or serum level of the element, the observed copper additions are negligible. On the contrary, the manganese additions from non-cleaned containers may equal or even exceed the normal mean natural concentration of the element. This table illustrates the vital importance of cleaning all such containers with extreme care; if only rinsing with water doubly distilled in quartz is used, errors of up to 15 or 20% for manganese may easily persist.

The values for manganese, copper and zinc listed in Table 5 give an impression of the errors to be

Table 3. Manganese, copper and zinc additions in blood samples*

Duration of contact, *min*	Stopper No.	Metal ion addition, *ng/ml*		
		Mn	Cu	Zn
30	1	0.129	11.2	21.3
	2	0.046	14.3	17.4
	3	0.292	23.5	15.5
120	1	0.135	17.8	11.0
	2	0.127	32.3	16.6
	3	0.064	18.5	15.5

*10-ml aliquots in contact with royal blue stoppers of Vacutainers®.

expected in serum samples collected and processed under suboptimal conditions. The values listed in subseries A were obtained for duplicate samples of healthy persons during the initial stage of our investigations, and those listed in subseries B after an improvement in our blood-sampling procedure (use of Spectrosil® quartz tubes and conventional polyethylene containers cleaned with meticulous care,[43–45,48,49] observance of stringent precautions against airborne contamination during sample collection and transport, sample manipulation and preparation under clean-room conditions). It is emphasized that the same radiochemical procedure was used for all analyses. It is evident that both the accuracy and precision of the manganese determinations of series A are extremely poor. The mean value is in error by about a factor of 10 (6.7 or 6.9 ng/ml *vs.* 0.63 or 0.64 ng/ml). It is interesting to note that the copper and zinc values of both series are roughly comparable. This illustrates that a sampling procedure may be adequate for analyses at the μg/ml level (serum

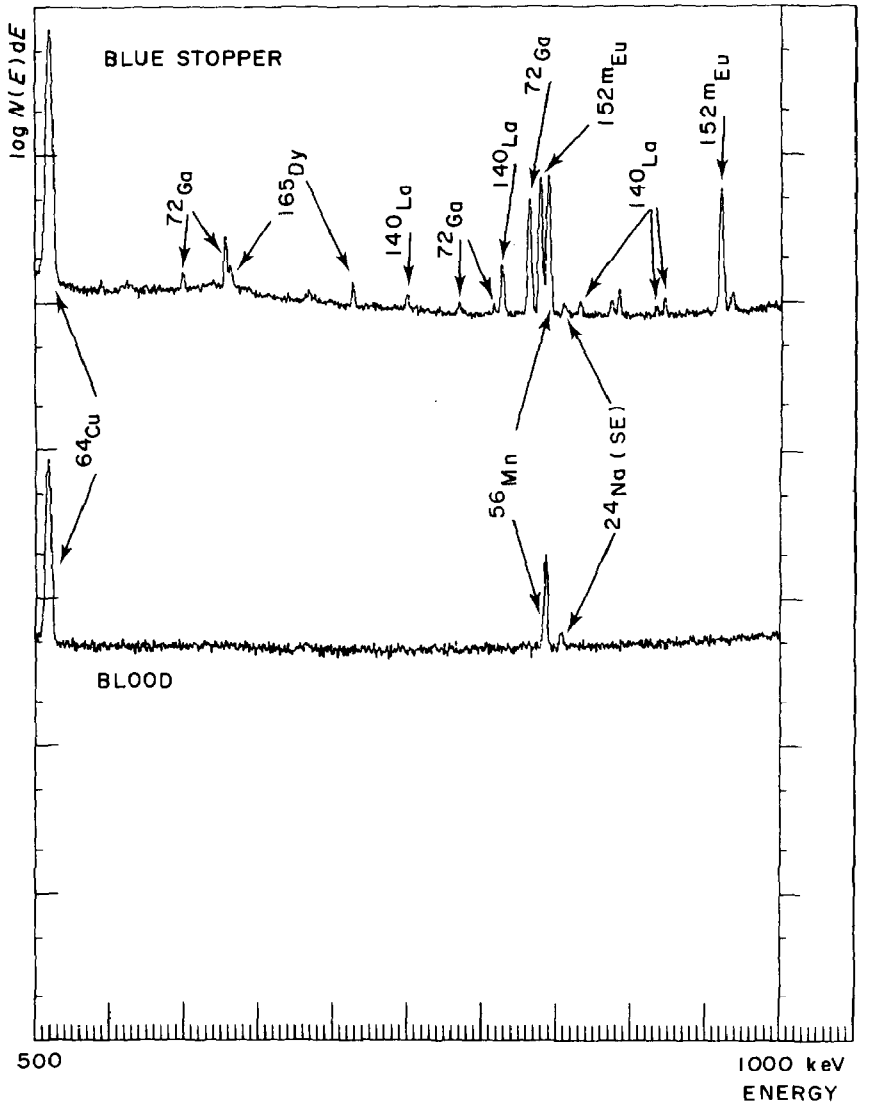

Fig. 3. Detail (500–1000 keV) of Fig. 2, showing that ^{24}Na, ^{56}Mn and ^{64}Cu appear in the sample, in contrast to ^{72}Ga, ^{140}La, ^{152m}Eu and ^{165}Dy.

Table 4. Manganese and copper additions in serum samples*

Polyethylene container No.	Pretreatment	Metal ion addition, *ng/ml* Mn	Cu
1	None	0.85	0.31
2		0.55	1.0
3		0.20	0.51
4		0.67	2.00
1	Briefly rinsed with	0.096	0.16
2	water doubly	0.018	0.26
3	distilled in quartz	0.12	0.10
4		0.10	0.55

*1-ml aliquots stored in polyethylene containers, diameter 9 mm, height 32 mm.

copper and zinc) but inadequate for analyses at the ng/ml level (manganese).

Table 6 compares the observed contamination in needle-aspiration biopsies of the liver with published information on the intrinsic concentrations of the elements. It is obvious that the additions are again extremely important. For iron, an error of some 5 or 10% may be found; for manganese, copper and zinc, 25 or 40%. For cobalt, and particularly for chromium and nickel, they may exceed the natural levels of the elements in the sample. For the same reasons as mentioned for the additions in blood samples, for scandium, silver, tin, antimony and gold the size of the error is difficult to estimate at present, but it is evident that it is far from negligible. Thus, needle-aspiration biopsies are unsuitable for the determination of several elements in normal human liver tissue.

Table 7 surveys the additions observed in wedge biopsies of liver. As expected on theoretical grounds (surgical wedge biopsies have a much more favourable volume-to-surface ratio than percutaneous needle biopsies), the errors are considerably smaller.

Table 5. Concentrations of manganese, copper and zinc determined in duplicate serum samples of healthy adults*

Series and No.		Subjects Age	Sex	Metal ion concentration, *ng/ml* Mn		Cu		Zn	
A	1	19	♂	2.8	5.7	720	750	1080	1180
	2	24	♂	3.8	3.4	860	910	1200	950
	3	27	♂	2.1	9.4	920	1000	1200	1340
	4	28	♀	24	6.3	1500	1530	1140	1230
	5	46	♂	1.9	8.5	1250	1210	900	860
	6	47	♀	4.6	2.3	620	690	990	1070
	7	51	♂	12	29	1100	1180	1350	1070
	8	55	♂	2.3	2.8	860	930	1110	1230
	9	58	♀	5.7	3.1	1010	930	1340	1100
	10	61	♀	1.3	1.8	1240	1340	1040	1050
	11	62	♀	12	6.1	1090	910	1300	1190
	12	74	♂	7.8	4.4	1080	1040	740	740
Mean				6.7	6.9	1020	1040	1120	1080
Standard deviation				6.6	7.3	240	240	180	170
B	1	17	♀	1.02	1.06	830	1000	900	1070
	2	19	♀	0.54	0.55	1010	1030	880	730
	3	20	♂	0.64	0.57	840	890	1140	1100
	4	22	♂	0.72	0.60	1120	1350	670	770
	5	23	♀	0.64	0.44	1540	1630	790	860
	6	32	♂	0.57	0.70	730	890	1040	1230
	7	45	♂	0.44	0.49	1010	1020	970	870
	8	51	♂	0.58	0.51	1010	1060	900	1050
	9	51	♀	0.56	0.58	930	930	720	650
	10	52	♂	0.64	0.78	1010	990	1050	980
	11	54	♂	0.65	0.81	1000	1180	1010	1090
	12	54	♀	0.60	0.60	700	850	780	740
Mean				0.63	0.64	980	1070	900	930
Standard deviation				0.10	0.14	220	220	140	180

*Series A: initial stage of investigation.
Series B: after substantial improvement in sampling procedure.
In both series analyses were performed by the same radiochemical technique.

Table 6. Trace element additions in aspiration biopsies of liver taken with Menghini needles, and literature values for intrinsic concentrations in liver tissue

Needle No.	Biopsy series	Concentration of metal ion* Sc ng/g	Cr μg/g	Mn μg/g	Fe μg/g	Co ng/g	Ni μg/g	Cu μg/g	Zn μg/g	Ag ng/g	Sn μg/g	Sb ng/g	Au ng/g
							Additions						
1	1			0.58				1.70					
	2			0.015				0.59					
2	1			0.64				1.23					
	2			0.54				2.70					
3	1			0.11				0.55					
	2			0.040				0.60					
4	1			0.10				0.89					
	2			0.022				0.51					
5	1	<0.58	8.5		20	230	6.2		17	12.0	0.46	69.0	<1.5
	2	<0.21	1.9		3.8	51	5.1		11	1.8	0.30	4.2	<120
6	1	<0.51	11.0		20	240	12.0		3.2	11.0	0.36	33.0	<1.5
	2	<0.057	0.48		0.99	15	1.2		2.5	0.61	0.042	5.5	<21.0
							Intrinsic concentrations						
References													
50		0.30		0.54	274	130		3.3	46		1.13	13.0	0.09
51			0.027		203	40		7.4	124			10.3	
52								7.5	85.0				
53					183	60		6.9	67				0.057
54			0.277	1.16		151	0.17	6.31	49.7	87.2			
55				1.10									
56		≤0.4	0.0054	1.41	205	34		5.98	59.0		≤0.32	11.0	
57			0.045	1.50	237	50		7.3	63.8		0.42		
58								7.46	78.2				
59				1.58	306			6.5	75				
60			0.0175	3.62	224	109	0.033	13.59	62.5	12.2	0.476		
61			0.0407	1.32	165.5	16.3		6.39	57.3			17.1	0.155
62				1.3	296				53			45.0	
63								10.2					
64			0.045	1.8		122		5	66				

*As fraction of wet weight.

Table 7. Trace element additions in wedge biopsies of liver taken with surgical blades

Blade No.	Biopsy series	Metal ion addition*											
		Sc ng/g	Cr μg/g	Mn μg/g	Fe μg/g	Co ng/g	Ni μg/g	Cu μg/g	Zn μg/g	Ag ng/g	Sn μg/g	Sb ng/g	Au ng/g
1	1			0.0035				0.0034					
2	1			0.0029				0.0047					
3	1			0.0034				0.018					
4	1			0.00091				0.0052					
5	1	0.0044	0.012		1.1	0.45	0.015		0.0055	0.050	<0.00096	0.34	0.0055
	2	<0.00072	0.010		0.34	0.095	0.0023		0.00044	<0.0051	<0.00062	0.072	<0.0025
6	1	0.010	0.020		4.6	1.51	0.064		0.01	0.064	<0.0021	1.1	0.015
	2	<0.0010	0.0028		0.40	0.14	0.0049		0.00089	<0.0066	<0.00054	0.090	<0.0033

*On basis of wet weight.

For manganese, iron, cobalt, copper and zinc they are practically negligible (less than 5%). The same conclusion probably holds for scandium, silver, tin and antimony. For gold, a contamination of about 10–25% is found. For chromium and nickel, the figures are even more significant. In some cases, however, some doubt remains about the normal level, thus hampering estimation of the error.

DISCUSSION

Several investigators have cautioned against the errors resulting from inadvertent sample contamination in trace-element research. Nevertheless, the real extent of the problem was slow in becoming fully recognized. One of the earliest warnings was issued by Thiers in 1957,[65] in the following terms: "unless the complete history of any sample is known with certainty, the analyst is well advised not to spend his time in analysing it." The very low serum-cobalt levels published by this investigator[42] (see Table 1) reflect his own care in sample collection and manipulation. They were largely ignored, and it was several years before they were supported by other results.[45,66–68] The precautions needed for obtaining reliable serum manganese and zinc values were outlined by Cotzias *et al.*[16] in 1966 and by Davies *et al.*[17] in 1968, but for both elements, more particularly for manganese, results obtained with apparently contaminated plasma or serum samples continued to be reported.[69–77] Heydorn and his associates[33,78] drew attention to the potential errors caused by airborne pollution in serum samples intended for manganese determinations. In recent years, several other studies have been published in which strong emphasis was laid on the importance of avoiding extraneous additions. Among these are the papers of Evenson and Patterson.[79,80] Their results suggest that most previously reported plasma-lead values are without any scientific significance because they are artificially elevated as a consequence of unsuspected contamination. Finally, Behne[81] reviewed the problems arising during sample collection and preparation. His paper includes a detailed discussion of the sources of contamination and the detection and prevention of resulting errors.

It is evident that the risk of obtaining grossly misleading figures owing to inadvertent extraneous additions is markedly higher for elements which occur at the ng/g level than for those occurring at the μg/g level and that the significance of a given addition will decrease as the concentration of an element in a matrix increases. Thus, for example, it is obvious that in Wilson's disease, primary biliary cirrhosis, or other conditions characterized by grossly increased liver-copper levels, the relative error in the result from a percutaneous needle aspiration biopsy will be lower than that when the liver-copper level is normal.

Our estimations of unwanted additions that may occur during collection and manipulation of biological samples corroborate the indirect measurements of

other researchers. Moreover, they illustrate that the resulting errors may be higher than frequently suspected, although it must be admitted that the irradiation damage to the instruments, particularly during long irradiations at high neutron-fluxes, may have increased the figures observed under our experimental conditions. There are further indications that the artefacts may be extremely important: first, although other sources of errors should not be overlooked, there are the widely divergent concentrations measured in normal human plasma or serum, varying over 2 or even 3 orders of magnitude,[47] and secondly, our own experience in determining manganese in serum (Table 5), proving that in suboptimally collected and handled samples the mean value may be increased by a factor of 10.

The risk of obtaining misleading serum zinc values because of extraneous additions from the rubber stoppers of Vacutainer® and of Venoject® tubes of various kinds has been identified by numerous investigators.[20–27,82,83] This prompted Becton-Dickinson to develop a new type of stopper for trace element studies, but our investigations show that it offers only a partial solution, as it causes considerable manganese contamination. Furthermore, its reliability in the determination of other low-level trace elements, such as vanadium, chromium, cobalt and nickel, remains to be investigated.

At a moment when sustained efforts have resulted in improving the sensitivity and specificity of analytical methods, a growing body of evidence suggests that inadequate sample collection and preparation may be the origin of errors more serious than those involved in any other step of the analytical procedure. Similar problems were encountered by researchers involved in analyses of fresh water[84,85] and lunar samples.[86,87]

Trace-element research imposes a stringent discipline on its practitioners. The analyses must be performed under rigorously controlled conditions to protect the samples from artefacts due to the containers, the reagents, or the ambient air. A detailed description of the different precautions to be observed is beyond the scope of this paper and we refer the reader to the many excellent publications on this subject.[88–104] The measures taken at the University of Ghent to preserve sample integrity are described in detail in previous publications.[43–45,48,49,105–110]

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CONTAMINATION AS A LIMITING PARAMETER IN TRACE ANALYSIS

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Summary—The relative importance of the main parameters determining systematic errors due to contamination in trace analysis is evaluated, with respect to the laboratory environment, tools, containers and reagents. Variability in the composition and quality of materials as well as of the relevant analytical data makes the treatment of the subject only semi-quantitative but the evidence suggests that control over contamination primarily depends on good laboratory practice. Among the appproaches applicable to trace concentrations, activation analysis gives results that are least affected by contamination. In other techniques, varying blanks and the need for their control make accurate analysis at the trace level demanding in terms of time and engagement; reliable data in routine determinations at levels much below 1 mg/kg can only be expected from specialized and experienced laboratories.

Contamination in trace analysis is always understood as the increase in the measured amount or concentration of a component, resulting from its introduction at various stages of the analytical procedure, from sources other than the sample.

There is only a minor group of elements which are difficult to control in the laboratory; it includes those occurring in high concentrations in working and living environments, such as aluminium, chlorine, chromium, copper, iron, nickel, lead, sodium and zinc, and those predominating in natural dusts, including aluminium, calcium, nitrogen (ammonium and nitrate), silicon and sulphur (sulphate). Concentrations of widely spread molecular pollutants, *e.g.*, pesticides, can be reduced in the laboratory by appropriate measures to levels not detectable by current methodologies, therefore they do not need a discussion in the context of this paper.

According to present needs and interests, data on elemental concentrations at, and below, 1 mg/kg are required for biological materials, foods and environmental media. They are also highly relevant in some contemporary technologies such as telecommunications and microelectronics. In the latter, tolerances for impurities in silicon and germanium are given in terms of the number of atoms per cm^3. These range from 10^{12} to 10^{15} atoms/cm^3, equivalent to concentrations between 0.1 and 100 μg/kg.[1] Upper limits for transition elements (cobalt, chromium, iron, manganese and nickel) in raw materials and glasses used in optical waveguides are in the 10 μg/kg range.[2] Data on toxic elements in human serum, water and basic foods are given in Table 1.

In rocks and soils concentrations of common elements below 1 mg/kg are exceptional, as can be seen from data on relevant reference materials.[3,4] In metallurgy, requirements are more specific and many elements do not create contamination problems.

Techniques applicable to the lowest trace levels include atomic-absorption spectrometry, spark-source and isotope-dilution mass-spectrometry, emission spectrometry based on excitation in a plasma (ICP), voltammetric techniques, and activation analysis.[5] The last-named technique by-passes the problem of contamination and can be considered as a reference trace technique for the elements which it covers. It should be emphasized, however, that the superiority of this technique only applies to contamination and that results grossly in error because of other positive systematic effects are not uncommon. Nevertheless, it is significant that not only have more than 50% of all certified values for trace elements been obtained by activation analysis,[6] but also that most of our knowledge of their normal concentrations in biological materials is based predominantly on data obtained by this technique.

Contamination in trace analysis is comprehensively dealt with in the book by Zief and Mitchell.[7] It initiated extensive follow-up work which has made an essential contribution to our understanding of the problem. Advances in contamination control are also the subject of a recent survey by Mitchell.[8] In their book, these authors differentiate between trace and ultratrace analysis, the former encompassing levels between 1 and 100 mg/kg and the latter the range below. Only concentrations lower than 1 mg/kg require a more detailed discussion, as frequency distribution plots of interlaboratory data in this range become asymmetric and skewed towards higher values. They imply the predominance of positive systematic errors which make the assignment of correct values on purely statistical grounds uncertain.

Several independent sources, besides the sample itself, add to the final signal for a particular constituent. These are the laboratory atmosphere and working areas, tools and apparatus associated with sam-

Table 1. Concentration ranges of trace elements in human blood serum, milk, water, wheat and meat

Material	Ranges, *mg/kg*				
	100–1	1–0.1	0.1–0.01	0.01–0.001	0.001–0.0001
Blood serum	K, Mg, P, Br	Cu, Zn, Fe, F	I, Se, Pb, Rb, Ba, Sr	As, Cd, Hg, Ni, Al	Mn, Mo, Co, Sn, V, Cr
Milk	Mg, Br, Zn	Cu, Al, Fe, Rb	I, Se, Pb, Ni, Sr, Ti	Cr, Mn, F, Mo	Cd, Co, Hg, As, Sb, Sn, V,
Tap water			Cl, Na, K	Cu, Pb, Zn, Mn, Fe	Ni, As, Cd, Cr, Hg, Sb, Co, Mo, Se, Sn, V
Wheat	Ca, Mg, K, Na	Cu, Mn, Zn	Cd, Pb, Ni, Co	As, Co, Sb, Hg, I	
Muscle tissue*					
Fish	Fe, Zn, Cu	As, Cr, Se, Ni, Mn	Pb, Hg, Co, Cd, Sb		
Pork	Fe, Zn, Ca, Rb, Na, Br, Cu,	Mn, Cs	Cr, Ni, Pb, I Mo, Se	Cd, Co, Hg, Sb, Sn, V	

*Concentrations refer to freeze-dried materials.
Data pooled from references 43–50.
Elements in concentrations exceeding 100 mg/kg not included.

pling and sample preparation, laboratory ware, and reagents. They will be discussed in sequence, mainly on the basis of recent data.

The laboratory environment

Ideally, trace analytical work depending on conventional techniques should be carried out in a high-class clean-air laboratory. According to Lievens *et al.*,[9] daily average fall-out in a laboratory is of the order of 1 $ng.cm^{-2}.day^{-1}$ for the ubiquitous elements Na, Fe, Al, one order of magnitude higher for chloride, and lower by a factor of around 10–100 for most other elements. Reported data are obviously valid only for a particular laboratory and for a given set of conditions. In the above measurements trace element levels were reduced by a factor of 10–100 in a dust-free room. Availability of clean-air facilities, however, does not in itself guarantee dust-free conditions. The placement of equipment or containers, too high exhaust velocities, room draughts, or an operator working near the fume-hood can distort laminar flow, cause turbulences and dramatically affect the performance of clean-air installations.[10,11]

Risks of contamination from laboratory construction materials and surfaces (walls, floor and ceiling) have been substantially reduced by introduction of special epoxy paints, fibreglass, disposable surface protectors, sealed plastic floors and tacky mats. The absence of metallic constituents in these materials makes it possible to adapt a conventional laboratory to trace analytical work without major investment and sophisticated equipment. Awareness and understanding of the problem are nevertheless essential for exploiting advances in technical approaches to it. Only at the lowest range of concentrations and in special operations involving exposure of the sample over a long period is the availability of an appropriate clean surface, or of one or more laminar-flow hoods providing air with less than 10^5 particles per m^3, or better, a prerequisite.

Dust and particles brought to, and released or created in the laboratory by the activities of personnel, appear a more abundant and critical source of contamination. In living quarters and in offices persistent contaminants (with regard to analytical data required at the trace level) are used in large amounts in the elemental or some other concentrated form. The list includes metallic objects made of or plated with copper, brass, lead, stainless steel and chromium. The last-named, for example, for which interlaboratory data on biological systems are particularly scanty, is used for coating the surfaces of tools and instruments, has compounds which form the basis of certain yellow and orange paints, occurs in leatherware at concentrations of up to several per cent, and is a major constituent of some cosmetics.

In the laboratory some contamination, *e.g.*, by ammonium salts, is unavoidable and in most instances is of marginal importance unless the analysis involves determination of the contaminant(s). Powdered re-

agents, however, and their concentrated solutions present a serious problem. They reflect particularities of the work in a laboratory and are frequently used in large amounts over extended periods. The experience from radiochemical laboratories shows how difficult it is to prevent spreading of powdered material or to remove the last residues from spills. Stable chemicals, in contrast to radioactive materials, cannot be traced with comparable efficiency. The risk can be greatly reduced by organizing the flow and sequence of operations so as to exclude critical phases involving concentrated chemicals from rooms and working areas reserved for trace determinations. Aspects of contamination related to the laboratory environment have been authoritatively treated by Hamilton.[12]

Sampling and sample preparation

Sampling for trace analysis is delicate in the case of certain types of compact solids, *e.g.*, metals, alloys, and rocks, which require crushing, drilling, sawing or turning. The degree of contamination obviously depends on the physical properties and chemical composition of the sample materials relative to those of the tools, and can be minimized by a proper selection of tools with respect to their composition and dimensions. Major problems are encountered in size reduction and homogenization of samples. Contamination with the constituents of the tools (blades, milling and vibrator balls, rotating parts) is unavoidable even in cases when very hard materials are used in homogenizing soft tissues or powders. Quantitative data obtained by Ure *et al.*[13] on the effect of milling techniques and materials on contamination of a liver sample, show that the levels of chromium and titanium are 20 and 3 times greater respectively when a Waring Blendor homogenizer with stainless-steel knife is used instead of an agate pestle and mortar. Replacing the stainless steel by titanium (containing 4.1 mg of Cr per kg) resulted in doubling the Ti concentration, but there was also a 50% increase in the apparent chromium level in the sample. In our own laboratory, homogenizing flour for a few minutes in a vibrator with a tungsten carbide ball was found to produce a tungsten contamination level approaching 100 ng/kg, as determined by activation analysis.

The problems encountered in collection or withdrawal of liquids (biological fluids, tap water) depend on the quality of the material used (tubing) and on the ratio between the surface area in contact with the liquid and the volume taken. From recent data by Lakomaa[14] for cerebrospinal fluid, and by Pietra *et al.*,[15] as well as from earlier results by Versieck and Speecke,[16] it follows that venepuncture needles, metallic cannulae and syringes used in blood sampling may increase the level of metals such as chromium, zinc, nickel or iron by as much as an order of magnitude. Quantitative data on the extent of metal release are not directly comparable because of variations in experimental approach, and there is evidence that neutron irradiation of materials for labelling potential contaminants enhances the release of certain constituents because of radiation damage. According to results by Vogt *et al.*,[17] contamination from the components of stainless steel in sampling blood is largely avoided by siliconing the needles; these authors claim that chromium levels between 0.1 and 0.5 mg/kg in blood serum have been found routinely.

Sampling, storage and sample preparation with special reference to biological materials have been discussed by Iyengar and Sansoni.[18,19]

Problems related to contamination in collection and storage of surface-water samples (rivers, lakes, brackish waters, open ocean) are dealt with in papers by Mart[20] and by Mart *et al.*[11] For sampling waters and liquids that can be drawn from taps, flushing has been shown to be efficient for removing accumulated corrosion products in pipes, *e.g.*, in the recent determination of nickel in the author's laboratory,[21] but this approach is not practicable when the volume withdrawn has to be kept small.

Laboratory ware

Materials used in the production of high-quality laboratory ware include polyethylene, polypropylene, Teflon, glass and silica. With appropriate treatment and handling in general they meet the standards of purity required for trace analytical work. Cleaning procedures for plastic ware used for storing water samples have been the subject of comprehensive investigations by Moody and Lindstrom[22] and very recently by Laxen and Harrison.[23] It is remarkable that among 13 methods recommended in the literature for analysis of surface waters, the latter authors found the simplest was the best suited for preliminary and routine cleaning, namely, soaking the plastic for 48 hr in dilute nitric acid, followed by thorough rinsing with purified water. Their observations can be extrapolated to cleaning plastic ware for other applications.

In all types of plastic less-expected contaminants sometimes occur as a result of particularities in the production process affecting certain batches. Salmela and Vuori[24] report the appearance of significant amounts of cadmium in solutions after cleaning Eppendorf pipette tips; presumably the source was the yellow pigment in the dye. Teflon has been found to be contaminated with stainless-steel particles embedded in the material during the manufacturing process.[25] Iron, nickel, chromium and molybdenum were leached out even after extended use. Lead was released from other products and in a certain batch of tubing used for making irradiation ampoules in the author's laboratory, mercury was found corresponding to 1 ng/cm^2; at the concentration in the sample (1 μg/kg) this could clearly give rise to an error of several hundred per cent. These examples emphasize the need for monitoring all plastic ware involved in an analysis, when very low levels of particular constituents are to be determined.

Table 2. Trace elements in laboratory ware

Material	Concentration range, *mg/kg*			
	100	10–0.1	0.1–0.01	0.01–0.001
Polyethylene and polypropylene	Na, Zn, Ca, (Al, Ti)*	K, Br, Fe, Pb, Cl, Si, Sr	Mn, Al, Sn, Se, I	Cu, Sb, Co, Hg
Poly(vinyl chloride)	Na, Sn†, Al, Ca	Br, Pb, Sn, Cd, Zn, Mg	As, Sb	—
Teflon	K, Na	Cl, Na, Al, W	Fe, Cu, Mn, Cr, Ni	Cs, Co
Polycarbonate	Cl, Br	Al, Fe	Co, Cr, Cu, Mn, Ni, Pb	—
Glass	Al, K, Mg, Mn, Sr	Fe, Pb, B, Zn, Cu, Rb, Ti, Ga (Cr, Zn)§	Sb, Rb, La, Au (As, Co)§	Sc, Tl, U, Y (In)§
Silica	—	(Cl)‡, Fe, K	Br, Ni, Cu, Sb, Cr	Sb, Se, Th, Mo, Cd, Mn, Co, As, Cs, Ag

*Al and Ti high in low-pressure polyethylene (used as catalysts).
†Heavy metal compounds used alternatively as stabilizers in certain types of PVC.
§Not certified elements in the NBS reference material 617; determined in this laboratory.
‡Chlorine high only in synthetic quartz.
Data compiled from references 19, 22 and 51.

Typical glasses used for manufacturing reagent bottles, beakers and flasks contain most trace elements in concentrations close to or above 1 mg/kg. On proper treatment in acid media glass usually improves, as trace constituents are leached out to leave a surface which is essentially silica. Experience with the determination of lead by dithizone illustrates that repeated applications of the reagent itself eliminate impurities from the glass surface. Blanks decrease and finally disappear after a series of samples have been treated in the same set of extraction funnels. Occasionally iron presents problems in working with glass, and Smith observed contamination by cadmium from glass ampoules used for decomposition under more severe conditions.[26] Tables 2 and 3 summarize data on impurities in materials used for making laboratory ware, and results on leaching experiments, respectively.

With silica apparatus, contamination problems are practically non-existent in the determination of most elements. Antimony appears to be a specific contaminant, but the degree to which it is leached out is measurable only by the activation technique, which possesses high sensitivity for this element.

The materials used for laboratory ware exhibit some adsorptive or exchange properties and they swell to some degree in organic solvents or reagents; this change in the fine structure of the surface makes the surface area much larger than would be expected from the dimensions of the container. It is therefore understood that flasks, reagent bottles, dishes or pipettes for handling samples and low-level reference solutions must never be used for transferring or processing stock solutions and concentrated reagents.

For protecting samples from contamination, flow-injection systems offer major advantages. Once dissolved, the sample and reagents are isolated from the laboratory environment; the only containers needed are reagent bottles and tubing, and these are continuously flushed. By injection of standards, the analytical performance is under constant control. The range of practical applications of the flow-injection technique is still rather limited, however, and the concentration levels covered are in the upper and intermediate part of the trace domain. Recently published methods for lead and cadmium with dithizone[27] and particularly the catalytic method for iodine in foods,[28] indicate the potential of this technique for routine analyses at the trace level.

Reagents and filtering media

Compared to other sources of contamination, contributions from reagents can be quantitatively measured (provided that the concentrations of impurities are known and the amount or volume added are recorded). Analysing reagents, however, adds much to the time and effort required and is only rarely justified.

An analysis normally involves the dissolution or the decomposition of the sample with either a solvent or reagent in a large excess. Residual concentrations of representative heavy metals in water (Cu, Fe, Mn, Pb, Zn) purified by various methods (including demineralization, distillation and adsorption, and combinations thereof) range from a few to a few hundred ng/kg. The differences in the data reflect the variability in materials (glass, quartz, polypropylene) and methods used, but the achievable purity appears to depend even more on parameters such as the period of storage and the degree to which a steady state has been reached in the operation of the unit. Since the concentrations of heavy metals in most commercial reagents of ultrapure quality are typically two or three orders of magnitude higher, water will not normally contribute significant amounts of any impurity if properly prepared and stored. It is perhaps worth mentioning that water purified in conventional

Table 3. Trace elements leached by nitric acid and hydrochloric acid from plastic containers after one week of contact (in ng/cm²)

Material \ Amount	6*M* HCl			9*M* HNO_3		
	10	10–1	1	10	10–1	1
Polyethylene (HP)	Na, Pb, Al	Tl, Cr, Zn	Ca, Sn, Cu, K, Mg, Ba, Ni, Cr, Cd, Sr, Se	Ca	Na, Fe, Se, Cu	Cr, Mg, Pb
(LP)	Ca	Zn, Na, Al, Fe, Cu, K, Ba	Sn, Co, Ni, Pb, Mg, Se, Cd, Sr	Na	Zn, Fe, K, Pb Ni, Sn, Sr	Mg, Ca, Cu, Se, Cr, Cd, Te, Ag, Ba
Polycarbonate	Fe, Ca, Sn, Pb	Na, Cu, Cr, Cd, Ba, Al	Mg, Te, Se, Ni, Sr	—	Al, Na, Fe, Ca, K	Cu, Zn, Ni, Se, Cr, Cd, Pb
Teflon	Fe	Cu, Zn, Cr, Al, Ba, Ca, Na, Pb, Te, K, Mg, Sn, Sr	Cd, Se, Ni	Ca, Fe	Mg, Al, Na, Zn, Pb, Ba, Cu, Ni, K, Sn	Cr, Te, Cd, Sr, Se

Elements are ranked in order of decreasing amounts introduced into the acid.
Data are compiled from reference 22. In the absence of a unified methodology, data in the literature cannot be compared, owing to variable and arbitrarily chosen parameters, (volumes, concentrations of acids, periods of contact).

Table 4. Trace elements in ultrapure acids (μg/l.) and reagents (μg/kg) at the given concentration ranges

Range	HCl	HF	HNO_3	H_2SO_4	$HClO_4$
1	Al, Si, S	B, Si, P, S	Si, S, K	Ca, Co, Cu, K, Mg, Na, (Se)	Cr, Fe, Na
1–0.1	Na, Mg, P, Ca, Fe	Na, Al, Ti, Ca, K, Fe, Cu, Zn	Al, Ti, Fe, Na, P, Mg, Ca, B, Cu, Cr	Mn, Ni, Sn, Sr, Tl	Ni, Sn, Br, K, Pb, Tl, Zn
0.1–0.01	B, Ti, V, Cu, Zn, Sn, Ba	Cr, Mn, Co, Zr, Cd, Pb	Zn, Ni, Ba, Pb, Cd		Cd, Sr
0.01	Mn, Co, Cr, Ni, Zr, Cd, Pb	Cd, Ba	Mn, Co, V	—	—

Upper limits for commercial ultrapure reagents range between 50 and 500 μg/l. for Ba, Cl and Na, and between 5 and 20 μg/l. for metals such as Cu, Fe, Mn and Pb. On extended storage, concentrations of most elements increase by a factor of up to 10 (*e.g.*, Fe). In solid reagents, *e.g.*, Na_2CO_3, KCl and NaOH, levels are slightly higher than for acids. Data compiled from references 19 and 25.

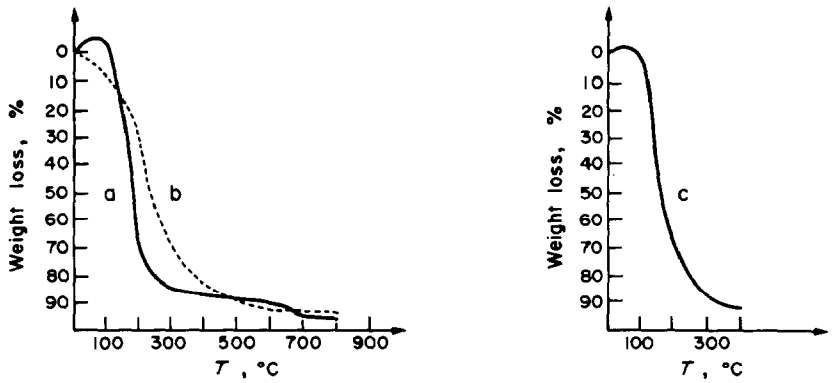

Fig. 1. Thermogravimetric curves: a,b—weight-loss of a grass sample (0.5 g) on heating in NO_2 (full line) and in air (dashed line), with temperature; c—ashing of a plant sample at constant temperature (300°C) as a function of time.

laboratories is not significantly different in quality from water produced in a clean-air area.

In total, nearly a hundred ultrapure reagents are available on the market. The extent to which concentrations of contaminants are reduced varies within wide limits and leaves much to be desired with respect to sodium, chlorine, potassium, phosphorus, silicon, sulphur, calcium and barium. Typical concentrations of copper, zinc and iron in commercial ultrapure acids, ammonia and organic solvents (which can be distilled) range between 5 and 50 μg/l. The same applies to ammonium salts and organic reagents which can be synthesized in the vapour phase or sublimed. Table 4 summarizes available data for mineral acids. Among solids, potassium chloride, and sodium carbonate and hydroxide can be purchased in comparable quality. In general, however, operations involving large amounts of solids (*e.g.*, fusion) are best avoided.

From results by Dabeka *et al.*[25] it follows that by distillation, either from quartz or polypropylene, mineral acids can be purified to a much higher degree. By use of a propylene sub-boiling still, the concentration of iron and zinc (the most persistent metals) was lowered in hydrochloric and hydrofluoric acids by a factor of 100–1000 compared to commercial acids of ultrapure grade. Considering the price of these acids it appears a sound investment to prepare some reagents, particularly mineral acids, in the laboratory.

Clean mineralization of organic samples and biological materials is feasible by ashing or by ignition in oxygen. To avoid losses a closed system is preferable; a substantial excess of gas, however, is required to prevent formation of tarry substances and deposition of carbonaceous materials. Therefore the advantage from the purity of the reagent is offset by the need for an ignition assembly with a large surface area. In our own experiments on determination of iodine and selenium by activation analysis, oxygen ignition proved very efficient,[29,30] but in determination of selenium by gas chromatography, the subsequent dissolution of the ash resulted in high blanks. On the other hand, the ignition can be done dynamically in a tube. Heng Bin Han *et al.*[31] recently reported the determination of selenium in rocks and soils at the trace levels by use of a dynamic system in a silica apparatus, as developed by Tölg.[32] Material losses to the gas stream may occur with some elements, according to experience in the author's laboratory.

A major drawback of direct ignition systems is the high temperature, at which some trace elements tend to form alloys or refractory compounds with the support material. Subsequently they do not dissolve to give a true ionic solution but remain in the form of non-reactive microaggregates and colloids. Recently, we obtained encouraging results in experiments where biological materials were heated in a slow stream of nitrogen dioxide.[33] Complete ashing was achieved in 2–3 hr at temperatures between 200 and 300°, at which the degree of dissociation of NO_2 increases from 4 to 16%.[34] The ashing effect is attributed to the oxygen formed. The diagrams in Fig. 1 show comparative thermogravimetric curves for the same material ashed in nitrogen dioxide and in air and a curve showing the rate of loss. The ignition system is shown in Fig. 2.

Ashing at low temperatures is important in techniques such as spark-source mass-spectrometry[13] since losses by volatilization are minimized or avoided, trace elements concentrated, and the risk of contamination greatly reduced, by making the electrodes directly from the solid residue.

Filtering media. In most instances the heavy metal content of filters is not negligible, but the risk of contaminating the sample by washing or leaching out the impurities is nevertheless low. Eventually, however, filters may become an integral part of the sample in the subsequent treatment or measurement. In such cases (*e.g.*, analysis of particulates, preconcentration of trace constituents on ion-exchange filter papers as in X-ray fluorescence analysis, ignition of carrier precipitates *etc.*) it is essential to know the concentrations of the relevant contaminants.

The high results obtained for selenium by gas chromatography, mentioned in the last column, were traced back to contamination from the filter paper (Schleicher & Schüll 598) used as the sample holder in the Schöniger combustion technique. Results from subsequent analyses of a set of conventional and membrane filters (Sartorius types 11106 and

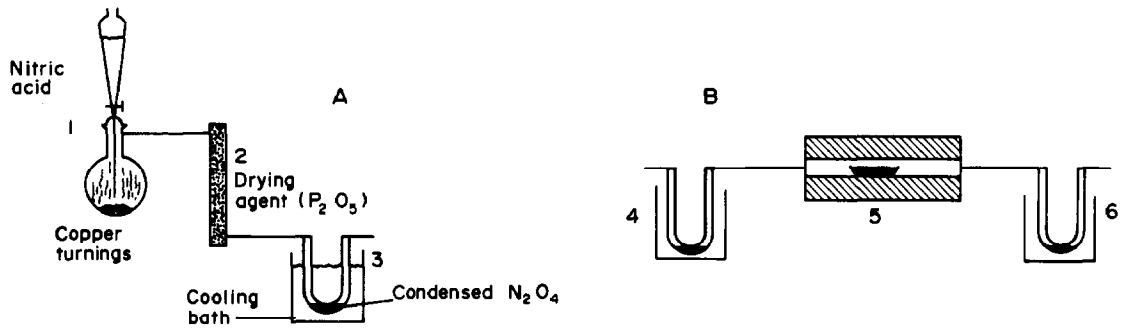

Fig. 2. A—preparation of N_2O_4 (1, reaction system; 2, drying column; 3, condensation trap). B—ignition system (4 and 6, traps; 5, ring-oven with the sample). It is noteworthy that tarry material or carbon does not form or deposit in the ignition tube or in trap 6.

11306) revealed variable concentrations of copper (0.3–3.5 ng/cm^2), manganese (0.4–20 ng/cm^2), mercury (0.1–0.6 ng/cm^2) and zinc (18–41 ng/cm^2). These levels approximately parallel those reported by other authors[35–38] for cellulose-ester and polycarbonate membrane filters. Similar figures apply for chromium, nickel and lead. Chatt and Kulathilake[35] analysed polycarbonate (Nucleopore) filters which they used for collecting microsamples of oceanic particulate matter. They determined a number of elements by photon-activation analysis. Using the same technique, Kato *et al.*[36] determined 18 elements in membrane filters based on cellulose esters (Millipore). According to their results, major contaminants such as chloride and sodium may exceed 1 μg/cm^2 levels, and calcium, potassium and magnesium occur in concentrations of several 100 ng/cm^2.

Chemically modified cellulose filters have a great tendency to attract particulate matter, owing to the static charge induced by handling. Some types have non-ionic detergents added as wetting agents. These may contain ammine salts and interfere with the determination of ammonia. Some additives absorb in the ultraviolet region and can affect results for nitrate.

Kingston and Pella[39] report preconcentration of heavy metals in sea-water on filter papers loaded with ion-exchanger before their determination by X-ray spectrometry. The absolute amounts of zinc and copper collected were around 1 μg, in which case contamination could be allowed for but the iron blanks were too variable to make the determination of iron possible.

Growing interest in determining heavy metal species associated with subcellular fractions of biological material requires data on contamination of the reagents used in gel filtration, ultrafiltration and ultracentrifugation. The amounts of reagents needed in some of these operations are large relative to the volume of the sample. Thus, the significant amounts of heavy metals found by Pietra *et al.*[15] in acrylamide gel, sephacryl and some buffers represent a major limitation.

Systems and techniques have recently been described which permit the removal of metallic contaminants from concentrated solutions of alkali-metal and transition-metal salts by use of a modified cellulose exchanger with selective chelating groups such as salicylic acid or 4-(pyridyl-2-azo)resorcinol attached. They have proved useful in lowering the contamination by elements such as copper, iron and zinc by orders of magnitude.[40–42]

In general, however, purification of solid, non-volatile reagents is still an open problem, since simple systems for removing a wide range of impurities simultaneously, without introducing other foreign material, would be needed.

Conclusions

The quality and selection of materials, and the facilities available, have considerably improved in recent years, thus minimizing contamination in analytical laboratories. With simple means and measures they make it possible to adapt conventional laboratories for trace analytical work within most of the practicable range.

Amounts of impurities introduced by reagents in the course of an analysis are measurable and constant for a given analytical procedure; other contributions are variable, but to ensure acceptable blanks, they have to be made low and constant. This is practicable only for absolute amounts of impurities of the order of a few ng, owing to limitations dictated by materials (containers, reagents, tools) in contact with the sample in the course of the analysis.

As to methods, the risk of contaminating the sample increases with their complexity. A corollary of the dependence on materials is the requirement for simple and direct methods involving only a few easily purifiable reagents and scaling down the system relative to the sample.

Contamination becomes the predominant limitation in all techniques, with the exception of activation analysis and tracer applications, at concentrations between tenths and hundredths of μg/kg, depending on the element and matrix. Compared with the performance of other multielement techniques applicable

directly (without preconcentration) to the lowest concentration range, spark-source mass-spectrometry is superior because it covers nearly all elements, and hence is currently widely used in tracing sources and levels of contamination. ICP emission spectrometry is highly sensitive, but there is a lack of data on its application to real complex samples. Atomic-absorption spectrometry, isotope-dilution mass-spectrometry and the newer voltammetric techniques have a limited coverage but proven performance in experienced laboratories. Certain developments, however, are in a less favourable direction: instead of reduction of other parameters relative to the sample size, the sample fraction measured is made smaller; to provide for loss of sensitivity, elaborate concentration procedures are sometimes introduced. They make these techniques more dependent on skill and expertise, and more prone to contamination. This is in agreement with the evidence from interlaboratory data, which suggests that in general the quality of trace analytical results has not improved in parallel with the advances in instrumentation and automation. Since contamination does not necessarily affect the precision of data, its importance has been underrated, but recently there has been a growing appreciation of the relevance of parameters other than the final measurement, including the requirement for identifying sources of blanks.

Control over contamination therefore rests more strongly on good laboratory practice and on the competence of the analyst than on facilities and instrumentation. At levels of around 1 ng or less, requirements for determining elements which are persistent contaminants necessitate an integrated analytical approach exploiting the specific potentials of individual techniques; economic factors may then become a restriction.

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PURIFICATION OF ANALYTICAL REAGENTS

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Summary—Considerable progress in the purification of reagent chemicals has been made during the past decade. Improved or newly developed methods and their application for producing analytical reagents of highest purity are described. Various techniques are evaluated critically with respect to convenience and efficiency of use in the trace analysis laboratory.

In modern ultratrace analysis rigid demands are imposed on the degree of purity required for the water, acids, bases, solvents, buffers, supporting electrolytes, fluxes, oxidants and reductants, chelating agents, and other reagent chemicals used in analytical work. Some of these needs for pure reagents have been met by commercial suppliers who have recently focused their attention on this problem. Because specific attention has been given to the proper handling, containing, analysis, and storage of reagents, levels of many trace elemental impurities in commercial lots of chemicals have been reduced considerably. Even though an overall improvement in the quality of several suppliers' chemicals has been accomplished, for most ultratrace analyses the analyst must still verify that the purity of any purchased reagent is sufficient for the intended analytical application. Reagent impurities capable of interfering with the analytical measurement should be so low that they give a blank value that is less than 10% of the analyte level. Although reagents sufficiently pure for many applications are available commercially, the analytical laboratory committed to ultratrace determinations of a variety of elements must also be able to produce a wide range of pure reagent chemicals itself. For many analytical problems the level of a specific contaminant of interest can be adequately controlled only by designing a special laboratory purification method. The basic analytical reagents can now be ultrapurified on the laboratory scale with relative ease by a number of techniques either introduced or significantly improved during the past decade.

Table 1 lists some of the techniques most widely used by analysts for laboratory-scale purification of reagents. By using one of these techniques or several in combination, analytical chemists have made significant advances in recent years. Discussion of the currently used techniques is provided in this report but the copious and critical details of the execution of various procedures to attain extraordinarily high degrees of purity for processed reagents are not included. It is presumed that the trace-element analytical scientist is aware that the purification apparatus should preferably be housed in an analytical cleanroom and has assimilated a basic knowledge of contamination-control techniques for trace analysis. These issues have been discussed previously[1,2] and are also reported in other papers in this "paper symposium".

Success in ultrapurification work depends critically on analytical techniques that establish the efficiency of the purification process and provide determinations of the purity level of the processed reagent. It is vital that the level of purity attained is verified by an unequivocally reliable and preferably direct quantitative analysis, which will usually be more difficult to execute reliably than the purification procedure. Detailed discussion of the excruciatingly careful procedures required in the reliable quantitative analysis of ultrapure reagents is beyond the scope of this report and is thus omitted. Analytical methods proven to be valuable for either monitoring the efficiency of purification processes or for characterizing the final product are mentioned frequently, however, and where possible, representative data are reported.

PURIFICATION METHODS

Ion-exchange

The primary use of the ion-exchange method in the analytical laboratory continues to be the demineralization of water. For this application it is accepted universally as one of the most effective means of removing dissolved ionic species from water.

Since ultrapure water is the most abundantly used analytical reagent and its purification by ion-

Table 1. Ultrapurification methods

Ion-exchange chromatography
Mercury-cathode electrolysis
Sub-boiling distillation
Isopiestic distillation
Low-temperature sublimation
Solvent extraction
Zone refining
Direct synthesis
Gas, liquid and column chromatography

Table 2. Levels (ng/ml) of Cu, Pb and Cd in water purified by different methods[3]

Element	Centrally demineralized	Demineralized and distilled in Pyrex	Doubly distilled in quartz	Distilled and Milli-Q purified
Cd	0.1–0.15	0.08–0.1	0.02–0.06	≤0.01
Pb	0.4–0.8	0.3–0.5	0.1–0.3	0.05–0.1
Cu	1.0–1.5	1.0–1.5	0.3–0.5	0.1–0.3

exchange is a perfectly straightforward application, each trace-analysis laboratory should easily have available a copious supply of this pure product. For most trace-metal analytical work demineralization of water in a mixed-bed ion-exchange column, followed by filtering and double distillation, in an all-quartz system, produces a satisfactory grade of water.[2] Prefiltering of distilled water through a charcoal cartridge followed by ion-exchange and filtering through μm pore-size Teflon filters is a suitable and more convenient alternative, since storage is obviated. In this case ion-exchange combined appropriately with prefiltering through charcoal or subsequent high-temperature pyrolysis is used to remove both trace metals and traces of organic impurities as well.

Recently Oehme and Lund[3] made comparisons of the quality of water prepared by (1) a central demineralization system, (2) distillation in a standard Pyrex still, (3) double distillation of tap water in an all-quartz still, and (4) a Milli-Q water system (Millipore Corporation). The water samples were analysed for copper, lead and cadmium by differential-pulse anodic stripping voltammetry. Their results[3] are reproduced in Table 2. Details of the analytical procedure were reported elsewhere.[4] These investigators found that centrally demineralized water and the product subsequently distilled in a normal Pyrex vessel were of comparable quality with respect to the metals tested. Double distillation of tap water in a quartz apparatus gave water that was substantially purer than the products from methods (1) and (2).

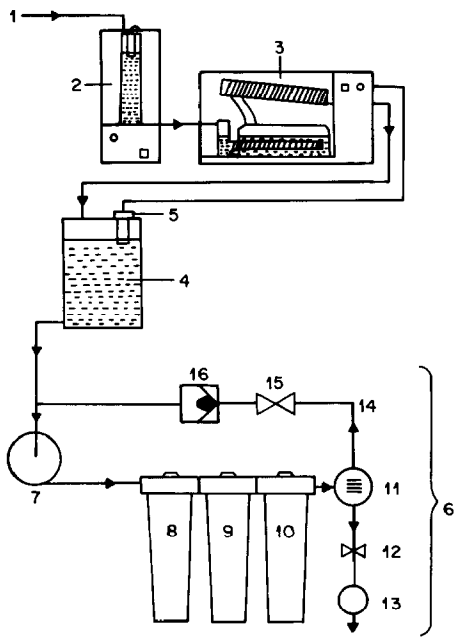

Fig. 1. Water purification system recommended by Oehme and Lund.[3] 1, Inlet for centrally demineralized water; 2, flow-controller; 3, conventional Pyrex water still; 4, polyethylene tanks as water reservoir; 5, pressure switch; 6, Millipore Milli-Q system; 7, pump; 8, activated-carbon filter cartridge; 9, 10, ion-exchange cartridges; 11, resistivity meter; 12, ball valve; 13, membrane filter; 14, recirculation path; 15, flow controller; 16, check valve.

It was stated (but not verified by analysis) that the trace organic impurity level of water produced by method (3) was also reduced. However, the relatively low production capacity of the double-distillation procedure (~2 l./hr) and interim storage of the water produced are cited as main disadvantages. The purest water with respect to the metals tested for was produced by the Milli-Q system. Oehme and Lund recommended the purification system shown schematically in Fig. 1. The feed water for the Milli-Q system was first centrally demineralized and then distilled in an ordinary still. Direct processing of centrally demineralized water through the Milli-Q system was condemned by these investigators, who experienced almost irreversible breakdown of the system, owing to suspended matter, gelatinous materials and slime from centrally demineralized water.

Mercury-cathode electrolysis

Electrolysis at the mercury cathode has been recognized for decades as being potentially extremely effective for purifying aqueous solutions of various reagents.[5] Practical use of the technique for reagent purification has increased considerably in recent years, owing to the availability of relatively inexpensive and convenient commercial apparatus optimized for treatment of several litres of reagent solutions. The ESA Model 2014P Reagent Cleaning System introduced by Environmental Sciences Associates is shown schematically in Fig. 2. The apparatus consists of a 4-litre reagent flask (usually a borosilicate glass vessel), a separate electronic console used to set the potential to values down to −1.5 V *vs.* the Ag/AgCl electrode, a reference electrode, and a counter-electrode. The nitrogen inlet passes a constant flow of inert gas through the solution to displace oxygen and

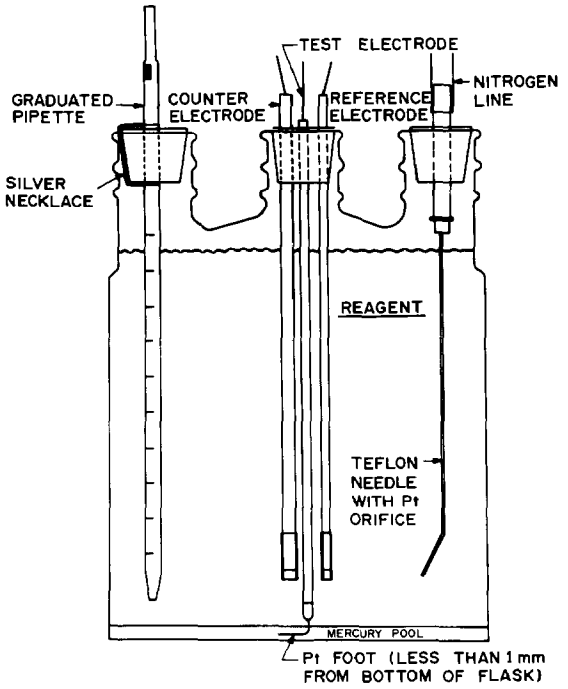

Fig. 2. Schematic diagram of mercury-cathode electrolysis apparatus.

to provide a non-contaminating method of mixing. Procedures for the use of this system and a comprehensive practical guide to reagent purification by mercury-cathode electrolysis were reported recently by Mitchell and McCrory.[6]

An improved apparatus, shown in Fig. 3, is provided by Princeton Applied Research, Princeton, New Jersey. This apparatus has the added conveniences of a levelling bulb for adjustment of the mercury volume, and stopcocks for removal of either mercury or pure reagent without opening the apparatus to the atmosphere or shutting off the applied potential. When the desired degree of purification has been obtained, the levelling bulb can be lowered so that there is no mercury in the side-arm of the solution drain. The solution can then be dispensed through the drain tube with the potentiostat still on and electrolysis maintained. In this way amalgamated impurities are prevented from being spontaneously released from the mercury pool to recontaminate the solution.

In principle, any reagent soluble in water can be purified by electrolysis at the mercury cathode, provided its component ions are not electroactive at the applied cathode potential required for reduction of the impurity ion. Electrochemical inertness at the anode potential is also required. Additionally, the reagent solution must be chemically inert with respect to reaction with mercury (including dissolution of the mercury). Solutes consisting of any combinations of the cations and anions in Table 3 can usually be purified.

Removal of electroreducible trace transition-metal cations from solutions of the salts of alkali, alkaline-earth and many rare-earth metals can be accomplished especially well. The cations listed in Table 4 can generally be effectively removed from aqueous solution by reduction to the metal and amalgamation at the mercury cathode. Electrolysis thus becomes the method of choice for the ultrapurification of various analytical buffers, fusion fluxes, masking agents, and supporting electrolytes. Sodium or potassium salts of acetic and boric acids, potassium hydrogen phthalate, mono- and dipotassium hydrogen phosphate, and the popular supporting electrolytes, sodium and potassium chloride and perchlorates can be ultrapurified. The concentrated solutions of the purified products are then used in chemical analysis procedures. The direct use of solutions eliminates the need to recover the solid product, a process plagued with problems of contamination from particulates and also from containers required for drying solids by heating.

In practice, effective electrochemical purifications can often be precluded by the chemical reactivity or excessive solubility of mercury in the reagent solution undergoing electrolysis, by simultaneous cyclic reactions of impurities at the cathode and anode, by nonformation of an amalgam, and by complexation of impurities to form difficultly reduced species.

Various techniques have been used to monitor mercury-cathode electrolysis and to establish its efficiency for removal of elemental impurities. Current–time monitoring,[5] gamma-ray spectroscopy of radioisotopes,[6] differential-pulse polarography[7] and stripping voltammetry[7] have been used to establish conditions for the purification of solutions of sodium hydroxide, sodium acetate, sodium carbonate, and components of phosphate buffer solutions.

Conditions found to be effective for impurity removal during small-scale radiotracer studies at the author's laboratory have been used on a larger scale for purification. Three litres of 1.0*M* sodium acetate solution were purified by using the apparatus shown in Fig. 2. The results reported in Table 5 demonstrate that the already reasonably pure reagents were further purified during a reasonably short electrolysis period of 40 hr. It has also been possible to prepare kg quantities of ultrapure sodium and calcium carbonates by mercury-cathode electrolysis of solutions from which these carbonates were subsequently precipitated by high-purity ammonium carbonate. More recently the mercury-cathode electrolysis of concentrated solutions of EDTA was monitored by induction-coupled plasma emission spectroscopy.[8]

To perform continuous rather than batchwise mercury-cathode purifications, Haapakka and Kankare[9] designed a flow-through cell and tested its efficiency for removing Cu, Pd, Cd and Zn from 0.1*M* sodium acetate buffer solutions. General applicability to electrolyte purification was suggested. However, the low throughput rate, 1 ml/min, of the flow-through cell offers little or no time advantage over the batchwise process, which produces up to 4 litres of purified solution within about 40 hr.

Table 3. Cations and anions purifiable by mercury-cathode electrolysis

Cations		Anions	
Al^{3+}	Na^{+}	CH_3COO^{-}	F^{-}
Ba^{2+}	Rb^{+}	NH_4^{+}	OH^{-}
Be^{2+}	Sc^{3+}	$HCOO^{-}$	PO_4^{-}
Ca^{2+}	Sr^{2+}	CCl_3COO^{-}	SO_4^{2-}
K^{+}	Y^{3+}	ClO_4^{-}	Tartrate
Li^{+}	La^{3+}	Citrate	
Mg^{2+}			

Table 4. Cations reduced and amalgamated at the mercury cathode

Ag^{+}	Co^{2+}	In^{3+}	Sn^{2+}
Au^{+}	Cr^{3+}	Ir^{3+}	Te^{+}
Au^{3+}	Cu^{2+}	Mn^{2+}	TiO^{2+}
BiO^{+}	Fe^{2+}	Ni^{2+}	VO^{2+}
Cd^{2+}	Fe^{3+}	Pb^{2+}	Zn^{2+}
Ce^{3+}	Ga^{3+}	Pd^{2+}	ZrO^{2+}

Sub-boiling distillation

The best method available for preparing concentrated, high-purity, high-boiling acids (nitric, sulphuric, hydrochloric, perchloric) was reported in 1972. The purification is based on quiescent evaporation of the liquid by infrared heating at the surface to prevent violent boiling. This technique, described in detail by investigators at the National Bureau of Standards, is commonly referred to as sub-boiling distillation.[10] A schematic diagram of the apparatus is provided in Fig. 4. Acids of extremely high purity are produced by multiple batchwise distillation of reagent-grade acids in the vitreous silica apparatus, which is placed in a laminar-flow hood. Hydrofluoric acid has been distilled in a still fabricated from polytetrafluoroethylene and heated by electric resistance heaters sealed inside a glass tube [inserted into a Teflon (TFE) rod].[11]

The efficiency of this process for removal of dissolved trace metals results from the preclusion of the formation of fine particles of spray or droplets of the originally impure liquid, which could otherwise be swept through the apparatus and contaminate the distillate. A high-purity product is thus afforded simply by the non-violent surface evaporation of the liquid. In this way any liquid reagent is vaporized completely before transport into the condenser.

Maintaining the purity of the distilled product requires careful execution of the entire process under controlled contamination conditions. Exhaustive cleaning of Teflon (FEP) bottles for containing the purified acids and storage at $-30°$ are required in order to retard contamination during storage. Under proper conditions, acids with residual impurities at the level of only fractions of 1 ng/g have been pro-

Table 5. Purification efficiency of Hg-cathode electrolysis

Reagent	pH	Electrolysis period, *hr*	Conc. of cation in solution,* *ng/ml*				
			Cr	Mn	Fe	Zn	Co
1.0*M* Sodium acetate	6.9	40	(7, <2)	(1, 1)	(30, <2)	(11, 4)	(<2, <2)
1.0*M* Sodium carbonate	11.1	40	(8, <2)	(7, <2)	(1, 1)	(1, 1)	(30, <2)

*Solution analysed by flameless AAS before and after electrolysis at -1.7 V (before, after).

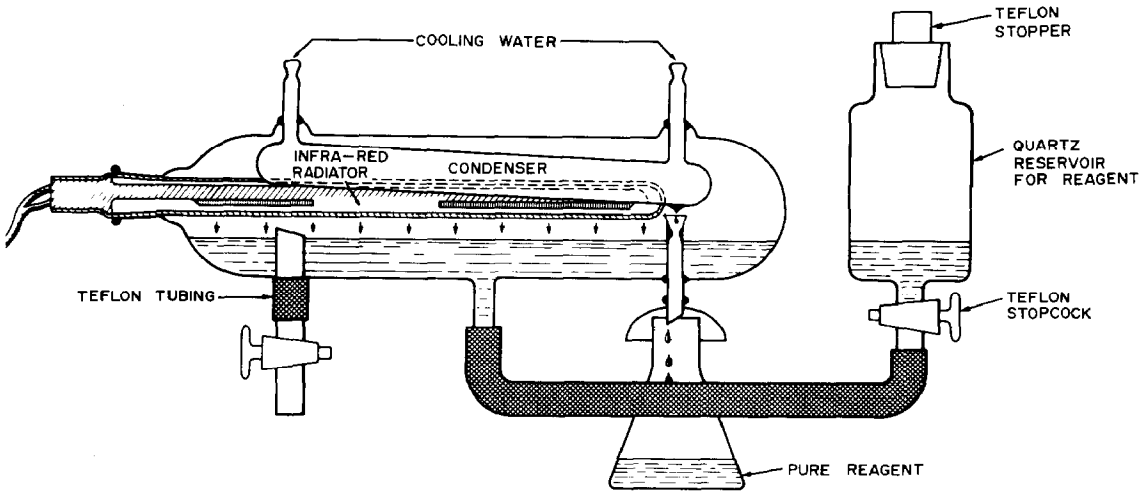

Fig. 4. Schematic diagram of sub-boiling distillation apparatus.

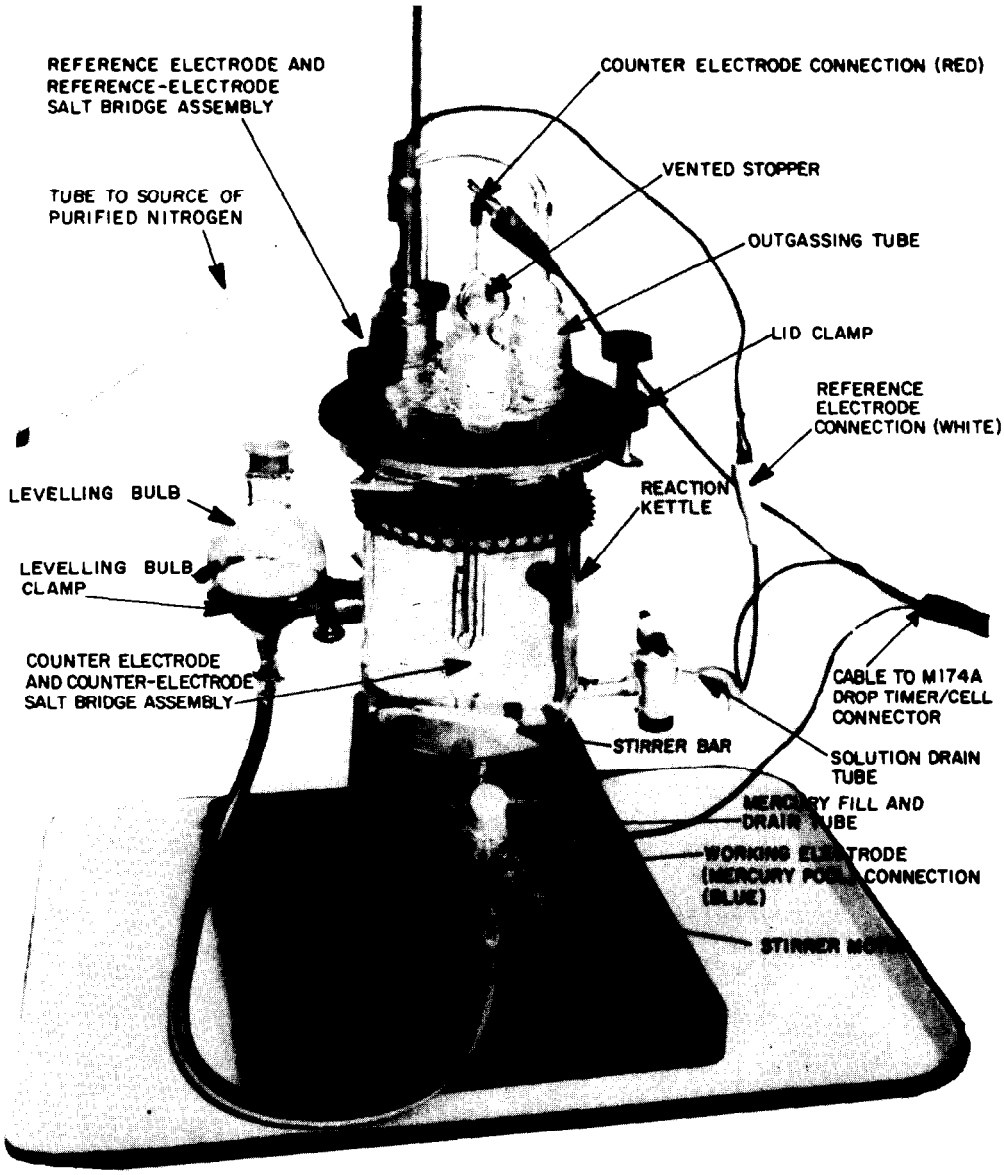

Fig. 3. Picture of Model 9500 electrolyte purification apparatus.

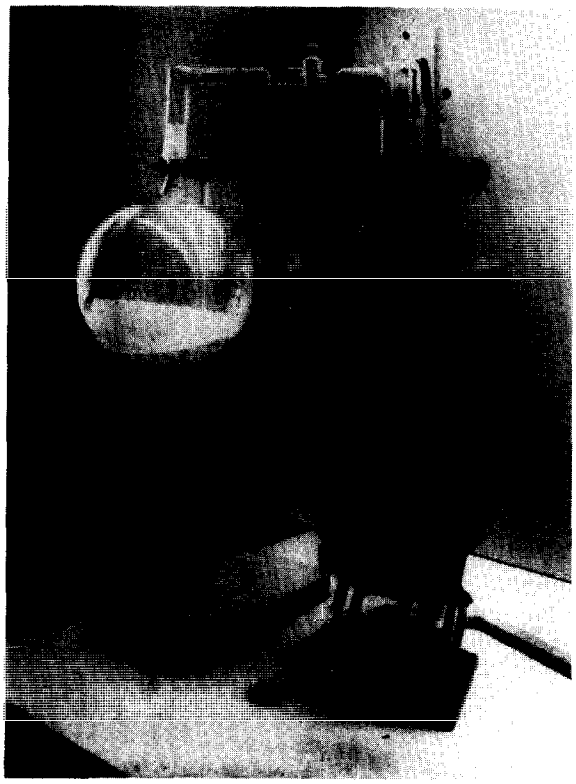

Fig. 5. Low-temperature sublimation apparatus for purification of liquid reagent chemicals.

Table 6. Impurity concentrations (*ng/g*) in sub-boiling distilled hydrochloric acid analysed by stable-isotope dilution/mass spectrometry[10]

Element	*ng/g*	Element	*ng/g*
Pb	0.07	Zn	0.2
Tl	0.01	Cu	0.1
Ba	0.04	Ni	0.2
Te	0.01	Fe	3.0
Sn	0.05	Cr	0.3
In	0.01	Ca	0.06
Cd	0.02	K	0.5
Ag	0.03	Mg	0.6
Sn	0.01	Na	1.0

duced (they were analysed by stable-isotope dilution mass spectrometry).[10] Data representative of the impurity level of various sub-boiling distilled acids are given in Table 6 for hydrochloric acid.

Isopiestic distillation

Isothermal (also called isopiestic) distillation[12,13] can produce volatile acids of medium concentration in high-purity form. Hydrochloric, hydrobromic, acetic and hydrofluoric acids can be produced. The pure acids are generated from reagent-grade material by placing an open container of concentrated reagent-grade acid adjacent to a container of pure water, within a closed system (such as a large desiccator). Acid vapours are continuously transferred into the pure water until equilibrium is obtained. This isopiestic distillation is the room-temperature version of the sub-boiling method (which it preceded) and produces a highly pure but medium strength product. Ammonia solution can also be produced, and preparation of 30% hydrofluoric acid (in 4 days), 6*M* hydrochloric acid (in 3 days) and 4.5*M* ammonia solution is routinely performed in our laboratory.

High-purity water can also be charged with pure hydrogen chloride, hydrogen bromide or hydrogen fluoride gas to prepare acids of any desired concentration. Standard apparatus constructed of polyfluorocarbon vessels has been reported for preparation of acids.[2] An essential component is a 0.45-μm Teflon filter at the outlet of the cylinder, to remove particulate matter. Preparation of hydrofluoric acid containing only 0.08 ng of lead per g has been described by Tatsumato.[14]

Low-temperature sublimation

With the exception of the lyophilization of water (freeze-drying) to obtain dry products, the potential of the sublimation technique for purifying reagents existing as liquids under ambient conditions was not recognized before the reports by Mitchell.[15] When confronted with the problem of ultrapurification of several extremely reactive, hygroscopic, corrosive, and toxic analytical reagents and research chemicals, Mitchell undertook a systematic investigation of the sublimation of liquids at temperatures and pressures below the triple point. Laboratory tests of a broad range of liquids showed that when frozen those in Tables 7 and 8 sublime easily, and in many cases as efficiently as the liquids can be distilled.

Table 7. Analytical reagents and solvents sublimable at low temperature

	M.P., °C	B.P., °C
Acetic acid (99%)	16.6	118.5
Ammonia (15%)	—	—
Benzene	5.5	80
Bromoform	8.3	149.5
Carbon tetrachloride	−23	76.8
Cyclohexane	6.5	80.7
Dioxan	11.8	101
Formic acid	8.4	110.7
Hydrazine (95%)	1.4	113.5
Hydrogen peroxide (15%)	0.5	150
Water	0	100

The primary practical advantages of low-temperature sublimation in comparison to distillation are all due to the much lower temperatures that are used. Major advantages include (1) significant reduction in problems of contamination by the container because of the greatly reduced chemical reactivity of liquids at low temperature, and (2) extraordinarily high decontamination factors for removal of dissolved ionic species from solution. Where applicable, such species can be captured on ion-exchange resins or complexed with chelating agents. The pure reagent or solvent can then be readily sublimed, leaving the impurities behind. Another practical and very important advantage is derived from the fact that any solid particulates originally present in chemically reactive or corrosive liquids remain undissolved during low-temperature sublimation, whereas they could dissolve under the conditions of distillation. A growing body of evidence has been reported from this laboratory, showing that a significant percentage of the total metallic impurity content of liquids and solids is due to impurities present in the particulate matter contaminating the reagent.[16–18]

Perhaps the most significant advantage of low-temperature sublimation is its simplicity. The apparatus shown in Fig. 5 is made of fused silica and Teflon. The reagent to be purified is frozen as a thin shell on the interior of the round-bottomed flask by rotating it in a suitable cooling bath. The flask is then attached

Table 8. Some inorganic halides sublimable at low temperatures by modified chemical vapour deposition

	M.P., °C	B.P., °C
Antimony pentachloride	2.8	79
Arsenic trifluoride	−8.5	—
Arsenic trichloride	−8.5	130.2
Bromine trifluoride	−2(8.8)	—
Phosphorus oxychloride	2.0	105.3
Silicon tetrabromide	5.4	154
Tungsten hexafluoride	2.5	17.5

to the remainder of the assembled apparatus, which is located in an all-plastic exhaust hood in a clean-room. The assembled vessel is then evacuated continuously with a mechanical pump which maintains an equilibrium pressure of a few mmHg. Under the continuous evacuation there is a mass-flow of vapour from the round-bottomed flask into the cylindrical vessel (submerged in a cooling bath) where the sublimed product is isolated. This self-contained reagent purification system can be easily fabricated in a modest glass-blowing shop and operated batchwise as easily as an ordinary distillation apparatus.

The efficiency of the method for the purification of hydrogen peroxide was determined experimentally by processing the 15% reagent doped with 1000 ng/ml of each of the impurities Co, Cr, Cu, Fe, Mn, and Ni.[19] X-Ray fluorescence analysis of the sublimed sample (8.5% H_2O_2), with use of a high-sensitivity co-precipitation method, showed all impurities to be below the detection limit of 0.1 μg/ml. A sample with the same carrier level of impurities (1000 ng/ml) was therefore doped with the radioisotopes, ^{51}Cr, ^{60}Co, ^{59}Fe, ^{54}Mn and ^{65}Zn and was found to contain these metals at less than 1-ng/ml level after a single sublimation. Direct analysis of another sample by neutron activation showed decontamination factors of 1100, 2700, and 320 for Mn, Co and As, respectively.

The exceptional purity and convenient processing available with this technique have been further demonstrated by treatment of aqueous hydrazine. Analytical data showing the reagent to be a high-purity and generally applicable reducing agent have been reported.[20] Analytical results obtained for analysis of several chemicals after purification by a single sublimation are reported in Table 9. This method is indeed a powerful purification tool for the trace analyst. As indicated, excellent purity is attained for hydrogen peroxide and hydrazine, two extremely useful analytical reagents for adjustment of oxidation states in trace analysis.

Purification of certain solids by sublimation under vacuum and at elevated temperature continues to be an extremely effective practical approach to the purification of various reagents. Free-flowing pure crystalline products are often generated by subliming solid chunks of reagent-grade material or a product prepurified by zone melting. The preparation of pure phosphoric acid from sublimed phosphorus pentoxide is a noteworthy analytical application. A novel low-cost procedure for the preparation of P_2O_5 in kg quantities has been reported.[21] A product was prepared which had elemental impurity levels in the ng/g range. Phosphoric acid of comparable purity was then obtained simply by dissolving the solid with a 3:1 (or higher) molar ratio of pure water.

Other techniques

Zone refining. Zone melting has not found widespread application in the trace analysis laboratory but is occasionally the method of choice for purification of selected reagents. A vertical zone melter suitable for the purification of organic and inorganic compounds melting at temperatures ranging from −10° to 300° has been designed.[22] Primary-standard benzoic acid[23] and 8-quinolinol[24] have been purified in this way. Systematic investigations of the use of zone melting to purify various metal chelates have also been conducted.[25]

Solvent extraction. Powerful broad-spectrum extractants find use in prepurification of aqueous reagent solutions for subsequent use in analysis. For example, a high-purity stock solution of dithizone in chloroform is an excellent extractant for the purification of aqueous salt solutions used as buffers or electrolytes in determination of trace elements reacting with dithizone. Reagent-grade salts of cations nonreactive with dithizone are purified by dissolution in high-purity water, filtration through 0.2-μm cellulose acetate membrane filters, and extraction two or three times in a Teflon (FEP) separatory funnel after proper adjustment of pH. After several washes of the aqueous solution with chloroform the salt solution has unusually low blanks for the elements Mn, Fe, Co, Re, Ni, Pd, Pt, Cu, Ag, Au, Zn, Ce, Hg, Ga, In, Tl, Sn, Pb, Sb, Bi, Se, Te and Pd. Aqueous solutions of alkali and alkaline-earth metal salts are especially well purified. Similarly, any reagent salts used in procedures for extractive photometry or atomic absorption can be purified first by extraction with the extractant to be used. This conventional use of extraction is a well known and often used approach to analytical purifications.[26]

Gas, liquid and column chromatography. Advances are still occurring in the design of laboratory-scale

Table 9. Results (*ng/ml*) of the analysis of singly sublimed reagents

Impurity	H_2O_2*		$POCl_3$†		NH_2NH_2†	
	Before	After	Before	After	Before	After
Co	1000	0.4	<0.4	ND	40	ND
Cr	1000	<2	28	ND	127	ND
Cu	1000	<2	ND	—	3	0.3
Fe	1000	<2	433 ± 6	15 ± 2	41	2
Mn	1000	0.9	14	ND	2	ND
Ni	1000	—	1245 ± 70	<1	13	ND

*Analysed by radioisotope techniques, reported in Ref. 18.
†Analysed by AAS, reported in Ref. 19 (ND = below detection limit).

Table 10. Fe levels* (*ng/ml*) in reagent solutions purified by chromatography on (1) coated and (2) uncoated columns of Amberlite XAD-2 co-polymer beads

Reagent Solution	Blank		10-ml sample		Standard†	
	(1)	(2)	(1)	(2)	(1)	(2)
10% $NH_2NH_2.HCl$	0.9 ± 0.1	0.6 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	1.5 ± 0.1	1.6 ± 0.1
10% CH_3COONH_4	0.7 ± 0.2	0.3 ± 0.1	0.6 ± 0.2	0.7 ± 0.1	1.1 ± 0.1	1.2 ± 0.2

*Average of four individual determinations, reported as mean ± 2σ.
†0.5 ng of Fe added to 10 ml of sample.

systems for isolating compounds by gas chromatography. However, there has not been even modest use of GC for preparatory-scale purifications in analytical laboratories, owing to several factors, including economics, convenience, and low production capacity. Scaled-up HPLC methods should, however, find applications for solving complex analytical purification problems. Useful applications for removal of by-products and other interfering trace impurities from spectrophotometric reagents can be predicted.

There have been many applications of conventional column (absorption or adsorption) chromatography for purification. Two similar approaches to the removal of ultralow levels of iron from reagent salt solutions were introduced by Schilt and Lundgren[27] and by Willis and Sangster.[28] The first method removed iron(II) from aqueous reagents by complexation with a highly selective ligand sorbed onto porous polystyrene–divinylbenzene co-polymer beads (coated column method). In the second method 1,10-phenanthroline was added to the aqueous solution containing iron impurities and the mixture was then flowed over the same type of porous polymer beads (uncoated column precomplex method).

Harris and Williams[29] recently evaluated both methods quantitatively by determination of iron in solutions of hydroxylamine hydrochloride and ammonium acetate purified by both methods. They were able to optimize conditions for the superior purification method (the precomplex procedure) because of their excellent performance of extremely precise and accurate quantitative determinations of iron at the sub-ng/ml level. Their results are shown in Table 10. These investigators concluded that (1) the precomplex procedure is a highly effective purification technique, (2) it should be readily applicable for removing iron from most non-complexing neutral salts, and (3) under proper conditions the general method can be made applicable to the removal of any cation for which a highly selective ligand exists that has sufficiently significant aromatic character for it and its metal chelate to be exclusively partitioned into the column-bead phase.

Direct synthesis. Occasionally analytical reagents can be synthesized directly under conditions that generate a solution of the ultrapure reagent. Ammonium 1-pyrrolidinecarbodithioate, an important extractant for preconcentrating trace elements for determination by atomic-absorption spectroscopy, is a typical example. We have synthesized this reagent in pure form from fractionally distilled pyrrolidine, carbon disulphide, and isopiestically distilled ammonia. The free acid has a much greater solubility in chloroform than any corresponding salt. The acid can be prepared conveniently by dissolving the purified reactants in chloroform.[30] When a few gaseous or liquid starting reagents can be easily prepurified and subsequently reacted to produce a desired product, direct synthesis under conditions of controlled contamination is a viable laboratory ultrapurification method.

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PURIFIED REAGENTS FOR TRACE METAL ANALYSIS

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Summary—Sub-boiling distillations have become a standard tool for the reduction of the inorganic analytical blank. More than 10 years of practical experience in the production of reagent acids is reviewed and a description is given of a new laboratory especially designed to permit trouble-free operation as well as ensure the continued high quality of the reagents produced.

In the present-day real world of trace element analyses, the theoretical or even practical sensitivity limit of the instrument is very often not the limiting factor in the accuracy of an analysis. Instead, the size and variability of the analytical blank is the principal limitation. Many techniques for improving analytical accuracy are concerned with the reduction and control of this blank through all steps of the process, from choosing containers, right through to the final analytical measurement. While many of the important individual steps in attempting to control the blank have been recognized and even practised for many years, it is the purpose of this paper to review the present state-of-the-art in contamination control in trace and ultratrace analytical chemistry with particular emphasis on the production of purified reagents.

As shown by Murphy,[1] sample-contamination may be caused by the method of handling or containment, by apparatus, by the use of analytical reagents, by the environment in which the sampling or analysis is performed, and even by the presence and influence of the chemist. By its very nature, trace or ultratrace analysis may be significantly influenced by all of these sources of contamination. The specific means used to reduce the analytical blank may vary from element to element but the use of clean-air Class 100 laboratories[2] and purified reagents has become a common denominator for present day analysis. A particularly useful compilation relevant to the use of apparatus in contamination control has been published by Zief and Mitchell.[3]

In general, all other factors being equal, there is rarely a trace analytical scheme which could not be improved by the use of better analytical reagents. Commercial high-purity acids may be better than reagent-grade acids but they are not sufficiently pure for the most demanding work.[1] The method of sub-boiling (or non-ebullient) distillation has been employed at NBS for more than 11 years in the production of more than 2800 litres of purified reagent acids and solvents.[4] Despite the appearance of a number of papers on the subject, we continue to receive numerous requests for reagents, information, and practical advice each year. Although the reagents to be described are prepared on a routine basis in a number of laboratories, it is obvious that the technique has not become widespread. It is hoped that the information presented here will enable the reader to prepare these high-purity reagents.

At least for trace analysis, the subject of analytical losses is related to the problem of contamination. A frequent mechanism of analytical losses is related to the acidification of a sample to stabilize it. Improperly acidified samples may lose cations through hydrolysis or adsorption on the container walls. Some aspects of this problem have been reviewed by Maienthal and Becker.[5] Most analysts hesitate to strongly acidify samples being held in storage, because of a blank problem associated with either the acidification or the subsequent neutralization. More strongly acidified samples also require more scrupulously clean apparatus and containers because of possibly increased rates of trace-element leaching. With a supply of reagents of adequate purity, the analyst does not hesitate to take the steps necessary to ensure stability relative to adsorption losses.

Sub-boiling distillation

Conventional fractional distillation presents a number of difficulties in purifying reagent mineral acids for trace-element analysis. Usually the apparatus itself will be constructed of glass, a material that is not conducive to the attainment of low trace-metal blanks. The most important parts of the still, in this respect, include the condenser, condensate feed line and the receiver. The use of quartz in these parts helps to reduce trace-metal blanks. However, the key to lower analytical blanks does not appear to lie in seeking a greater number of theoretical plates but rather in seeking a different distillation process.

Most problems associated with the distillation process are mechanical in nature. Among these is the "creep" of liquids up the walls of the still-pot, where they may be partially entrained by vapours rising into the condenser. The boiling process itself is violent in nature, producing an aerosol of the still-pot liquid which is then entrained into the condenser. By either

means, the result is the contamination of the distillate with liquid directly from the still-pot.

One means that has been used to overcome this difficulty is isothermal distillation.[6] This process was used at NBS before the advent of sub-boiling distillation and may still be useful to many laboratories today. In principle, if containers of volatile acid and of distilled water are placed in a sealed chamber, acid vapour will be absorbed in the distilled water until an equilibrium vapour pressure is reached. The purity of the distilled water and purity of the container largely determine the quality of reagent produced.

In a more practical sense, reagents which can be produced by this method are dilute and the "distillation" rate is rather slow, being a function of the volatility of the acid. Purified hydrofluoric acid can be produced in a plastic chamber. Obviously, the use of any metal parts anywhere around the apparatus will lead to corrosion products which ultimately contaminate the acid produced.

In 1971, scientists at NBS and the Carnegie Institution became aware of a new still being produced by the French firm of Quartz et Silice*. Although initially marketed for the production of high-purity distilled water, the still became known for its ability to produce reagent acids of the highest purity. The term sub-boiling distillation was coined and the first publication was produced by 1972.[4] Since then, a number of publications have dealt with some aspect of the non-boiling principle, including the "two bottle" still by Mattinson[7] and the polypropylene still by Dabeka *et al.*[8] More recently, a Teflon analogue of the quartz sub-boiling still has become commercially available. Very similar to the original NBS design, the present version is based on the work of Tschöpel *et al.*[9]

Production and analysis of sub-boiling distilled acids

The Quartz et Silice stills have been sold in the U.S.A. under the model designations of PB-5, PB-10, and PB-15. All three stills have been used at NBS, the only significant difference between them being that of size and the resulting rate of distillation. The model PB-15 is used as our primary source of distilled water. By using a variable autotransformer it is possible to vary the distillation rates over a wide range to suit needs, or to select a distillation rate which will fill a container in a given period of time. Distilled water prepared in this manner is stored in 8-litre quartz flasks. With an input power of ~500 W it is possible to prepare about 20 litres of distilled water per day.

Normally, the rate of distillation is kept at a lower level that approximately matches the rate of consumption. The PB-15 is used solely as a redistiller to "polish" a supply of distilled (not demineralized) water which has trace-element blanks at the ng/g level or below. For this reason, it is also the only distillation process at NBS which does not run on a batch basis. After a period of trial and error, we have found that a Teflon needle valve may be used to regulate the flow of ordinary distilled water into the PB-15 still. The valve is adjusted to give an overflow from the still at 3–4 times the rate of distillation at the highest routine rate from the PB-15 still. Under these conditions, the quiescent conditions of the sub-boiling distillation process are preserved. Too great a rate of overflow causes a marked decrease in the rate of distillation since most of the heat is being conducted to the drain. In over 5 years of continuous use under these conditions, absolutely no build-up of scale or dirt has been found in the still-pot. Of course, a poorer quality of distilled water feedstock could change this observation. The still itself is supported by "Plexiglas" inside a metal-free Class 100 clean-air chamber.

Table 1 summarizes the power input and other requirements for the distillation of the common mineral acids. The power requirements are slightly different from those published by Kuehner *et al.*[4] and the distillation rates listed are also different for some reagents. Part of this difference is because the present rates are calculated for a double distillation process whereas the original values were for a single distillation.

The high-purity acids are all produced from reagent-grade acids and the distillates have essentially the same concentration as the reagent-grade acids, except for hydrochloric acid and sulphuric acid. To distil hydrochloric acid, dilution with distilled water is required to avoid the violent evolution of hydrogen chloride. In the beginning, these distillations were made with the constant boiling (6*M*) acid but experience showed that this concentration could be raised safely to 10–10.5*M*. At higher concentrations than this bubbling can be observed during the distillation, and contaminates the reagent produced.

Sulphuric acid is distilled as the concentrated reagent, but it has such an affinity for water that the

Table 1. Summary of experimental conditions for producing doubly distilled high-purity acids by sub-boiling distillation

Reagent	Concentration of still feed acid, %w/w	Still power,* W	Cooling-water flow,* l./hr	Production rate, ml/24 hr
HCl	32	200	20	2000
HNO_3	70	90	20	750
$HClO_4$	70	280	20	800
H_2SO_4	96	480	20	200
HF	48	42	10	100

*Required for each still.

*Certain commercial equipment, instruments, or materials will be named in this report to specify adequately the experimental procedure. This does not imply recommendation of endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment named will necessarily be the best available for the purpose.

slow permeation of water vapour through the Teflon walls of the storage container soon causes the dilution of the acid to a slight degree. In addition, both nitric acid and perchloric acid may undergo some degree of decomposition if elevated power levels are used. At the recommended power levels, the degree of decomposition is slight and no instance has been reported of an interference in trace analytical procedures.

All distillation rates are nominal and have been observed to change considerably during the year as the temperature of the tap water used for cooling the condensers varies (by about 15° from summer to winter in Washington, DC). As might be expected, the distillation rates and/or the power levels required are in accordance with the volatility of the acid being distilled. Distillation rates for hydrofluoric acid are limited by the temperature limit of the Teflon used to enclose the heating element.

It should be noted here that the original NBS Teflon still had a serious design flaw. In time, sufficient hydrogen fluoride gas diffused through the Teflon heater to attack the quartz tube used to contain the Nichrome heating element. As the quartz was dissolved, the hot metal came in contact with the Teflon, causing it to cold-flow and ultimately perforate about 3 years after it was constructed. A modified heater has been successfully used for the past 8 years. A nickel liner is inserted inside the Teflon heater tubes and then the Nichrome wire heating-element is placed inside a quartz tube to insulate it electrically from the nickel insert. An additional safety factor of lower power settings may also have been of benefit, but in any case no cold-flow has so far been observed in the heater elements.

For sulphuric acid, pieces of platinum foil have been used to reflect infrared radiation back into the still and enhance the rate of distillation. In addition the angle of the condenser relative to the liquid surface has been increased slightly to permit the more viscous sulphuric acid to flow more easily down the condenser to its tip.

A two-stage or double-distillation technique was started in 1971, became routine by 1974, and has been the only method of production since 1976. Figure 1 details this procedure. One still is modified slightly to accept a Teflon (FEP) tube which is run to the next still through a Teflon T-valve. In use, the first still is charged with reagent acid and the product is distilled into the second still. When the second still has become charged with sub-boiling acid from the first still, it is turned on and the distillate is collected in a 2-litre Teflon (FEP) bottle.

A 2.7-litre reagent-acid bottle provides sufficient acid to charge the first still and enough distillate is produced to charge the second still and yield 2 litres of doubly distilled acid. Afterwards, both stills are drained and the process is repeated. The rates of distillation in Table 1 are about 15% less than would be achieved by a single-stage distillation. All of these distillations are batch distillations.

We are frequently asked if the use of commercial high-purity acids as starting materials would result in the production of higher purity acid. The two-stage distillation permits a direct answer to this question since the results of this distillation process are not discernibly better than the single-distillation results reported in 1971.[4] Thus, the use of better starting materials will not alone guarantee better results. The double-distillation process was adopted as a means of guaranteeing that no accidental contamination of the starting material (because of deteriorating laboratory conditions) or human error could affect the quality of the distilled acids. This approach appears to be valid since in the entire 11 years of production not a single instance of trace-element contamination has been discovered. For some elements, such as lead, the double-distillation process is believed to be of some benefit. In theory, the factors limiting the quality of the dis-

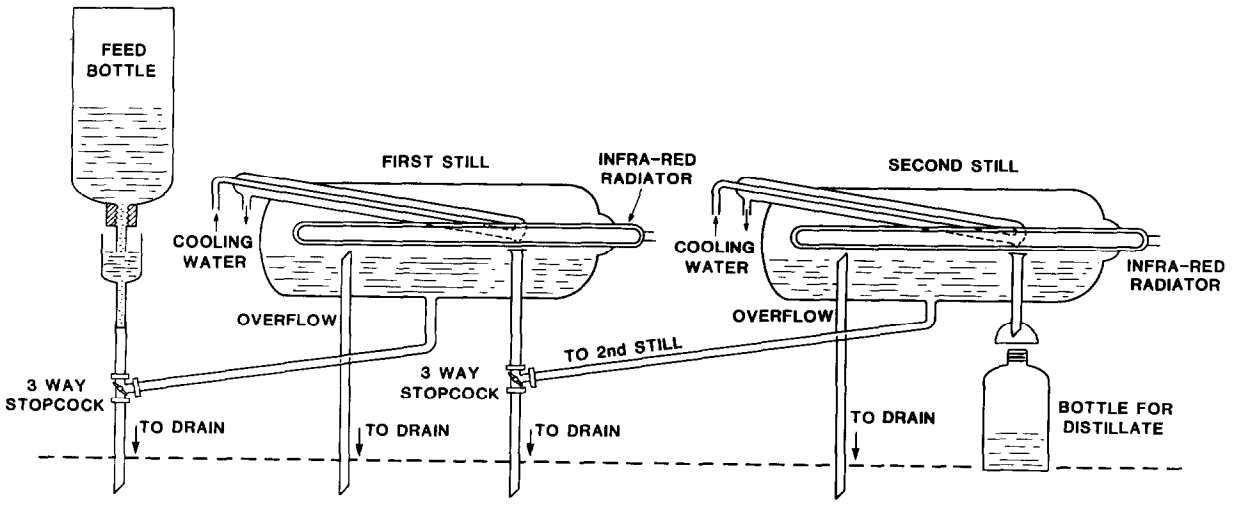

Fig. 1. Schematic diagram of apparatus for double sub-boiling distillation.

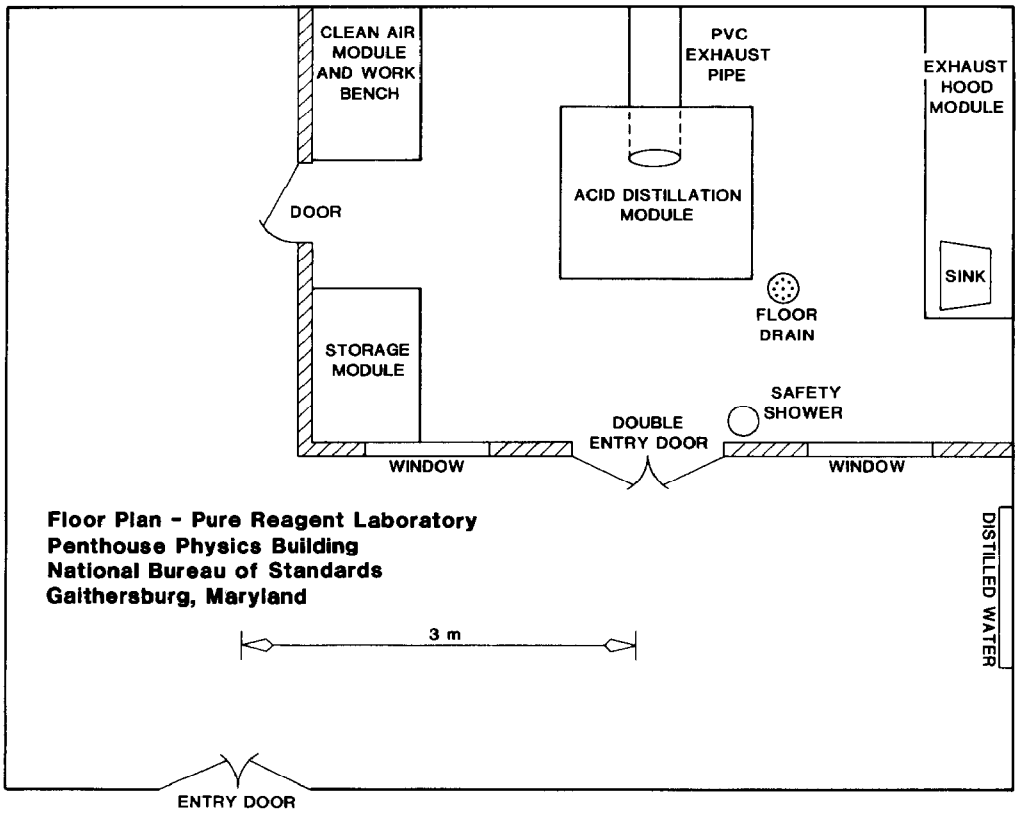

Fig. 2. Floor-plan of pure-reagent laboratory.

tilled reagents should be the purity of the quartz condenser and the Teflon storage container, and the volatility of some inorganic compounds.

Nearly all our stills have benefited from many years of leaching by acids and many of our Teflon containers have had the same continuous leaching by high-purity acids. There has even been a significant improvement in the past few years in the apparent quality of the commercial Teflon (FEP) bottle, as judged by the reduced number of inclusions observed within the bottle walls. Despite all this and the double-distillation process, no general improvement in the quality of the purified acids has been found. As described by Kuehner *et al.*,[4] the method of analysis for trace metals continues to be isotope-dilution spark-source mass-spectrometry (ID–SSMS). Some 18–23 elements are determined simultaneously, this number being limited only by the number of separated isotopes available. The results of the double-distillation process shown in Table 2 require some explanation relative to the method of analysis.

Isotope-dilution analysis is generally considered to be one of the most accurate methods available for the concentration levels concerned. For the analysis of these high-purity materials, a fixed amount of 0.1 μg of every separated isotope of interest is added to 100 ml or more (for higher purity materials) of the sample. The mixture of sample and the spike isotopes is evaporated to a single drop under Class 100 conditions. This drop is transferred to gold electrodes or mixed with gold powder to form a suitable electrode for use in SSMS. For many elements, no detectable

Table 2. Average impurity concentrations in doubly distilled acids (*ng/g*)

Element	HCl	HNO_3	$HClO_4$	HF	H_2SO_4
Pb	0.02	0.03	0.22	0.1	0.3
Tl	0.04	0.06	0.07	0.07	0.1
Ba	0.04	0.02	0.17	0.09	0.3
Te	0.02	0.03	0.05	0.05	0.09
Sn	0.12	0.02	0.14	0.15	0.12
Cd	0.03	0.02	0.05	0.05	0.16
Ag	0.21	0.06	0.06	0.18	0.3
Sr	0.03	0.02	0.02	0.03	0.16
Se	0.03	0.06	0.5	0.21	—
Zn	0.13	0.06	0.2	0.19	2.5
Cu	0.08	0.05	0.10	0.26	0.14
Ni	0.12	0.08	0.37	0.45	0.12
Fe	2.5	0.35	1.2	2.5	3.7
Cr	0.16	0.06	3.2	2.1	0.12
Ca	0.12	0.11	0.6	1.3	1.2
K	0.5	0.17	0.37	0.9	2.3
Mg	0.27	0.09	0.12	1.0	2
Al	1.0	0.7	0.4	0.85	1
Na	0.8	0.7	1.2	4.0	6
Total	6.0	2.7	9.0	14.4	20.6

amounts of natural (non-spike) isotopes are found. In such a case, the ratio of noise level of the baseline (electrical detection) or the fog level of the photoplate (photographic detection) and the measured intensity of the separated isotope is used to calculate a concentration.

Such results are called upper limit numbers, which is to say that the true trace-impurity levels are less than the number reported. Discrepancies between the results shown in Table 2 and those reported by Kuehner *et al.*[4] are quite likely, for this reason. When necessary, the exact concentrations of a number of elements such as Pb, Cd, Ag, U, have been determined and found to be as low as a few pg/g (10^{-12}g/g). Even then, many of these results may be distorted by the evaporation blank, which is not easily evaluated. Rather than make possibly exaggerated claims for a given lot, we give the results reported in Table 2 as conservative averages of 10 years of determinations. Routine analytical blank determinations for our analytical work provide an excellent means of constantly monitoring the high-purity reagents between the periodic ID–SSMS analyses.

Preparation of clean bottles, and reagent storage

Relatively little has changed in the past 11 years in this regard, although some experimental work by Moody and Lindstrom[9] has shown that the cleaning methods used are adequate. Many who undertake the production of their own reagents fail to take into account the time and the extent of cleaning necessary to produce the requisite clean Teflon (FEP) (fluorinated ethylene–propylene) bottles. A similar effort is necessary to ensure quality control throughout the cycle of reagent production, storage, and use. For example, we currently have almost 850 Teflon (FEP) bottles in circulation, used solely for high-purity acids.

New bottles are rinsed with alcohol or acetone followed by distilled water to remove surface grease and dirt. They are then immersed in glass vats in boiling hydrochloric acid (1 + 1) for about 2 weeks. Next they are transferred to glass vats filled with nitric acid (1 + 1) and boiled for about 2 weeks. After this preliminary cleaning, the bottles are filled with high-purity distilled water and placed in a metal-free storage cabinet. This preliminary filling with distilled water leaches nitric acid from the Teflon and is presumed to perform some slight leaching of trace metals as well. The distilled water is changed once or twice over a period of several months. The bottles are then emptied, numbered serially by scratching the outside, and filled with the desired reagent. For quality-control purposes, the bottle is dedicated to that one reagent. Reagents are always poured from the 2-litre Teflon (FEP) bottles used to catch the distillate into one of a large number of clean bottles used for the storage of reagents. Transfers are then made from these stock bottles to the new bottles or to other bottles being returned for refill. This process guards against the accidental spread of contamination from a dirty bottle to other clean bottles of purified reagent and promotes the rotation of stock.

After the initial filling with purified acid, the bottle is placed in storage for one or more months to leach additional trace metals from the container wall. Afterwards the acid is discarded or redistilled and the bottle is refilled with freshly distilled acid. This process is repeated a total of three times before a bottle is considered to be clean enough for use. The complexity and the length of time involved in the complete process are somewhat arbitrary, but less than in some laboratories. In any event, the process does assist in the rotation of stock.

All the stills are run all day for more than 350 days per year. The constant demand for acid to be used for bottle cleaning assists in the rotation of stock and the maintenance of a fresh supply. Since our distillation capacity is greater than 1000 litres per year and our actual consumption is only ~250 litres per year, there is a temptation to distil only on demand, which would increase the age of the stock considerably. Although there is no direct evidence indicating that blank levels do increase with age, it seems prudent to avoid unnecessarily long storage times for any purified reagent. Moody and Lindstrom[10] obtained some data which would tend to support the likelihood of continued leaching. Ideally, any purified reagent should be consumed as quickly as possible.

Because our laboratory serves about 50 scientists at NBS and at least an equal number in other laboratories, it is not always possible to predict the rate of usage. We have compromised by keeping a modest stock which is easily maintained in fresh condition and which provides a buffer against temporarily high rates of usage. Fortunately, all this is consistent with keeping an adequate supply of clean Teflon bottles available for reagents or other analytical purposes. Complete records are kept of the history of usage of each bottle, to enable any potential source of contamination to be traced.

Laboratory and other refinements

During the past 11 years, we have prepared distilled acids in several NBS laboratories, and, as might be expected from the quantity of production, not without considerable difficulty. Nearly all problems have been associated with corrosion of metals within the laboratory, and a considerable effort was required to continue production without contaminating the reagents produced. The need for a specialized laboratory was clearly recognized by the mid-70s. Construction began in 1980 with completion in 1981. The new laboratory has been successfully operated for more than a year, so some useful comparative information can be given.

In the earliest days of acid production at NBS, metal clamps covered with Teflon tape backed by aluminium foil were used to support the stills, in combination with a similarly coated ring stand. This arrangement proved to be very unsatisfactory because

the metal soon corroded, requiring replacement in just a few weeks. Similar problems were observed for the clamps supporting the constant-level feed and reagent bottle. More recently, boxes were fabricated from "Plexiglas" to support the constant-level device and reagent bottle as well as to confine the fumes from them. These devices were serviceable but were difficult to adjust to compensate for changes in reagent-bottle sizes and hence the resulting liquid level within the still. In addition, the Plexiglas gradually darkened and deteriorated after prolonged exposure to perchloric acid.

These problems had to be overcome in the design of the new laboratory. The problem of adjusting the liquid levels within the stills was solved by using large poly(vinyl chloride) (PVC) bolts to adjust the height of a pair of bakelite brackets which were fabricated to support the stills. The thick bakelite does not appear to be adversely affected by years of exposure to fumes, does not produce particles, and tolerates (with some relatively inconsequential charring) the high temperatures required to distil sulphuric acid. Plexiglas and other thermoplastics may be used if the operating temperatures are kept below the softening points. The new arrangement permits the adjustment both of elevation and side-to-side as well as front-to-rear inclination of the stills. The maximum rate of distillation is achieved when the liquid level within the still is kept as close to the infrared heaters as possible.

Acid spills from the feed bottles, stills, and collector bottles were a major problem. In any operation of this type, it should be assumed that such spills are always possible. Because of human error, breakage, or electrical failures caused by corrosion, many spills occurred overnight and were not discovered for many hours. This caused further damage by contact with both liquid and fumes from the acids. A means had to be found to control or even prevent spills for the sake of both safety and equipment reliability.

The flooring in the new laboratory is a seamless resilient type chosen especially for its resistance to acid contact. A PVC material manufactured in the United Kingdom for clean-rooms would have been a better choice but it was prohibitively expensive. At the walls the covering rises 10 cm from the floor to contain spills and there are even raised coved entrance thresholds. The only penetrations through the floor are a floor drain and a glass pipe servicing a sink drain. The walls are made of concrete block sealed with epoxy paint, both for resistance to acids and to prevent particulation. A safety shower and a rubber garden hose for the rapid dilution and disposal of a major spill in the laboratory complete the safety equipment.

Other features of the laboratory are borrowed from experience with clean-laboratory design. A suspended ceiling with supports made of polyurethane-coated aluminium and panels made of plastic-laminated wood products is used. Light fixtures are made of aluminium painted with polyurethane paint. All furniture and storage cabinets are made of plastic-laminated wook products. All parts which might contact liquid, including the distillation module and a 2.5-m fume hood, are made of white PVC. A final precaution included the installation of all electrical equipment outside the laboratory, with vinyl-covered low-voltage wiring to power the stills and the hot plates for bottle cleaning.

Ventilation requirements

The original NBS acid laboratory had an air-exhaust capacity of $\sim 60\ m^3$/min, which gradually decreased to considerably lower values. This figure has proved to be grossly inadequate for the scope of work described here. After 9 years of experience in dealing with painted and coated metal surfaces in the presence of recirculating acid fumes, ample proof was obtained that no recirculation of air could be permitted and no metal surfaces could be adequately protected against prolonged exposure to acid fumes. Stainless steel exposed to acid fumes will corrode and produce particles within days. Even the air-filtration equipment, which was well protected, had a half-life of about 3 months when exposed to uncontrolled acid fumes in the laboratory.

Two basic principles were employed in the design of air-handling equipment for the new laboratory. First, the entire exhaust–air system was made of PVC for assured corrosion-resistance and long life. Commercial versions of PVC fans are available both in the United States and Europe. Commercial ductwork available in the United States is designed to be solvent-welded. Similar materials available in Europe are of at least equal quality and use flanges and Teflon gaskets to join sections together. Some of the solvent-welded joints have already proved to be leaky. In all other respects the system has been service-free and has performed as expected.

The second principle involved the means and balance of introduction of air into the laboratory. Three modular units each capable of supplying up to $75\ m^3$/min of Class 100 filtered air were integrated into the ceiling of the laboratory. The total exhaust capacity of the laboratory was fixed at $150\ m^3$/min by virtue of the fan-belt drives. The modular clean-air unit has adjustable solid-state speed controls. Two of the units were placed over the distillation module to create a Class 100 vertical air-flow area measuring 1.5×1.8 m. The total air-flow was adjusted to $\sim 80\ m^3$/min to give the distillation modules a slightly positive pressure with respect to the laboratory. All the modules were constructed of polyurethane-painted aluminium and had high-efficiency particulate (HEPA) filters with plywood frames and metal-free design. Thus, from the supply of air at the HEPA surface until the air is exhausted through the roof of the building there is no metal whatever in the system. The overpressure of Class 100 air ensures that no contamination can be introduced into the distillation module

when the doors to the distillation module are opened to service the stills.

A third clean-air module was used to form a vertical-flow clean-air bench for packaging. The air from this bench, plus the slight amount of leakage from the distillation module, serves as the supply air for a 2.5-m PVC exhaust hood. The air-flow from the third clean-air module was adjusted until the entire laboratory was at slightly negative pressure with respect to its surroundings. Although this causes the leakage of dirty air into the laboratory, it does positively prevent the leakage of acid fumes from the laboratory (which would recreate the same old problems). Thus, it is this balance of the air-flows which ensures that the lifetime of electrical equipment will be normal and that corrosion outside the laboratory will be minimal. In a large laboratory with very expensive equipment, this latter assurance is perhaps even more important than all other considerations.

To summarize, all the air entering the laboratory, except for small amounts of deliberate leakage, is filtered through HEPA filters. All of this air makes a single pass through the laboratory and is then totally exhausted. Linear air-velocities within the acid-distillation module are about 25 m/min and the flow is reasonably laminar, which assists in the prompt removal of fumes from this module. Overall, these characteristics have effectively reduced free fume levels in the laboratory to very low values, which ensures the safety of the chemists and the operational life of the equipment both inside and outside the laboratory.

Production of other reagents

Over the years a combination of need and curiosity has led us to attempt the sub-boiling distillation of a number of other reagents. Usually these reagents were characterized for only one or two trace elements. Thus, no general claim can be made for the total impurity level. However, since the distillation did improve the analytical blanks for the elements examined, it seems reasonable to expect a similar improvement for other elements as well.

The single-stage sub-boiling process has been used to purify the following reagents at NBS: acetic acid, hydrobromic acid, ethanol, methanol, isopropyl alcohol, benzene, pyridine, acetone, methyl isobutyl ketone, and small amounts of a few other solvents. It should be remembered that many organometallic compounds are reasonably volatile and thus the sub-boiling process may be considerably less advantageous than for mineral acids. Because all these materials are reasonably volatile, power inputs of about 100 W are more than adequate to produce distillation rates of 1–2 litres per day.

The so-called "two bottle" still described by Mattinson[7] could easily be called a poor man's sub-boiling still. The process involved is the same but the rates of distillation are much lower. Because the bottles are closed, a Class 100 clean-air environment is not necessary, although it would be helpful to have such an environment in which to open the "still". Fume production is also much less, but Teflon does "breathe", so the "stills" should be kept in a fume exhaust hood. Since the quality of the reagent produced is comparable with that from the sub-boiling still, only the slow rate of distillation has prevented a more general use of the device at NBS.

Occasionally such a small-scale need does arise; for example, we have attempted the preparation of fuming sulphuric and fuming nitric acid for more rapid wet digestions. By charging the receiver bottle with sub-boiling distilled acid and the still-pot bottle with reagent grade fuming acid, an equilibrium between the two is reached in a few days. By changing the charge of fuming reagent acid several times it is possible to obtain purified fuming acid with approximately 75% of the free SO_3 or NO_2 content of the reagent grade fuming acid.

However, there were several problems with the apparatus in this application. First, the Teflon walls of the bottles are so permeable to water vapour that the consequent dilution of the reagent is more than is tolerated for the intended use. In addition, both fuming reagents seemed to attack the Teflon slightly, since the residues left by evaporation were viscous and black, and considerable amounts of organic material were observed during the mass-spectrometric analysis of these acids. If this idea were to be pursued further, a quartz two-bottle still would be a logical choice.

High-quality ammonia solution is produced by passing filtered ammonia gas into a Teflon bottle of distilled water chilled in ice. If a 1-litre Teflon bottle is filled with about 600 ml of distilled water, it will be nearly full when the water is saturated with ammonia. When this material is combined with sub-boiling distilled acetic acid, solutions of acetate buffers of very low trace-metal content may be prepared. Reagents of this type are valuable in the use of Chelex resins as reported by Kingston *et al.*[11]

Summary and conclusions

This paper has addressed a particular aspect of the problem of analytical contamination. Contamination control for the reduction of analytical blanks has been recognized by a diverse group of authors over a number of years and the publications of Mitchell[3,12] and Murphy[1] exemplify this trend. Whereas Mitchell's contribution to this symposium reviews many methods of purification, this paper concentrates on the details necessary for a single method. We do not mean to suggest that sub-boiling distillation is a panacea for all analytical problems. However, the use of high-purity reagent acids is almost certainly universal among trace-element laboratories. No laboratory operating today can claim to be in the forefront if it does not either produce or have access to these reagents. It was for this reason that it was believed that an update of the paper by Keuhner *et al.*[4] would be

useful. We hope that sufficient practical detail has been given to enable any laboratory to duplicate our work without suffering through a learning experience.

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CAUSES AND ELIMINATION OF SYSTEMATIC ERRORS IN THE DETERMINATION OF IRON AND COBALT IN AQUEOUS SOLUTIONS IN THE ng/ml AND pg/ml RANGE

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Summary—The determination of very low levels of elements, in the ng/g and pg/g range, is very closely linked with different systematic errors causing considerable discrepancies in the analytical results for real samples. Main sources of these errors are blanks as well as losses of elements and compounds by volatilization and adsorption. In this paper, systematic errors arising from contamination of the sample solution by the vessels and the dust content of the air are demonstrated for determination of iron and cobalt at pg/ml and ng/ml levels in pure aqueous solutions by AAS with electrothermal atomization. Effective cleaning procedures are described for different vessel materials such as PTFE, quartz and glass. Blanks due to airborne dust can be substantially reduced by working on clean-benches in clean-rooms.

In the determination of extremely low absolute amounts of elements or element concentrations in the ng/ml and pg/ml range, *e.g.*, in high-purity materials, biological tissues and fluids, environmentally relevant samples or even in pure aqueous solutions, various systematic errors generally arise, such as blanks caused by contamination of the sample solution, mainly by the vessel itself, the reagents and airborne dust, or losses of trace elements by volatilization or by adsorption on the surface of the vessel.[1–6] With decreasing concentration of the element to be determined, the systematic error can prevail to such an extent that the analytical results become incorrect by orders of magnitude, as can clearly be seen from interlaboratory comparative control studies.[3,5] In addition, we have to take into account the ubiquity of the elements in question, and the anthropurgic contamination of the environment by technological and scientific activity.

Our knowledge of the sources of systematic errors and their effective elimination[7–10] is by no means sufficient, and there is an almost total lack of systematic investigation in this field. Some hints to the problems are hidden in the analytical literature, but are difficult to find.

In addition we have to learn that we are not allowed to transfer the knowledge gathered by solving one analytical task to another analytical problem or to other elements. Generalization is strictly prohibited in extreme trace analysis. Therefore, the analyst is faced with the troublesome, difficult and laborious job of gathering information piece by piece and using observations and experience in order to form a mosaic that reflects the situation clearly.

Though it is impossible to give here a fully comprehensive report on systematic errors, we should at least try to give an introduction to this field in connection with the so-called "multi-stage combined procedures" consisting of decomposition, separation, preconcentration and determination steps. These sophisticated procedures, which can easily be calibrated with aqueous standard solutions, are often used if there is a lack of suitable standard reference materials covering the extreme trace range.

The most important sources of systematic errors[4,6] inherent in these procedures are:

(1) incorrect sampling and sample storage;
(2) contamination by vessels, reagents and airborne dust;
(3) adsorption and desorption effects at the surface of the vessel;
(4) volatilization of elements, *e.g.*, mercury, arsenic, selenium, cadmium, and compounds such as oxides, halides, hydrides;
(5) matrix effects during excitation of the analytical signals for the determination;
(6) signal interferences by the background and signals from other compounds;
(7) errors in calibration from use of incorrect standards, unstable standard solutions and blanks.

Systematic errors are by no means easy to recognize and to reduce, when standard reference materials are not available. Interlaboratory comparisons, independent procedures with different techniques, testing of the procedure with radio-tracers, and use of absolute analytical procedures which need not be calibrated are helpful approaches.

Table 1. Spectrometric conditions for ETA–AAS

	Fe	Co
Wavelength, *nm*	248.3	240.7
Slit-width, *nm*	0.2	0.7
Expansion	1 or 5	1 or 5
HCL current, *mA*	15	12

Temperature programme*	Dry	Ash	Atomize	Clean out
Temp., °C	150	700	2600	2600
Time, *sec*	20	20	5	3

*Same conditions for Fe and Co.

As the most important systematic error is due to contamination, this paper is mainly concerned with this broad field.

Contamination

Contamination is mainly due to the reagents, the vessels and dust in the air. The possibilities for purifying solid reagents are very limited because laborious and sophisticated procedures are required, and the levels of only a few elements are decreased, while those of others might well be increased. Besides gases, only the acids commonly used in the laboratory can be easily purified to give very low blanks (in the range below 1 ng/ml), sub-boiling point distillation being used for the purpose.[11–14] In order to avoid blanks from the vessels we must use only very pure materials such as quartz, glassy carbon,[15] PTFE or polypropylene, and must avoid glass, a very impure material with a few main components and a lot of elements as impurities in the μg/g range. In addition, the surfaces of the vessels have to be cleaned very carefully by special techniques such as steaming[12,13] or leaching in very pure acids for some hours.[16–20] Rinsing with acids or water is not sufficient,[12,13] as will also be shown in this paper.

Blanks from airborne dust are restricted to a very low level by the use of clean-benches and clean-rooms[12,13,21–23] in which the air is filtered through a high-efficiency particulate-filter, reducing the dust level by 3–4 orders of magnitude compared with the air of a normal laboratory.

In continuation of an earlier investigation on zinc and magnesium[12,13] with respect to blanks from vessels, reagents and dust, element losses due to adsorption, and contamination of the sample solution during storage or simple working steps, *e.g.*, pipetting, filtration or evaporation, an examination of iron and cobalt, two elements with different levels of ubiquity, is described here. This work especially aims at the examination of the cleaning procedures for vessels made of different materials such as borosilicate glass, quartz and polytetrafluoroethylene (PTFE). Sources of errors and their elimination, simple working steps such as the filling and emptying of flasks by different techniques, as well as contamination during storage, are also investigated.

EXPERIMENTAL

Instrumentation

Perkin–Elmer model 430 double-beam atomic-absorption spectrometer, with HGA 76 graphite furnace and HGA 76B control unit, AS-1 automatic sampler (10 and 20 μl) and Perkin–Elmer 56 recorder, hollow-cathode lamps for Fe (Oriel, Darmstadt) and Co (Dr. Kern and Sprenger GmbH, Göttingen), and Perkin–Elmer graphite tubes, coated with pyrolytic graphite in our own laboratory. (For instrument settings see Table 1.) The vessels (see Fig. 1) for the automatic sampler had a volume of 2 ml and were made of borosilicate glass, quartz and PTFE in our own workshops. Isostatically pressed PTFE vessels were made by the Venus Kunststoff GmbH, Heidenheim, FRG. Each vessel was provided with a PTFE cover (see Fig. 1).

All experiments—if not stated otherwise—were done in clean-rooms on vertical-flow clean-benches with exhaust[12,13] or on a small horizontal-flow clean-bench (SLEE, London, U.K.). The sample vessels were handled with a pair of tweezers with special half-round grips made of PVC. The vessels were stored within a dust-proof "Plexiglas" box, fitted into holes in a PVC plate, and transported from the clean-room to the AAS room in a desiccator.

Standard solutions were dispensed from automatic micropipettes (Eppendorf Varipetten, Eppendorf Gerätebau, Hamburg, FRG). The solutions were homogenized (20–30 sec) by a small stirrer (type CuV-O-Stir, model 338, Hellma, Mühlheim, FRG) provided with a quartz rod (length 38 mm, diameter 3 mm) which was rinsed with doubly distilled water before and after use, and stored in a

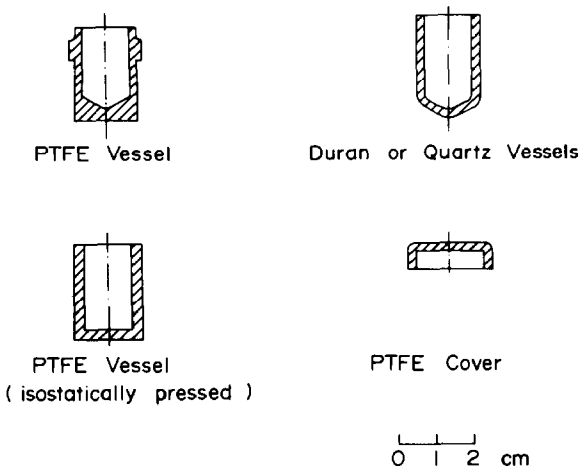

Fig. 1. Sample vessels (2 ml) of PTFE, Duran glass and quartz.

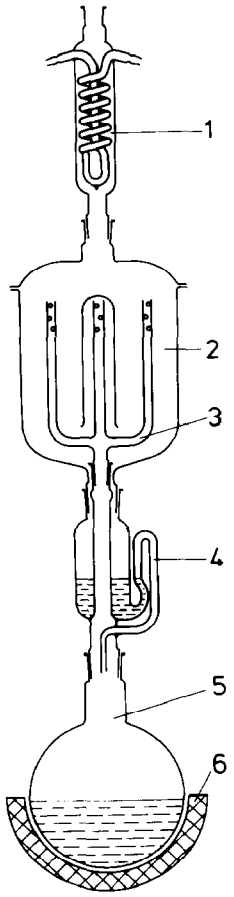

Fig. 2. Steaming apparatus for the cleaning of vessels in acid and water vapour. 1, Reflux condenser; 2, steaming chamber; 3, vapour tubes; 4, over-flow; 5, round-bottomed flask; 6, heating device.

quartz tube in a clean bench to avoid contamination. All preparation and handling was done on a clean bench unless mentioned otherwise.

Cleaning

All laboratory ware such as beakers, vessels, flasks, bottles, pipettes, stirrers *etc.* was cleaned by means of a special steaming apparatus,[12,13] shown in Fig. 2. In a 1–2 litre round-bottomed flask (6) acid (nitric or hydrochloric) or water is heated to boiling. The vessels to be cleaned are hung open end downwards on top of the quartz tubes (3) through which the vapour passes, thus washing the inner surfaces continuously, with fresh acid vapour for 4–6 hr and subsequently with water vapour for 1–2 hr. By this process the surfaces are not only cleaned very effectively but in addition are preconditioned in such a manner that the adsorption of traces of elements is substantially inhibited.

In some cases an ultrasonic cleaner (type TT3, 35-kHz, Bandelin Electronic K.G., Berlin, FRG) was used for cleaning purposes.

Table 2. Blanks

	[Fe], *ng/ml*	[Co], *ng/ml*
16*M* HNO_3 (subb.)	0.2	≤0.3
10*M* HCl (subb.)	0.6	≤0.3
H_2O doubly distilled	0.3	≤0.3
Detection limit	0.1	0.3

Reagents

Standard solutions, 2 mg/ml. Prepared in 500-ml quartz flasks from Titrisol solutions, (Merck, Darmstadt) and stored in polyethylene bottles.

Stock solutions, 1 and 0.01 μg/ml. Prepared daily by dilution of the standard solutions, in 20-ml quartz flasks.

Hydrochloric and nitric acids (subb.). Purified by sub-boiling point distillation[11–14] (quartz sub-boiling still, Heraeus Quarzschmelze GmbH, Hanau); for specification of the purity see Table 2.[12]

Doubly distilled water. Prepared by means of a quartz double still, type BD15, with quartz storage bottle (10 litre), (Westdeutsche Quarzschmelze, Geesthacht).

All vessels were cleaned by steaming.

RESULTS

Calibration curves for iron and cobalt

The sample solutions were prepared directly from the stock solution by dilution in the sample vessels with water and 10*M* hydrochloric acid (subb.) respectively. For each concentration a separate quartz beaker (2 ml), cleaned by steaming and then rinsing 3 times with doubly distilled water, was used. All measurements were done by AAS with electrothermal atomization and signal recording, and evaluation of the peak height of the signal relative to the mean value of the background.

The calibration graphs were linear in the range between 0.1 and 25 ng/ml. The reproducibility of the calibration graphs over a period of 4 months is given in Table 3, in the form of the slopes.

Table 3. Reproducibility of the AAS-calibration curves over a period of 4 months

	Fe		Co	
	Exp.* 1	Exp.* 5	Exp.* 1	Exp.* 5
Concentration range, *ng/ml*	0–25	0–2,5	0–25	0–2.5
Number of measurements	13	18	4	4
Mean slope, $mm.ml.ng^{-1}$	3.4	19.1	2.06	10.5
Standard deviation of slope, $mm.ml.ng^{-1}$	0.14	0.65	0.01	0.4
Relative standard deviation, %	4.2	3.4	0.7	3.8

*Expansion

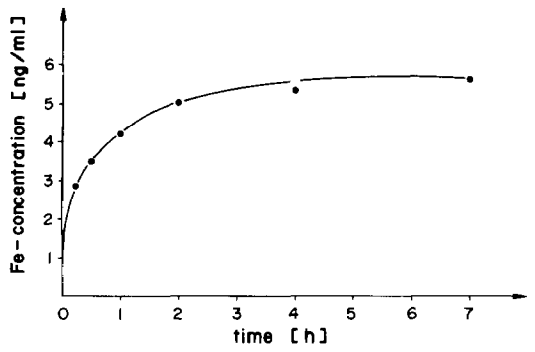

Fig. 3. Contamination of 150 ml of 2*M* HCl with Fe by 20 pipette tips.

Contamination by the pipette tips

To get an indication of the blanks arising from the tips of the automatic micropipettes[12] during dispensing of solutions, 20 tips (1000 µl) were stored for several hours in 150 ml of 2*M* hydrochloric acid (subb.). Figure 3 shows the increasing Fe content of the liquid. Within 3 hr the concentration was as high as 5 ng/ml, so each tip gave up to 37.5 ng of iron to the solution. Though during pipetting the solution does not come into contact with the whole surface of the tip, nevertheless the tips should be cleaned before use by immersion in 2*M* hydrochloric acid (prepared from the 10*M* "sub-boiling" acid) for two 24-hr periods) then rinsing with doubly distilled water and doubly distilled acetone and drying.

Contamination by vessels

In spite of very careful and punctilious cleaning of the vessels, further contamination of the sample solutions cannot be avoided during the analytical procedure or during storage for several days.

The concentration of the blank depends on various parameters such as the element itself, the kind of vessel material, its purity, the pretreatment and the cleaning procedure. The following cleaning techniques were therefore investigated for Duran glass, quartz and PTFE:

(*a*) steaming with concentrated nitric acid, hydrochloric acid or water, in the apparatus described elsewhere[12,13] (see Fig. 2);
(*b*) leaching in 150 ml of 2*M* nitric acid (subb.) or hydrochloric acid (subb.) in a 250-ml conical flask (Duran glass), previously steamed with nitric acid and water;
(*c*) ultrasonic treatment in a bath of 2*M* hydrochloric acid (subb.) and 0.1*M* EDTA. For this purpose the vessels were immersed in 100 ml of the cleaning solution contained in a conical flask which was dipped into the water-filled ultrasonic bath for 5–10 min.

After the cleaning the vessels were rinsed with doubly distilled water, closed with a PTFE cover and transported in a desiccator into the clean-room. Here the vessels were again rinsed, filled with 2 ml of 1*M* hydrochloric acid (subb.) from a quartz pipette and covered. Before measurement the solution was homogenized by stirring for 20–30 sec. During storage of up to 3 days the vessels were allowed to stand in a "Plexiglas" box in the clean-room.

Figures 3–6 show the results of the experiments for iron. Even the first measurement after about 90 min shows the appearance of considerable Fe blanks, whereas for Co no measurable contamination occurred even after 8 days' storage.

For Duran glass and quartz vessels the most effective cleaning was achieved by steaming with nitric or hydrochloric acid for 4 hr. Longer steaming times had no additional effect.

The PTFE vessels show totally different behaviour, depending on the method of producing the PTFE and the vessels (*e.g.*, extruded rod from which the vessels are machined on a lathe, or isostatic pressing of the vessel directly from PTFE powder). After tooling on a lathe all the surfaces appear very uneven and fissured when examined under a microscope. On treatment with hot acids at 120° the roughness increases.[3] The sample solution can then penetrate the pores of the material and hence can dissolve impurity particles in the bulk.[25–29] Therefore steaming is not necessarily the optimal cleaning procedure.

Storage of the PTFE-vessel in 5*M* hydrochloric acid (subb.) at 20° for 24 hr had a better effect as regards Fe. The surface of the material was scarcely attacked by this treatment. Figure 5 again shows that the amount of the blanks extracted by the sample solution from the PTFE may differ considerably from batch to batch of PTFE made by a given manufacturer or between the products of different makers. In some of the extruded PTFE, dirt particles can clearly be observed with the naked eye.

The isostatically pressed vessels have a much smoother surface than the extruded PTFE (lathe-made) containers and can be cleaned very effectively by steaming with nitric or hydrochloric acid (see Fig. 6).

In the ultrasonic cleaning method differences again appeared between the extruded PTFE vessels and the isostatically pressed ones (Fig. 4).

Filling and emptying of calibrated flasks[12,13]

Unused 100-ml flasks of different materials and of different pretreatment were used. Flasks made of Duran glass or quartz, provided with a ground-in stopper, were steamed with nitric acid and water, and flasks made of polypropylene (PP) with PP stoppers were stored in 5*M* hydrochloric acid (subb.) for 24 hr and then rinsed three times with doubly distilled water.

On a clean bench all flasks were rinsed again with doubly distilled water, filled with Fe or Co solution (10 ng/ml) and stoppered. They were then stored for up to 12 days. After vigorous shaking of the flasks 2-ml samples were taken by different techniques after

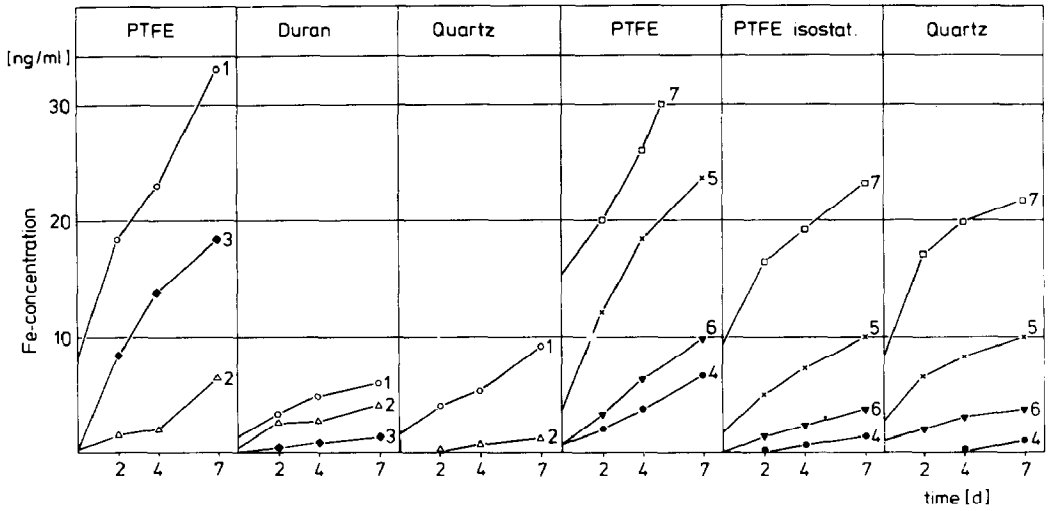

Fig. 4 Contamination of 2 ml of 1*M* HCl with Fe by vessels, cleaned by different techniques (with no. 3 and quartz vessels, no contamination was detected). 1. Rinsed with 10*M* HCl (subb.) and doubly distilled H_2O. 2. Stored in 5*M* HCl (subb.) for 24 hr, rinsed with doubly distilled H_2O. 3. Steamed with HNO_3 for 4 hr, rinsed with doubly distilled, H_2O. 4. Stored in 5*M* HCl (subb.) for 24 hr, rinsed with doubly distilled H_2O. 5. Stored in 0.1*M* EDTA for 24 hr, rinsed with doubly distilled H_2O. 6. Treated in an ultrasonic cleaner with 5*M* HCl (subb.), rinsed with doubly distilled H_2O. 7. Treated in an ultrasonic cleaner with 0.1*M* EDTA rinsed with doubly distilled H_2O.

2 hr, 4, 8 and 12 days. The solutions were homogenized in a small quartz beaker with a quartz stirrer before measurement. For each sampling technique and each solution a separate unused flask was used.

Figure 7 shows occurrence of a considerable contamination of up to several ng/ml.[18,30–32] In addition to the blanks (gains) there are probably losses of iron by adsorption on the surface of the vessels, as is also to be seen in Fig. 7 by the steep decline of the curves. With Co only negligible deviations from the theoretical value were established, probably because of the low concentration and ubiquity of this element. Similar contaminations are also shown in Tables 4 and 5 for the blank concentrations of Fe and Co in different acids and ammonia solutions that have been stored for periods of up to several months.

Contamination by airborne dust

Dust in the air also causes considerable blanks, depending on the element. To investigate the parameters influencing the iron and cobalt blanks, carefully cleaned quartz beakers (volume 20 ml, cross-section 16 cm^2) were filled with 10 ml of 1*M* hydrochloric acid (subb.) and left uncovered in different

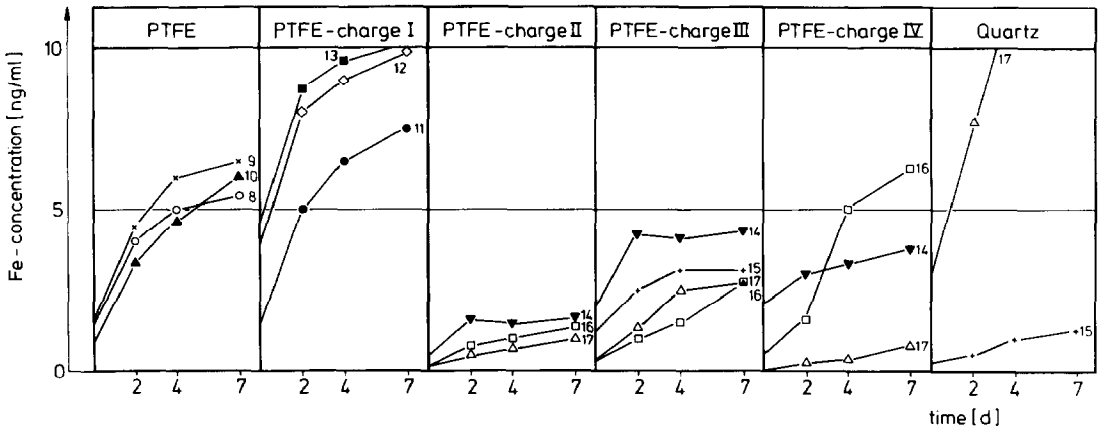

Fig. 5. Contamination of 2 ml of 1*M* HCl with Fe by vessels cleaned by different techniques (with nos. 13, 15 and 16 and vessels of PTFE charge II and IV, and with nos. 8–14 and 16 and quartz vessels, no contamination was detected). 8. Steamed with HNO_3 for 4 hr and H_2O for 1 hr. 9. Steamed with HNO_3 for 6 hr and H_2O for 1 hr. 10. Steamed with HNO_3 for 6 hr and H_2O for 1 hr. 11. Steamed with HCl for 1 hr and H_2O for 1 hr. 12. Steamed with HCl for 3 hr and H_2O for 1 hr. 13 and 14. Steamed with HCl for 5 hr and H_2O for 1 hr. 15. Stored in 5*M* HCl (subb.), rinsed with H_2O. 16. Steamed with HNO_3 for 5 hr and H_2O for 1 hr. 17. Stored in 5*M* HNO_3 (subb.), rinsed with doubly distilled H_2O.

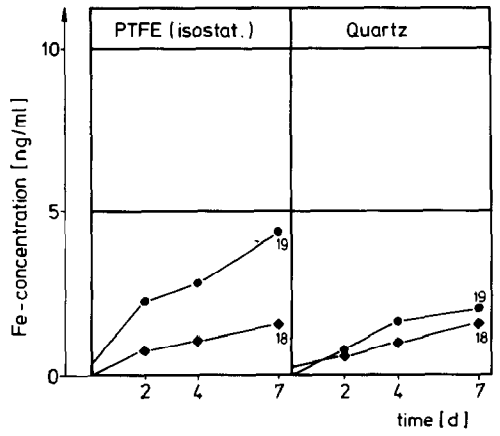

Fig. 6. Contamination of 2 ml of 1*M* HCl with Fe by vessels of isostatic PTFE and quartz (for both materials, after steaming with HNO_3 or HCl, no Fe was detected). 18. Stored in 5*M* HCl (subb.) for 24 hr, rinsed with H_2O. 19. Stored in 5*M* HNO_3 for 24 hr, rinsed with H_2O.

laboratories and on a clean-bench for 6 hr. Figure 8 shows as an example the contamination of the solution in the AAS laboratory on different days.

The dust content of the air is not constant but is influenced by several factors,[12,13] *e.g.*, the condition and history of the laboratory, the kind of air supply and ventilation, the number of persons in the laboratory, and the kind of their activity. The weather also plays an important role. If it is raining the air is generally purer than when it is dry, as is shown by Fig. 8.

For Co no real blanks could be measured, whereas the amount of Fe collected in the 10 ml of acid over a period of 6 hr was up to more than 30 ng. With respect to the surface area exposed, the uptake rate for Fe was up to 2.5 ng/cm^2.

To exclude falsification of the results by blanks from the vessels themselves, identical experiments were made simultaneously on a clean-bench, but no measurable Fe and Co blanks were found.

DISCUSSION

The aim of this contribution is to show the real limits of extreme trace analysis with modern techniques and instruments, by means of the two elements iron and cobalt, which have very different levels of ubiquity. To be able to work in the ng/ml and pg/ml range several conditions have to be fulfilled.

(1) All materials used for apparatus and tools must be as pure and inert as possible. Laboratory ware of, for example, quartz, PTFE, PP, must be cleaned very carefully, for the most part by steaming.

(2) Reagents have to be used in the purest form; acids and most other liquids have to be purified by sub-boiling point distillation.[23] Reagents that can be purified only to a limited degree or by lengthy and sophisticated procedures (and this holds for most solid salts) have to be avoided.

(3) Contamination from the air has to be substantially excluded by use of clean-benches and clean-rooms.

(4) Most important is exact knowledge of the sources of error as well as very careful and accurate work, with special attention paid to all the small but decisive manipulations during the work, which are generally taken no notice of.

Whereas for zinc and magnesium steaming of the vessels with nitric and hydrochloric acid for 4–6 hr and then with water for 1 hr is the optimal cleaning procedure irrespective of the materials used,[12,13] with iron there is a difference in the behaviour of vessels of quartz, Duran glass, isostatically pressed PTFE and extruded PTFE.

For quartz, Duran glass and isostatically pressed PTFE containers, steaming with nitric or hydrochloric acid is the best procedure (see Figs. 4 and 5). Soaking the vessels in acids or EDTA solution for 24 hr or treatment in an ultrasonic bath results in unequivocally poorer cleaning.

All PTFE vessels were contaminated with iron whereas no Co blanks could be detected. Impurities

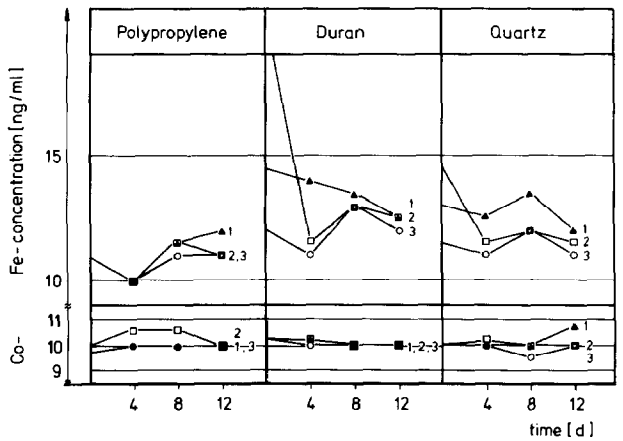

Fig. 7. Errors in different techniques for sampling a 10-ng/ml solution of Fe or Co from 100-ml flasks. 1. Solution poured out. 2. Pipetted by a 1-ml PE pipette. 3. Pipetted by a quartz pipette.

Table 4. Iron blanks of different acids and aqueous ammonia solution, as a function of storage time

Sample	Storage vessel	[Fe], *ng/ml*
1*M* HCl p.A. freshly prepared	2.5-l. bottle (Merck original)	3.9
1*M* HCl p.A. recently prepared	1-l. bottle (Merck original)	3.1
1*M* HCl, Titrisol, Merck 12 months old	1-l. PE bottle	5.5
1*M* HCl z.A. 3.5 months old	100-ml flask (Duran)	14.7
10*M* HCl (subb.) freshly prepared	250-ml quartz bottle, sample taken by quartz pipette	0.6
1*M* HCl (subb.) freshly prepared	250-ml quartz bottle, sample taken by pouring	1.5
15*M* HNO_3 p.A. 1 month old	1-l. bottle (Merck original)	4.2
1*M* HNO_3 p.a. 3.5 months old	100-ml flask (Duran)	38
conc. H_2SO_4 2 months old	1-l. bottle (Merck original)	12.1
conc. NH_3 solution p.A. 1 month old	1-l. bottle (Merck original)	1.4
4*M* NH_3 solution freshly prepared by isothermal distillation	200-ml PTFE bottle	1.3

*Detection limit 0.1 ng/ml

in the bulk of the material and the surface were lower for isostatically pressed PTFE. For vessels made of extruded PTFE a bath in 5*M* hydrochloric acid (subb.) for 24 hr was the best cleaning procedure. Storage in 5*M* nitric acid (subb.) was less effective whereas EDTA gave bad results (see Figs. 4 and 5). Ultrasonic treatment during this soaking process reduces the cleaning time necessary to 10–20 min. PTFE vessels made of extruded material and steamed with hydrochloric acid, nitric acid and water give distinctly higher contamination of pure solutions than untreated containers do. The longer the steaming time the higher the blanks (see Fig. 5).

Figures 4 and 6 show very clearly that vessels manufactured by isostatic pressing of PTFE powder under controlled purity conditions possess much better properties than those made of extruded PTFE. Unfortunately, laboratory ware of this kind is not readily available commercially and can only be obtained by close co-operation between the manufacturer and the analytical laboratory.

As the PTFE powder itself is very pure, owing to the production process, additional protection of the pressing tools, the PTFE powder and the working area against dust, by use of a clean-bench during the critical phase of the production process, should result in apparatus and containers comparable to quartz with respect to purity and contamination. This is es-

Table 5. Cobalt blanks of different acids and aqueous ammonia solution, as a function of storage time

Sample	Storage vessel	[Co],* *ng/ml*
1*M* HCl p.A. 3.5 months old	100-ml flask (Duran)	1.0
1*M* HCl, Titrisol, Merck 12 months old	1-l. PE bottle	1.3
1*M* HCl p.A. freshly prepared	2.5-l. bottle (Merck original)	DL
1*M* HCl (subb.) freshly prepared	200-ml quartz bottle	DL
1*M* HNO_3 p.A. 3.5 months old	100-ml flask (Duran)	DL
4*M* NH_3 solution freshly prepared by isothermal distillation	200-ml PTFE bottle	DL

*Detection limit (DL): 0.3 ng/ml

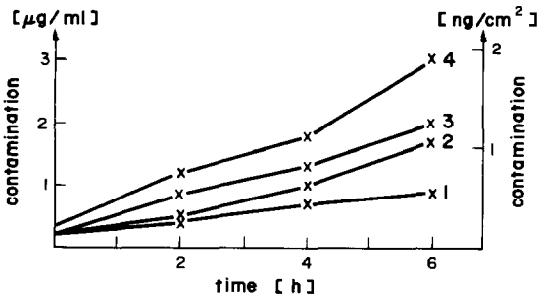

Fig. 8. Contamination of 10 ml of 1*M* HCl (subb.) with Fe by airborne dust in AAS laboratory on different days. 1 and 2, rainy weather; 3 and 4, dry weather. No contamination was detected when a clean-bench was used.

pecially important, since nowadays PTFE products increasingly contain impurities, which occur in different colours and forms such as clouds or sharply bordered inclusions, which can be very clearly seen under a microscope.

As seen in Fig. 8, remarkable amounts of impurities are brought into the sample solution from the air, especially iron. But here, too, generally valid statements are not possible because of the large number of parameters both inside and outside the laboratory that influence the dust content of the air and the composition of the dust particles. The lower the concentration of the element to be determined, the more severe will be the interferences to be expected. Blanks from airborne dust can very easily be avoided by using clean-benches, but because contamination of the sample solution by the vessel is much higher than that by the dust, the use of the expensive and sophisticated clean-room technique is only reasonable if the necessary attention also is paid to careful cleaning of all the containers and instruments.

Another part of our investigations was the sampling of solutions from calibrated flasks made of different materials, by different techniques such as pouring the solution out of the flask, and pipetting by micropipettes (PE-tips) or by quartz pipettes.

Contamination by Co is negligible (<1 ng/ml), but iron blanks (especially with Duran glass and quartz flasks with ground joints) are particularly high, and independent of the sampling technique. With PP-flasks, where the surface of the joint area is smooth, iron blanks occurred only when the solution was poured out of the flasks.

Only a small amount of our experience with very small amounts of elements and low concentrations can be dealt with in this paper, and many phenomena are still unexplored or even undiscovered. Once again, however, it becomes very clearly manifest that, in spite of both very careful working and protective measures, contamination of the sample can by no means be totally avoided. The limitations of modern trace analysis are really set by this.

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GAINS OR LOSSES OF ULTRATRACE ELEMENTS IN POLYETHYLENE CONTAINERS

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Summary—The extent of elimination of losses and reduction of blank values in ultratrace elemental analysis can only be ascertained by comprehensive investigations for each element separately. Different, and partially conflicting precautions are found to be needed in the determination of manganese, copper, selenium, and mercury by neutron-activation analysis when polyethylene irradiation containers are used.

Many problems in elemental analysis are associated with the level of concentration to be determined, and it is therefore expedient to classify the elements according to their content in a sample.[1,2]

Major and minor elements: 0.01–100%
Trace elements: 0.01–100 mg/kg
Ultratrace elements: <0.01 mg/kg

In the determination of major or minor elements the problem of the blank value can usually be disregarded, and in trace analysis it is normally easy to keep the blank value small relative to the content in the sample. In ultratrace analysis, however, the blank value is quite often comparable with the total amount present in the sample, and any uncertainty in the blank is directly reflected in the analytical result.

According to their uncertainty we may distinguish three types of blank:[3]

(*a*) known blanks for which *correction* is *possible* with complete confidence and often without significant loss of precision;

(*b*) estimated blanks, which depend on supplementary information on the particular sample analysed; correction is more uncertain, but a *maximum error* can usually be given; and

(*c*) unknown blanks, for which *no correction* can be applied even when potential sources of blank errors are known; the only solution to this problem is to reduce the individual blank contributions so that they are insignificant.

Different analytical methods have different blank problems, but one is common to all, namely the influence of the container holding the sample. In neutron-activation analysis almost all other blank problems have been eliminated, and this method is therefore particularly amenable to the study of container blank problems.

Neutron-activation analysis is the most sensitive analytical method available for more than 30 trace and ultratrace elements, while it is virtually insensitive to carbon, oxygen, and hydrogen. Polymers, particularly polyethylene, are therefore widely used as a container material for many different types of samples during irradiation in neutron-activation analysis, and some of the blank problems associated with the use of polyethylene in ultratrace analysis deserve consideration.

MATERIALS AND METHODS

In many respects polyethylene is an excellent material for trace element analysis. Its mechanical properties are satisfactory at normal temperatures, and it is very resistant to mineral acid and alkalis, as well as to many solvents. No significant adsorption or leaching takes place in most dilute aqueous solutions, which may therefore be stored without detectable changes for a considerable length of time.[4] Polyethylene is more resistant to radiation than most other polymers and as a container it is available in a variety of sizes and types. The pure form is polymerized at high pressure, without a catalyst, and this low-density form of polyethylene is a preferred material for irradiation containers in neutron-activation analysis.

One such container,* with a useful volume of approximately 1.2 ml and a weight of 1.1 g, has been widely used in the determination of trace elements by neutron-activation analysis; it therefore seemed logical to make a further study of its applicability as an irradiation container for ultratrace analysis.

The trace element levels in these polyvials were determined by instrumental neutron-activation analysis of a number of containers from different batches delivered at different times over a period of several years. Considerable variation was found for most elements, and mean values were not constant from batch to batch; only the order of magnitude is therefore given for the results presented in Table 1.

*The 0.4-dram polyvial, manufactured by Ron McIntosh, Santa Monica, California, USA.

Table 1. Trace elements in 0.4-dram polyvials (type I) determined by instrumental neutron-activation analysis

Elements in order of concentration	Total quantity,* μg
Fe	1–10
Cl, Na, K, Al, Zn	0.1–1
Cu, Cd, Cr, Br, Mn	0.01–0.1
Sb, W, Co, As, Au	0.001–0.01
Se, V, La, Ag, Sc	<0.001

*Weight of container 1.1 g.

In determination of all the major and most of the minor elements in a 1-g sample, the contribution from the polyethylene container under consideration can be ignored. Many trace elements may be determined without significant loss of precision and accuracy by counting the irradiated container with and without the sample. In this way an accurate correction is made for the individual blank value.

Trace element concentrations comparable with those of the irradiation container are more difficult to correct for the influence of the container. The uncertainty in the determination of the container blank now has a direct influence on the precision of the analysis and may be decisive for the attainable detection limit. Transfer of the sample to an unirradiated container before counting, particularly when combined with a radiochemical separation, will reduce the detection limit considerably.

This may, however, introduce both positive and negative blank values from interactions between the sample and container; the true blank value is affected in one direction by leaching of trace elements from the polyethylene and in the other by adsorption of trace elements from the sample. An estimate of the maximum blank errors is made by analysing samples stored in the container for different periods of time, supplemented by analysis of redistilled water blanks.

Leaching of the container with strong mineral acids before irradiation removes trace elements from the surface: Mitchell[5] found no release of the elements manganese, copper, *etc.* from polyethylene after treatment with 50% nitric acid. Robertson[6] recommended soaking in 8*M* reagent-grade nitric acid for several days followed by rinsing with doubly-distilled water, and Karin *et al.*[7] found a 3-day leach optimal for removal of trace elements, with no difference between use of concentrated and 8*M* nitric acid.

When treated in this way the polyethylene container does not interact significantly with most samples, and removing the sample from the container does not introduce any container blank with respect to trace elements.

Under the influence of radiation, however, some elements form volatile compounds which may be lost during transfer from the irradiation container to the counting vial. This applies to the halogens[8] and to mercury,[9] but may also be expected for selenium, arsenic, *etc.* Wherever possible these elements should be determined by counting the irradiated sample before transfer, and then the container blank afterwards. Although mercury is not found in the container before irradiation, some is often found in the container blank after transfer of an irradiated sample containing this element.[3]

In ultratrace analysis correction for the blank from the container is usually impossible, and perhaps the best way to eliminate the problem is to irradiate the sample without contact with the container. This requires irradiation of samples from which the surface layers can be removed before measurement; in the case of liquids, the temperature must be kept below the freezing point until irradiation is completed and the surface layers subsequently melted away.

This method is often impracticable, however, and the alternative is to evaluate the actual positive and negative blank errors for each element of interest under real conditions. Some experiments have, therefore, been carried out for the elements copper, manganese, mercury and selenium.

EXPERIMENTAL

Irradiation containers

I. Standard 0.4-dram polyvials from Ron McIntosh, Santa Monica, California, (*a*) cleaned by rinsing with redistilled water only, (*b*) leached with 8*M* nitric acid for 3 days, or (*c*) soaked in 3% hydrogen peroxide overnight.

II. Special 1.5-ml polyvials, courtesy of Dr. Nic Spronk, Vrije Universiteit, Amsterdam.

III. New 5-ml polyethylene ampoules from Atomic Industrial Co., Tokyo.

All containers were cleaned with redistilled water immediately before use. After filling, they were sealed by radiant heat and leak-tested by immersion in ethylene glycol and evacuation according to ANS/N 542.[10]

Reagents

Redistilled water, conductivity <4 μS.

Dimethyl selenide, Alfa Inorganics, Beverly, Massachusetts.

Radioactive tracers

Mercury metal, labelled with ^{203}Hg, 60 MBq/g.

Mercury(II) nitrate, 5 mg/l. in 0.1*M* nitric acid, labelled with ^{203}Hg, 60 MBq/l.

Determination of container blank

Correction for redistilled-water blanks was made by analysing duplicate samples of freshly redistilled water, taken from the same bottle at the same time and transferred to polyethylene irradiation containers in a clean-room. One container was treated according to the planned investigation, the other was cooled by immersion in a mixture of ethanol and solid carbon dioxide, so that the water was frozen immediately on contact with the container wall. Both samples were irradiated with thermal neutrons at a flux of approximately 7×10^{12} n.cm^{-2}.sec^{-1} for 1 hr. The container with frozen sample in it was surrounded by solid carbon dioxide during the entire irradiation, and afterwards the frozen sample was removed from the container so that the surface layer could be allowed to melt away. The solid core of ice that had not been in contact with the container wall was analysed and used to give the blank values for the redistilled water used in the experiment.

Analytical method

Copper, manganese and selenium were determined by neutron-activation analysis with radiochemical separation.[11] All counting of radioactive indicators was done with 3 × 3 in. NaI(Tl) scintillation detectors.

Container permeability

Standard 0.4-dram polyvials were exposed to ^{203}Hg-labelled mercury vapour in a desiccator containing 10 mg of mercury at room temperature and pressure. The ^{203}Hg activity was measured after different exposure times, to find the rate of diffusion of mercury into the polyethylene. All results were normalized to 1 ml of ^{203}Hg standard aqueous solution in a sealed Pyrex glass container with the same dimensions as the polyvials.

Standard 0.4-dram polyvials containing approximately 1 ml of ^{203}Hg-labelled mercuric nitrate solution were kept on an open bench in the laboratory under normal room ventilation and temperature. Measurements were made exactly as above to determine losses of mercury as a function of time.

A new 5-ml polyethylene ampoule (type III) containing an approximately 100-ppm aqueous solution of dimethyl selenide was stored at −20°. The total selenium content was determined after different lengths of time by instrumental neutron-activation analysis with ^{77m}Se ($t_{1/2}$ = 17.4 sec) as indicator;[12] with a total analysis time of less than 90 sec, the sample remained in the frozen condition for the duration of the experiments.

RESULTS

Adsorption and desorption

As shown in Table 1, selenium and mercury were not found in the standard polyethylene containers used for irradiation, whereas manganese and coppper were present at trace levels. Container blanks were therefore determined for these two elements after cleaning by the three different methods, and the mean values and standard deviations based on results for 10–15 different containers are shown in Table 2.

Up to 10% of the manganese present could be released to the water during irradiation, and the variability of this blank, *a*, exceeds the normal level of manganese in human serum.[13] Treatment with nitric acid, *b*, reduced the total manganese present by about 25%, in good agreement with the investigations of Karin;[7] at the same time the maximum release of manganese to the water dropped by a factor of more than two. The variability in the amount released is, however, still comparable with the total level to be determined. Hydrogen peroxide was found to be just as effective as nitric acid for reducing the blank value, *c*, and much better for reducing its variability.

A significant positive correlation was found between the total amount of manganese in the polyvial and the quantity released to the redistilled water blank, and a search was made for polyethylene vials with lower manganese content.

Containers of type II, with only about half as much manganese, were tested in the same manner, and the mean and standard deviation for manganese in redistilled water blanks from 10 containers are given in Table 3. Only about 1% of the manganese was released during irradiation of this type of container, and no correlation with total manganese was observed. The contribution from the container still exceeds the concentrations usually found in the redistilled water, and the variability severely limits the precision.

Unlike the other two, containers of type III could not be analysed for manganese by instrumental neutron-activation analysis: the total content was therefore determined by radiochemical separation[12] only after the redistilled water blanks had been measured in 10 containers. In this case the mean value and standard deviation of the redistilled water blank are almost indistinguishable from the concentrations found in the redistilled water alone; both adsorption and desorption can, therefore, be disregarded at the 0.01-ng/ml level for this container.

It is obvious from these results that the total manganese content of the irradiation container is not a useful guide to the selection of containers for ultra-trace analysis.

The results for coppper given in Table 2 contrast sharply with those for manganese. No reduction in copper content is brought about by leaching with nitric acid; on the contrary, the release of copper into the water is increased by an order of magnitude. Hydrogen peroxide has a similar, but less pronounced effect, and the lowest blank value is obtained when only redistilled water is used for cleaning the container. In fact, the redistilled water blank is not significantly greater than the concentration of copper found in the redistilled water alone, so neither nett adsorption or desorption takes place at the 0.4-ng/ml level in water.

Gains and losses

The absence of selenium and mercury from the irradiation container does not rule out the presence of a blank value for these elements.

Table 2. Blank values for 0.4-dram polyethylene irradiation containers (type I) with 1.1 ml of redistilled water

		Irradiation container blank, *ng*		Redistilled water blank, *ng/ml*	
Cleaning	Method	Mn	Cu	Mn	Cu
H_2O	*a*	24 ± 7	91 ± 41	1.1 ± 0.7	3.4 ± 0.4
HNO_3	*b*	18 ± 5	101 ± 15	0.4 ± 0.4	33 ± 9
H_2O_2	*c*	22 ± 8	105 ± 49	0.5 ± 0.1	10 ± 4

Table 3. Manganese blank values for different polyethylene irradiation containers

Irradiation container Type	Origin	Mn blank, *ng*	Redistilled water blank Volume, *ml*	Manganese, *ng/ml*
I	California	22 ± 8	1.1	0.5 ± 0.1
II	Amsterdam	14 ± 3	1.1	0.15 ± 0.05
III	Tokyo	19 ± 11	4.5	0.02 ± 0.01

An empty, closed polyvial (type I) absorbs up to approximately 300 ng of mercury when exposed to mercury vapour at room temperature. Initial permeation proceeds as shown in Fig. 1 with a diffusivity of approximately 1 μm^2/sec whereas desorption takes place much more rapidly, as shown in Fig. 2.

The coefficient of diffusion is thus concentration-dependent, perhaps in direct proportion to the amount of mercury present in the polyethylene. Equilibrium with the environment is reached within approximately one week, and the content of mercury in the polyvial then responds rapidly to temperature change or other causes of change in the partial vapour pressure.

This dynamic equilibrium, however, applies only to an empty container, where the mercury partial pressure is the same inside and outside. If the sample in the container may react with the mercury no such equilibrium is reached.

Mercury may be removed by oxidation with nitric acid, and Fig. 3 shows the uptake of mercury during exposure to ^{203}Hg mercury vapour at room temperature, as a function of time for various media. Samples A and B both contain 1 ml of 10*M* nitric acid with 5 and 1 μg of non-radioactive mercury(II), respectively; C and D contain 1 ml of 0.1*M* nitric acid with the same amounts of mercury.

The higher uptake by A and C relative to B and D shows that mercury(II) participates in the oxidation process:

$$Hg^{2+} + Hg \rightarrow Hg_2^{2+} + 12 \text{ kJ/mole} \quad (1)$$

The higher uptake by A and B relative to C and D is caused by the subsequent oxidation of mercury(I) to mercury(II) by the more concentrated nitric acid. This gives rise to an exponential increase in the mercury content, according to reaction (1), but even after an uptake of more than 80 μg of mercury by A the original ratio of approximately 5 between the total contents in A and B is preserved.

In the dilute nitric acid no oxidation takes place, and in C and D the uptake of mercury according to reaction (1) is limited by the amount of mercury(II) present from the beginning. However, the limiting uptake in C was only between 1 and 2 μg of mercury, which means that some mercury(II) has been reduced by other processes.

This is confirmed by Fig. 4 which shows the loss of mercury from a ^{203}Hg-labelled 0.1*M* nitric acid solution of mercury(II) (5 μg/ml), freshly dispensed and sealed in a polyvial. No loss is observed during the first 2–3 weeks, but then more than 3 μg is lost over a period of 7–8 weeks. Similar losses are observed for higher concentrations of mercury and nitric acid. Reduction of mercury(II) to mercury must therefore be caused by the polyethylene container.

Losses of selenium have been observed from solutions containing dimethyl selenide,[12] which is produced in organisms during the metabolism of selenium. In order to reduce these losses, samples of

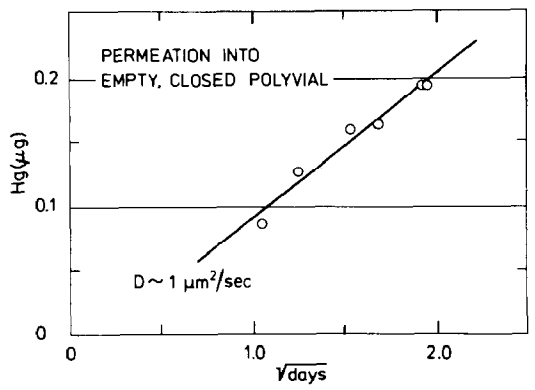

Fig. 1. Diffusion of mercury into a 0.4-dram polyethylene container (type I) exposed to mercury vapour at room temperature.

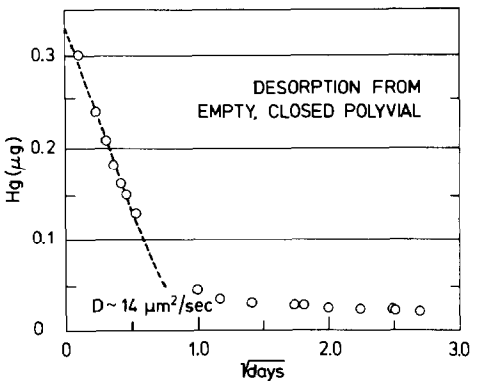

Fig. 2. Diffusion of mercury out of a 0.4-dram polyethylene container (type I) saturated with mercury at room temperature.

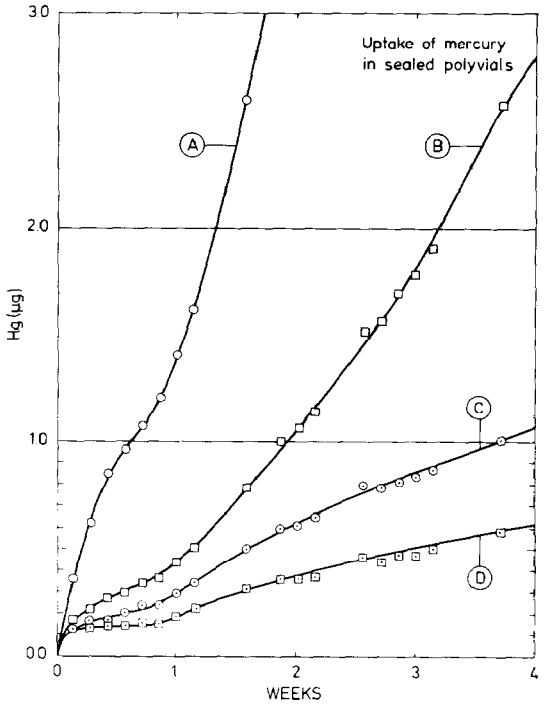

Fig. 3. Uptake of mercury in 0.4-dram polyethylene containers (type I) exposed to mercury vapour at room temperature, initially filled with solutions of mercury(II) nitrate in nitric acid. A, 5 µg Hg in 10*M* HNO_3; B, 1 µg Hg in 10*M* HNO_3; C, 5 µg Hg in 0.1*M* HNO_3; D, 1 µg Hg in 0.1*M* HNO_3.

serum are stored at −20°, and Fig. 5 shows the losses observed over a period of almost 3 months for a polyethylene container of type III. The initial losses are approximately 5 µg of selenium per day.

DISCUSSION

The release of manganese and copper from a polyethylene irradiation container to a sample has been investigated by several authors.[7,14,15] In contrast to the present study the container was irradiated empty, and inactive sample material was added only after completion of neutron activation.

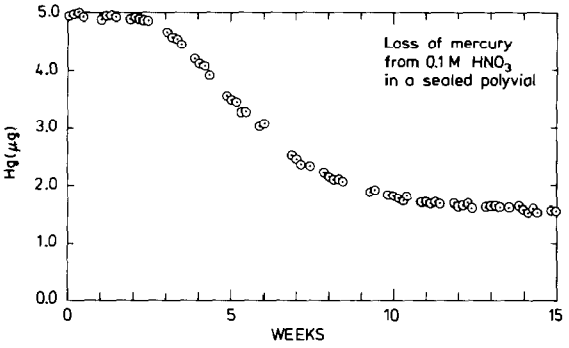

Fig. 4. Loss of mercury from a 0.4-dram polyethylene container (type I) initially filled with 5 µg of Hg as mercury(II) nitrate in 0.1*M* nitric acid.

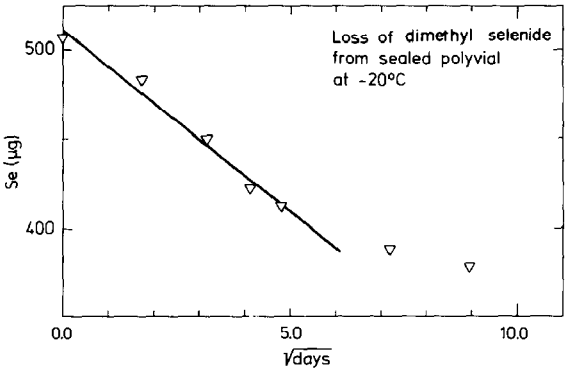

Fig. 5. Loss of selenium at −20° from a polyethylene container (type III) initially filled with approximately 5 ml of aqueous dimethyl selenide solution.

Brune[14] observed an average release of 0.07 ng of Mn and 6 ng of Cu per cm^2 of polyethylene which has been leached with nitric acid before irradiation. This is in remarkably good agreement with the results in Table 2(b) for the containers of type I, which have an inner surface area of about 5 cm^2 in contact with the water. Karin *et al.*[7] found that much higher amounts could be leached, but did not perform any leaching before irradiation. In both cases the contents of manganese and copper in the containers were higher than in those used in the present investigation.

Versieck[15] determined the actual blank value for the two elements by shaking the irradiated polyvial with lyophilized serum. In this way only a minor fraction of the leachable manganese and copper is transferred to the sample.

All investigations indicate that the manganese and copper are released from the inner surface of the container; it is therefore surprising that complete elimination of such blank contributions cannot be achieved by thorough cleaning before irradiation.

The effects of irradiation include the Szilard–Chalmers effect, or nuclear recoil, which may liberate activated nuclides from chemical bonds. However, calculations by Brune[14] clearly show that this effect could explain only a small fraction of the quantities released. Other chemical effects of radiation include the formation of hydrogen peroxide in aqueous media, which may be responsible for the dissolution of some manganese in the form of MnO_2. This and other chemical effects of reactor irradiation depend strongly on the composition of the sample being irradiated and therefore make a blank correction impossible.

Under the optimum conditions determined separately for copper and manganese, no release could be detected by using redistilled water as a sample and therefore no blank correction should be made for levels exceeding 0.01 and 0.4 ng/ml respectively.

Loss of mercury from solutions in polyethylene containers was originally attributed to adsorption[16] or volatilization,[17] and should be prevented by the

addition of mineral acid, often in combination with oxidants.[18–23] The addition of sulphide, cysteine,[24] thiocyanate[25] or certain sulphur-containing organic compounds[26] has been found to reduce or prevent losses. Mahan and Mahan[27] noted, in agreement with Fig. 4, that losses began to accelerate by the second day and recommended that analysis be done on the day the sample was collected.

Bothner and Robertson[28] found gains of mercury in acidified sea-water stored in polyethylene containers, and Cragin[29] reported uptakes of up to 200 ng per day in an oxidizing pure-water sample in a 1-litre polyethylene bottle stored in a Class 100 clean-air cabinet.

Similar effects may be responsible for the poor agreement observed in an intercalibration of mercury in sea-water.[30] While most biological samples do not sustain losses of mercury,[26] they do pick up mercury from the air, even through a sealed polyethylene envelope.[31]

Increased losses of mercury after neutron activation in polyethylene containers were reported by Bate[9] for a 1.5*M* nitric acid solution, but McFarland[32] and Larson and Tandeski[33] found no losses during irradiation of solutions containing more concentrated nitric acid or oxidizing agents. Guinn[34] proposed that radiolysis of the water causes reduction to elementary mercury, which then diffuses through the polyethylene. This mechanism was confirmed later[35] by irradiating sealed polyvials of type I containing ^{203}Hg-labeled mercury(II) nitrate solutions, with a linear accelerator. Precipitation of metallic mercury was here observed at doses as low as a few kGy.

At temperatures of $-20°$ neither gains[29] nor losses of mercury[26] have been detected, and this is probably the best method of circumventing the diffusion of mercury during storage. However, irradiation in a nuclear reactor is seldom possible at low temperatures, and transfer to quartz containers is advisable.

Diffusion of selenium through polyethylene from solutions of dimethyl selenide is so rapid at room temperature that trace analysis is virtually impossible. Storage at low temperature is therefore imperative, but in contrast to mercury the losses continue at a significant rate. Only by analysis on the day of collection can reliable results be expected.

CONCLUSION

The reduction to insignificance of gains and losses associated with the use of polyethylene containers in ultratrace analysis cannot be achieved by adherence to any standard procedure. Large systematic errors can be avoided only by carefully evaluating for each element all possible sources of positive or negative contributions to a blank value.

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LOSSES OF METABOLICALLY INCORPORATED SELENIUM IN COMMON DIGESTION PROCEDURES FOR BIOLOGICAL MATERIAL

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Summary—Two common procedures for wet destruction of biological materials for subsequent determination of selenium have been investigated. Rat organs and biological fluids were endogenously labelled with ^{75}Se to monitor losses during the procedures. Addition of nitric and perchloric acids with gradual heating up to 210° seemed to be the best method: at this temperature the labelled selenium was still recovered quantitatively, and the destruction was fast and efficient.

Since the discovery that selenium is essential to human health, the need for information about the concentration of this element in human tissues has become more urgent. For the determination of trace elements in biological samples a pretreatment is needed which will destroy all organic matter but retain the element of interest. As some selenium species are known to be volatile at higher temperatures, it is necessary to investigate possible losses at every step of the determination procedure.

For the drying step, Behne and Matamba[1] found no differences were caused in the selenium concentrations in blood serum by drying at 90°. Fourie and Peisach[2–4] studied the loss of selenium metabolically incorporated in zoological specimens and found no significant losses caused by drying at up to 105°. Iyengar *et al.*[5] studied the retention of radioactive selenium incorporated into biological tissues when different drying procedures were used and proved that, with heating at up to 120°, the loss was less than 5%. This evidence indicates that the drying of biological material is not a critical step.

For the decomposition step, Hall and Gupta[6] found selenium losses from 10% up to 60% during wet ashing with nitric and perchloric acids at temperatures from 150° up to 200°. We therefore first tested a digestion procedure with 110° as the highest temperature. A predigestion step at a lower temperature was included in this procedure, as suggested by Olson,[7] with the aim of hydrolysing easily oxidizable biological material. The temperature was then gradually increased in the presence of a sufficient amount of nitric acid to prevent charring of the organic material and consequent evolution of H_2Se from the digestion mixture.[8–11] Olson[7] stated that since some organoselenium compounds can resist acid attack by perchloric acid up to 200°, the destruction has to proceed at above this temperature for at least 15 min. After recovery experiments with the $(CH_3)_3Se^+$ ion, Olson *et al.*[10] extended the heating period to 30 min. Hall and Gupta[6] warned against using temperatures above 230°. Therefore in the second digestion procedure tested, the final temperature was set at 210°. In a recent recommendation for the determination of selenium in biological materials[12] sulphuric acid is added, but only after complete oxidation of the biological matrix, since it is especially useful for driving off the remaining nitric and perchloric acids, both of which interfere in the recommended fluorimetric determination procedure. However, since sulphuric acid enhances the risk of charring the biological matrix,[11,13,14] it was not added in the two digestion procedures used in this study.

All the published studies on the recovery of selenium during wet destruction have made use of spikes, sometimes containing radioactive tracers, which were added to the samples on the assumption that the matrix selenium and the spike, mostly added in the inorganic form, would behave similarly during the matrix decomposition.[13,15,16] This approach is questionable, however, since the concentration ranges of the trace elements in biological tissues vary over several orders of magnitude and especially since it has not been confirmed that the inorganic form and the organically bound selenium behave in the same way in the decomposition procedures.

A better appraisal is obtained by the analysis of standard reference materials, which have been analysed for selenium by a non-destructive technique, and which resemble the various types of biological samples as closely as possible. In another approach, possible losses can be studied during digestion of tissues in which radioactive selenium has been biologically incorporated.[17]

In the present investigation the radioactive ^{75}Se isotope was injected intraperitoneally into rats in

order to incorporate this element into the body tissues during normal metabolic functioning and to study the recovery of the selenium in its naturally occurring form, when the two different wet digestion procedures were applied.

EXPERIMENTAL

Reagents and apparatus

All reagents were of analytical grade. In all experiments ^{75}Se ($t_{1/2}$ = 120 days; E_γ = 265 keV) was used. Sodium selenite with initial specific activity 5.9 μCi per μg of Se, and sodium selenate with initial specific activity 6.3 μCi per μg of Se, were obtained from the Radiochemical Centre, Amersham, England. The various organs and biological fluids were decomposed in a 20-position Tecator automatic digestion apparatus (Höganäs, Sweden), including a No. 1005 heating unit and a No. 1008 control unit. The total height of the digestion tubes and specially constructed condensers was 55 cm. In the first set of experiments, the gamma-emitting nuclide was counted on a Ge(Li) detector with a 4096-channel analyser. In the second experiment a Berthold automatic gamma sample-changer unit LB MAG 312 (Wildbad, Germany) was used for quantitatively analysing the radioactivity. The detector consists of a 3 × 3 in. NaI(Tl) well-type scintillation crystal with a well diameter of 22 mm and is rather insensitive to small variations in counting geometry.

Procedure

Two adult Wistar rats with average body weight of 250 g were injected intraperitoneally with 4 μg of selenate (25 μCi) and 4.25 μg of selenite (25 μCi). After injection the rats were placed in metabolism cages for collection of urine and faeces. Standard food was supplied and they received water *ad libitum*. Every 24 hr, urine and faeces were collected and after 9 days the animals were killed. Samples of whole blood, brain, heart, kidney, liver and spleen were removed. The initial radioactivities of ^{75}Se were measured in all samples. The biological tissues were taken for destruction and the residual activity in the empty vials was checked. The digestion procedure was based on the comprehensive literature evaluation of Verlinden.[18] Concentrated nitric acid (5 ml per g of sample) was added to react with the organs overnight at room temperature; subsequently the solution was heated at 70° for 23 hr and then at 100° for 20 hr. The condensers were then removed, the volume was reduced to a few ml and 2 ml of perchloric acid were added. The mixtures were heated for 1 hr at 107° with the condensers replaced. After cooling and addition of 1 ml of concentrated hydrochloric acid the mixture was heated for 5 min at 100° to reduce all selenium to the quadrivalent state, so that it could be determined by common analytical procedures. The radioactivity of the solutions obtained after the destruction of organic matter was measured with the same geometry in the Ge(Li) detector.

In a second experiment the rats were kept alive for 30 days after injection of 33 μg of ^{75}Se (80 μCi). Half of the selenium was added as selenite, the other half as selenate. This higher amount of selenium is still only one thirtieth of the reported minimum lethal dose of selenate or selenite injected into rats,[19,20] so it is improbable that the metabolic incorporation was occurring under pathological conditions. The rats were killed, and the initial radioactivity was measured in the whole blood, brain, heart, kidney, liver, lung, muscle, spleen and thyroid. Urine and faeces were collected during the 30 days and were also included in this tracer experiment. The digestion scheme finally adopted, following recommendations in the literature,[6,7,9,10,13,14,18,21] involved addition of concentrated nitric acid (10 ml per g of sample) to the organs and predigestion overnight at room temperature; heating at 75° for 24 hr and then at 120° for 6 hr until the solution volume was reduced to 4 ml; addition of 5 ml of perchloric acid (70%) and heating (up to 215°) for 30 min; cooling and reduction of selenium to the quadrivalent state by heating for 5 min with concentrated hydrochloric acid. A standard solution containing 0.66 μg of ^{75}Se (0.34 μg of selenium as selenite and 0.32 μg as selenate) was also taken through the entire decomposition procedure. The radioactivities before and after destruction were measured in the NaI(Tl) detector.

RESULTS AND DISCUSSION

Table 1 summarizes the results for the retention of the radioactive isotope after decomposition at the lower temperature ($\leqslant$110°) of heart, liver, spleen, kidneys, brain, blood, urine and faeces of the two rats following 9 days of metabolic incorporation. For the biological fluids, the recovery is nearly complete and the reproducibility satisfactory. For some of the tissues the results are apparently too high and the reproducibility is somewhat poor. This is due to some uncertainties in the counting geometry, that could not be avoided during the counting of the whole organs and faeces because, in view of the low subtoxic quantities of selenium used, the gamma-activities were low, necessitating measurement close to the Ge(Li) detector. Still, it seems that, in the low-temperature decomposition process, the selenium is retained virtually quantitatively in the final solution. However, some lipid material remained and the faeces were not completely destroyed in this procedure. Verlinden[18] also found low selenium concentrations for blood and serum by AAS after this decomposition step.

In the second experiment, the incorporation of a higher amount of selenium took place over a longer period, and muscle and lungs were also investigated because selenium is known to affect muscle dys-

Table 1. Recovery of ^{75}Se, metabolically incorporated for 9 days, after decomposition at 110°

Samples	Recoveries,* %
Heart	90, —
Liver	95, 126
Spleen	95, 111
Kidney	112, 95
Brain	117, 107
Blood	95, 95
Urine	
(after 1 day)	93, 97
(after 6 days)	94, 102
(after 8 days)	102, —
Faeces	
(after 1 day)	103, —
(after 6 days)	103, —
(after 8 days)	114, —
Overall mean	102 ± 10

*Individual values, measured by Ge(Li) spectrometry, for two rats.

Table 2. Specific activity of ^{75}Se metabolically incorporated for 1 month, in different organs and biological fluids, and its recovery after decomposition by the extended wet-acid procedure at high temperature (210°)

Samples	Specific ^{75}Se activity, *cpm/g*	Recovery,* %	Concentration found, *μg/g wet weight*
Heart	18.0×10^3	101, —	0.33
Liver	38.4×10^3	—, 91	1.23
Spleen	28.5×10^3	95, —	0.42
Kidney	58.0×10^3	107, 102	1.21
Brain	7.8×10^3	99, 102	0.14
Blood	26.1×10^3	110, 96	0.36
Urine (1 week)	18.4×10^3	89, —	
(2 weeks)	13.0×10^3	82, —	
(1 month)	8.5×10^3	102, —	
Thyroid	15.1×10^3	84, —	0.35
Muscle	10.0×10^3	109, 90	0.11
Lung	17.1×10^3	111, 96	0.32
Standard ^{75}Se solution	6.5×10^8	97, 100	
	Overall mean: 97 ± 8		

*Individual values, measured by NaI(Tl) and Ge(Li) spectrometry, respectively, for one rat.

trophy, and selenium accumulation in the lungs has been reported.[22,23] The samples were also measured with a well-type NaI(Tl) detector that is less sensitive to geometry effects. Before measurement of the initial radioactivity on the Ge(Li) detector, the organs and fluids were allowed to stand for 24 hr in 10–20 ml of concentrated nitric acid, so that a more comparable geometry was obtained before and after the digestion. Table 2 summarizes the results for the recovery of incorporated ^{75}Se after the extended wet destruction with nitric and perchloric acids, and with heating to 210°.

The highest specific activities were found in kidney, liver, spleen and blood, while brain showed the lowest activity. These findings agree quite well with the literature data on the distribution of subtoxic amounts of ^{75}Se in the tissues of rats.[24] Liver and kidney seem to be the target organs in exposure of rats to selenium,[23,25] whereas in man the lungs appear to accumulate the selenium.[22,23] Harr *et al.*[25] stated that the liver-to-kidney selenium ratio is dependent on the level of selenium supply, so after addition of higher doses, higher liver-to-kidney ratios are found. This could be explained by the fact that the conversion of selenite into volatile selenium compounds takes place largely in the liver,[26] and that these can be exhaled by the rats.[27] Another way of eliminating high amounts of selenium is in the urine, in which the trimethylselenonium ion is the major metabolite excreted:[28–31] hence, the kidneys will also show higher selenium concentrations.

From Table 2 it can be concluded that even at the high decomposition temperature of 210° the recovery of ^{75}Se is almost complete for the different organs. The clear solutions obtained after digestion also point to a complete destruction of fatty materials. Hence this method seems excellent for determination of selenium in biological samples. Only thyroid and some urine samples showed a somewhat lower recovery. No explanation can yet be offered for the lower recovery from thyroid tissue. For urine, losses of selenium after wet or dry ashing are widely reported in the literature. Schwarz[32] recommended a closed system for accurate analysis, since serious losses of the element, as much as 75–84%, were observed during open wet digestion. In a dry ashing method for urine, Roquebert and Truhaut[33] found serious losses. Probably incomplete digestion of the volatile selenonium ion is the major reason for these losses. Geahchan and Chambon,[31] however, found no difference in selenium recovery when they compared destruction of urine in a closed system (Teflon Parr bomb), with an open digestion method (using a nitric–perchloric acid mixture) similar to the procedures presented above.

CONCLUSION

Selenium-75 was injected intraperitoneally to incorporate the element into the various organs of rats in a natural physiological form. The highest specific activity was found in kidney, liver, spleen and blood, while brain accumulated the least activity.

Heating with nitric–perchloric acid mixture up to 210° appeared to be a most convenient method for decomposing the different organs and biological fluids: the matrix was completely destroyed, and no significant losses of the incorporated selenium could be observed (except for thyroid and some urine samples). The decomposition procedure is rather complex and long, but can easily be performed in an automatic digestion apparatus. The resulting solutions can be used for selenium determination by, for example, hydride-generation atomic-absorption spectrometry[18] or energy-dispersive X-ray fluorescence analysis after selective reduction and preconcentration on activated charcoal.[34]

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POTENTIAL INTERFERENCES INHERENT IN NEUTRON-ACTIVATION ANALYSIS OF TRACE ELEMENTS IN BIOLOGICAL MATERIALS

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Summary—A comprehensive review is given of how neutron-activation analysis for trace elements in biological matrices can be jeopardized by radiation damage, by the impurities present in the packing material or by nuclear interferences of major elements. Systematic errors during the counting process and the quantitative interpretation of the γ-ray spectra should not be disregarded.

The difficulties of obtaining a biological sample which remains unchanged during sampling, sample treatment and storage are many and various.[1] If the original trace element content remains representative of the material to be analysed, reactor neutron-activation analysis (RNAA) is an ideal technique. It possesses an alleged freedom from blank corrections and is a multi-element method, often a purely instrumental one. Using RNAA, most chemical treatments can be postponed till after irradiation, so that the hazard of contamination prior to irradiation can be controlled. However, biological samples undergo minute or pronounced radiation damage which may affect some particular trace element determinations. A further drawback, innate to RNAA, arises from the impurities present in the materials of construction of the irradiation vials. In addition, some matrix components or major elements occurring in biological material can give rise to nuclear interferences, putting the exact determination of certain trace elements into jeopardy. The analyst should also be aware of those different systematic errors which are proper to activation analysis.[2] A crucial point is the correct measurement of the radiation.

This paper reviews the main difficulties liable to occur in the RNAA of biological materials.

PRINCIPLE OF REACTOR NEUTRON-ACTIVATION ANALYSIS (RNAA)

RNAA consists of neutron bombardment of a given material, followed by measurement of the induced radioactivity. The most usual type of activation is done with thermal or epithermal neutrons in a nuclear reactor, according to the reaction ${}^{A}_{Z}X(n,\gamma)^{A+1}_{Z}X$. The total number of radioactive atoms $^*N$ of a particular nuclide is given by equation (1):

$$^*N = \phi_{th} \cdot \sigma_{th}\left(1 + \frac{\phi_{epi} I_0}{\phi_{th}\sigma_{th}}\right)\frac{N \cdot S}{\lambda} \qquad (1)$$

where σ_{th} is the cross-section for the (n,γ) reaction with thermal neutrons (unit barn $= 10^{-24}$ cm^2), I_0 the activation resonance integral (unit barn), ϕ_{th} the thermal neutron flux (n.cm^{-2}.sec^{-1}), ϕ_{epi} the epithermal neutron flux (n.cm^{-2}.sec^{-1}), N the number of atoms of a certain nuclide ($= 6 \times 10^{23} P\theta g/A$), ($P$ = per cent of the element in the compound; θ = isotopic abundance of the target nuclide; g = weight of the compound; A = atomic weight of the element), λ, the disintegration constant ($= 0.693/t_{\frac{1}{2}}$; $t_{\frac{1}{2}}$ = half-life of the radioactive isotope), and s ($= 1 - e^{-\lambda t}$) is the saturation factor (t = irradiation time).

The amount of the element is experimentally deduced from the ratio of the radioactivity of the particular isotope in the sample to that of a standard, irradiated simultaneously, *i.e.*,

$$\frac{\text{weight of element in sample}}{\text{weight of element in standard}} = \frac{\text{radioactivity of isotope in sample}}{\text{radioactivity of isotope in standard}}.$$

The standard can be successfully replaced by a multi-comparator.[3] It is assumed that all the parameters (θ, σ, ϕ, t, counting efficiency) remain identical for the unknown and the standard and that the radionuclide is not formed from another nuclide in the sample (*i.e.*, no nuclear interference).

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Neutron-activation analysis combined with high-resolution γ-spectrometry is an excellent multi-element method. Radiochemical separations may be necessary, depending on the element and the matrix. The possibilities of RNAA for the elemental analysis of biological materials have been outlined by Guinn and Hoste[4] and by Erdtmann and Nürnberg.[5]

EFFECT OF THE IRRADIATION PROCESS ON BIOLOGICAL SAMPLES

As a consequence of the Szilard–Chalmers process, recoil energy up to several MeV is transmitted to the newly formed nucleus. The result is a change in the chemical bond as well as in the position of the nucleus in the matrix. High gamma dose-rates lead to a rise in temperature and to radiolysis of organic compounds and of water. This in turn may result in the loss of some elements by volatilization or diffusion. The sample gradually decomposes and is eventually completely charred. It is evident that in these circumstances only irradiation in sealed containers (usually made of quartz) can ensure a quantitative recovery of the trace elements. As there is a continuous build-up of internal pressure, there comes a point when the ampoule will burst. This is the case, for example, when 100 mg of lyophilized serum is irradiated for 12 days at 10^{14} n.cm^{-2}.sec^{-1}. The explosion hazard can be avoided by dry-ashing the sample in the quartz vial at up to 450° before the irradiation, but this is only applicable to the determination of non-volatile elements. For safety reasons, the irradiated ampoules should never be opened unless they have been thoroughly cooled in liquid nitrogen.

One of the earlier analytical applications of the Szilard–Chalmers process was developed by Comar and Le Poec[6] for the determination of iodine in biological fluids. It is based on the observation that during irradiation most of the linkages between the proteins and activated iodine are broken, so all the radioactive iodine can be fixed quantitatively on an anion-exchange resin. The same radiation effect also governs standard iodide solutions. Only a strongly alkaline medium appears reliable, as the free ^{128}I-atoms are then trapped. Neutral or acid media cannot prevent the loss of iodine by either adsorption onto the container wall or by volatilization. This phenomenon may also give rise to serious cross-contamination.

The most difficult element to determine accurately is mercury. It is common knowledge that mercury solutions contained in sealed polyethylene containers may undergo significant losses. To a limited extent this also applies to mercury in powders. Although this phenomenon occurs in unirradiated polyethylene vials at room temperature, several authors have mentioned an additional effect that is proportional to the irradiation dose.[7–10] In all cases these losses can be prevented by sealing the samples and the standards in quartz ampoules.

RNAA of biological material should therefore never be described as a non-destructuve method. There is always some radiation damage, although sometimes only to such a very minor degree that a purely instrumental procedure remains feasible.

BLANK VALUES CAUSED BY THE IRRADIATION PROCESS

A very small number of atoms of various impurities present in the irradiation vials become radioactive during neutron bombardment and are liable to be ejected from their original sites.[11] However perfectly the surface of the vials has been cleaned, radioactive isotopes of the impurities may end up in the sample as a result of this recoil effect. However, only determinations at the nano- and subnanogram levels are at risk, as the quartz and organic polymers used should be of the highest purity available. This particular kind of contamination hazard becomes more obvious when the samples have to be washed out of the containers after the irradiation. Although originally representative, the samples will yield erroneous values because of the addition of extraneous radioactive atoms.

Typical trace element concentrations in various container materials are given in the literature.[12–18] Every known container material contains detectable trace element impurities. Therefore any application of instrumental RNAA to ultratrace elements irradiated in quartz or plastic vials is open to doubt. This statement is supported by comparison of the figures given in Table 1, for the concentration of some impurities present in quartz and polyethylene, with those for normal human serum.

Several reports have appeared on the effect of such impurities.[15–17,22,31,32] Moody and Lindstrom[15] studied the amounts of impurities leached from various plastic containers by different solvents, as well as the quantities of Na, Al, K, Co, Zn, Br, Sb, La, W and Au removable from plastics after short and long irradiation times. Only minor amounts of trace elements were found to be leached out by the acids, implying that they are distributed throughout the matrix.

The interference of an impurity present in polyethylene can be exemplified by the results obtained for iodine in the IAEA V-5 wheat flour,[31] after radiochemical separation of ^{128}I. The mean value of 2.2 ± 0.4 ng/g was obtained only after switching from polyethylene to high-purity quartz vials. Analyses of the wheat flour irradiated in polyethylene yielded values up to 10 ng/g. The results appeared to be affected by the 20 ng of iodine present in the container, some of which formed part of the induced ^{128}I activity measured. The poor reproducibility of the results is typical of this kind of contamination.

Another example shows the extent to which the trace analysis of lyophilized urine is affected by impurities in the polyethylene container material.[32] Measurements of the γ-activity showed the presence of ^{110m}Ag, ^{198}Au, ^{82}Br, ^{51}Cr, ^{59}Fe, $^{122+124}$Sb, ^{46}Sc

Table 1. Comparison of the concentrations of trace elements in serum with those of impurities present in quartz and conventional polyethylene

	Serum		Quartz		Polyethylene	
Element	*ng/ml*	Reference	*ng/g*	Reference	*ng/g*	Reference
Al	4	19			182	18
					500	15
					5400–7000	12
As	1.07	20			1.8	18
Br	$2.13.10^3$	21			>20	15
Co	0.108	22	0.13	16	10–370	12
			<1	17		
			95.2	14		
Cs	0.74	23	136	14		
Cr	0.16	22	1.26	16	180–1500	12
Cu	$1.07.10^3$	24			91	18
Hg	1.8	25				
Mn	0.54	20	32	17	18	18
			$4.62.10^5$	14		
Mo	0.58	26				
Ni	2.0	27				
Rb	$1.7.10^2$	23				
Sb	0.52	28	0.099	16		
			0.4	17	5	15
			118	14	<10	12
Se	$1.3.10^2$	23			<3	18
Sn	30	29				
V	0.031	30			0.9	18
Zn	$0.94.10^3$	23	0.30	16	300	12
			<10	17		

and ^{65}Zn. The highly hygroscopic and electrostatic nature of lyophilized urine necessitates a thorough rinse-out of the container and this enhances the contamination hazards. A blank value has to be taken into account for Cr and Mn (concentrations in urine 1 and 0.1 ng/ml respectively), but appears negligible for Zn (up to 0.5 μg/ml).

High-purity quartz also yields some relatively important blank values. Part of this is the result of long irradiations at high neutron fluxes (*e.g.*, 1–12 days at 10^{13} or 10^{14} n.cm^{-2}.sec^{-1}). A further part of the blank is created when the tip of the quartz ampoule is sealed by heating in a flame at 1500–1900°, as described by Mazière *et al.*[17] The impurities volatilized from the silica are deposited on the walls of the tube, and whereas those condensed on the exterior of the tube are eliminated during the cleaning of the ampoule after irradiation, the impurities condensed on the interior are only dissolved once the ampoule is opened. As the ashed or lyophilized biological material undergoes serious radiation damage, it must always be dissolved with hot, strong acids, and this procedure inevitably enhances the blank value.

Versieck *et al.*[22] investigated the Cr and Co radioactivity leached out of "Spectrosil" quartz ampoules opened by snap cutting. The corresponding concentration represented about 30% of the mean value found by RNAA for these elements in serum. The apparent mean contaminations per ml of serum were estimated as 0.0478 ng of Cr (range 0.0262–0.074 ng) and 0.0267 ng of Co (range 0.0167–0.0338). Heraeus "Suprasil" yielded lower blank values, 0.020 and 0.0075 ng/ml of serum for Cr and Co respectively.

Mazière *et al.*[17] studied the possible influence of the "wet ashing" blank on the constituents present in 100 μl of serum. A 1-g fused quartz ampoule ("Quartex") was irradiated for 1 day at 2.5×10^{14} n.cm^{-2}.sec^{-1} and the amounts of the elements leached out with a mixture of nitric acid and hydrogen peroxide were compared with those expected in 100 μl of serum. Of the 39 elements investigated, only Ba, Br, K, Na, P, Rb, Se and Sr gave blank values that could be neglected (<5%). Since then it has become common practice to use quartz of a much higher purity than that experimented on by Mazière *et al.* The list of elements giving negligible blanks can now be extended. Nevertheless, contamination with Cr, Co and Sb, caused by wet-ashing of biological samples irradiated in quartz, constitutes a real problem for ultratrace element determinations in difficult matrices such as serum.[33]

Tjioe *et al.*[34] evaluated the highly variable blank values from irradiated quartz vials, by crushing the irradiated containers in a pneumatic press and processing the sample together with the quartz splinters. The blank values were investigated for vials, made of either "Vitrosil" or "Spectrosil" quartz. The comparison of their blank values with the expected values[33] per 100 mg of lyophilized or ashed serum, points to unacceptable interferences for As, Cd, Co, Cr, Fe, Mo and Sb, but none for Br, Cu, Hg, Se and Zn.

Bereznai *et al.*[16] have also reported wet-ashing

values for 0.5 g of Heraeus "Suprasil" ampoules, amounting to 5×10^{-5} ng of Sc, 5×10^{-4} ng of Cr, $1\text{–}4 \times 10^{-2}$ ng of Co, 9×10^{-3} ng of Sb and 0.2 ng of Zn per vial.

It is evident from the various data cited that the purer quartz materials are liable to influence only the results for ultratrace levels of certain elements in specific biological matrices. In all cases requiring the subtraction of a blank, however, the accuracy is lower, because the blanks, even under similar experimental conditions, cover wide ranges.

NUCLEAR INTERFERENCES

Systematic errors, including those due to different fluxes in samples and standards, interfering nuclear reactions and self-shielding, have been discussed by De Soete *et al.*[2] It is beyond the scope of this paper to review all these aspects, which are intrinsic to the correct application of activation analysis. It may be useful, however, to draw attention to one of these errors associated with activation, *viz.* nuclear reactions jeopardizing ultratrace element determinations in biological matrices.

The principle of the RNAA method given here assumes that the radioisotope can only be formed through an (n, γ) reaction. The reactor neutron spectrum also contains fast neutrons, which can give rise to threshold reactions of the type (n, p), (n, α) and (n, 2n). Thus,

thermal neutrons yield

$$^{A}_{Z}X(n, \gamma)^{A+1}_{Z}X$$

and fast neutrons yield

$$^{A+1}_{Z+1}Y(n, p)^{A+1}_{Z}X$$

$$^{A+4}_{Z+2}Z(n, \alpha)^{A+1}_{Z}X$$

$$^{A+2}_{Z}X(n, 2n)^{A+1}_{Z}X$$

Hence the degree of interference depends mainly on the flux ratio $\phi_{thermal}/\phi_{fast}$, and the cross-sections and concentrations of neighbouring elements in the periodic table. The cross-sections of these threshold reactions appear to be a few orders of magnitude smaller than those for (n, γ) activation. Therefore this interference only becomes measurable when the concentration of the interfering element exceeds that of the element to be determined by several orders of magnitude.

The organic matrix of the biological material (as far as C, H, N, O are concerned) causes none of these interferences, but some major elements such as P might do so. The interference is expressed as an apparent concentration *i.e.*, the concentration of the analyte element yielding the same radioactivity through its (n, γ) reaction as that given by the threshold reaction of the interfering element.

$\bar{\sigma}_f$ = cross-section for fast neutron capture.

A limited list of possible interferences in determination of trace elements in biological material is given in Table 2. The apparent concentrations can be estimated from the nuclear characteristics of the isotopes,[35] the matrix composition and the thermal:fast neutron ratio, but the exact amount has to be determined experimentally, as there is a large spread in the published cross-sections for threshold reactions.[36]

Three practical examples of this limitation are now described in more detail, for Al in serum and Mn and Cr in red blood cells.

The amount of P in serum is about 132 μg/ml. The interference reaction $^{31}P(n, \alpha)^{28}Al$ ($\bar{\sigma}_f$ = 1.9 mb)* in a thermal:fast flux ratio of 6.4 produces an apparent Al concentration of about 180 ng/ml, completely masking the 4 ng/ml actually present.[19] It is improbable that a neutron flux could be found, so well thermalized that this interference could be eliminated.

Red blood cells contain about 1025 μg of Fe per g. The interference reaction $^{56}Fe(n, p)^{56}Mn$ ($\bar{\sigma}_f$ = 1.07 mb) in a thermal:fast flux ratio of 6.4 produces an apparent Mn concentration of about 11 ng/g, seriously masking the 15 ng/g actually present. Irradiation in a well thermalized position reduces this interference. The Cr determination based on the $^{50}Cr(n, \gamma)^{51}Cr$ reaction becomes unreliable in the presence of large amounts of Fe, because of the

Table 2. Nuclear interferences by fast neutron threshold reactions

Element	Thermal neutron activation reaction	Interfering reaction
Al	$^{27}Al(n, \gamma)^{28}Al$	$^{28}Si(n, p)^{28}Al$ $^{31}P(n, \alpha)^{28}Al$
Co	$^{59}Co(n, \gamma)^{60}Co$	$^{60}Ni(n, p)^{60}Co$ $^{63}Cu(n, \alpha)^{60}Co$
Cr	$^{50}Cr(n, \gamma)^{51}Cr$	$^{54}Fe(n, \alpha)^{51}Cr$
Cu	$^{63}Cu(n, \gamma)^{64}Cu$	$^{64}Zn(n, p)^{64}Cu$
K	$^{41}K(n, \gamma)^{42}K$	$^{42}Ca(n, p)^{42}K$
Mg	$^{26}Mg(n, \gamma)^{27}Mg$	$^{27}Al(n, p)^{27}Mg$ $^{30}Si(n, \alpha)^{27}Mg$
Mn	$^{55}Mn(n, \gamma)^{56}Mn$	$^{55}Fe(n, p)^{56}Mn$ $^{59}Co(n, \alpha)^{56}Mn$
Na	$^{23}Na(n, \gamma)^{24}Na$	$^{24}Mg(n, p)^{24}Na$ $^{27}Al(n, \alpha)^{24}Na$
Ni	$^{64}Ni(n, \gamma)^{65}Ni$	$^{65}Cu(n, p)^{65}Ni$ $^{68}Zn(n, \alpha)^{65}Ni$
P	$^{31}P(n, \gamma)^{32}P$	$^{32}S(n, p)^{32}P$ $^{35}Cl(n, \alpha)^{32}P$
Se	$^{80}Se(n, \gamma)^{81m}Se$	$^{81}Br(n, p)^{81m}Se$
Si	$^{30}Si(n, \gamma)^{31}Si$	$^{31}P(n, p)^{31}Si$ $^{34}S(n, \alpha)^{31}Si$

reaction $^{54}Fe(n, \alpha)^{51}Cr$. Such might be the case for the analysis of red blood cells for Cr. The Fe present already yields an apparent Cr value of 11 ng/g ($\bar{\sigma}_f = 0.74$ mb, $\phi_{th}/\phi_f = 6.4$), which is of the same order of magnitude as the actual Cr content.

Another group of nuclear interferences is due to second-order nuclear reactions, through chains of the type

$$^{A}_{Z}X(n, \gamma)^{A+1}_{Z}X \xrightarrow{\beta\text{-decay}} {}^{A+1}_{Z+1}Y(n, \gamma)^{A+2}_{Z+1}Y.$$

The possible impact of this type of nuclear reaction can be assessed from a compilation by Op de Beeck.[37] The minor contribution of such an interference can be exemplified by the second-order interference of Fe in the determination of Co in red blood cells. The nuclear reaction

$$^{58}Fe(n, \gamma)^{59}Fe \xrightarrow{\beta\text{-decay}} {}^{59}Co(n, \gamma)^{60}Co$$

on 1025 μg of Fe per g of red blood cells, irradiated for 10.7 days at 5×10^{12} n.cm^{-2}.sec^{-1} would yield an apparent Co content of 5.2×10^{-4} ng/g. This is negligible in comparison with the 0.11–0.22 ng/g reportedly present.[38]

A good rule to go by is to precede any new analysis by an investigation of the different possible nuclear interferences for each element in its particular matrix. Such a short preliminary study could prevent many an erroneous analysis result.

MEASUREMENT OF THE RADIATION

Nearly all the radioactivity measurements for RNAA are based on γ-detection (and a few on β-detection), as this allows the simultaneous determination of several isotopes. This necessitates the use of a high-resolution Ge(Li) γ-ray detector coupled to a 4000-channel analyser. Important sources of systematic errors reside in the counting process and the quantitative interpretation of the γ-spectra. Crucial points are geometry factors, γ-attenuation, losses due to dead-time, pulse pile-up phenomena, spectral interferences and peak area calculations (particularly prone to errors when the peak-to-background ratio becomes small). These problems have been thoroughly investigated by Hertogen[39] and Baedecker.[40]

The neutron activation of biological material generally yields major γ-radioactivities of ^{24}Na ($t_{\frac{1}{2}}$ 15.02 hr), ^{38}Cl ($t_{\frac{1}{2}}$ 37.2 min), ^{42}K ($t_{\frac{1}{2}}$ 12.36 hr), ^{80}Br ($t_{\frac{1}{2}}$ 17.7 min), ^{82}Br ($t_{\frac{1}{2}}$ 35.3 hr) and an important bremsstrahlung of ^{32}P (pure β-emitter, $t_{\frac{1}{2}}$ 14.28 days). These high-energy dominant activities limit the sensitivity of the method of many trace elements with comparable or shorter-lived radioisotopes. The solution is a radiochemical separation of the element of interest. Such a procedure is necessary to improve the accuracy of low radioactivity measurements of long-lived isotopes hardly discernible from the Compton scattering of higher-energy γ-rays or from bremsstrahlung. This applies, for example, to the measurement of ^{51}Cr activities in irradiated normal serum. Only a radiochemical separation of Cr (by distillation as CrO_2Cl_2) enables accurate measurement to be made of the 320-keV photopeak, free from the background Compton continua of ^{59}Fe, ^{60}Co, ^{65}Zn, ^{86}Rb, ^{134}Cs and from the ^{32}P bremsstrahlung.

CONCLUSION

RNAA is a very sensitive technique for many trace and ultratrace elements in biological materials.[4] The accuracy of the analytical results depends upon an appreciation of the basis of the activation process. Such a scrutiny may reveal errors associated with activation (flux inhomogeneities, self-shielding, interfering reactions), as well as losses due to high radiation doses and blanks originating from the activated container material. The last-named phenomenon should not be underestimated in ultratrace determinations requiring long irradiations at high neutron fluxes. A considerable amount of trace-element analysis in biological material has become purely instrumental. Radiochemical separations, however, constitute the major part of the analytical procedure and make possible many ultratrace element determinations by separating the radioisotope of interest from an overwhelming or interfering matrix activity.

The detection of the radioisotopes is commonly performed by γ-ray spectrometry with Ge–Li detectors, followed by computer evaluation of the γ-ray spectra. The main sources of systematic errors may reside in the counting process and in the quantitative interpretation of the γ-ray spectra.

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MINIMIZATION OF ACCURACY RISKS IN VOLTAMMETRIC ULTRATRACE DETERMINATION OF HEAVY METALS IN NATURAL WATERS

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Summary—This paper describes precautions aimed at reducing the magnitude of blanks during sampling, sample pretreatment and voltammetric determination of tracemetals, including cadmium, lead, copper, mercury, nickel and cobalt. The general approach of working in controlled clean areas and performing all manipulations outside them within closed systems is described. The voltammetric determination has been adapted to clean-bench working.

As a consequence of the ecotoxicological impact of certain heavy metals,[1,2] even at trace levels in natural waters, their determination has become an important subject of environmental trace analytical chemistry.[3–6] It is also of key significance for fundamental studies in chemical oceanography and limnology of trace metals. This is reflected in the considerable number of publications dealing with methods for the determination of trace-metal levels in the environment. Confusion about these trace levels, mainly in natural waters, was only resolved in the mid-seventies, when it turned out[7–11] that trace-metal levels are really several orders of magnitude lower than had been presumed and found before. Before these recent careful and reliable investigations, only a few analysts had taken into consideration problems arising from contamination in field work[12] and in laboratories.[13–15] Progress in electronics has considerably enhanced the determination capability, while development of high-capacity filtration units has simplified upgrading of the laboratory atmosphere. Consequently sources of error can now be clearly defined and minimized.

This paper summarizes the ultimate factors limiting accuracy, consisting mainly of blanks from inadequate sampling methods or airborne contamination. Figure 1 demonstrates that from the preparation of the sampling bottles and the sampler to the determination itself, risks of contamination by dust from the environment can be of very different magnitudes. Some manipulations can be performed in controlled dust-free areas but others have to take place in extremely contaminated surroundings. The only way of keeping the samples unaltered by uncontrollable blanks will be by working in quasi-closed compartments, as will be described later. It is assumed that the determination method applied is voltammetry. However, the general precautions for avoiding risk of contamination and other sources of error during sampling, sample pretreatment and sample handling remain relevant when other methods of determination are used.

CONTROLLED ATMOSPHERE

The laboratory

Generally an existing laboratory has to be converted into a clean-room. This has usually to be achieved with a minimum of alterations and at reasonable cost. The high cost of a class 100 laminar-flow room, according to US Fed. Stand. 209, and the extreme precautions for maintaining this standard during operation, can be avoided in a pragmatic manner by adopting a less stringent approach:[13] the laboratory is flushed with a non-laminar flow of filtered air, and within this "clean" area, several laminar-flow boxes, class 100, provide dust-free conditions for all critical manipulations. Further precautions are required, such as elimination of corroding metal components and unnecessary instrumentation, and also covering walls and ceilings with a non-shedding paint.

The effectiveness of upgrading the ambient laboratory air with respect to the dust level was checked by monitoring dust particles with a particle-counting device, the Partoscope R (Kratel KG, Stuttgart, Germany). Measurements in ordinary laboratories without these provisions generally yielded around 2×10^5 particles (with a diameter of $>0.5\ \mu m$) per cubic foot (28 litres). This corresponds to about 6 μg of dust per cubic foot. Background levels (per cubic foot) during the night ranged up to 1×10^5 particles with a diameter above 0.5 μm and a few particles larger than 5 μm. Unrestricted coming and going, and in particular smoking, increased the particle numbers to levels that could no longer be counted correctly by the Partoscope as they were beyond the limit of the measurement range (2×10^5). In the dust-controlled labora-

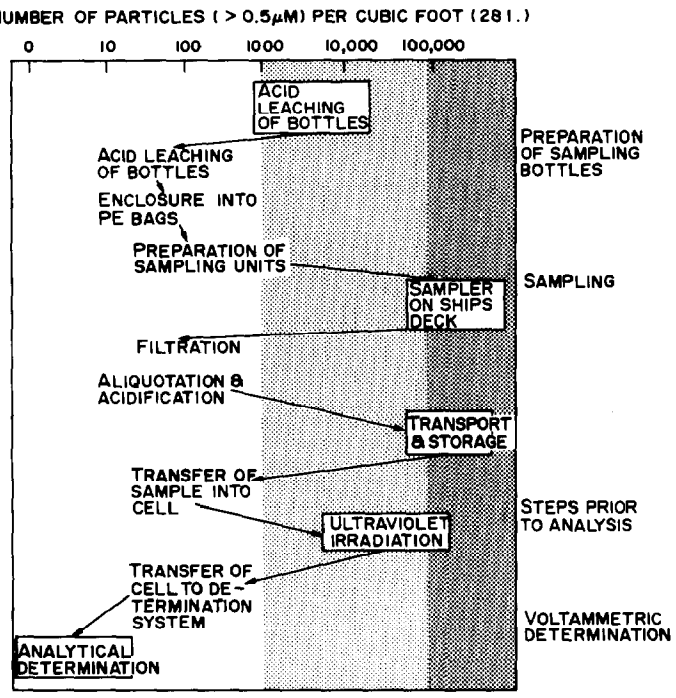

Fig. 1. Risks by dust contamination during preparative steps, sampling and determination. Principle of working either in clean areas or in closed systems.

tory, however, the particle count decreased to about 800 particles (diameter 0.5–5 μm) per cubic foot while no particles above 5 μm in diameter were present. The level generally becomes higher by a factor of about 10 if two staff members are working in the dust-controlled laboratory. At the laminar-flow clean bench the level is normally below 10 particles (0.5–5 μm) per cubic foot and can increase to 100–200 particles per cubic foot during manipulations, but only for a few seconds.

Of course, such low levels of dust can only be maintained if the staff wear protective clean-room overalls and arm sheets, together with polyethylene gloves for manipulations at the clean bench. Normal clothing increases the dust particle numbers considerably.

The laboratory in field missions

Reduction of blanks, a persistent problem in trace metal analysis, is far more difficult during field work, *e.g.*, during sampling missions on a research vessel. Laboratory air on board is severely polluted with trace metals such as lead, nickel and zinc. It is well known from numerous observations that during a journey there will frequently be work on paint-scrubbing and maintenance with lead-based anticorrosion paints. This of course creates serious problems of heavy contamination of ambient air with lead. One of the best ways of arranging to work in a clean area is to instal a clean-room container. This approach is limited only by the cost of installation and of transport to the port of departure of the research vessel. Once again, alterations to an existing laboratory may have to be restricted. Creation of a controlled atmosphere will involve reducing airborne contaminants in the form of dust and soot. The normal fresh air supply should, if possible, be cut off. Dirt particles introduced by shoes can be captured with a sticky floor covering[16] such as the plastic mats made by Dycem Ltd., Bristol (the Dycem Control Screen). A transportable clean-bench with horizontal laminar air-flow corresponding to US Fed. Stand. 209 (K. Bleymehl, Reinraumtechnik, Jülich, Germany) provides a clean working area of about 1 × 0.6 m at a height of 0.8 m. Horizontal air-flow is preferable for clean working with deep-sea sampling units. In mobile laboratories in vans for field missions on land, the installation of a clean-bench again creates satisfactory working conditions for ultratrace determination of heavy metals.[17]

EXPERIMENTAL REQUIREMENTS

Ultrapure water

High-purity water can be supplied from the reagent-grade ion-exchange unit Milli-Q (Millipore, Bedford, Mass., U.S.A.).[15] Demineralized water from a central supply is used for feeding a further ion-exchange cartridge. This water, with a conductivity of about 0.2 μS/cm is used for feeding the Milli-Q system. Water from this system yields the following low blanks: Cd < 0.1 ng/kg, Pb < 2.0 ng/kg, Cu < 10.0 ng/kg.

The lead blank can be further lowered by using the adsorption capacity of polyethylene surfaces for lead uptake from the demineralized water prepared as described above. For this purpose, water from the Milli-Q system is stored for some days in polyethyl-

ene bottles that have been leached in acid media for several days, as described later.

This system can easily be used on board a ship. The separate ion-exchange cartridge can also be fed with tap water, but this, of course, will reduce its capacity to about 500 litres.

Reagents

Hydrochloric, nitric and sulphuric acids are commercially available in high purity, *e.g.*, Suprapur® (Merck, Darmstadt). Usually the trace-metal levels are lower by a factor of 10 than those indicated on the label. In the acidification of samples to pH 2–3 (50 μl of concentrated acid added per 50 ml), the lead level in water samples is increased by 0.3 ng/kg. This blank need only be taken into consideration in deep-sea water analyses.

Reagents used in the determination of nickel and cobalt,[15] such as ammonium chloride and ammonia (both Suprapur®, Merck), and dimethylglyoxime (reagent grade) give a constant nickel blank of 4 ng/kg. Hydrogen peroxide (Suprapur®, Merck), is added as oxidant only to estuarine or polluted coastal waters[19] with higher trace-metal levels, and gives a blank of about 5 ng/kg when 200 μl are added to a 50 ml sample. Standard solutions (Titrisol, Merck) give no measurable blanks.

Cleaning of laboratory ware and sampling bottles

For sampling and storage, bottles and flasks from high-pressure (low density) polyethylene should be used in most cases. They are subjected to the following treatment: first, the bottles are carefully degreased with detergent in a washing machine, then pollutant trace metals, from the manufacture or incorporated into the raw material, are leached by soaking the bottles in an acid bath at medium temperature (60°) (Fig. 2). The acid bath has to be changed several times, finally to ultrapure water acidified to pH 2 with pure acids, *e.g.*, Suprapur® hydrochloric acid. The last manipulation, leaching in a very pure acid bath, has to be done at a clean-bench, to avoid contamination by dust particles. The sampling bottles and flasks thus prepared are filled with acidified ultrapure water (pH 2), wrapped inside two clean polyethylene bags and stored until required. Details of cleaning procedures have been described elsewhere.[16] The method of heating a large number of sampling bottles under contamination-controlled conditions is depicted in Fig. 2. Laboratory ware for mercury determinations has to be cleaned in separate containers which have not previously been loaded with laboratory ware or cells that have been used in work with mercury electrodes.

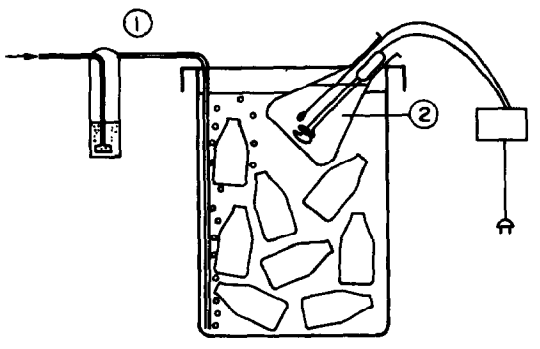

Fig. 2. Acid leaching of large quantities of bottles.[16] 1, Bubbling nitrogen avoids build-up of temperature gradients; 2, water-bath with temperature-controlled immersion heater (60–70°). (Reproduced by permission of the copyright holders, Springer-Verlag.)

Cleaning methods have recently been systematically investigated.[20]

Storage of samples

A steady state of non-leaching or non-adsorption will never be reached by cleaning or treating a surface, a fact that must be taken into account when samples have to be stored.[16,21] Keeping acidified solutions in (pH 2) polyethylene bottles at normal room temperature will increase the lead content by about 1 ng/kg per 2 weeks, because of leaching from the container walls. This effect, which mainly affects the lead blank, can be avoided by reducing the storage temperature. The best method of sample storage is deep-freezing to about −20°, which eliminates leaching of trace metals from the container walls and also losses by adsorption.

Adsorption of some trace metals, such as lead, occurs mainly in samples stored at neutral pH values. This adsorbed lead can be redissolved by acidification to pH 2. In general, samples such as sea-water, with large amounts of alkali and alkaline earth metal ions competing for adsorption sites, are less affected by losses through adsorption than are fresh water samples.

In all kinds of samples mercury is very sensitive to adsorption onto polyethylene. Thus subsamples for the determination of mercury have to be stored in glass or quartz bottles at low temperatures (4°) in a refrigerator. Of course, subsampling for mercury should be done as soon as possible, in order to avoid longer contact of the water with the plastic parts of a sampler.[22]

Collection of water samples

Surface water from rivers, lakes and the sea. Collection of pure surface water directly from a large ship is practically impossible, although it has been attempted again and again. The only safe way is to use a small rubber boat to leave the heavily contaminated area around the ship, and to collect water (in a clean bottle) at the bow of the boat whilst rowing upwind.[23] The same principle can be used on smaller ships (10–20 m length) by using a telescopic bar 3.5 m long. By means of this extension a sampling bottle can be dipped under the surface of the water while the ship proceeds at low speed.[23] Of course, precleaned polyethylene gloves are required for all manipulations with the sampling bottle.

Deep-sea water. In the last year it has turned out

that conventional deep-sea sampling methods using commercially available samplers clamped to the hydrowire may introduce large amounts of trace-metal contamination, especially of lead. Once again the determination of lead is particularly seriously affected, as it seems to be the only trace metal of interest present at extremely low levels in deep-sea waters, *e.g.*, about 1–10 ng/kg.[24–26] A recent investigation[27] has shown that no commercially available sampler for collecting water samples will give a lead blank below 10 ng/kg, thus making these samples suitable only for requirements less stringent than deep-sea sampling. Contamination problems with other trace metals are somewhat less severe than those with lead.

Contamination from the outer parts of the sampler or from the hydrowire can be avoided by using dynamic sampling, *e.g.*, collecting water with the sampler continually being lowered into virgin water during sampling. This was first achieved with the CIT sampler (California Institute of Technology).[27] Dynamic sampling, together with sophisticated precautions against contamination, has been used by us in the development of a deep-sea sampler automatically collecting three samples at different depths.[28] All manipulations such as preparation of the sampling units, that can be inserted into a main frame, and draining of samples into ultraclean bottles, are carried out within the dust-free area of a transportable clean-bench. When handled on the deck of the ship, the sampling units are hermetically sealed and thus protected from airborne contamination. Details of the sequence of operations during sampling have been published elsewhere.[25,28] A new approach to the collection of bottom-water samples at 1 m above the sea floor has been described elsewhere.[29]

Rain and snow. Rain and snow are sampled by automatic devices.[30] A humidity sensor controls the motor-activated lid of the sampler which consists of a large polyethylene funnel with a 0.45 μm filter at the bottom. The rain or snow collected is stored in a polyethylene bottle. When the rain stops the lid over the funnel closes automatically thus avoiding contamination by airborne dust during dry periods.

To our knowledge, there does not yet exist a reliable way of sampling rain or snow from a large ship during oceanic cruises. The surroundings on board ship are extremely contaminated and almost everywhere the air flow is turbulent, impeding dust-free sampling for any period longer than a few minutes. These findings have been confirmed recently by a short investigation on sampling of snow from an icebreaker in the Arctic Sea.[25] Uncontaminated snow could only be collected on ice floes upwind and at a distance from the ship.

Filtration of water samples

Apart from dealing with rain, as indicated before, filtration should only be used for turbid coastal waters, in order to distinguish so-called "dissolved" trace metals from those adsorbed on or incorporated into particulate matter. This fractionation is achieved by filtration through a membrane filter (0.45-μm pore size), according to a common convention in aquatic chemistry.

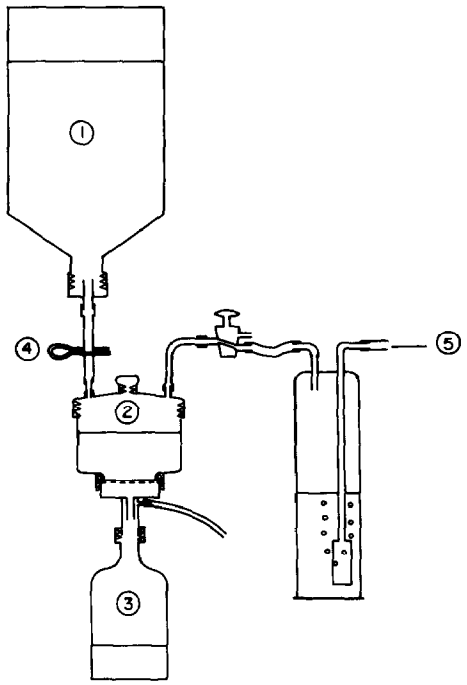

Fig. 3. Filtration unit.[16] 1, Freshly collected sample; 2, filtration unit; 3, polyethylene bottle for collection of filtered sample; 4, pinch clamp; 5, nitrogen-inlet for build-up of pressure. (Reproduced by permission of the copyright holders, Springer-Verlag.)

Membrane filters must be carefully precleaned in acid baths, to avoid leaching of trace metals during filtration. Filtration should be performed in the clean bench area, in order to avoid blanks from airborne contamination during change of filter. The filtration unit (Sartorius, SM 16511) is virtually a closed system, activated by nitrogen pressure (Fig. 3). Details of the sequence of operations during filtration have been published elsewhere.[16]

Ultraviolet irradiation

Ultraviolet irradiation, which is, apart from acidification, the only preliminary preparative step in the voltammetric determinations, may be required for degradation of organic substances binding trace metals as inert complex species. Analysis of samples from estuaries, rivers or waste waters might be significantly affected by the binding capacity of such dissolved organic substances. Irradiation requires ultraviolet lamps which often have corroding connections and soldering. The risk of contaminating samples during irradiation is thus quite significant. Samples for the determination of cadmium, lead, copper, nickel and cobalt have to be irradiated in a quasi-closed system, as depicted in Fig. 4. At a clean-bench, a subsample in a Teflon measuring cell is covered with a quartz beaker. A water-bath separates the cell and

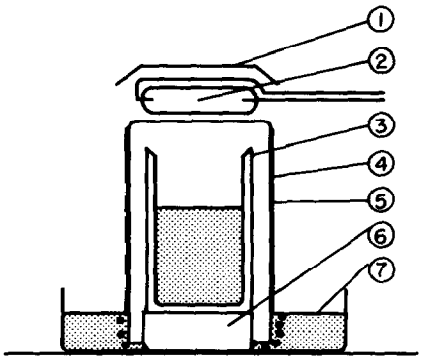

Fig. 4. Device for ultraviolet irradiation.[19] 1, Reflector; 2, ultraviolet lamp; 3, Teflon voltammetric cell filled with sample; 4, quartz beaker; 5, aluminium foil, the area of which determines the temperature under the beaker; 6, distance block; 7, glass dish with water for separating the cell from the outer atmosphere. (Reproduced by permission of the copyright holders, Springer-Verlag.)

the sample from the outer highly polluted atmosphere during irradiation.[19] After this operation, the outside of the apparatus is rinsed and the device is then opened at the clean-bench. The irradiated sample, that has boiled down to about half or one third of its original volume, can be diluted with ultrapure water, or measured out directly if it is desired to use this preconcentration factor of about 3.

For a more rapid oxidative photolytic decomposition, 200 μl of hydrogen peroxide (Suprapur®, Merck) are added to 50 ml of sample from estuaries, rivers and waste waters.[31] Heating samples to above 70° by ultraviolet irradiation must be avoided for determination of mercury: such samples are treated in closed quartz ampoules, cooled by a fan.[32]

Decomposition of particulate matter

Particulate matter collected on filters can be decomposed with particularly low contamination risks by low-temperature ashing (International Plasma Corp., Heyward, CA., U.S.A., Model 4005 B 448 AN). A prerequisite for low blanks is once again use of a controlled (essentially dust-free) atmosphere and clean combustion dishes made from quartz. Trace-metal losses seem to be negligible, as the ashing temperature is below 150°. After decomposition (3 hr) the residues are dissolved with 50 μl of pure acid and made up to 20 or 50 ml, depending on the working electrode used, *i.e.*, the hanging mercury drop or the thin mercury film on a glassy-carbon support.

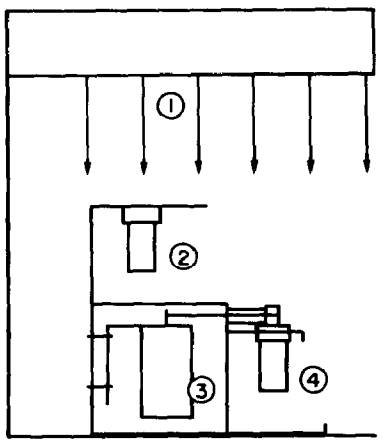

Fig. 5. Rotating electrode designed for clean-bench working. 1, Laminar clean-air flow; 2, cell for preliminary outgassing with nitrogen; 3, motor and electrical connections, completely separated from clean-air area; 4, voltammetric cell for determination.

Voltammetric determinations

The most sensitive electrochemical determination of cadmium, lead, copper, bismuth, and zinc involves preconcentration by deposition into a mercury film plated *in situ* on the glassy-carbon support of a rotating electrode.[8,19] Mention will be made here only of differential-pulse anodic-stripping voltammetry (DPASV), used for the ultratrace determination of the metals mentioned and the preferred technique for trace levels below 1 μg/kg.

We use two different sets of electrodes, one with a set of 8 electrolytic cells for work in our home laboratory[19] and a set with 2 electrodes for work during field missions.[25] Contamination that may arise with commercially available electrodes is avoided by careful design. Driving motors and electric connections are enclosed in a separate housing (Fig. 5). The cell and the electrodes, described elsewhere,[19] as well as manipulations such as changing cells in the laminar flow of the clean-bench, give rise to no measurable blanks (*i.e.*, below 0.1 ng/kg).

The determination of nickel and cobalt after interfacial preconcentration by adsorption of their dimethylglyoxime complexes at a hanging mercury drop[18] is not seriously affected by the reagent blank of 5 ng/kg for nickel. Care must be taken not to use Vycor tips (Corning Glass Corp.) to separate reference electrodes from sample solutions. Substantial amounts of nickel and cobalt may be leached from this kind of porous glass and severely contaminate the sample.

The determination of mercury at a rotating gold electrode[32] should be done in a separate laboratory. Experience over the years has shown that electrochemical laboratories usually have measurable levels of mercury vapour in the air, normally below the maximum tolerable levels for human health, but nevertheless critical for the determination of the element at ultratrace levels.

CONCLUSION

When all the precautions discussed in this article are taken, both high precision and extremely low limits of detection can be attained in the voltammetric determination of cadmium, lead, copper, mercury, nickel and cobalt (see Fig. 6). The accuracy is also good, as far as can be seen from a comparison of measurements on some sea-water samples. Table 1 summarizes the results of a first test organized by

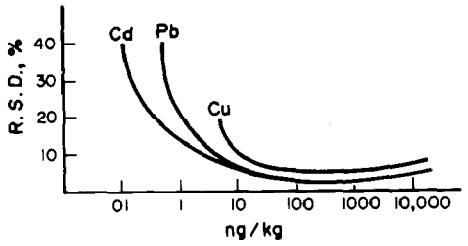

Fig. 6. Precision and actual determination limits for cadmium, lead and copper. For nickel and cobalt see ref. 18. For mercury see ref. 22.

Table 1. Interlaboratory comparison of analytical results for a deep-sea water sample from 1000 m depth, 120 km off the Californian coast, collected with the CIT sampler (Schaule and Patterson)

	Cd	Pb	Cu
Investigators, determination methods		*ng/kg*	
C. C. Patterson and B. Schaule, Caltech, Pasadena; CH_3Cl–dithizone extraction and isotope dilution mass spectrometry		3.3	
K. Bruland, University of California, Santa Cruz; APDC–DDDC extraction and electrothermal AAS	105		115
L. Mart, Nuclear Research Centre, Jülich; DPASV	101	2.9	110

Schaule and Patterson. A more recent interlaboratory comparison for cadmium in 10 sea-water samples organized by Danielsson at the University of Göteborg, yielded results comparable with those of the previous test, with a deviation of results below 10% and thus within the relative standard deviation of the methods used.

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ROLE OF RADIOTRACERS IN THE DEVELOPMENT OF TRACE ELEMENT ANALYSIS

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Summary—This article discusses the contribution of the tracer technique to the present state of development of trace element analysis. It reviews the use of radiotracers for testing the individual steps of the analytical process, especially with regard to the sources of systematic error. The major subjects considered are: sampling, storage and pretreatment of samples, sample decomposition, separation and preconcentration, and the measurement stage.

For the determination of trace elements in various matrices, an impressive number of powerful methods based on various physical principles is at the disposal of the analyst. These analytical techniques can be classified into two groups: (1) direct instrumental or single-stage techniques, and (2) multistage or combined techniques.

The direct techniques, which include optical emission spectrometry with spark or arc sources, X-ray spectrometry, spark-source mass-spectrometry and activation analysis, are in many instances the optimum choice. In general, however, the combined techniques can be taken as models for the methodology of trace element analysis. A typical analytical process consists then of the following steps: sampling, sample preparation, decomposition, separation (preconcentration), and measurement. Each of these steps can be a source of systematic error. Thus, the principal problem in any combined procedure is to convey the very small amounts (from pg to μg) of the determined elements through all steps of the procedure, from sampling to detection, without introducing systematic errors, such as contamination or loss.

Radioactive tracers have proved to be an excellent means for examining the individual steps of the analytical process and revealing the concomitant sources of systematic error. To a considerable extent the progress achieved in trace element analysis is the result of studies using the radiotracer technique. In most cases it is probably the best approach, and some problems cannot be solved by other means. However, it should be emphasized that the radiotracer technique cannot itself provide the solution to all aspects of accurate analysis, but rather should be considered as one of the necessary tools for achieving this end. For instance, it can only make a limited contribution to the solution of such problems as calibration and standardization. A clean working atmosphere, high-purity reagents, and standard reference materials are also principal requirements if accuracy is to be achieved.

In this paper, the unique capabilities of the radiotracer technique for studying the individual steps of trace analysis and identifying the sources of systematic error are discussed. The most important applications are surveyed, and illustrated with suitable examples.

SAMPLING AND SAMPLE HANDLING

The first and perhaps the most critical step in an analysis is sampling and sample handling. It includes taking the sample, and transporting, storing and processing it before the decomposition step or direct instrumental analysis. All these stages must be considered as possible serious sources of error.

Sample homogeneity

The fundamental requirement of sampling bulk material is that the proportion of the component of interest is, within the limits of error, the same in the sample as in the whole.[1,2] No difficulties are encountered in obtaining representative samples of liquid materials. On the other hand, sampling is an extremely difficult problem in the case of solid materials because of their considerable lack of homogeneity. Close attention must be paid to the possible segregation of components. The homogeneity has to be considered not only with regard to appearance, but also to how well the sample for analysis represents the whole. The sample homogeneity requirements can also depend on the method used for determination. Solution techniques normally utilize relatively large samples, from which homogeneous fractions are analysed, resulting in the reduction of inhomogeneity errors. However, in a number of direct techniques, such as spark-source mass-spectrometry, direct X-ray fluorescence spectrometry, optical emission spectrometry and charged-particle activation analysis, only a small volume fraction of the sample is normally involved in the excitation of element-specific signals,

and thus the homogeneity of the sample is of great importance. Inhomogeneity can become a severe problem when the sample consists of particles of different sizes and the content of the trace component in question varies with particle size, as is often the case in airborne particles, geological and industrial materials. Considerable inhomogeneities also occur in biological matrices.

The radiotracer technique has proved to be well suited for examining the course of homogenization in an arbitrary mixing process.[3,4] The system to be investigated can be labelled either by the addition of a radiotracer to the component of interest, or by radioactivation. The material is then sampled at various stages of homogenization, *e.g.*, at various intervals of time, and the radioactivity of the samples (normalized to sample weight) are related to the relevant parameter, usually time. The closer together the specific activities of the samples, the greater the degree of homogeneity (f_H) of the material. We can express f_H quantitatively by means of the coefficient of variation V:

$$f_H = (1 - V)100\% \quad (1)$$

and

$$V = \sqrt{\frac{\Sigma(a_i - \bar{a})^2}{(n-1)\bar{a}^2}} \quad (2)$$

where a_i is the relative specific activity of sample i, and $\bar{a}$ the average relative activity of the n samples (taken after different homogenization periods).

The inhomogeneity problem is very serious in the analysis of compact solids such as metals or semiconductors.[5,6] In the case of metals in particular, the usual treatment for preparation of a homogeneous sample, *i.e.*, crushing, grinding and mixing, is not feasible. In many instances, a homogeneous sample can only be prepared by dissolution.

Radiotracers and autoradiography have been widely applied to homogeneity studies on solids and have contributed greatly to study of the distribution of trace admixtures in materials. Significant progress in the evaluation of two-dimensional distributions has been achieved by colour autoradiography[7] based on the relationship between radioactivity and colour shade.

Inhomogeneous distribution is evidently caused by limited solubility or lack of solubility of the trace components in the matrix, as the insoluble elements tend to form agglomerates. However, even soluble elements can be distributed inhomogeneously because of segregation during the crystallization process, whereby the elements may accumulate at the phase boundary or inside the grain, as was demonstrated by segregation of labelled copper and tungsten in an Fe–C alloy.[8] It is also possible that the concentration changes from the grain boundary to the interior were the result of heat treatment, as observed for bismuth distribution in copper grains.[9] An impressive example of an extremely inhomogeneous distribution of a trace impurity in the matrix is shown in Fig. 1. It can be seen that the tantalum (labelled as ^{182g}Ta by activation), the main impurity, is concentrated in an inclusion located approximately in the middle of the niobium disc. As a consequence of this inhomogeneity large errors can occur in bulk analysis if the sample is not excited uniformly over the whole volume when a direct instrumental technique is used, or if only a small-volume sample is taken for dissolution.

In addition to metals, semiconductors have been extensively investigated by autoradiography with regard to distribution of impurities and doping elements.[10] Radioactive tracers have also been used for studying some aspects of sampling and processing of geological materials.[11]

Contamination problems

The tracer technique offers a valuable tool for examining difficult contamination problems. For example, in the determination of essential or toxic elements in biological fluids and tissues at the ng/g level, contamination-free sampling, sample-handling and storage prior to analysis, are essential for accuracy, if activation analysis, which is a blank-free method, is applied.

In contrast to compact solids, the surface contamination introduced into these samples during sampling and storage cannot be removed by etching. These contaminations can easily exceed the actual contents by several orders of magnitude. This explains the continuous decrease in the "normal values" of several essential elements during recent years, *e.g.* for cobalt and chromium in serum and plasma, from about 10 ng/ml some years ago down to 0.1 ng/ml today.[12,13] Even if the composition of the surgical and container materials used is known, it is very difficult to establish the extent of this contamination.

The tracer technique has contributed to present knowledge of these contamination problems. The surgical instruments have been labelled *in situ* by neutron-activation and then used for *in vitro* experiments simulating medical practice, and the contamination introduced has been measured by means of its radioactivity.[14–16] The results for Co and Cr, expressed as "apparent concentrations", are given in Table 1. It can be seen that this particular type of needle cannot be used if these two elements are to be determined.

The extent of the iodine contamination of biological material in neutron-activation analysis done with irradiation containers made from polyethylene has been tested,[16] and that from containers made from high-purity quartz has been examined for 37 elements[17,18] by irradiating the cleaned quartz capsules, simulating a wet-ashing with a mixture of nitric acid and hydrogen peroxide and counting the activity of the corresponding radionuclides. In the case of Au, Cd, Co, Cr, Mn, Sc, the wet-ashing blank is either at

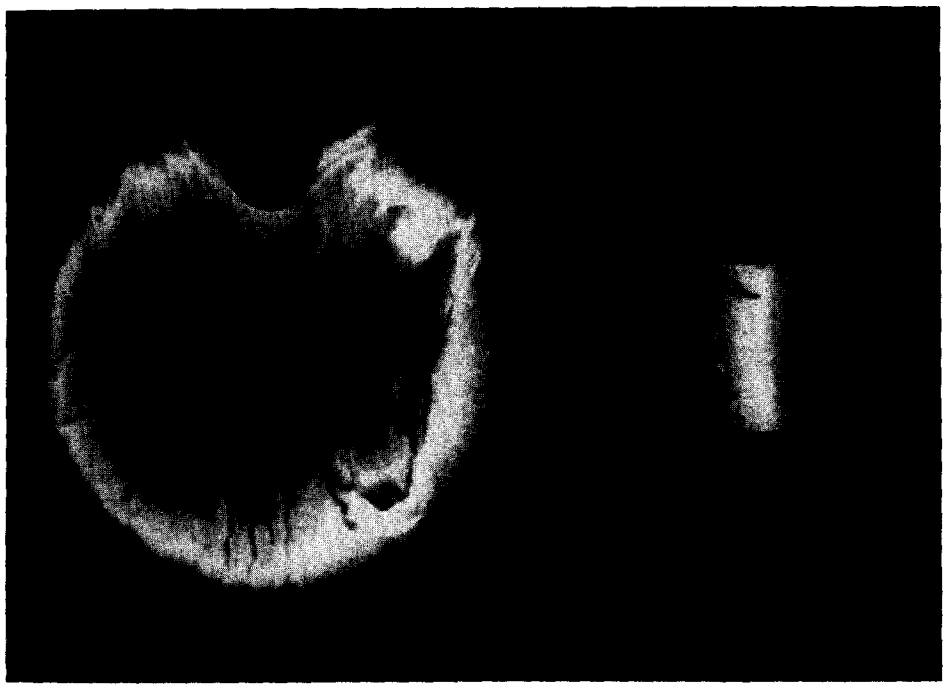

Fig. 1. Distribution of tantalum in a niobium disc, (2.5 cm diameter) determined by *in situ* labelling and autoradiography.[7] (By permission of the copyright holders.)

Table 1. "Apparent concentrations" in serum, caused by contamination introduced in taking successive 20-ml blood samples with disposable needles[16] (reproduced by permission of the copyright holders)

Element	Normal range, *ng/ml*	Sample	Apparent concentration, *ng/ml*
Co	0.1–0.3	1	0.9
		2	0.2
		3	0.1
		4	0.2
Cr	0.2–0.7	1	85
		2	12
		3	10
		4	15

the same level as the mass of these elements present in 100 μl of serum, or up to one order of magnitude higher. Thus, these contaminations can be a considerable source of error. However, if a surface layer about 20 μm thick is removed by etching before the ampoule is sealed, the wet-ashing blank can be significantly reduced.

In analysing solids of extremely high purity by activation analysis, special attention must be paid to the removal of any surface contamination which has occurred during sample preparation and irradiation. In some cases, surface decontamination can itself create problems.[19] In the determination of very low contents of chromium and iron in pure niobium by proton-activation analysis,[20] the removal of the appropriate indicator radionuclides from the surface in the post-irradiation etching was checked by the tracer technique. The sample surface was deliberately contaminated with metallic and ionic chromium and iron, and the radiotracers were produced *in situ* by irradiation with protons under the same conditions as used in irradiation for analysis. As can be seen from Fig. 2, complete removal of contaminants is achieved by the etching procedure used; the total maximum matrix loss was found to be 0.08 mg/cm^2 (corresponding to a thickness of 3×10^{-4} mm) and could therefore be neglected.

Adsorption losses

Storage of liquid samples and standards may be one of the most important sources of error in an analytical procedure, but the danger of these errors is all too often underestimated or even totally neglected. Radioactive tracers undoubtedly provide the best technique for investigation of losses of trace elements by adsorption. The adsorption losses can be determined either by counting the solution before and after the adsorption, and calculating the amount adsorbed by difference, or by directly counting the radioactivity of the solid adsorbent. Although a large amount of experimental information has been accumulated on the adsorption of inorganic species on glass, quartz, plastics and other materials[21,22] satisfactory systematic evaluation has not been possible, as the exact nature of the adsorption phenomena is in many cases not clear. Consequently, most elements do not behave predictably under all circumstances. The conclusion is that adsorption of trace elements is a very complex process. It can be affected by many factors, of which the most significant are the kind of material and its pretreatment, the nature of the ion, the pH-value, the presence of other electrolytes, the temperature and the time. The best understanding of the mechanism and course of adsorption has been obtained for glass as the adsorbent. One important type of adsorption of cations is ion-exchange with the groups ≡SiOH or ≡SiOMe (Me = alkali or alkaline-earth metal). An interesting characteristic of this adsorption is the possibility of reducing it by acidifying the solution, so that the metal cations are replaced by protons. Anion-exchange, *e.g.*, of anionic metal complexes, can also take place on glass. In this case, an increase in hyd-

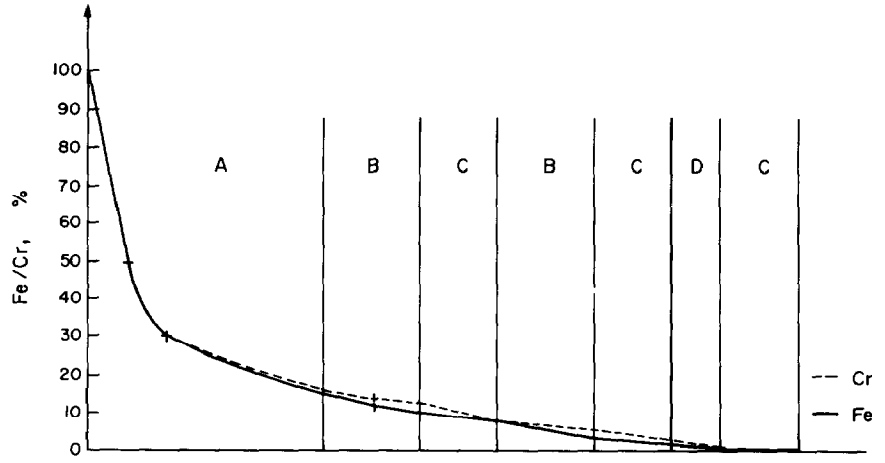

Fig. 2. Percentage removal of the indicator radionuclides ^{52}Mn and ^{56}Co produced from surface contamination of a niobium matrix with Cr and Fe during the etching procedure.[20] A—3 hr in 10*M* HCl at 65°; B—1 min in HF/HNO_3/H_2O (9:1:10); C—1 hr in 10*M* HCl at 65°; D—30 sec in HF/HNO_3/H_2O (9:1:10). (By permission of the copyright holders, Elsevier Sequoia and Akademiai Kiàdo.)

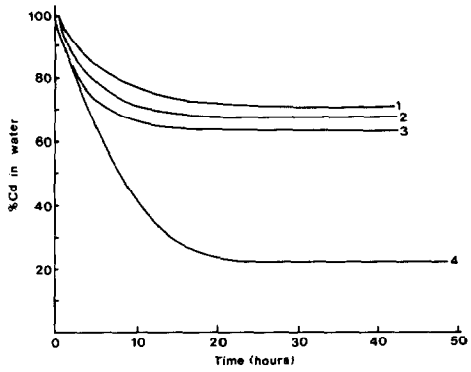

Fig. 3. Decrease of cadmium concentration in water at pH 10 during storage in a soft glass container, determined by using ^{109}Cd as radiotracer.[23] [Cd], ng/ml: 1, 200; 2, 100; 3, 25; 4, carrier-free ^{109}Cd. (Reproduced from *Anal. Chem.*, 1974, **46**, 771, with permission. Copyright the American Chemical Society).

roxide-ion concentration will reduce the adsorption. However, products of metal ion hydrolysis and colloidal particles can also be involved in adsorption.[22]

From the example shown in Fig. 3, it can be seen that the adsorption losses of cadmium on glass can reach considerable levels within a few hours and depend on the cadmium concentration; the lower the concentration the larger the relative loss.[23] The adsorption properties of various materials (glass, quartz, plastics, Teflon) have been investigated with ^{110m}Ag and ^{111}Ag as tracers.[24,25] None of the materials tested was found suitable for long storage of liquids containing traces of silver. However, the adsorption can be made negligible (<1%) by addition of sodium thiosulphate.

Several tracer studies have been devoted to investigation of losses of trace mercury from solution. By use of ^{197}Hg as tracer, severe adsorption losses on polyethylene were observed for both ionic mercury (up to 74% within 48 hr) and organomercury (up to 30% within 48 hr) for unacidified samples.[26] The results of another study,[27] with ^{203}Hg, showed that evaporation can also contribute to losses of inorganic mercury from stored aqueous solutions. On storage of a solution containing 2 ng of Hg per ml in 0.5*N* nitric acid in PTFE, about 25% of the mercury is lost in 10 hr by volatilization, whereas the losses are significantly lower from hydrofluoric acid solution (~4%). Losses are negligible from both solutions if potassium iodide or potassium cyanide is added. The utilization of ^{203}Hg as tracer in a recent study on the behaviour of mercury chloride and methylmercury chloride in inland water and sea-waters[28] revealed that the loss of mercury observed upon storage of unacidified sea-water samples in polyethylene bottles was due to both adsorption and to diffusion of metallic mercury through the container wall. For the chemical speciation of mercury compounds, time and the nature of the storage were found to be of paramount importance. For example, in three days' storage in brown glass bottles, 47% of $^{203}HgCl_2$ added to sea-water became reduced to mercury, but complete reduction with tin(II) chloride could not be achieved. Large adsorption losses have also been observed for many other elements, *e.g.*, gold up to 100% on polyethylene,[29] barium up to 70% on glass,[30] and cobalt up to 95% on glass.[31]

In analysing water samples, it is important to differentiate between the trace contents in solution and in the suspended matter. For this purpose, the samples may be filtered through a 0.45-μm membrane filter. Thus, when radiotracers (^{115}Cd, ^{60}Co, ^{59}Fe, ^{197}Hg, ^{99}Mo, ^{212}Pb, ^{65}Zn) were added to a filtered sea-water sample and the solution was repeatedly filtered through a fresh membrane filter, serious adsorption losses on the filter were found for iron (up to 33%),

Table 2. Losses of trace elements occurring during oven drying, freeze drying and dry ashing, determined by the radiotracer technique (after Sansoni and Iyengar[32])

Process	Element	Matrix	Temperature, °C [Pressure, *mm Hg*]	Time, *hr*	Loss observed, %
Freeze drying	Hg	Human urine	[0.05]	48	2
		Blood	[0.05]	24	9
		Water	[0.01–0.05]	48–72	39
	Se	Human urine	[0.05]	48	3
Oven drying	Hg	Human urine	105	24	15
		Rat brain	120	24	5–16
	I	Rat blood	120	24	7
		Rat kidney	120	24	15
	Pb	Oyster	100	48	17
	Se	Human urine	105	24	30–50
Dry ashing	As	Rat blood	450	16	86
	Co	Molluscs	450	?	26
	Cr	Rat liver	500	16	6
		Rat blood	700	16	51
	Na	Human rib	600	16	10
	Sr	Rat blood	450	16	16

lead (up to 11%) and mercury (up to 19%). The losses depended on both the volume filtered and the concentration of the trace elements.[26] In this way, it was shown that it is a mistake to apply filtration in the determination of mercury in water unless the adsorption is prevented by lowering the pH of the solution.

Volatilization losses during drying and ashing

Before instrumental measurements or decomposition, biological and environmental samples are often dried. This can be done by simple drying in air (not recommended), in an oven, or by freeze drying. It has been revealed with the aid of radiotracers that surprisingly large losses of several elements can occur not only during oven drying at temperatures between 70 and 120° but also during freeze drying.[32] Table 2 gives examples of losses occurring during the sample pretreatments discussed above.

A serious reduction in accuracy may result from losses during evaporation of solutions, often an essential step in analytical procedures. The tracer technique is the routine method for investigating such losses.

SAMPLE DECOMPOSITION

One step in which severe losses can obviously be expected is the decomposition of the sample. Dry ashing in a muffle furnace (at temperatures between 400 and 500°) is still often used for the decomposition of organic material, because of its simplicity and suitability for dealing with large samples and large numbers of samples. However, numerous radiotracer studies indicate that severe losses of several elements may occur during this ashing procedure, even of those elements for which no loss would normally be expected. In addition, the extent of the volatilization loss depends on the nature of the biological material. Some examples of significant losses detected by the tracer technique are included in Table 2. Because of the danger of volatilization losses, dry ashing and wet decomposition in open systems should be avoided unless such losses have been checked for the given element and matrix. On the other hand, with the help of radiotracers, decomposition procedures using closed systems have been developed which avoid losses even for the more volatile elements. For example, good recoveries of mercury and selenium are achieved in the decomposition of biological materials with nitric acid under pressure in a Teflon tube,[33] or with oxygen activated in a microwave discharge,[34] as can be seen from Table 3.

Valuable results were obtained in an investigation (by the tracer technique)[35] of the behaviour of trace platinum elements during chemical dissolution of aluminium and nickel matrices. Aluminium and nickel samples doped with Ru, Os and Ir were labelled *in situ* by irradiation with reactor neutrons. The labelled samples were dissolved, and by means of chemical separations, were tested to determine whether the doped trace constituents had dissolved. The results showed that sample decomposition can have a decisive effect. The three trace elements were not dissolved during decomposition of the aluminium matrix, either in acid or alkaline solution. Even after further treatment, iridium could not be completely dissolved, and then separated quantitatively, but in the case of osmium and ruthenium this was possible by oxidation followed by distillation. On the other hand, Os, Ru and Ir were dissolved during the decomposition of the nickel matrix in nitric acid. These results are similar to those obtained when attempting to determine iridium and other elements in niobium,[36] when low yields and poor reproducibility were a serious problem. These results are of great importance. If a matrix is to be decomposed for the purpose of trace impurity determination, it is necessary to know the chemical state of the trace constituents of interest after the decomposition of the samples.

Table 3. Recovery of some volatile elements in decomposition of samples in closed systems (after Tölg and co-workers[33,34])

Decomposition	Element/ radioisotope	Amount applied, *ng*	Recovery, %
Wet, with HNO_3 under pressure	Be/^{7}Be	5–100	97.5
	Se/^{75}Se	1–10	98.5
	I/^{131}I	5–100	97.5
	Hg/^{203}Hg	3–80	98
Dry, with activated oxygen	Se/^{75}Se	31–250	98.5*
	Zn/^{65}Zn	60	100†
	Hg/^{203}Hg	0.5–60	91§

*Dissolved in 0.1*M* HNO_3.
†Dissoved in 1*M* HNO_3.
§Dissolved in concentrated HNO_3.

SEPARATION AND PRECONCENTRATION PROCEDURES

Analytical procedures performed without chemical separations can only be successful when the signals due to the elements to be determined are not interfered with by other elements or the matrix, or when adequate corrections can be made for any interference. In many instances, it is necessary either to remove the major components or to separate the element(s) of interest from the sample. Sometime preconcentration is also required. The tracer technique is well suited for the rapid and accurate evaluation of the distribution of the elements of interest in a separation process. In fact, most of the separation data for trace elements have been obtained by using radiotracers. These data include the distribution coefficient, capacity factor, separation factor, separation efficiency and recovery factor. Radiotracers have been used extensively in all types of separation research, such as separation by phase change (volatilization, precipitation, electro-deposition, liquid-extraction,

ion-exchange, adsorption chromatography) and separation by difference in mobility (paper chromatography and electrophoresis). Some examples are given below. By means of radioactive tracers it was shown that selenium (as matrix) can be removed practically completely (>99.999%) by distillation as the tetrabromide, while the impurities Na, K, Sc, Cr, Fe, Co, Cu, Zn, Ga, Ag, Cd, La, Ta and Au remain (with good recoveries) in the residue.[37] On the other hand, trace selenium may be separated from biological materials, rocks and soils directly in the decomposition (by combustion in oxygen) under dynamic conditions in a special apparatus.[38] While concomitant elements forming relatively non-volatile oxides remain in the ash on the sample holder, selenium dioxide can be volatilized and then condensed on a cold-finger. It may then be dissolved with hydrochloric or nitric acid, by boiling under reflux. The recovery determined by using ^{75}Se as radiotracer was found to be 97%, regardless of the matrix.

Sixty-three radiotracers were used for labelling in an extensive study of the retention of ions from different acid media on columns of eleven ionic precipitates.[39] The results of about 2000 adsorption experiments enabled many new separations of great practical significance to be realized. For example, sodium and tantalum can be separated quantitatively on hydrated antimony pentoxide from 58 other cations in 12*M* hydrochloric acid medium. From 14*M* nitric acid, quantitative retention of Na, Ge, As, Se, Nb, Mo, Ag, Ta and Pa may be achieved.

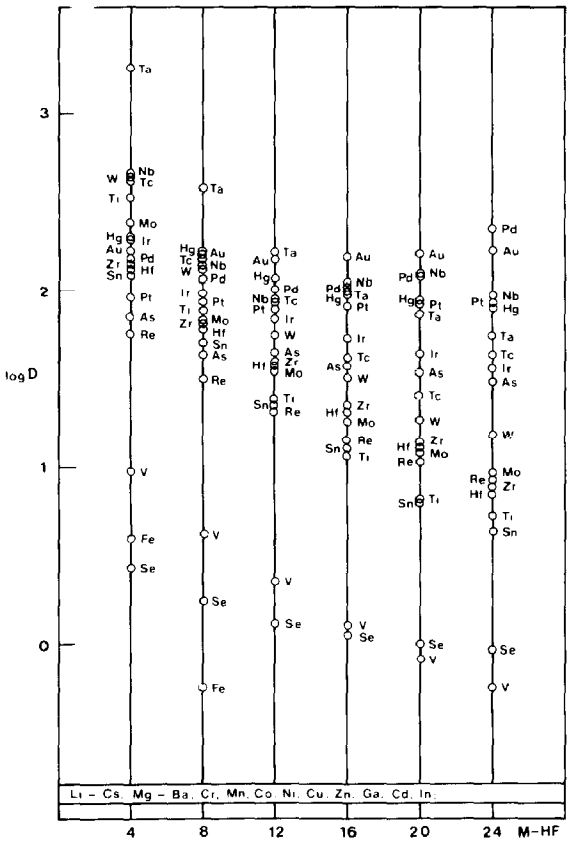

Fig. 4. Distribution coefficients for Dowex 1 × 8 resin and HF solutions, determined by the radiotracer technique (excluding Li and Mg).[41] (Reproduced from *Anal. Chem.*, 1981, **53**, 1719, with permission. Copyright by the American Chemical Society).

The radioisotopes ^{59}Fe, ^{60}Co, ^{65}Zn and ^{207}Bi have been used to study the deposition of the corresponding elements on a graphite cathode. After separation the deposited elements can be determined by AAS or optical emission spectroscopy.[40] Amounts of a few ng at the <10 ng/ml level could be separated on the graphite tube with yields above 98%.

The radiotracer method has also been used to study the adsorption of 31 elements on Dowex 1 × 8 from hydrofluoric acid and hydrofluoric acid/nitric acid media under static conditions.[41] These media are suitable for the decomposition of refractory metals, glasses and geological samples. The adsorption of the elements from hydrofluoric acid solution is illustrated in Fig. 4. The results obtained for hydrofluoric acid/nitric acid systems show that with increasing nitric acid concentration there is a continuous decrease in the *D* value for all elements tested. These distribution data allow useful separation procedures to be worked out; for example, in the determination of Zr, Mo, Hf and W in niobium and tantalum by X-ray fluorescence spectrometry.[42] One of the main steps of this procedure is, in addition to the separation of the niobium or tantalum matrix by extraction with diantipyrylmethane, the preconcentration of Zr, Mo, Hf and W on a strongly basic anion-exchange paper from hydrofluoric acid solution, followed by measurement with a wavelength-dispersive X-ray fluorescence spectrometer, with limits of detection below 0.3 ppm for all four elements.

Radioactive indicators have also been widely used in other separation techniques and their application reviewed.[21,43]

DETERMINATION

The tracer technique offers unique possibilities for clarifying what actually happens to the elements of interest during the determination stage. This knowledge often reveals sources of systematic error and facilitates optimization of the determination. The potential of the tracer technique for this purpose is exemplified by the determination of mercury by flameless atomic-absorption spectrometry.

Flameless AAS should be a suitable technique for the determination of trace mercury but its application has been limited because of significant losses of mercury occurring in the preatomization treatment in the graphite tube, *i.e.*, in the drying and pyrolysis steps. Investigations using ^{203}Hg as radiotracers have contributed to the understanding of these processes.[44] From the results given in Table 4 it can be seen that from certain media 40–90% of the trace mercury can be lost during drying at 70° whereas non-detectable

Table 4. Mercury losses during drying* (Reproduced from *Anal. Chem.*, 1982, **54**, 579 by permission. Copyright the American Chemical Society)

Solution	Mercury loss, %
2% HNO_3	77.7 ± 4.7
2% H_2SO_4	86.7 ± 4.3
2% H_2O_2	42.7 ± 3.1
2% HCl	49.3 ± 5.1
2% HNO_3 + 2% H_2O_2	48.7 ± 4.0
2% H_2SO_4 + 2% H_2O_2	44.3 ± 5.7
2% HCl + 2% H_2O_2	not detectable
2% HCl + 2% H_2O_2 + 2% HNO_3	not detectable

*In a pyrolytically coated tube at 70°, Hg 0.3 μg/ml, ramp-time 20 sec, hold-time 50 sec.

losses occur from others at temperatures up to 250°. Similar stabilization effects have been achieved by formation of mercury sulphide with hydrogen sulphide in the graphite tube before drying. A technique for the determination of mercury in biological material based on these stabilization effects has been worked out. It involves decomposition with nitric acid in a pressure bomb, adjustment of the solution with hydrochloric acid/hydrogen peroxide mixture and analysis by flameless AAS. Similar investigations on other elements are in progress in our laboratory.

Radioactive tracers have been used for investigation of a number of basic problems in atomic-emission spectrometry. For instance, the thermochemical behaviour of various elements in graphite electrodes on d.c. arc excitation was studied by the tracer technique.[45,46] Experiments with ^{59}Fe and ^{110m}Ag for measurements of the evaporation rate and the activity distribution in the electrodes led to a better understanding of the transport mechanism in the arc. With the aid of these measurements it was possible to demonstrate the influence of the cathode shape and the form of the gas discharge chamber on the evaporation of the components. On the basis of the tracer studies, two rotating plasmas within the graphite tube were applied, and this improved the detection limits.

With the help of radiotracers (^{59}Fe, ^{60}Co, ^{95}Zr and ^{45}Ca) the processes taking place in rotating disc electrodes and their influence on spectrochemical analysis in solution have been investigated.[47] Owing to the chromatographic effect, there is variation in the depth of penetration by the four elements.

The results suggest that the spectral parameters of the rotating disc electrodes depend not only on the physical properties (density, porosity, grain size, electrical and thermal conductivity) but also on the chemical properties.

CONCLUSION

In this review, an attempt has been made to show the versatility of the radiotracer technique as a tool for the investigation of all aspects of trace element analysis, especially with reference to gains and losses of determined.

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THE ORIGIN OF SYSTEMATIC ERRORS IN BACKGROUND MEASUREMENTS IN ZEEMAN ATOMIC-ABSORPTION SPECTROMETRY

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Summary—The problem of background measurement in Zeeman atomic-absorption spectrometry is discussed. The background measurement may be more or less faulty if the background is caused by line-rich electron excitation spectra of molecules. Molecules also such as OH, NO, NO_2 and SO_2 show a Zeeman effect. Therefore, the background measured in two different polarization planes or measured with and without a magnetic field present may differ. The danger of systematic errors in background measurement is less if the background is caused by polyatomic molecules. Rotational structure is less marked or not at all observable.

One of the most significant and difficult problems in atomic-absorption spectrometry is to measure the true background. Correct background measurement is especially important if the addition method of calibration has to be applied, *e.g.*, if the matrix material of the analytical sample cannot be obtained completely free from determinand, in which the calibration graph has to be extrapolated to zero net absorbance. However, in order to do that, the background absorbance caused by radiation scattering and atomic or molecular absorption by the matrix material must be found. This cannot be done by merely pushing the knob marked 'automatic zero".

The problem of background measurement is mainly to determine the spectral background underlying the very narrow resonance line, and cannot be resolved by the monochromator. The normal method of background measurement or background compensation with a continuum source of radiation is correct only when the background is a spectral continuum, *e.g.*, when it is caused by radiation scattering or photodissociation of molecules. The background measurement may be more or less faulty if the background is due to line-rich electronic excitation spectra of molecules. The actual background is entirely dependent upon whether or not a rotational line of the molecular spectrum coincides with the atomic-absorption line.[1] It is very difficult to deduce this from measurements obtained with the commercial atomic-absorption instruments normally used.

In the course of the past years, the most important step forward in atomic-absorption spectrometry was the application of the Zeeman effect to the quasi-simultaneous measurement of atomic and background absorption. Nowadays, three types of commercial instruments are already available which use different Zeeman techniques. However, we do not know how these techniques compare in respect to accuracy of background measurement and how they compare with commonly used techniques of background measurement with a continuum source of radiation.

SYSTEMATIC ERRORS OF BACKGROUND MEASUREMENT WITH A CONTINUUM SOURCE OF RADIATION

The best "guinea-pig" to show the origin of systematic errors due to structured background is the OH-band in the wavelength region near the bismuth resonance line at 306.8 nm. Figure 1 shows the absorption spectrum of an acetylene–air flame in the region of the bismuth line (lower spectrum). The spectrum has been recorded with a continuum source of radiation in combination with a high-resolution instrument. The practical resolution is approximately $R = \lambda/\Delta\lambda = 2.5 \times 10^5$. The absorption scale is in arbitrary units, because the transmission T_p of the photoplate has been recorded.

The upper spectrum shows the profile of the bismuth line recorded with the same resolution. As a result of nuclear-moment splitting, the bismuth line has two components. The wavelength difference between them is approximately 0.01 nm. It can be seen that two rotational lines coincide almost exactly with the bismuth components. Therefore, the actual background under the bismuth components is very high. This cannot be deduced from background measurements with low-resolution atomic-absorption spectrometers. If a deuterium lamp is used and measurements are made with a spectral bandwidth (halfwidth of the transmission profile of the monochromator as indicated in Fig. 1) of, for example, $\Delta\lambda = 0.5$ nm, we measure only an arbitrary mean value, at large wavelength intervals. This value is far too low in comparison with the actual background

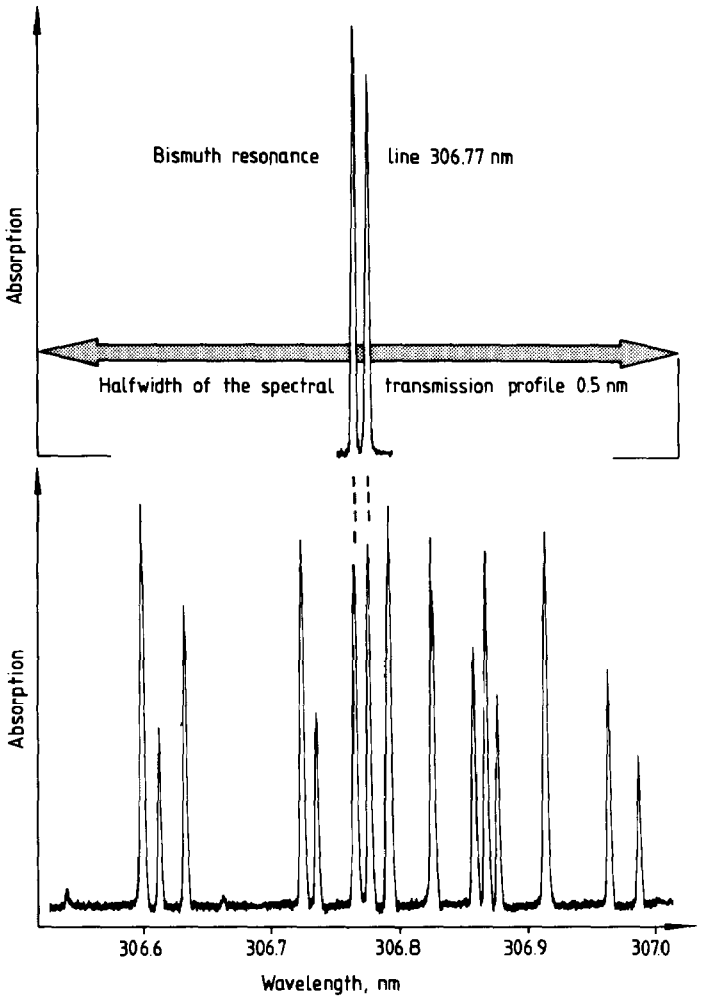

Fig. 1. Absorption spectrum of the OH-radical in an acetylene–air flame (lower spectrum) and of the 306.77-nm Bi line (upper spectrum). Absorption scale in arbitrary units. The two components of the Bi line coincide nearly exactly with two rotational lines of OH.

absorbance. This can easily be demonstrated by measuring the actual background absorbance, using a bismuth hollow-cathode lamp. Figure 2 shows the absorbance signals of an acetylene–air flame. The absorbance has been measured at 306.8 nm with a bismuth hollow-cathode lamp as source. The base-line (zero absorbance) was recorded without the flame.

It would be expected that with background compensation no change in absorbance would occur after ignition of the flame. However, the "compensated" absorbance signal of the stoichiometric flame (10-cm slot burner) is about 0.18 absorbance units, the absorbance signals of a reducing flame being lower and those of an oxidizing flame higher.

If we compare the absorbance signals of the stoichiometric flame with and without background compensation we can see only a small difference. That means that in this case background compensation is highly ineffective.

In fact, this is an extreme example. The 306.8-nm bismuth line is not suitable for determination of bismuth by flame atomic-absorption spectrometry. It may be the preferred line in furnace atomic-absorption spectrometry, where OH-interferences do not generally occur. The sensitivity of the 223.1-nm line is somewhat higher, but background interferences at 306.8 nm are much lower in general.

However, a warning must be given at this point that background measurement is not all that easy to perform. Generally, it can be stated that the measurement may be faulty in all cases, if the atomic absorption is measured in a wavelength region where molecules of the matrix cause a structured background.

The most frequently observed background interferences caused in flames as well as in furnaces by structured absorption of molecules are those of the pyrolysis products of SO_4^{2-}, NO_3^- and PO_4^{3-}. Diatomic molecules such as SO, NO and PO show very sharp rotational structure in the ultraviolet wavelength region.[1] Otherwise, for large molecules the density of the energy states is generally so high that under the conditions of atomic-absorption spectrometry (atmos-

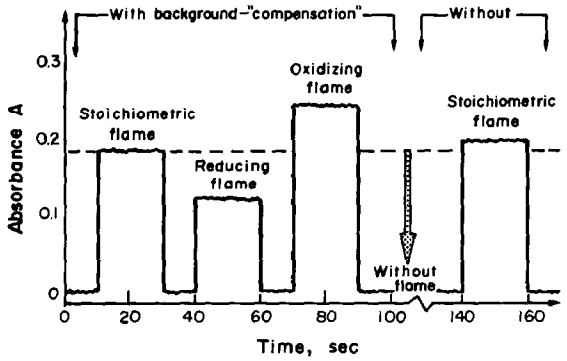

Fig. 2. Absorbance signals of an acetylene–air flame measured at 306.8 nm with and without "background compensation". The primary source was a bismuth hollow-cathode lamp. The base-line (zero absorbance) was recorded without the flame.

pheric pressure, temperature 2000–3000 K) rotational structure cannot be observed.[2] Even in this case, however, the vibrational band-edges may be very sharp. Therefore—but rarely—at some wavelengths, correct background measurement with a continuum source of radiation may be dubious.

ZEEMAN TECHNIQUES FOR QUASI-SIMULTANEOUS MEASUREMENT OF ATOMIC AND BACKGROUND ABSORPTION

The application of the Zeeman effect in atomic-absorption spectrometry for the correction of the spectral background was proposed as early as 1969.[3] Since then, many papers have appeared. Different techniques of Zeeman atomic-absorption have been applied. Yasuda *et al.* published an excellent review in 1980.[4]

Figure 3 shows the principle of some Zeeman techniques for quasi-simultaneous measurement of atomic

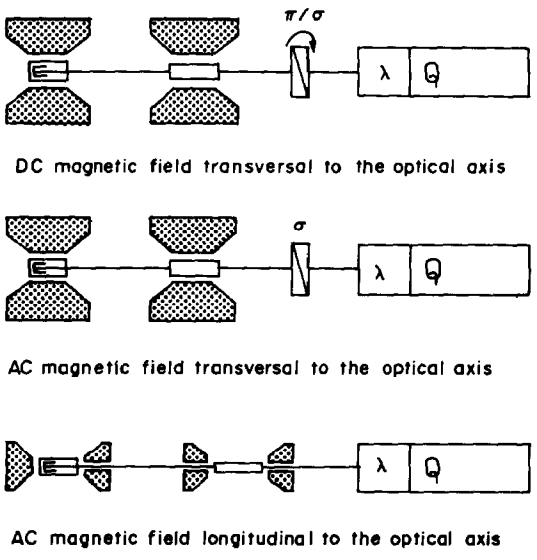

Fig. 3. Zeeman techniques for quasi-simultaneous measurement of atomic and background absorption.

and background absorption. The magnetic field can be applied either to the primary source or to the absorption cell. It may be either a constant field or a periodically modulated field. The direction of the field lines may be either transversal or longitudinal to the optical axis. Depending on which technique is used, the figures of merit such as sensitivity, precision, accuracy of background measurement *etc.*, may be widely different. In this paper, only three techniques will be discussed, those which are already realized in commercial atomic-absorption instruments.[5] In addition, these techniques are discusssed in respect to one figure of merit only: the accuracy of background measurement. These techniques are as follows.

1. Constant magnetic field transversal to the optical axis. Field applied to the absorption cell. Measurement of atomic and background absorption in two different polarization planes. (Hitachi, Ltd., Tokyo, Japan.)

2. Constant magnetic field transversal to the optical axis. Field applied to the primary source of radiation. Measurement of atomic and background absorption in two different polarization planes. (Erdmann & Grün, Wetzlar, Germany.)

3. Modulated magnetic field transversal to the optical axis. Field applied to the absorption cell. Measurement of atomic and background absorption with and without the magnetic field. (Perkin-Elmer Corporation, Norwalk, U.S.A.)

With technique 2, the background is measured on both sides of the analytical line, but—unlike measurements with a continuum source—at definitive wavelengths in close proximity to the line. In special cases, this may be a decisive advantage.

With techniques 1 and 3, the background is measured at exactly the wavelength of the analytical line. This is the dream of the spectroscopist, namely, to measure the background "under " the line, but even if this could be realized, we could not be sure that the background measured in two polarization planes or measured with and without the magnetic field would be the same. We know that molecules show the Zeeman effect, too.[6]

In molecular spectroscopy, the Zeeman effect is a powerful diagnostic tool, *e.g.*, in spin–rotation analysis. The Zeeman effect of di- and tri-atomic molecules has been extensively studied: OH,[7] NO_2,[8,9] NO,[10] SO_2,[11,12] SH,[7] NH_3, H_2O, OCS,[13] ClO_2.[14]

In order to study the influence of Zeeman splitting of sharp rotational lines of diatomic molecules in atomic-absorption spectrometry, the "guinea-pig" 306.8-nm bismuth line was used again for demonstration purposes. The magnetic field was applied to an acetylene–air flame transversal to the optical axis. Figure 4 shows the relative absorbance of the OH rotational lines at the wavelength of the 306.8-nm bismuth line as a function of the magnetic flux density.

The primary source of radiation was a bismuth hollow-cathode lamp. The measurements were made in a

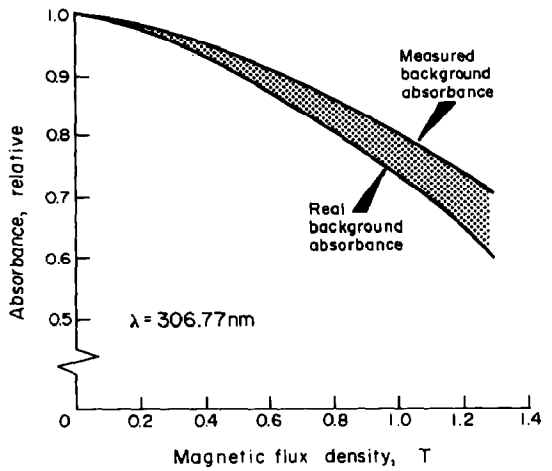

Fig. 4. Measured and real background absorbance in an acetylene–air flame at 306.77 nm as a function of the magnetic flux density (magnetic field transversal to the optical axis).

polarization plane parallel to the field lines (real background absorbance) and perpendicular to the field lines (measured background absorbance). As seen from Fig. 4, with increasing flux density, both absorbance signals decrease, but in a different way. The difference between the measured background absorbance and the real background absorbance gradually increases. At a magnetic flux density of 1 Tesla, we observe a value of the measured background exceeding 10% even at that early stage. From this experiment it can be deduced that the Zeeman technique 1 causes systematic errors in background measurement if coinciding rotational lines of molecules show Zeeman splitting.

In addition, this experiment allows predictions about the accuracy of background measurements with the Zeeman technique 3.

If background absorbance without the magnetic field is compared with the measured background absorbance at a flux density of 1 Tesla, a systematic deviation of as much as 20% can be observed in this case. This deviation is negative and has an absolute value twice as great as that with the field applied. From this experiment, it can again be deduced that the Zeeman technique must cause serious errors in background measurement if coinciding rotational lines of molecules show Zeeman splitting. Figure 5 shows the systematic deviations between the measured and the real background in an acetylene–air flame at 306.8 nm as a function of the magnetic flux density. The background absorption was measured with a constant magnetic field transversal to the optical axis (technique 1) and a periodically modulated magnetic field (technique 3). This is an example of systematic errors caused by structured background absorption due to the sharp rotational lines of diatomic molecules.

The danger of systematic errors due to structured background is much less if the background is caused by polyatomic molecules. Rotational structure is less marked or even not observable. An example of the absorption spectrum of a polyatomic molecule is shown in Fig. 6. Iron in xylol was to be determined by graphite furnace AAS. The spectrum in the neighbourhood of the 271.9-nm iron line was recorded photographically. An HGA 74 graphite furnace was used at a temperature of 2600° and a xenon-flash lamp was the primary source of radiation. The spectral resolution of $R = 10^5$ is sufficiently high for the real physical background structure of xylol at this temperature (and pressure) to be seen—but not the physical profiles of the atomic resonance lines of iron.[15] The latter cannot be fully resolved with a practical spectral resolution of only 10^5.

The edge of the vibrational band of xylol at 272.2 nm is very sharp. Background measurements in the

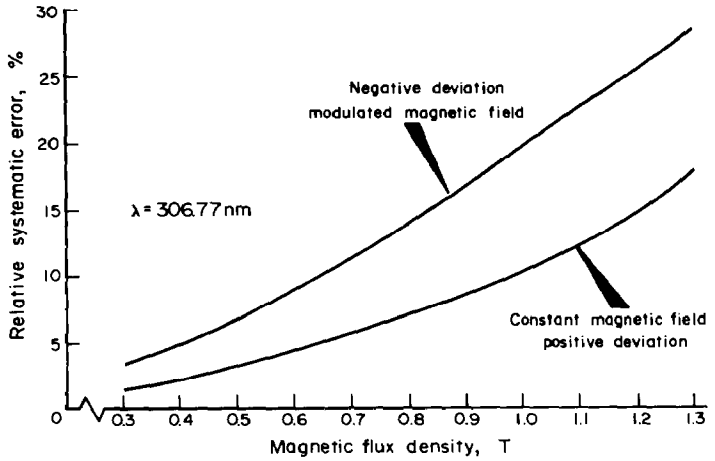

Fig. 5. Systematic errors of background measurement (relative to the height of background absorbance in an acetylene–air flame at 306.77 nm) observed with technique 1 (constant transversal magnetic field) and technique 3 (modulated transversal magnetic field), as a function of the magnetic flux density.

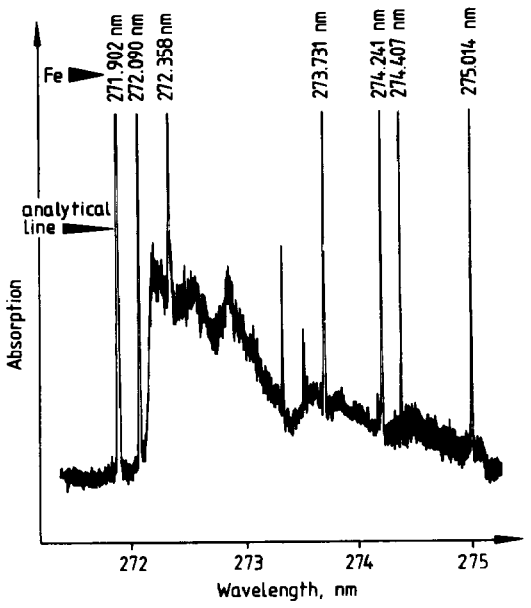

Fig. 6. Atomic-absorption spectrum of iron and molecular-absorption spectrum of xylol recorded photographically with a spectral resolution of approximately $R = 10^5$ (graphite furnace HGA 74, temperature 2600°).

neighbourhood of such edges may be problematic. The distance between the iron line at 271.9 nm and the band-edge at 272.2 nm is only about 0.3 nm. Background measurements with a deuterium lamp cannot give correct results. On the other hand, the distance between the analytical line and the band-edge is great enough to allow correct background measurements by the three Zeeman techniques mentioned. No systematic deviations between the measured and the real background could be observed.

CONCLUSION

Background measurement by Zeeman techniques in atomic-absorption spectrometry may cause serious systematic errors if the spectral background is due to sharp rotational lines of molecules.

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MEASUREMENTS OF ORGANIC LEAD IN AIR—A REVIEW

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Summary—Mounting concern over the presence and the role of organic lead compounds in the environment and living systems has resulted in the development of selective and sensitive measuring techniques. In the last decade considerable efforts have been devoted to the collection of data on the levels of the pollutant in environmental air. The procedures used and the chemical and instrumental problems associated with them, as well as the most important analytical results, are reviewed.

Organic lead compounds have been used since 1923 as additives to petrol as a convenient and economic way of increasing the octane rating of fuels for use in high-compression internal combustion engines. Commercially, five tetra-alkyl-lead (TAL) compounds containing methyl and/or ethyl groups are of importance: tetramethyl-lead (TML), trimethylethyl-lead (TMEL), dimethyldiethyl-lead (DMDEL), methyltriethyl-lead (MTEL) and tetraethyl-lead (TEL). They are all clear, colourless liquids with a fairly high vapour pressure and a fruity smell, rather insoluble in water, and very soluble in most organic solvents. Because of the thermal instability of these substances and the additional inclusion of ethylene dichloride and dibromide as scavengers in leaded fuels most of the lead exhausted is in the form of halide salts attached to small particles. Pollution by organic lead has therefore for a long time been regarded as a secondary problem compared to pollution by inorganic lead. However, as a result of spillage of leaded petrol, evaporative losses from the fuel tank and carburettor, and emission in the exhaust gases through incomplete combustion, considerable quantities of gaseous TAL appear to be emitted into the atmosphere. This knowledge, added to the hypothesis of the existence of a large-scale natural source of TML in the sea,[1] has caused in the past few years an increased concern about the organic lead levels in ambient air. In a recent review by Grandjean and Nielsen,[2] the ecologic implications were strongly emphasized.

Although the fate of TAL compounds in the environment is as yet only little understood, Röderer[3] and De Jonghe *et al.*[4] independently suggested the existence of a (bio)geochemical cycle for lead which includes TAL. Figure 1 constitutes a summary of these so far still hypothetical main pathways. Convincing evidence[5–9] indicates that TAL compounds in the environment are converted eventually into inorganic lead through tri- and dialkyl-lead salts (TriAL and DiAL). These solid compounds are very soluble in water, and therefore should be expected to undergo both dry and wet precipitation processes in the atmosphere. Hence, they enter the aquasphere, where they are subject to further decomposition reactions. Most probably the lead compounds can be taken up by aquatic organisms and can become accumulated through the food chain.[10–16] A small part of the bioaccumulated lead is withdrawn from the aquatic system, and can be ingested by humans. The remaining lead pool gradually deposits with excreta, dead organisms, or as particulate-bound lead.

Although there is *in vitro* evidence for the biological[17–22] and chemical[23–27] alkylation of lead salts to form TAL, it still remains obscure whether such mechanisms exist in natural environments. Dissolution of any TAL produced would be low, but may be increased by turbulence and emulsification. Evaporation of TAL from either solutions or emulsions is facilitated by turbulence and vertical water movements. These steps would be largely irreversible because of the hydrophobic nature of TAL. In view of the apparent lability of all alkyl-lead compounds it is questionable, however, whether these (bio)alkylation processes may indeed create significant amounts of organolead and thus increase the anthropurgic contribution to natural environments. Nevertheless, the conclusion must be drawn that all organisms in TAL-polluted biotopes could come into contact not only with TAL, but also with the highly toxic TriAL and the other derivatives.. This has to be taken into account when evaluating the potential toxic hazards arising for humans, from a TAL-polluted biotope.

It is therefore of considerable importance to be able not only to detect small amounts of organic lead but also to identify the specific types of compounds, as the environmental impact depends strongly on the state of chemical combination. Even within a certain class of organolead compounds (TAL, TriAL or DiAL) there are appreciable differences in environmental behaviour and toxic potential. TEL, for instance, appears to be significantly more toxic than TML, and the same is true for the corresponding TriAL dealkylation products.[12,28–33] In living systems the conversion of TAL into TriAL appears to be essential for the toxic effects to arise.[8,28] Thus, analytical methods capable of distinguishing between inorganic lead and

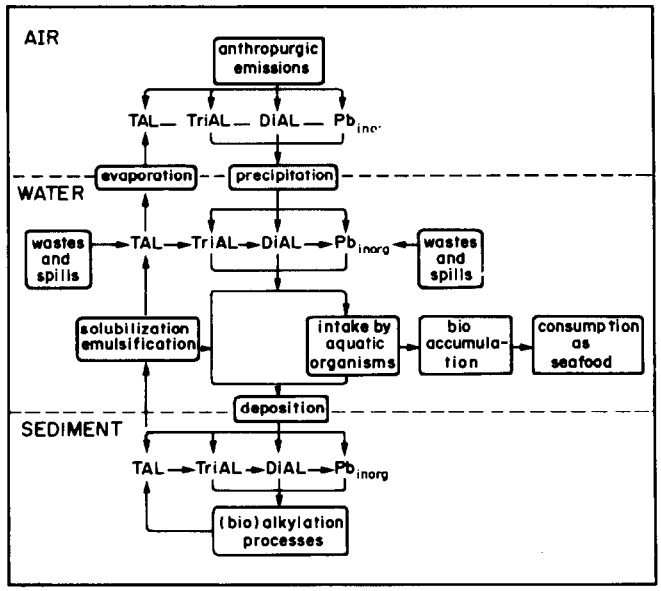

Fig. 1. Simplified (bio)geochemical cycle for TAL compounds in the environment. The actual pathways are likely to be more complex in view of disproportionation reactions[81] possibly occurring at different stages of the cycle, and the equilibria that may exist between organic lead in the particulate matter and in the gas phase.[35,36,78]

the different forms of organic lead are of special interest in environmental toxicology.

In most of the procedures commonly used for the determination of traces of organic lead in the atmosphere, separation of organic from inorganic lead is based on filtration. The particulate matter, containing most if not all of the inorganic lead, is assumed to remain quantitatively on the filter while the organic lead vapours pass on for concentration and subsequent analysis of the non-filterable lead.

In what follows we have made a distinction between the different organolead compounds: TAL, which can be supposed to be present in the gas phase only, and TriAL and DiAL compounds which may be present partly in the gas phase and partly in the particulates. Three different analytical methodologies can be distinguished: (*i*) methods that provide information on the total organolead content of the non-filterable fraction of the air-particulate system (these procedures are collected under the general heading "total organolead"), (*ii*) procedures that are based on the determination of the volatile lead compounds, termed "volatile organolead", and (*iii*) speciation methods for individual TAL compounds. The distinctions, especially between (*i*) and (*ii*) are not clear cut. Usually it is not clearly stated to which category the results of a particular method belong. A number of methods suffer from lack of specificity for organic lead, and at present the reported concentrations are probably too high, because of inclusion of inorganic lead that escapes trapping by the prefilter. Discrepancies could also be expected between the results obtained with short-term sampling procedures and those obtained with extended sampling techniques, in view of the marked diurnal fluctuations in concentration.[34] A direct comparison of the results obtained with different analytical procedures is therefore not straightforward. Finally, it should be emphasized that nearly all methodologies that have so far been developed for measuring airborne alkyl-lead pay attention exclusively to the non-filterable species, though there are indications that a fraction of the organic lead present in the atmosphere may be associated with the particulate matter.[35–38] This fraction probably contains the degradation products TriAL and DiAL, and not TAL itself.[39,40] The term "total" organolead should hence be used with caution when referring to results based only on the non-filterable fraction.

The aim of this review is to present an updated and extended version of the 1977 survey by Harrison and Perry.[41] The various analytical procedures that have been used for measuring the organic fraction of the non-filterable lead content of the air are critically described. A list of the reported concentrations is included.

Total organolead methods

With the exception of one procedure,[42] in which the air to be sampled is supplied directly from the atmosphere to the burner of an atomic-absorption spectrometer, in all methods a collection step (which is also a concentration step) and a determination step can be distinguished.

One of the approaches for collecting organic lead from air consists of the conversion of the volatile TAL compounds into non-volatile lead salts through a

series of reactions with iodine: $PbR_4 \rightarrow PbR_3^+ \rightarrow PbR_2^{2+} \rightarrow Pb^{2+}$. The reaction between TEL and iodine proceeds rapidly enough to allow quantitative collection of TEL by passing the air under test through an aqueous iodine solution.[43–46] The other TAL species react less readily, however, and thus more severe conditions are required for the collection of all five TAL compounds.

The first method with practical applicability was described by Snyder and Henderson.[47] Organic lead vapours were removed from the atmosphere by passage over iodine crystals, and were measured colorimetrically after a dithizone extraction. Although not very sensitive, the method was adopted a few years later by de Treville *et al.*[48] to evaluate the hazard in occupational exposure to TML, and by Cholak[49] to study the organic lead pollution of the air in Cincinnati, U.S.A. Using the same principle for collection, Walker[50] developed a more sophisticated procedure in which a transparent tube filled with iodine crystals was combined with a photo-optical sensor. The presence of alkyl-lead in the atmosphere could be detected by means of the yellow coating of lead iodide on the inner surface of the tube. Moreover, by connecting the photocell into an appropriate circuit, an alarm could be triggered by a predetermined amount of yellow coating. As no information was reported with regard to the sensitivity, it is difficult to ascertain the value of the latter procedure in environmental research.

In general, the use of solid iodine has a number of disadvantages. The main ones are the difficulty in avoiding channelling of the air through the tube, which results in incomplete collection, the variability of the blank values from one adsorption tube to another, and the risk of volatilization of iodine from the collecting tube during sampling. According to Linch *et al.*[51] a solution of iodine in methanol is as efficient as solid iodine for collecting TAL from the air. It is clear, however, that owing to the volatility of the solvent, monitoring with this technique is restricted to limited periods of time. For the same reason the use of a solution of iodine in carbon tetrachloride, as proposed by Nagasawa and Funakubo,[52] has little practical significance.

Moss and Browett[53] used a solution of iodine monochloride in hydrochloric acid for the collection; the TAL compounds were quantitatively converted into the corresponding dialkyl-lead salts without any further reaction to give inorganic lead. The subsequent analysis then involved extracting the collected lead at high pH as dialkyl-lead dithizonate into chloroform/carbon tetrachloride and matching the colour with a standard disc. With sampling periods of 8 hr or more, organic lead concentrations down to 10 $\mu g/m^3$ could be measured. Purdue *et al.*[54] used the same collecting agent but performed the measurement by atomic-absorption spectrometry (AAS) after extracting the dialkyl-lead with ammonium pyrrolidinedithiocarbamate (APDC) into methyl isobutyl ketone (MIBK). For a 24-hr sampling period, the minimum detectable quantity was about 0.2 $\mu g/m^3$. A similar AAS procedure was adopted by Colwill and Hickman,[55] but these authors extracted the dialkyl-lead with dithizone rather than with APDC and used considerably longer sampling periods of several days. The iodine monochloride method was also adopted by Cope *et al.*[56] and by the U.S. Environmental Protection Agency[57] for estimating the TAL emission from alkyl-lead manufacturing plants. In the latter case, the AAS analysis was performed directly on the absorbing solution without any prior treatment.

At this point it should be emphasized that the methods described so far are, strictly speaking, not specific for organic lead. They all rely on filtration for its discrimination from inorganic lead. Most commonly, the lead particulates are removed from the air stream by a membrane filter of pore size around 0.4 μm, positioned before the collection system for the organic lead. A significant proportion of the particulate atmospheric lead may escape collection by such filters[58,59] and can hence cause serious interference in the measurement of organic lead. An additional complication, encountered with any non-specific method, is that because of the widespread dispersion of lead, blank values tend to be relatively high.

Hancock and Slater[60] were able to eliminate these problems by an elegant modification of the iodine monochloride method. After sampling, the organic lead was extracted into carbon tetrachloride with dithizone, any inorganic lead being kept in the aqueous phase by masking with EDTA. The organic lead was then stripped from the carbon tetrachloride extract with nitric acid/hydrogen peroxide solution, the lead concentration in which was then determined by graphite-furnace AAS. The procedure of Hancock and Slater is sensitive and also specific for organic lead compounds, with a very low and easily repeatable blank value. For a 1-hr sampling period the limit of detection is 0.04 $\mu g/m^3$. The method has been successfully adopted and applied by several other workers,[61–64] giving detection limits ranging between 8 and 0.25 ng/m^3 for sampling periods from 6 to 48 hr.

Organic lead compounds may also be collected quantitatively by adsorption on activated carbon. In the method developed by Snyder,[65] a large volume of air (100–200 m^3) is drawn through a bed of activated carbon. After sampling, the carbon is digested in nitric acid/perchloric acid to remove the adsorbed lead. The extracted lead is then measured colorimetrically with dithizone. Owing to a significant blank level of lead in the carbon, very lengthy sampling periods of up to 2 weeks are necessary in order to obtain meaningful results. Despite this drawback and the problem of non-specificity, a similar method was adopted by Cope *et al.*[56] and by the American Society for Testing and Materials.[66] The use of AAS for the analysis, as suggested by Jacobs,[67] should allow greater sensitivities.

In the initial development of the sampling method, Snyder[65] found an irreversible adsorption of TML on

the carbon, but Lizetskaya *et al.*[68] have since reported that the adsorbed TEL can be quantitatively eluted with ethanol. As extraction of inorganic lead can be avoided in this way, lower blanks are obtained and a more sensitive measurement is possible: sampling for 6–7 hr is sufficient to allow the detection of TEL down to concentrations of 5 ng/m^3. Experiments performed by Zemskov and Stepanov[69] have indicated, however, that during prolonged sampling a considerable fraction of the adsorbed TEL can be mineralized, owing to the interaction with oxygen. The use of activated carbon for the collection of organic lead has also been described by other workers[70,71] but in their procedures the lead was determined by direct X-ray fluorescence spectrometric analysis of the graphite. Rabinowitz *et al.*[72] similarly employed an activated charcoal scrubber for the collection of vapour-phase lead, without specifying the method of measurement.

A very convenient way of determining the organic lead content in air by means of activated carbon sampling was recently reported by Birnie and Noden.[73] These authors found that the alkyl-lead compounds were retained quantitatively on a iodized glass-fibre carbon filter. Treatment of the commercially available glass-fibre carbon filters with a methanolic iodine solution was necessary to maintain a satisfactory collection efficiency under humid sampling conditions. Earlier, Carboni and De Lindemann[71] had noticed that impregnation with iodine improved the adsorption characteristics of the activated carbon. After sampling, the lead collected is extracted from the filter with a nitric acid/bromine mixture, after which the analysis can be completed by flameless AAS.[73] According to the authors the method appears to be especially suitable for personal monitoring, though with a detection limit of 4 μg/m^3 for a sampling period of 1 hr, application to ambient air samples does not seem plausible.

Methods for volatile organolead

The results obtained by any of the methods for total organolead may be inaccurate as a measure for tetra-alkyl-lead compounds, owing to the possible presence of other organic lead compounds in the atmosphere, *e.g.*, the breakdown products TriAL and DiAL. If present, these may be expected to exist in both the vapour phase and the particulate matter of small diameter. Since the collection of these less volatile organolead species by filters is therefore unlikely to be quantitative, the concentration data are probably too low to represent "total" organic lead but also too high to represent only TAL.

A number of techniques capable of measuring "volatile" organic lead have been developed, however. A rather simple and yet very sensitive procedure of this kind was reported by Harrison *et al.*[74,75] The gaseous lead alkyls were efficiently trapped from the air by means of a short adsorption tube filled with GC-column packing material and cooled in liquid nitrogen. Subsequent heating of the tube followed by nitrogen elution allowed desorption of the volatile compounds, which were then passed into the combustion air stream of an atomic-absorption spectrometer, set up for lead measurement. Although the question has not been examined systematically, it appears improbable that lead compounds other than organic lead, if collected, would be eluted in a similar way during this procedure. Hence the method can be considered as specific for volatile organic lead. According to the authors a sampling period of 30 min is sufficient for the determination of concentrations down to 3 ng/m^3. This method has been developed further by Rohbock *et al.*[76–78] To remove water vapour that might block the adsorption tube, these authors first pass the air through a cold trap at -78°. The airborne lead alkyls are then collected on GC-column packing material at liquid-air temperature. For the subsequent AAS-analysis, the compounds are thermally desorbed and flushed by argon carrier-gas into the graphite furnace of an electrothermal atomization unit. Interference by other substances is eliminated by deuterium background correction. The cold trap makes it possible to raise the sampling volume to about 200 litres of air, so a detection limit of 1 ng/m^3 can be obtained with a sampling period of about 2 hr. In addition, as the sub-μm lead particles that pass the filter act as nuclei for freezing the water vapour and are deposited in ice particles, analysis of the water retained in the trap furnishes a direct measure of the inorganic lead passing through the filter.

It was claimed that the cold-trap was completely by-passed by the lead alkyls in this procedure, but experiments by De Jonghe *et al.*[79,80] indicated that at a temperature of -78° a fraction of the less volatile TAL species TEL and MTEL was retained. Moreover, according to these authors, in general, removal of water by a cold-trap is only quantitative at temperatures so low that some TAL will also be retained, which is not surprising in view of the rather similar vapour pressure.[81] These authors preferred, therefore, to trap both the water and the lead alkyls in the same tube by using a temperature of -130°.[79] This required the use of a more loosely packed material than GC-supports, *e.g.*, 4-mm diameter glass beads, to avoid rapid clogging of the pores by deposition of ice. Such a collection system was applied in the procedure described by Jiang *et al.*[82] After sampling, the cold-trap was warmed up slowly to volatilize the accumulated lead alkyls, which were subsequently collected in a nitric acid/hydrogen peroxide mixture. By a simple additional treatment it thus became possible to perform the graphite-furnace AAS determination of airborne volatile organolead with aqueous solutions of lead nitrate. The detection limit for a 1-hr sampling was quoted as 0.04 μg/m^3.

This problem of water condensation was also successfully circumvented by Coker,[83] who used polymer beads for the collection, at room temperature. Volumes of at least 10 litres of air could be taken without breakthrough losses, allowing sampling

periods of up to 8 hr. The analysis was completed by direct thermal desorption of the trapped compounds into the burner of an atomic-absorption spectrometer. As the absolute detection limit of the method is 10–20 ng of lead, application of this method is restricted to highly polluted environments.

Species-specific TAL methods

The species-specific measurement of airborne TAL compounds implies the use of chromatographic separation techniques. Owing to the favourable vapour pressures,[81] gas–liquid chromatography has invariably been considered to give a suitable separation. Cantuti and Cartoni[84] described a procedure for the direct collection of TEL from polluted air and its GC-determination at ppm levels by electron-capture detection. The air to be analysed was passed through a sampling trap consisting of a short tube packed with normal gas chromatographic support and liquid phase. When enough air had been passed through to saturate this trap, there was equilibrium between the concentrations in the gas phase and the liquid phase. The collected compounds were introduced into the gas chromatograph column by flash heating the sample tube to about 130° in a small electric furnace positioned immediately above the injection port. The method was successfully employed for the measurement of the pollution level in the air around a factory where TEL was produced. It was later modified and improved by Tausch[85] to allow its application to the analysis of city air. In this way detection limits of about 0.05 and 0.5 $\mu g/m^3$ were obtained for the determination of TEL and TML. Gas chromatography with electron-capture detection was also used in the procedure of Corrin and Menne.[86,87] Sampling was accomplished in this case with hexane bubblers at −78°. The hexane solutions were concentrated under vacuum at −78° to approximately 2% of the original volume before injection into the gas chromatograph. As environmental samples often contain a variety of organic compounds with high electron affinity[88,89] these methods presumably lack the specificity required for detecting ultratrace quantities of TAL.

The widespread acceptance of gas chromatography as the analytical method for separating atmospheric alkyl-lead substances follows from the work of Laveskog,[90,91] who applied the technique to a detailed study of the TML and TEL concentration levels in street air. To ensure complete collection of the organic lead, instead of using equilibration between the compounds in the air and those adsorbed on the GC packing material, this author kept the adsorption tube at about −80° during sampling. It may be mentioned here that most other cryogenic trapping techniques for TAL are direct descendants of Laveskog's original system. The analysis involved desorption by heating, and transport of the desorbed compounds with an inert gas into a gas-chromatograph/mass-spectrometer combination (GC/MS). TML and TEL were detected in sequence at $m/e = 237$ [$(CH_3)_2{}^{207}Pb$ or $C_2H_5{}^{208}Pb$]. A limit of detection of 10 pg could be achieved, which for a 1-litre sample corresponded to 0.01 $\mu g/m^3$ for an individual compound in air and was attainable in about 15 min. This sensitivity is extremely high and is unlikely to be attainable with all GC/MS instruments.[41]

The use of a low to medium resolution mass spectrometer, as used by Laveskog together with gas chromatographic separation, is also unlikely to give total freedom from the mass-spectral interferences that can arise from organic materials in the atmosphere and from substances bleeding from the GC column.[41,92] However, the same method was adopted later by Allvin and Berg.[93] Nielsen *et al.*[94] recently reported a refined version of the technique. These authors collected the TAL compounds at ambient temperature on co-polymers of ethylvinyl- and divinylbenzene. After sampling was completed, the analytes were thermally desorbed and collected at −80° on a small adsorption tube containing GC column packing material to concentrate the sample in a small volume before its introduction into the GC/MS. Use of the isotope-dilution technique, accomplished by adding known amounts of deuterated TML and TEL in advance to the sampling tubes, made it possible to correct for decomposition during sampling and/or the analytical procedure. Recovery studies with standards revealed a significant loss of TEL during sampling, possibly as a result of reaction with ozone. TML, on the contrary, was recovered quantitatively and could be determined down to concentrations as low as 20 pg/m^3, with a sampling period of 24 hr. It can be mentioned that a preliminary study by Perry *et al.*[95] has indicated that the adsorption tubes used in cryogenic trapping can equally well be used for direct analysis for TAL by mass spectrometry, without prior GC-separation.

Chau *et al.*[96] used collection on cooled GC-column packing material, followed by determination of all five TAL compounds by GC/AAS. The adsorption tubes were connected to a 4-way valve between the carrier-gas inlet and the column injection-port, so that the sample could be swept into the GC column by the carrier gas. The GC was interfaced by stainless-steel tubing to the burner-nebulizer, so that conventional flame AAS could be used. Consequently, the measurement was highly specific: only lead-containing components in the GC effluent were detected. Moreover, if the AA recorder was started simultaneously with the sample introduction, the retention time could be used for identifying the different species. The method was primarily developed for the study of the biological conversion of lead compounds into TML in aquatic systems. The low analytical sensitivity (the detection limit for an individual species is about 0.08 μg) combined with a very slow air-sampling flow-rate (130–150 ml/min) makes this procedure unsuitable for the analysis of environmental air. In a later modification,[97–99] the authors were able to improve the sensitivity by three orders of magnitude by using an elec-

trically heated silica furnace in the AAS unit. In this case, the burning of hydrogen, introduced at the centre of the furnace, was claimed to improve the atomization efficiency.

Several authors[100–102] have pointed out that graphite-furnace AAS (GFAAS) has excellent performance characteristics when used as a metal-specific detection system for TAL compounds separated by gas chromatography. Radziuk *et al.*[92] applied this approach to the analysis of air samples, in combination with a collection system similar to the one described by Chau *et al.*[96–99] An empty glass tube at $-15°$ was incorporated in the sampling train to condense the moisture. The GC/GFAAS interface was achieved by passing the effluent from the GC-column through an electrically heated, Teflon-lined, aluminium transfer tube to a tantalum connecting piece, friction-fitted into the injection port of the graphite furnace. The silica windows were removed from the furnace assembly to allow optimal gas flow. During the analysis, the graphite furnace was continuously heated at 1500° for 20 min. The system described permits the determination of individual TAL compounds down to concentrations of 0.5 ng/m^3, with a sampling period of about 18 hr. Interfering absorbance signals may be observed, however, as a result of decomposition of the TAL compounds in the hot part(s) of the transfer tube, giving rise to deposition and remobilization of lead during subsequent runs (memory effect).

These problems were not encountered with the GC/GFAAS apparatus employed by Robinson and coworkers,[103–105] because the column effluent was conducted by means of a heated stainless-steel transfer line to the bottom of a specially designed "hollow-T" carbon atomizer. Effects resulting from the decomposition of TAL were avoided, as no part of the interface was exposed to extreme temperatures. The sensitivity was very good: for TML a detection limit of 0.04 ng was reported.[103] Alkyl-lead compounds from the atmosphere were collected on Tenax-packed glass columns at liquid-nitrogen temperature, allowing volumes of about 1 m^3 of air to be sampled. Despite these favourable conditions, the authors were usually not able to detect any organic lead.[104–106] They claimed that this was probably due to the gaseous fraction of atmospheric lead being predominantly inorganic in nature (instead of organic) and hence not determinable by GC/GFAAS.[105–108] In our view this assumption contradicts the results obtained by a number of other authors (see Tables 1–3) and we believe that their failure to detect organic lead is attributable to losses of collected TAL before the GC/AAS analysis, and is associated with the explosive evaporation of the accumulated gases from the adsorption tube, as may occur on removal from the liquid nitrogen.[79]

GC/GFAAS also provided the basis for the procedure reported by De Jonghe *et al.*,[40,79] who interfaced conventional GC and AAS units by introducing the GC-column effluent into the graphite furnace through the inner gas-flow entrances, thus avoiding any drastic modifications to either of the instruments. The flow of GC carrier-gas followed the same pattern as the normal gas flow (which it replaced) inside the graphite tube;[102] deuterium-lamp background correction was sufficient to correct for interfering absorbance signals. By an appropriate choice of the instrumental parameters, the GC/GFAAS analysis time could be reduced to about 6 min. The gaseous alkyl-lead compounds were collected from the air by cryogenic trapping on glass beads at $-130°$. The collected TAL species were then thermally desorbed from the trap and transferred to a short adsorption tube filled with GC packing material, to allow rapid injection into the GC/GFAAS apparatus. In general, air samples were taken for a period of 1 hr, corresponding to a volume of 360 litres; under these conditions the lowest detectable concentration was about 0.2 ng/m^3. Despite the speed of the sampling and the analytical procedure the application of the technique was quite critical and cumbersome.

Reamer *et al.*[109,110] developed a method using a gas chromatograph/microwave plasma detector (GC/MWPD) for the analysis. The TAL species were collected from the air in a cold-trap containing GC packing material at $-80°$ and were then removed from the sorbent by a freeze-drying technique. The volatile alkyl-lead compounds were concentrated in an organic solvent before separation and determination. For the background correction, a technique based on wavelength modulation was applied. With 2-hr sampling periods, the detection limit for an individual TAL compound was about 0.5 ng/m^3. The lengthy period necessary for the analysis (more than 12 hr) makes this method rather impractical for pollution-control measurements. Moreover, the GC/MWPD combination is instrumentally complex and not routinely available in analytical laboratories.

Air concentration levels

Tables 1–3 summarize the main analytical results from surveys performed with the different methods described. Usually the concentration found for organic lead is expressed as a fraction of the total lead concentration, as this figure is less dependent than the absolute level on the total lead use at a particular location and the sampling site topography and meteorology.[41] However, the total composition of atmospheric lead is not yet definitively known: to date, reliable data are available only for filter-collected particulate lead and gaseous lead alkyls in ambient air. The variable proportions of gaseous inorganic lead[105–108] and non-filterable particulate lead in Aitken particles,[111] as well as the organic lead present in the particulate matter, have not yet been sufficiently investigated. As total lead is thus more or less a fictitious quantity, it is better to use the ratio of non-filterable organic to filtered particulate lead.[112]

It appears from Tables 1–3 that a direct comparison of the alkyl-lead data so far available is still

Table 1. Reported concentrations of alkyl-lead in ambient air, as obtained with "total organolead" methods†

Location and date	Ref.	Type of site	No. of samples	Averaging time	Organic lead conc., ng/m^3		Organic/particulate lead, %	
					Range	Mean	Range	Mean
San Francisco 1963*	46	Petrol station	266	15–30 min	N.R.	6710	N.R.	238
Los Angeles 1963*	46	Petrol station	321	15–30 min	N.R.	2120	N.R.	86
Cincinnati 1964*	49	Urban	3	15–30 min	100–200	200	N.R.	1.3
6 U.S. cities 1973*	54	Urban	54	1 day	100–800	230	2.0–72	12
		Parking garage	5		1800–2200	1900	18–22	20
London 1973*	55	Highway	6	3–6 days	470–3570	1365	3.6–27	12
		Tunnel	4	5–9 days	750–1720	1238	6.7–37	15
		Petrol station	8	4–11 days	460–1540	889	39–144	82
London 1975	60	Works entrance, morning	3	25 min	⩽100–300	⩽200	N.R.	N.R.
		Works entrance, evening	9	10 min	⩽100–2500	⩽770		
Lancaster 1978	61	Rural	33	1–2 days	0.5–230	20	1.5–49	11
		Residential			1.7–20	9	0.8–16	4.7
London 1980	62	Urban, 5 m height	7	1 day	24–190	94	3.3–11	6.6
		Urban, 14 m height			16–130	65	3.6–15	7.2
Antwerp 1980	63	Urban, 1 m height	3	6 hr	77–262	166	14–26	20
		Urban, 20 m height	2		76–112	94	19–24	22
		Residential	5		8–20	13	2.6–13	7.9
		Rural	4		⩽8	⩽8	⩽5.7–8.7	⩽7.5
		Tunnel	2		99–112	106	1.7–6.1	3.9
		Petrol station			192–213	203	31–32	32
Glasgow 1981	64	Urban, 0.3 m height	1	1 day	—	96	—	11
		Urban, 7.5 m height	28		7.2–7.7	32	2.5–25	6.7
		Residential	10		1.5–54	16	0.9–6.7	3.0
		Rural	8		1.6–6.5	3.9	1.5–64	14
		Urban, overnight (1800–0800)	5	14 hr	2.7–30	11	0.8–4.2	2.5
		Urban, daytime (0800–1800)		10 hr	21–144	53	2.2–6.4	4.0
		Urban, 3 m height	8	7 hr	37–110	73	2.5–6.3	3.7
		Urban, 12 m height	5		31–53	43	2.5–6.0	3.5
		Urban, 5–30 m height	6		2.9–52	24	1.7–8.1	4.9
		Petrol station	3		47–274	195	15–47	25
Los Angeles 1967	65	Highway	6	4–15 days	47–108	78	1.6–4.4	2.6
Delaware, Pa 1976	67	Roadside by bridge	10	days	⩽6–210	83	0.3–1.8	0.8
Los Angeles 1977	72	Urban area	1	5–20 days	—	100	—	5.3

†The results for the locations indicated with an asterisk are probably unreliable owing to interference by inorganic lead.
N.R. = not reported.

Table 2. Reported concentrations of alkyl-lead in ambient air, as obtained with "volatile organolead" methods

Location and date	Ref.	Type of site	No. of samples	Averaging time	Organic lead conc., ng/m^3 Range	Mean	Organic/particulate lead, % Range	Mean
London 1974	74, 75	Urban	4	30 min	40–110	78	0.9–3.4	1.7
		Tunnel	1		—	20	—	0.1
		Petrol station	2		240–590	415	4.1–11	7.3
Frankfurt 1980	77	Urban, 3.5 m height	40	2–3 hr	5–170	45	N.R.	7.1
		Urban, 20 m height	20		2–130	20		9.0
		Residential	30		1–90	24		7.8
		Rural	4		1–7	3		3.5
		Highway	10		3–15	8		0.7
		Parking garage	4		450–1000	678		28
Antwerp 1981	82	Petrol station	3	30–60 min	210–260	239	N.R.	N.R.

N.R. = not reported.

Table 3. Reported concentrations of alkyl-lead in ambient air, as obtained with "species-specific" TAL methods

Location and date	Ref.	Type of site	No. of samples	Averaging time	Organic lead conc., ng/m^3 Range	Mean	Organic/particulate lead, % Range	Mean
Fort Collins, Colorado 1977	86	Highway	6	N.R.	33–180 TML	109	2.8–30	14
Stockholm 1970	90, 91	Urban	6*	2–24 hr	220–950 TML, TEL	480	6.5–31	15
		Urban	11	1–13 hr	100–1760 TML, TEL	637	N.R.	N.R.
Stockholm 1977	93	Urban	5	3–8 hr	120–1300 TML, TEL	508	N.R.	N.R.
		Parking garage	3	2–5 hr	560–3400 TML, TEL	2007		
		Petrol station	1	4 hr	— TML, TEL	700		
Stockholm 1981	94	Urban	12	10 hr	11–77 TML	39	N.R.	N.R.
Copenhagen 1981	94	Urban	2	2 hr	185–195 TML, TEL	195	N.R.	N.R.
		Residential	6	2–26 hr	5.3–60 TML, TEL	34		
		Rural		16–26 hr	0.5–2.5 TML	1.5		
Toronto 1979	92	Urban	N.R.	18 hr	N.R. TMEL, DMDEL MTEL, TEL			
Antwerp 1981	40	Urban	9	1 hr	49–109 TML, . . . , TEL	83	4.6–12	8
		Residential	7		3.2–14 TML, TMEL, DMDEL	7	0.6–3.4	2
		Highway	10		14–44 TML, . . . , TEL	24	0.8–3.0	1.7
		Tunnel	15		12–162	39	0.2–1.4	0.5
		Car-repair shop	6		100–290	205	5.9–14	10
		Petrol station	21		17–410	149	2.7–35	12
		Rural	6		0.3–3.9 TML, TMEL	2	0.1–0.7	0.5
Baltimore 1978	110	Highway	6	2 hr	26–75 TML, . . . , TEL	53	1.4–5.2	3.2
		Tunnel	9		57–130	92	0.4–0.8	0.6

*Each sample period is made up of sequential 10–15 min samples; individual samples show much wider ranges of concentrations.
N.R. = not reported.

rather problematic. Mean concentrations in ambient air range from 2 ng/m^3 at a rural site to up to 2000 ng/m^3 in a parking garage, whilst individual measurements at specific sites cover a wide range. Owing to the diurnal fluctuations, there is a far greater variability in the concentrations measured over short(er) averaging periods. Factors affecting roadside concentrations include the traffic density and the driving mode: cold engines running on the choke, cruising vehicles with warm engines, idling vehicles, *etc.*, apparently differ in emission factor. For instance, individual 20-min kerbside samples alongside a set of traffic lights during a period when cold vehicles (with choke-running) were leaving a factory, ranged up to 2500 ng/m^3, well above the typical kerbside values of about 100 ng/m^3 when the traffic is free-flowing.[60] Concentrations further depend on the proximity to the vehicular emissions. Measurements by Birch *et al.*[62] in a central London street showed a 30–35% decrease in the TAL concentrations found, when the sampling height was increased from 5 m to 14 m above the street, with a similar decline for particulate lead. For evaporative emission sources, the meteorological conditions should also have a profound influence on the levels measured. Similarly, the concentration and type of alkyl-lead compounds in the petrol used in the area of investigation is crucially important.

The data suggest an average alkyl-lead concentration in urban air of about 100 ng/m^3, typically representing 5–10% of the particulate lead level. The main part of the investigations carried out in the U.S. and Canada shows that the proportion of organic to particulate lead is of the order of 1:100–1:20. Since the legislation on emission control systems in cars is more stringent in the U.S.A. than in other countries of the Western World, the level of organic lead is probably 5–15% of the particulate lead content in the urban air in the latter countries. Tables 1–3 further indicate that in an urban environment a background organic lead level of 10–20 ng/m^3 is present. It is air with this composition that will be convected to rural sites and there give rise, after dilution and physicochemical transformations, to concentrations of several ng/m^3.

The question of possible methylation of inorganic lead in the environment is still unanswered. So far only Harrison and Laxen[1] have obtained indications for naturally produced organic lead in samples near the intertidal flats on the West Coast of England. When the sampled air had passed over the sea and the coastal area, organic to particulate lead ratios of up to 50% were reported. Unfortunately, the method of analysis used by these authors could not discriminate between the different alkyl-lead species, so it is impossible to verify whether such elevated ratios are accompanied by exceptionally high TML contributions. Measurements of organic lead with the species-specific technique of De Jonghe *et al.*[79] in the atmosphere at rural sites near Beijing, China,[113] were all below the detection limit of 0.1 ng/m^3, thus precluding terrestrial sources as its origin.

Another problem of present interest is the separate determination of the TAL-degradation products (TriAL and DiAL) in the atmosphere. So far, no such method has been reported. The discrepancies between the total organolead results and those obtained with species-specific TAL methods can support the idea that there are substantial quantities of TriAL and DiAL compounds in the air.[40] Nevertheless, the measurements by Nielsen *et al.*[114] appear to be the only direct evidence so far for their occurrence in natural samples. These authors found that the brain tissues of individuals residing in the lower floors of buildings in a city have a TriAL content of the order of 40 ng/g. Attempts to determine these compounds in water samples have been unsuccessful, probably because the analytical method employed[115] was not sensitive enough.

Although, in laboratory studies, individual TriAL species have allegedly been determined by gas chromatography with an element-specific detector,[116–118] the use of ion chromatography or HPLC might also be advantageous for sorting out the lower lead alkyls. Pioneering experiments of the latter kind have been done by Potter,[119] but the lack of a specific detector prohibited application. Interfacing of the HPLC system with AAS, MWPD or ICP should be a possible solution to this problem.

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DETERMINATION OF SILVER IN ORES, CONCENTRATES, ZINC PROCESS SOLUTIONS, COPPER METAL AND COPPER-BASE ALLOYS BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY AFTER SEPARATION BY EXTRACTION OF THE TRIBENZYLAMINE–SILVER BROMIDE COMPLEX

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Summary—A method for determining ~0.1 μg/g or more of silver in ores and concentrates and ~0.001 μg/ml or more of silver in zinc process solutions is described. Silver is separated from the matrix elements by chloroform extraction of the tribenzylamine–silver bromide ion-association complex from 0.08*M* potassium bromide–2*M* sulphuric acid and stripped with 9*M* hydrobromic acid. This solution is evaporated to dryness and organic material is destroyed with nitric and perchloric acids. Silver is determined by atomic-absorption spectrophotometry in an air–acetylene flame, at 328.1 nm, in a 10% v/v hydrochloric acid–1% v/v diethylenetriamine medium. Cadmium, bismuth and molybdenum are partly co-extracted but do not interfere. The method is also applicable to copper metal and copper-base alloys. Results obtained by this method are compared with those obtained by a fire-assay/atomic-absorption method.

A current CANMET project involves a study of the behaviour of silver in conventional and proposed zinc hydrometallurgical processes (designed to recover metallic zinc from zinc ores and concentrates) with the objective of increasing the recovery of silver in Canadian zinc plants. As part of this project, a simple, sensitive, relatively rapid and reliable atomic-absorption spectrophotometric (AAS) method was needed for the routine determination of small amounts of silver in zinc process solutions containing sulphuric acid, ferric sulphate up to ~25 g/l., zinc sulphate at ~200 g/l. and moderate amounts of various other elements. The method should also be applicable to sulphide concentrates and other processing products. Because large amounts of zinc suppress the absorbance of silver in an air–acetylene flame owing to reduction of the atomization rate, because of the high viscosity of the solution, it was considered that a suitable method would involve a reasonably selective solvent extraction–preconcentration step.

Recently, solvent extraction–AAS methods for silver have been reviewed and, from this work and studies of the literature cited, it was considered that methods based on chelation systems (phenylthiourea, ammonium pyrrolidinedithiocarbamate and other dithiocarbamates, dithizone, xanthate and 8-hydroxyquinoline)[1] including iso-octyl thioglycollate,[2] were not the most promising for the present work because of their lack of selectivity. Methods based on the extraction of silver into solvents containing tri-iso-octyl thiophosphate, triphenylphosphine and petroleum sulphides are more selective[1] but these reagents were not immediately available. Furthermore, many of these methods involve the extraction of silver from a nitric acid medium which, in the presence of chloride as a contaminant, results in incomplete extraction of the silver because of the formation of silver chloride.[1] Many recent extraction methods, with AAS and other finishes, are based on the extraction of anionic chloro-, bromo- or iodo-silver complexes as ion-association compounds from halide media into organic solvents containing high molecular-weight amines and quaternary ammonium salts such as tricaprylylmethylammonium chloride (Aliquat 336),[3,4] tricaprylylamine (Alamine 336),[4] tri-n-octylamine (TOA),[5] trialkylbenzylammonium chloride[6] and tri-n-octylmethylammonium bromide.[7] In most of these methods, and in studies of the extraction behaviour of silver with TOA,[8,9] tri-n-dodecylamine,[10] trilaurylamine,[11] tetra-alkylammonium salts[12] and various other basic extractants,[13,14] silver is extracted from dilute hydrochloric acid or from hydrochloric acid–potassium iodide media, from which zinc and usually iron are also largely co-extracted. However, the extraction curves shown in a recent study[15] of the extraction of various elements from sulphuric acid–potassium iodide media into *o*-xylene containing TOA suggested that it might be possible to separate silver from zinc and iron by extracting silver from a relatively dilute halide–sulphuric acid medium. Con-

sequently, the applicability of this type of separation was investigated in the present work. Although in most of the extraction methods mentioned above the organic extract is sprayed into the flame, an aqueous medium was chosen for this work because of the greater ease of preparation and convenience of aqueous calibration solutions for routine work.

The proposed method separates the silver from matrix elements by extraction from 0.08*M* potassium bromide–2*M* sulphuric acid into chloroform containing tribenzylamine. Silver is then stripped with 9*M* hydrobromic acid. Results obtained for ores and concentrates by this method are compared with those obtained by a fire-assay/AAS method. The applicability of the method to copper metal and copper-base alloys is shown.

EXPERIMENTAL

Apparatus

A Varian-Techtron model AA6 spectrophotometer, equipped with a 10-cm laminar-flow air–acetylene burner and a silver hollow-cathode lamp, was used under the following conditions.

Wavelength: 328.1 nm
Lamp current: 3 mA
Spectral band-pass: 0.20 nm
Height of light-path above burner: 4 mm
Acetylene flowmeter reading: 2.0 (~2.0 l./min)
Air flowmeter reading: 6.5 (~13.0 l./min)
Flame: strongly oxidizing
Aspiration rate: 2 ml/min

Reagents

Standard silver solution, 100 μg/ml. Dissolve 0.1000 g of pure silver foil in ~25 ml of 25% v/v nitric acid, add ~1 ml of 50% v/v sulphuric acid and evaporate the solution to dryness. Dissolve the salts in ~50 ml of concentrated hydrochloric acid and transfer the solution to a 1-litre standard flask containing ~350 ml each of concentrated hydrochloric acid and water. Allow the solution to cool to room temperature, then dilute to volume with water. This solution is stable for at least 2 months. Prepare a 10-μg/ml solution by diluting 10 ml of this stock solution and 36 ml of concentrated hydrochloric acid to 100 ml with water. Prepare this diluted solution fresh as required.

Diethylenetriamine. A 10% v/v solution in water.

Bromine. A 20% v/v solution in carbon tetrachloride.

Potassium bromide, 20% solution.

Tribenzylamine. A 3% solution in chloroform.

Sulphuric acid, 50% v/v.

Sulphuric acid 5% v/v. Store in a plastic squeeze-type wash-bottle.

Hydrobromic acid, 50% v/v. Store in a plastic squeeze-type wash-bottle.

Ammonia, 25% v/v solution. Add 50 ml of concentrated ammonia solution to 150 ml of water. Store in a plastic squeeze-type wash-bottle.

Ethyl acetate. Analytical reagent-grade.

Procedures

Calibration solutions. Add 10 ml of 10% diethylenetriamine solution to each of eleven 100-ml standard flasks; then, from a burette, add to the first five flasks 2, 4, 6, 8 and 10 ml, respectively, of 10-μg/ml standard silver solution. To the next five flasks, add 1.5, 2, 2.5, 3 and 4 ml, respectively, of 100-μg/ml standard solution. The last flask contains the zero calibration solution. Add sufficient concentrated hydrochloric acid to each flask for the final solution to be 10% v/v hydrochloric acid, then dilute each solution to volume with water.

Ores and concentrates. Transfer up to 1 g of powdered sample, containing up to ~500 μg of silver and not more than ~350 mg of lead, to a 250-ml Teflon beaker, cover and add 5 ml of 20% bromine solution in carbon tetrachloride (Note 1) and 15 ml of concentrated nitric acid. Mix and allow the solution to stand for ~15 min, then heat gently to remove the bromine and carbon tetrachloride. Add 30 ml of 50% sulphuric acid, heat until the evolution of oxides of nitrogen ceases, then remove the cover and wash down the sides of the beaker with water. Add 5 ml of concentrated hydrofluoric acid and carefully evaporate the solution until copious fumes of sulphur trioxide are evolved. Cool, wash down the sides of the beaker with water (Note 2) and evaporate the solution to fumes of sulphur trioxide again to ensure the complete removal of hydrofluoric and nitric acids. Cool, add ~40 ml of water and heat to dissolve the salts. Cool the solution and, if necessary, filter it (Whatman 11-cm No. 40 paper) into a 125-ml separatory funnel marked at 100 ml. Wash the beaker and the paper once each with water, then wash the beaker twice with ~5-ml portions of 25% ammonia solution (Note 3). Wash the paper and residue three times with ~5-ml portions of 25% ammonia solution followed by ~10 ml of 5% sulphuric acid, then discard the paper. Dilute the solution to ~95 ml with water.

Add 5 ml of 20% potassium bromide solution and 20 ml of 3% tribenzylamine solution (Note 4) to the resulting solution, stopper the funnel and shake it for 2 min. Allow several min for the layers to settle, then drain the chloroform phase into a 60-ml separatory funnel. Extract the aqueous phase twice more by shaking for 2 min and 1 min, respectively, with 5-ml portions of tribenzylamine solution. Wash the stem of the funnel with 1 ml of methanol after the last extraction step (Note 5). Add 15 ml of concentrated hydrobromic acid (Note 6) to the combined extracts, stopper, and shake for 3 min (Note 7). Add 5 ml of ethyl acetate, shake gently for ~5 sec and allow the layers to separate. Drain the lower aqueous phase into a 100-ml beaker and wash the stem of the funnel with 50% hydrobromic acid. Wash the chloroform phase twice (Note 8), by shaking it for ~1 min and ~30 sec, respectively, with 5-ml portions of concentrated hydrobromic acid and add the washings to the beaker. Wash the stem of the funnel with 50% hydrobromic acid each time. Cover the beaker, heat the resulting solution to remove methanol and residual chloroform, then remove the cover and evaporate the solution to dryness. Cover, add 5 ml of concentrated nitric acid and 2 ml of concentrated perchloric acid and heat until fumes of perchloric acid are evolved. Remove the cover and evaporate the solution to dryness. Depending on the expected silver content, add sufficient concentrated hydrochloric acid and 10% diethylenetriamine solution for 1 ml of each to be present for each 10 ml of final solution (Note 9). Transfer the solution to a standard flask of appropriate size (10–200 ml) and dilute to volume with water.

Measure the absorbance at 328.1 nm when this solution is aspirated into a strongly oxidizing air–acetylene flame (Note 10). Calculate the silver content (in μg) from the sample absorbance and those obtained concurrently for calibration solutions that bracket the sample concentration.

Zinc process solutions. Transfer up to 75 ml (Note 11) of the solution, containing up to ~500 μg of silver, to a 125-ml separatory funnel marked at 100 ml and add sufficient concentrated or 50% sulphuric acid for the final concentration to be ~2*M*. Dilute the solution to ~95 ml with water and allow it to cool to room temperature. Add 5 ml of 20% potassium bromide solution and 20 ml of 3% tribenzylamine solution and proceed with the extraction and subsequent determination of silver as described above.

Copper metal and copper-base alloys. Transfer up to 1 g of sample, containing up to ~500 μg of silver and not more than ~350 mg of lead, to a 400-ml beaker. Cover the

beaker, add 15 ml of concentrated nitric acid, heat gently until the sample is decomposed, then add 30 ml of 50% sulphuric acid and heat until the evolution of oxides of nitrogen ceases. Remove the cover, wash down the sides of the beaker with water and evaporate the solution until copious fumes of sulphur trioxide are evolved. Cool, wash down the sides of the beaker with water (Note 2) and evaporate the solution to fumes of sulphur trioxide again to ensure the complete removal of nitric acid. Cool, add ~40 ml of water, heat to dissolve the salts, then proceed with the filtration of the solution, if necessary, and the extraction and determination of silver as described above.

Notes

1. Bromine is added to oxidize sulphur and sulphides to sulphate.
2. If more than ~2 or 3 mg of antimony is present, add 5 or 10 ml of concentrated hydrobromic acid at this stage. Evaporate the solution to fumes of sulphur trioxide, then wash down the sides of the beaker with water and repeat the evaporation step.
3. Washing with ammonia solution is necessary to dissolve any silver chloride produced by contamination of the solution with chloride, any silver bromide resulting from the decomposition step or from the volatilization step described in Note 2, and any silver retained by lead sulphate. The beaker should be washed with ammonia solution even if the filtration step is unnecessary. To prevent the neutralization of too much sulphuric acid, the total volume of ammonia solution used should not exceed ~25–30 ml.
4. In the first extraction step, it is recommended that potassium bromide solution should be added to only two solutions at a time, followed by the extraction—within ~5 min—of the tribenzylamine–silver bromide complex. The extraction may be incomplete if the solution is allowed to stand for too long after the addition of the bromide solution. The second and third extraction steps can be applied to a series of solutions.
5. Methanol dissolves any dried tribenzylamine–silver bromide complex remaining in the stem of the funnel, but not more than ~1 ml should be used or the organic and aqueous phases may become miscible.
6. It is recommended that yellow hydrobromic acid containing bromine should be used, because it is easier to see the interface between the aqueous and organic phases.
7. A wrist-action shaker can be used, with elongated pear-shaped separatory funnels, if a heavy rubber band is stretched around the stopper and stopcock to hold the stopper in tight to prevent leakage.
8. Some tribenzylamine may separate in the chloroform phase during the washing steps but this does not interfere with the separation of silver.
9. For final sample solution volumes of 10, 25, 50, 100 and 200 ml, add 1, 2.5, 5, 10 and 20 ml, respectively, of each solution. If the residue contains molybdenum, indicated by blue salts, add the diethylenetriamine solution first and, if necessary, warm the solution to dissolve the salts.
10. Scale expansion (~3–5-fold) is recommended for the determination of ≤ 1 μg of silver per ml.
11. Up to ~100 ml of the solution can be taken if the required volume of concentrated or 50% sulphuric acid is added and the solution is evaporated to ~75 ml and then transferred to the separatory funnel (Note 3).

RESULTS AND DISCUSSION

Calibration solutions

Calibration solutions for silver determination by AAS often contain ~30–50% by volume of concentrated hydrochloric acid to keep the silver in solution. This also avoids the error resulting from contamination with chloride, which can occur in nitric acid media. However, considerably less hydrochloric acid can be employed if a soluble amine such as diethylenetriamine (DTA) is used to complex the silver.[16] This helps to reduce burner corrosion. Although DTA can also be used in conjunction with hydrobromic acid, hydrochloric acid was used for convenience in this work because concentration of the final hydrobromic acid solution by evaporation, and destruction of the excess of tribenzylamine, are usually necessary before the AAS determination. Calibration solutions containing 10% and 1% by volume of concentrated hydrochloric acid and DTA, respectively, and ≤ 1 μg of silver per ml are stable for at least 2 weeks. Solutions of higher concentration must be prepared fresh every 4 or 5 days because the absorbance slowly decreases on standing.

Separation of silver by tribenzylamine-chloroform extraction

Recently, Spivakov *et al.*[15] investigated the effects of sulphuric acid and potassium iodide concentration on the extraction of silver, zinc, iron and various other elements into *o*-xylene containing TOA. Their extraction curves suggested that it might be possible to separate silver from zinc by extracting silver from $<0.2M$ potassium iodide–sulphuric acid. They also reported that iron(III) is not extracted from up to at least $2.5M$ sulphuric acid–$1M$ potassium iodide solutions. In preliminary tests, tribenzylamine (TBA) and chloroform were chosen as extractant and diluent because of their ready availability and the high density of the mixture. Silver was added in the form of a dilute sulphuric acid solution, the extracts were evaporated to dryness, and the residues were treated with hydrogen peroxide, nitric and perchloric acids, followed by evaporation of the solution to dryness to destroy TBA. The results showed that up to 500 μg of silver can be completely extracted, in a triple extraction, into chloroform containing 1.5% TBA, from 100 ml of $0.05M$ potassium iodide–$2M$ sulphuric acid and separated from 20 g of zinc sulphate and 2.5 g of ferric sulphate. In these tests, ~1 g of ascorbic acid was added to reduce iron(III) and prevent its reaction with iodide. However, this approach was ultimately abandoned because of the difficulty of destroying large amounts of TBA and because silver could not be readily stripped from the chloroform phase. It could not be stripped with concentrated hydrochloric acid, and the use of concentrated nitric acid resulted in the formation of silver iodide in the separatory funnel. Furthermore, tests showed that small amounts of copper interfered with the extraction of silver because of the formation of insoluble cuprous iodide.

Tests involving the extraction of the silver chloro- and bromo- ion-association complexes from sodium chloride–sulphuric acid and potassium bromide–sulphuric acid media, respectively, showed that although both complexes can be readily stripped from the extracts with the corresponding halide acids, it is more convenient to strip the bromo-complex. In the

chloride system, not all the silver is stripped in one step, which means that because of the greater density of the chloroform phase the use of two separatory funnels is required for complete back-extraction of silver. Conversely, in the bromide system, the stripping step can be performed in one funnel because the density of concentrated hydrobromic acid is almost the same as or slightly greater than that of the chloroform extract. However, the addition of a solvent of low density such as ethyl acetate is required to further decrease the density of the organic phase and aid in phase separation. On the basis of these findings, the extraction of silver from a potassium bromide–sulphuric acid medium was investigated in this work.

Preliminary tests with pure silver solutions showed that up to at least 500 μg can be quantitatively extracted, in three extractions, from 100 ml of $\sim$0.2–5M sulphuric acid/$\sim$0.02–0.08M potassium bromide by shaking with $\geq$3% TBA in chloroform, as described in the procedure. The extraction of silver at this level is incomplete if less than 20 ml of TBA solution or less than a 2-min shaking period is used in the first extraction step. Extraction at higher acid concentrations was not tested because of the slowness of phase separation. The optimum sulphuric acid concentration is $\sim$0.2–2.5M. At higher acidities, TBA may precipitate in the second and third extracts but this does not interfere with either the extraction or the back-extraction of silver. Silver is not extracted in the absence of sulphuric acid. The optimum TBA concentration is $\sim$3%. Extraction is incomplete at concentrations of $\sim$2% or less and, at $>$3%, too much TBA precipitates in the chloroform phase. Concentrations of $\sim$2M sulphuric acid and 0.08M potassium bromide were chosen for subsequent work. Under these conditions, up to 500 μg of silver in $\sim$100 ml of solution can be completely separated from the amounts of zinc sulphate and ferric sulphate mentioned above. At lower potassium bromide concentrations the extraction of silver in the presence of the stated amounts of zinc and iron sulphate is incomplete.

Effect of diverse ions

Under the conditions used for the extraction of silver, bismuth—at the 25-mg level—is $\sim$84% extracted as a yellow bromo- ion-association complex into chloroform containing TBA, cadmium is $\sim$35% extracted at the 100-mg level and molybdenum(VI) is $\sim$3.5% extracted at the 200-mg level. However, at these levels, these elements will not interfere in the extraction of silver, and the amounts co-extracted will not affect its determination by AAS. More than about 25 mg of bismuth interferes in the extraction step because it forms a sticky yellow material that is relatively insoluble in chloroform and may clog the separatory funnel. A low result is obtained for silver under these conditions. The presence of molybdenum is indicated by the yellow colour of the hydrobromic acid after the stripping step and by the bluish salts obtained when the final solution is evaporated to dryness.

Up to at least 8 g of zinc, 1 g of copper(II), 700 mg of iron(III), 500 mg of nickel, 200 mg of arsenic(III), arsenic(V), chromium(III), vanadium(V), phosphorus as phosphate, manganese(II), cobalt(II), indium(III) and tin(IV), 100 mg of antimony(III), titanium(IV) and zirconium(IV), 25 mg of tellurium(IV), tellurium(VI), selenium(IV) and selenium(VI) and 10 mg of tungsten(VI) will not interfere in the determination of silver by the proposed method. Mercury(II) forms a white precipitate with TBA but up to 50 mg will not interfere. More than $\sim$25 mg of tellurium(IV) and tellurium(VI) will cause a low result for silver, and more than 10 mg of tungsten(VI) interferes by causing emulsification in the chloroform phase. Antimony(V) interferes because it hydrolyses in the solution before the extraction step. Interference from antimony(V) can be avoided by removing it by volatilization as the bromide during the sample preparation step. Arsenic, germanium, tin, selenium and mercury will also be removed under these conditions. The effects of gold, platinum and palladium, which would probably also be partly co-extracted with the silver,[4,5] were not investigated because the amounts of these elements present—relative to silver—in zinc process solutions or in a 1-g sample of ore or concentrate would not be expected to interfere in the extraction of silver or to cause error in the AAS determination.[17] Chromium(VI) would be partly extracted[18] but it would not usually be present in zinc process solutions or in the sample solution obtained after the proposed decomposition step.

Up to at least 100 mg of chloride will not interfere in the extraction of silver because silver is also extracted into chloroform as an anionic chloro-TBA ion-association complex. However, if precautions are not taken, any chloride contamination in the initial sample solution will cause a low result for silver because of the precipitation of silver chloride. This will be retained in the beaker and on the filter paper before the extraction step. This source of error can easily be eliminated by washing the beaker and the filter paper with 25% ammonia solution to dissolve the silver chloride. This washing step also eliminates error resulting from possible bromide contamination from the decomposition step and error caused by the retention of silver by lead sulphate.[19] Under these conditions, up to $\sim$350 mg of lead can be present in the sample without causing significant error in the result. Nitric acid must be absent during the extraction step.

Applications

To test the reliability of the proposed method, it was applied to a series of synthetic zinc process solutions, in which the silver, added in the form of a dilute sulphuric acid solution, was varied from 2 to 500 μg. It was also applied to Canadian Certified Reference Materials Project (CCRMP) certified refer-

Table 1. Recovery of silver from synthetic zinc process solutions

Matrix	Total Ag present, μg	Ag found, μg
2*M* sulphuric acid solution (~95 ml) containing 20 g of $ZnSO_4$ and 2.5 g of $Fe_2(SO_4)_3$	3.0_3	2.9_6
	6.0_3	5.9_9
	51.0	$51._5$
	101.0	$101._8$
	251.0	$253._5$
	501.0	$501._9$

Duplicate determinations of silver in the zinc sulphate (without added silver) by the proposed method gave 1.2_0 and 0.8_6 μg.

ence ores and concentrates and to National Bureau of Standards (NBS) and British Chemical Standards (BCS) copper metal and copper-base alloys. The results of these analyses, which are the means of four or five AAS measurements, are given in Tables 1, 2 and 3. All the results shown are for individual samples. The results obtained at CANMET, during the interlaboratory certification programmes, for the CCRMP ores and concentrates by a fire-assay/AAS method[20,21] are also given in Table 2.

Precision

The precision for silver at about the 4–1100 μg/g levels was tested by analysing six samples each of three certified CCRMP ores. The results of these tests, which are the means of four or five AAS measurements, are given in Table 4. The results obtained for these ores at CANMET by a fire-assay/AAS method during the interlaboratory certification programmes are also given in Table 4.

Table 1 shows that the results obtained for the synthetic zinc process solutions agree favourably with the calculated amount of silver present. Except for PTM-1, the results obtained for the CCRMP reference ores and concentrates (Table 2) are in excellent agreement with the certified values and with those obtained at CANMET by a fire-assay/AAS method.[20,21] The results obtained for PTM-1 by the proposed method agree with those obtained at the National Institute of Metallurgy in South Africa[2] by wet-chemical AAS methods involving leaching of the sample with nitric acid and either subsequent extraction of silver into toluene containing iso-octyl thioglycollate (74.4 μg/g), or direct determination of silver

Table 2. Determination of silver in CCRMP reference ores and concentrates

Sample	Nominal composition, %	Certified value and 95% confidence limits, Ag, μg/g	Ag found, μg/g	
			Fire-assay/AAS*	Proposed method
CCU-1 Copper concentrate	24.7 Cu, 3.2 Zn, 30.8 Fe, 35.6 S, 2.6 SiO_2	139 (136–142)	142.1 (141–144)	139.4, 140.9
CPB-1 Lead concentrate	64.7 Pb, 4.4 Zn, 8.4 Fe, 17.8 S, 0.7 SiO_2	626 (620–632)	620 (604–631)	624.0, 622.0
CZN-1 Zinc concentrate	44.7 Zn, 7.5 Pb, 10.9 Fe, 30.2 S, 1.0 SiO_2	93 (90–95)	93.0 (89–96)	95.4, 93.9
SU-1a Nickel-copper-cobalt ore	~20 Fe, ~10 S, ~38 SiO_2, 1.3 Ni, 1.2 Cu, 5.0 Al, 3.5 Ca, 3.0 Mg	4.3 (4.1–4.6)	4.3 (4.1–4.5)	4.3, 4.6
PTC-1 Noble metals-bearing sulphide concentrate	5.2 Cu, 9.4 Ni, 26.9 Fe, 23.5 S	5.8 (5.5–6.2)	6.0 (5.8–6.3)	5.6, 6.3, 5.7
PTM-1 Noble metals-bearing nickel-copper matte	30.2 Cu, 44.8 Ni, 1.6 Fe, 21.6 S	66 (59–73)	68.6 (67.6–70.7)	74.7, 75.0, 75.2
KC-1 Zinc-lead-tin-silver ore	20.1 Zn, 6.9 Pb, 16.1 Fe, 28.1 S, 11.1 Si, 0.7 Sn	1120 (1110–1130)	1110 (1101–1118)	1109, 1115
MP-1 Zinc-tin-copper-lead ore	15.9 Zn, 1.9 Pb, 5.7 Fe, 11.8 S, 19.4 Si, 3.6 Al, 3.4 Ca, 2.1 Cu, 0.8 As, 2.4 Sn	57.9 (55.7–60.1)	54.7 (52.5–56.2)	59.6, 58.9
MP-1a Zinc-tin-copper-lead ore	19.0 Zn, 4.3 Pb, 6.2 Fe, 12.7 S, 41.8 SiO_2, 1.4 Cu, 1.5 Ca, 1.3 Sn	69.7†	67.0 (66.5–67.9)	68.2‡, 67.0, 66.7

* The results shown (except for MP-1a—mean of 5 values) are the means of 10 values, varying over the ranges shown in parentheses, obtained at CANMET during the respective interlaboratory certification programmes.

† Consensus mean of 90 results reported during the interlaboratory certification programme.

‡ 50 mg of antimony (as potassium antimonyl tartrate) added before the decomposition step. Antimony was subsequently removed by volatilization as the bromide, as described in Note 2.

Table 3. Determination of silver in NBS and BCS copper metal and copper-base alloys

Sample	Nominal composition, %	Certified value and range, Ag, %	Ag found, %
NBS-62B Manganese bronze	57.4 Cu, 38.0 Zn, 1.0 Sn, 0.8 Fe, 1.3 Bi, 1.0 Al	0.005 (0.003–0.006)*	0.0059, 0.0060
NBS-63B Phosphor bronze	78.0 Cu, 0.7 Zn, 9.4 Pb, 9.8 Sn, 0.5 Fe, 0.4 P, 0.5 Sb	0.04†	0.044_2‡, 0.044_7
NBS-124D Ounce metal	83.6 Cu, 5.0 Zn, 5.2 Pb, 4.6 Sn, 1.0 Ni, 0.2 Sb	0.02†	0.020_0, 0.019_7
BCS-197d Pure copper	99.9 Cu	<0.005§	0.0011, 0.0010
BCS-207 Bronze "C"	86.8 Cu, 2.5 Zn, 9.8 Sn	0.02	0.019_5, 0.020_1

* Certified value based on the 2 results shown in parentheses.
† NBS provisional result.
‡ Antimony removed as described in Note 2.
§ Result obtained by spectrographic analysis.

with compensation for viscosity and matrix effects (73.8 μg/g). The results obtained for the NBS and BCS copper metal and alloys (Table 3) are also in good agreement with the certified values.

Table 4 shows that the precision of the results for silver by the proposed method at about the 4–1100 μg/g levels is reasonably good. Although the relative standard deviations obtained are slightly higher than those calculated for the fire-assay/AAS results, they still compare favourably with them. However, because sample inhomogeneity can occur in ores, particularly when the noble metals are present in the native form,[22] the precision of the fire-assay/AAS results would be expected to be slightly better than that of the proposed method because of the much larger sample weight taken, *i.e.*, ~15–30 g compared with ~0.4–1 g.

In the proposed method, it may be possible to extract silver into methyl isobutyl ketone (MIBK) followed by its direct determination in the extract by AAS. Probably this would also result in an increase in sensitivity, depending on the volume of MIBK required for complete extraction. However, this approach was not investigated because of the noxious nature of MIBK and because aqueous calibration solutions are considerably easier to prepare and more convenient to use for routine work. Also more elements (*e.g.*, iron, antimony, indium and tin) would be expected to be co-extracted because of the formation of oxonium-type ion-association bromo-complexes with the MIBK.[3] The proposed method has some advantages over other methods involving the extraction of silver from hydrochloric[1,3–5] and hydrobromic acid media[7] with amines and quaternary ammonium salts, because of the wide range of sulphuric acid concentration that is applicable. The use of sulphuric acid is also advantageous for sample preparation and, unlike hydrochloric acid and probably hydrobromic acid, it is not co-extracted into chloroform containing TBA.[18] Furthermore, unlike methods based on the extraction of silver from nitric acid media with reagents such as iso-octyl thioglycollate,[2] petroleum sulphides, triphenylphosphine and tri-iso-octyl thiophosphate,[1] a relatively large amount of chloride will not interfere in the extraction step. The advantages of extracting silver from a potassium bromide–sulphuric acid medium rather than from a potassium iodide–sulphuric acid medium are that copper will not inter-

Table 4. Precision for silver in ores

	Ag found, μg/g					
	By fire-assay/AAS*			By proposed method		
	SU-1a	MP-1a	KC-1	SU-1a	MP-1a	KC-1
	4.2	66.9	1108	4.29	66.7	1109
	4.5	66.5	1114	4.58	67.0	1115
	4.2	67.9	1113	4.78	66.7	1111
	4.1	67.3	1117	4.95	69.6	1118
	4.5	66.5	1111	4.72	65.0	1128
	4.1		1106	4.31	69.8	1119
	4.5		1104			
	4.1		1103			
	4.3		1118			
	4.1		1101			
Mean	4.26	67.0	1110	4.60	67.5	1117
Standard deviation	0.18	0.59	5.99	0.26	1.87	6.77
Relative standard deviation, %	4.2	0.9	0.5	5.6	2.8	0.6

* Results obtained at CANMET during the interlaboratory certification programmes.

fere because it forms a soluble bromide, ascorbic acid is not required to reduce iron(III), antimony(III) is not co-extracted and, in general, fewer elements form extractable bromo-complexes than form iodo-complexes.[4]

The proposed method is suitable for silver in ores and related materials at levels as low as ~0.1 μg/g and in zinc process solutions down to the ~0.001-μg/ml level. Solutions containing ≥2 μg of silver per ml can be analysed directly by AAS, with reasonable accuracy, after tenfold dilution of the solution to reduce viscosity and matrix effects. The same amounts of concentrated hydrochloric acid and diethylenetriamine as in the calibration solutions should be added to the diluted sample solution. The method can also be used to determine microgram quantities of silver in lead chloride after treatment of a suitable sample (up to ~0.5 g) with 30 ml of 50% sulphuric acid, followed by chloride removal by double evaporation of the solution to fumes of sulphur trioxide, the sides of the beaker being washed down with water between the evaporations.

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PREPARATION AND CHARACTERIZATION OF GRAPHITE-COATED METALLIC ELECTRODES: THE GRAPHITE-SPRAYED ELECTRODE*

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Summary—Graphite-coated metal electrodes were constructed and evaluated for use in voltammetry. Aluminium, copper and platinum electrodes were sprayed with a colloidal solution of graphite particles dispersed in methyl methacrylate polymer. The polishing step was omitted for anodic stripping voltammetry with a mercury film. The resistance is about 1 Ω. Electrode areas are readily reproduced by utilizing metal supports with equal areas. Background currents are very low and useful potential ranges are extended, with −1.3 to +1.7 V *vs.* SCE being possible, depending on the electrolyte. It is possible to achieve ±0.1% precision for the peak-currents in the electrochemical oxidation of ferrocyanide. For phenol, which is strongly adsorbed, a precision of 2.5% can be achieved by polishing the electrode before each determination.

The literature on modified electrodes is becoming extensive and is growing rapidly.[1] The reason for this increasing interest is that most solid electrodes do not fulfil the requirements of good conductivity, high overpotential for hydrogen and oxygen evolution, chemical and electrochemical inertness, and low background current over the whole useful range of potential.

Among the materials most used, carbon in various forms is well suited for making electrodes for voltammetric and stripping analyses. Glassy-carbon and carbon-paste electrodes are generally employed in voltammetric experiments. Each type has its own advantages and has been used with varying degrees of success. For example, the very hard mirror-like and conductive glassy-carbon material exhibits higher background currents and lower useful potential ranges than carbon-paste electrodes,[2–6] and the latter exhibit electrochemical properties that depend on the paste used and the pasting formulation.[3,6,7]

Various modifications have been tried in attempts to build non-porous surfaces in order to diminish background currents and improve the carbon-electrode response. Pyrolytic-graphite and ordinary graphite electrodes have been modified either physically or chemically by using wax or various polymers as impregnators or binders.[8–27] The techniques generally consist of immobilizing any of a wide variety of chemical species onto conventional electrode materials, either by covalent bonding, adsorption or physical coating onto a solid electrode surface. Unfortunately, there are often practical difficulties that limit the ease with which the electrodes can be routinely prepared, utilized and renewed for subsequent use. We have recently described the modification of either a carbon-paste or glassy-carbon electrode by spraying a graphite powder on the surface to create a thin film of uniform particles, the "graphite spray electrode".[28] Though the working potential range was somewhat narrowed, the background currents were decreased.

In the present work, we describe a simple, very rapid and convenient means of making composite graphite electrodes for general voltammetric applications. The technique does not require the time-consuming impregnation procedure, but uses a colloidal solution of graphite particles dispersed in a suitable polymer for coating the planar disk surfaces of metal electrodes (platinum, copper, aluminium) with an adhering graphite-based conductive layer. The working potential-ranges are enhanced and the electrode areas are highly reproducible.

EXPERIMENTAL

Apparatus

Voltammetric measurements were made on a Brucker E 100 polarograph with a Hewlett-Packard type 7004 B recorder. Cyclic voltammetric measurements were made by use of a triangular-signal generator constructed in our

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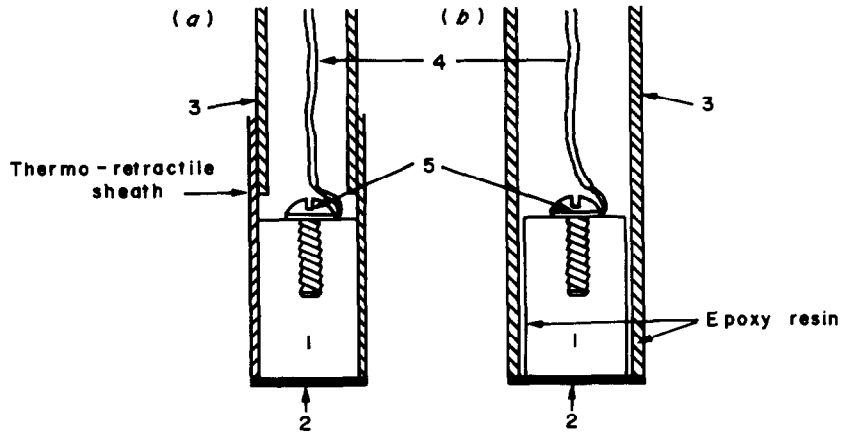

Fig. 1. Two ways of making the spray-coated electrode. (*a*) The metal rod is sheathed with thermo-retractile material; (*b*) the metal rod is cemented in a glass tube with epoxy resin. 1, metal rod; 2, graphite-based conducting coating; 3, glass tube; 4, conducting wire; 5, connecting screw.

laboratory and coupled with the recorder. A three-electrode cell thermostatically controlled at 25.0 ± 0.1° was used.

The reference electrode, a saturated calomel electrode, was placed inside a salt bridge filled with the electrolyte under investigation. A platinum wire served as counter-electrode.

The working electrodes were home-made metal electrodes (see below). Deoxygenation was accomplished by passing purified nitrogen through the cell, and nitrogen was passed over the solution in the cell throughout the experiments. Stripping analyses were done by the standard-additions method, Hamilton microsyringes being used for making the additions.

Reagents and solutions

All reagents were of analytical grade (Merck). Mercury(II) nitrate solution (0.1*M*) was prepared from highly purified mercury, dissolved in nitric acid. Metal solutions more dilute than $10^{-3}M$ were prepared just before use. Water was demineralized and then distilled twice, the first time from permanganate, and stored in polyethylene bottles.

Electrode fabrication

Two procedures were investigated and gave satisfactory results. In the first (Fig. 1*a*), a collar of heat-shrinkable tubing was shrunk over the metal rod (diameter 9 mm) and the glass tube. The rod and sheath were then cut to the desired electrode length. Finally, a flat surface was obtained by manually polishing the electrode on metallographic paper (Carbinet 600, Buchler, Evanston, Illinois).

The second procedure (Fig. 1*b*) consisted of sealing the metal rod (diameter 8 mm), carefully centred, in an appropriate glass tube with epoxy resin, and then polishing the metallic disk and the glass surrounding it to a very flat surface with metallographic paper.

The modification procedure consisted of applying a graphite layer onto the polished electrode surface by means of short bursts of spray from an aerosol can of graphite solution (Acheson, Dag 40*) until the disk and the sheath (glass or heat-shrinkable tubing) appeared completely coated when inspected with a hand lens. The sheath of the electrode body was cleaned with acetone, so that only the planar surface of the electrode was coated with graphite. Between bursts of spray, the surface was heated with a hair-drier to remove any traces of organic solvent.

This procedure gives, in about 5 min, electrodes ready to use. The graphite layer is hard and tight, owing to the inherent stability and durability of the acrylic polymers. Moreover, poly(methyl methacrylate) films are extremely resistant to alkaline saponification and are relatively unaffected by acids. The total electrode assembly has a resistance of about 1 ohm, as measured with a mercury pool contact at the face of the disk.

The electrodes are ready for use in ASV measurements, but polishing is necessary in organic voltammetric analyses in order to improve the electrode response (see Fig. 4 below). This can be done by carefully polishing the graphite layer with successively finer abrasives, and finally finishing with abrasive strips of the type used for polishing ion-selective electrodes (Tacussel or Orion). The surface of an electrode prepared in this manner appears mirror-like and is sufficiently durable to permit re-use several times, with polishing before use. Poorer looking and less reproducible waves can result from breaking and chipping of the graphite layer by extensive polishing, but fortunately, respraying of the damaged surface restores the original behaviour.

Procedures

Voltammetric measurements. Cyclic and linear-scan voltammetry were applied to the oxidation of ferrocyanide dissolved in 0.5*M* potassium chloride and phenol dissolved in 0.1*M* sulphuric acid. These compounds were selected as model depolarizers, the first showing reversible behaviour on platinum, in contrast to the second, which exhibits irreversible behaviour and electrode filming or poisoning phenomena.[4,29]

Anodic stripping measurements. A concentration of $2 \times 10^{-4}M$ mercury(II) was added to all test solutions for *in situ* mercury-film deposition. The experiments were done with various media: ammonia buffer of pH 8.5, 0.1*M* tartaric acid or 0.1*M* sulphuric acid, but the simultaneous determination of Zn, Cd and Pb was done in acetate buffer, pH 4.7. The mercury film and the three metals to be determined were deposited at a potential of −1.25 V (*vs.* SCE), with magnetic stirring. At the end of the deposition period, the stirring was stopped, and after a 15-sec rest period, the metals were stripped from the mercury film by applying an anodic potential scan, in either the linear or the differential pulse mode. The scan was stopped at +0.05 V (*vs.* SCE)

*Acheson Dag 40 (Acheson Company, 1020 Brussels, Belgium) is a dispersion of colloidal graphite (30%) in poly(methyl methacrylate) dissolved in butyl acetate. The propellent gas is Freon 12.

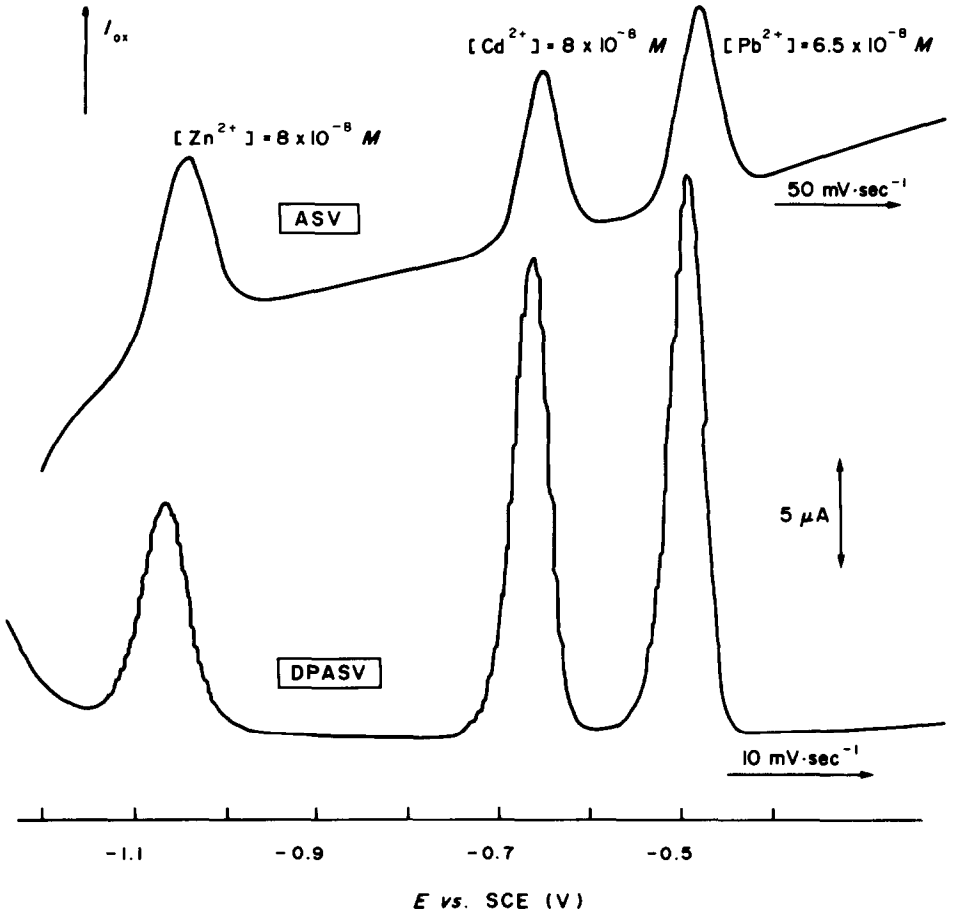

Fig. 2. Typical anodic stripping voltamperograms with the modified aluminium electrode: 5-min deposition at -1.25 V; $[Hg^{2+}] = 2 \times 10^{-4} M$; supporting electrolyte HAc/Ac$^-$ 0.1M; pH = 4.7

and this potential was maintained for 1 min before the next run was performed. In agreement with the results obtained by Florence[30] with the glassy-carbon electrode, we found that the first scan always gave lower peak-heights than the following scans and served to condition the graphite-coated electrode. The concentrations of the metals in the sample were determined by the standard-additions method, without removal of the electrode from the solution.

At the conclusion of the analyses, the graphite layer was completely removed by wiping the surface with a filter paper soaked in acetone, and the metal disk surface was then ready for respraying. Other instrumental parameters were: d.p. amplitude 40 mV; pulse repetition rate 0.5 sec; pulse duration 55 msec.

RESULTS AND DISCUSSION

Anodic stripping voltammetry

In our previous reports, we have demonstrated that it is possible to improve the surface characteristics of carbon-paste electrodes for use as thin mercury-film electrodes (TMFE) in making ASV measurements.[28,31] The technique consisted of coating the carbon-paste surface by spraying it with a graphite-based conductive solution. The resulting electrode, when electrochemically coated with mercury, exhibited good stability in various supporting electrolytes. The reproducibility of the measurements of ASV currents with freshly prepared surfaces was better than that for the simple carbon-paste electrode, but the working potential-range was decreased.

In an attempt to extend use of the spray technique to other supports than carbon, metal electrodes (Al, Cu, Pt) were investigated. The use of these conductive supports obviously requires a uniform coating and complete tightness of the graphite layer, in order to avoid any contact of the metal with the solution to be analysed. Figure 2 illustrates typical anodic stripping voltamperograms for simultaneous determination of zinc, cadmium and lead with an aluminium graphite-coated electrode. Both stripping modes employed, linear scan (LSASV) and differential pulse (DPASV), performed well, but the latter gave better sensitivity, as shown by the slopes of the base current for the supporting electrolyte at the stripping potential for lead and cadmium; viz. 10 μA/V for LSASV and 0.7 μA/V for DPASV.

The stability of the sprayed layer was confirmed by the good linearity of plots of peak currents *vs.* concentration of metal ions [for use of the standard-addition

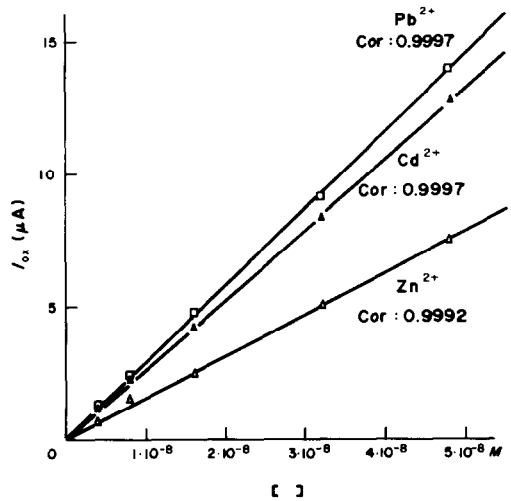

Fig. 3. Differential-pulse anodic stripping voltammetry with the modified aluminium electrode. Calibration curves for Pb, Cd and Zn; 5-min depositions at −1.25 V; $[Hg^{2+}] = 2 \times 10^{-4}M$; supporting electrolyte HAc/Ac^- 0.25M; drop time 0.5 sec; pulse amplitude 40 mV; scan-rate 10 mV/sec.

method without removal of the electrode from the solution (Fig. 3)].

With Florence's criteria,[32] the limits of detection were estimated to be $10^{-10}M$ for cadmium and lead and $4 \times 10^{-10}M$ for zinc (10-min deposition time).

In view of the cyclic voltammetric results (see below), we compared the ASV performances of polished-surface electrodes with those of unpolished sprayed-surface electrodes. As shown in Table 1, the results with both electrodes compared favourably for lead and cadmium. There is, however, a slight increase in peak-currents on the rough electrode as expected, due to the larger electrochemically active area. In contrast, peak-currents for zinc were lower on rough electrodes; this lack of sensitivity was related to the increase in peak half-width ($b_{1/2}$) and could presumably be attributed to diminution of the reversibility of the electrode process, *i.e.*, slower charge-transfer at the electrode.

The present results obtained with graphite-covered metal electrodes compare favourably with the DPASV data reported for various carbon mercury thin-film electrodes. As shown in Table 1, there is no great advantage in polishing the electrode for ASV measurements, since polishing introduces a risk of contamination. Moreover, the graphite-spray coating has the advantage of being easily renewable, ready for use, without any memory effects for several metals (mercury in particular) following removal of previous coatings, with acetone or benzene.

Cyclic and linear-scan voltammetry

The good behaviour of the modified metal electrodes in ASV has focused our interest on evaluating the electrochemical properties of the graphite coating for utilization in cyclic and organic voltammetry. The cyclic voltamperograms reported in Fig. 4 clearly show that polishing the graphite layer is necessary in order to improve the performance of the electrode. Figure 4*b* displays a typical cyclic voltamperogram of a 1mM ferrocyanide solution in 0.5M potassium chloride, obtained with a polished graphite-coated aluminium electrode. As a result of the polishing, the rate of charge-transfer is greatly improved, giving rise to a smaller ΔE_p and larger peak-currents. The peak-potential (+0.280 V *vs.* SCE) and peak-potential separation (ΔE_p 120 mV) compared favourably with the results reported for other carbon electrodes.[2,4] Over the whole range of scan-rates investigated (10–300 mV/sec) the I_a/I_c ratio was in accord with theory for a reversible system, and the peak-currents were diffusion-controlled, as shown by the linearity of plots of current *vs.* the square root of the scan-rate.

The potential-ranges available with the polished electrode were determined. The cyclic voltammetric residual currents for a typical modified aluminium electrode in common mineral acids are illustrated in Fig. 5. The background currents were very low and the useful potentials significantly extended, compared to those for other solid electrodes.

Two aspects of the reproducibility of peak-current and peak-potential measurements were investigated. First the reproducibility of a given surface was examined. This was accomplished by scanning the linear current–voltage curve for the oxidation of 1mM ferrocyanide in 0.1M potassium chloride, briefly stirring the solution, and rescanning the I–E curve. The relative standard deviation (rsd) of peak-currents for 9 replicate runs was lower than 0.1%, and the peak-potentials were almost constant. The second aspect concerned variations from surface to surface. A phe-

Table 1. Differential-pulse anodic stripping on polished and unpolished graphite-sprayed aluminium electrodes: acetate/acetic acid buffer pH 4.7; Hg^{2+} $2 \times 10^{-4}M$; deposition time 5 min

	E_p, *mV*		I_p/C, *A.l.mole*$^{-1}$		$b_{1/2}$, *mV*		
	Unpolished	Polished	Unpolished	Polished	Unpolished	Polished	rsd*, %
Pb	−500	−505	345	220	43	43	0.7
Cd	−670	−675	254	215	45	45	1.0
Zn	−1070	−1090	95	160	55	45	1.3

*Seven replicates.

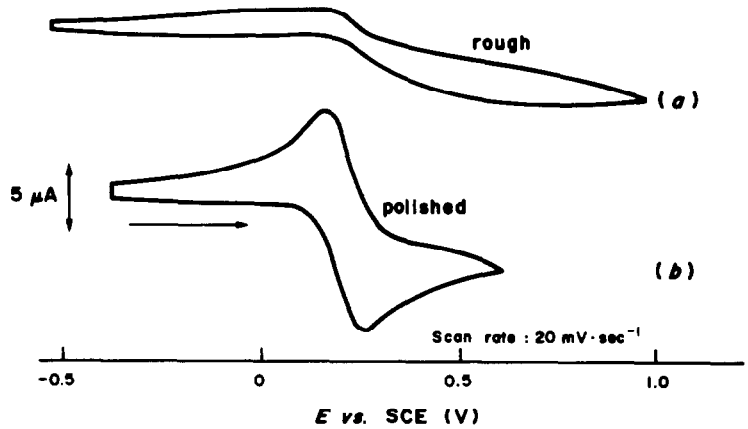

Fig. 4. Cyclic voltamperograms of 1m*M* ferrocyanide in 0.5*M* potassium chloride, at the modified aluminium electrode.

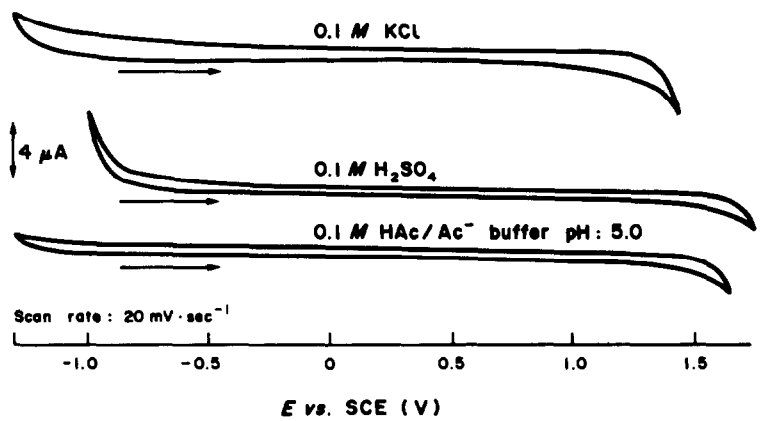

Fig. 5. Background cyclic voltamperograms for the modified aluminium electrode in several media.

nol solution was used, as it is known[4,29] to poison electrode surfaces drastically and in such cases, polishing between runs is necessary. A typical linear-scan voltamperogram is illustrated in Fig. 6. The rsd of peak-currents for 9 different surfaces was 2.5% with peak-potentials reproducible within ± 2 mV.

CONCLUSIONS

The graphite-coated metal electrode represents a new type of solid electrode, which exhibits almost ideal behaviour. It has high electrical conductivity, good mechanical strength, low residual currents, wide operating voltage range, and highly reproducible area and performance. The electrode is very rapidly prepared, easily renewable, inexpensive and machinable into various shapes. Several electrodes of equal electrochemical area and response can be readily prepared simply by machining metal supports of equal cross-sectional area and then spraying them with graphite. Copper, platinum or aluminium supports work equally well, and the less expensive metals allow construction of electrodes at very little cost. With proper coating of the supports, no metallic contamination of the solutions has been found by means of ASV. Performances reported in this work were obtained with aqueous solutions, but aqueous methanol solutions have also been successfully used in the determination of pharmaceutically interesting organic compounds. Further studies are in progress on the utilization of these electrodes in non-aqueous media.

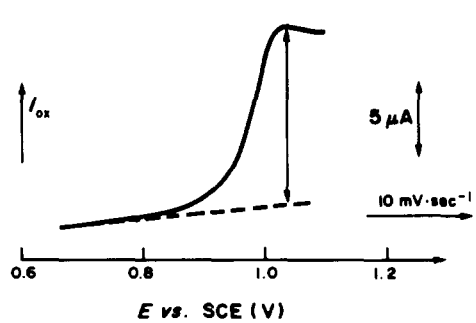

Fig. 6. Current–voltage curve from 1×10^{-4}*M* phenol in 0.1*M* sulphuric acid, at the modified aluminium electrode.

Preliminary studies have indicated that the electrode is not affected by acetonitrile, but acetone and benzene cause the polymer to dissolve or separate from the electrode.

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STUDIES ON POTENTIOMETRIC STRIPPING ANALYSIS

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Summary—Theoretical and experimental investigations have been made on the method of potentiometric stripping analysis. By solving Fick's second law of diffusion for the metal in the amalgam, it was found that $\tau = C_R^0 l/k[Ox]$, where τ is the elapsed time, l is the thickness of the mercury film, C_R^0 is the concentration of the metal in the amalgam, [Ox] is the concentration of the oxidant in the solution and k is a constant. Since C_R^0 is proportional to the concentration of that particular ion in the solution under given pre-electrolysis conditions, the relations given by the equation above can all be verified experimentally. The equation of the potential–time curve and the effect of complex formation on the elapsed time were also investigated and discussed. In the absence of complexation reactions in the solution and with proper control of the concentration of the oxidant, the lowest limit of detection for Pb was found to be $10^{-12}M$ for a 4-min pre-electrolysis, with dissolved oxygen as oxidant.

Potentiometric stripping analysis, recently proposed by Jagner,[1,2] is an analytical method for determining certain metal-ion concentrations in very dilute solutions. This method consists of two steps: the pre-electrolysis step and the oxidation step. In the pre-electrolysis step, metal ions are reduced at constant potential on a thin mercury film (attached to a glassy-carbon electrode) to form amalgams. This pre-electrolysis step is similar to that in anodic or chronopotentiometric stripping, but in the oxidation step, a suitable oxidant is used for stripping the amalgam instead of use of a potential sweep as in anodic stripping, or a constant current as in chronopotentiometric stripping. During the course of oxidation, the potential of the glassy-carbon electrode varies with time, and the potential–time curve is recorded. The shape of this curve is similar to those in chronopotentiometry or chronopotentiometric stripping. The potential of the electrode remains almost constant from the beginning of the oxidation of a certain metallic species in the amalgam until the amalgam is stripped of such a species. The potential then varies rapidly with time until the oxidation of the next species begins. The time of oxidation of a certain species, called the elapsed time τ for that species, can be derived from the curve and is related to the concentration of the species in the solution before pre-electrolysis.

The novelty of this method is that there is virtually no current passing through the electrode during the entire period of oxidation. Thus the problem of charging or capacitative current no longer exists. Any blank that might occur is entirely due to impurities present in the water or reagents used and these can be completely removed by experimental means. Therefore, under properly controlled experimental conditions, the sensitivity of this method could be much enhanced. In order to verify this point, we did some theoretical and experimental investigation on this method. The following is a report of this work so far. Some data have been published in Chinese scientific journals.[3,4] The cation investigated was lead.

EXPERIMENTAL

Apparatus

A model 75-3 rapid scan polarograph (Amoy, China) was used for constant-potential electrolysis. The oxidation curve was recorded with an XWX-2042 recorder (Shanghai, China). A rotating glassy-carbon electrode BDX-1 (Changtsun, China) was used as the working electrode; it had a diameter of 4.5 mm and was rotated at 2000 rpm. A spiral Ag/AgCl electrode was used as the reference electrode and a saturated potassium nitrate or chloride agar salt bridge for connection. A CY-Z oxygen meter (Shanghai, China) was used for determining oxygen in the solutions.

Reagents

All reagents used were of the general or analytical reagent grade. Any lead in these reagents was exhaustively removed by cathodic mercury-pool electrolysis at -1.8 V to give the least possible blank. The water used was first treated by reverse osmosis to remove most of the ions (except those of water, of course) and practically all of the undesirable neutral or charged particles, then passed through a cation-exchange column and a Millipore 0.45-μm filter and finally distilled in quartz before use.

All solutions were kept in plastic bottles to avoid contamination from lead. The nitrogen used for deaeration was purified by passage through vanadium(II) chloride solution.[5]

Preparation of the working electrode

A clean glassy-carbon electrode is immersed in 50 ml of 0.1M hydrochloric acid containing 25 mg of mercury(II) nitrate per litre and connected to an Ag/AgCl electrode by a saturated agar bridge, and electrolysis is performed at -0.5, -0.6, -0.7 and -0.8 V for 30 sec at each voltage and then at -0.9 V for 2 min. The total electrolysis time is 4 min. The time of electrolysis can be varied to give mercury films of different thickness. The electrode is rotated during electrolysis. The rotation is stopped when the elec-

trolysis is over and at the same time the working and reference electrodes are connected to a recorder and the film is stripped. The electrode is then rinsed with distilled water and stored in distilled water for later use.

Procedure

Measure 50 ml of a lead solution which is 0.5M in sodium nitrate, 0.05M in nitric acid and $2 \times 10^{-5}M$ in mercury(II) into an electrolysis cell. Deaerate with purified nitrogen for 60 min. After that let the stream of nitrogen pass over the surface of the solution to prevent oxygen from redissolving in the solution. Immerse a glassy-carbon electrode with a mercury film in the solution and complete the cell with an Ag/AgCl reference electrode. Electrolyse for 4 min at -1.1 V with the electrode rotating. Then stop the rotation and at the same time connect the electrodes to a recorder to trace out the potential–time curve.

RESULTS AND DISCUSSION

Theoretical considerations

Let the thickness of the mercury film be l and the co-ordinate system of the working electrode be as given in Fig. 1. The oxidation reaction proceeds at the surface of the mercury film, *i.e.*, at $x = l$. Since the Pb^{2+}/Pb couple is reversible, the rate of oxidation is controlled by the rate of diffusion of lead in the mercury film. Assuming Fick's second law to hold in the mercury film, we have

$$\partial C_R/\partial t = D_R(\partial^2 C_R/\partial x^2) \quad 0 < x < l \tag{1}$$

where D_R is the diffusion coefficient of lead in mercury, C_R is the concentration of lead in the mercury and t is the time of oxidation. We also have the following initial and boundary conditions.

Initial condition:

$$t = 0; \quad C_R = C_R^0; \quad 0 < x < l \tag{2}$$

where C_R^0 is the concentration of lead in the mercury film before oxidation begins.

Boundary conditions:

$$t > 0; \quad x = 0; \quad D_R(dC_R/dx) = 0 \tag{3}$$

$$t > 0; \quad x = l; \quad D_R(dC_R/dx) = -k[Ox] = -\beta \tag{4}$$

where k is the rate constant of the oxidation reaction and [Ox] signifies the concentration of lead in the solution. The initial condition states that the concentration of lead in the mercury film is C_R^0 after the pre-electrolysis. The first boundary condition states that the diffusion of lead is limited within the mercury film. The second boundary condition states that at $x = l$ lead is oxidized by the oxidant. If the oxidant is in large excess, [Ox] may be considered as constant, and so k[Ox] is also constant and can be represented by a constant β. Since the lead ions no longer stay within the mercury film, β is preceded by a minus sign to show that the concentration gradient is negative with respect to x.

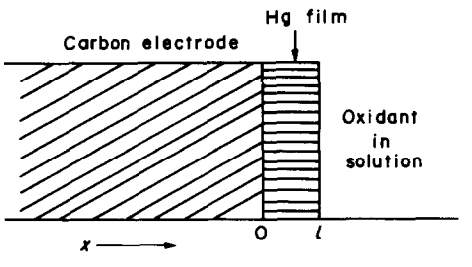

Fig. 1. The co-ordinate system of the working electrode.

We can find a solution to this problem by using the Laplace transform,[6] *i.e.*,

$$C_R = C_R^0 - \frac{\beta l}{D_R}\left\{\frac{D_R t}{l^2} + \frac{3x^2 - l^2}{6l^2} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2} \times \exp(-D_R n^2\pi^2 t/l^2)\cos\frac{n\pi x}{l}\right\}. \tag{5}$$

At $x = l$, this becomes

$$C_{R(x=l)} = C_R^0 - \frac{\beta l}{D_R}\left\{\frac{D_R t}{l^2} + \frac{1}{3} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2} \times \exp(-D_R n^2\pi^2 t/l^2)\cos n\pi\right\}. \tag{6}$$

In our experiments, l was of the order of 10^{-5} cm and D_R of the order of 10^{-5} cm^2/sec. The maximum value of t (*i.e.*, τ) is of the order of 10^2 sec. Therefore the exponential term in equation (6) can be neglected and we have

$$C_{R(x=l)} = C_R^0 - \beta t/l - \beta l/3D_R. \tag{7}$$

At the end of the oxidation reaction, $C_{R(x=l)} = 0$ and $t = \tau$, so we have

$$\tau = lC_R^0/\beta - l^2/3D_R. \tag{8}$$

This means that under a given set of experimental conditions τ is a linear function of C_R^0. Furthermore, if C_R^0 is large enough, then

$$lC_R^0/\beta \gg l^2/3D_R,$$

and we have

$$\tau = lC_R^0/\beta = lC_R^0/k[Ox]. \tag{9}$$

The diffusion of lead ions in the solution after oxidation is given by[7]

$$C_{0(x=l)} = 2\beta(t/\pi D_0)^{1/2} \tag{10}$$

where $C_{0(x=l)}$ is the concentration of lead ion at $x = l$ and D_0 is the diffusion coefficient of lead ions in the solution.

Since the Pb^{2+}/Pb couple is reversible, the potential of the working electrode is governed by the Nernst equation, *i.e.*,

$$E = E^0 + \frac{RT}{2F}\ln\frac{C_{0(x=l)}}{C_{R(x=l)}} \tag{11}$$

where R, T, F and E^0 have their usual meaning. If $l^2/3D_R$ is negligible, substituting equations (9) and (10)

Table 1. τ values in different solutions (corrected for blanks)

$[Pb^{2+}]$, M	0.5*M* $NaNO_3$–0.05*M* HNO_3 pH = 1	0.5*M* NaCl–0.05*M* HCl pH = 1	0.5*M* NaAc–0.6*M* HNO_3 pH = 1	0.5*M* $Na_2C_4H_4O_6$–1*M* HNO_3 pH = 1
10^{-10}	1.0			
10^{-9}	1.8	0.8		
10^{-8}	3.2	1.9	1.7	
10^{-7}	15.1	8.2	6.0	5.3
2×10^{-7}	30.0			
5×10^{-7}	60.0			
8×10^{-7}	102.7			
10^{-6}	125.0	64.6	28.7	25.6

into (11) gives

$$E = E^0 + \frac{RT}{2F}\ln[2l/(\pi D_0)^{1/2}] + \frac{RT}{nF}\ln[t^{1/2}/(\tau - t)]. \quad (12)$$

This is the equation of the potential–time curve.

CORRELATION BETWEEN THEORY AND EXPERIMENT

The relation between τ and C_R^0

From equations (8) and (9), we can see that τ varies linearly with C_R^0, but it is well known that in stripping analysis, for a given set of experimental conditions, C_R^0 is directly proportional to the concentration of lead in the solution before pre-electrolysis. Therefore τ should also vary linearly with the original concentration of Pb^{2+} in solution. Table 1 gives the τ values for four sets of solutions of different composition. We can easily see that for a linear relationship holds for the chloride and nitrate systems, but for the acetate and tartrate solutions the sensitivity is too low to show the linearity clearly. The reason for the lower sensitivity will be discussed below. Figure 2 gives a typical set of stripping curves for different solutions.

Relation between τ and β

Equation (9) also shows that for constant C_R^0 and l, τ is inversely proportional to β, *i.e.*,

$$\tau \propto 1 \Big/ \sum^{n} k_n[\mathrm{Ox}]_n. \quad (13)$$

This means that the elapsed time increases with the decrease of oxidant concentration in the solution. This relation is shown in Figure 3. The presence of $2 \times 10^{-5}M$ Hg^{2+} in the solution is to maintain a large excess of oxidant.

The relationship between time of deaeration and the concentration of oxygen is given in Table 2. Figure 3 shows that τ increases linearly with deaeration time up to about 50 min. After that the increase in τ is lower and τ remains constant for deaeration times over 60 min. This can be explained by the fact that after about 50 min the removal of oxygen is more difficult and after about 60 min the concentration of oxygen remains constant despite further purging.

Relation between elapsed time and film thickness

According to equation (9), τ should be proportional to l at constant C_R^0, but since C_R^0 is itself inversely proportional to l, the net result is that τ is independent of l. Table 3 shows such a relation: τ is independent of l over a fivefold range of l values. The l values are calculated from the equation

$$l = 10^{-6}\, iwt/nFdr^2 = 2.43 \times 10^{-11}\, it/r^2 \quad (14)$$

where i = plating current (μA), w = atomic weight of mercury, t = deposition time (sec), n = number of electrons involved in reduction of Hg^{2+}, d = density of mercury (g/cm^3), r = radius of mercury film (cm).

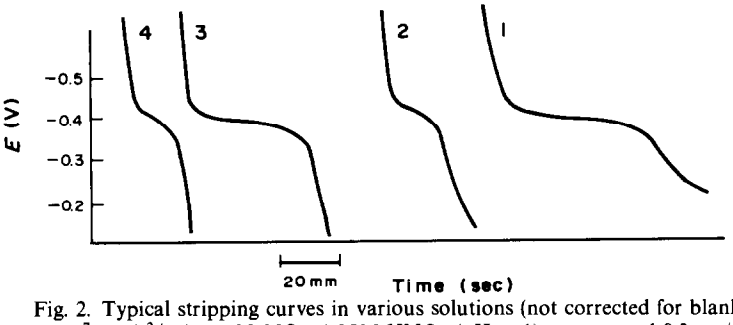

Fig. 2. Typical stripping curves in various solutions (not corrected for blanks).
1. $10^{-7}M$ Pb^{2+}, 0.5*M* $NaNO_3$–0.05*M* HNO_3 (pH = 1) paper speed 0.3 sec/mm.
2. $10^{-7}M$ Pb^{2+}, 0.5*M* NaCl–0.05*M* HCl (pH = 1) paper speed 0.3 sec/mm.
3. $10^{-7}M$ Pb^{2+}, 0.5*M* NaAc–0.6*M* HNO_3 (pH = 1) paper speed 0.15 sec/mm.
4. $10^{-7}M$, Pb^{2+}, 0.5*M* $Na_2C_4H_4O_6$–1*M* HNO_3 (pH = 1) paper speed 1.5 sec/mm.

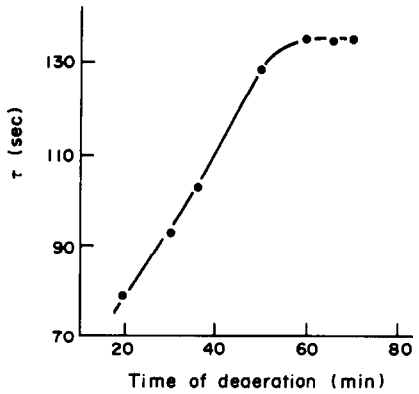

Fig. 3. Increase of τ with increasing time of deaeration. $10^{-6}M$ Pb^{2+}, 0.5*M* NaCl, 0.05*M* HCl, $2 \times 10^{-5}M$ Hg^{2+}. Pre-electrolysis time 4 min, nitrogen flow-rate 10 ml/sec.

The experimental verification of equation (12)

The simplest way of verifying equation (12) is to plot $\log[t^{1/2}/(\tau-t)]$ against E. This should give a straight line with a slope of 2.3 RT/nF (0.03 V at 25° for Pb), and this was found experimentally for nitrate and chloride solutions (slopes 0.033 and 0.030 V respectively).

In Fig. 4 we can see that the linear relationships are very well demonstrated. The slope of the line for nitrate solution is 0.033 V while that for chloride solution is 0.030 V, both are in good agreement with the theoretically predicted value.

Influence of solution composition on elapsed time and the potential–time curve

Table 1 also demonstrates the influence of solution composition on the elapsed time τ. With the same amount of lead in the solution ($[Pb^{2+}] = 10^{-6}M$) and under the same experimental conditions, the four different solutions give four different elapsed times. In our opinion, this is due to complexation reactions in the solution. In the reaction

$$Pb \rightleftharpoons Pb^{2+} + 2e^{-}.$$

Complexation decreases the concentration of lead ions, thus increasing the rate of ionization of lead and decreasing the elapsed time τ. For the solution, we

Table 2. The relationship between the time of deaeration and the partial pressure and concentration of the remaining oxygen

Time of deaeration, *min*	P_{O_2}, *mmHg*	$[O_2]$, M
10	58	1.06×10^{-4}
20	40	7.29×10^{-5}
30	30.5	5.56×10^{-5}
40	22	4.01×10^{-5}
50	13.9	2.53×10^{-5}
60	12.5	2.28×10^{-5}

Table 3. The relation between τ and *l* for various plating times

Plating time, *min*	*l*, *cm*	τ, *sec*
4	3.0×10^{-5}	49.6
6	3.5×10^{-5}	48.7
9	6.3×10^{-5}	46.3
12	7.5×10^{-5}	48.2
15	15.0×10^{-5}	49.1

can write the side-reaction coefficient α for this complexation:

$$\alpha_{Pb(L)} = C_{Pb^{2+}}/[Pb^{2+}] = 1 + \beta_1[L] + \beta_2[L]^2 + \ldots \beta_n[L]^n \quad (15)$$

where $C_{Pb^{2+}}$ is the total lead concentration, $[Pb^{2+}]$ the free ion concentration, [L] the free ligand concentration and the β_n values the overall stability constants of the complexes. It is easy to see that as $\alpha_{Pb(L)}$ increases, $[Pb^{2+}]$ will decrease and so will the elapsed time τ.

On the other hand, in this case the E *vs.* t curve will be given by the expression

$$E = E^0 + \frac{RT}{nF}\ln\frac{C_O/\alpha_{Pb(L)(x=l)}}{C_{R(x=l)}}$$

$$= E^0 + \frac{RT}{nF}\ln[2l/(\pi D_O)^{1/2}] - \frac{RT}{nF}\ln\alpha_{Pb(L)} + \frac{RT}{nF}\ln[t^{1/2}/(\tau - t)]. \quad (16)$$

When $\ln[t^{1/2}/(\tau - t)] = 0$, we have

$$E = E^0 + \frac{RT}{nF}\ln[2l/(\pi D_O)^{1/2}] - \frac{RT}{nF}\ln\alpha_{Pb(L)}. \quad (17)$$

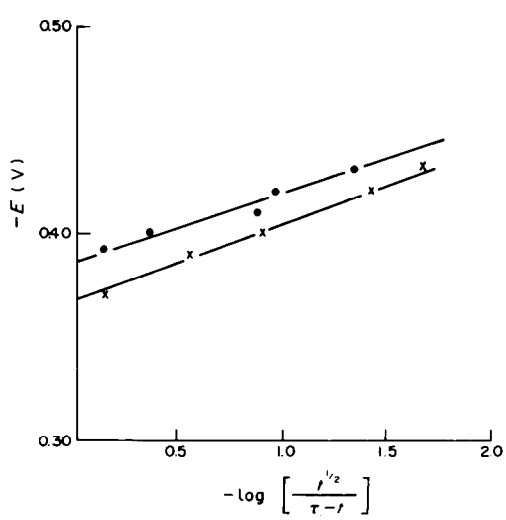

Fig. 4. The linear relationship between E and $\log[t^{1/2}/(\tau - t)]$.

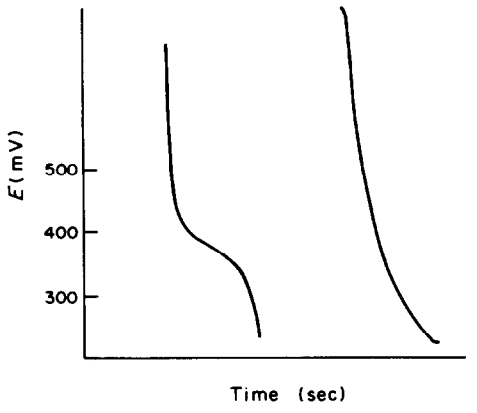

Fig. 5. The stripping curve for $10^{-12}M$ Pb, and the corresponding blank.

If we can assume that there is no complexation, as will be the case for lead and nitrate, we have $\alpha = 1$, and then

$$E_{(NO_3^-)} = E^0 + \frac{RT}{nF} \ln[2l/(\pi D_0)^{1/2}]. \quad (18)$$

When there is complexation, however, as for lead and chloride,

$$E_{(Cl^-)} = E^0 + \frac{RT}{nF} \ln[2l/(\pi D_0)^{1/2}] - \frac{RT}{nF} \ln \alpha_{Pb(Cl)}. \quad (19)$$

If we subtract equation (18) from (17) and assume that E^0 and D_0 are the same in both equations, we have, for the same l, the expression

$$E_{(NO_3^-)} - E_{(Cl^-)} = \frac{RT}{nF} \ln \alpha_{Pb(Cl)}. \quad (20)$$

The right-hand side of equation (20) is therefore equal to the difference between the intercepts on the E axis in Fig. 4, 0.02 V. From the literature[8] the value of β, for lead and chloride is $10.^{1,2}$ Since the concentration of chloride in the solution is approximately $0.5M$, $(RT/nF) \ln \alpha_{Pb(Cl)}$ can be calculated:

$$\alpha_{Pb(Cl)} \approx 10^{1.2} \times 0.5$$

$$\frac{RT}{nF} \ln \alpha_{(Cl)} \approx 0.027 \text{ V}$$

in fair agreement with the experimental value of 0.02 V, taking into consideration the difference in ionic strength and temperature.

It should be emphasized that equations (15)–(20) are valid only for rapid complexation reactions; otherwise equilibrium is not attainable in the course of the oxidation, and the equations are no longer valid.

Sensitivity

From the discussion above, it can be concluded that if there is no complexation of the analyte in the solution, the sensitivity of the method can be much enhanced by controlling the quantity of oxidant present. This is indeed the case. In a nitrate solution, if the amount of dissolved oxygen is reduced to a minimum by deaeration with nitrogen for 60 min and there are no other oxidants present, the maximum sensitivity of this method is $10^{-12}M$ for Pb. A series of 13 determinations done at different times gave an average result of 1.04 sec with a standard deviation of 0.24 sec. The stripping curve and the corresponding blank are given in Fig. 5. It should be noted that the time of pre-electrolysis in these experiments is only 4 min. The sensitivity could be further enhanced by prolonging the pre-electrolysis.

For routine analysis, deaeration for 60 min is too time-consuming. Investigations are under way to reduce the time required for the removal of oxygen, and the results will be given in later communications.

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SIMULTANEOUS DETERMINATION OF ARSENIC, ANTIMONY AND TIN BY FAST-SCAN DIFFERENTIAL-PULSE POLAROGRAPHY AND ITS APPLICATION TO ALLOY STEELS

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Summary—Arsenic, antimony and tin can be determined in the presence of one another by fast-scan differential-pulse polarography (FSDPP) on a single mercury drop in 1.5*M* hydrochloric acid. The reduction of As(III), Sb(III) and Sn(IV) to the metals is practically reversible and the reduction peaks are sufficiently separated. The lower limits of determination are 0.05, 0.005 and 0.001 μg/ml for As, Sb and Sn respectively, the calibration graphs exhibit very good linearity and the peak heights are reproducible. For application to alloy steels employed in nuclear technology, the elements to be determined must be separated from the matrix by extraction as their covalent bromides into toluene from concentrated sulphuric acid and back-extracted into the base electrolyte (1.5*M* HCl + 0.012*M* Br^- + 0.03*M* hydrazinium sulphate). The recoveries of As, Sb and Sn are 100, 95 and 92% respectively and the relative error of determination is a few per cent.

In the manufacture of alloy steels for use in nuclear technology, trace levels of arsenic, antimony and tin (and of some other elements) are of great importance. Typical values are 50–250 ppm As, 10–100 ppm Sb and 50–250 ppm Sn. Polarographic methods for determination of these elements are generally more sensitive than flame AAS, comparable with electrothermal AAS and are simpler.[1–6]

Arsenic, antimony and tin have been polarographically determined many times, on the basis of the reduction of As(III), Sb(III) and Sn(IV) in base electrolytes containing inorganic or organic acids (*e.g.*, As,[4,7] Sn,[5,8,9] Sb[10]). However, a reliable method for simultaneous determination of all three elements has not been described.

Arsenic, antimony and tin must be separated from the more complex matrices before polarographic determination. Extraction in the form of covalent halides into a non-polar solvent is especially suitable for steels, as it is very selective;[11,12] the test elements are oxidized to their highest oxidation states during the sample decomposition, but the arsenic and antimony are simultaneously reduced to the tervalent states required for polarographic determination[7] and the extraction efficiency is high, increasing from chloride to iodide.[12] This extraction has been applied to steels[13] and alloys of tin[14] and copper.[15]

In the present work we employ fast-scan differential-pulse polarography (FSDPP) recently developed[16] in Laboratorní Přístroje, Prague, using a single stationary mercury drop, which permits the polarogram to be recorded within 40 sec, with a sensitivity and resolution better than those of conventional differential-pulse polarography (DPP). The instrument polarizes the electrode with pulses of an amplitude adjustable from 12.5 to 100 mV, in either direction from the d.c. voltage ramp. The pulse duration is 100 msec and the interval between successive pulses is also 100 msec. The sampling windows before the pulse and at the end of the pulse are 20 msec wide. The scan-rate can be varied from 0.5 to 500 mV/sec. Two systems of sample-and-hold circuits can be used, one with the time constant, RC, equal to 100 msec, the other with RC = 10 msec. The static mercury-drop electrode (SMDE) has a capillary which can be closed by a needle operated by an electronic circuit, and the time of opening of the capillary, which determines the drop size, can be varied from 40 to 160 msec. The whole operation of drop-formation, potential-scan with recording, and drop-disconnection is automated.

EXPERIMENTAL

Apparatus

The FSDPP measurements were done with a PA-3 polarographic analyser, a three-electrode circuit consisting of an electronically controlled static mercury-drop electrode, a saturated calomel reference and a platinum

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auxiliary electrode, and a 4103 X-Y plotter (all from Laboratorní Přístroje, Czechoslovakia). The solutions were deaerated with prepurified nitrogen.

Small solution volumes were measured with Eppendorf micropipettes (FRG). The extractions were performed in 25-ml standard flasks and back-extractions in 10-ml standard flasks, fitted with polyethylene stoppers.

Reagents

All chemicals used were of *p.a.* purity from Lachema (Czechoslovakia) and Merck (FRG) and were not further purified.

A standard solution of As(III) (0.1 mg/ml) was prepared by dissolving 0.1320 g of arsenious oxide in 20 ml of 2*M* sodium hydroxide, diluting with water to about 100 ml, acidifying with 10 ml of dilute sulphuric acid (1 + 5) and diluting with water to 1000 ml. Standard solutions of Sb(III) and Sn(IV) (0.1 mg/ml) were prepared by dissolving the pure metals (99.99%) in 20 ml of hot concentrated sulphuric acid and diluting with dilute sulphuric acid to 1000 ml.

Cyclohexane, benzene, toluene and *o*-xylene (*p.a.*) were used for the extractions. The hydrochloric acid was standardized by acid–base titration.

All measurements were made at room temperature and all potentials referred to the SCE.

RESULTS AND DISCUSSION

Selection of the base electrolyte

From the literature survey, base electrolytes containing hydrochloric or oxalic acid might be suitable for simultaneous determination of the three elements. Therefore hydrochloric acid at various concentrations, its mixture with oxalic acid, oxalic acid alone and a mixture of oxalic acid with Methylene Blue were tested. The last electrolyte was selected because it was reported[5] to be advantageous for a.c. polarographic determination of tin. The FSDPP polarograms of the three elements in these base electrolytes are shown in Figs. 1 4. The Sn(IV) → Sn(II) reduction peak (which generally occurs[1] at between 0 and −0.2

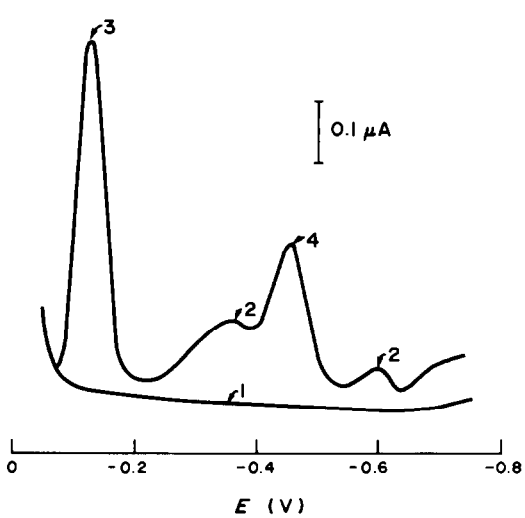

Fig. 1. The FSDPP curves for 1.5*M* HCl medium. 1—Base electrolyte, 2—As, 3—Sb, 4 Sn. c_M = 1 μg/ml, potential scan-rate = 20 mV/sec, pulse amplitude = −50 mV, RC = 10 msec.

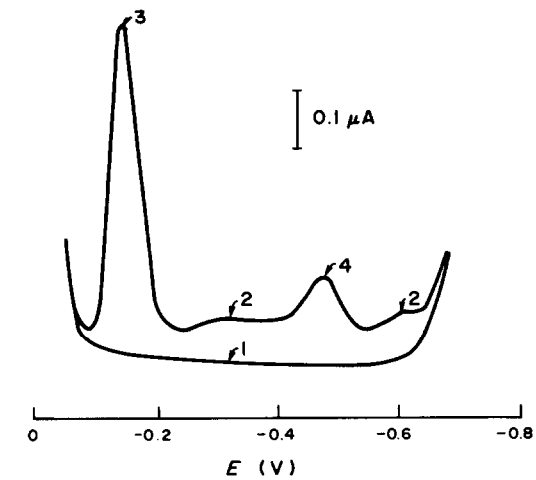

Fig. 2. The FSDPP curves for 1.5*M* HCl + 0.1*M* oxalic acid medium. For conditions see Fig. 1.

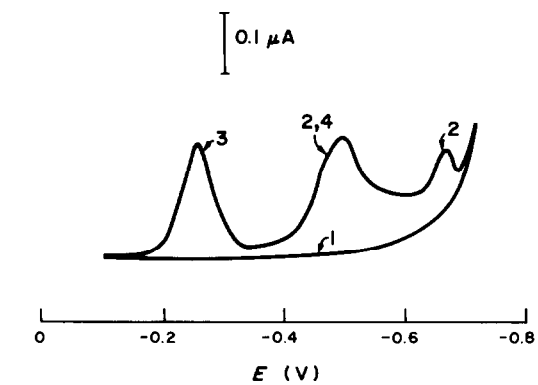

Fig. 3. The FSDPP curves for 0.5*M* oxalic acid medium. For conditions see Fig. 1.

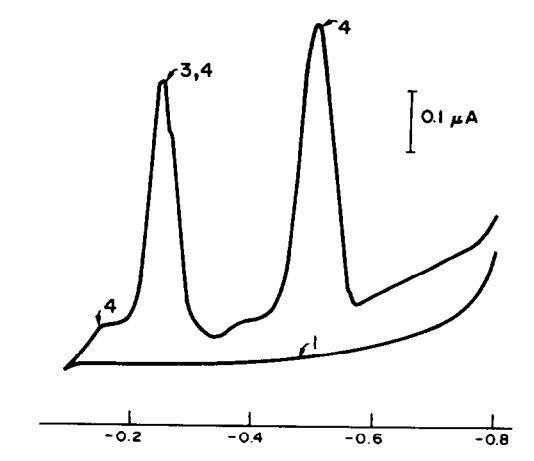

Fig. 4. The FSDPP curves for 0.5*M* oxalic acid + 5 × 10^{-4}*M* Methylene Blue medium. For conditions see Fig. 1.

Table 1. Tests for reversibility of the electrode reactions As(III) → As, Sb(III) → Sb and Sn(II) → Sn: c_{metal} = 1 μg/ml, potential scan-rate = 20 mV/sec, pulse amplitude = ±50 mV, RC = 10 msec; E_p^+ and I_p^+ are the peak potential and current, respectively, for the pulse in a positive direction, E_p^- and I_p^- are those for the pulse in a negative direction[17]

Test metal	Base electrolyte	$E_p^- - E_p^+$, mV	$\lvert I_p^+/I_p^- \rvert$	Electrode process
As	1.5*M* HCl	55	1.00	Reversible
Sb		45	1.03	Reversible
Sn		50	0.98	Reversible
As	1.5*M* HCl + 0.1*M* oxalic acid	40	1.43	Quasi-reversible, $\alpha < 0.5$
Sb		35	1.02	Quasi-reversible, $\alpha < 0.5$
Sn		40	0.90	Quasi-reversible, $\alpha \geqslant 0.5$
As	0.5*M* oxalic acid	45	1.05	Reversible
Sb		55	0.97	Reversible
Sn		50	0.95	Reversible
As	0.5*M* oxalic acid + Methylene Blue	65	0.91	Irreversible
Sb		65	0.83	Irreversible
Sn		55	1.05	Reversible

V) does not appear in the given potential range except for the base electrolyte containing Methylene Blue, thus only the Sn(II) → Sn reduction peak is generally recorded. In hydrochloric acid arsenic yields three peaks:[4] the first, at about −0.4 V, corresponds to the As(III) → As reduction; the second, at about −0.6 V, has the character of a polarographic maximum and disappears at arsenic concentrations below 0.3 μg/ml; the third, corresponding to the As → As^{3-} reduction, at a potential of −0.84 V, does not appear in the given potential range. The first peak is proportional to the arsenic concentration and strongly depends on the hydrochloric acid concentration, apparently as a result of changes in the distribution of the various chloride and hydroxide complexes of arsenic.[7]

Tests for reversibility were done with the peaks for Sb and Sn and the first peak for As, by the method of Birke *et al.*[17] The results are given in Table 1.

It is evident that hydrochloric acid solutions are best suited for the simultaneous determination of the three elements. In 0.5*M* oxalic acid, antimony and tin could be simultaneously determined, and tin alone could be determined in 0.5*M* oxalic acid containing Methylene Blue at the $5 \times 10^{-4}M$ level.

The effect of hydrochloric acid concentration on the peak heights and potentials is shown in Fig. 5. A graphy under these conditions are compared in Fig. 6. It can be seen that the FSDPP method is more selectivity of measurement. The results obtained by FSDPP, conventional DPP and sampled d.c. polarography under these conditions are compared in Fig. 6. It can be seen that the FSDPP method is more selective than DPP, and more sensitive for Sb and Sn, but somewhat less sensitive for As.

The parameters of the calibration regression lines are given in Table 2. The calibration graphs were obtained both for the three elements separately (calibration A) and in mixtures (calibration B). The limit of determination was taken as three times the standard deviation of the baseline signal. It can be seen that the calibration graphs exhibit very good linearity and that the limit of determination is sufficiently low for the given purpose.

The effect of bromide and iodide

In view of the possible use of bromide or iodide for extraction of the test elements, their effect on the FSDPP curves was studied. It was found that the presence of bromide in the base electrolyte at concentrations up to 0.015*M* leads to only a small shift in the peak potentials (by −20 mV for arsenic and +20 mV for antimony and tin) and to a maximum decrease in the peak height of about 10% with arsenic

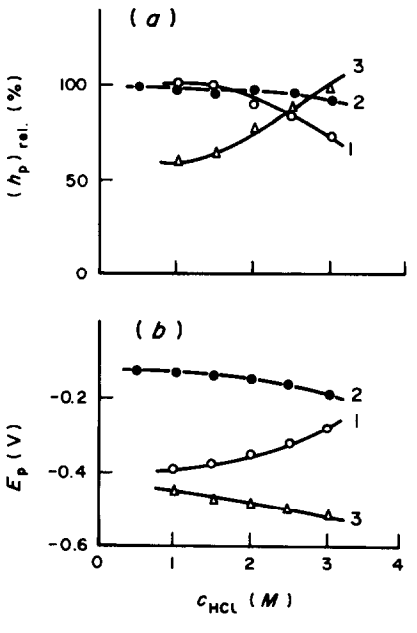

Fig. 5. The effect of HCl concentration on the height h_p (*a*) and the potential E_p (*b*) of the peaks of As (1), Sb (2) and Sn (3). $c_{As,Sn}$ = 1 μg/ml, c_{Sb} = 0.5 μg/ml, for other conditions see Fig. 1.

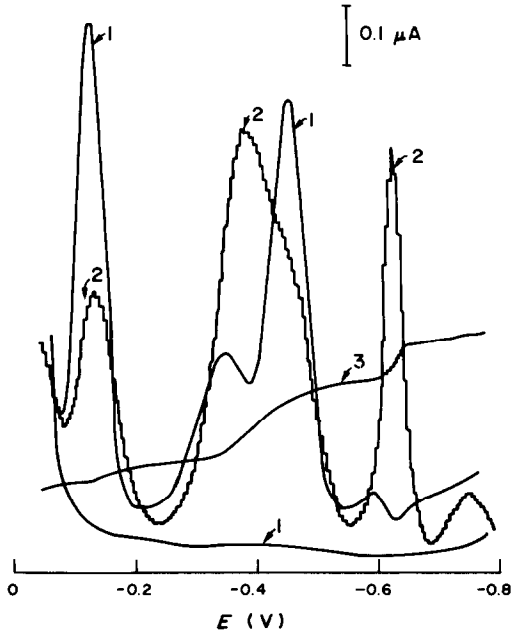

Fig. 6. Comparison of the FSDPP (1), DPP (2) and sampled d.c. polarographic (3) curves for As, Sb and Sn in 1.5*M* HCl. $c_{As,Sn}$ = 1 μg/ml, c_{Sb} = 0.5 μg/ml, potential scan-rate = 5 mV/sec, pulse amplitude = −50 mV, RC = 100 msec, drop-time with DPP and sampled d.c. = 1 sec.

and tin and about 20% with antimony. Further, it was found that the concentration of bromide extracted from sulphuric acid into toluene varied from less than 10^{-4} *M* for 6.4*M* sulphuric acid to 8.8×10^{-3} *M* for 11.7*M* acid (the bromide was back-extracted into 0.1*M* sodium hydroxide and after pH adjustment was potentiometrically titrated with 0.02*M* silver nitrate, a silver ion-selective electrode being used). As this concentration will be further decreased on back-extraction of the sample into the base electrolyte, it will not exceed 5×10^{-4} *M* and thus the addition of enough bromide to the base electrolyte to give a concentration of 0.012*M* will swamp the effect of this extracted bromide (there will be a relative change of only 4% in the bromide concentration after the extraction). The calibration graphs obtained with base electrolyte that was 0.012*M* in bromide gave a linearity as good as that in the absence of bromide, with limits of determination of 0.03, 0.03 and 0.04 μg/ml for arsenic, antimony and tin, respectively.

On the other hand, even low concentrations of iodide strongly affect the peaks of antimony and tin. Therefore, removal of iodide by precipitation with excess of silver nitrate was attempted. However, the FSDPP peaks of the three elements, especially arsenic, decreased with increasing amount of precipitate, owing to adsorption, and thus the use of iodide for the extraction was found unsuitable.

For further measurements, 1.5*M* hydrochloric acid–0.012*M* bromide base electrolyte was therefore used. In this electrolyte, the three elements were simultaneously determined at various concentrations, and it was found that the individual elements could be determined without increase in measurement error in the presence of a tenfold ratio of the other two, except that arsenic could tolerate only a fourfold ratio of tin.

Extraction of arsenic, antimony and tin bromides

Cyclohexane, benzene, toluene and *o*-xylene were tested as extractants. The elements were extracted from 8.3*M* sulphuric acid–0.21*M* sodium bromide mixture in a 25-ml standard flask with 1 ml of solvent and back-extracted into the base electrolyte in a 10-ml standard flask. The extraction time was 5 min. Under these conditions, toluene and *o*-xylene yielded the highest recoveries, about 100% for arsenic and 47% for antimony. Tin was not extracted. Toluene was therefore used in subsequent experiments.

The effect of the bromide concentration on the extraction of the elements was examined at three different concentrations of sulphuric acid. The results are shown in Fig. 7. The extraction of tin was always negligible, that of arsenic decreased above a certain concentration of bromide, whereas that of antimony increased and approached a constant value.

With further increase in the sulphuric acid concentration, extraction of all the three elements was increased, becoming practically complete from the 12*M* acid (see Fig. 8). The bromide concentration has little effect at this acid concentration. Under these con-

Table 2. Characteristics of the calibration regression lines: $c_{As,Sn}$ = 0.2–2.4 μg/ml, c_{Sb} = 0.1–0.9 μg/ml, potential scan-rate = 20 mV/sec, pulse amplitude = −50 mV, 25 nA/cm; equation: $h_p = bc_M + a$, where h_p = peak height in cm

Calibration	Element	No. of measurements	$b \pm s_b$, $cm.ml.\mu g^{-1}$	$a \pm s_a$, *cm*	Corr. coeff.	Limit of detn., μg/ml
A	As	6	$5.9_4 \pm 0.1_0$	0.1 ± 0.1	0.9994	0.07
	Sb	6	22.2 ± 0.2	1.0 ± 0.1	0.9998	0.01
	Sn	6	$9.2_3 \pm 0.0_7$	-0.3 ± 0.1	0.9999	0.03
B	As	7	$6.2_3 \pm 0.0_9$	0.0 ± 0.1	0.9994	0.05
	Sb	6	22.1 ± 0.1	0.6 ± 0.0	0.9999	0.005
	Sn	7	$10.1_1 \pm 0.0_5$	-0.1 ± 0.1	0.9999	0.01

Table 3. Dependence of the degree of extraction, E, on the number of extractions: $2.2 \times 10^{-5}M$ As(III), $3.4 \times 10^{-6}M$ Sb(III), $7.0 \times 10^{-6}M$ Sn(IV), $12M$ H_2SO_4, $0.01M$ NaBr, toluene, $V_{aq} = 24$ ml, $V_{org} = 1$ ml, extracted for 5 min

Extraction No.	E, %		
	As	Sb	Sn
1	98	95	95
2	0	7	3
3	0	0	0
4	0	0	0

ditions, the rate of equilibration between the phases and the number of extractions required for complete extraction were tested. The equilibrium is established within 2 min, but an extraction time of 5 min is used, to give a safety margin. The effect of the number of extractions is shown in Table 3, which shows that two suffice for complete extraction. The optimum conditions for selective and group extraction of the elements are summarized in Table 4.

Because arsenic and antimony are oxidized to the quinquevalent state in the decomposition of steel samples, but must be in the tervalent state for extraction as the bromides, we tested the extraction of As(V) and Sb(V) under the same conditions. Results identical with those for As(III) and Sb(III) were obtained, which indicates that As(V) and Sb(V) are reduced to the tervalent state during the extraction, presumably by hydrogen bromide in the aqueous phase. This would lead to the formation of a small amount of bromine, which would also be extracted. To prevent any possible interference, we decided to add $0.03M$ hydrazinium sulphate to the base electrolyte.

The calibration graphs for the three elements were then obtained after extraction under the conditions above. The parameters of the regression lines are given in Table 5, and show that the extraction does not introduce additional errors into the procedure.

Application to alloy steels

Two standards that had been analysed at various laboratories by various methods were used. The sample decomposition with a mixture of hydrochloric and nitric acid[18] was selected to suppress volatilization of the chlorides of the test elements. To prevent

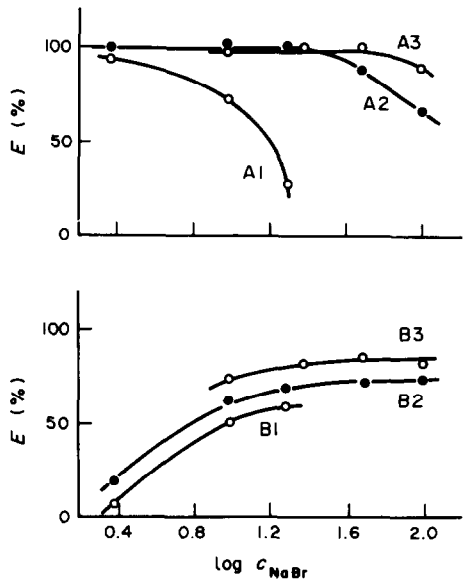

Fig. 7. The effect of the Br^- concentration and the acidity on the extraction E of arsenic (A) and antimony (B). $2.2 \times 10^{-5}M$ As(III), $3.4 \times 10^{-6}M$ Sb(III). 1—$7.3M$ H_2SO_4, 2—$8.3M$ H_2SO_4, 3—$9.4M$ H_2SO_4; toluene, $V_{aq} = 24$ ml, $V_{org} = 1$ ml, extracted for 5 min.

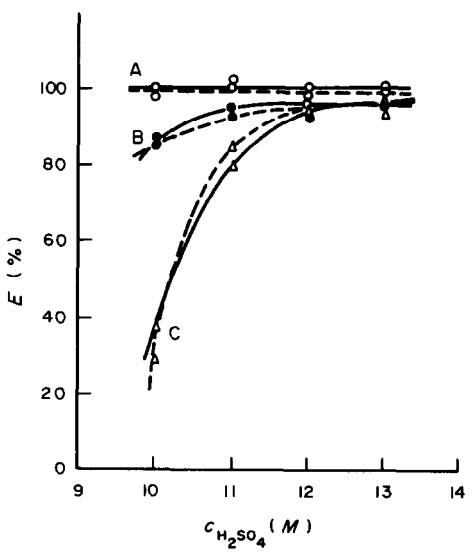

Fig. 8. The effect of the aqueous phase acidity on the extraction of arsenic (A), antimony (B) and tin (C). $2.2 \times 10^{-5}M$ As(III), $3.4 \times 10^{-6}M$ Sb(III), $7.0 \times 10^{-6}M$ Sn(IV). Solid line—$0.042M$ NaBr, dashed line—$0.010M$ NaBr; toluene, $V_{aq} = 24$ ml, $V_{org} = 1$ ml, extracted for 5 min.

Table 4. Optimal conditions for selective and group extraction of arsenic, antimony and tin: toluene, $V_{aq} = 24$ ml, $V_{org} = 1$ ml, extracted for 5 min

Element	$c_{H_2SO_4}$, M	c_{Br^-}, M	E, %	log D_c
As	$\leqslant 7.3$	$\geqslant 0.5$	100	$\geqslant 3.0$
Sb	$\leqslant 7.3$	< 0.04	60	1.6
As + Sb	9.5	0.03	100 + 82	$\geqslant 3.0 + 2.0$
As + Sb + Sn	11.7	0.02	100 + 95 + 92	$\geqslant 3.0 + 2.7 + 2.4$

Table 5. Characteristics of the calibration regression lines after the extraction: aqueous phase 11.7*M* H_2SO_4 + 0.02*M* NaBr + 0.03*M* hydrazinium sulphate, 24 ml; organic phase toluene, 1 ml; extracted for 5 min; 0.5 ml of organic phase back-extracted into 10 ml of the base electrolyte (1.5*M* HCl + 0.01*M* NaBr + 0.03*M* hydrazinium sulphate); potential scan-rate 5 mV/sec, pulse amplitude −50 mV, RC 100 msec, 500 nA/cm (Sb), 100 nA/cm (As), 250 nA/cm (Sn), 8 measurements

Element	$b \pm s_b$, *cm*/%	$a \pm s_a$, *cm*	Corr. coeff.	Limit of detn., %†
As	610 ± 3	0.11 ± 0.05	0.9999	0.0002
Sb	659 ± 4	0.03 ± 0.06	0.9999	0.0003
Sn	563 ± 10	−0.2 ± 0.2	0.9991	0.0009

†Calculated for 0.2 g sample weight.

Table 6. Characteristics of the calibration regression lines for the determination in steel: for the conditions see Table 5; a 0.2-g sample of pure iron is dissolved and analysed; 8 measurements

Element	$b \pm s_b$, *cm*/%	$a \pm s_a$, *cm*	Corr. coeff.	Limit of detn., %
As	610 ± 3	0.11 ± 0.05	0.9999	0.0002
Sb	641 ± 6	−0.04 ± 0.1	0.9998	0.0005
Sn	409 ± 12	−0.1 ± 0.2	0.9973	0.0015

precipitation of ferric sulphate, excess of nitric acid was removed chemically, by addition of formic acid. The problem of precipitation of ferric sulphate also occurs in the extraction from fairly concentrated sulphuric acid, where part of the As, Sb and Sn can be lost by adsorption on the precipitate. However, it was found that these losses are small and constant and their effect can be eliminated, if necessary, by using the standard-addition method. To suppress the possible oxidation of the test elements by bromine, hydrazinium sulphate was added both to the aqueous solution before the extraction and to the base electrolyte.

For 0.2-g samples, the calibration graphs are linear from 0.001 to 0.03% As, Sb and Sn, the detection limits being 2×10^{-4}, 3×10^{-4} and 9×10^{-4}% for As, Sb and Sn respectively.

Procedure

A 0.200-g sample is dissolved in 10 ml of a 3:1 v/v mixture of concentrated hydrochloric and nitric acids in a covered tall beaker. The dissolution can be hastened by cautious heating, but evaporation must be suppressed to prevent volatilization of the As, Sb and Sn. After the dissolution, 20 ml of 14*M* sulphuric acid are added and the mixture is carefully evaporated to the first appearance of white fumes. The beaker walls are rinsed with a small amount of water, 2 ml of formic acid are added and the evaporation is repeated. A ferric sulphate precipitate must not appear, since tin would be adsorbed on it. The hot solution is transferred to a 25-ml standard flask, and cooled with water to room temperature. Then 1.0 ml of a 1.3% solution of hydrazinium sulphate in 0.5*M* sodium bromide is added, followed by 1.00 ml of toluene. The whole is diluted with water to the mark, the flask is shaken for 5 min, the phases are allowed to separate and 0.500 ml of the organic phase is pipetted into 10.0 ml of the base electrolyte (1.5*M* HCl + 0.012*M* NaBr + 0.03*M* hydrazinium sulphate) in a 10-ml standard flask. The flask is shaken for 1 min, the organic phase is separated, and the elements are determined by FSDPP over the range of potential from −0.05 to −0.6 V.

The parameters of the calibration graphs for this procedure, obtained by analyses of synthetic standards made from pure iron (free from the test elements) are summarized in Table 6. Comparison with Table 5 indicates that losses of arsenic and antimony are small, but almost 30% of the tin is lost during the procedure. However, this is a systematic error that can be considerably suppressed by using a suitable calibration procedure or the standard-addition method. It is due to loss of tin(IV) chloride during the decomposition, and could perhaps be avoided by use of a decomposition method not needing hydrochloric acid.

The method was tested on two reference materials. The precision of the determination is summarized in Table 7. It is highest for antimony, 1.5–2.7% relative error; for arsenic and tin the values are 4.1–4.6% and 7.4–8.2% respectively. As seen from Table 8, except for one determination of arsenic, the accuracy of the method is satisfactory, and the precision is the same as or better than that of the other methods listed.

In view of the high selectivity of the extraction method (only selenium and germanium are co-extracted under the given conditions[19]) the method can be useful in analysis of a great variety of materials, from alloys to organic substances.

Table 7. The precision of the determination of the elements in two reference materials ($\alpha = 0.05$)

Material	Volume of extract used, *ml*	No. of measurements	Element	$\bar{x} \pm (st_s)/\sqrt{n}$, *ppm*
1	0.5	5	As	63.5 ± 2.9
			Sb	115.9 ± 1.8
			Sn	71.1 ± 5.8
2	0.2	5	As	201.5 ± 8.2
			Sb	214.5 ± 5.7
			Sn	512 ± 38

Table 8. Comparison of the determination of arsenic, antimony and tin in two reference materials with the results obtained by other methods in various laboratories

	Metal content, *ppm*					
	Material 1			Material 2		
Laboratory	As	Sb	Sn	As	Sb	Sn
A	80[a]	110[f]	60[f]	220[a]	230[f]	460[f]
B	95[a]	118[b]	75[e]	—	—	—
C	—	115[f]	70[f]	—	—	—
D	70[a]	110[f]	58[f]	—	—	—
E	85[g]	120[h]	115[i]	—	—	—
F	75	95	71	240	200	570
G	79[b]	106[b]	55[b]	267[b]	204[b]	523[b]
H	45[d]	107[c]	—	181[d]	209[c]	—
The present method	64 ± 3	116 ± 2	71 ± 6	202 ± 8	215 ± 6	512 ± 38

Methods: [a]spectrophotometry, molybdenum blue, distillation; [b]AAS with electrothermal atomization; [c]spectrophotometry, Rhodamine B, extraction; [d]potentiometric titration with bromate, distillation; [e]spectrophotometry, phenylfluorone, extraction; [f]AAS of an extract; [g]spectrophotometry, molybdenum blue, extraction; [h]spectrophotometry, Malachite Green, extraction; [i]polarography, co-precipitation.

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SYNTHESIS OF SOME NEW *o*-THIOAZO LIGANDS AND EVALUATION OF THEIR METALLOCHROMIC PROPERTIES

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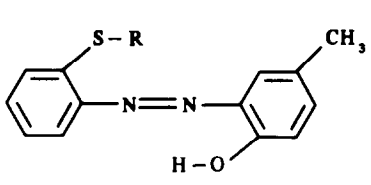
Summary—The synthesis of several new *o*-thioazo derivatives of *p*-cresol and 2-naphthol is reported, as well as their spectral properties, acid dissociation constants, and potential as metallochromic reagents. All the ligands form complexes with Cu^{2+} and Ni^{2+}. *o*-Mercaptoazo complexes of Fe^{3+} and Cu^{2+} with molar absorptivities of 3.83×10^4 and 3.58×10^4 l.mole^{-1}.cm^{-1}, respectively, are described.

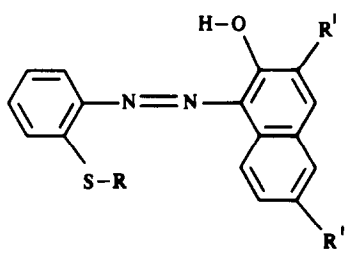
Sulphur-containing chromogenic agents such as dithizone and the dithiocarbamates are well known to analytical chemists. Other sulphur-containing ligands such as 8-mercaptoquinoline[1] and 6-mercaptopurine[2] have been studied and have found limited use as analytical reagents. There are some sulphur-containing azo derivatives such as 1-(2-thiazolylazo)-2-naphthol[3] and 2-(2-thioazolylazo)-5-dimethylaminophenol[4] that have been explored as metallochromic reagents, but in both the sulphur is part of the heterocyclic thiazol ring. Azo dyes in which the sulphur is bonded directly to one or both of the aromatic rings have not been explored to any great extent. Burawoy *et al.*[5–7] reported on the synthesis of a number of *o*-mercaptoazo compounds and indicated that many of these formed precipitates with a variety of transition metal ions, but very little has been done on the potential of such compounds as colorimetric reagents. Koch and Pringle[8] reported the synthesis of bis-(2[(tetrahydro-2H-pyran-2-yl)thio]phenyl)diazene and its potential as a colorimetric reagent for Hg(II). This reagent, with the sulphur atoms protected by pyranyl groups, is indefinitely stable in solution, contrary to the azomercaptans reported by Burawoy, and other thiol ligands.

The purpose of this paper is to report the synthesis of the compounds shown in Table 1, evaluate their metallochromic behaviour, and compare the effects of the various sulphur-blocking groups. The blocking groups (R) were selected to provide a variety of steric effects and also, as in the case of compounds **I** and **II**, a comparison of a mercapto group with a thioether group. The acetic acid grouping was introduced into compounds **IV** and **VI** to increase the solubility and also to provide a blocking group that could enter into complex formation. A compound similar to **IV** and **VI**, 2-aminophenylthioacetic acid,[9] has been shown to act as a uni-, bi- or terdentate ligand and to complex with Cu^{2+}, Ni^{2+} and Cd^{2+}.

I and **V**, which proved to be the most difficult to synthesize, were prepared by coupling diazotized 2-benzylthioaniline with *p*-cresol or 2-naphthol and then debenzylating with anhydrous aluminium bromide. The debenzylation and final acidification were done in a nitrogen atmosphere. The other dyes were easily produced by diazotizing the appropriately

Table 1. Structures of thioazo ligands





I. R=H
II. R=CH_3
III. R=$CH_2C_5H_6$
IV. R=CH_2COOH

V. R=H R'=H
VI. R=CH_2COOH R'=H
VII. R=CH_3 R'=SO_3H

blocked *o*-alkylthioaniline and coupling with *p*-cresol, 2-naphthol or 2-naphthol-3,6-disulphonic acid. After suitable purification and the determination of the acid dissociation constants, the ligands and their metal complexes were investigated spectrophotometrically.

EXPERIMENTAL

Syntheses

Preparation of [*2-(methylthio)phenyl*](*2-hydroxy-5-methylphenyl*)*diazene* (*II*). Diazotized *o*-methylthioaniline[10] (6.2 ml in 2 ml of concentrated sulphuric acid, with 2.3 g of sodium nitrite) was added dropwise to 4.5 g of *p*-cresol in 1800 ml of water containing 5.0 g of sodium hydroxide and 140 g of sodium carbonate. The reaction mixture was stirred continuously and maintained at 0°. The product was filtered off and recrystallized from methyl alcohol, yielding 4.5 g (43%) of orange-red needles, m.p. 103–105°. Calculated for $C_{14}H_{14}N_2SO$: C, 65.09%; H, 5.46%; N, 10.85%; S, 12.41%; found: C, 64.9%; H, 5.5%; N, 10.9%; S, 12.4%. ^{1}H NMR ($CDCl_3$): δ 2.4 (s, 3, $ArCH_3$), 7.3 (m, 7, ArH), and 12.6 (br. s, 1, OH).

Preparation of [*2-(benzylthio)phenyl*](*2-hydroxy-5-methylphenyl*)*diazene* (*III*). 2-Benzylthioaniline,[11] 2.0 g, was dissolved in 10 ml of 95% ethanol and diazotized by the addition of 5.0 ml of 4*M* hydrochloric acid containing 1.0 g of sodium nitrite, both solutions being at 0°. The diazonium salt was immediately added to a 10% sodium carbonate/10% sodium hydroxide solution containing 1.5 g of *p*-cresol and cooled to 0°. After 30 min of stirring 1.7 g (55%) of crude product were recovered by filtration. Recrystallization from ethanol gave orange-red needles, m.p. 101–102° (lit.[12] 102–103°). ^{1}H NMR ($CDCl_3$): δ 2.4 (s, 3, $ArCH_3$), 4.2 (s, 2, $ArCH_2S$), 7.3 (m, 7, Ar), and 12.4 (s, 1, OH).

Preparation of (*2-mercaptophenyl*)(*2-hydroxy-5-methylphenyl*)*diazene* (*I*). One g of **III** was placed, under nitrogen, in a double Schlenck flask and dissolved in 40 ml of deaerated anhydrous benzene. Anhydrous aluminium bromide, dissolved in 40 ml of benzene, was then added. The solution, which immediately changed from orange-red to violet, was stirred for 24 hr at room temperature. The product was collected on the glass frit in the flask and washed with 40 ml of benzene. After removal of the last traces of benzene by evacuation, 40 ml of 1*M* hydrochloric acid were added. This resulted in a yellow product which was again collected within the flask and washed twice with deaerated water. The product was dissolved in 50 ml of benzene, the solution was filtered and the benzene removed under reduced pressure. The product, a yellow powder, m.p. 121–123°, was stored under nitrogen. ^{1}H NMR ($CDCl_3$): δ 2.4 (s, 3, $ArCH_3$), 7.3 (m, 8, ArH and SH), and 12.0 (s, 1, OH).

Preparation of sodium o-aminophenylthioacetate.[9,13] Two g of sodium metal, cut into small pieces, were slowly added to 10 ml of *o*-aminothiophenol dissolved in 25 ml of absolute ethanol. Monochloroacetic acid, 8.0 g, neutralized with 4*M* sodium hydroxide, was added dropwise. After refluxing for 1 hr, the solution was cooled to room temperature and used for subsequent syntheses with no further isolation.

Preparation of [*2-(carboxymethylthio)phenyl*](*2-hydroxy-5-methylphenyl*)*diazene* (*IV*). Twenty-five ml of the *o*-aminophenylthioacetate solution were diazotized by addition to a solution of 3.2 g of sodium nitrite in 10 ml of water and 20 ml of 5*M* hydrochloric acid at 0°. The diazonium salt solution was then added to 5.0 g of *p*-cresol dissolved in 300 ml of water containing 2.0 g of sodium hydroxide and 70.0 g of sodium bicarbonate. After 30 min of stirring the solution was acidified with concentrated hydrochloric acid to pH < 2. The crude product was recrystallized from 95% ethanol, giving 5.5 g (47%) of orange needles, m.p. 146–148°. Calculated for $C_{15}H_{14}N_2SO_3$: C, 59.58%; H, 4.67%; N, 9.27%; S, 10.6%; found: C, 60.0%; H, 4.8%; N, 8.4%; S, 11.9%. ^{1}H NMR (acetone, d_6): δ 2.4 (s, 3, $ArCH_3$), 4.0 (s, 2, $SCH_2C{=}O$), 6.1 (br.s, 1, COOH), 7.4 (br.m, *ca.* 8, ArH), and 12.0 (br.s, *ca.* 1, OH); infrared (KBr) 3300–2300 (acid O—H) and 1690 (C=O) cm^{-1}.

Preparation of [*2-(carboxymethylthio)phenyl*](*2-naphthol*) *diazene* (*VI*). Twenty-five ml of diazotized *o*-aminophenylthioacetate solution, prepared as above, were added to 300 ml of an aqueous solution containing 5.76 g of 2-naphthol, 5.0 g of sodium hydroxide and 70.0 g of sodium bicarbonate, both solutions being cooled to 0° both before mixing and during the 30-min reaction time. The mixture was then acidified with concentrated hydrochloric acid to pH < 2. The dark red precipitate was isolated and recrystallized from glacial acetic acid. The yield was 6.0 g (46%) of greenish prisms which had a metallic lustre, m.p. 167–169°. Calculated for $C_{18}H_{14}N_2SO_3$: C, 63.89%; H, 4.17%; N, 8.28%; S, 9.47%; found: C, 63.6%; H, 4.3%; N, 8.1%; S, 9.6%. ^{1}H NMR (acetone, d_6): δ 4.0 (s, 2, $SCH_2C{=}O$), 5.2 (br.s, 1, COOH), 7.9 (m, 10, ArH), and 15.8 (s, 1, OH); infrared (KBr) 3400–2200 (acid O—H) and 1705 (C=O) cm^{-1}.

Preparation of (*2-mercaptophenyl*)(*2-naphthol*)-*diazene* (*V*). [2-(Benzylthio)phenyl](2-naphthol)diazene (0.5 g, prepared by diazotizing 2-aminophenylbenzylsulphide and coupling with 2-naphthol) was placed in one arm of a double Schlenk flask and dissolved in 40 ml of deaerated anhydrous benzene under an atmosphere of nitrogen. Addition of 0.60 g of freshly sublimed anhydrous aluminium bromide, dissolved in 10 ml of benzene, caused the red solution to change immediately to violet. After stirring for 24 hr at room temperature, the product was collected on the glass frit of the flask and washed with 50 ml of benzene. The benzene was removed by syringe and evacuation. The resulting solid was treated with 1*M* hydrochloric acid, collected on the frit, and washed with 50 ml of deaerated water. The product was dissolved in 50 ml of benzene, the solution was filtered, and the benzene removed under reduced pressure. The final product was a reddish-orange powder, sintering at 113–114° (lit.[14] 118°). ^{1}H NMR ($CDCl_3$): δ 3.30 (s, 1, SH), 7.2 (m, *ca.* 11, ArH) and 15.8 (br.s, 1, OH).

Preparation of [*2-(methylthio)phenyl*](*2-naphthol-3,6-disulphonic acid*)*diazene, monosodium salt* (*VII*). Diazotized 2-methylthioaniline (4.2 g dissolved in 20 ml of 1:1 ethanol–water mixture containing 4.1 g of sodium nitrite, followed by addition of 15 ml of 4*M* hydrochloric acid to the mixture, cooled to −5°) was added to 12 g of 2-naphthol-3,6-disulphonic acid, disodium salt, dissolved in 30 ml of 20% sodium hydroxide solution. The reaction temperature was kept at 0°. After 1 hr the mixture was acidified with 6*M* hydrochloric acid, cooled and filtered. The crude product was crystallized three times from 95% ethanol and finally extracted with acetone in a Soxhlet extractor. After stripping of the acetone, 4.0 g (15%) of a deep purple solid were recovered. Analysis with titanium(III) chloride showed that the product was 87% pure, on the basis of its azo content.

K_a *determinations*

The K_a value for the OH and/or SH group of each dye was determined spectrophotometrically. The absorbance of the dye, taken over a number of closely-spaced pH values, was plotted *vs.* pH; the inflection point of this curve served as the K_a value. The methods of Clark and Lubs, and of Bates and Bower, as found in Bates,[15] were used to prepare the buffered solutions. Because of solubility difficulties, many of the K_a values were determined in aqueous ethanol media. The K_a values reported are based on the apparent pH values measured in the media used, and may not agree with the K_a values measured in purely aqueous medium.

Table 2. Properties of the thioazo ligands

Ligand	λ_{max}, nm (ϵ, $l.mole^{-1}.cm^{-1}$) pH < 7	7 > pH < 12	pH > 12	pK_a values
I	425 (4.30 × 10^3)[a]	555 (4.4 × 10^3)	470 (3.30 × 10^3)	SH, ~4.5[d] OH, ?[d]
II	415 (1.00 × 10^4)[a]		485 (1.62 × 10^4)	OH, 11.75[a]
III	415 (9.50 × 10^3)[a]		485 (1.06 × 10^4)	OH, 11.15[c]
IV	409 (9.00 × 10^3)[b]		490 (9.50 × 10^3)	COOH, 5.38[a] OH, 11.25b
V	488 (1.35 × 10^4)[a]	545 (1.08 × 10^4)	455 (1.70 × 10^4)	SH, 5.64[a] OH, ~12.7[d]
VI	490 (1.90 × 10^4)[b]		460–490 (1.37 × 10^4)	COOH, 5.30[a] OH, 12.25[b]
VII	500 (1.00 × 10^3)[c]		513 (6.50 × 10^2)	SO_3H, 2.54[c] OH, 10.97[e]

[a]50% ethanol. [b]40% ethanol. [c]20% ethanol. [d]Too unstable toward air oxidation to make suitable estimates. [e]Water.

Furthermore, the K_a values reported for **V** and **VI** are most likely only approximate, as they were determined in very basic solutions with the attendant alkaline errors in the pH measurements. The K_a values for the carboxylic acid protons in compounds **III** and **IV** were determined by a pH-titration with 0.1*M* sodium hydroxide.

Mole-ratio determination

The combining ratio of dye and metal ion was determined by the standard spectrophotometric mole-ratio method. The pH used for each determination was selected by study of the pH-dependence of the absorbance for each complex.

RESULTS AND DISCUSSION

With the exception of **I** and **V**, the azo ligands were stable and could be stored indefinitely both as the solid and in solution. **I** and **V**, however, were difficult to synthesize, needing a nitrogen atmosphere, and were very unstable in solution. A chloroform solution of **I** would slowly change from yellow to green over a period of two days. A solution of **V** made up in absolute ethanol which had been purged with nitrogen showed signs of deterioration after only an hour or two.

Table 2 shows the spectral characteristics of each of the azo ligands as well as the values for their acid dissociation constants. Since compounds **I**–**VI** showed little solubility in water, the spectral data were obtained by use of ethanol–water mixtures as solvents. **VII**, however, was very soluble in water. Most of the compounds were yellow in acidic media and changed to orange or red with increasing pH. Compounds **I** and **V** showed an intermediate colour change, from yellow to violet and then to orange.

The K_a values for the OH proton fell into two categories. The phenolic protons of **I**–**IV** had pK_a values of about 11.5, whereas the naphthol protons of **V**–**VII** had values near 12.5. The NMR-shift values also showed a similar effect in that the phenolic protons had a chemical shift of approximately 12.3 ppm and the OH on the naphthol derivatives had a shift of about 15.8 ppm. The K_a values of the COOH group in **IV** and **VI** were determined potentiometrically because the dissociation was not accompanied by a spectral change, presumably because the carboxylic acid group is not conjugated to the aromatic system. The pK_a values for the SH groups are only approximate, because of the unstable nature of their solutions.

Each of the ligands was tested for complex formation with a variety of metal ions, which included Ag^+, Ba^{2+}, Bi^{3+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Hg(I), Hg(II), Ni^{2+}, Pb^{2+} and Zn^{2+}. Table 3 summarizes the results. Copper and nickel reacted with all the ligands, though in the case of **VI**, the copper complex was only partially soluble in 50% aqueous ethanol and the copper and nickel complexes of **V** were both insoluble in water–ethanol mixtures. **VI** was unique in that it formed fluorescent precipitates with Ba^{2+}, Cd^{2+} and Mg^{2+}. Attempts to dissolve these precipitates in water, carbon tetrachloride, ethanol, methanol, cyclohexene, and acetone were all unsuccessful.

The ligands in which the sulphur was present in a thioether group (**II**, **III**, **IV**, **VI** and **VII**) all formed 1:1 complexes with copper and nickel. In an attempt to find the extent of involvement of the sulphur atom in the bonding, the oxygen analogue of **III**, [(2-benzoxy)phenyl](2-hydroxy-5-methylphenyl)diazene, and phenyl(2-hydroxy-5-methylphenyl)diazene were prepared, and their copper complexes studied. The benzoxy and phenyl diazenes both formed 3:1 complexes with copper, in contrast to the 1:1 complex of **III**. This would indicate that the sulphur atom is unquestionably involved in the bonding, most likely though strong $d\pi$–$d\pi$ interaction with the metal ion.

From an analytical point of view, **I** and **V** are perhaps the most interesting. When a chloroform solution of **I** is shaken with an aqueous solution of Cu^{2+}, Ni^{2+}, Hg(II) or Ag^+, an intensely blue complex is extracted into the chloroform layer. The molar absorptivity of the copper complex is 3.58 × 10^4 $l.mole^{-1}.cm^{-1}$. The complex obeys Beer's law up to a copper concentration of 2.5 ppm, but the plot does not pass through the origin, probably because the oxidation product of the uncomplexed ligand interferes. The exact nature of the complexes of **I** has yet to be studied, because of the lack of stability of the dye, although once the complex is formed, its colour seems to be stable indefinitely.

V proved to react with the greatest number of metal ions. A solution of **V** in absolute ethanol was added to a test solution of each of the metals listed

Table 3. Properties of thioazo complexes

Ligand	Metal(s)	Mole ratio (L:M)	pH range	Colour	λ_{max}, *nm*	ϵ, $l.mole^{-1}.cm^{-1}$
I	Cu^{2+}	—	[a]	Blue	570	3.58×10^4
	Ni^{2+}, Hg(II), Hg(I)	—	[a]	Blue	580	—
II	Cu^{2+}	1:1	5–10[b]	Pink	520	1.40×10^4
	Ni^{2+}	—	—	Pink	510	6.70×10^3
III	Cu^{2+}	1:1	7.5–11[c]	Orange	520	1.06×10^4
	Ni^{2+}	—	—	Orange	510	9.00×10^3
IV	Cu^{2+}	1:1	6–9[b]	Pink	520	1.05×10^4
	Ni^{2+}	—	—	Pink	510	9.00×10^3
	Ag^{+}[d]					
V	Fe^{3+}	2:1	4–5[c]	Rose	525	3.83×10^4
	[Ba^{2+}, Ca^{2+}][f]					
	[Ni^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+}, Hg(II), Pb^{2+}, Cu^{2+}][g]					
VI	Cu^{2+}[h]	—	—	Pink	525	—
	Ni^{2+}	1:1	7	Pink	520	2.02×10^4
	[Ba^{2+}, Cd^{2+}, Mg^{2+}][i]					
VII	Cu^{2+}	2:1	9–11	Red	513	2.00×10^3
	[Fe^{3+}, Ca^{2+}][j]					

[a]Complex was extracted into $CHCl_3$ for acidic solution of the metal ion. [b]Acetate buffer. [c]Carbonate buffer. [d]Complex forms only in the presence of a large excess of Ag^{+}. [e]Perchloric acid–sodium carbonate buffer. [f]Show evidence of very weak complex formation. [g]Form chloroform soluble precipitates of various colours. [h]Only sparingly soluble. [i]Form fluorescent precipitates which are insoluble in common solvents. [j]Quenched the dye's fluorescence.

and then buffered at pH 5.6, the final ethanol–water ratio being 1:1. Under these conditions a number of transition metals gave highly coloured complexes, Ba^{2+}, Ca^{2+} and Fe^{3+} formed soluble complexes. The Fe^{3+} complex seemed the most highly coloured and stable. The molar absorptivity was 3.83×10^4 $l.mole^{-1}.cm^{-1}$, and the combining ratio was 2:1 (L:M). The complex formed very slowly, but once formed, it also decomposed very slowly. Figure 1 shows the mole-ratio data for this complex as well as the time-dependence for its formation and decomposition. As in the case of **I**, the complex of **V** is far more stable than the ligand itself.

The ligands in which the sulphur is present in a thioether group, although more stable and easier to work with, do not have exceptionally high molar absorptivities (*ca.* 10^4 $l.mole^{-1}.cm^{-1}$), nor do they show large enough bathochromic shifts to make them attractive analytically. The ligands in which the sulphur is present as a thiol group, however, have high molar absorptivities and show large bathochromic shifts when complexed. Successful attempts to stabilize thioazo ligands should prove useful in producing new chromogenic agents.

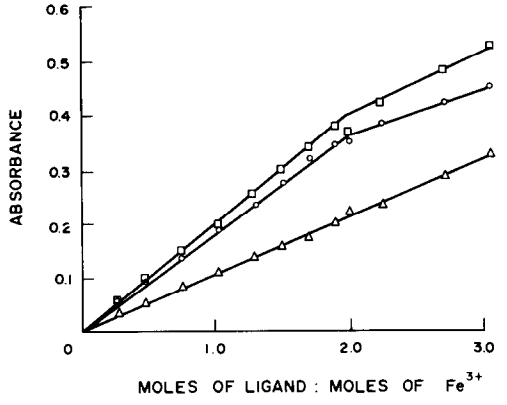


Fig. 1. Mole-ratio plot for Fe^{3+} and V, △, immediately after mixing Fe^{3+} and ligand; □, 4 hr after mixing; ○, 4 days after mixing. Final Fe^{3+} concentration, $1.00 \times 10^{-5} M$; wavelength, 525 nm.

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A SURVEY OF THE INFORMATION ON CO-ORDINATION COMPOUND FEATURES WHICH CAN BE GAINED FROM ELECTROANALYTICAL METHODS*

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Summary—The information provided by modern electroanalytical techniques on co-ordination compounds is surveyed. The problem of the interaction between the electrode and intermediates or products is also briefly considered; it is pointed out that the electroanalytical approach can be successfully employed to provide new insight into chemical properties of metal complexes only when weakly interacting species are involved. The information obtainable is considered under the following headings: (*i*) mechanistic studies on metal complexes and electroanalytical evidence for their reactivity and stability; (*ii*) structural features of co-ordination compounds in solution; (*iii*) feasibility of electrochemical syntheses; and (*iv*) stability of intermediate oxidation states with reference to the nature of the ligands co-ordinated to the metal.

Recent years have seen a growing interest in the application of electroanalytical techniques for investigation of the redox properties of co-ordination compounds.[1–4] Most of the work seems to have been concerned with correlations between the electronic configuration of metal complexes and their oxidation and reduction potentials, proof of existence of a co-ordination compound under given experimental conditions and study of the equilibria in which it is involved. However, electroanalytical methods allow many other interesting pieces of information to be obtained. In the last decade, this type of investigation has received considerable impetus from wider knowledge of the more subtle criteria for interpreting the responses of dynamic electroanalytical techniques and from the greater availability of theoretical tools for quantitative treatment of the experimental data.[5,6]

The responses of the dynamic techniques are more meaningful than those of the static methods (*e.g.*, potentiometry). This is because the electrode may be used as a tool able to produce a variable but precisely known amount of reactive species in a small volume surrounding the electrode surface (by control of the current or the electrode potential) and at the same time monitor the chemical reactions in which such species are involved. Consequently, these dynamic techniques provide not only information on the electrochemical quantities typical of the redox process, but also allow investigation of the chemical reactions coupled with the charge-transfer step; moreover, since the relevant responses can be obtained within a few msec after stimulation of the electrode, they may be used for studying mechanisms involving very fast reactions, allowing detection of short-lived transient intermediates.

*This topic was the subject of a plenary lecture presented by M. F. at the XIV National Meeting of the Italian Chemical Society (Catania, September 1981).

It is because these techniques are able to provide such a wealth of information that they are among the best tools for the *in situ* characterization of co-ordination compounds; unlike other methods they do not require preliminary separation of the species of interest.

The most widely used of these techniques is cyclic voltammetry, which is unmatched in its ability to provide qualitative information with a modest expenditure of time and effort in the acquisition and interpretation of data. When quantitative kinetic data are desired, supplementary techniques such as chronoamperometry, and rotating disk, ring–disk and a.c. voltammetry are often preferred because of their greater accuracy.

The aim of this survey is not to review all the electroanalytical work on metal complexes, but to discuss the considerable insight which can be gained through electroanalytical techniques into the chemical properties of co-ordination compounds. We wish, in particular, to refute the opinion, rather common amongst inorganic chemists, that the conclusions drawn about the nature of co-ordination compounds from electroanalytical investigations are always ambiguous and speculative unless accompanied by various kinds of spectroscopic evidence. We feel that in many cases electroanalytical findings can easily give an adequate description of these species, provided the data are properly interpreted. Only when detailed information cannot be gathered by electroanalytical techniques alone (for instance, in the exhaustive description of an intermediate species), does combination with non-electroanalytical methods become advisable or profitable. It is the existence of this comparatively small area in which conclusive statements cannot be inferred by the electroanalytical approach on its own, that has led to the rapid growth of modern spectro-electrochemical methods.[7]

In any case, electroanalytical investigations, either alone or coupled with other measurements, can provide some types of chemical information which are difficult or tedious to gather otherwise.

The information obtainable will be dealt with under four headings: (*i*) mechanistic studies, (*ii*) structural features of species in solution, (*iii*) electrochemical synthesis, (*iv*) stabilization of intermediate oxidation states.

First, however, it is worthwhile considering the nature of electrode reactions with reference to degree of interaction of reactants, intermediates and products with the electrode surface. When the interactions are very strong, the species are bonded to the surface or even, as in electro-deposition and electrodissolution, are really part of the electrode. Weaker interactions cause chemical or physical adsorption of the species and hence affect the relevant responses both thermodynamically and kinetically. Only when very weak interactions are present, are the reactants, intermediates and products freely diffusing solution species, the properties of which are unaffected by the presence of the electrode.

It is only in this last case that the thermodynamic and kinetic conclusions drawn from electroanalytical data correctly describe the properties of the species under ordinary solution conditions.

Therefore, the strength of the interactions should be checked whenever information gained by the electroanalytical approach is to be used for outlining chemical, physicochemical and configurational properties of co-ordination compounds.

In this connection, it should be remarked that one of the advantages of non-aqueous solvents over water is the very rare occurrence of adsorption processes.

MECHANISTIC STUDIES

The elucidation of the mechanism of catalytic reduction of oxygen in biological and non-biological systems can be taken as a representative example of electroanalytical studies on this subject. It was achieved[8,9] by investigating the electrochemical reduction of molecular oxygen in aprotic solvents in the presence of a series of cobalt chelates which are known as oxygen-carrier model systems. The prototype chelate is *N,N'*-ethylenebis(salicylideneaminato)-cobalt(III) $[Co(salen)]^+$. Voltammetric measurements have allowed it to be ascertained that the overall process occurs through the sequence:

Oxygen reacts with the cobalt(II) complex obtained as the primary reduction product, yielding an oxygen adduct within which charge transfer occurs from the metal to the O_2 ligand. Remarkably less energy is required for the reduction of O_2 to the superoxide ion in this step than in the direct process, thanks to the formation of the cobalt–oxygen bond. Subsequently, interaction of the superoxide ion with the acceptor M^+ (transfer of activated oxygen) allows renewal of the depolarizer Co(III), completing the catalytic cycle.

Electroanalytical techniques were used for determining the extent of oxygen activation by the metal co-ordination and to gain information on the nature of the ligands which favour the process thermodynamically and kinetically.

The electroanalytical approach can also give information concerning metal-promoted organic syntheses, not only with respect to catalytic activity in a reaction mechanism but also with regard to improving the yield.

An example is the promotion of the coupling reaction of organic halides by the triphenylphosphine complex of nickel(0):[10]

$$2RX + Ni^0 \rightarrow R\text{–}R + Ni^{II}X_2 \qquad (5)$$

An appropriate combination of voltammetric, chronoamperometric and coulometric findings has shown the possibility of continuously recycling the nickel(0) complex by cathodic reduction and that the coupling product R–R is formed by the reaction scheme shown in Fig. 1.[11]

It seems worthwhile to describe this scheme in some detail to illustrate the depth of insight into charge-transfer mechanisms which can be gained by use of electroanalytical techniques.

The nickel promoter $[Ni^0L_4]$ (L = triphenylphosphine), obtained by cathodic reduction of the nickel(II) species initially present in the solution, undergoes oxidative addition of organic halides (RX) leading to σ-bonded organometallic nickel(II) derivatives having stabilities which depend on the nature of the co-ordinated organic group.

Aryl groups give thermally stable derivatives and the nickel–carbon bond can be cleaved only by a further reduction, which can occur in two cathodic steps, at different potentials (see route 2). In both steps the coupling product is formed (in the first by a

$$[Co^{III}(salen)]^+ + e^- \rightleftarrows [Co^{II}(salen)] \qquad (1)$$

$$[Co^{II}(salen)] + O_2 \rightleftarrows [Co^{II}(salen)\,O_2] \qquad (2)$$

$$[Co^{II}(salen)\,O_2] \rightarrow [Co^{III}(salen)\,O_2^-] \qquad (3)$$

$$[Co^{III}(salen)\,O_2^-] + M^+ \rightarrow \underline{[Co^{III}(salen)]^+} + MO_2 \qquad (4)$$

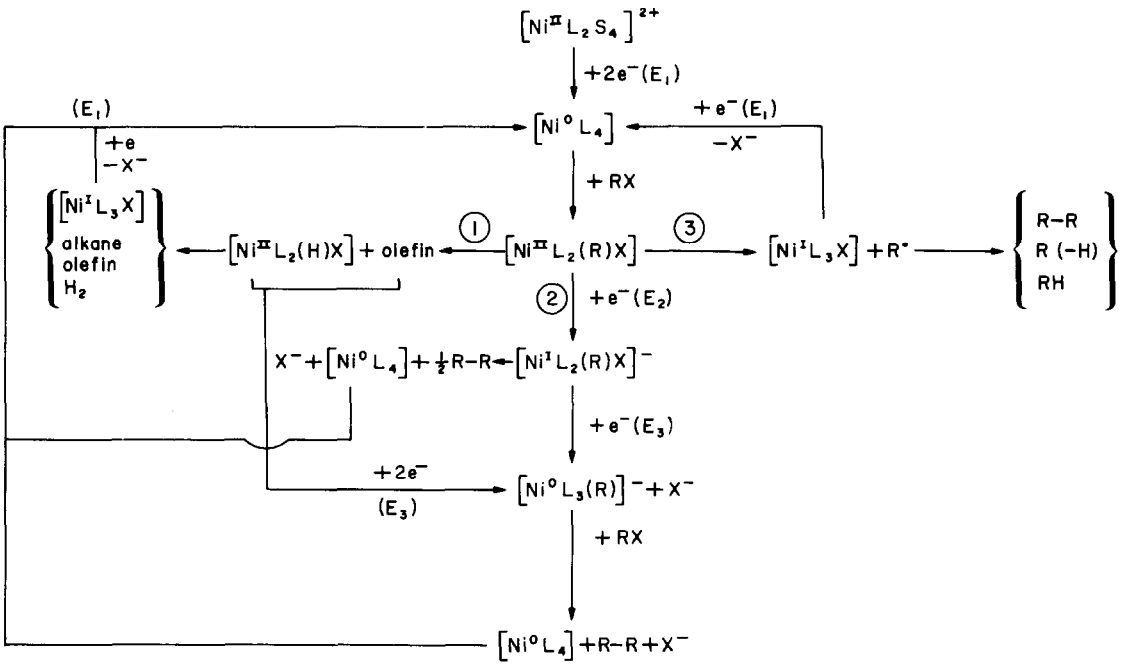


Fig. 1. Reaction scheme for the coupling of organic halides electrocatalysed by the Ni–PPh_3 system. (L = PPh_3; S = CH_3CN; X = Cl, Br or I; R = alkyl, aryl or aralkyl group). $E_1 > E_2 > E_3$.

radical dimerization, in the second by formation of an organic anion) together with the nickel(0) species which immediately reacts with the organic substrate, completing the electrocatalytic cycle.

In contrast the alkyl derivatives undergo thermal decomposition at room temperature. This decomposition involves reductive elimination of the organic moiety by two pathways, depending on whether a hydrogen atom is present or not in the β-position of the organic group. In the case of alkyl halides not bearing hydrogen atoms in the β-position, a simple homolytic fission takes place, yielding the coupling product by dimerization of the organic radicals formed and nickel(I) (see route 3). The nickel(I) is then reduced to the nickel(0) promoter at the same potential as that used for reduction of the nickel(II) initially present, again allowing cyclic regeneration of the inorganic promoter.

If, however, the organic group has a hydrogen atom in the β-position, the thermal decomposition of the organonickel intermediate occurs by a β-elimination reaction (see route 1) to give the corresponding olefin and an unstable nickel hydride which decomposes, yielding nickel(I) reducible at the working potential, again renewing the nickel(0) promoter and completing the electrocatalytic cycle.

In this last case it is also possible to obtain the coupling product by cathodic reduction at more negative potentials at which the nickel hydride intermediate can also be reduced. In this way, nickel(0) is regenerated by an electrocatalytic pathway partially overlapping with route 2. In this case it is therefore possible to pilot the process towards the generation of the desired products simply by choosing the appropriate working potential.

In connection with this subject, the basic contribution recently made by Saveant[12] to the understanding of the reaction pathways involved in an electrocatalytic process, must be acknowledged.

The next example illustrates the information which can be gained on the existence of previously unknown complexes and on the procedures for their preparation.

The cathodic reduction of nickel(II) in acetonitrile in the presence of triphenylphosphine may be considered as a reference point for this discussion. An appropriate combination of voltammetric and spectrophotometric work has established that this reduction occurs through the following reaction sequence:[13]

$$[Ni^{II}L_2S_4]^{2+} + 2e^- \xrightarrow[-4S]{+2L} [Ni^0L_4] \quad E_1 \qquad (6)$$

$$[Ni^0L_4] + [Ni^{II}L_2S_4]^{2+} \xrightarrow[-4S]{+2L} 2[Ni^{I}L_4]^{+} \qquad (7)$$

$$[Ni^{I}L_4]^{+} + e^- \rightleftharpoons [Ni^0L_4] \quad E_2 > E_1 \qquad (8)$$

L = triphenylphosphine; S = CH_3CN = solvent

Nickel(II) undergoes a direct two-electron reduction which is affected by a quite high overvoltage because of the significant molecular rearrangements accompanying such a charge-transfer step. The nickel(0) complex obtained then reacts with the depolarizer, giving nickel(I) which is reducible in turn to nickel(0) in a reversible electrode reaction at the working potential.

Consequently, the nickel(II) is not really reduced to nickel(0) in a simple two-electron electrode reaction which requires a very high activation energy, but by an autocatalytic process. In the overall electrochemical reaction the activation energy is considerably lowered thanks to the catalytic activity displayed by the nickel(0) produced in the electrode process. In other words, during the reduction, the slow Ni(II) → Ni(I) transfer at the electrode is substituted for the fast occurrence of the same redox reaction in the homogeneous solution phase.

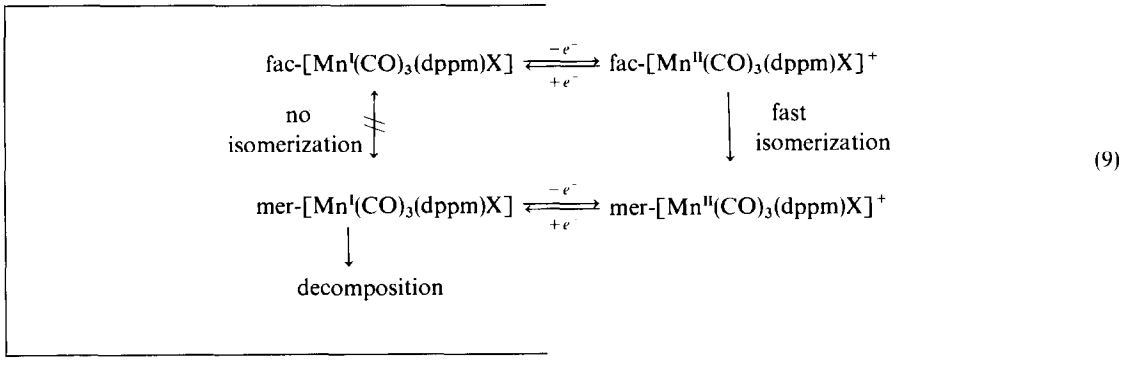
Among the findings provided by this mechanistic study, we may emphasize the discovery of the previously unknown nickel(I) complex $[NiL_4]^+$ and the useful suggestions that can be provided for its preparation. It has been found possible, in fact, to prepare this complex by reacting equimolar amounts of the corresponding nickel(0) and nickel(II) species (see step 7).

Although this statement is about 30 years old, electroanalytical methods have seldom been employed for deducing the structure of co-ordination compounds; nevertheless, the degree of reversibility is a good basis for predicting the configurational features of metal complexes in solution. In this connection, it should be emphasized that the usual methods of structure determination, which require preliminary separation of the co-ordination compound investigated, are only seemingly more direct procedures, since in many cases the geometric and/or ligand configuration may not be the same in solution and in the solid state. In recent years, however, there has been more interest in use of electroanalytical techniques for tackling this problem.

For example, consider the structural study of the octahedral manganese complexes with the metal (in both the +1 and +2 oxidation states) of the type $Mn(CO)_3(dppm)X$, where dppm = diphenylphosphinemethane and X = Cl, Br,[15] for which the two isomers shown in Fig. 2 can be envisioned.

Previous work in this field allowed characterization of only the facial isomer for Mn(I), and no information was available on the isomers formed with Mn(II).

By cyclic voltammetry of solutions of the previously characterized fac-$[Mn^I(CO)_3(dppm)X]$ complex, the charge-transfer reactions and chemical events in the following scheme could be identified.



(9)

SOLUTION STATE OF METAL COMPLEXES

Electroanalytical techniques can also be used for inferring structural properties of co-ordination compounds and to obtain valuable information concerning these species in solution, because the overvoltage of an electrochemical process reflects all changes accompanying the electron-transfer reaction, including changes in structure and in stoichiometric composition during the electrode reaction.

This connection between the degree of reversibility characterizing an electrode process and the configurational features of the redox partners involved is analogous to Libby's symmetry principle[14] which states that a reversible electrode reaction is observed when the co-ordination spheres of the oxidized and reduced forms are identical.

To account for the simplicity of the conceptual approach used to gain this information, let us consider the relevant cyclic voltammetric behaviour reported in Fig. 3.

At low temperature (−35°), a one-electron anodic peak was observed, with which only one cathodic peak was associated (full line). This redox system was found to be quite reversible, indicating that under the experimental conditions the oxidation product was the isostructural fac-Mn(II) complex, sufficiently stable not to decompose during the time-span of the voltammetric experiment at this temperature.

When the temperature was raised to 22° the cathodic peak was decreased but at the same time a new cathodic–anodic system located at less positive potentials appeared and increased with temperature (dashed line in Fig. 3). This behaviour showed that the stability of the fac-Mn(II) complex is lost at room temperature; under these conditions the charge-

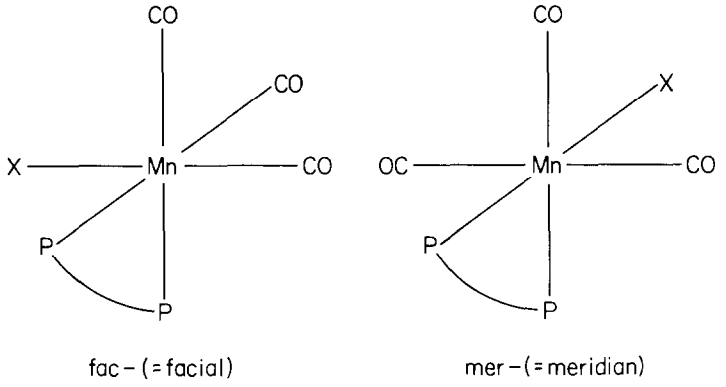


Fig. 2. Isomeric forms for the octahedral manganese complexes with the ligand set $(CO)_3$ (dppm)X.

transfer step is apparently followed by a chemical reaction (E.C. process) which has to be a fac/mer isomerization since it appears to involve no ligand-loss. The new redox system which became evident at room temperature also exhibited reversible character, suggesting the same structural features for the redox partners [mer-Mn(II) + $e^- \leftrightarrows$ mer-Mn(I)]. Finally, by controlled-potential electrolyses it was possible to confirm all these findings, as well as to check the thermal instability of the mer-Mn(I) species.

The correctness of all these conclusions was then confirmed by infrared and e.p.r. spectroscopy. A comparison between the two approaches shows that the electroanalytical method provides the desired information in a simpler and more straightforward way.

Another example is a voltammetric and chronoamperometric study of nickel halide phosphine complexes of the type $[Ni^{II}(PPh_3)_2X_2]$ (PPh_3 = triphenylphosphine; X = Cl, Br).[16]

The conclusions drawn were based not only on the degree of reversibility of the processes involved, but also on the kinetic character of the relevant responses. It was found that such nickel(II) complexes undergo the following ligand-disproportionation equilibria in polar solvents:

$$2[Ni^{II}(PPh_3)_2X_2] \rightleftarrows [Ni^{II}(PPh_3)_3X]^+ + [Ni^{II}(PPh_3)X_3]^- \quad (10)$$

$$3[Ni^{II}(PPh_3)X_3]^- \rightleftarrows [Ni^{II}(PPh_3)_3X]^+ + 2[Ni^{II}X_4]^{2-} \quad (11)$$

the first being almost completely shifted towards the right-hand side, and the second occurring quite

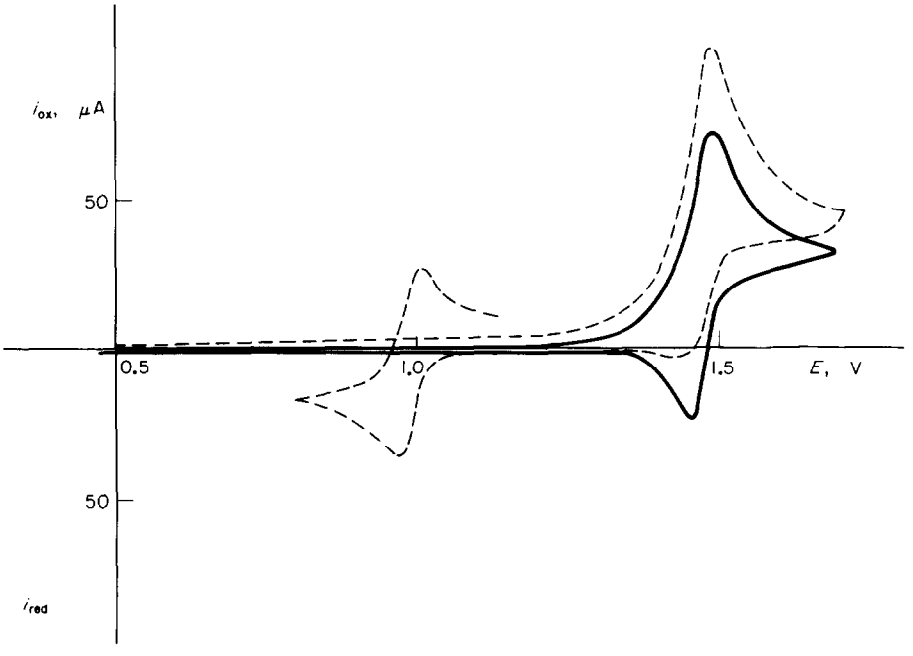


Fig. 3. Cyclic voltammetric curves recorded with a platinum microelectrode for a CH_3CN solution containing fac-$[Mn^I(CO)_3(dppm)X]$ ($2 \times 10^{-3}M$) and $[NBu_4^+][ClO_4^-]$ (0.1M); (——) at −35°C; (– – – –) at 22°C. Scan-rate: 0.5 V/sec.

slowly. Moreover, two different nickel(I) reduction products were found, in equilibrium with each other according to the equation

$$[Ni^{I}(PPh_3)_4]^+ + X^- \rightleftarrows [Ni^{I}(PPh_3)_3X] + PPh_3 \quad (12)$$

This ligand-exchange reaction was also quite slow.

Another electroanalytical investigation on nickel complexes with a series of phosphine ligands[17] by a combination of voltammetric and spectrophotometric measurements made it possible to state that four phosphine molecules could enter the co-ordination sphere of nickel(II) only if they were small; at the most two molecules of the bulkier phosphines could be co-ordinated to the nickel(II). Voltammetric work has also shown the existence of stable nickel(I) complexes with all the phosphine ligands used. The geometric configuration of these previously unreported nickel(I) complexes was inferred from the degree of reversibility of their electrode processes.

ELECTROCHEMICAL PREPARATIVE PROCEDURES

Another useful application involves the use of electroanalytical techniques to optimize the conditions for electrochemical synthesis. Such optimization requires a profound knowledge of the reaction mechanism involved, so a new electrochemical synthesis should be always preceded by a careful electroanalytical investigation.

Most electrochemical syntheses established in this way are competitive with those done by the usual chemical methods, but sometimes they are the only known preparative procedures.

It should be pointed out here that the electrochemical methods have the advantage of giving purer products than chemical methods can, because of the absence of by-products due to the use of chemical reactants. Moreover, higher selectivity can be achieved by control of parameters such as the working potential.

For example, consider the synthesis of rhodium and iridium organometallic derivatives of the type $[M^{I}(R)(CO)(PPh_3)_2]$ (M = Rh, Ir; R = alkyl, aryl; PPh_3 = triphenylphosphine).[18]

Their chemical preparation is very difficult since rather unstable (and hence hard to handle) organolithium and organomagnesium species are used as reducing agents, and a tedious purification procedure is required to separate the oxidation products of such reagents from the desired compounds.[19,20] These derivatives can be easily prepared, however, by the electrochemical route:

$$\begin{array}{l} [M^{I}(CO)(PPh_3)_2Cl] + RX \longrightarrow [M^{III}(R)(CO)(PPh_3)_2ClX] \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \Big\downarrow +2e^- \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad [M^{I}(R)(CO)(PPh_3)_2] + Cl^- + X^- \end{array} \quad (13)$$

The products are synthesized by cathodic reduction of M(III) species, which are obtained *in situ* by simple oxidative addition of the appropriate organic halide (RX) to the corresponding M(I)–chloride complex.

Alternatively, if the organic halide has poor reactivity in the oxidative addition step, the steps in scheme (13) can be transposed. That is, the oxidation state of the metal in the starting material is first lowered to −1, so that it becomes more prone to undergo oxidative addition of RX, according to the scheme:

$$\begin{array}{l} [M^{I}(CO)(PPh_3)_2Cl] + PPh_3 + 2e^- \longrightarrow [M^{-I}(CO)(PPh_3)_3]^- + Cl^- \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \Big\downarrow +RX \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad [M^{I}(R)(CO)(PPh_3)_2] + X^- + PPh_3 \end{array} \quad (14)$$

When these preparative methods are used, there is no problem in recovering the products, since they are not soluble in the solvent employed (acetonitrile). Of course, a detailed electroanalytical investigation able to give complete insight into the processes involved was necessary for designing the procedures.

The synthesis of the tetrakis(triethylphosphite)cobalt(0) complex is an example of an electrochemical procedure representing the sole available preparative method.

This compound has been obtained[21] through the following sequence of anodic and cathodic processes, in the first of which a cobalt(I)–phosphite complex is generated by oxidation of metallic cobalt in the presence of the phosphite ligand:

$$\begin{array}{l} Co_{(s)} + 5P(OEt)_3 - e^- \longrightarrow (Co^{I}[P(OEt)_3]_5)^+ \\ (Co^{I}[P(OEt)_3]_5)^+ + 2e^- \longrightarrow (Co^{-I}[P(OEt)_3]_4)^- + P(OEt)_3 \\ (Co^{-I}[P(OEt)_3]_4)^- - e^- \longrightarrow (Co^{0}[P(OEt)_3]_4) \end{array} \quad (15)$$

A facile recovery of the product is again possible owing to its low solubility in the acetonitrile medium.

All the electroanalytical studies in this field indicate that electrochemical preparations can be performed by following the two basic reaction pathways summarized in the scheme:

$$M_{(s)} - ne^- \longrightarrow M^{n+} \begin{cases} \xrightarrow[-ne^-]{+mL} M^0L_m & (a) \\ \xrightarrow[+mL]{} M^nL_m^{n+} & (b) \end{cases} \quad (16)$$

The route is chosen according to the oxidation state desired for the metal in the co-ordination compound synthesized.

In both cases the metal is anodically dissolved, thus yielding the corresponding cation. In route (*a*), the cation is returned to the metallic state by cathodic reduction in the presence of the appropriate ligand. In this way the "bare" metal atoms generated at the electrode surface are trapped by the ligand before the metallic lattice has time to grow.

In route (*b*), on the contrary, the electrogenerated cation reacts directly with the ligand. Of course, if the oxidation state for the metal in the desired co-ordination compound is different from that exhibited by the electrogenerated cation, the complex first obtained can be further electrochemically oxidized or reduced.

Route (*b*) is used, for instance, in the above-mentioned synthesis of tetrakis(triethylphosphite)cobalt(0), and route (*a*) in the synthesis of the catalytically active cyclo-octatetraene nickel(0) complex.[22]

STABILITY OF "INTERMEDIATE" OXIDATION STATES

This topic is of considerable importance in co-ordination chemistry. It is known that metals in high oxidation states give stable complexes with strongly basic ligands (*i.e.*, σ-donors), and that the lowest oxidation states are stabilized by ligands able to promote back-donation of charge (π-acceptor ligands), but a considerable uncertainty exists about the behaviour of the so-called "intermediate" oxidation states.

An unstable intermediate oxidation state will undergo disproportionation reactions. On the other hand, a stable intermediate oxidation state can be formed by interaction of higher and lower oxidation states. Therefore, electroanalytical techniques appear to be valuable tools for gaining information on this aspect of co-ordination chemistry, thanks to their ability to detect such chemical reactions when they are coupled with charge-transfer steps, and to predict these reactions on the basis of the redox potentials.

In spite of this ability, however, most of the electroanalytical investigations on intermediate oxidation states appear to be concerned with determining the electronic configurations[23] (that is, the molecular orbital occupied by the electron added or removed in the charge-transfer reaction, and the relevant spin state). Very few studies dealing with the stability of an intermediate oxidation state towards disproportionation have been reported. Amongst them, however, we may mention an extensive study concerning the stability of nickel(I) complexes in the presence of substituted phosphorus ligands.[17]

The relevant findings point out that the +1 oxidation state for nickel is stabilized relative to the +2 and 0 states only when ligands with properly balanced σ-donor/π-acceptor properties are present in the co-ordination sphere of the metal. Thus, nickel(I) is fully destabilized when phosphite ligands (π-acceptor ability much higher than σ-donor) are present, whereas the existence of stable nickel(I) complexes is made possible by the presence of phosphines, which have more suitable electronic features. Moreover, on the basis of the thermodynamic data obtained, it was possible to estimate the relevant disproportionation constants as well as to account for the failure of reported attempts to prepare phosphite–nickel(I) complexes.[24]

The already mentioned electroanalytical investigations leading to the synthesis of the tetrakis(triethylphosphite)cobalt(0) complex,[21] constitute another example.

CONCLUSIONS

The investigations cited above represent only a fraction of the reported electroanalytical studies concerned with co-ordination compounds, but this survey was not intended as a complete review of the field, but only to indicate what chemical information can be obtained by such an approach.

We think that the survey has shown that many electrode reactions fall into the class of weakly interacting systems in which the electrochemically generated intermediates and/or products diffuse into solution, where they display their inherent chemical properties. Consequently, electroanalytical techniques are able to provide thermodynamic and kinetic information concerning the species participating in the reaction and in this way give new insight into chemical properties.

Finally, it should be emphasized that the growing interest in the application of these methods to chemical and physicochemical characterization of co-ordination compounds, clearly apparent in the literature, is promoting progressive extension of the range of information which they are able to provide. It is this increase in information which is making the electroanalytical approach more competitive with the usual investigation methods such as the various types of spectroscopy.

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SHORT COMMUNICATIONS

HOMOGENEOUS ENZYMATIC FLUORESCENCE IMMUNOASSAY OF SERUM IgG BY CONTINUOUS FLOW-INJECTION ANALYSIS

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Summary—A new technique for automated homogeneous immunoassay has been developed and applied to the determination of serum IgG. An enzyme label, horseradish peroxidase (HRP), conjugated to the antibody (anti-human IgG) was inhibited on immunochemical association. The inhibition of activity was monitored as a decrease in the laser-induced fluorescence of dichlorofluorescein, produced by the HRP-catalysed oxidation of leuco-diacetyldichlorofluorescein by hydrogen peroxide. The entire procedure was performed by flow-injection analysis at a rate of 60 samples per hour. Serum IgG concentrations from 1.4 to 25 mg/ml could be determined after a 1:700 dilution, with a within-run precision of ±9.8%.

Automation in analytical chemistry has greatly benefited from the introduction and development of flow-injection analysis (FIA),[1–5] and recent reviews[6–9] of the theory, methodology and applications of this technique have emphasised its ease of use and versatility. In addition, competitive protein-binding techniques have added a new dimension to clinical chemical analysis. The use of immunochemical reactions has resulted in significant improvements in sensitivity, speed and specificity of measurements.

Immunoassays generally involve the measurement of a label molecule chemically bound to the antigen or antibody of interest. By monitoring of the tag and its changes in location and/or properties, the extent of reaction may be quantified. Homogeneous immunoassays are emerging as preferred methods.[10] Here, binding of the label alters the properties used for monitoring, in such a way that physical separation of the bound and free fractions of the labelled reactant (which is required in heterogeneous methods) is unnecessary. As a result, homogeneous methods are generally simpler and faster than the corresponding heterogeneous immunoassays.

Many analytical techniques (*e.g.*, radiochemistry, fluorescence spectrometry, nephelometry, enzyme analysis and electrochemistry) may be used to monitor immunochemical reactions.[11] Radioactive techniques are most common and provide the most sensitive immunoassay procedures. Radioimmunoassays, however, are heterogeneous, time-consuming, expensive, and potentially hazardous. Fluorescence and enzymatic methods offer a wide variety of rapid, inexpensive, and sensitive techniques that can be either heterogeneous or homogeneous.[10,12]

In spite of recent improvements in homogeneous immunoassay techniques, many methods are still time-consuming and may require relatively large quantities of expensive reagents. The successful application of continuous-flow FIA to immunoassays would allow rapid measurements of microsamples with minimal consumption of reagents. Lim *et al.*[13] recently described the FIA determination of albumin by use of energy-transfer immunoassay[14] for fluorescence detection. It was necessary to employ a stopped-flow step in the measurements.

In this paper we introduce a new homogeneous immunoassay system that utilizes horseradish peroxidase (HRP) as the antibody label, and the first application of continuous-flow FIA for measurement of an immunochemical system. The enzyme catalyses a recently developed indicator reaction[15] in which oxidation of leuco-diacetyldichlorofluorescein (LDADCF) by hydrogen peroxide in the presence of HRP produces the highly fluorescent dichlorofluorescein (DCF). The binding of the labelled antibody to the antigen, IgG in this case, partially inhibits the enzyme activity, resulting in a decrease in the fluorescence intensity produced by the indicator reaction. A dual-channel, dual-reagent FIA system automatically performs the appropriate sequence of reactions before the detection by a laser filter fluorimeter with a sheath flow-cell.[15] Measurements are made at a rate of 60 samples per hour.

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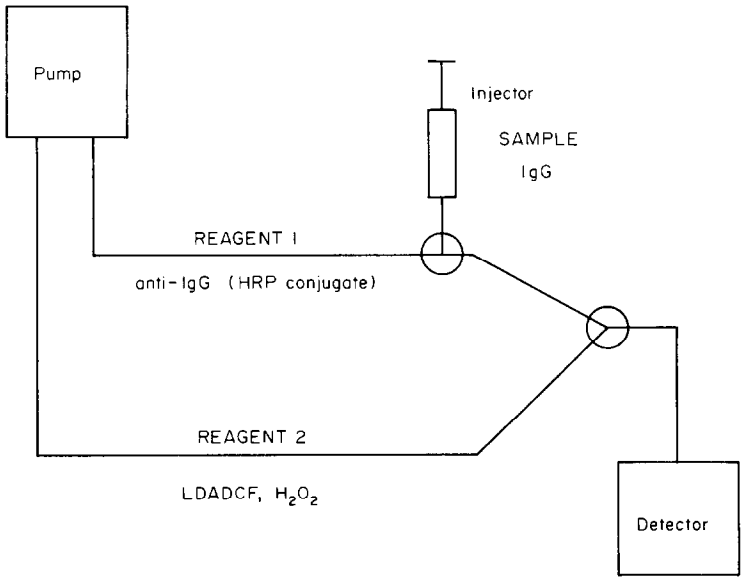

Fig. 1. Dual-reagent flow-injection analysis system.

EXPERIMENTAL

To study the binding equilibria of the antigen–antibody reaction and the activity of the conjugated enzyme, a static spectrofluorimeter (Perkin–Elmer 650–10S) was used, as described in previous studies.[15] Phosphate-buffered saline (PBS) solution (0.02*M* phosphate, 0.15*M* sodium chloride, 1% PEG-6000 and pH 7.4, boiled to prevent bacterial degradation of the proteins) was used to make all the solutions and dilutions. Successive additions of reagents or sample were made as follows, with the resultant change in fluorescence intensity monitored for each step. Step (*a*) was the addition of 2.8 ml of human IgG solution (25 μg/ml, Sigma Chem. Co. I-4506) to the cell. Next, in (*b*), various volumes of either HRP (35 IU/ml) or anti-human IgG conjugated with HRP (Miles-Yeda, Ltd. 61–130; 1:20 dilution) were added. After various periods of time (from 30 sec to 6 min), 50 μl of activated LDADCF ($10^{-5}M$) were added in step (*c*). Finally, in step (*d*), 50 μl of hydrogen peroxide ($10^{-5}M$) were added to obtain the final indicating response. Except for step (*c*), the interval between addition and subsequent fluorescence monitoring was 30 sec.

For the flow system in this study (Fig. 1), Teflon tubing (0.8 mm bore, 1.0 m long) with a 25-μl loop injector was used.[15] A dual-reagent system was incorporated. One line carried the antibody through the injector, where the antigen (standard or sample) was injected for binding to occur, before mixing in the second reagent line with the dye and hydrogen peroxide for the fluorescence indicator reaction (at a point half-way to the detector). The first reagent was a 1:250 dilution of anti-human IgG(goat) conjugated with HRP. The second reagent was $10^{-5}M$ LDADCF, activated with dilute base,[15] and hydrogen peroxide ($10^{-4}M$).

Aqueous standards were prepared with purified crystalline human IgG at appropriate concentrations. For accuracy, recovery, and comparison studies, serum samples (already analysed by radial immunodiffusion, from Calbiochem, Tri-Partigen) were diluted 700-fold with the PBS reagent before injection. By adjustment of the stream pressures to 0.36 and 0.32 atm for the streams of reagent 1 and reagent 2, respectively (reagent 2 and the sheath-stream for the detector[15] were driven by the same pressure), flow-rates of 0.50 ml/min for both reagent streams and 1.60 ml/min for the detector sheath-stream were obtained.

The fluorimeter instrumentation was used as previously described.[15] Fluorescence was excited by an argon-ion laser at 488 nm, and the emission intensity was monitored at 525 nm. The emitted light was passed through a 520-nm cut-off antifluorescent filter to remove scattered radiation.

RESULTS AND DISCUSSION

Reaction conditions

The following reaction sequence was employed:

$$\mathrm{Ag} + \mathrm{Ab\text{–}HRP} \rightleftharpoons \mathrm{Ag \cdot Ab\text{–}HRP} \quad (1)$$

$$\mathrm{LDADCF} + \mathrm{H_2O_2} \overset{\text{Ab HRP}}{\rightleftharpoons} \mathrm{DCF} + 2\mathrm{H_2O} \quad (2)$$

where Ag represents the antigen and Ab–HRP the antibody–HRP conjugate. The two reactions were evaluated initially with a static spectrofluorimeter before use in the FIA system. The particular conditions used were those generally established as optimal for immunochemical reactions,[16] and worked well for the indicator system employed. The antigen and antibody dilutions were made according to the general recommendations of the manufacturers. Enzyme activities of the free HRP solutions were adjusted to reproduce the activities quoted for the conjugated enzyme.

Response behaviour: precipitin curve

Several specific features were observed in the response behaviour. In the absence of IgG, the activities of the free and conjugated enzymes were equal. When IgG was present, binding to the antibody was found to inhibit the activity of the conjugated enzyme. The extent of inhibition varied with the concentration ratio of antigen to antibody. The inhibition served as

a means of obtaining a "precipitin curve" (Fig. 2) indicative of the binding equilibrium and stoichiometry of this immunochemical reaction. The binding observed here was association and aggregation between free antigen and antibody molecules, with no significant precipitation, because it took place in less than the 30 sec necessary to measure the reaction. The inhibition of fluorescence under these conditions makes possible the successful use of continuous-flow FIA. Longer times (up to 6 min) were found to cause little enhancement of the signals (less than 10%). The precipitin curve was obtained by subtracting the signals of HRP-conjugated antibody bound to antigen from the signal for free HRP alone. The maximum of the precipitin curve occurred at an HRP concentration of 0.56 IU/ml, corresponding to a 1250-fold dilution of the antibody, and an IgG concentration of 25 μg/ml or a 700-fold dilution of serum containing high IgG levels. These concentrations were used as a guide to the conditions for use with the flow system. They do not necessarily represent the optimal conditions for FIA, because there is greater dilution of the reagent by the sample.[15]

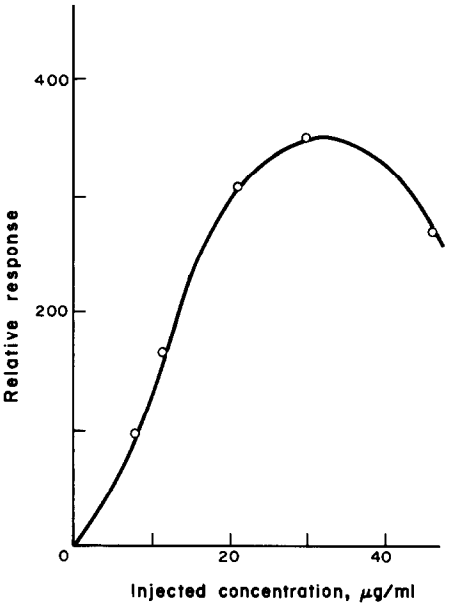

Fig. 3. Relationship of fluorescence response to concentration of IgG serum samples.

Analytical range

Since the conditions chosen for FIA correspond to the maximal inhibition of HRP activity for high IgG levels, the entire normal range of serum IgG levels (8–14 mg/ml, or 11–20 μg/ml after dilution) should fall within the region of the precipitin curve in which the antibody is in excess, and allow for straightforward quantification. Pathologically elevated IgG levels would correspond to the region of the precipitin curve where the antigen is in excess and would give rise to signals that could be misinterpreted as corresponding to an antigen level much lower than the true value, and to resolve this possible ambiguity the sample would have to be further diluted and re-analysed. A maximum response was observed at 31 μg/ml IgG (Fig. 3). Calibration was possible over the range 2–30 μg/ml. The concentrations listed correspond to those of the 1:700 diluted IgG serum samples which were injected into the flowing stream. The minimum detectable IgG concentration was 2 μg/ml ($1.25 \times 10^{-8} M$ IgG, or 50 ng in the 25-μl sample injected, corresponding to 1.4 mg/ml in the undiluted serum.

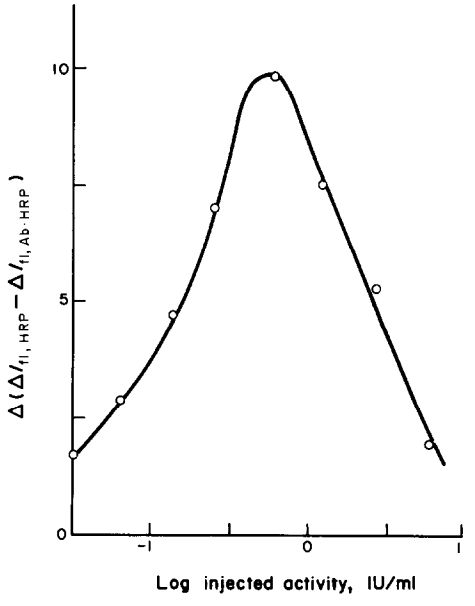

Fig. 2. Fluorescence intensity change with HRP activity. The response is the difference between the signals obtained with free HRP and with HRP conjugated to bound antibody, corresponding to a precipitin curve.

Accuracy and precision

To determine the accuracy of the assay system, recovery and comparison studies were performed. Twelve serum samples analysed by radial immunodiffusion (Tri-Partigen from Calbiochem) were analysed in triplicate, with use of a calibration curve made with aqueous standard. The average serum IgG response was 96.7% of the corresponding standard response. An average recovery of 103% from serum spiked with IgG was observed.

The flow system exhibited a within-run precision of ±9.8% for the determinations run at a rate of 60 samples per hour. The precision was adversely affected by a back-pressure developed during the sample injection. This was perhaps compounded by the viscosity of Reagent 1 being higher than that of Reagent 2, because of the presence of the PEG-6000. The PEG-6000 was necessary to minimize blank readings in the indicator reaction,[11] and this surfactant is also used to accelerate antigen–antibody complexation.[17]

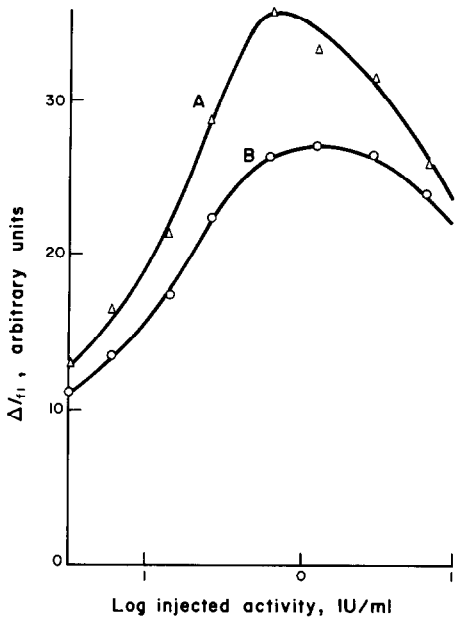

Fig. 4.

Either a by-pass stream for injection or a modified pumping system should result in significantly improved precision. Also, introduction of PEG into both streams might serve to equalize the viscosities and result in somewhat improved precision.

Time of reaction

At the pressures used, it took the sample plug 45 sec to reach the detector. Half that time was spent in the 0.5 m length of tubing before the second reagent stream was introduced. The last 0.5 m of tubing after the T-connector and before the detector gave time for the indicator reaction to take place.

Reagent consumption

Because of the rapid sequential injection of samples, reagent consumption per sample was small, about 500 μl of each reagent, amounting to a total cost of less than 8 cents per analysis. By use of the merging-zone technique,[13,18,19] this could be further reduced.

The system described here gives considerable improvement in analysis time and sensitivity, because of the choice of reaction sequence . This is apparently the first example of a non-competitive system for antigen assay, in which enzyme conjugated to the antibody is inhibited upon immunochemical reaction, opening a new area for homogeneous immunoassays.

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DETERMINATION OF INDIUM IN ALUMINIUM ALLOYS BY FLAME ATOMIC-ABSORPTION SPECTROSCOPY AND FLAME ATOMIC-EMISSION SPECTROMETRY*

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Summary—The use of flame atomic-absorption and atomic-emission spectrometry for the determination of indium in aluminium alloys is described. Two types of flame are used: air–acetylene and nitrous oxide–acetylene. The effect of other ions, especially aluminium, has been studied, and the use of lanthanum as a releasing agent is proposed for both techniques, the amount used depending on the amount of aluminium present.

Since 1926, when Paez introduced sodium metal to obtain a finer grain in alloy castings, and the consequent creation of ALPAX, various elements have been added to aluminium to try to improve its properties for specific industrial purposes.[1] Indium is a very interesting element for the metallurgical industry, owing to its high plasticity, low melting point, relative stability and low toxicity. Its recent use in aluminium alloys, to take advantage of its lubricating action, its capacity to withstand the acids produced by the oxidation of lubricating oils, and its effect of lowering the melting point in welding and increasing the corrosion resistance of alloys, unfolds a promising future for this element in such alloys. Hence it is important to establish methods for determination of indium in these alloys, usually at the trace level. In such investigations the effect of the matrix elements, in particular aluminium, must be examined. The recentness of this exploitation of indium explains the paucity of references to its determination in aluminium alloys, and high-purity aluminium.[2]

As part of a line of research on the analysis of aluminium alloys,[3] this paper describes two methods for the determination of indium, by absorption and mination in aluminium alloys, and high-purity aluminium.[2]

*Taken in part from the Ph.D. work of A. J. Aller.

EXPERIMENTAL

Apparatus

A double-beam Pye-Unicam SP-1900 atomic-absorption spectrometer was used for both methods, with an indium hollow-cathode lamp. Table 1 lists the operating conditions.

Reagents

Standard indium solution. Dissolve 1 g of indium in 6*M* hydrochloric acid and dilute to 1 litre with distilled water.

Table 1. Operating conditions for the determination of indium by flame atomic-absorption and atomic-emission spectrometry

	Atomic absorption		Atomic emission	
	air–C_2H_2	N_2O–C_2H_2	air–C_2H_2	N_2O–C_2H_2
C_2H_2 flow-rate, *l./min*	0.8	4.2	0.8	4.4
Support gas flow-rate, *l./min*	5	5	5	5
Burner slot, *cm*	10	5	Méker type (13 small holes)	5
Burner slot-width, *mm*	0.7	0.7	—	0.7
Lamp current, *mA*	5	5	—	—
Wavelength, *nm*	303.97	303.97	451.13	451.13
Slit, *mm*	0.2	0.2	0.15	0.20

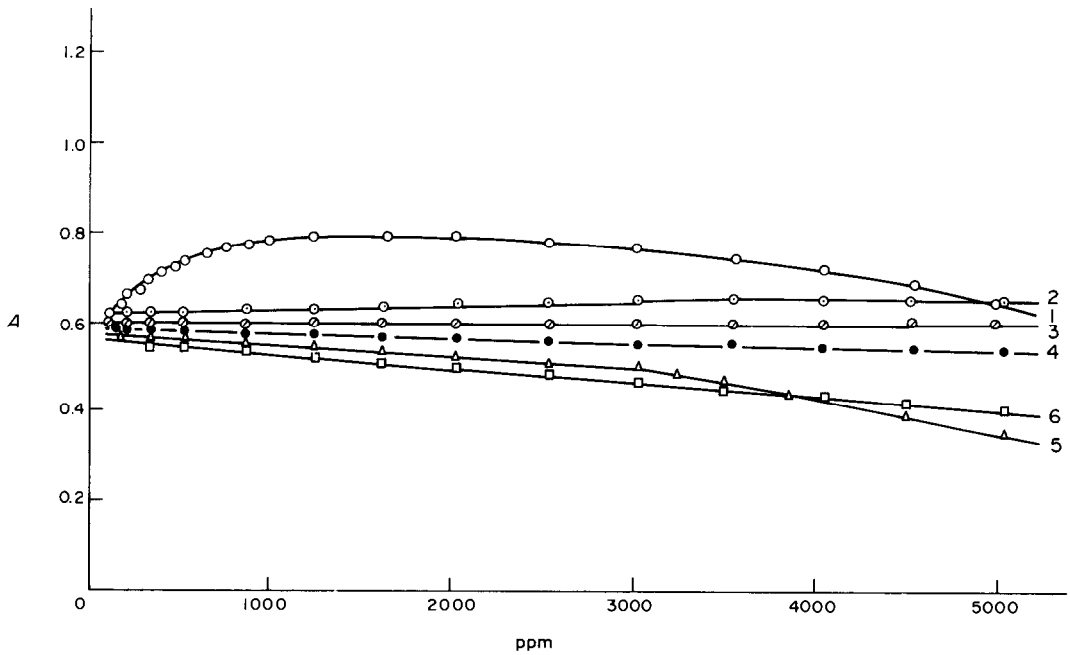

Fig. 1. Effect of various elements on the AAS signal from 5 ppm of indium, in an air–acetylene flame. 1, O——O Ca^{2+}; 2, ⊙——⊙ Bi^{3+}; 3, ⊘——⊘ Na^{+}, Sr^{2+} and La^{3+}; 4, ●——● Al^{3+}; 5, △——△ Co^{2+}, Mg^{2+}, Ni^{2+}, Fe^{3+}, Mn^{2+}, As^{3+} and Sb^{3+}; 6, □——□ V (V), W (VI), PO_4^{3-}, Cr^{3+}, Ti^{4+} and Mo (VI).

Aluminium solution. Dissolve 10 g of aluminium in 6*M* hydrochloric acid and dilute to 1 litre with distilled water.

For study of interferences, commercial 5000-ppm standard solutions for atomic-absorption spectrometry (AAS) were used.

Procedure

Dissolve the sample [large enough to give a final indium concentration of 3–5 ppm, but not less than 0.5 g (to avoid problems of inhomogeneity)] in 15 ml of 6*M* hydrochloric acid, heating slightly if necessary. Add the necessary amount of interference corrector according to the method to be used and the aluminium content expected. Dilute to 100 ml with distilled water and determine the indium content, using the conditions given in Table 1.

RESULTS AND DISCUSSION

Effect of foreign ions

Atomic-absorption spectroscopy. Solutions of composition similar to that of the sample to be studied were prepared, all with an aluminium content of 5000 ppm, and the concentrations of other ions varied from 1 to 5000 ppm, first individually and then jointly (provided that they were chemically compatible), and the difference between the indium concentration taken (1–10 ppm) and that found by AAS was investigated. Figure 1 shows the results for 5 ppm of indium.

The following effects were observed for the air–acetylene flame. Calcium gave non-linear enhancement by up to 20%, possibly by initial suppression of ionization, offset at higher concentration levels by formation of thermally stable compounds. Bismuth had a weak enhancing effect (5% at 5000 ppm) perhaps of excitation type in cases of maximum content. Sodium, strontium and lanthanum had no effect, but aluminium had a weak depressive effect (6% at 5000 ppm), probably due to formation of thermally stable compounds in the flame. There was a medium depressive effect by Co^{2+}, Mg^{2+}, Ni^{2+}, Fe^{3+}, Mn^{2+}, As^{3+} and Sb^{3+} (16% at 5000 ppm), again presumably by stable-compound formation. A strong depressive effect was given by V(V), W(VI), PO_4^{3-}, Cr^{3+}, Ti^{4+} and Mo(VI) (30% at 5000 ppm) through formation of refractory compounds.

However, these interferences were only serious for higher concentrations of the interferents, and at the usual concentration range of these elements in aluminium alloys, the effect is generally negligible (< 3%), except for that of calcium.

When several of these elements are added jointly the effect is similar, but much more difficult to predict.

In the nitrous oxide–acetylene flame the effect of all these ions is generally less marked, which seems to confirm that most of the interferences are due to formation of thermally stable indium compounds; aluminium gives about 2% depression of the signal. There is practically no influence by up to 5000 ppm of Sn^{2+}, Fe^{3+}, Cd^{2+}, Zn^{2+}, Hg^{2+}, Te(VI), Ce^{3+}, Ba^{2+}, Na^{+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Bi^{3+}, Cr^{3+}, As^{3+}, Tl^{+}, Li^{+}, Mo(VI), V(V), La^{3+}, PO_4^{3-}, Ti^{4+}, Sb^{3+}, Zr^{4+}, Ca^{2+}, Pb^{2+}, Ni^{2+}, Sr^{2+}, Mn^{2+} and W(VI).

In both types of flame weaker effects are observed for low indium concentrations, and this should be taken into account for sampling.

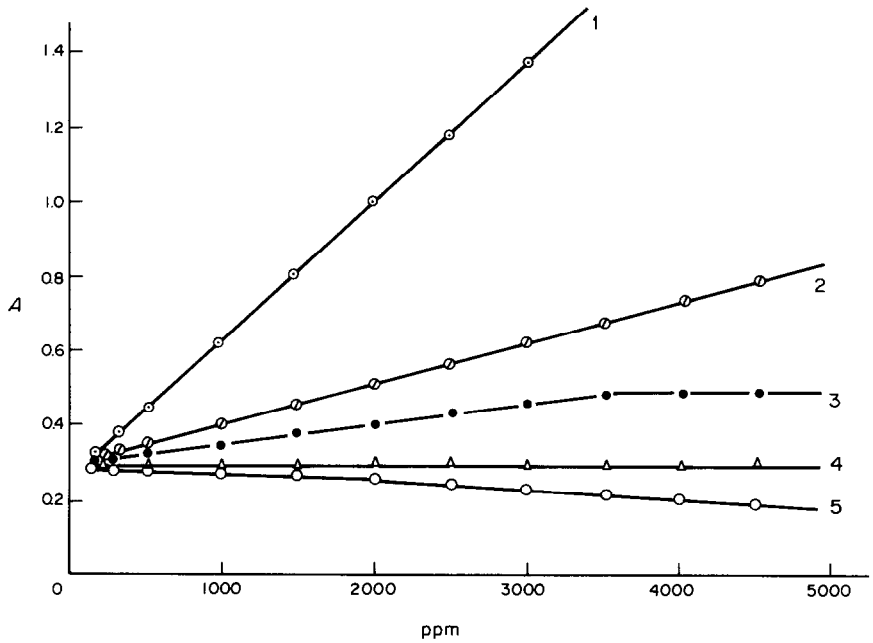

Fig. 2. Effect of various elements on the flame emission signal from 5 ppm of indium in an air–acetylene flame. 1, ⊙——⊙ W(VI), V(V), Mo(VI), La^{3+} and Ti^{4+}; 2, ∅——∅ Ce^{3+}, Sr^{2+}, Zr^{4+}, Al^{3+} and PO_4^{3-}; 3, ●——● Ca^{2+} and Na^{+}; 4, △——△ Li^{+}, Bi^{3+}, Cd^{2+}, Te^{4+}, Tl^{+}, Ni^{2+}, Fe^{3+}, Zn^{2+}, Pb^{2+} Co^{2+}, Mn^{2+}, Cr^{3+}, Cu^{2+}, As^{3+}, Sb^{3+}, Mg^{2+} and Sn^{2+} 5, O——O Hg^{2+}.

The following methods of correcting interferences were studied: the use of matrix-matched standards; the method of standard additions; the use of releasing agents. The last was found to be the easiest to use. La^{3+}, Sr^{2+} and Ca^{2+} have been used as releasing agents, and the optimum addition ratios (with respect to the aluminium present) were: Al/La = 12/1; Al/Sr = 10/1; Al/Ca = 10/1. The use of lanthanum is recommended.

Atomic-emission spectroscopy. With an air–acetylene flame and experimentation similar to that for the atomic-absorption work the results shown in Fig. 2 were obtained for 5 ppm of indium.

There was strong enhancement by W(VI), V(V), Mo(VI), La^{3+}, Ti^{4+}, Ce^{3+}, Sr^{2+}, Zr^{4+}, Al^{3+} and PO_4^{3-}, moderate enhancement by Ca^{2+} and Na^{+}, no effect by Li^{+}, Bi^{3+}, Cd^{2+}, Te(IV), Tl^{+}, Ni^{2+}, Fe^{3+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, Cu^{2+}, As^{3+}, Sb^{3+}, Mg^{2+} and Sn^{2+}, and a depressive effect by Hg^{2+}.

Again, the levels of these elements in alloy samples would be low enough for their effects to be negligible. It is very difficult to decide the reasons for the various effects, some of which are curious. For example, Na^{+} increases the readings but Li^{+} does not (both should give suppression of ionization). The enhancement observed at high interferent levels may be due to an effect of the concomitant elements on the flame background, combined with the effect of the high concentration of salts in the solution.

With the nitrous oxide–acetylene flame less marked effects are generally observed, but most of the elements cause a slight increase in the indium signal.

Interference correction was examined as before and again the use of releasing agents was considered best. The optimum ratios are Al/La = 12/1, Al/Sr = 10/1 and Al/Ca = 5/1, the use of lanthanum being recommended. We emphasize the peculiarity of the

Table 2. Analytical and statistical characteristics of both techniques

	Atomic absorption		Flame photometry	
	air–C_2H_2	N_2O–C_2H_2	air–C_2H_2	N_2O–C_2H_2
Linear calibration range, *ppm*	1–40	1–30	1–10	1–8
Detection limit, *ppm*	0.7	0.9	0.7	0.4
Sensitivity, *ppm*	0.2	0.4	0.05	0.02
Coefficient of variation, %	0.8	1.5	0.2	0.6

Table 3. Determination of indium in aluminium alloys*

Sample	Certified In content, %	In found, %			
		AAS		AES	
		air–C_2H_2	N_2O–C_2H_2	air–C_2H_2	N_2O–C_2H_2
L-3710	0.028	0.028	0.028	0.027	0.028
L-3721	0.026	0.025	0.024	0.026	0.027
L-3731	0.025	0.026	0.026	0.027	0.025
L-3741	0.028	0.029	0.027	0.028	0.028
L-3751	0.030	0.030	0.031	0.030	0.030

*Courtesy of E.N.D.A.S.A.

counter-interference effect of these pairs of elements which, separately, have an enhancing effect.

Characteristics of the two methods

Table 2 summarizes the main features of both methods, showing the optimum range, detection limit and sensitivity, and a summary of the statistics for ten analyses of an aluminium alloy containing 0.030% of indium.

Application to metallurgical samples

The accuracy of the method was assessed by determination of indium in aluminium alloys (Pechiney). Table 3 shows the results obtained with both techniques, which confirm the validity of the methods.

The two techniques give very similar results, but the analytical and statistical characteristics are better for emission spectrometry. However, in the presence of large amounts of elements other than aluminium, the atomic-absorption method should be preferred.

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FLUORIMETRIC DETERMINATION OF SELENIUM IN SOME MARINE MATERIALS AFTER DIGESTION WITH NITRIC AND PERCHLORIC ACIDS AND CO-PRECIPITATION OF SELENIUM WITH LANTHANUM HYDROXIDE

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Summary—A modified spectrofluorimetric method for the determination of selenium in some marine tissues is described. Selenium is isolated from potentially interfering elements by co-precipitation, then a piazselenol is formed between selenium and 2,3-diaminonaphthalene at pH 1.0 and extracted into cyclohexane. The selenium is determined fluorometrically (λ_{ex}, 377 nm; λ_{em}, 519 nm) in the extract. The detection limit is 3.6 ng (6 times the standard deviation of the blank) and the coefficient of variation is 4% at the 10-ng level.

Of the methods available for the determination of selenium at trace levels in biological tissues, fluorimetry of the selenium–2,3-diaminonaphthalene complex is frequently used and has proved to be sensitive, precise and accurate.[1–3]. Previously reported methods, however, suffer from several limitations. Most lack simplicity of operation, as several transfers of liquid are often required. Moreover, the acidity of the sample digest is usually adjusted by the addition of ammonia solution before the formation of the piazselenol, which leads to variability in the matrix in which the complex is formed and from which it is subsequently extracted for determination.

In the procedure reported here, co-precipitation is used to isolate selenium from possible interferences and to provide, on dissolution, a constant matrix for the formation and extraction of the piazselenol. Also, all operations (sample decomposition, co-precipitation, piazselenol formation and extraction) are performed in the same centrifuge tube to reduce liquid transfers and equipment requirements.

EXPERIMENTAL

Apparatus

All fluorescence measurements were made with a Perkin–Elmer 3000 spectrofluorimeter.

Reagents

Standard selenium solutions. Stock solutions (1000 μg/ml), of selenium(IV) and selenium(VI) were prepared by dissolving analytical-reagent grade selenious acid and sodium selenate respectively in 0.01*M* hydrochloric acid. Aliquots of these solutions were diluted as required with 0.01*M* hydrochloric acid, to give working solutions in the μg/l. range. Organoselenium standards were prepared by dissolving diphenyl diselenide and *N*,*N*-dimethylselenourea in methanol. The solutions were standardized by atomic-absorption spectrometry, by comparison with standards made by dissolving the pure element in hydrochloric acid.

Lanthanum (III) solution. Prepared by dissolving 5 g of lanthanum chloride in 100 ml of distilled water.

2,3-Diaminonaphthalene (DAN) solution. Prepared by dissolving 0.1 g of DAN in 100 ml of 0.1*M* hydrochloric acid containing 0.5 g of hydroxylamine hydrochloride. This solution was purified by heating it at 50° in a water-bath for 25 min and then extracting with three 10-ml portions of cyclohexane; it was then stored at 2°, protected from light. The purified solution was stable for at least 48 hr.

EDTA-hydroxylamine solution. A masking solution was prepared by dissolving 3 g of EDTA (disodium salt) and 5 g of hydroxylamine hydrochloride in 50 ml of distilled water.

Procedure

Samples were freeze-dried and ground (to <200 μm) before analysis. A weighed sample (<0.5 g) was placed in a 30-ml Pyrex centrifuge tube, 5 ml of concentrated nitric acid were added and the mixture was allowed to stand for at least 12 hr at room temperature to ensure complete dissolution (this avoids foaming during heating). The tube was then placed in an aluminium heating block and refluxed until the evolution of brown fumes ceased. The solution was cooled, 2 ml of 72% perchloric acid were added and heating was continued for at least 30 min after the appearance of dense perchloric acid fumes. To convert selenium(VI) into selenium(IV), 1 ml of concentrated hydrochloric acid was added and the digest was heated at 70° for 30 min. Selenium was then isolated by co-precipitation).[4,5] One ml of lanthanum solution and one drop of phenolphthalein indicator were added with stirring, followed by addition of 25% ammonia solution until the solution was pink. The lanthanum precipitate (containing selenium) was separated by centrifugation, then dissolved in 5 ml of DAN solution; after addition of 0.5 ml of masking solution the tube was placed in a water-bath at 50° for 25 min. The piazselenol formed was extracted into 5 ml of cylcohexane by means of a vortex mixer (extraction time 1.5 min) and a portion of the cyclohexane layer was transferred into a 1-cm quartz cuvette. The fluorescence of the extract was

measured at 519 nm after excitation at 377 nm. Calibration graphs of fluorescence intensity *vs.* amount of selenium (0–0.1 μg and 0–1 μg) were prepared by using selenium(VI) standards carried through the entire analytical procedure.

RESULTS AND DISCUSSION

Isolation of selenium and formation of piazselenol

Only selenium(IV) will form the piazselenol.[6] Preliminary experiments also showed that selenium(VI) is not quantitatively co-precipitated with lanthanum hydroxide. Therefore any selenium(VI) initially present or produced during the digestion must be reduced to selenium(IV). The method selected was to heat the digest with hydrochloric acid. The optimum heating time was 20–30 min at 70° and the optimum hydrochloric acid concentration 6–10*M*.

To isolate selenium from other material present, co-precipitation with lanthanum hydroxide was used. Selenium was collected quantitatively only if the pH was adjusted to between 9 and 10.

Dissolution of the precipitate in the DAN and EDTA–hydroxylamine reagents resulted in a solution of pH 1.0. At this pH, the optimum conditions for formation of the piazselenol are heating for 25 min, at 50°.

Sample decomposition

The efficiency of the acid digestion for converting some organic selenium compounds into inorganic selenium was also investigated (Table 1). The recommended decomposition procedure was used, with a spike of selenium compound added to 5 ml of concentrated nitric acid and refluxed before addition of 72% perchloric acid. In both cases the recovery was greater than 97%, showing that this acid mixture should be suitable for converting the organoselenium compounds investigated, if present in marine tissues, into inorganic selenium without serious loss. The high recoveries of inorganic selenium also indicated that loss by volatilization was negligible.

Interferences

A number of ions (Ag^+, Al^{3+}, As^{3+}, Ba^{2+}, Bi^{3+}, Ca^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sb^{3+}, Sn^{4+}, Te^{4+}, Zn^{2+}) were tested and caused no significant interference when present in amounts of up to 1000 μg.

Table 1. Recovery of selenium from inorganic and organic selenium compounds after acid digestion

Compound*	Recovery, %†
Se(IV)	98.6 ± 1.1
Se(VI)	98.8 ± 0.8
$C_{12}H_{10}Se_2$	97.2 ± 1.5
$(CH_3)_2NCSeNH_2$	97.7 ± 2.3

*Amount taken was equivalent to 0.5 μg of selenium.
†Means of four replicates.

Table 2. Recovery of selenium added to selected biological tissues

Sample	Selenium, *ng* Added	Found*	Recovery, %
Macroalga			
lobospira bicuspida	0	69 ± 2	—
(0.5 g)	50	116 ± 2	97
	100	167 ± 4	99
Pisce			
Sillaginodes punctatulus	0	76 ± 2	—
(0.2 g)	50	127 ± 3	101
	100	173 ± 4	98
Scallop			
Pecten alba	0	190 ± 3	—
(0.25 g)	200	378 ± 5	97
	400	577 ± 6	98

*Means of 4 analyses.

Accuracy, precision and detection limit

The accuracy of the method was assessed by recovery experiments and the analysis of a standard reference material (Orchard Leaves, NBS SRM 1571). As shown in Table 2, complete recovery of added selenium was obtained within experimental error for the selected marine tissues. The selenium concentration obtained by replicate analysis of the orchard leaves (76 ± 2 ng/g) was in agreement with the certified value (0.08 ± 0.01 μg/g).

The precision was estimated from 5 replicate analyses of a 10-ng Se(VI) standard carried through the entire procedure. The relative standard deviation was 4% (6 determinations).

The standard deviation of the blank (6 determinations) corresponded to 0.6 ng of selenium. The source of the blank (9 ng) was mainly the DAN reagent.

Conclusion

The procedure described allows the determination of down to 10 ng of selenium with a relative standard deviation of less than 4%. The advantage of this procedure is primarily the use of a co-precipitation step to provide a constant matrix for the formation and extraction of the piazselenol. The reduction in equipment and labour required, owing to the elimination of most liquid transfers, also makes the method particularly suitable for use in routine analyses in which selenium is to be determined in large numbers of samples.

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SENSITIVE SPECTROPHOTOMETRIC DETERMINATION OF TRACES OF ZIRCONIUM WITH 2-(6-BROMO-2-BENZOTHIAZOLYLAZO)-5-DIETHYLAMINOPHENOL IN THE PRESENCE OF SODIUM LAURYL SULPHATE

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Summary—A simple and highly sensitive procedure for spectrophotometric determination of zirconium has been developed. At pH 4.6, zirconium reacts with 2-(6-bromo-2-benzothiazolylazo)-5-diethylaminophenol in the presence of sodium lauryl sulphate to form a red-violet complex, which has an absorption maximum at 520 nm. The molar absorptivity at 520 nm is 4.4×10^5 l.mole^{-1}.cm^{-1}. Beer's law is obeyed for 0.25–1.50 μg of zirconium in 25 ml of solution. The method has been used in the determination of zirconium in aluminium alloy and steel samples.

Arsenazo III and some azo-dyes[1] have been used as spectrophotometric reagents for determination of micro amounts of zirconium. Recently, owing to the use of surfactants,[2] the sensitivity of the determination of zirconium has been raised. It has been briefly reported that 2-(6-bromo-2-benzothiazolyazo)-5-diethylaminophenol (Br-BTAE) forms a violet-red complex with cadmium,[3] and we have now found that it can be used as a chromogenic reagent for zirconium. This paper describes the synthesis of the reagent and its use for the spectrophotometric determination of zirconium in aluminium alloy and steel samples in the presence of an anionic surfactant, sodium lauryl sulphate (NaLS).

EXPERIMENTAL

Reagents

Zirconium solution. Dissolve 1.766 g of pure zirconyl chloride octahydrate in 20 ml of hydrochloric acid (1 + 1), and dilute to 500 ml with the same concentration of acid. Standardize the solution by EDTA titration. Dilute the stock solution with 2*M* hydrochloric acid to give a 1.00-μg/ml working standard.

Br-BTAE solution, 1.00mM. Dissolve 0.0405 g of the purified 2-(6-bromo-2-benzothiazolylazo)-5-diethylaminophenol in 100 ml of *N,N*-dimethylformamide (DMF).

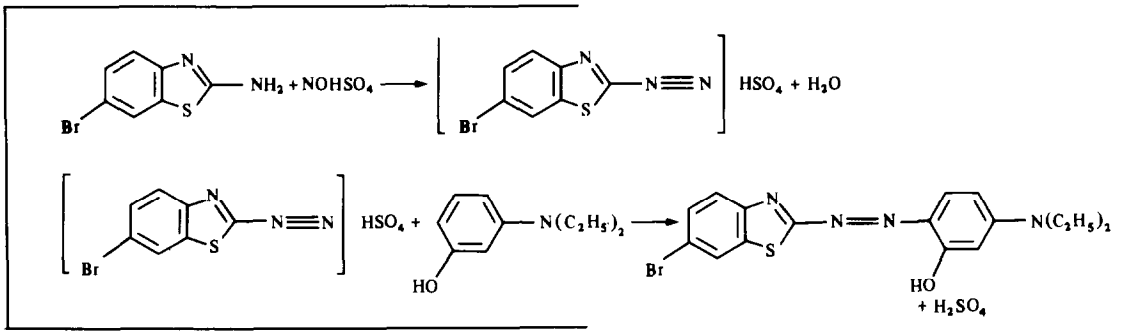
NaLS solution, 1.50%

Sodium acetate buffer (pH 4.6). Dissolve 68 g of sodium acetate trihydrate in water, add 45 ml of glacial acetic acid and dilute to 1000 ml.

Synthesis of Br-BTAE

The synthesis is based on the general procedure for synthesis of benzothiazolylazo dyes.[4] In an acidic solution, nitrosylsulphuric acid is reacted with 6-bromo-2-aminobenzothiazole to produce a diazonium salt, which is then coupled with *m*-diethylaminophenol to form the coloured azo dye.

$$2NaNO_2 + 3H_2SO_4 \rightarrow 2NOHSO_4 + Na_2SO_4 + 2H_2O$$



Sodium nitrite (2.8 g, 0.04 mole) is added portionwise with stirring and cooling to 30 ml of concentrated sulphuric acid and 16 ml of water at 20°. The pale green solution of nitrosylsulphuric acid obtained is cooled to 0–5°, and a solution of 4.6 g (0.0201 mole) of 6-bromo-2-aminobenzothiazole in 30 ml of *N,N*-dimethylformamide is added dropwise at 15–18°.

The mixture is stirred for 2 hr at 0–5°, then 1.6 g (0.016 mole) of sulphamic acid, and 10 min later, 4.6 g (0.0272

mole) of *m*-diethylaminophenol dissolved in 30 ml of *N,N*-dimethylformamide are added at 0–5°. The mixture is stirred for 1 hr at 0–5°, 120 g of sodium acetate in 200 ml of water are added, then the cooling is stopped, and the mixture is stirred for 2 hr and filtered. The product is washed with water and dried, yielding 3 g of red precipitate (36% yield).Recrystallization from *N,N*-dimethylformamide gives a material with m.p. 208.5–209.5°.

Analysis gave C 50.1%, H, 4.2%, N, 13.8%, Br, 20.1%: $C_{17}H_{17}ON_4BrS$ requires C 50.38%, H 4.23%, N, 13.82%, Br 19.71%.

Procedure

Transfer a sample containing not more than 1.5 μg of zirconium into a 50-ml beaker. Add in sequence, with swirling, 1.0 ml of 0.1*M* EDTA, 0.2 ml of Br-BTAE solution, 2.0 ml of NaLS and 1.0 ml of ethanol. Adjust to pH 4.6 with 0.1*M* sodium acetate and 0.1*M* hydrochloric acid, and then add 2.0 ml of sodium acetate buffer (pH 4.6). Transfer the solution into a 25-ml standard flask, dilute to the mark with water and mix well. Measure the absorbance at 520 nm in a 1-cm cell against a reagent blank, after 30 min. Prepare a calibration graph for the range 0.25–1.5 μg of zirconium in 25 ml of final solution.

Analysis of aluminium alloy and steel samples

Weigh a 0.1000-g sample into a 100-ml beaker and dissolve it in 10 ml of 6*M* hydrochloric acid, with heating if necessary. Filter off any residue on a small (9 cm) filter paper, and wash the residue and paper 4 or 5 times with 2*M* hydrochloric acid, collecting the filtrate and washings in a 250-ml standard flask.

Place the residue and paper in a silica crucible and dry and ignite in the usual way. Fuse the residue with a small amount of potassium pyrosulphate and cool. Add 10 ml of 6*M* hydrochloric acid and warm to dissolve. Transfer the solution to the flask containing the original filtrate. Dilute to the mark with 2*M* hydrochloric acid.

Transfer an aliquot containing not more than 1.5 μg of zirconium into a 50-ml beaker, and analyse as already described.

For samples containing a large amount of nickel, extract the nickel with α-furildioxime. For steel samples add acetylacetone to mask Fe(III).

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of Br-BTAE and its zirconium complex at pH 4.6 in the presence of NaLS are shown in Fig. 1. The absorption maximum of the reagent lies in the range 510–540 nm and that of the zirconium complex at 520–530 nm, but the molar absorptivity of the reagent (2×10^4 l.mole^{-1}.cm^{-1}) is much smaller than that of the complex. The absorbance of the reagent blank is stable.

Effect of variables

A study of the effect of pH on the complexation of Br-BTAE with zirconium in the presence of NaLS shows that the coloured complex has constant absorbance in the pH range 1.5–5.5. However, in more acidic solution, the absorbance of the reagent blank is increased.

To decrease the effect of foreign ions, pH 4.6 is preferred. The absorbance values at pH 4.6 of 25 ml of solution containing 1.0 μg of zirconium and increasing amounts of Br-BTAE and NaLS solutions show that maximum absorbance is reached with amounts of the reagents in the ranges 0.17–1.0 and 0.7–3.0 ml respectively. To accelerate the complexation and stabilize the reagent blank, 0.5–1.5 ml of ethanol should be added. Temperature affects the time required for full colour development of the complex. In the temperature range 17–35°, the solution should be kept for 30 min for completion of colour development; the colour intensity remains virtually constant for 2 hr.

Effect of foreign ions

The following ions, when present up to the amounts (in mg) shown in brackets, do not interfere in the determination of 1.0 μg of zirconium: Na^+ (40), K^+ (20), NH_4^+ (20), Ag^+ (0.01), Ca^{2+} (5), Ba^{2+} (5), Mg^{2+} (2), Be^{2+} (1), Pb^{2+} (1), Mn^{2+} (0.1), UO_2^{2+} (0.05), Co^{2+} (0.05), Cu^{2+} (0.02), Zn^{2+} (0.02), Ni^{2+} (0.01), Cd^{2+} (0.01), Al^{3+} (1), Fe^{3+} (0.1), Bi^{3+} (0.1), Cr^{3+} (0.1), La^{3+} (0.01), Ge (IV) (0.1), Sn^{4+} (0.1), Th^{4+} (0.01), Ce^{4+} (0.025), Nb(V) (0.02), Ta(V) (0.02), V(V) (0.01), Mo(VI) (0.05), W(VI) (0.04), Cr(VI) (0.01), Cl^- (50), CH_3COOH^- (50), NO_3- (20), CNS^- (10), ClO_4^- (10), Br^- (2), I^- (1), F^- (0.09), SO_4^{2-} (20), $B_4O_7^{2-}$ (20), $P_2O_7^{2-}$ (10), SiO_3^{2-} (10), $C_2O_4^{2-}$ (10), $S_2O_3^{2-}$ (1), citrate (20), tartrate (20), S^{2-} (0.17), PO_4^{3-} (1), As(V) (0.05). The interference of Ti(IV), (0.02) and Tl(III) (0.01) can be prevented with DCTA and lactic acid respectively.

Under the experimental conditions used, hafnium behaves similarly to zirconium, but the molar absorptivity is higher.

Some transition elements such as Cu, Ni, Fe interfere in the reaction with zirconium, because they react with the reagents used. For example Ni^{2+} reacts with Br-BTAE and NaLS at pH 9.5 (molar absorptivity 2.5×10^5 l.mole^{-1}.cm^{-1}), and even at pH 4.6 nickel

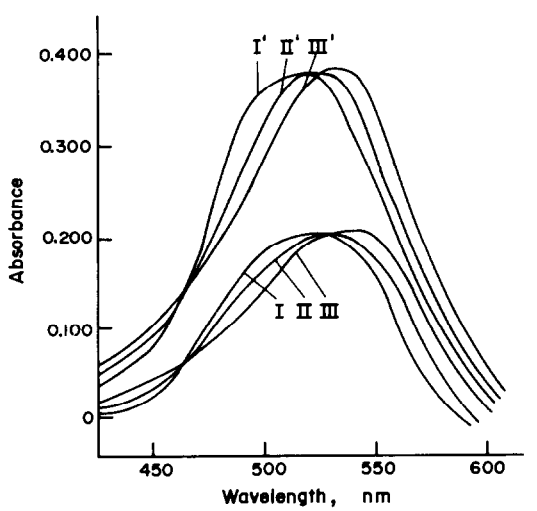


Fig. 1. Absorption spectra at different pH values (1-cm cell). Reagent *vs.* water: I—pH 2.8, II—pH 3.9, III—pH 5.1; Zr-complex *vs.* water: I'–pH 2.8, II'—pH 3.9, III'—pH 5.1.

Table 1. Determination of zirconium in aluminium alloy and steel samples

Sample	General composition, %	Zr, %	
		Present method	Spectrographic method
Aluminium alloy	Cu, 0.0073; Mg, 8.57; Mn, 0.26; Si, 0.17; Fe, 0.092; Ni, 0.093; Sb, 0.025; RE, 0.13	0.13, 0.12, 0.12, 0.11	0.12
Steel 1	Si, 0.52; Mn, 1.37; P, 0.033; S, 0.006; Nb, 0.87; Mo, 0.54; Ti, 0.095; V, 0.097; RE, 0.025	0.098, 0.099, 0.099, 0.098	0.098
Steel 2	C, 0.14; Si, 0.54; S, 0.009; Ti, 0.14; Mn, 0.87; V, 0.36; Nb, 0.034	0.20, 0.19, 0.19, 0.21	0.21
Steel 3	Ti, 0.06; Ta, 0.02; Nb, 0.02; V, 0.05; Co, 0.01; Ni, 0.01; Ce, 0.02	0.049, 0.050, 0.048, 0.052	0.050
Steel 4	Th, 0.05; Mo, 0.1; W, 0.1; Sn, 0.1	0.050, 0.051, 0.049, 0.048	0.050

still causes obvious interference. When the quantity of nickel is too high, its extraction with α-furildioxime is necessary: at pH 4.6, 5 ml of 1% α-furildioxime solution in choloroform will extract 1 mg of nickel.

The interference of Fe(III) can be eliminated with acetylacetone; 1.0 ml of 5% acetylacetone solution will mask 1 mg of Fe(III) under the conditions for determination of zirconium.

Composition of the complex

The Zr:Br-BTAE ratio has been found to be 1:2 by the continuous-variation and the mole-ratio methods, and the ratio Zr:LS 1:2 by the slope-ratio method, so the complex should be Zr (Br-BTAE)$_2$ (LS)$_2$.

Table 2. Sensitivities of methods for the spectrophotometric determination of zirconium

Methods	λ_{max} nm	ϵ, 10^5 l.mole^{-1}.cm^{-1}	Reference
TAM/Zephiramine	595	1.05	2
PADAP/antipyrine	560	1.06	5
3,5-diBr-PADAP/ antipyrine	600	1.35	5
Arsenazo III	665	1.2	6
Chlorophosphonazo III	675	2.1	7
Picramine-ε/ Ethylrhodamine B	560	3.2	8
Br-BTAE/NaLS	520	4.4	Present method

Analysis of samples

Table 1 gives results for some typical samples, showing the method is satisfactory.

Comparison with other zirconium reagents

This is one of the most sensitive reagents for zirconium. The proposed method is simple, rapid and fairly free from interference in the presence of EDTA, lactic acid, DCTA and acetylacetone, or after extraction with α-furildioxime. The sensitivities of various methods are listed in Table 2.

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BIACETYL MONOXIME GLYCINIMINE AS A SELECTIVE REAGENT FOR PALLADIUM AND NICKEL

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Summary—Biacetyl monoxime glycinimine is proposed as a new reagent for the selective gravimetric and extractive photometric determination of Pd(II) and Ni(II). The reagent forms yellow and rose-red water-insoluble complexes with Pd (pH 0.5–5.5) and Ni (pH 5.0–11.2) respectively. The complexes can be used for direct gravimetric determination or extracted with molten naphthalene. The solidified naphthalene–complex mixture is dissolved in chloroform and measured photometrically. The effects of experimental variables and diverse ions are reported. The proposed reagent offers better selectivity than dimethylglyoxime.

Dioximes are one of the most selective groups of reagents. Dimethylglyoxime (DMG) is specific for precipitation of nickel and palladium.[1] There are many observations[2–7] indicating that DMG forms highly stable complexes with all transition metal ions from manganese to zinc, and it is well established that the nitrone form of the oxime is responsible for the complex formation.[8,9] As early as 1927, Pfeiffer *et al.*[10,11] showed that if one of the oxime groups is replaced with an imino or methylimino group, complex formation can occur in the same fashion, but only three such compounds have been reported as analytical reagents. Mashima[12,13] used *N,N'*-ethylene-(4-methoxy-1,2-benzoquinone-1-oxime-2-imine) as an extractive photometric reagent for transition metals. Mathur and Narang[14] used bis(biacetylmonoxime)-*o*-phenylenedi-imine and its ethylenedi-imine analogue as gravimetric reagents for Ni(II) and Pd(II). As a result, it was decided to investigate some imine oximes to determine their reactivity with metal ions.[15,16] This paper presents the results of a study of biacetyl monoxime glycinimine for the separation, and gravimetric and photometric determination of Pd(II) and Ni(II).

The reagent (**I**) precipitates Pd(II) quantitatively as a yellow complex at pH 0.5–5.5, and Ni(II) as a rose-red complex at pH 5.0–11.2. The complexes have composition $M(C_6N_2O_3H_9)_2$ and after drying for 2 hr at 110–120° can be weighed for determination of Pd(II) and Ni(II).

EXPERIMENTAL

Reagents

Biacetyl monoxime glycinimine was synthesized by heating equimolar solutions of biacetyl monoxime and glycine on a boiling water-bath for 3 hr. The crude sample was recrystallized from ethanol until the m.p. was constant (234–235°). Elemental analysis gave C 45.7%, N 17.6%, H 6.4%; $C_6H_{10}O_3N_2$ requires C 45.56%, N 17.71%, H 6.37%. It is a white crystalline solid, sparingly soluble in water but readily soluble in ethanol and acetone. A 0.05*M* solution was prepared in aqueous ethanol (1 + 1). A 0.02*M* palladium chloride or nitrate solution and a 0.1*M* nickel ammonium sulphate solution were prepared, and standardized with dimethylglyoxime.

Determination of Pd

A known volume of Pd(II) solution was taken in a 400-ml beaker, diluted to about 100 ml and adjusted to pH 0.5–1.5 with dilute hydrochloric or nitric acid. A threefold excess of reagent solution was added with stirring. The complex was digested on a water-bath at 50–60° for about 30 min, allowed to cool and filtered off on a porosity-4 sintered-glass crucible. The precipitate was washed with hydrochloric or nitric acid (1% v/v) followed by 10% aqueous ethanol to remove the excess of reagent, and dried at 110–120° to constant weight.

Separation and determination of Pd and Ni

Pd(II) was precipitated first at pH 1.5 and dealt with as above. The filtrate was concentrated to about 100 ml and adjusted to pH 9.0–11.0 with ammonia. A threefold excess of reagent was added with constant stirring, then the precipitate was digested for 30 min, filtered off, washed with 10% aqueous ethanol and dried at 110–120° to constant weight.

Effect of diverse ions on the gravimetric determination of Pd

Interferences were prevented by pH control. At pH 0.5–1.5, 3 g of Cl^-, Br^-, F^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, CH_3COO^-, citrate, tartrate and EDTA, and 0.3 g of Pb^{2+}, Hg^{2+}, As^{3+}, Cd^{2+}, Al^{3+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, Mn^{2+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Ag^+ (in the absence of chloride), Pt(IV), Au(III), Rh(III), Os(VIII) and Ir(III) did not interfere.

Effect of diverse ions on the gravimetric determination of Ni

Three g of Cl^-, Br^-, F^-, I^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, CH_3COO^-, citrate, tartrate, NH_4^+, Ba^{2+}, Ca^{2+}, Ag^+, did not interfere. Hg^{2+}, Pb^{2+}, Cd^{2+}, Al^{3+}, Zn^{2+}, Fe^{3+}, Bi^{3+}, Cr^{3+}, Mn^{2+} were precipitated. Co^{2+}, Cu^{2+}, Fe^{2+}, and Pd^{2+} gave soluble complexes in ammoniacal medium at pH 9.0–11.0. EDTA interfered.

The interference of Fe^{3+}, Cr^{3+}, Al^{3+}, Cd^{2+}, Bi^{3+} and Zn^{2+} can be avoided by precipitating the nickel at pH

Table 1. Spectral characteristics of the Pd(II) and Ni(II) complexes

Complex	λ_{max}, nm	ϵ, $l.mole^{-1}.cm^{-1}$	Extraction pH	Validity of Beer's law, μg/ml	Optimal concn.* μg/ml	Std. devn. μg/ml
Pd(II)-BAMGI	375–380	2.1×10^3	0.5–5.5	0–90	2–9	0.009
Ni(II)-BAMGI	380–385	5.2×10^3	5.0–11.2	0–18	10–40	0.007

*For: 6.0 ppm of Ni(II) or 21.3 ppm of Pd(II); 10 determinations.

5.0–5.5 (acetate buffer) in the presence of tartrate or citrate and ammonium chloride, after removal of Pd(II), if present, at pH 0.5–1.5.

Extractive photometric determination of Pd and Ni

The complexes can be only partially extracted into chloroform, and at high concentration of the metals the complexes accumulate at the water–chloroform interface, so the technique of using molten naphthalene[17–21] was tried and proved successful.

Procedure

To the nickel or palladium sample solution, in a tightly stoppered Erlenmeyer flask, add 2.5 ml of 0.05*M* reagent and adjust to the appropriate pH. Dilute to 25–30 ml. Mix well, and warm on a water-bath at about 60°. Add 2.0 g of naphthalene and warm the mixture in a water-bath at 90° to melt the naphthalene. Shake vigorously till the naphthalene solidifies, forming microcrystals, and allow to cool to room temperature. Again warm to melt the microcrystals slowly, and let them grow to give a coarser deposit. Cool to room temperature. Collect the solid on a filter paper, wash with water, remove the surplus water with dry filter paper, and air-dry the residue. Dissolve the solid in chloroform and dilute to volume in a 10-ml standard flask. Measure the absorbance at 380 nm in a 10-mm cell against a reagent blank prepared similarly. Prepare a calibration curve by applying the procedure to suitable standards.

DISCUSSION

Experimental factors

The nickel and palladium complexes in chloroform solution give maximum absorption at 380–385 and 375–380 nm, respectively. The reagent does not absorb at these wavelengths.

The extraction is quantitative at pH 0.5–5.5 and 5.0–11.2 for palladium and nickel respectively.

The essential spectral data are given in Table 1. Digestion is not necessary but heating for up to 60 min does not affect the results. The method of continuous variations in a two-phase system[22] showed that both complexes have a metal to ligand ratio of 1:2. The absorbance remains constant for several hours.

Varying the amount of naphthalene from 0.5 to 3.0 g does not affect the extraction. The volume of chloroform required to dissolve 1.0 g of naphthalene is 2.0 ml. Tolerance levels for various ions (< 3% error) are given in Table 2.

Applications

Two mg or more of palladium or nickel can be determined gravimetrically with a precision of 0.4 and 0.5%, respectively.

The reagent is suitable for determination of palladium in hydrogenation catalysts, and dental or ornamental alloys, and readily separates it from gold, platinum, silver, copper and zinc. Synthetic solutions

Table 2. Interferences in the extraction method

Tolerance limit, [Ion]/[M(II)]	Ions
Effect of foreign ions on the determination of Pd(II) at pH 1.5	
>10000	Cl^-, F^-, Br^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, CH_3COO^-, $C_2O_4^{2-}$, $S_2O_3^{2-}$, EDTA, citrate, tartrate
300	Hg(II), Pb(II), As(III), Cd(II), Al(III), Zn(II), Co(II), Ni(II), Fe(III), Mn(II), Ba(II), Ca(II), Mg(II), Cr(III)
25	Ag(I), Au(III), Pt(IV), Rh(III), Os(VIII), Ir(III)
Effect of foreign ions on the determination of Ni(II) at pH 9.0–11.0	
>10000	Cl^-, Br^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, CH_3COO^-, $C_2O_4^{2-}$, $S_2O_3^{2-}$ citrate, tartrate
200	Hg(II), Ca(II), Cd(II), Ag(I), Pd(II), Pb(II), Cd(II) Al(III), Zn(II), Fe(III), Cr(III), Mn(II), Co(II) Cu(II)
	EDTA interfered seriously

Table 3. Gravimetric determination of Pd in dental alloys

Constituent	Amount taken, mg	Pd found, mg
1. Pt(IV)	21.30	
Pd(II)	21.35	21.32 (0.03_6)
Au(III)	10.65	
Cu(II)	42.60	
Zn(II)	10.65	
2. Pt(IV)	42.60	
Pd(II)	10.65	10.59 (0.05_6)
Au(III)	21.30	
Cu(II)	10.65	
Zn(II)	21.30	
3. Pt(IV)	10.65	
Pd(II)	85.20	85.3_3 (0.08)
Au(III)	42.60	
Cu(II)	21.30	
Zn(II)	10.65	

*Standard deviation in parentheses.

Table 4. Extractive photometric determination of Pd and Ni

Samples	Nominal content, %	Found, % Present method	Found, % Polarographic method[25]
$CaCO_3$ + Pd (Merck)	5.0	4.9$_0$%	4.8$_8$
Active carbon + Pd (Merck)	10.0	9.7$_2$%	9.6$_8$
Steel BCS 335	9.47	9.6$_0$%	9.5$_6$

simulating the composition of dental alloys were analysed gravimetrically for palladium and results are given in Table 3. The photometric technique was applied for the determination of palladium in two catalysts and nickel in a standard steel. The results are summarized in Table 4.

Advantages of the reagent

There are a few drawbacks in the use of dimethylglyoxime as a selective reagent for palladium[23,24] and modified procedures have to be adopted when metals such as Pt, Rh, Ir, Au and Ni are present. The new reagent is more selective towards palladium and nickel, and does not require chemical separation of palladium from other noble metals.

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ANALYTICAL DATA

STUDY OF SEMICARBAZONES AND THIOSEMICARBAZONES DERIVED FROM 1,2-NAPHTHOQUINONE, AS ACID–BASE INDICATORS: EVALUATION OF THEIR TRANSITION LIMITS THROUGH THE CHROMATICITY CO-ORDINATES

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Summary—The use of 1,2-naphthoquinone-2-semicarbazone, 1,2-naphthoquinone-2-semicarbzone-4-sulphonic acid and 1,2-naphthoquinone-2-thiosemicarbazone-4-sulphonic acid as acid–base indicators has been studied. The sharpness of the indicator transitions has been investigated by means of photometric titrations and the colour quality specified with the aid of the CIE chromaticity system. The results show that the three substances are satisfactory as neutralization indicators.

Semicarbazones and thiosemicarbazones have not been studied previously as neutralization indicators.[1] However, both groups show a resonance structure with an acidic hydrogen atom, and if conjugated with aromatic systems that can exist in benzenoid form and quinonoid form, can have a visible-region absorption spectrum that is pH-dependent.

The study began with the 1,2-naphthoquinone-2-semicarbazone (NQS) and the good results obtained led to the comparative study of the 4-sulphonic acid derivative (NQS4S), which is more soluble in water, and finally, to study of 1,2-naphthoquinone-2-thiosemicarbazone-4-sulphonic acid (NQT4S).

The present work deals with the synthesis and spectrophotometric study of these compounds, determination of their ionization constants and their analytical applications as acid–base indicators. The colours observed during the transitions of the indicators are described in terms of the CIE chromaticity systems and with the RUCS system co-ordinates, and the complementary chromaticity co-ordinates of the transition limits are given.

EXPERIMENTAL

Apparatus

An Acta M-VII spectrophotometer and a Radiometer pH-meter (Model PHM64) with glass/calomel electrodes were used. The pH-meter was standardized with potassium hydrogen phthalate solution (pH 4.008) and Na_2HPO_4/NaH_2PO_4 solution (pH 6.865).

Chemicals

NQS. Prepared by condensing semicarbazide hydrochloride with 1,2-naphthoquinone in acidic medium. The product was recrystallized and tested for purity by TLC (Merck silica gel; butanol–acetic acid–water, 5:4:1). Required for $C_{11}H_9N_3O_2$: 61.4% C, 19.5% N and 4.2% H. Found: 61.9% C, 20.2% N and 4.6 H. $\bar{\nu}$ (KBr) 3450, 3250 and 3150 cm^{-1} (NH, NH_2), 1720, 1680 and 1634 cm^{-1} (CO). The n.m.r. spectrum agrees with the literature data.[2]

NQS4S and NQT4S. Prepared by condensing semicarbazide hydrochloride or thiosemicarbazide hydrochloride with sodium 1,2-naphthoquinone-4-sulphonate in acidic medium. The products were recrystallized and tested for purity by TLC. The first is red and the second orange in solid form. The elemental analysis of the compounds shows that both crystallize with two molecules of water (confirmed by n.m.r.).

NQS4S.$2H_2O$ requires (for $C_{11}H_9N_3O_5S.2H_2O$): 39.8% C, 12.7% N, 3.4% H and 9.7% S. Found: 39.4% C, 12.6% N, 3.3% H and 9.8% S. $\bar{\nu}$ (KBr) 3430 cm^{-1} (NH), 3075 cm^{-1} (ArH), 1700 and 1633 cm^{-1} (CO) and 1220 and 1060 cm^{-1} (SO_2) agree with Ueda *et al.*[2] NQT4S.$2H_2O$ requires (for $C_{11}H_9N_3O_4S_2.2H_2O$) 38.0% C, 12.1% N, 3.8% H and 18.5% S. Found: 37.7% C, 11.9% N, 3.5% H and 18.0% S. $\bar{\nu}$ (KBr) 3400, 3230 and 3160 cm^{-1} (thiosemicarbazone group), 3080 (ArH), 1422, 1490, 1340 and 1312 cm^{-1} (NCS) and 1185 and 1055 cm^{-1} (SO_2) agree with Ueda *et al.*,[2] Grecu and Neamtu[3,4] and Gingras *et al.*[5] The n.m.r. spectra of NQS4S and NQT4S (in DMSO with TMS as reference) agree with those in the literature[2] but both had a new peak at 3.25 ppm, corresponding to the presence of approximately two molecules of water.

Aqueous solutions ($10^{-3}M$) *of NQS, NQS4S and NQT4S. Buffer solutions*[6] *pH 2.2–11.6 and* I = *0.01.*

Analytical grade reagents and doubly-distilled water were used for the standard solutions.

Procedure

The ionization constants were determined spectrophotometrically by the Albert and Serjeant method.[7] The pH of each solution was measured, and the absorbance was recorded over the range 250–650 nm. At wavelengths longer than 650 nm the absorbance was negligible. The stability of NQS, NQS4S and NQT4S solutions with time

was determined by measurement at the absorption maximum in acidic, neutral and basic media. The colour-change pH ranges and their sharpness were evaluated by titration of 0.1 and 0.01*M* hydrochloric acid and acetic acid with 0.1 and 0.01*M* sodium hydroxide. The titrations were followed simultaneously by potentiometry and by continuous measurement of the absorbance at 494 nm for NQS; 481 nm for NQS4S and 515 nm for NQT4S (the wavelengths of maximum absorption in basic medium). The titration solution was continuously circulated between the titration vessel and the spectrophotometer cuvette by a peristaltic pump. The solutions were stirred with a stream of nitrogen. The temperature was 25 ± 1°.

The colour of the buffered indicator solutions ($10^{-5}M$) at different pH values was evaluated, generally at 0.1-pH intervals. The transmittance was measured at 10-nm intervals from 350 to 700 nm in 10- and 50-mm cuvettes (the transmission was 100% at $\lambda > 700$ nm).

RESULTS AND DISCUSSION

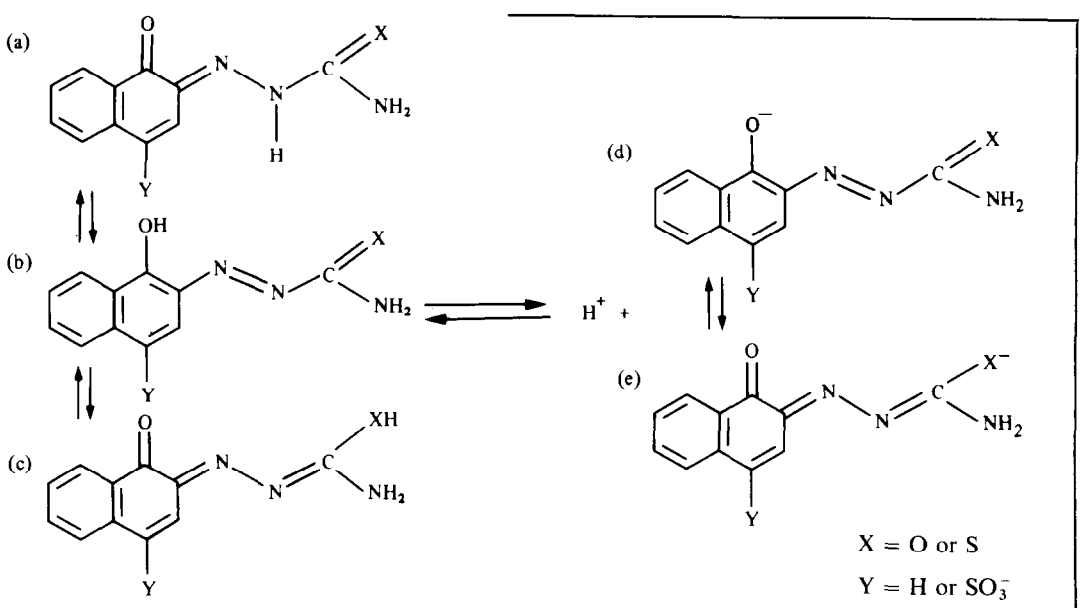
The absorption spectra of the indicators in aqueous solution at different pH values show different forms in equilibria in the pH range studied, and several isosbestic points (Table 1).

The ionization equilibria corresponding to deprotonation of the semicarbazone or thiosemicarbazone group can be complicated by the tautomeric equilibria of the neutral or monoanionic forms of the indicator in acidic medium and mono or dianionic forms in basic medium. This can probably be explained in the same way as by Gingras *et al.*[5,8] and de Castro *et al.*[9] for similar substances. The equilibria can be written as follows.

Table 1. Spectral characteristics

Substance	Medium	λ_{max}, nm	Log ε
NQS	HCl 0.1*M*	440	3.987
	and H_2O*	320	4.302
		270	4.504
	NaOH 0.1*M*	494	4.219
		304	4.052
NQS4S	HCl 0.1*M*	430	3.876
	and H_2O*	320	4.189
		272	4.372
	NaOH 0.1*M*	481	4.277
		308	4.137
		273	4.324
NQT4S	HCl 0.1*M*	455	4.932
	and H_2O*	323	4.612
		279	5.118
	NaOH 0.1*M*	515	4.630
		312	4.348

*The spectrum was the same in both media.



In all probability the main form in acid medium is (*b*), because the spectra show the "leuco" form, normally associated with benzenoid forms, and the presence of the quinonoid form (*e*) in basic medium is the cause of the colour. For NQT4S (*e*) is slowly transformed into (*d*), as demonstrated by the time stability study, since in basic medium there is a decrease in absorbance in the visible together with a displacement of the absorption maximum to shorter wavelengths and an increase in the absorbance maximum in the ultraviolet. The times needed for 10% decrease in absorbance for the indicators in acid (basic) medium were 30 days (8 hr) for NQS, 30 days (4 hr) for NQS4S and 30 hr (1 hr) for NQT4S.

Seven concordant data points were used for calculating each pK_a value (NQS 9.82 ± 0.02; NQS4S 8.94 ± 0.06 and NQT4S 8.46 ± 0.05). The pK_a value for the sulphonic group in NQS4S and NQT4S was not determined.

Table 1 shows that the neutral and anionic forms of NQS and the mono and dianionic forms of NQS4S

Table 2.

Substance	Colour-change pH-range	Acidic colour	Basic colour
NQS	9.3–10.3	Greenish-yellow	Orange
NQS4S	8.0–9.4	Yellow	Orange
NQT4S	8.1–9.0	Yellowish-orange	Red

and NQT4S exhibit different colours, which can be observed at very low indicator concentration.

The results of the photometric titrations show the good behaviour of these products as indicators for the determination of strong and moderately strong acids. Table 2 shows the limits of the colour-change pH-range for these indicators.

However, these indicators give soluble coloured complexes[2] with Cu^{2+}, Ni^{2+}, Bi^{3+}, Fe^{2+}, Fe^{3+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Co^{2+}, Zn^{2+}, Ag^{+} and Ce^{3+}, so these ions interfere and must be removed beforehand if present in the sample to be titrated.

Colour-change evaluation

The description of the colour change of indicator solutions before, during and after the colour-change pH-range is given by their chromaticity co-ordinates calculated by the weighted ordinate method ($\Delta\lambda = 10$ nm). The results are described through the x–y [CIE (1931)], u–v [CIE (1960)] and u'–v' [CIE (1976)] systems, the last two involving more uniform colour spacing. The evaluation was based on the CIE table of coefficients for standard illuminant C.[10]

U–V co-ordinates on the basis of the RUCS system of Breckenridge and Shaub[11] were also calculated because they permit comparison with the colour data for an important group of neutralization indicators, the phthaleins,[12] and some others.[13] The U–V values were calculated from the following relations

$$U = 0.075 - \frac{0.823(x + y - 1)}{x - 7.053y - 1.640}$$

$$V = \frac{3.697x - 5.077y - 1.369}{x - 7.053y - 1.640} - 0.500.$$

The results are given in Table 3, and are represented in Figs. 1 and 2. The scatter at the lower end of the curve c' in Fig. 2 is a consequence of the lower precision of measurement at high absorbance.

Table 3. Chromaticity co-ordinates for the colour change of the indicators at some pH-values in the colour-change pH-range

		CIE x–y (1931) system						RUCS U–V system			
		d = 10 mm			d = 50 mm			d = 10 mm		d = 50 mm	
Substance	pH	Y	x	y	Y	x	y	U	V	U	V
NQS	7.872	0.964	0.338	0.357	0.866	0.425	0.461	0.009	0.005	0.054	−0.021
(3.0 × $10^{-5}M$)	8.225	0.958	0.339	0.356	0.844	0.430	0.458	0.009	0.004	0.054	−0.026
	8.970	0.926	0.346	0.355	0.744	0.459	0.447	0.010	−0.001	0.057	−0.052
	9.394	0.882	0.355	0.353	0.644	0.492	0.432	0.011	−0.010	0.060	−0.084
	9.936	0.825	0.368	0.350	0.557	0.523	0.417	0.013	−0.023	0.063	−0.117
	10.070	0.798	0.375	0.349	0.529	0.535	0.412	0.014	−0.029	0.064	−0.130
	10.559	0.758	0.386	0.347	0.493	0.548	0.405	0.015	−0.039	0.065	−0.146
	10.890	0.745	0.389	0.346	0.484	0.551	0.402	0.016	−0.043	0.065	−0.150
NQS4S	7.795	0.973	0.330	0.346	0.892	0.401	0.436	0.004	0.008	0.044	−0.013
(2.0 × $10^{-5}M$)	8.094	0.963	0.330	0.347	0.858	0.414	0.438	0.005	0.006	0.047	−0.022
	8.410	0.947	0.338	0.349	0.809	0.433	0.440	0.006	0.002	0.051	−0.036
	8.843	0.920	0.347	0.352	0.747	0.462	0.443	0.010	−0.003	0.057	−0.056
	9.213	0.896	0.355	0.355	0.703	0.481	0.444	0.012	−0.009	0.061	−0.070
	9.580	0.882	0.360	0.357	0.681	0.491	0.444	0.014	−0.012	0.062	−0.077
	9.893	0.864	0.365	0.360	0.637	0.502	0.444	0.016	−0.015	0.064	−0.085
	10.033	0.868	0.364	0.360	0.625	0.500	0.444	0.015	−0.015	0.641	−0.085
NQT4S	7.425	0.843	0.371	0.375	0.524	0.528	0.441	0.021	−0.015	0.069	−0.108
(3.7 × $10^{-5}M$)	7.827	0.770	0.373	0.357	0.384	0.563	0.398	0.016	−0.024	0.067	−0.163
	8.113	0.736	0.378	0.350	0.331	0.582	0.380	0.015	−0.031	0.066	−0.193
	8.509	0.635	0.381	0.323	0.260	0.604	0.344	0.006	−0.049	0.063	−0.245
	8.930	0.553	0.389	0.298	0.227	0.607	0.320	−0.002	−0.070	0.057	−0.272
	9.204	0.520	0.391	0.286	0.215	0.604	0.311	−0.006	−0.078	0.053	−0.279
	9.564	0.497	0.392	0.279	0.204	0.604	0.304	−0.008	−0.084	0.051	−0.288
	9.842	0.483	0.394	0.276	0.198	0.605	0.303	−0.010	−0.089	0.051	−0.288

$$u = \frac{4x}{-2x + 12y + 3}, \quad v = \frac{6y}{-2x + 12y + 3}; \quad u' = u, \quad v' = 1.5v.$$

Table 4. Complementary chromaticity co-ordinates

Indicator	pH	Q_x	Q_y	J^*	K_λ (λ, nm)
NQS	8.400	0.146	0.117	0.087	1.035 (440)
($3.0 \times 10^{-5}M$)	12.067	0.136	0.256	0.214	0.907 (490)
NQS4S	7.695	0.145	0.064	0.120	0.899 (440)
($2.0 \times 10^{-5}M$)	10.860	0.129	0.171	0.313	0.928 (480)
NQT4S	7.452	0.154	0.150	0.394	1.119 (460)
($3.7 \times 10^{-5}M$)	10.020	0.194	0.410	0.132	1.183 (520)

*The J values refer to 10-mm path-length.

The values obtained are a function of the experimental conditions (concentration c, and path-length d). To obtain colour points before and after the colour-change pH-range, which are independent of c and d, the complementary chromaticity co-ordinates Q_x and Q_y have been calculated by the weighted ordinate method[14–16] ($\Delta\lambda = 10$ nm) on the basis of the absorbance values (Fig. 1).

The "colour concentration", J, related to the complementary stimuli, was evaluated by means of the relation $J = 2.303S_A/S_a$,[14] where S_a is a constant and S_A is calculated by the weighted ordinate method ($\Delta\lambda = 10$ nm) especially recommended by Kotrlý and Vytřas.[16] The J values also permit K_λ values for maximum absorption wavelengths to be obtained. The results are given in Table 4.

The results show that the three substances have satisfactory behaviour as neutralization indicators and that NQT4S gives the greatest sharpness. NQS and NQS4S exhibit similar sharpness to that found by King[13] for Methyl Red and Tashiro's indicator and NQT4S is comparable to some phthaleins studied by Bhuchar.[12] The criterion for sharpness was the intensity of the change in the chromaticity co-ordin-

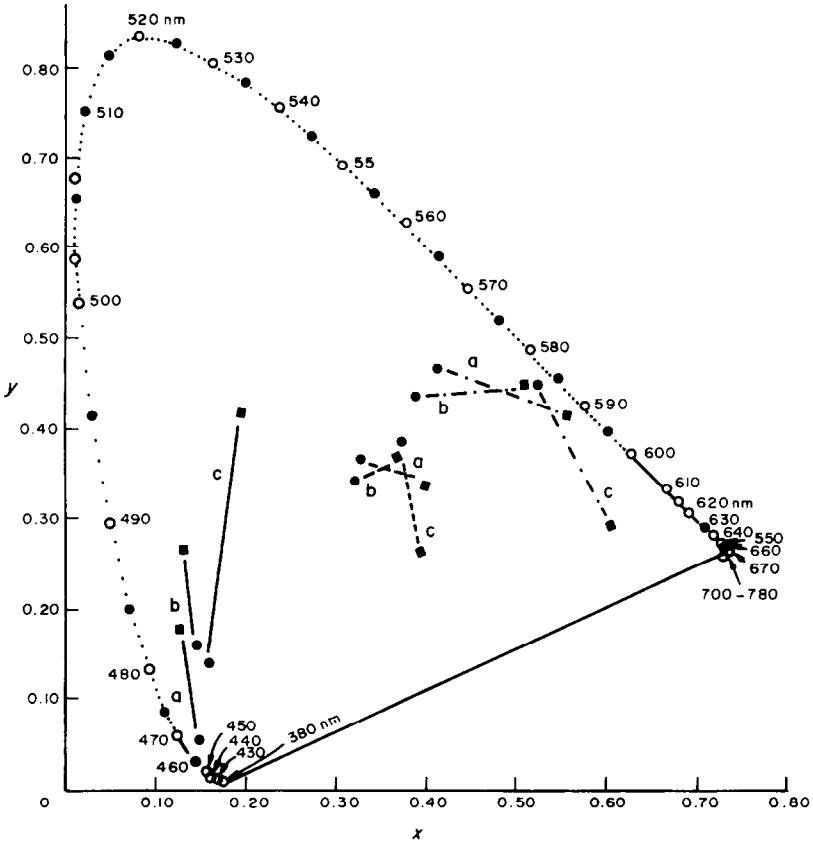


Fig. 1. Colour points in the CIE (x, y) chromaticity system, for acid–base titrations using (*a*) NQS, (*b*) NQS4S and (*c*) NQT4S as indicators. ●, Colour point of acid form of indicator; ■, colour point of basic form of indicator; - - - -, colour points ($d = 10$ mm); ·-·-·-, colour points ($d = 50$ mm); ——, complementary colour points. The co-ordinates of CIE standard source c are $x = 0.310$ and $y = 0.317$.

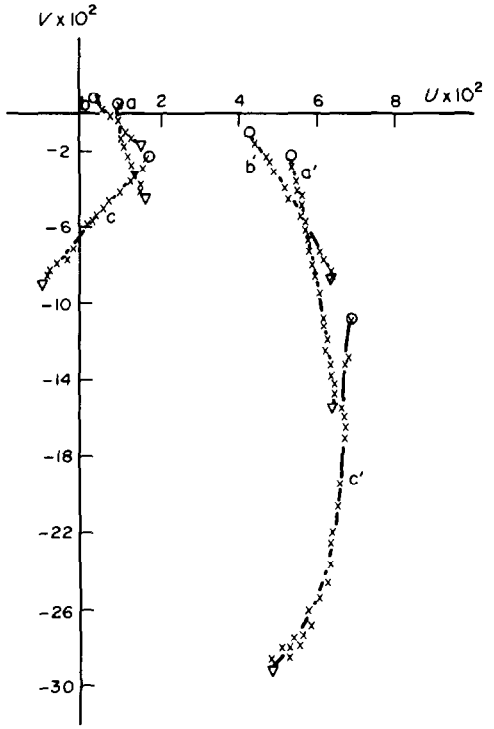


Fig. 2. The U–V co-ordinates (RUCS System) of the colour change with pH of (*a*) NQS, (*b*) NQS4S and (*c*) NQT4S (d = 10 mm) and (*a'*) NQS, (*b'*) NQS4S and (*c'*) NQT4S (d = 50 mm). ○, Colour point of acid form of indicator; ▽, colour point of basic form of indicator.

ates of the forms of the indicator at each end of the transition.

We agree with other authors that the CIE colour specification should be included among basic data on chemical acid–base indicators because they permit establishment of comparative criteria for sharpness of the colour change of indicators. Furthermore, the complementary chromaticity co-ordinates can also be used for the calculation of indices of colour quality, and for evaluation of the constants for the equilibria involved in the indicator transition.[17]

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ANNOTATIONS

CHANGE IN CONSISTENCY AND COMPOSITION OF TRICHLOROETHYLENE- AND TRICHLOROETHANE-TREATED ASPHALTS

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Summary—Solvent extraction of asphalt from pavement mixtures is a technique used to study the change in asphalt during service. Rheological measurements indicate that asphalts recovered from trichloroethylene or trichloroethane are markedly hardened. Compositional studies on asphaltic fractions reveal a notable decrease in saturates, naphthenes, H, N, Ni and V, and increase in polar aromatics and asphaltenes along with incorporation of Cl in all fractions. These structural changes are responsible for the hardening of the asphalt and are interpreted in terms of cyclization of saturates, aromatization of naphthenes, coupling of free radicals with neutral species to give a high content of asphaltenes, and in terms of loss of the relatively volatile Ni and V porphyrins during the extraction–recovery process.

Since it is not feasible to study *in situ* the effect of aging on asphalt during service, the practical alternative is solvent extraction of asphalt from pavement samples and its subsequent recovery. The validity of this method depends on whether the solvent causes any significant change in the properties of the asphalt. Although benzene is the solvent most widely used in asphalt technology, chlorinated hydrocarbons are also used by some asphalt research laboratories. In a study surveying the effect of the solvents generally used in the recovery process, trichloroethylene and trichloroethane were reported to cause marked hardness in the recovered asphalts.[1]

Analysis of asphalt before and after recovery would help understanding of the fundamental changes involved and, accordingly, how the asphalt hardness came about. Compositional studies of asphalts have been conducted by several investigators[2–4] but the methods of analysis used were quite detailed. The development of a simplified analytical procedure,[5] whereby an asphalt can rapidly be fractionated into distinct classes of components, makes it possible to characterize individual fractions and to follow any change in asphalt composition.

This investigation was undertaken in an examination of the role of chlorinated solvents in hardening recovered asphalts and reports the results in terms of change in properties, as obtained by rheological measurements on the whole asphalts and compositional analysis of the fractionated asphalts. The work reported was done with a Kuwaiti asphalt, but similar effects have been observed with an Egyptian Western Desert asphalt as well as those reported on earlier,[1] so the effect seems a general one.

EXPERIMENTAL

The asphalt used was an atmospheric heavy-end fraction of Kuwait oil and all chemicals were reagent grade. Asphalt samples (200 g) were each treated four times with trichloroethylene or 1,1,1-trichloroethane according to a reported procedure.[1] The solvent was then distilled in a rotary flash-evaporator at 100° to avoid any oxidation or overheating of the asphalt.

The softening point and penetration of the original and recovered asphalts were measured by standard methods.[6] The asphalts were then separated into their major components by precipitation of asphaltenes with n-hexane, and fractionation of petrolenes by adsorption chromatography.[5] The asphaltic fractions were analysed for C, H, N and Cl.

Vanadium and nickel were determined by electrothermal atomic-absorption spectroscopy with a Perkin–Elmer 300S spectrometer with HGA-72 graphite furnace and deuterium background-correction. Teflon bombs were used for the decomposition of samples at 190° with a mixture of hydrochloric, nitric and perchloric acids. The standards were aqueous solutions of sodium metavanadate and nickel ammonium sulphate at an acidity similar to that of the test solutions. Blanks were obtained by treating pure paraffins in the same way to give a similar matrix. The metals were determined, in an argon atmosphere, at the resonance wavelengths 318.5 nm (V) and 232 nm (Ni).

The n.m.r. spectra were measured on a Varian-A60 spectrometer, with tetramethylsilane as internal standard.

Table 1. Rheological and compositional change in treated asphalts

Property	Asphalt		
	Untreated	Trichloro-ethylene-treated	Trichloro-ethane-treated
Softening point, °C	50	54	62
Penetration at 25°C (100/5)	52.3	44.3	42.7
Petrolenes, % w/w			
Saturates	16.5	13.0	10.6
Naphthene-aromatics	37.0	31.8	30.2
Polar aromatics	32.0	33.0	35.0
Asphaltenes, % w/w	11.5	20.3	21.8

RESULTS AND DISCUSSION

To find whether the consistency of asphaltic materials has changed, rheological properties such as softening point and penetration are usually measured. Differences in these properties for trichloroethylene- and trichloroethane-treated asphalts are given in Table 1 along with the properties of untreated asphalt, as reference. The increase in softening point and decrease in penetration are indicative of hardening in the treated asphalts. The asphalt recovered from trichloroethane is much harder than that recovered from trichloroethylene. Table 1 also gives the relative proportions of the major fractions of the asphalts. The effect of the chlorinated solvents is manifested by the decrease in saturates and naphthene-aromatics, slight increase in polar aromatics and substantial increase in asphaltenes. The effect is more pronounced with trichloroethane.

Investigation of the chemical characteristics of the complex matrix of whole asphalts is undoubtedly difficult. To obtain useful results more easily, only the asphaltic fractions were investigated. The change in their carbon and hydrogen content is shown in Table 2. The slight increase in the C/H ratio for all fractions in going from untreated asphalt to trichloroethylene-treated to trichloroethane-treated asphalts reflects the chemical changes that might have taken place during the extraction with chlorinated solvents. The reactions most likely to be responsible for such changes are partial cyclization of saturates and aromatization of the naphthenes formed and of those already present. The latter reaction may offer an explanation for the decrease in naphthene-aromatic fraction (Table 1), which is due to aromatization of the naphthenes present in that fraction.

The structural changes in the asphaltic fractions were examined by n.m.r. spectroscopy. The chemical shifts for hydrogen, seen in the spectra of saturates (Fig. 1) are: H_a, 0.88; H_b, 1.23; H_c, 2.35 (untreated asphalt)–2.50 (treated asphalts); H_d, 7.33 ppm. Signals of type a are assigned to the CH_3-groups of saturated hydrocarbons or side-chains farthest from aromatic rings, whereas signals b and c represent CH_3, CH_2 or CH-groups of saturated hydrocarbons, or side-chains that are β and α, respectively, to aromatic rings. Signals of the type d are due to aromatic protons.

The decrease in signals a and b for the recovered asphalts is accompanied by a slight increase in signals c and d. Corresponding changes in the integrated signals were observed. These spectral differences were interpreted as due to a decrease in both side-chain length and degree of substitution on aromatic rings.[7] The change in integrated signal with treatment of the asphalts was taken to imply a decrease in the total hydrogen content of the saturates in the treated asphalts. This indicates that the composition of the samples shows a trend during extraction and recovery, for conversion of saturates into more condensed ring structures.

The n.m.r. spectra of the other components showed similar patterns revealing increased cyclization and aromatization. The spectra of the asphaltenes separated from trichloroethane-treated asphalt showed more increase in signal d than any other fraction, reflecting a higher degree of aromatization.

Analysis showed that chlorine appears to be incorporated in the fractions from the treated asphalts. The results, in terms of Cl/H ratio, are listed in Table 2. The higher ratio for polar aromatics than for asphaltenes is consistent with the higher aromaticity of the latter fraction. The data suggest that a free radical chlorination may occur during the extraction. The notable hardness of the treated asphalts and the significant increase in their content of asphaltenes can be explained as due to free radicals coupling with other

Table 2. Change in carbon–hydrogen content and incorporation of chlorine in components of treated asphalts

Components	Asphalt					
	Untreated		Trichloroethylene-treated		Trichloroethane-treated	
	C/H	Cl/H	C/H	Cl/H	C/H	Cl/H
Saturates	6.5_0	—	6.5_2	0.017	6.7_5	0.028
Naphthene-aromatics	8.6_9	—	8.8_0	0.042	9.1_0	0.079
Polar aromatics	9.1_4	—	9.1_5	0.096	9.5_4	0.115
Asphaltenes	11.0_6	—	11.1_0	0.063	11.3_7	0.090

Table 3. Effect of chlorinated solvents on the nitrogen and trace metal contents of asphaltic components

Element	Saturates	Naphthene-aromatics	Polar aromatics	Asphaltenes
		Untreated asphalt		
N, %	0.07	0.59	0.81	0.87
V, *ppm*	—	79	184	350
Ni, *ppm*	—	15.5	23	100
V/Ni	—	5.1	8.0	3.5
		Trichloroethylene-treated asphalt		
N, %	0.01	0.53	0.75	0.76
V, *ppm*	—	65	170	298
Ni, *ppm*	—	14.7	19	90
V/Ni	—	4.4	8.9	3.3
		Trichloroethane-treated asphalt		
N, %	—	0.41	0.60	0.70
V, *ppm*	—	54	116	249
Ni, *ppm*	—	14	21	82
V/Ni	—	3.9	5.5	3.0

free radicals or with neutral species, producing high molecular-weight materials in either reaction.

An esr investigation on the role of radicals in the aging of coal liquids[8] showed that some oil components were converted into asphaltenes during aging. Those components were rich in free spins and free radicals were also characterized in the asphaltenes formed. Another esr study has reported that the concentration of free radicals was observed to increase with increasing degree of cyclization and aromatization in asphaltenes.[9] These findings suggest that the increase in the content of asphaltenes and hence in the hardness of the recovered asphalts is very probably due to increased aromaticity and to combination of free radicals with neutral species.

Although it has been indicated that characterization of petrolenes contributes a great deal more toward characterizing the whole asphalt,[10] asphaltenes have recently been reported as significant in characterizing asphalts, and hence parent crudes, by means of their trace metal content.[11] Accordingly, analysis for vanadium, nickel and nitrogen (since the two metals mostly occur as metalloporphyrins in oils *etc.*) might help to give a composite picture of the structure of the whole asphalt. The effect of chlorinated solvents on trace-element content should therefore also be considered in attempts to detect any structural differences between the original and treated asphalts. The analytical data are given in Table 3. Each metal concentration is an average of five results and the relative standard deviation ranges from 5% for Ni to 7% for V. Recovering the asphalts from the chlorinated solvents decreased the levels of these elements, the effect of trichloroethane again being greater than that of trichloroethylene. The decrease in the trace metal content is associated with decrease in nitrogen content, indicating that during the course of extraction and recovery, the prophyrin content and consequently the level of porphyrin–metal complexes decrease. This is in agreement with the fact that a significant proportion of the metallic constituents of petroleum is relatively volatile.[12] The effect is particu-

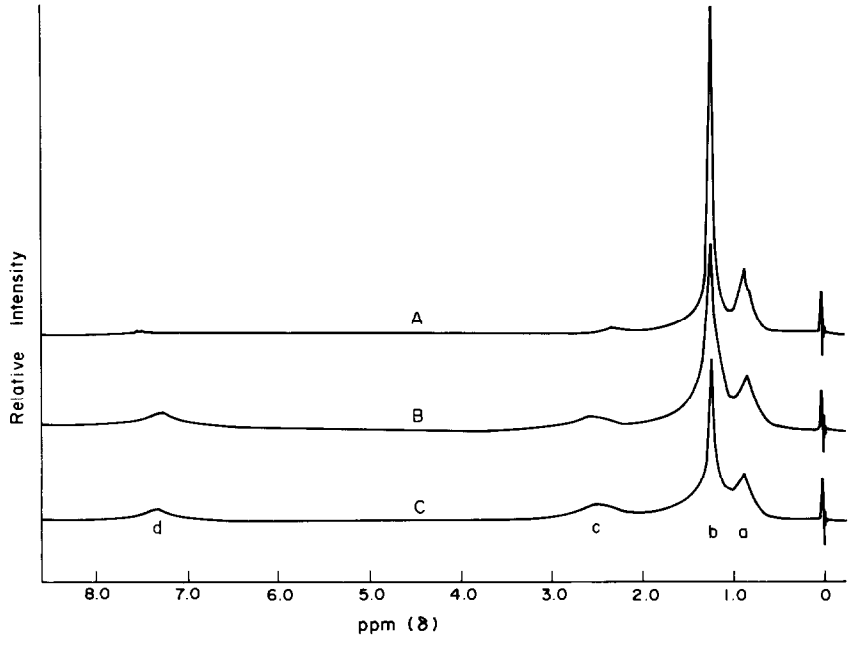

Fig. 1. n.m.r. spectra of saturated fractions of asphalts: A, untreated; B, trichloroethylene-treated; C, trichloroethane-treated.

larly apparent in the asphaltenes, for which the V/Ni index is comparable despite the variation in the individual concentrations of the two metals. The marked difference in V/Ni ratio between the naphthene-aromatics and polar aromatics suggests that the vanadium compounds are much more volatile than the nickel compounds in these fractions, whereas there is little difference for those in the asphaltenes. The V/Ni ratio may therefore be a significant characteristic for asphaltic heavy-end fractions and should perhaps be related to the study of asphalt composition.

CONCLUSIONS

Extraction of asphalts from pavement samples with trichloroethylene or trichloroethane usually causes a change in their rheological and chemical properties, rendering them markedly harder. Such changes are undesirable as they will certainly interfere in investigation of the effect of aging on the properties of asphalts.

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A SOLVENT EXTRACTION–SPECTROPHOTOMETRIC DETERMINATION OF BISMUTH(III) AS TETRA-n-BUTYLAMMONIUM TETRAIODOBISMUTHATE

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Summary—A solvent extraction–spectrophotometric method has been developed for determination of bismuth in the form of tetra-n-butylammonium tetraiodobismuthate(III). The effects of pH, the concentrations of tetra-n-butylammonium and iodide ions, and the nature and amount of the organic solvent have been studied. Tetra-n-butylammonium was found to be the most useful cation for extraction of tetraiodobismuthate with chloroform, giving quantitative extraction. The optimum pH is <3, and the extracted species is stable for at least a day at room temperature. Bismuth can be determined at the $10^{-5}M$ level in aqueous solution, the relative standard deviation being 1.7%.

Bismuth compounds are used in pharmaceuticals and cosmetics, but there are a few reports on determination of bismuth in these materials.

The reaction between iodide and bismuth(III) in aqueous solution has been discussed in the literature[1,2] but reactions between the tetraiodobismuthate anion and high molecular-weight cations have been comparatively little studied. Buděšínský and Vaníčková[3,4] have used tetraiodocadmate(II) and tetraiodobismuthate(III) (BiI_4^-) as counter-ions for ion-pair formation with organic bases such as caffeine, hexamethylenetetramine and antipyrine, and have determined these organic compounds in this way gravimetrically. Matsuo *et al.*[5] have also used the ion-pair complex of BiI_4^- with tetradecyldimethylbenzylammonium ions for the determination of bismuth. Methods involving ion-pair formation are very useful because they can provide simultaneous concentration and separation of chemical species from the matrix. The present work aimed at finding the most suitable and effective counter-ion for the separation and determination of bismuth by extraction of a BiI_4^- ion-association complex.

EXPERIMENTAL

Reagents and materials

The bismuth solution was prepared from analytically pure $Bi(NO_3)_3 \cdot 5H_2O$ and standardized complexometrically with Xylenol Orange as indicator. All solvents were purified according to Weissberger.[6] Analytical-reagent grade potassium iodide, tetra-n-butylammonium iodide (Bu_4NI), cetylpyridinium chloride, and Zephiramine® (benzyldimethyltetradecylammonium chloride, Dojin Chem. Co.) were used without further purification. All the other chemicals were of analytical-reagent grade. Two kinds of medical cream were used as test samples.*

*The sale of creams containing bismuth salts is now prohibited by law in Japan.

Apparatus

Absorbances were measured on a Shimadzu UV-200 double-beam spectrophotometer with 1-cm glass cells. A Hitachi-Horiba glass-electrode M-5 pH-meter was used.

RESULTS AND DISCUSSION

Effect of pH and acid concentration

At pH >3, there is a decrease in extraction yield with increasing pH, probably because of precipitation of bismuth as hydroxo-species.[7] At pH <3 there is no shift in the wavelength for maximum absorption (λ_{max}) and the absorbance at λ_{max} remains constant.

Matsuo *et al.*[5] used Zephiramine as counter-ion for ion-pair formation with BiI_4^- and reported that the absorbance of the methylene chloride extract remained unchanged even when the extraction was from an aqueous solution of pH 5. We also have studied various high molecular-weight ions. Zephiramine, cetyltrimethylammonium, or cetylpyridinium easily form emulsions with tetraiodobismuthate(III) and chloroform, and difficulties arise in separation of the phases. On the other hand, when Bu_4N^+ is used as the counter-ion there is a clean separation of the two layers and the absorbance is reproducible. Tetramethylammonium, which has smaller formula weight than Bu_4N^+, gives a lower molar absorptivity, probably because of the difference in molecular cross-section. The pH is not critical provided it is not above 3.

Effect of Bu_4N^+ concentration

For concentrations of bismuth(III) and iodide of $5 \times 10^{-5}M$ and $0.1M$, respectively, in the aqueous phase, the absorbance of the extract is constant if the Bu_4N^+ concentration is more than $7 \times 10^{-4}M$. Therefore, the concentration of Bu_4N^+ should be at least 14 times the concentration of bismuth(III).

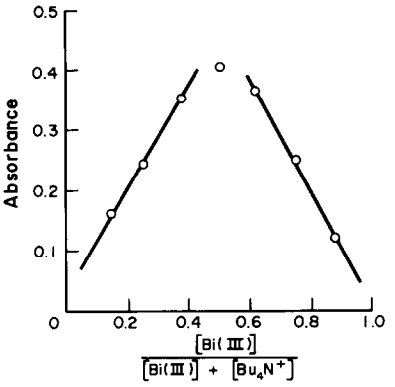

Fig. 1. Continuous variations plot. $[Bi(III)]_{aq} + [Bu_4NI]_{aq} = 8.0 \times 10^{-5}M$; $[KI]_{aq} = 2 \times 10^{-2}M$; pH 0.90. Volume of aqueous phase, $V_{aq} = 10$ ml; volume of organic phase, $V_{org} = 5.0$ ml; wavelength, $\lambda_{max} = 490$ nm.

Composition of the ion-pair complex

The continuous variations method with measurement at λ_{max} (490 nm), showed that a 1:1 complex was formed (Fig. 1). The small absorbance observed even at zero mole fraction of bismuth might be due to formation of $Bu_4N^+I_3^-$, some iodide being oxidized to I_3^- by contaminants. This oxidation reaction can be satisfactorily eliminated by the addition of phosphinic acid as a reducing agent.[8,9] A similar result was obtained by the mole-ratio method. The ion-pair complex, $Bu_4N^+ \cdot BiI_4^-$ predominates under the given conditions, making it advantageous for analytical use.

Organic solvent

Matsuo *et al.*[5] used methylene chloride for the extraction, but this solvent is much more volatile than the other solvents, and also more difficult to handle than chloroform, 4-methyl-2-pentanone (MIBK), *etc.* However, chloroform was chosen rather than MIBK as the extraction solvent because of the good extraction yield and ease of handling.

Effect of the phase-volume ratio

When other conditions were kept within the optimal ranges, the absorbance was almost independent of the aqueous:organic phase-volume ratio if this was less than 12, but increased with increasingly higher ratios. This is attributed to dissolution of some of the chloroform in the aqueous phase, thus decreasing the effective volume of the chloroform extract. Therefore the volume ratio should be less than 12. However, if the water used is presaturated with chloroform there should be less loss of the solvent.

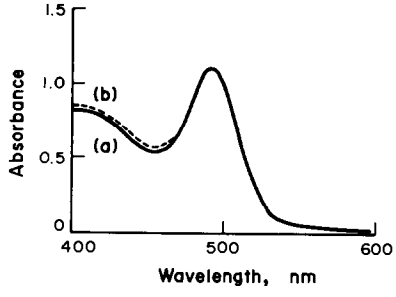

Fig. 2. Absorption spectra of $[BiI_4^-][Bu_4N^+]$ complex in chloroform extracts. Conditions: $[Bi(III)]_{aq} = 5.0 \times 10^{-5}M$; $[KI]_{aq} = 2.0 \times 10^{-2}M$; $[Bu_4NI]_{aq} = 2.5 \times 10^{-3}M$; $[H_3PO_2]_{aq} = ca.\ 1\%$; $V_{aq} = 25$ ml; $V_{org} = 5.0$ ml; pH 1.0. (a) Extract *vs.* reagent blank. (b) Extract *vs.* $CHCl_3$.

Effects of shaking time and standing time

The absorbance of the extract remained constant for shaking times from 30 sec to 6 min (at ~250 strokes/min), and at room temperature showed no change with storage time of up to a day. The yield in a single extraction (shaking for 3 min) was almost quantitative, 98% for a volume ratio of 5:1. A second extraction makes the yield completely quantitative.

Absorption spectra

The absorption spectrum of $Bu_4N^+ \cdot BiI_4^-$ in chloroform extracts is shown in Fig. 2. Beer's law is obeyed up to an absorbance of 1.55 for $7.00 \times 10^{-5}M$ bismuth(III) in the aqueous phase by using chloroform extraction. The corresponding linear regression equation and the coefficient of correlation, r, are

$$\text{Absorbance} = 0.0106X + 0.0013$$
$$r = 0.999$$

where X is the weight (μg) of bismuth extracted into 5.0 ml of chloroform. The conditional molar absorptivity, found by using $2.00 \times 10^{-5}M$ bismuth solution was 1.13×10^4 l.mole^{-1}.cm^{-1} at 490 nm in chloroform (*cf.* 8.2×10^3 l.mole^{-1}.cm^{-1} at 340 nm in aqueous solution[10]).

Effect of foreign ions and materials

Several cosmetics such as skin creams and nail enamels may contain bentonite, silicic acid, aluminium oxide, calcium oxide, magnesium oxide and so on. However, the presence of these materials in 1000-fold concentration did not interfere with the measurement of bismuth, because they did not react with iodide to form a corresponding complex anion, but Hg(II), Cd(II) and Pb(II) interfered with determination of bismuth.

Elimination of organic components

Skin creams usually contain various organic components, including beeswax, white vaseline, isopropyl myristate, glycerol monostearate and so on. In order to determine bismuth in the creams, we had to eliminate these organic compounds because they interfered in formation of the ion-pair complex. Attempts to extract the inorganic compounds into an aqueous phase at pH 1 from solutions of the samples in n-hexane, diethyl ether or chloroform failed because some of the organic compounds also dissolved in the aqueous phase and then interfered with formation of the ion-pair complex. Thus, we removed the organic compounds by a wet-combustion procedure; the

Table 1. Recovery of bismuth added to skin creams (free from bismuth salts) prior to wet combustion; single extraction

Run	Bismuth added, μg	Found, μg	Recovery, %
1	52.3	51.2	98.0
2	52.3	49.8	95.3
3	52.3	50.7	97.1
4	104.5	101.4	97.1
5	104.5	101.9	97.5
6	104.5	102.4	98.0

Sample size: 1 g of skin cream. Aqueous phase 25 ml, organic phase 5 ml.

Table 2. Determination of bismuth in cosmetic cream

Sample*, g	Absorbance	Found, μg	Content, μg/g
0.4801	1.100	103.4	215
0.5091	1.200	112.8	222
0.2532	0.605	56.9	225
0.2558	0.600	56.4	220

*Weight of wet sample.

sample was easily decomposed with a mixture of concentrated nitric acid (10 volumes) and perchloric acid (1 volume). The decomposition was very slow if only the nitric acid was used, but the sample decomposed rapidly with the acid mixture. The caramel-like residue was easily decomposed by further addition of nitric acid.

Procedure

Weigh the sample (~1 g) accurately into a porcelain evaporating dish. Add concentrated nitric acid (10 ml) and perchloric acid (1 ml) and about 0.1 g of potassium sulphate to avoid spitting during the heating. Heat on a sand-bath until the mixture becomes dry. Repeat the treatment with the acid mixture 3 times. Dissolve the final residue in 10 ml of 2*M* nitric acid and transfer the solution into a separatory funnel. Add 2*M* potassium iodide (0.2 ml), 50% v/v phosphinic acid solution (0.5 ml), 0.1*M* Bu_4N^+I (0.25 ml), and chloroform (10 ml), and shake the funnel vigorously for 3 min. Measure the absorbance of the extract at 490 nm *vs.* chloroform. Determine the amount of bismuth by means of the calibration graph or the linear regression equation.

Recovery and sample analysis

Results obtained by the procedure described are given in Tables 1 and 2. The 95% confidence limits[11] calculated from the values in Table 2 are 213–227 μg.

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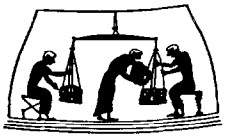

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Formation constants for the levulinate– and acetate–proton, –manganese(II), –cobalt(II), –nickel(II), –copper(II) and –zinc systems: PETER W. LINDER, RALPH G. TORRINGTON and UTE A. SEEMANN. (14 September 1982)
Spectrophotometric determination of lithium in blood serum: JAY K. TRAUTMAN, VICTOR P. Y. GADZEKPO and GARY D. CHRISTIAN. (15 September 1982)
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A titrimetric method for the determination of thiocarbonate sulphur in the presence of sulphite, thiosulphate and thiocyanate: K. SINGH and B. A. FODEKE. (7 October 1982)

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A highly sensitive spectrophotometric method for determination of trace amounts of arsenic in water: QIAN-FENG WU and PENG-FEI LIU. (9 July 1982)

A kinetic study of the oxidation of phenols and chlorophenols by metaperiodate: N. G. BUCKMAN, R. J. MAGEE and J. O. HILL. (9 July 1982)

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A sensitive and specific resin-bead test for histidine, and its spectrophotometric determination with potassium bromate as reagent: K. G. VARSHNEY, S. ANWAR and S. Q. QURESHI. (16 July 1982)

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OBITUARY

R. BELCHER
(1909–1982)

Ronald Belcher, Professor Emeritus, Birmingham University, died on 29 June 1982 after being ill for some months.

He was born in Nottingham in 1909. As a young assistant in coal chemistry research at Sheffield University from 1927, he was involved in analysis and was greatly stimulated by a period of training in microchemical techniques at the Pregl Laboratory in Graz. He studied part-time for the Associateship of the then Institute of Chemistry, passing the examinations in 1939 and being elected to the Fellowship in 1942.

From these modest beginnings, an interest in teaching analytical chemistry was aroused by a spell on the staff at Rotherham Technical College in the early 1940s and from 1946 at Aberdeen University. In 1948 he moved to Birmingham University and with the help of a few young colleagues, fired by his own enthusiasm, quickly established a large research team. Classical type investigations on indicators, etc., broadened over the ensuing thirty years to cover a host of subjects such as new organic reagents, submicro methodology, gas chromatography of metal chelates, membrane electrodes, kinetic methods, MECA spectroscopy.

Some 500 research papers, books and monographs testify to the prodigious work of Belcher, who not only inspired many from within Britain but attracted considerable numbers of postgraduate and postdoctoral students from abroad. Former pupils and collaborators now occupy prominent positions in various countries. He received the D.Sc. degree in 1955 and was appointed Professor of Analytical Chemistry in 1959.

Belcher's influence extended to active involvement in the affairs of numerous national and international bodies, notably the former Society of Analytical Chemistry and new Royal Society of Chemistry and in the International Union of Pure and Applied Chemistry. He received many honours and was invited to lecture extensively throughout the world. A fuller account of his career was given in *Talanta*, 1969, **16**, 757.

He will be missed, not least for his leadership as Chairman of the Editorial Board of *Talanta*, the journal he was instrumental in starting.

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THE LOUIS GORDON MEMORIAL AWARD

The Editorial Board and Publisher of *Talanta* take great pleasure in announcing that the Louis Gordon Memorial Award for 1981 (for the paper judged to be the best written of those appearing in *Talanta* during the year) will be made to Dr. Lars Kryger, of Aarhus University, Denmark, for his paper "Interpretation of Analytical Chemical Information by Pattern Recognition Methods—A Survey" (*Talanta*, 1981, **28**, 871).

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Synthesis of some new *ortho*-thioazo ligands and evaluation of their metallochromic properties: D. L. PRINGLE, M. T. MOHABBIS, M. H. MAHONEY, C. H. SOTAK and J. M. SULLIVAN. (8 February 1982)
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A survey of the information on co-ordination compound features that can be gained by the electroanalytical approach: GINO BONTEMPELLI, MILLA ANDREUZZI-SEDEA and MARIO FIORANI. (10 February 1982)
Formation constants and thermodynamic functions for some tervalent rare-earth metal complexes with *p*-sulphosalicylidene sulphanilamide: G. P. SENGUPTA and C. R. BERA. (12 February 1982)
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Validation of the sulphur concentration of selected iron-base NBS standard reference materials by isotope-dilution spark-source mass spectrometry: R. W. BURKE, P. J. PAULSEN, E. J. MAIENTHAL and G. M. LAMBERT. (16 February 1982)
Ternary complexes of Cu(II) and Ni(II) chelates of EDTA and DCTA with cyanide and ethylenediamine: J. KORSSE, L. A. PRONK, C. VON EMBDEN, G. LEURS and P. W. F. LOUWRIER. (24 February 1982)
Titrimetric microdetermination of certain sulphur-containing organic compounds by oxidation with alkaline potassium permanganate: K. K. TIWARI and R. M. VERMA. (25 February 1982)
Radiochemical determination of cobalt in environmental samples: C. D. JENNINGS and T. M. BEASLEY. (20 December 1981)

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Studies on the nature of reactions and species involved in potentiometric determination of uranium: S. G. MARATHE, B. N. PATIL, VEENA BHANDIWAD and KESHAV CHANDER. (15 October 1981)
Origin of double peak signals for trace lead, bismuth, silver and zinc in a microamount of steel in atomic-absorption spectrometry using direct electrothermal atomization of solid samples in a graphite-cup cuvette: KUNIO TAKADA and KICHINOSUKE HIROKAWA. (15 October 1981)
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Titration of vanadium(IV) with cerium(IV) sulphate, with ferroin as indicator, in aqueous alcohol as a facile reaction medium: K. SRIRAMAM, N. RAMABRAHMA SASTRY and G. S. N. SASTRY. (6 November 1981)

Performance of the Orion 97–70 total residual chlorine electrode at low concentrations and its application to the analysis of cooling waters: N. A. DIMMOCK and D. MIDGLEY. (30 October 1981)

Spectrophotometric determination of some phenols with sodium metaperiodate and aminophenols: C. S. PRAKASA SASTRY, K. E. RAO and U. V. PRASAD. (6 November 1981)

Adsorption of chromium(VI) on silica gel impregnated with Adogen 464 and its determination by furnace atomic-absorption: P. BATTISTONI, S. BOMPADRE, G. FAVA and G. GOBBI. (6 November 1981)

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TALANTA ADVISORY BOARD

The Editorial Board and the Publisher of *Talanta* take pleasure in welcoming the following new members of the Advisory Board of the journal.

D. T. BURNS
E. M. DONALDSON
R. J. MAGEE
F. PELLERIN
S. B. SAVVIN

They also wish to record their sincere thanks for the help given by

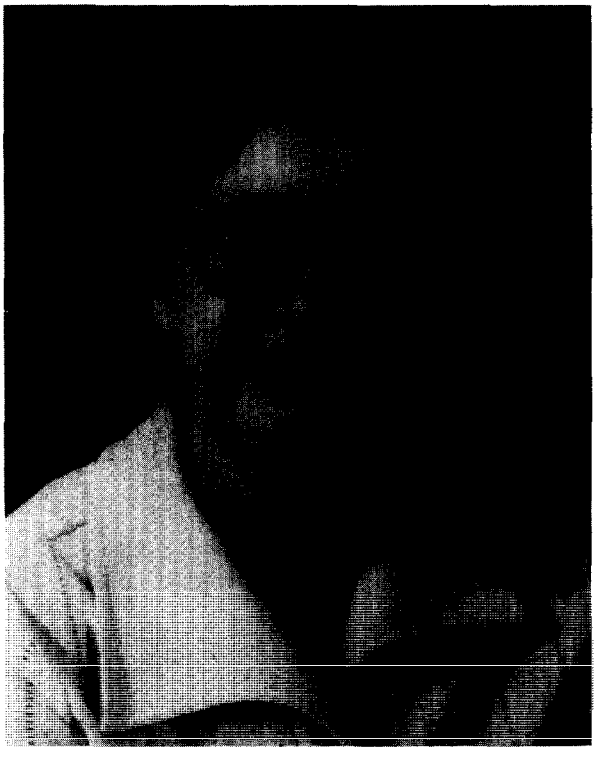
J. BARTOS
C. B. BELCHER
A. CORSINI
F. KILLER
C. LITEANU

who retire from the Advisory Board.

Biographical notes on some of the new members (and Professor Shu-Chuan Liang, who was appointed to the Board in 1981) appear below. Professor Savvin's notes will follow later.

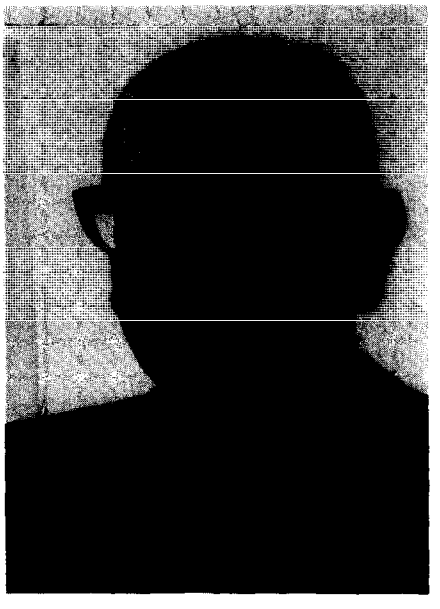
PROFESSOR BURNS graduated in chemistry at Leeds University (1955) where he stayed on to do research in physical chemistry, obtaining a Ph.D. in 1959. His first full time appointment, in 1958, was to Medway College of Technology as Assistant Lecturer in Physical Chemistry, and he became Lecturer in physical chemistry and also an Associate of the Royal Institute of Chemistry in 1959. In 1963 he joined the staff of the late A. I. Vogel at Woolwich Polytechnic as Senior Lecturer in Inorganic and Analytical Chemistry. He joined the Society for Analytical Chemistry and returned to the Midlands in 1966 upon appointment to Loughborough University of Technology as Senior Lecturer and Head of the Analytical Chemistry Section. He became Reader in 1971 and obtained the first substantive D.Sc. of Loughborough in 1972. In 1975 he was appointed to the established Chair of Analytical Chemistry in the Queen's University of Belfast. He became a Fellow of the Royal Institute of Chemistry in 1968, and the Institute of Chemistry in Ireland in 1976, was awarded the James Taylor Prize of

the Sheffield Metallurgical and Engineering Society in 1970, gave the 31st Pearson Lecture at Whitcliffe Mount School in 1975, the Opening Lecture at Euroanalysis III 1978, and is to give the Royal Society of Chemistry's endowed Theophilus Redwood Lecture in 1982. Professor Burns has been active in the Analytical Division of the Royal Society of Chemistry and its forebears as follows: past member of Council and Chairman of the Midlands Region, past Chairman and past Secretary of the Education and Training Group, past Chairman of the Northern Ireland Sub-Committee of the Scottish Region; he has also assisted in the organization of several conferences, and is currently a member of Council as Honorary Secretary to the International Affairs Committee, and a member of the Northern Ireland Region and the Microchemical Methods Group Committees. He is an associate member of IUPAC Commission V/1. Current research interests include ion-association extraction systems, elemental analysis, HPLC of organometallics, anions, medicinal compounds and their metabolites, and characterization of poteen and fire-accelerant residues. He has co-authored several books and published over 170 research and review papers including several on his hobby—the study of the history of analytical chemistry. Professor Burns' main administrative contribution to the University is concerned with Health, Safety and Welfare at Work as Chairman of the University Safety Committee, *ex officio* membership of subcommittees, and a watching brief over 32 area committees.

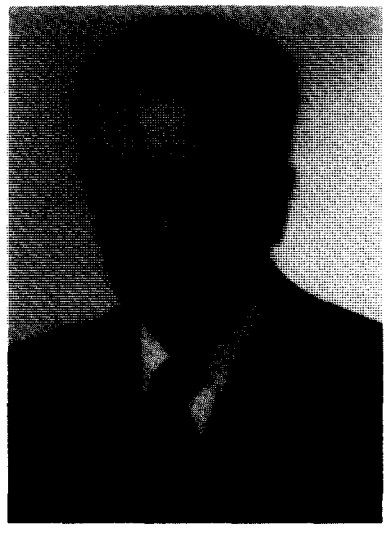


ELSIE M. DONALDSON was born in 1929 in Winnipeg, Manitoba, Canada, and obtained her B.Sc. degree in chemistry from the University of Manitoba in 1951. In August 1951, she joined the staff of the Mines Branch of the Department of Mines and Technical Surveys in Ottawa, Ontario (now the Canada Centre for Mineral and Energy Technology, Department of Energy, Mines and Resources) where she is currently the head of the Research and Special Projects Group of the Mineral Sciences Chemical Laboratory. Her research interests are in solvent extraction and analytical chemistry with special emphasis on the development of instrumental methods for the determination of trace and small amounts of elements in rocks and in diverse ores and alloys. She has published about 40 papers and reports in these fields and has authored a book dealing with the analysis of rocks, ores and related materials. A second edition of this book is in progress. She is a member of the Chemical Institute of Canada and was a joint winner in 1971 of Talanta's Louis Gordon Memorial Award.

PROFESSOR SHU-CHUAN LIANG was born in 1912 in Chefoo, China. He obtained his B.Sc. from Yenching University, Peking in 1933, then studied for his Dr. phil. nat. (1937) at the University of Munich, Germany. After a short stay in the laboratory of Professor (at that time Dozent) F. Hecht in Vienna, Dr. Liang returned to China to teach analytical chemistry at West China Union University and then at Chungking University. In 1947 he joined Academia Sinica, and was elected a member of the Academy in 1955. From 1955 to 1965 he served as editor-in-chief of the journal *Acta Chimica Sinica*. He is a member of the editorial boards of *Oceanologia et Limnologia Sinica* and *Fenxi Huaxue* (*Chinese Journal of Analytical Chemistry*). He is particularly interested in the analytical chemistry of rare elements and also micro and trace analysis. He is the author of more than seventy papers.



PROFESSOR R. J. MAGEE is a native of Northern Ireland, and holds the degrees of B.Sc., M.Sc., Ph.D., D.Sc. He is also a Fellow of the Royal Australian Chemical Institute, the Institute of Chemistry in Ireland, and the Royal Chemical Society. He has held posts ranging from Assistant Lecturer, through all grades up to Professor. He is currently Foundation Professor at La Trobe University, in the Department of Inorganic and Analytical Chemistry. He has held visiting professorships at the Case Institute of Technology, Trinity College Dublin, Carleton University, Graz University, Athens University, the Indian Institute of Science and the National University of Malaysia. He is interested in research on inorganic and analytical chemistry, including thermal, spectral, magnetic and structural studies on metal complexes, with particular reference to sulphur ligand complexes, studies on the electrochemistry of transition-metal systems and the kinetics and mechanisms of electrode processes, and the use of electroanalytical and thermoanalytical methods in the determination of potentials in natural waters and the atmosphere. He has published some 280 papers on the main areas of his research interests.



PROFESSOR PELLERIN was born in 1923 in Normandy, and is now Professor of Pharmacy in the University of Paris (South). His research activities in analytical chemistry have developed along two lines. The first is organic functional group analysis, including study of organic solvents for protometric and functional analysis reactions in two-phase solvent systems, and various studies on the reactivity and stability of organic molecules as a function of structure. The second line involves studies on analytical chemistry applied to pharmaceuticals, including quality control, study of additives (colours, preservatives, antioxidants), and studies of stability. The results obtained in this last branch of work have led him to consider the plastics used in the pharmaceutical and surgical fields. The interaction between plastics and pharmaceuticals or biological media originates (and finds its explanation) in the relation between the physicochemical properties and the structure and composition of the materials. In this domain, Professor Pellerin has been especially concerned with the role of the reactivity of organic molecules and the chemical properties of the functional groups. Professor Pellerin is the Secrétaire Technique de la Commission de Pharmacopée Française, Member of the National Academy of Pharmacy, and President of the Analytical Division of IUPAC.

SUBJECT INDEX

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NOTICE

AMERICAN VACUUM SOCIETY

29TH NATIONAL VACUUM SYMPOSIUM AND SHOW

15–19 NOVEMBER 1982

Baltimore Convention Center, Hyatt Regency Hotel, Baltimore, MD

The 29th National Symposium of the American Vacuum Society will be held at the Baltimore Convention Center, Baltimore, Maryland, 15–19 November 1982. Topics include Electronic Materials and Processing, Surface Science, Thin Films, Vacuum Technology, Fusion Technology, Vacuum Education and Vacuum Metallurgy. There will also be a comprehensive exhibit of related equipment and a number of short courses.

SESSION TOPICS

ELECTRONIC MATERIALS AND PROCESSING
Metal-Semiconductor Interfaces
Atomic and Electronic Structure of Semiconductor Surfaces
Growth and Structure of Interfaces of Electronic Materials (Poster Session)
Epitaxy and Growth (MBE)
Beam Processing
Oxide–Semiconductor Interfaces
Dry Processing
Microanalysis of Electronic Devices

SURFACE SCIENCE
Reactions and Interactions at Surfaces
Electronic Structures and Bonding
Surface Geometries
Radiation-Induced Surface Chemistry and Physics
Vibrational Properties
Absorption and Defects
Exciting New Directions
Surface Science (Poster)
Post-Deadline Discoveries (Special Evening Session)

FUSION TECHNOLOGY
Design and Construction of Large Vacuum Systems
Leak Testing and Leak Repair of Plasma Devices
Plasma Diagnostics
Plasma–Wall Interactions
Vacuum Materials Development
Inertial Confinement Target Development
Handling, Storage and Recovery of Radioactive Gases
Neutral and Negative Ion Beam Injection Technology
Fueling of Plasma Devices
Industrialization of Fusion Power (Special Evening Session)

THIN FILMS
Ion Beam Sputtering and Deposition
Ion Beam Induced Chemical Sputtering
Techniques for Modifying Thin-Film Properties
Sputtering of Multicomponent Materials
Surface Preparation for Thin Film Deposition
Thin Films for Biomedical Applications
Ohmic Contacts and Metallization Thin-Film Physics (Special Poster Session)

VACUUM TECHNOLOGY
Mechanical Motion in Vacuum
Reliability of Vacuum Measurements
Computer Uses in Vacuum Equipment and Processes
Vacuum Joints, Bonding and Seals
Storage Rings
Vacuum Processes for Microelectronics
High Vacuum Insulation and Switch Design
Microbalance Techniques

FUSION TECHNOLOGY/VACUUM TECHNOLOGY
Pumping and Pressure Measurements in Fusion Devices
ASTM (E-42 Committee)
Quantitative Interface Analysis

EDUCATION

VACUUM METALLURGY

For further details contact:

American Vacuum Society
335 East 45th Street
New York, New York 10017
U.S.A.

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NOTICE

1983 PITTSBURGH CONFERENCE AND EXPOSITION ON ANALYTICAL CHEMISTRY AND APPLIED SPECTROSCOPY

The 34th Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy will convene 7–12 March 1983 in Atlantic City, New Jersey, USA. Atlantic City was chosen as the meeting site for the 4th consecutive year because of the very successful 1982 Conference and Exposition.

PAPERS ARE REQUESTED IN THE FOLLOWING CATEGORIES:

01. Air Pollution Analysis
02. Atomic Absorption Spectroscopy
03. Atomic Fluorescence Spectroscopy
04. Automated Analysis—
    a. Laboratory
    b. Plant
05. Biochemical Pharmaceutical
06. Biomedical Pharmaceutical
07. Classical Chemical Analysis
08. Clinical Chemistry
09. Computer Applications
10. Electrochemistry
11. Electron Spin Resonance
12. Emission Spectroscopy
13. Flame Emission Spectroscopy
14. Fluorescence-Luminescence
15. Food Analysis
16. Forensic and Drug Analysis
17. Fourier Transform Methods—
    a. Infrared
    b. NMR
18. Gas Chromatography—
    a. Applications
    b. Instrumentation
19. Gel Permeation Chromatography
20. General Analysis
21. Infrared Spectroscopy
22. Ion Chromatography
23. Liquid Chromatography—
    a. Applications
    b. Instrumentation
24. Mass Spectrometry
25. Microwave Spectrometry
26. Microscopy
27. Mössbauer Spectrometry
28. New Instrumentation
29. New Instrument Concepts
30. Nuclear Magnetic Resonance Spectroscopy
31. Pesticide Analysis
32. Photoacoustic Spectroscopy
33. Plasma Emission Spectrometry—
    a. ICP
    b. DC
34. Polarography
35. Polymer Analysis
36. Process Stream Analysis
37. Raman Spectroscopy
38. Selective Ion Electrodes
39. Surface Analysis
    a. Auger
    b. ESCA
    c. SIMS
40. Thermal Analysis
41. Thin Layer Chromatography
42. Toxicological Analysis
43. Trace Analysis
44. UV–VIS Spectrophotometry
45. Water Pollution Analysis
46. X-Ray Diffraction/Emission Spectroscopy
47. Other (Please Specify)

Papers may be contributed in all areas of the disciplines of Analytical Chemistry and Applied Spectroscopy.

Those authors wishing to present papers at the 1983 Pittsburgh Conference should submit five copies of a 300-word abstract to:

Mrs. Linda Briggs, Program Secretary
Pittsburgh Conference
437 Donald Road, Department J-029
Pittsburgh, PA 15235, U.S.A.

The abstract should be complete and show:

(a) The title of the paper.
(b) The name of the author(s), the organization(s) in whose laboratory the work was done, and the address(es). Provide the *COMPLETE* mailing address of each author, including department and any other mailing code.
(c) The name of the author who will present the paper must be *underlined.*
(d) Sign and date the abstract page in verification that the paper and all material therein has not been published or previously presented.

The final date for receipt of abstracts is 15 August 1982. Abstracts received after this date cannot be guaranteed consideration for inclusion in the 1983 Technical Program.

In 1982, the Modern Laboratory Equipment presented at the Conference totalled 560 exhibitors occupying 1380 booths and 35 seminar rooms in which was displayed the latest equipment available in the areas of Analytical Chemistry and Spectroscopy. Anyone desiring to reserve exhibit space or to obtain additional information regarding the 1983 Exposition should contact:

Mr. Paul E. Bauer, Exposition Chairman
Pittsburgh Conference
437 Donald Road, Department J-029
Pittsburgh, PA 15235, U.S.A.

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PROFESSOR WIKTOR KEMULA ON HIS 80th BIRTHDAY

Professor Wiktor Kemula started his research and teaching activity in 1925 in the Jan Kazimierz University in Lwów. In 1939 he was nominated as Professor of Inorganic Chemistry at the University of Warsaw, but he was unable to take up this post until 1945, when he contributed significantly to the reconstruction of the activity of the University Chemistry Section, of which he was Chairman for many years. In 1956–58 he was the Vice-Rector of the University. In 1969 he was moved to the Institute of Physical Chemistry of the Polish Academy of Sciences. Since 1956 he has been a member of the Polish Academy of Sciences. For many years Professor Kemula was very active in the Polish Chemical Society, being its President in 1955–60. In 1976 he was honoured by being elected the Honorary President of the Society. In 1955 he initiated the Committee for Analytical Chemistry of the Polish Academy of Sciences, and was its Chairman until 1980, when he was distinguished with the title of Honorary Chairman of the Committee. His activity also extended abroad. Since 1947 Professor Kemula has been a member of IUPAC, and from 1969 to 1972 was the President of the Analytical Chemistry Division.

The scientific achievements of Professor Kemula cover many aspects of physical and analytical chemistry. He has worked in spectroscopy, photochemistry, electrochemistry and chromatography, but his finest achievements are in anodic stripping polarography and chromatopolarography—in both fields he was considered the founder and best expert. He also obtained excellent results in HPLC and application of clathrates for separation of organic compounds. In total he has published more than 400 scientific papers and a few academic textbooks. He has presented the results of his studies in numerous international conferences and during his scientific travels to all significant centres in the world. From his former students twenty are presently professors working at universities and in scientific institutes. He has been awarded the State Prize several times, and also many honours abroad.

He is a member of The German Academy "Leopoldino". The Czechoslovak Chemical Society, The Royal Chemical Society and the New York Academy of Sciences, and an honorary member of the Japan Society for Analytical Chemistry and of the Société de Chimie Industrielle.

In spite of his 80 years Professor Kemula is still very active, participating in meetings, and helping his younger colleagues and co-workers with advice and experience. He always has at heart the development of science and the interests of his country. In his life he does not exclude other fields of human activity. He is a well-known connoisseur of music and the fine arts. He has a broad knowledge of history and is a brilliant source of information for all who have the opportunity to talk with him. On the occasion of his 80th birthday his former students, and his co-workers and colleagues wish him long years of further successes and good health.

ADAM HULANICKI

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PUBLICATIONS RECEIVED

The Principles of Ion-Selective Electrodes and of Membrane Transport: W. E. MORF, Elsevier, Amsterdam, 1981. pp. 416. DF1.170, US$83.00.

This is a book full of equations describing the behaviour of ion-selective electrodes, backed up by judicious comment on the relationship of the equations to practical experience. Mathematical results, rather than proofs, are presented and the great advantage of the book is that it sets out rival theories in a common notation and so makes comparison much simpler. It is not a book for the practising analyst, but I do strongly recommend it for the post-graduate student and full-time researcher in ion-selective electrodes.

The first 160 pages gives a general discussion of membrane potentials and membrane transport, including diffusion potentials and liquid-junction potentials. The rest of the book uses the theory to illustrate the behaviour of ion-selective electrodes in regard to response, limit of detection (to some extent) and especially selectivity. The chapters on solid-state (actually only silver compounds), liquid ion-exchange and neutral carrier electrodes are all very good. Glass electrodes are also covered and two rather make-weight chapters discuss time responses and gas-sensing and enzyme electrodes.

The text is a direct copy from double-spaced typescript but is reasonably legible. It is regrettable that the publishers did not employ an English editor to remove a few minor but quite frequently occurring Germanicisms: Morf's style is lucid and deserves better than this petty economy.

DEREK MIDGLEY

Membrane Filtration—Applications, Techniques and Problems: BERNARD J. DUTKA (editor) Dekker, New York, 1981. Pp. XI + 612. Price

This book, volume 17 in a series on Pollution Engineering and Technology, presents a detailed, up-to-date review of membrane filter technology. There are 21 chapters, written by 31 contributors from Brazil, Canada, Japan, New Zealand, South Africa, the U.K., and the U.S.A. The text is authoritative and international; four of the chapters present the current European, Japanese, New Zealand and South African views of membrane filtration, resulting from the differences in legislation and water purity standards that exist internationally.

This does not set out in any sense to be an analytical chemistry text, yet its contents are of great importance for analytical chemists engaged in a wide range of environmental and industrial applications involving water purification and processing. The first chapter gives an excellent account of the history of development of membrane filters, their modes of preparation, and characteristics. Other chapters are distinctly microbiological or bacteriological, reflecting the now established practice of removing coliforms and other organisms of faecal origin from potable water supplies by means of membrane filtration. The final chapter gives a good account of the extent to which membrane filtration has already become established in the fermentation, pharmaceutical, and other industries. There is clearly a lot of development potential left in this new technology; the importance for analytical chemists lies in the extent to which standards of water purity *etc.* will change for the better with a consequent demand for analytical methods of greatly improved sensitivity and specificity for all types of aqueous systems.

D. M. W. ANDERSON

Treatise on Analytical Chemistry, 2nd Ed., Part I, Vol. 5: P. J. ELVING, E. CRUSHKA and I. M. KOLTHOFF (eds.), Vol. 7: P. J. ELVING, E. J. MEEHAN and I. M. KOLTHOFF (eds.), Wiley-Interscience, New York, 1981. Vol. 5: pp. xxix + 668, £48.00. Vol. 7: pp. xxviii + 816, £48.00.

The superlatives that have already been lavished on the first edition of the treatise continue to be deserved by the second edition. These two volumes, like the preceding two issued (Vols. 1 and 2), show the great advances made in analytical science since 1959. Volume 5 deals with decomposition of samples, giving a brisk survey of the field, but most of the book (over 90%) deals with separation techniques, some of which were virtually undeveloped at the time of the first edition of the treatise. There are surveys of mechanical methods, membrane processes, ultrafiltration membranes, liquid membranes, crystallization and precipitation, bubble methods, distillation, solvent extraction, and countercurrent distribution, with a general introduction by Calvin Giddings. Volume 7 deals with optical methods, covering principles and equipment, luminescence methods (fluorimetry and phosphorimetry), infrared spectroscopy, emission spectroscopy, flame emission spectrometry, and atomic-absorption methods. As expected, the individual contributions are authoritative, well documented, and up to date, but the index is rather on the short side in view of the contents, and there is not enough cross-indexing or choice of key words. The reader looking for information on diode arrays as detectors, for example, would need to know that they were included under vidicons in the index or would need to scan the contents list rather carefully. This is a minor criticism, however, and analysts will turn as eagerly to this edition as they did to the first, for rapid and reliable orientation in any field of analytical chemistry.

R. A. CHALMERS

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NOTICES

ELECTROANALYSIS SYMPOSIUM

CARDIFF, WALES, 5–8 APRIL 1983

An international symposium on Electroanalysis in Biomedical, Environmental and Industrial Sciences, organized by the Electroanalytical Group and Western Region of the Analytical Division of the Royal Society of Chemistry, is to be held at UWIST, Cardiff, from Tuesday 5 April to Friday 8 April 1983. The first circular is now available and may be obtained from the Short Courses Section, UWIST, Cardiff, U.K.

CHEMOMETRICS IN ANALYTICAL CHEMISTRY

PETTEN, THE NETHERLANDS

15–17 SEPTEMBER 1982

The application of chemometrical techniques in analytical chemistry is of growing importance. However, there is still a gap between chemometrics and its application in daily analytical practice. Accordingly, the theme of the conference will be "Chemometrics in Analytical Chemistry", with special emphasis on practical applications. The scientific committee expects the selected topics to be of benefit to scientists active or interested in the introduction of chemometrics into analytical chemical practice. The scientific program will include invited plenary lectures, invited and submitted research papers.

Topics

Application and development of formal techniques for design, optimization and evaluation of analytical procedures and results.

Application of systems theory, operations research, information theory, statistics, and other chemometrical techniques in analytical chemistry.

Data retrieval, pattern recognition, artificial intelligence, etc.

Computerized signal- and data processing, optimum filtering techniques, noise reduction methods.

Education in chemometrics.

Secretariat address

International Conference on Chemometrics in Analytical Chemistry,
CAC-Holland Laboratory for Analytical Chemistry,
University of Amsterdam, Nieuwe Achtergracht
166, 1018 WV Amsterdam, The Netherlands

9th INTERNATIONAL SYMPOSIUM ON MICROCHEMICAL TECHNIQUES

AMSTERDAM, 28 AUGUST–2 SEPTEMBER 1983

ISM 83 will cover both pure and applied aspects of analytical chemistry related to microchemical techniques and trace-analysis, similar to the preceding symposia. Special attention will be paid to modern techniques as well as to newer fields of application such as flow-analysis; analysis of microvolumes, surfaces and thin films; analysis of aerosols, coal and fly-ash.

Symposium Secretariat

c/o Municipal Congress Bureau,
Oudezijds Achterburgwal 199
1012 DK Amsterdam, The Netherlands

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CHEMOMETRICS IN ANALYTICAL CHEMISTRY

PETTEN, THE NETHERLANDS

15–17 SEPTEMBER 1982

The application of chemometrical techniques in analytical chemistry is of growing importance. However, there is still a gap between chemometrics and its application in daily analytical practice. Accordingly, the theme of the conference will be "Chemometrics in Analytical Chemistry", with special emphasis on practical applications. The scientific committee expects the selected topics to be of benefit to scientists active or interested in the introduction of chemometrics into analytical chemical practice. The scientific program will include invited plenary lectures, invited and submitted research papers.

Topics

Application and development of formal techniques for design, optimization and evaluation of analytical procedures and results.

Application of systems theory, operations research, information theory, statistics, and other chemometrical techniques in analytical chemistry.

Data retrieval, pattern recognition, artificial intelligence, etc.

Computerized signal- and data processing, optimum filtering techniques, noise reduction methods.

Education in chemometrics.

Secretariat address

International Conference on Chemometrics in Analytical Chemistry,
CAC-Holland Laboratory for Analytical Chemistry,
University of Amsterdam, Nieuwe Achtergracht
166, 1018 WV Amsterdam, The Netherlands

9th INTERNATIONAL SYMPOSIUM ON MICROCHEMICAL TECHNIQUES

AMSTERDAM, 28 AUGUST–2 SEPTEMBER 1983

ISM 83 will cover both pure and applied aspects of analytical chemistry related to microchemical techniques and trace-analysis, similar to the preceding symposia. Special attention will be paid to modern techniques as well as to newer fields of application such as flow-analysis; analysis of microvolumes, surfaces and thin films; analysis of aerosols, coal and fly-ash.

Symposium Secretariat

c/o Municipal Congress Bureau,
Oudezijds Achterburgwal 199
1012 DK Amsterdam, The Netherlands

Talanta, Vol. 29, p. ii, 1982
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NOTICES

ELECTROANALYSIS SYMPOSIUM

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Talanta, Vol. 29, p. iii, 1982
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PUBLICATIONS RECEIVED

Ion-Selective Electrodes, 3: E. PUNGOR (ed.), Elsevier, Amsterdam, 1982. pp. 428. $100.00.

This book consists of the papers presented at a conference held in Mátrafüred, Hungary in October 1980. These papers are divided into four plenary lectures, five keynote lectures and twenty-three discussion papers. The first two groups of lectures constitute approximately one third of the book and true to the pattern of this style of lecture most specialist workers will have read or heard most of it all before. Each of the discussion papers is followed by an account of the questions and answers that arose from the presentation. Regardless of Professor Pungor's claim of equal division of time between presentation and discussion, I get the impression that question time was occasionally moribund and I feel that its inclusion adds little to the book. The discussion papers are predominantly theoretical and those directly concerned with analysis are in the minority. These papers are mainly from Eastern European countries and they give a fair indication of their current interests in ion-selective electrodes.

I consider this book contains too few interesting papers, and it could only be recommended to the specialist intent on complete coverage of his field.

K. TORRANCE

Separation and preconcentration methods in inorganic trace analysis: J. MINCZWESKI, J. CHWASTOWSKA and R. DYBCZYNSKI, Horwood, Chichester, 1982. pp. xi + 543. £37.50.

The aim of the authors in writing this book was to provide a text for both experienced and uninitiated analysts who work with methods for separating and preconcentrating trace constituents. Within the constraints that they have set themselves on the subject, they have succeeded, and produced a useful book. The authors have dealt with methods based on precipitation, co-precipitation, volatility of constituents, extraction, ion-exchange and reversed-phase chromatography. These aspects of analysis are dealt with in general terms and in sufficient detail for the book to be a practical aid in the laboratory. Each chapter reviews the subject and is followed by a full international and up to date collection of references which more than covers any detailed procedures which have been omitted from the text. The theoretical principles on which the methodology is based are discussed in sufficient detail to permit the application of the theory to practice.

The book is arranged in seven chapters. The first two deal with problems and working techniques in trace analysis. The third chapter discusses the problems associated with methods of precipitation and the application of these methods to trace analysis. Chapter 4 is concerned with volatilization of substances as an analytical technique and the problems of volatility associated with various ashing techniques. The fifth chapter is concerned with liquid–liquid extraction and the application of extractions in separation processes. A comprehensive review of the subject is dealt with in the text and in tabular form. Chapter 6 is the largest and perhaps the most detailed in the book and deals with the materials, theory and practice of ion-exchange chromatography. Various application methods are dealt with textually and in tabular form. The seventh is a brief chapter dealing with extraction chromatography, again theoretically and applied to practice.

Because of the field in which the authors work, there is slight bias towards the examination and solution of problems of trace analysis in nuclear research, but the book loses nothing by this and is easily read, a compliment to the authors and the translator.

AUBREY GELMAN

Talanta, Vol. 29, p. iii, 1982
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Talanta, Vol. 29, p. iii, 1982
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PUBLICATIONS RECEIVED

Liquid Chromatography of Polymers and Related Materials III (Chromatographic Science Series, Volume 19): JACK CAZES (ed.), Dekker, New York, 1981. Pages viii + 299. S.Fr. 108.

This third volume in the series comprises fourteen papers presented at the International GPC Symposium, October 1980. As in earlier volumes in the series most of the contributions deal with practical aspects of gel permeation chromatography (GPC). This volume includes such topics as characterization of oligomers, polyphosphazines and branched block copolymers, and applications of GPC to polyolefins and poly(olefin-sulphone)s. Two new developments in GPC columns are described—coiled microcolumns for fast GPC and polystyrene bonded silica as a column packing.

G. GORDON CAMERON

Nothing but Motion: DEWEY B. LARSON, North Pacific Publishers, Portland, Oregon, 1979. Pages xvi + 292. $9.50.

This book is an introduction to a new theory which is said to be applicable to the whole field of physical science. As the title implies, the author regards the entire universe as composed of motion and material objects as manifestations of this motion. While the notion of a "universe of motion" has a certain appeal in connection with the relativistic quandaries of space and time, this "reciprocal theory" does not look quite as satisfactory when applied to the more mundane problems of chemical science. This is in part due to the "college text" chemistry which is dealt with, and also to the unfamiliar notation which the new theory requires for its application in this area. Whether this book is Science Fact or Science Fiction is impossible for this reviewer to decide—but it certainly is thought-provoking.

R. ALAN HOWIE

Analysis of Pesticide Residues: H. ANSON MOYE (ed.), (Chemical Analysis, Vol. 58), Wiley, New York, 1980. Pages viii + 467. £25.75.

One might well ask 'Why write a monograph on this topic which is already covered by several well established handbooks?' The various authors have, I think, had different aims in mind, but each has contributed something useful to this volume. The chapter on chromatographic columns says much that will not be found in the standard reference texts and proves the necessity of saying it by analysing the performance of laboratories regularly engaged in pesticide analysis. This excellent introduction is followed by an updating review on detectors which also aims to assist the newcomer in choosing the most appropriate one for his problem. Shorter chapters on the application of TLC and HPLC to pesticide residue analysis are followed by one on the determination of chlorinated hydrocarbons. The chapter on acidic herbicides includes a number of detailed procedures, which is not the policy adopted in the other chapters, while chapter eight is almost a monograph in itself on organophosphorus pesticides, including much useful information on common and trade names, chromatographic data and so on. Carbamate insecticides are considered largely in terms of their determination by GLC after derivatisation, and then a small group of insect pheromones is dealt with, where the problem is one of resolving stereoisomers by GLC. An interesting and useful final chapter discusses U.S. Government requirements for pesticide residue analysis—this is a good guide to an administrator's jungle.

There is much useful information here, presented clearly and readably, though somewhat unevenly. A brief introduction covering classification of pesticides, their cycles in the environment, toxicities and uses, scale of production and persistence would have been a useful addition to what will certainly become a valuable complementary text to the official handbooks on the subject.

IAIN L. MARR

Colorimetric Chemical Analytical Methods: L. C. THOMAS, G. J. CHAMBERLIN and G. SHUTE, 9th Ed., Tintometer, Salisbury; Wiley, New York, 1980. $85.00.

This ninth edition of a book first introduced in 1953 still provides a useful series of methods relying on simple colorimetric equipment. The list of methods clearly includes many used by current users of the Lovibond Tintometer equipment: indeed one method (albeit with clear warning notices) still uses benzidine, at the insistence of an overseas user. The initial sections provide an introduction to the methods, including descriptions of ion-exchange resins, buffer solutions and the Lovibond range of apparatus. A section on the colorimetric determination of pH is followed by a section of some 27 organic methods, including ones for alcohol, amines, dichlorophen and sugars. Half of the book is devoted to the next section, on the determination of metals and other inorganic species. This section contains many well-known and useful methods. The next sections are concerned with methods used in biochemistry and related areas, and of toxic substances in air. The final section, entitled "Colour grading and quality tests", is a mixture of methods on colour grading of a variety of foods as a means of quality control, and of quality control tests such as the COD test.

This book is an invaluable source of simple methods which are suitable for use by lowly-qualified staff, and contains much information of value to the hardened analyst.

A. G. FOGG

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ERRATA

In the paper by Faix and Krivan in the April issue (p. 285) the heading of the last column in Table 1 should be

Proton energy
at σ_{max},
MeV

In the book review of "*Membrane Filtration*" in the May issue (p. ii), the price was omitted. It is US$69.50.

Talanta, Vol. 29. p. iii. 1982
Pergamon Press Ltd. Printed in Great Britain

PUBLICATIONS RECEIVED

Solid Surface Luminescence Analysis: ROBERT J. HURTUBISE, Dekker, New York, 1981. pp. xi + 274. S.Fr. 94.00.

This book is the first of a series of monographs devoted to recent advances in the field of analytical chemistry. As such, it must be considered as an extended review of publications over the last two decades, rather than an authoritative textbook of standard reliable methods. Many may wonder about the necessity of bringing together in one volume information about so many disparate subjects. Among the subjects covered are the design of commercial and research instruments, the theory of scattering from solid surfaces, spectroscopic considerations and analytical applications in a number of quite different disciplines. In many cases the substances being analysed do not constitute an integral part of a surface, but are merely components of a mixture separated on some chromatographic matrix, so that the interest lies in the separation, rather than the nature of the surface. Further, many of the identifications, or determinations made on the surface, are familiar from similar measurements made in solution. Nevertheless, the text will be useful to the analytical chemist in providing a guide to the possibilities of working with samples in the solid phase, without the necessity of time-consuming and irreproducible extraction procedures.

J. R. MAJER

Handbook of Practical Organic Microanalysis: S. BANCE, Horwood, Chichester, 1980. pp. 206. £18.50.

This is a really practical book which gives readers the benefits of the author's many years experience in the microanalytical laboratory of a major industrial organisation. It is meticulously compiled and covers practical details and hints which would be invaluable to anyone setting up a traditional microanalytical laboratory. Of necessity, the accent is on traditional methodology, on tried and proven methods, some of which have now been overtaken by instrumental techniques, but nevertheless have their place in a well organized and comprehensive microanalysis laboratory. It is well indexed. Each chapter relates to a particular element or group of elements and opens with a general discussion on the method and the theoretical background. It then proceeds to a detailed account of the apparatus and method with practical hints which are not often seen even in a practical chemist's text book. Altogether the book is a mine of information and knowledge gained by first hand experience and should prove to be a most useful addition to any library.

DORIS BUTTERWORTH

Analysis of Non-Metals in Metals, GUNTHER KRAFT (ed.), Walter de Gruyter, Berlin, 1981. pp. xiv + 546. DM 148.

This book is a record of the Proceedings of the International Conference held in West Berlin, 10–13 June 1980. There are 36 papers printed in French, German and English, the French and German papers being provided with short summaries in English. In addition to communications describing analytical problems using conventional techniques, there are some which are concerned with more recent instrumental methods. There is a large section devoted to activation analysis, the activation being realized by neutron bombardement, by radiation with charged particles and by exposure to high-energy gamma-rays. Among the physical methods involving the use of large instruments included in the text are spark-source mass spectrometry, plasma emission spectrography, electron microscopy and Auger electron spectroscopy. The methods used are applied to the determination of non-metals in both ferrous and non-ferrous metals. The final paper is the one which will be of most general interest in that it discusses the provision of reference samples for the analysis of non-metals in metals.

J. R. MAJER

Talanta. Vol. 29, p. III, 1982
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OBITUARY

G. GOPALA RAO
(1908–1981)

G. Gopala Rao, retired Professor of Chemistry, Andhra University, died on October 26, 1981 after a prolonged illness.

After his early education at the Rajah's College, Kakinada and Maharajah's College, Vizianagaram, Gopala Rao proceeded to Allahabad, where he took his M.Sc. degree in 1929. He then joined for research under Professor Nil Ratan Dhar and received the D.Sc. degree in 1932. When Andhra University organized the Honours courses in Chemistry in 1932, Gopala Rao joined the Chemistry Department as a founder member of the Faculty and rose to be the Professor in 1946 and Head in 1949.

Gopala Rao's early research interests were in the field of photochemistry, with particular reference to the development of analytical methods by application of the chemical action of light. During the course of these studies, his research interests moved to redox analytical processes, in which his contributions are recognized as very profound. Simultaneously, Gopala Rao also pioneered studies in catalysis in analytical processes, which was a precursor of the kinetic methods in analysis, a topic of current interest. His outstanding contributions in these fields were acknowledged by his being invited to act as a Regional Editor of *Talanta*.

Gopala Rao had taken a very active interest in initiating innovative academic programmes. The credit for organizing a full-fledged MSc. Analytical Chemistry course in India in 1957, goes to him. He encouraged research activities among his junior colleagues, which helped to sustain active research in Andhra University in the field of Analytical Chemistry, right up to the present.

After retirement from service in November, 1968 Professor Gopala Rao continued active research work as a University Grants Commission Retired Scientist and as Emeritus Professor in the Chemistry Department till March, 1979. The fact that as many as 40 research papers on the Analytical Chemistry of selenium, tellurium, arsenic *etc*., were published during this period, shows his dedication for research. The total number of research papers published by him is about 250. An unfinished programme undertaken by him was the writing of a monograph on the role of complexation methods in analytical procedures.

Professor Gopala Rao was awarded the Honary Degree of Doctor of Science by Andhra University on the occasion of its Golden Jubilee celebrations, for his outstanding contributions to teaching and research.

Professor Gopala Rao is survived by his wife, four sons and three daughters.

M. N. SASTRI

Talanta, Vol. 29, pp. iii to iv, 1982
Pergamon Press Ltd. Printed in Great Britain

PUBLICATIONS RECEIVED

Chlorinated Dioxins and Related Compounds: Impact on the Environment: Edited by O. HUTZINGER, R. W. FREI, E. MERIAN and F. POCCHIARI, Pergamon Press, Oxford, 1982. Pages 624, £37.50.

This is Volume 5 of the Pergamon Series on Environmental Science. It contains the proceedings of a workshop held in Rome in 1980—an attempt to bring together scientists of different disciplines in order to introduce a multidisciplinary and systematic approach to an emotive subject.

This major book succeeds admirably in presenting an up-to-date account of major environmental aspects of polychlorinated dibenzodioxins (PCDD) and chemically related compounds. The well laid-out text contains 52 refereed papers covering analytical methodology, the environmental fate and levels of PCDD, incineration, toxicology and metabolism as well as observations in man. The relationship between incineration and PCDD formation is particularly well documented, as are the scientific aspects of environmental findings around the town of Seveso after the accidental release of tetrachlorodibenzodioxin (TCDD).

Diagrams, graphs, photographs and tables are well reproduced and laid-out. The papers are well written and contain adequate up-to-date references. The summary and conclusions of the workshop and a good index serve to make this specialist text not simply a collection of important original papers, but more of a textbook covering all aspects of a topical subject of extreme importance. Purchase of this excellent book is recommended for all scientists interested in the environment.

D. M. W. ANDERSON

Undergraduate Instrumental Analysis, 3rd Ed.: JAMES W. ROBINSON, Dekker, New York, 1982. Pages xvii + 550. SFr. 75.

This book was originally written to provide undergraduates with a basic but comprehensive guide to instrumental analytical techniques. Its success is shown by the appearance of a third edition within 12 years. Although many books on instrumental techniques have been published in the last decade, only a few are really useful for a newcomer to the field. This one describes the principal features of most of the commonly encountered techniques in a simple and informative but sometimes idiosyncratic manner. Generally a non-mathematical approach has been adopted, except for a few simple and unavoidable mathematical equations, which makes the book easy to understand and probably helps to keep the student's interest in the text.

The latest edition consists of fifteen major chapters and an introductory section. The first chapter deals with the general concepts and definitions in analytical chemistry. The second and third chapters contain the basic laws and theories of spectroscopy, and provide a theoretical background for the next eight chapters, which are devoted to the different spectroscopic techniques. The last four chapters deal with chromatography, thermal analysis, mass spectrometry and electrochemistry. Each chapter ends with a bibliography, simple problems and some suggestions for experiments to familiarize the student with instrument handling. Most of the chapters have been revised and improved by adding recent developments in various techniques, and this has resulted in a significant expansion in the present edition.

It seems surprising, however, that the book contains little about atomic-fluorescence spectroscopy, which is an important and fully developed technique in analytical chemistry. Statements such as "A disadvantage of the method is that only one element can be determined at a time" and "solid samples must first be dissolved and the solution then analysed" (both from the atomic-absorption chapter) seem useful generalizations but may misguide the reader, since multichannel (or at least dual-channel) instruments are available and solid samples can also be analysed with carbon atomizers. Tables 7.1 and 9.2 (on flame temperatures) are not mutually consistent. The proof-reading seems rather careless.

However, these are minor points and do not affect the overall balance of the book, though they may cause problems for the student.

J. ANWAR

Advanced Mass Spectrometry: U. P. SCHLUNEGGER, Pergamon Press, Oxford, 180. Pages xii + 143. £12.50

Although completely new types of spectroscopy to aid structure elucidation of organic compounds are now rare, developments in existing methods continue and this book describes one of them. DADI (direct analysis of daughter ions)/MIKE (mass-analysed ion kinetic energy) spectrometry provides a method of deducing the origin of fragment ions in the mass spectrometer without recourse to traditional and time-consuming isotopic labelling.

The early chapters in the book describe ionization methods, basic concepts of mass spectrometry such as metastable ions, collisional activation and collision-induced dissociation and the design of single and double focusing mass spectrometers. Knowledge of these subjects is necessary to understand the basis of MIKE spectrometry in which the conventional Nier–Johnson geometry of the double-focusing mass spectrometer has been reversed, *i.e.*, the ions pass through the magnetic sector before the electrostatic sector. Certain ions such as the molecular ion are separated in the magnetic analyser and fragmented by collision with an inert gas in the second field-free region between the magnetic and electrostatic analysers. The electrostatic analyser separates the fragment ions and so a clear picture of the fragmentation of the ion initially separated in the magnetic analyser is obtained. A closely related technique—"linked scan"—is also described, and it too gives a "cleaner" picture of fragmentation patterns.

Chapters 6 and 7 (more than half of the book) provide a wide selection of examples of the power of DADI/MIKE spectrometry. Elucidation of the structure of complex natural molecules, including peptides (sequencing), is illustrated with many good examples. Frequently encountered isomerizations and rearrangements of ions in the mass spectrometer can also be detected and elucidated by using this technique.

The book is timely and coincides with the author's review in *Topics in Current Chemistry*, Vol. 95, in which the same subject is treated in less depth. The translation is generally good and the book should stimulate further interest in the subject. Non-specialists with a working experience of mass spectrometry should have little difficulty in understanding the concepts and hence broadening their view of mass spectrometry as an analytical tool.

A. R. FORRESTER

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PUBLICATIONS RECEIVED

Inorganic Reaction Chemistry, Vol. 2, Reactions of the Elements and Their Compounds, in two parts, A: **Alkali Metals to Nitrogen,** B: **Osmium to Zirconium:** D. T. BURNS, A. TOWNSHEND and A. H. CARTER. Ellis Horwood, Chichester, 1981. A, pp. 300, £27.50; B, pp. 280, £27.50.

These books are published as part of the Ellis Horwood Series in Analytical Chemistry and are linked to an earlier volume in the same series, "Systematic Chemical Separation" by Burns, Townshend and Catchpole, which is referred to as Volume 1 on the topic of Inorganic Reaction Chemistry. The reviewer has not yet seen Volume 1, which was published in 1980, but judged by the quality of the present volumes, the series of three books should provide an excellent up to date treatment of the separation reactions used in qualitative analysis and the specific reactions used for the identification of inorganic ions. Compared to older texts on this subject, the treatment contains much more detailed information on reactions with inorganic and particularly organic reagents, as well as a useful breakdown of reactions for the different oxidation states of relevant elements. All three books are products of the efforts, involving the detailed examination of suitable reactions, carried out by members of the Midlands Association for Qualitative Analysis. Since most reactions have been experimentally verified by members of the association, the practical detail is a valuable bonus, and the end result is an authoritative treatise on inorganic reactions which is much wider than that required for qualitative analysis procedures. As such the volumes will represent a laboratory handbook of considerable practical application in laboratories which suffer the submission of unknown substances for analysis.

The structure of the book as indicated by the subtitles, follows the alphabetical order of the elements. In a few cases such as the alkali metals, niobium and tantalum, rhenium and technetium, etc. elements have been grouped together because their chemistry and reactions are similar. Each chapter contains a brief summary of the chemistry of an element and its typical compounds, followed by a description of selected reactions of the element in the various forms in which it commonly exists. The selection of reactions for inclusion has been made after rejection of reactions considered unreliable. The reactions are simple and straightforward and can be carried out with apparatus no more extensive than a test-tube or spot-plate. The full range of reactions would, however, require an extensive collection of inorganic and organic reagents, although since numerous alternative reactions are given for each element or species, an exhaustive collection of reagents would rarely be needed. Remarks are also included on the toxicity of relevant elements and compounds. Numerous references to the reactions are given, and lists of the organic reagents referred to and useful solubility products are given in Appendixes. The two volumes are intended to go together, and as an aid in their use, the pages have been numbered consecutively through both volumes (apart that is from the pages at the beginning of each chapter which, although included numerically, have not been numbered). Advantage has been taken of this consecutive numbering, by the introduction of an index in volume 2, which covers both books. These volumes are obviously more suited to laboratory than library use and should be popular with both students and practising analytical chemists. A cheaper paperback version would be of great benefit and would ensure the wider appreciation of the valuable work carried out over many years by the authors and their colleagues in the Midlands Association for Quantitative Analysis.

J. M. OTTAWAY

Analysis of Drugs and Metabolites by Gas Chromatography–Mass Spectrometry, Vol. 7. B. J. GUDZINOWICZ and M. J. GUDZINOWICZ, Dekker, New York, 1980, pp. x + 557. S. Fr. 146.

This latest addition to the series devoted to descriptions of the analytical successes of the gas chromatograph–mass spectrometer combination deals with a recurrent contemporary problem, *viz.* the examination of inhaled smokes. The first section is concerned with the identification of the products of pyrolysis of tobacco, and also the analysis of the constituents of the original tobacco leaf. It is interesting that in the study of the polynuclear aromatic hydrocarbon fraction of cigarette smoke, the measurement of retention volumes plays a more significant part in the identification of individual compounds than does the mass spectrum.

The second section describes the application of the same techniques to the analysis of the chemical composition of the marijuana plant and the products of pyrolysis of marijuana. In the case of both intoxicants there are full descriptions of the methods used in the determination of metabolic products. As in previous volumes, there are always full experimental details of the apparatus used and precise summaries of results.

J. R. MAJER

Progress in Analytical Atomic Spectroscopy, Volume 2: C. L. CHAKRABARTI (ed.), Pergamon Press, Oxford, 1981, pp. v + 386. £33.00.

The second volume in this series contains five reviews, all written to a high standard by international authorites. "Atomic Fluorescence Spectrometry: Basic Principles and Applications", by Omenetto and Winefordner takes up almost half of the volume, and is an excellent comprehensive monograph on AFS. As might be expected, theoretical principles and recent novel developments and applications are particularly well covered, but early work too is adequately treated, much use having been made of clear and concise tables. "Trace Element Analysis of Food and Beverages by AAS", by Fricke, Robbins and Caruso, covers analytical atomic spectroscopy-based procedures, arranged by element, for the analysis of samples falling in this category. This review includes useful background information on how elements enter the food chain and, where appropriate, their role in nutrition, as well as extensive tables of published procedures. Subramanian and

Chakrabarti's "Determination of Trace Metals in Ultrapure Water", although one of the shorter contributions, is a useful concise introduction to possible problems and pitfalls for those contemplating (or completing) determinations at very low concentrations. "Interferences in Flame Spectrometry, their Elimination and Control", by Rubeška and Musil is a valuable account of current ideas on the nature of such interferences and underlying theoretical concepts. This contribution is to be commended for the way it brings together information from so many diverse sources to produce a concise and readable account which still manages to cover the topic in depth. Finally "Emission Spectroscopic Analysis Using Cool Flames, Part I" by Henden, Pourreza and Townshend is a summary, primarily based on the work done at Birmingham University, of external vapour generation systems for MECA. This review is however of value to anyone interested in vapour generation systems, regardless of whether they intend to use MECA or flame or plasma OES.

The book is well presented from camera-ready copy, and good value at the price by today's standards.

M. S. CRESSER

The Analysis of Explosives, JEHUDA YINON and SHMUEL ZITRIN, Pergamon Press, Oxford, 1981, pp. xii + 310. £9.35.

It may be considered that in the present disturbed era the appearance of a book devoted to the analysis of explosives is most timely, more particularly so when the book contains a chapter describing methods of detection of hidden explosives. In this modestly sized volume the authors have chosen to review all significant methods and analytical procedures for the determination of explosive materials. As a result, the treatment is often uneven, so that column chromatography methods, surely of only historical interest, are described in some detail, while the section on thermal methods consists only of a brief outline of the principles involved. Nevertheless, the text does contain a review of the more recent work on mass spectrometric methods. Apart from the familiar electron impact spectra, attention is drawn to spectra using alternative methods of ionization, such as field desorption and chemical ionization. Some reference is made to the production of negative ions from explosive materials, and to the less familiar fields of ionization at atmospheric pressure and plasma chromatography. While it is unlikely that many practising analytical chemists will be required to carry out routine examinations of explosive materials, it remains important that they should be capable of making such examinations when required, and therefore this text should be on their bookshelves.

J. R. MAJER

Analytical Isotachophoresis: F. M. EVERAERTS (ed.), Elsevier Amsterdam, 1981, pp. xi + 234. $58.50.

This book constitutes a record of the Proceedings of the 2nd International Symposium on Isotachophoresis held at Eindhoven, 9–11 September 1980. There are 29 short papers describing advances in the theoretical aspects of the subject, instrumentation and data handling, and applications to analytical problems. The versatility of the isotachophoretic method may be gauged by the diversity of the samples which have been submitted to analysis. These range from medium and high molecular-weight materials of biological origin to simple mixtures of condensed phosphates or carboxylate ions. The automation of the technique and the reduction of data with the aid of microprocessors is also considered. The book provides an overview of the state of development of a rapidly expanding analytical technique.

J. R. MAJER

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GAINS AND LOSSES—ERRORS IN TRACE ANALYSIS

Talanta. Vol. 29, p. iv, 1982
Pergamon Press Ltd. Printed in Great Britain

NOTICES

SECOND INTERNATIONAL CONFERENCE on CARBONACEOUS PARTICLES IN THE ATMOSPHERE

11–14 September 1983

Linz, Austria

organized by the
"Österreichische Gesellschaft für Mikrochemie und Analytische Chemie" in the
"Gesellschaft Österreichischer Chemiker"

CALL FOR PAPERS

The purpose of this conference is to provide an international forum for reviewing current research on characterization, sources, transport, formation, transformation, and effects of carbonaceous particles in the atmosphere. In the context of this conference, the term "carbonaceous particles" is used to describe organic and inorganic carbon-containing species associated with suspended particles.

Carbon is the largest elemental fraction of aerosol particles, and carbon-containing particles may influence global chemistry, cause loss of visibility, and adversely affect ecological systems, including human health. Carbonaceous particles and their gaseous organic precursors may also play an important role in cloud chemistry and physics, *e.g.*, in the formation of acid rain, and are important ingredients in the chemistry of polluted atmospheres.

During the last decade, there has been a dramatic resurgence of interest in this pollutant, as evidenced by the extent and diversity of research presented at the First Conference on Carbonaceous Particles in the Atmosphere, held at Lawrence Berkeley Laboratory, Berkeley, California, in 1978. That conference attracted 157 participants and 45 presentations.

The Second International Conference on Carbonaceous Particles in the Atmosphere (Linz, Austria) takes place 5 years after the first conference in Berkeley and focuses on the same general subject—carbonaceous particles.

Both invited and contributed presentations for this conference should be focused on (but not limited to) the following topics.

Sources, transport and atmospheric chemistry
Climate effects, visibility degradation and other physical effects
Analytical chemistry and measurement techniques, including sampling
Ecological, biological and human health effects, effects on materials

Conference Chairman:
Prof. Dr. H. Malissa

Conference Vice Chairman:
Prof. Dr. T. Novakov

Further information may be obtained from the Conference Secretary: Dr. H. Puxbaum, *Institute for Analytical Chemistry, Technical University of Vienna, Getreidemarkt 9, A-1060 Wien, Austria.*

3rd CONGRESS OF FUNDAMENTAL AND APPLIED MASS SPECTROMETRY

Palaiseau, France, 5–8 April 1983

This Congress is organized jointly by the Groupement pour l'Avancement des Méthodes Spectroscopiques et Physico-chimiques d'Analyse (G.A.M.S.) and the Ecole Polytechnique, Palaiseau.

Themes covered by papers in the fundamental field will include:

Negative Ions
The Chemistry of Positive Ions (Structure, Fragmentation Mechanisms, Kinetic Energy, *etc.*)
Photoionization by Laser and Collisional Excitation and in the applications field:

Identification and Analysis of Substances Affecting Man
Secondary Ion Mass Spectrometry
Applications of Isotopic Analysis

Details may be obtained from *G.A.M.S., 88 Boulevard Malesherbes, F 75008 Paris, France.*

Talanta. Vol. 29, p. iv, 1982
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Talanta, Vol. 29, p. v, 1982
Pergamon Press Ltd. Printed in Great Britain

EDITORIAL

This special issue on gains and losses in trace analysis is intended to form a "Paper Symposium", in which a number of papers on a common theme, written by invited experts in the field, are gathered together to give a survey of current developments and thinking. The idea of such an issue was long cherished by the late Professor Belcher, who was associated with *Talanta* from the inception of the journal, and we would therefore like to dedicate it to his memory. He was the moving spirit behind the issue, and though, unfortunately, he did not live to see it appear, he knew that all had been prepared. The organization of the symposium was very capably undertaken by Dr. Peter Tschöpel in Europe, with assistance from Dr. John Moody in the United States.

The gains and losses that arise in trace analysis can stem from any stage of the analysis, from sampling through to calculation and reporting of results, and can arise from the materials and equipment used, and from the techniques themselves. Practically all these aspects are dealt with in the symposium, and the perceptive reader will readily see that some sources of error are less obvious than others, the background errors in Zeeman atomic-absorption spectrometry being a case in point.

Devotees of dictionaries and analytical conferences will know that a symposium is a drinking party. Extraction methods are frequently used in trace analysis. We suggest that readers of this Paper Symposium on trace analysis may help this issue live up to its name, by drinking in the information given in it, and extracting the wisdom from its pages.

Talanta, Vol. 29, p. v, 1982
Pergamon Press Ltd. Printed in Great Britain

ERRATA

In the paper by K. K. Stewart in the November issue (*Talanta*, 1981, **28**, 789), equation (2) on page 795 should read

$$t(\text{eq}) = k_1 \ln (C_a/nC_b) + k_2$$

The name of the third author of the paper "Spectrophotometric determinations of isoniazid with metol and vanadate, ferricyanide or iron(III)", *Talanta*, 1981, **28**, 477, was incorrectly spelt, and should be S. S. N. Murthy.

Talanta, Vol. 29, pp. v to vi, 1982
Pergamon Press Ltd. Printed in Great Britain

NOTICES

INTERNATIONAL CONFERENCE AND EXHIBITION ON ANALYTICAL CHEMISTRY (SAC 83)

The University, Edinburgh, Scotland, July 17–23 1983

SYMPOSIUM ON "ANALYTICAL INORGANIC MASS SPECTROMETRY"

This Symposium is organized, on behalf of the delegates to the Symposium on Inorganic Mass Spectrometry held at EUROANALYSIS IV in Helsinki 1981, by Professor F. Adams and Dr. A. M. Ure in collaboration with the Analytical Division of the Royal Society of Chemistry.

The Symposium will take the form of a one-day programme of invited and contributed lectures on the themes of "ANALYTICAL APPLICATIONS AND METHODOLOGY" and "NEW TECHNIQUES AND INSTRUMENTATION". A poster session will also be provided and the Symposium will be concluded with an Open Forum Discussion Meeting on "FUTURE NEEDS AND DEVELOPMENTS". The Symposium will form a part of the wide-ranging Programme of SAC 83.

Contributed papers on any aspect of Analytical Inorganic Mass Spectrometry including IDMS, FTMS, LAMMA, PSMS, SIMS, SSMS, TSMS *etc.* will be welcomed.

All those interested in attending this Symposium and SAC 83 should request a copy of the SAC 83 2nd circular from The Secretary, Analytical Division, Royal Society of Chemistry, Burlington House, London W1V 0BN, U.K. Please also indicate your interest in the Symposium and/or your intention to present a paper at it if appropriate. The 2nd circular will be available in June 1982.

THE 23rd COLLOQUIUM SPECTROSCOPICUM INTERNATIONALE INCLUDING THE 10th INTERNATIONAL CONFERENCE ON ATOMIC SPECTROSCOPY

Amsterdam, 26 June–1 July 1983

Coverage of the following techniques is envisaged:

1. Atomic-emission spectroscopy
2. Atomic-absorption spectroscopy
3. Atomic-fluorescence spectroscopy
4. X-Ray spectroscopy
5. Infrared and Raman spectroscopy
6. Ultraviolet and visible spectroscopy
7. Methods for surface and thin film analysis
8. Mass spectroscopy for inorganic analysis
9. Laser spectroscopy

The fundamental and instrumental aspects of these techniques as well as their applications will be dealt with in either specialized sessions or more general symposia with a special emphasis on the following issues:

A. Detection systems
B. Standard reference materials and methods
C. Environmental analysis
D. Biological, clinical and pharmaceutical analysis
E. Analysis of metals and industrial materials

F. Geochemical and petrochemical analysis
G. Agricultural and food analysis

Internationally recognized authorities in these fields will review the current trends and future perspectives in the respective fields. Major efforts will be undertaken to promote interdisciplinary coverage and informal contacts. Plenary lectures will run consecutively and parallel sessions of oral presentations will be minimized by emphasizing the role of poster presentations.

An instrument exhibition will be held in the lounges around the lecture halls.

The conference languages are English, French and German.

Social events including a reception in the Rijksmuseum, a conference banquet, and an excursion featuring characteristics of the Netherlands will be organized.

Accompanying persons can choose from a variety of daily excursions.

For further details write to:

23rd CSI
c/o Organisatie Bureau Amsterdam BV
Europaplein
1078 GZ Amsterdam
The Netherlands

1st INTERNATIONAL SYMPOSIUM ON DRUG ANALYSIS: FROM PHARMACEUTICAL PREPARATION TO DRUG MONITORING

Sponsored by the F.I.P.

Free University of Brussels (U.L.B.), Campus Plaine-1050 Brussels, 7–10 June 1983

The purpose of the symposium is to bring together people from Industry, Universities, Control laboratories and Hospitals to discuss the current status of analytical techniques, including instrumental applications as well as theoretical developments. The topics are as follows

(1) Naturally occurring drugs.
(2) Analytical problems in the development of drug formulation.
(3) Control of pharmaceutical specialities.
(4) Determination of drugs in food products for human or animal use.
(5) Determination of drugs in biological media, including drug monitoring.
(6) Research into toxic reactions due to drugs.

A limited number of plenary and keynote lectures will be given by invited speakers. Contributed papers will be either poster presentations or oral presentations.

Panel discussions may be organized.

The official languages of the conference are English, French and Dutch.

For further information please contact:

Ms C. Van Kerchove (Secretary)
Société Belge des Sciences Pharmaceutiques—Belgisch Genootschap voor Pharmaceutische Wetenschappen
rue Archimedesstraat 11
B-1040 Brussels
Belgium
Tel. (02) 733 98 20 ext. 33

Talanta, Vol. 29, pp. V to VI, 1982
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PROFESSOR RONALD BELCHER

Professor Belcher, Co-Chairman of the Advisory Board and Chairman of the Editorial Board of *Talanta*, was awarded an Honorary Doctorate of Science by the University of Saarbrücken in February 1981. The presentation was made by Professor Friedrich Tomi, Dean of the Faculty of Mathematical Sciences.

Die

Mathematisch-Naturwissenschaftliche Fakultät

der

Universität des Saarlandes

verleiht

unter der Präsidentschaft des Professors für Biogeographie
Dr. rer. nat. Paul Müller
und unter dem Dekanat des Professors für Mathematik
Dr. rer. nat. Friedrich Tomi

Herrn Ronald Belcher D. Sc.

Professor em. für Analytische Chemie
an der Universität Birmingham
die Würde eines

Ehrendoktors der Naturwissenschaften (Dr. rer. nat. h. c.)

Sie zeichnet damit einen vielseitigen Forscher aus, der bahnbrechende Methoden auf dem Gebiet der organischen und anorganischen Mikroanalyse entwickelt hat. Er bemühte sich um die Ausarbeitung eines umfassenden Systems der quantitativen Ultramikroanalyse, und es war ihm möglich, viele Probleme zu bewältigen, die mit der Herabsetzung vom Milligramm- in den Mikrogrammbereich verbunden sind. Mit der MECA-Spektroskopie gelang ihm der Vorstoß in den Nanogramm-Bereich.

Sein unermüdliches, höchst lebendiges Wirken verhalf der Analytik auf vielen Gebieten der Chemie und der Pharmazie weltweit zu Ansehen und Geltung.

Saarbrücken, den 9. Februar 1981

Der Präsident
der Universität des Saarlandes

Der Dekan
der Mathematisch-Naturwissenschaftlichen Fakultät

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G. Agricultural and food analysis

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WORKING PARTY ON ANALYTICAL CHEMISTRY (WPAC) FEDERATION OF EUROPEAN CHEMICAL SOCIETIES (FECS)

European Analytical Column 5

By January 1982 the WPAC consisted of 27 national chemical societies of 21 European countries, represented by 26 delegates. Among the FECS member societies only the Portuguese Chemical Society, the Pancyprian Union of Chemists and the Turkish Chemical Society are not represented in the WPAC. The Romanian Chemical Society sent an observer to the last WPAC meeting.

The 12th meeting of the WPAC was held in Helsinki, 23 August 1981, on the occasion of the Euroanalysis IV conference. At this meeting Professor E. Pungor (Budapest) was elected as WPAC chairman for the period 1981–1984, and the following issues were discussed.

1. *Euroanalysis IV, Helsinki, 25th event of the FECS*

This conference was organized on behalf of the WPAC by the Association of Finnish Chemical Socieites as a broad spectrum conference and was attended by 750 participants (including 100 accompanying persons) from 39 countries. The number of papers presented was well over 250, including 17 invited plenary and keynote lectures, the latter to be published in a joint east–west issue of the series "Reviews on Analytical Chemistry" with the title "Euroanalysis IV", publishers: Akadémiai Kiadó (Budapest), Chemical Publ. Co. (Helsinki). The conference programme also included three "Special Sessions" (1, Analytical Chemistry, the Analyst and Society; 2, Symbolism in Analytical Chemistry; 3, Mass Spectrometry in Inorganic Analysis) and a commercial exhibition of analytical equipment and literature.

2. *FECS lecture 1981*

An FECS lecture, which is delivered annually on the occasion of a major event of FECS, was granted in 1981 to Professor Bengt Samuelson (Karolinska Institutet, Stockholm), who presented, at the invitation of the Association of Finnish Chemical Societies, an outstanding survey on "Leukotrienes: A New Group of Biologically Active Compounds" on 25 August 1981, at the Finlandia Hall, Helsinki, on the occasion of Euroanalysis IV.

3. *Euroanalysis V, 1984*

The next conference was announced as to be held in Cracow, Poland, 26–31 August 1984, at the Jagellonian University and the Technical University Cracow. The decision will be considered at the next WPAC meeting on 26 April 1982 in Munich. The topics of the special sessions will be decided, according to the report of the Polish delegation.

4. *Euroanalysis VI, 1987*

According to a decision at the last WPAC meeting, Euroanalysis VI will be organized on behalf of the WPAC by the French Chemical Societies in 1987 in France. The location is the subject of a later decision.

5. *Euroanalysis VII, 1990*

There has been an official bid of the Austrian Society of Microchemistry and Analytical Chemistry to hold this conference in 1990 in Vienna.

6. *COBAC II* (FECHEM Conference on Computer-Based Analytical Chemistry), *Munich* 28–29 April 1982

Organized by WPAC in co-operation with the Analytical Chemistry Division of the Gesellschaft Deutscher Chemiker. The following topics will be discussed.

On-line coupling of computers and analytical equipment, integrated process control systems, peripheral equipment.
Desk computers: application in computer-based interpretation of spectra.
Library search procedures, identification of substances by means of spectroscopic data.
Programs for analytical chemistry.
Pattern recognition.
Present status of computerization in clinical analysis.
Interpretation of clinical data.
Control of significance of clinical data.
Role of computerization in the bill of costs of a large laboratory.

7. *COBAC III*

This FECHEM conference is planned to be held as a special session of the Euroanalysis V conference. Professor Hippe (Rzeszow, Poland) has been proposed as conference chairman. Scientists interested in this

active field of research are invited to contact the secretary of WPAC (Professor R. Kellner, Institut für Analytische Chemie, Technische Universität Wien, A-1060 Wien, Getreidemarkt 9, Austria).

8. *Education in Analytical Chemistry*

As part of the preparation for the next FECHEM conference on Education in Analytical Chemistry (in connection with Euroanalysis V) a questionnaire was prepared to find out about the new situation in this field in Europe after the last FECHEM conference in Vienna 1980. All institutions active in the field of higher education in Analytical Chemistry are invited to apply for a copy of the questionnaire. Please write to the secretary of WPAC if you have not received your own copy.

9. *Chemometrics and Analytical Chemistry*

A definition of Chemometrics (as approved by the Chemometrics Society) and a model curriculum for chemometrics studies were presented by Dr. Christie (Oslo) and will be further discussed at the next meeting.

10. *Pending FECS sponsorship*

At the request of the new chairman, Professor Pungor, WPAC should ask for FECS sponsorship for

(*a*) Symposium on "Electrochemical Detection in Flow-Analysis", Matrafüred (Hungary), 17–20 October, 1982;

(*b*) Symposium on "Pattern Recognition", Matrafüred (Hungary), 20–22 October, 1982;

(*c*) FECHEM conference on GLP (date and location to be specified).

11. *Next (13th) meeting of the WPAC*

The Fachgruppe Analytische Chemie of the Gesellschaft Deutscher Chemiker invited WPAC to hold the 13th meeting in Munich in connection with the "Biochemische Analytik" and the "Analytika" on Monday, 26 April 1982.

AUTHOR INDEX