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COMBINED MICROASSAY AND DETERMINATION OF BIOACTIVITY OF *N*-ACETYLNYSTATIN BY FLOW MICROCALORIMETRY

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Summary—The application of flow microcalorimetric bioassay for polyene antibiotics to nystatin and its *N*-acetyl derivative, and its extension to the measurement of their relative bioactivities, are reported. The responding organism was *Saccharomyces cerevisae*. The bioactivity of the *N*-acetyl derivative is shown to be less than that of the parent antibiotic and to be concentration-dependent. A limited diffusion bioassay study supports these conclusions.

Polyene macrolide antibiotics have been used for many years in the treatment of systemic mycoses.[1] More recently, they have been found to be active in the therapy of benign prostatic hypertrophy[2] and in reducing serum cholesterol levels.[3] Owing to the high toxicity,[4] side-effects[5,6] and poor aqueous solubility of these antibiotics, attempts have been made to synthesize derivatives that do not possess these disadvantages but which are still bioactive.

The *N*-acetyl derivative of nystatin (Fig. 1) is an example of such a derivative which retains some bioactivity and is rather more water-soluble than its parent compound.[7]

Improvements in classical microbiological assay methods for the polyenes have recently been reported.[8] However, the trend in bioassay procedures for this class of antibiotics is toward physico-chemical methods, *viz.* high-pressure liquid chromatography,[9,10] planet coil centrifugation,[11] atomic-absorption spectroscopy,[12] ion-selective electrodes,[13] radioisotope methods[14] and flow microcalorimetry.[15,16] The flow microcalorimetric method has been shown to be the most reproducible and sensitive assay system so far described for the polyene antibiotics.[15,16]

The quantitative assay of *N*-acetylnystatin and the measurement of its bioactivity have not been reported previously. This paper reports measurements of these parameters by flow microcalorimetry. The technique relies, in part, for its reproducibility and accuracy upon the use of inocula of the responsive organism *Saccharomyces cerevisae* NCYC 239 that have been stored in liquid nitrogen.[17]

EXPERIMENTAL

Materials

Nystatin was obtained from Squibb and Sons Ltd., Merseyside (U.K.). *N*-Acetylnystatin was prepared by the method of Mechlinski and Schaffner.[7] The chromatographic and ultraviolet spectroscopic properties of the derivative were in accord with the literature values.[7] The same batch of nystatin was used for assay of the parent compound. All other chemicals used were of analytical reagent grade.

Saccharomyces cerevisae (NCYC 239) was obtained from the National Collection of Yeast Cultures (Brewing Industry Research Foundation, Nutfield, Surrey). *Saccharomyces cerevisae* (SC1600) was obtained from Squibb & Sons Ltd. It is the Squibb internal house standard for assays of nystatin by the plate agar diffusion method.

The preparation, storage, recovery and assay of inocula of *Saccharomyces cerevisae* NCYC 239 and of *Saccharomyces cerevisae* SC 1600 were as previously described.[17]

Yeast suspensions of *Saccharomyces cerevisae* NCYC 239 and *Saccharomyces cerevisae* SC 1600 were prepared as follows. Broth[17] was inoculated from a transfer slope and incubated for 24 hr at 30° on a reciprocating shaker (90 strokes/min). A subculture (5 ml) into fresh medium was then incubated for 16 hr. A further transfer (10 ml) was then made and incubated for 12 hr. The cells were in the early stationary phase at this point. They were harvested by centrifugation and suspended in sterile saline. The calorimetric activity of the cell preparation was determined by respiration experiments as described below (see also Newell[18]), before freezing[17] in ampoules containing 2.0 ml portions of the cell suspension (equivalent to approximately 10^7 cells/ml).

The flow microcalorimeter (LKB type 10700-1, LKB Produkter, Bromma, Sweden), its design[19] and operation have been described.[17] The microcalorimeter was operated in the flow-through mode at 30° (the air-bath temperature was maintained at 30 ± 0.005°) in a room maintained at 25 ± 0.5°.

Procedure

The storage and preparation of solutions of antibiotics and the design of the assay experiment using flow microcalorimetry have been described previously in detail.[17]

For a typical microcalorimetric incubation a glucose solution in phthalate buffer (0.05*M* potassium hydrogen phthalate, 0.01*M* sodium hydroxide; pH 4.5; 0.01*M* glucose) was passed through the microcalorimeter flow-through cell to establish a steady base-line deflection at an amplifier sensitivity equivalent to 157.0 μW for full-scale deflection on the recorder (Philips PM 8000). An ampoule of yeast, stored in liquid nitrogen and containing 10^7 cells/ml, was thawed at 40° for 3 min; 2 min after comple-

tion of the thawing 1.0 ml of the thawed yeast suspension was inoculated into 50.0 ml of the glucose/buffer solution. Another 170 sec later, antibiotics or buffer/dimethylformamide solutions (as controls) were added to the incubation vessel in 1.0 ml portions. The efflux from the microcalorimeter was recycled into the incubation vessel. The incubations were done anaerobically by flushing the incubation vessel with nitrogen both before the inoculation with yeast cells and during the microcalorimetric experiments. After each incubation the microcalorimeter cell and tubing were sterilized by flushing with 0.1*M* sodium hydroxide followed by distilled water.

The nystatin agar diffusion method was used for comparative purposes to assay nystatin and *N*-acetylnystatin as follows. After dissolution of the sample in dimethylformamide, dilutions were made in phosphate buffer (10%; pH 6.2) to concentrations of approximately 80 and 20 units/ml. A Squibb internal nystatin standard (NY 3) was similarly prepared. These solutions were spotted (50 μl) into wells punched into B.B.L. agar plates and inoculated with a cryogenically stored inoculum of *Saccharomyces cerevisae* SC 1600. The standard assay procedures, including the inoculum, were those routinely employed by Squibb & Sons Ltd.[20]

RESULTS AND DISCUSSION

Nystatin is a mixture of at least three components which are chemically closely related and have not been separated and resolved structurally, designated A_1, A_2 and nursimycin.[15] The bioactivities of the components are consequently difficult to establish. The nystatin referred to and used in pharmaceutical preparations is the mixture of the materials as described.

The differences between A_1, A_2 and nursimycin are believed not to involve the site of *N*-acetylation. Moreover, since the nystatin raw material cannot be adequately purified and is known to contain in addition ~6% of material other than A_1, A_2 and nursimycin, then *N*-acetylnystatin must also be impure. The exact nature of the impurities is unknown, but they are thought to result from aerial oxidation of the antibiotics. The assumption is made in this study that since impurities are present in both preparations they will have a negligible effect on comparisons of the bioactivities of the antibiotics.

It is currently suggested[21–23] that the mycosamine residue (3-amino-3,6-dideoxy-D-mannopyranose; Fig. 1) plays an important part in the mode of action of those polyenes which possess this moiety. In *N*-acetylnystatin this moiety has been modified.

Figure 2 shows the response obtained in flow microcalorimetry from (*a*) yeast alone, (*b*) yeast in the presence of nystatin, and (*c*) yeast in the presence of *N*-acetylnystatin. Only the yeast cells yield a thermogram which arises from the metabolic activities (largely respiration) of the cells.

The reproducibility of the control thermogram was found to be $\pm 2.5\%$ (by using inocula stored in liquid nitrogen) over a period of at least two years. Hence the establishment of a dose–response curve for a given batch of inocula could suffice for large numbers of assays without recourse to recalibration. Probably only occasional checks on the performance of the inoculum are required.

The addition of antibiotics (at the concentration levels used) results in a decrease in metabolic activity and an eventual return of the thermogram to the base-line level. This base-line level represents the cessation of respiration.

Previous work[16] has established that the time T_x (Fig. 2) from the initial thermal response to the return of the thermogram to its base-line level is an appropriate measure of the antibiotic concentration (other relationships do not yield simple dose–response curves). No formal mathematical model has been found to account for this response. It has, however, been found to be applicable to a wide range of antifungal antibiotics over wide concentration ranges. That the kinetics of the antibiotic/cell interaction process are important in determining the "response" measured for a particular antibiotic concentration is made apparent in comparisons of assay experiments performed at 25° and 30°. This small change in temperature resulted in somewhat different thermograms, the one at 30° decreasing linearly[16] (see Fig. 2) whereas that obtained at 25° did so non-linearly.[15] Whilst this difference is undoubtedly connected with the enormous complexity of the cell/antibiotic reaction (and as yet no explanation is available) the use of the simpler response at 25° is proposed here. For solutions of equimolar concentrations, the response times (T_x) may therefore be compared directly as measures of "potency" or bioactivity.[16] Furthermore,

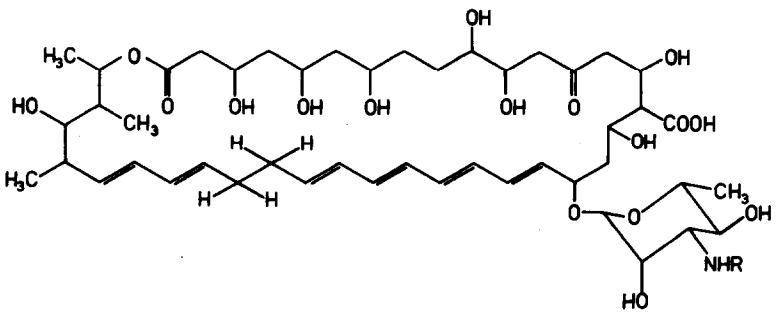

Fig. 1. Formulae of antibiotics used: nystatin, R = H; *N*-acetylnystatin, R = $COCH_3$.

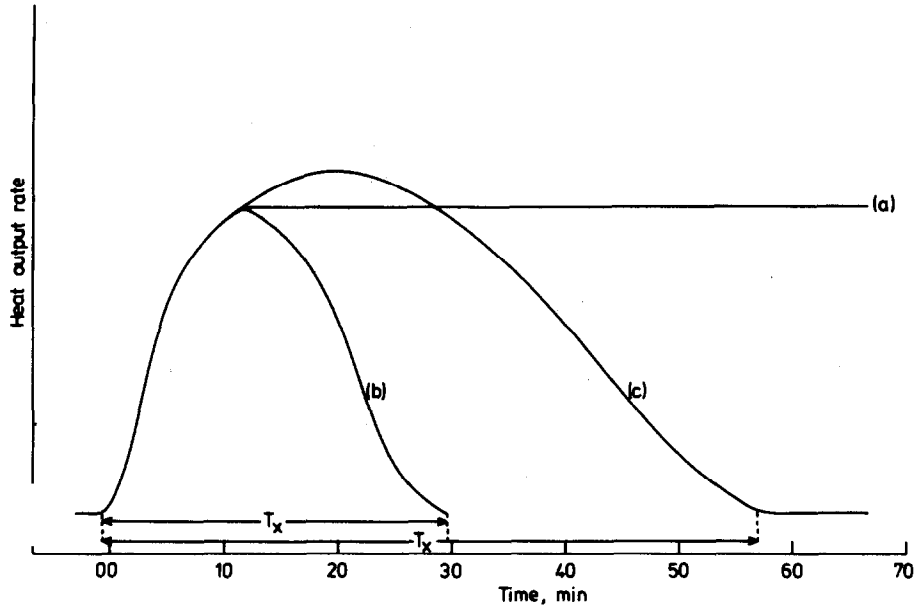

Fig. 2. Flow microcalorimetric response obtained from (*a*) yeast alone, (*b*) yeast–nystatin interaction, (*c*) yeast–*N*-acetylnystatin interaction under the experimental conditions used. The concentration of both antibiotics is $5 \times 10^{-6}M$. The yeast concentration is 10^7 cells/ml.

since time is easily measured electronically, the possibility of a simple automated system exists.

The shape of the thermograms obtained at 25° and reported by Beezer *et al.*[15] revealed a maximum heat output higher than that for yeast cell controls when low or medium concentrations of nystatin were incubated with yeast cells. In the present study conducted at 30° and that reported by Beezer *et al.*[16] this maximum was not observed for nystatin. The maximum observed previously was believed to be dependent on the kinetics of the cell/antibiotic interaction. Since nystatin is membrane-active[21–23] it is suggested that low concentrations of this drug modify the membrane in such a way as to permit easier transport of glucose, thus increasing briefly, until death processes become dominant, the respiration role. This phenomenon is observed here with *N*-acetylnystatin and could therefore be due to the lower activity of *N*-acetylnystatin producing a similar effect.

The results obtained in this study are presented in Fig. 3 and Table 1. The dose–response relationship is linear for both antibiotics over the concentration range 9×10^{-7}–$6 \times 10^{-6}M$. The microcalorimeter technique used in the work reported here has been shown to be more rapid (sample throughput time ~1 hr), sensitive and reproducible (±3% compared with ±5–10%) than the conventional agar plate diffusion assay. Some of the alternative physico-chemical methods cited in the introduction are not as general in application as microcalorimetry is, *e.g.*, potassium ion efflux is not observed with the polyene antibiotic A-435.[24] In addition, flow microcalorimetry offers the advantage that no separation of cells from suspension is required before measurement. This is not the case, for example, for measurement of potassium efflux by atomic absorption[12] or of amino-acid efflux.[25,26]

Flow microcalorimetry, as has been suggested, but not evaluated previously,[16] permits assessment of the relative bioactivities of different antibiotics. Other physico-chemical methods do not permit evaluation of this "potency" so readily, if at all. Hammond *et al.*[27] noted, for example, that different polyene antibiotics yield similar patterns of potassium ion efflux. No relationship was defined between bioactivity and the rate and/or extent of the efflux of potassium ions from yeast cells treated with polyene antibiotics. However, comparison of the response of a general reaction property, the enthalpy change, does permit judgements on the relative potency of nystatin and its *N*-acetyl derivative.

From Fig. 3, comparison of the response times T_x (as the simple ratio T_x for *N*-acetylnystatin/T_x for nystatin) at equimolar concentrations of each antibiotic between 6×10^{-6} and $9 \times 10^{-7}M$ indicates that the relative bioactivity of *N*-acetylnystatin changes from 65% to 42% of that of nystatin over this concentration range.

An alternative way of representing the difference in bioactivity may be deduced from Fig. 3. The concentration of nystatin required to produce an arbitrarily chosen response, *e.g.*, $T_x = 50$ min, is measured ($8.5 \times 10^{-7}M$); to achieve the same value of T_x for *N*-acetylnystatin would, by extrapolation, require a concentration of *N*-acetylnystatin equal to $7.0 \times 10^{-6}M$ (*i.e.*, an increase in concentration by a factor of ~80). If both compounds occupy identical sites on the cell membrane then this latter assessment of bioactivity may be more revealing.

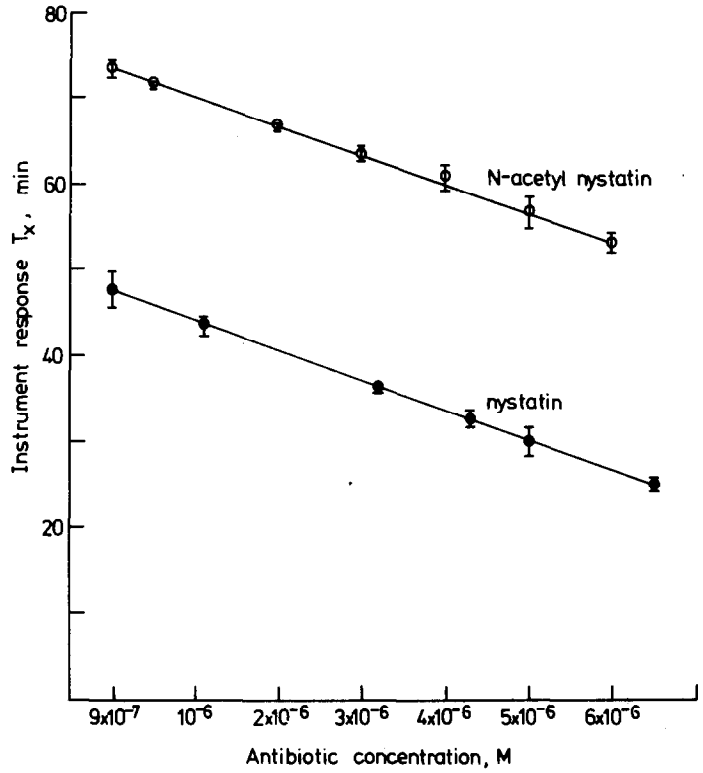

Fig. 3. Flow microcalorimetric response of interaction of *N*-acetylnystatin and nystatin over the concentration range 9×10^{-7}–$6.5 \times 10^{-6} M$, from Table 1. The instrument response represents the time T_x in Fig. 2.

With a different yeast, *Saccharomyces cerevisae* SC 1600 (Squibb Culture Collection), and the agar plate diffusion technique,[28] the activities of the two materials (relative to a nystatin "standard") were found to be 4750 units/mg for nystatin and 2450 units/mg for *N*-acetylnystatin (both values have a reproducibility of ±4% and inocula stored in liquid nitrogen were used[20]). This represents a reduction of 49% in bioactivity. Only one sample was assayed in this examination. The result does, however, support the conclusion reached by the more extensive microcalorimetric study.

The pH employed and strain of *Saccharomyces cerevisae* used in the flow microcalorimetric assay and

Table 1. Results of flow microcalorimetric assay for nystatin and *N*-acetylnystatin

Concentration of nystatin, *M*	T_x, *min*						Mean, *min*	Std. devn., *min*
9×10^{-7}	49.0	48.0	48.0	49.0	47.5	46.5	48.0	±1.9
1.2×10^{-6}	44.0	44.0	44.0	43.5	44.0	44.5	44.0	±0.6
3.2×10^{-6}	37.0	37.3	36.9	37.3	36.6	36.9	37.0	±0.5
4.2×10^{-6}	33.5	33.5	33.5	33.5	34.0	33.0	33.5	±0.6
5.1×10^{-6}	30.0	31.0	30.5	30.5	29.0	29.0	30.0	±1.6
6.6×10^{-6}	25.0	25.0	25.0	25.4	24.7	24.9	25.0	±0.5
Concentration of *N*-acetylnystatin, *M*								
9×10^{-7}	73.0	72.5	73.0	73.5	73.7	72.3	73.0	±1.1
8.8×10^{-6}	70.5	70.5	70.5	70.5	70.6	70.4	70.5	±0.1
2.2×10^{-6}	76.0	66.5	66.9	67.5	67.0	67.1	67.0	±0.6
3.2×10^{-6}	63.0	62.0	63.5	63.0	63.5	63.0	63.0	±1.1
4.2×10^{-6}	59.3	60.7	60.0	60.3	60.8	58.9	60.0	±1.5
5.2×10^{-6}	57.0	56.0	57.5	57.5	58.0	56.0	57.0	±1.7
6.1×10^{-6}	53.5	53.2	53.7	54.0	52.7	53.9	53.5	±1.0

the agar diffusion assay differ. Cosgrove,[29] however, has shown that the bioactivity of the two antibiotics is similar for both micro-organisms. Furthermore the comparative study was conducted only to show a general pattern of behaviour and not to establish absolute comparability. Circumstances prevented the use of each inoculum in each experiment to establish this comparability.

Mechlinski and Schaffner[7] observed that the *N*-acetyl derivative of amphotericin B (a polyene antibiotic closely related to nystatin) had a reduced bioactivity relative to the parent compound as measured by minimum inhibitory concentration (MIC) methods. However, the use of MIC methods in testing antibiotics has been questioned.[30] In contrast, MIC measurements of the relative bioactivities of the methyl ester of amphotericin B and amphotericin B itself showed no difference.[7] Measurement of the relative bioactivities by flow microcalorimetry may prove worthwhile since it has been noted[16,31,32] that antibiotics which have, apparently, the same MIC values do not always reveal the same relative bioactivity in microcalorimetric experiments. This could be due to the complex physical process(es) underlying the agar diffusion method (diffusion rate of the antibiotic through agar being an important parameter) whereas the general technique of calorimetric observations on homogeneous aqueous suspensions gives results which are more easily interpreted.

The estimation of the relative bioactivity of other polyene macrolide antibiotic derivatives[33–36] by flow microcalorimetry is recommended.

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EXTRACTION OF PLATINUM METALS FROM HYDROCHLORIC ACID MEDIUM WITH TRIPHENYLPHOSPHINE SOLUTION IN 1,2-DICHLOROETHANE

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Summary—Extraction of platinum metals with TPP in 1,2-dichloroethane from hydrochloric acid medium has been examined. At hydrochloric acid concentrations higher than 6*M*, palladium, platinum and osmium are extracted, whereas at low acidity only palladium is quantitatively extracted. Addition of stannous chloride as labilizing agent makes possible a group separation of platinum metals (except osmium). Possible extraction mechanisms are discussed.

Triphenylphosphine and other phosphines (arsines and stibines) form with platinum metals complexes of the type $MeCl_nL_m$. These compounds have been known for a long time and many of them have been synthesized.[1] The reactivity of phosphines with platinum metals conforms with Pearson's rule[2] of affinity of "soft" bases or "soft" (or intermediate) acids. Phosphines are soft bases, and platinum metals in their lower oxidation states can be considered as soft acids.

The possibility of extracting palladium with triphenylphosphine was first pointed out by Senise and Levi.[3] Extraction–spectrophotometric methods for determination of palladium, based on extraction from iodide[4] or chloride media[5] were developed. Other studies showed that silver and gold could also be extracted with triphenylphosphine. This was used to advantage for separation of these metals before their determination by atomic absorption.[6–9] Ruthenium(III) can also be extracted with triphenylphosphine,[10] which is also used as synergistic agent in the extraction of chelate complexes of metals.[6–11]

In view of the extraction of silver, gold and palladium with triphenylphosphine it seemed of interest to examine the extraction of the platinum metals with this reagent. 1,2-Dichloroethane was chosen as the diluent.

EXPERIMENTAL

Reagents

Triphenylphosphine (TPP) was crystallized twice from hot ethanol. Its m.p. (81–82°) was in agreement with the published data.

Preparation of standard solutions of palladium and platinum was as described previously.[12] Standard 0.005*M* solutions of other platinum metals in 6*M* hydrochloric acid were freshly prepared from appropriate salts such as K_2RuCl_6, $RhCl_3 \cdot 3H_2O$, K_2OsCl_6, K_2IrCl_6. Other reagents used were of analytical purity.

Procedure

Equal volumes of the aqueous and organic phases were shaken for 60 min (10 min when stannous chloride had been added to the aqueous phase). The initial metal concentration in the aqueous phase was $10^{-3}M$ at a given hydrochloric acid concentration. A 0.1*M* TPP solution in dichloroethane or pure dichloroethane was used as the organic phase. After extraction the phases were separated and the metal concentration in one or both of the phases was determined spectrophotometrically[13] after evaporation or mineralization if necessary. Palladium was determined by the α-furildioxime method and platinum and rhodium by the stannous chloride method. Iridium was determined on the basis of the absorbance of $IrCl_6^{2-}$. Osmium and ruthenium were determined with thiourea. All experiments were carried out at 20 ± 2°.

RESULTS AND DISCUSSION

Extraction with TPP solution in dichloroethane

The dependence of the extraction of platinum metals on the hydrochloric acid concentration is shown in Fig. 1. The extraction of palladium from 1–8*M* acid is quantitative. Above an acid concentration of 6*M* osmium is practically quantitatively extracted. At high acid concentrations (8*M*) platinum and (in part) iridium are extracted. Ruthenium and rhodium are practically not extractable with TPP solution from 1–8*M* hydrochloric acid.

Two mechanisms are possible for extraction of platinum-metal chloride complexes with triphenylphosphine:

(*a*) solvation

$$MeCl_p^{(p-n)-} + mTPP_{(0)} \rightleftharpoons MeCl_n(TPP)_{m(0)} + (p-n)Cl^-$$

(*b*) ion-exchange

$$MeCl_p^{(p-n)-} + (p-n)(TPPH^+ \cdot Cl^-)_{(0)} \rightleftharpoons (TPPH^+)_{p-n}MeCl_{p(0)}^{(p-n)-} + (p-n)Cl^-.$$

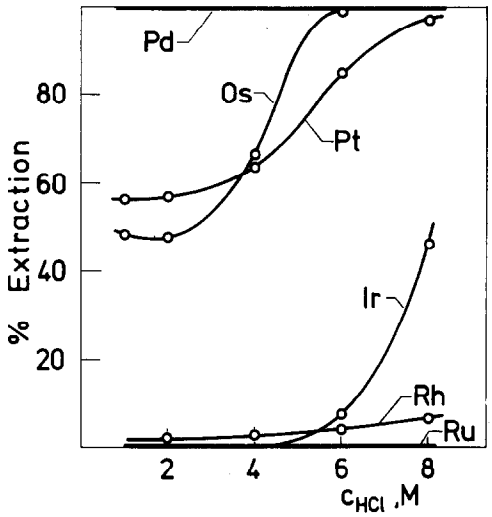

Fig. 1. Dependence of the extraction of platinum metals on acid concentration for 0.1*M* TPP in dichloroethane and 0.001*M* metal concentration.

In the solvation mechanism the extraction efficiency should increase with the stability of the bonding between the phosphine and the central ion (the softer the metal ion, the higher the stability). Another favourable factor is a small difference in the stability of the complexes $MeCl_p^{(p-n)-}$ and $MeCl_n$, because $(p - n)$ chloride ions must be detached from the initial complex. The chloride ligands must be replaced at a sufficient rate.

These conditions are well satisfied in the case of palladium. It can be supposed that the poor extraction of Pt and Os and non-extraction of Ir, Ru, Rh at low hydrochloric acid concentrations is due mainly to the inertness of the chloride complexes. This was partly confirmed by the results of further studies.

In strongly acid medium triphenylphosphine, being a weak base (much weaker than tertiary amines), forms a cation $TPPH^+$ which can participate in extraction by the ion-exchange mechanism. The high extraction of osmium, iridium and platinum at high acid concentrations indicates that this is the operative extraction mechanism. In this case inertness of the chloride complexes is not a limiting factor, because there is no exchange of the ligand in the central ion.

In the extraction of platinum metals with triphenylphosphine account has to be taken of the oxidation of the reagent to phosphine oxide and reduction of the metal to a lower oxidation state. Though triphenylphosphine is more resistant to oxidation than trialkylphosphines, this phenomenon was observed in the extraction of gold.[9] The results obtained indicate that during extraction with triphenylphosphine the platinum(IV) in the aqueous phase is quantitatively reduced to platinum(II). Similar phenomena can be expected to occur in the extraction of other platinum metals. Formation of triphenylphosphine oxide, which extracts platinum fairly well for example, can influence the overall effectiveness of extraction.

The considerations above indicate that it is difficult to interpret unequivocally the extraction of platinum metals with triphenylphosphine. It is only certain that palladium is extracted as $PdCl_2(TPP)_2$.[10]

The results obtained demonstrate that palladium can be separated from iridium, rhodium and ruthenium by extraction with triphenylphosphine from a hydrochloric acid medium less than 4*M* in concentration.

Extraction from 8*M* hydrochloric acid gives separation of palladium, platinum and osmium from rhodium and ruthenium.

Extraction with TPP solution in the presence of stannous chloride

It has long been known that the inertness of chloride complexes of platinum metals decreases after addition of stannous chloride to the solution.[14,15] Tin-containing complexes of the type $MeCl_k(SnCl_3)_l^{n-(k+l)}$ are then formed in which the replacement of $SnCl_3^-$ by another ligand is not subject to such kinetic difficulties as is the replacement of chloride as ligand. The labilizing effect of stannous chloride in the extraction of platinum metals can be well observed in the diphenylthiourea system[16,17] and in the present studies advantage has been taken of the methods used in those works.

The extraction of platinum metals with triphenylphosphine in the presence of stannous chloride was examined by using various procedures described below. The dependence of the extraction of individual platinum metals, at constant stannous chloride concentration in the aqueous phase, on the hydrochloric acid concentration is shown in Fig. 2. In the next experiment, the results of which are shown in Fig. 3, after addition of stannous chloride solution the aqueous phase was heated on a boiling water-bath for

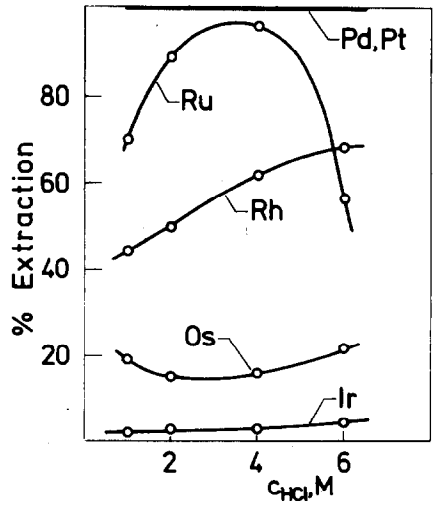

Fig. 2. Dependence of the extraction of platinum metals on acid concentration in presence of $SnCl_2$ (0.1*M*) for 0.1*M* TPP in dichloroethane and 0.001*M* platinum metal concentration.

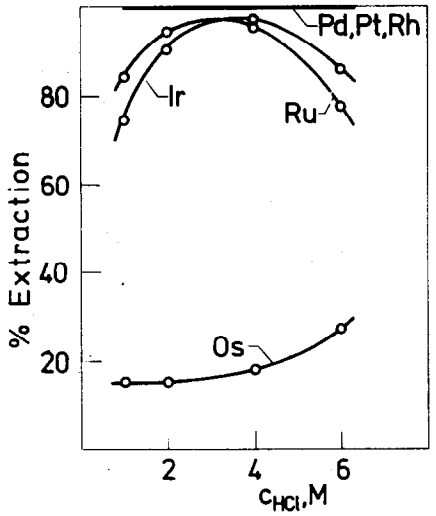

Fig. 3. Dependence of the extraction of platinum metals on acid concentration in presence of $SnCl_2$ with heating before extraction. Other conditions as for Fig. 2.

30 min, and the solution was cooled and extracted with a TPP solution in dichloroethane. In the third variant of the procedure the aqueous phase containing stannous chloride was heated and a TPP solution in acetone was added (at a concentration sufficient to ensure homogeneity). The solution was then heated again, allowed to cool, and extracted with pure dichloroethane. The results obtained are shown in Fig. 4.

In all cases platinum and palladium were quantitatively extracted and the equilibrium was attained in less than 3 min. The addition of stannous chloride results in better extraction of all the platinum metals except osmium, which is extracted more efficiently in the absence of stannous chloride. Except for osmium

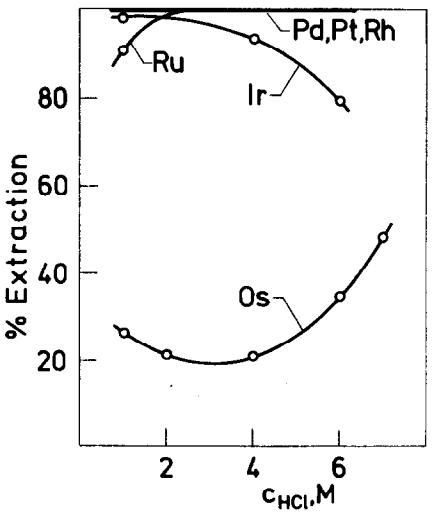

Fig. 4. Dependence of the extraction of platinum metals after addition of TPP in acetone (0.01*M*) and $SnCl_2$ (0.1*M*) to the aqueous phase. Heating before extraction.

all platinum metals can be jointly extracted from 2–5*M* hydrochloric acid in the presence of stannous chloride if the aqueous phase is heated before the extraction. When TPP in acetone is added to the aqueous phase, group extraction of all platinum metals except osmium is possible from 1–4*M* hydrochloric acid.

The addition of stannous chloride to the aqueous phase results in the formation of a variety of platinum metal species which may be extracted. Except for palladium(II) and platinum(II) the oxidation states of the platinum metals in the complexes are not known. It has been found, by using the atomic-absorption method, that the organic phase after extraction does not contain tin. This indicates that the stannous chloride is a labilizing agent, which is involved in the platinum-metal complexes only in the aqueous phase.

For the stannous chloride systems there are still two extraction mechanisms possible:

(*c*) solvation

$$MeCl_k(SnCl_3)_l^{n-(k+l)} + mTPP_{(0)} + (n-k)Cl^- \rightleftharpoons MeCl_n(TPP)_{m(0)} + lSnCl_3^-$$

(*d*) ion-exchange

$$MeCl_k(SnCl_3)_l^{n-(k+l)} + lCl^- + (p-n)(TPPH^+Cl^-)_{(0)} \rightleftharpoons [MeCl_p^{(p-n)-}(TPPH^+)_{(p-n)}]_{(0)} + lSnCl_3^- + (k+l-n)Cc^-.$$

The significant increase in degree of extraction after addition of stannous chloride seems to indicate that the solvation mechanism (*c*) predominates. Had the ion-exchange mechanism been involved, no significant difference in the effectiveness of extraction would have been observed, because in that case the ligand exchange (between chloride and phosphine) in the coordination sphere of the metal would have not have taken place.

In oxidation states lower than (III), osmium forms neither stable chloride complexes with phosphines nor stable electronegative chloride complexes. Hence addition of stannous chloride results in poorer extraction of this metal.

The changes in the effectiveness of extraction of other platinum metals with TPP, found in further experiments, indicate the liability of the platinum metal complexes to be Ir < Rh < Ru < Pt < Pd. This order of increasing lability is in agreement with that established in the extraction of platinum metals with diphenylthiourea.[16,17]

CONCLUSIONS

Triphenylphosphine is known to be an effective extractant for palladium, The results presented here indicate that other platinum metals are also extracted

with triphenylphosphine, if stannous chloride is added to the aqueous phase and the solution is heated. In the absence of stannous chloride it is possible to extract palladium, platinum and osmium quantitatively at hydrochloric acid concentrations higher than 6*M*.

The extraction from 1–3*M* hydrochloric acid (addition of $SnCl_2$, TPP in acetone, heating) gives good separation from iron and some non-ferrous metals. At higher hydrochloric acid concentrations these metals also form stable electronegative complexes and can be extracted as well.

The inertness of the chloride complexes of platinum metals results (except for Pd) in their poor extraction with TPP from solutions of low acidity, in the absence of stannous chloride, because under these conditions the solvation mechanism predominates. A significant increase in extraction at higher acid concentrations indicates that the ion-exchange mechanism is then involved in the extraction.

In the extraction of platinum metals with triphenylphosphine stannous chloride is a labilizing agent and does not enter into the extracted species. Except for palladium, which is extracted as $PdCl_2(TPP)_2$, it is difficult to determine the composition of the extracted species. Detailed studies are necessary to elucidate this problem.

The extraction with triphenylphosphine can be used to advantage for a group concentration of the platinum metals for analytical purposes, *e.g.*, before determination by atomic absorption.

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THE INFLUENCE OF pH AND COMPLEX FORMATION ON THE ASV PEAKS OF Pb, Cu AND Cd

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Summary—The effects of pH, electrolyte composition and complex formation on the size and position of the ASV peaks of Pb, Cu and Cd have been systematically evaluated, with an instrument equipped with a mercury thin-film electrode and by applying a linear ramp voltage scan. The peak heights change with pH and the magnitude of the pH effect varies with base electrolyte composition. Anions such as chloride and acetate reduce the signal, as does the presence of excess of ligands such as 2,2′-bipyridyl, NTA and EDTA. Formation of stable chelates (*e.g.*, with EDTA) can lead to total loss of signal, but dissociation of labile complexes can be enhanced by reducing the pH and/or increasing the magnitude of the applied deposition potential, thus producing measurable peaks. The peak potentials vary with pH, and in copper systems there are additional shifts in the presence of citrate, 2,2′-bipyridyl and chloride. With the last two, double peaks are formed and these are attributed to the formation of both Cu(I) and Cu(II) oxidation products. The varied response, particularly in the case of copper, which can follow changes in the base electrolyte composition, supports the need for careful control of the chemical environment in quantitative determination, and raises some queries about the feasibility of using direct ASV for speciation purposes.

The range of application of anodic stripping voltammetry (ASV) has expanded[1,2] to embrace the determination of μg/l. levels of over 20 different elements, and consideration[3–6] of the basic processes has led to the derivation of equations which aptly summarize many of the parameters which influence the response of ASV procedures.

For example, the amount of metal reduced at the working electrode (usually a mercury drop or thin mercury film), at the end of the deposition step, is given by Faraday's laws. Oxidation, or anodic stripping, of this deposited metal in the second stage of the procedure yields the current signal which is used for quantitative evaluation. Different potential–time wave-forms can be used in this step, but with the most widely applied (a linear ramp potential change), and a mercury thin film electrode (MTFE), the magnitude of this current has been shown to be proportional to both the quantity of metal to be oxidized and the scan-rate.

Combination of the deposition equations with an expanded stripping-current relationship such as that of Roe and Toni[4] yields an expression which highlights a number of the aspects which must be controlled in order to ensure that the current measured is directly related to the component sought.

Other authors have suggested that the stripping current can be a function of chemical factors such as pH, presence of ligands, and concentration of base electrolyte.

The role of these chemical factors has not been so systematically studied, and this paper summarizes the effects noted when such variables were investigated with a commercial instrument, thin mercury film electrodes, and solutions containing μg/l. amounts of Pb, Cu and/or Cd.

It has also been proposed that the potential at which a stripping peak appears can be indicative of the initial chemical form of the metal ion, and further experimental confirmation of this contention was sought in this study. For a mercury thin film electrode, this appearance potential has been shown to vary with factors such as thickness of film, rate of diffusion of oxidized species, thickness of the surface diffusion layer (influenced by stirring rate) and scan-rate. Any influence of chemical environment on the appearance potential must therefore arise from variations in the conditional electrode potential of the redox system.

EXPERIMENTAL

Residual metal ions in the demineralized water supply were removed by passage through an Elga Model B116 Cartridge Deionizer before being used in the dissolution of the analytical-grade metals or reagents required for preparing the test solution. Metal impurities in the base electrolyte solutions were removed, when necessary, by treatment in an ESA Model 2014P Reagent Cleaning System.

The ESA Model 2014 Anodic Stripping Analyzer used in this study has four test cells, each containing a wax-saturated graphite rod (precoated with a thin mercury film), a platinum counter-electrode and an Ag/AgCl reference electrode. A nitrogen flow, monitored by an in-line ball flowmeter, serves to deoxygenate and stir each 5.0 ml of test solution.

The performance of the test cells was evaluated by using a sodium acetate (1*M*)-sodium chloride (0.2*M*) base solution containing 100 μg of lead per litre, and it was found that small differences in electrode geometry or film thick-

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ness led to slightly different current responses. Accordingly, all comparison studies were based on the use of a single cell. The ASV traces were recorded on a fast response Rickadenki Chart Recorder, Model B-181 H.

To evaluate the effect of pH on stripping current, 1*M* sodium acetate base solutions, containing 100 μg of Pb, Cu or Cd per litre were adjusted to various pH values within the range 2–9, by addition of either nitric acid or sodium hydroxide solution (pH measurements were made on a Philips Meter, Model PW 9418). Each solution was then repeatedly passed through the selected deposition–stripping cycle, the main operating parameters being a deposition potential of −900 mV applied for 10 min, a nitrogen flow-rate of ~40 ml/min, a stripping scan speed of 50 mV/sec, and a cut-off potential of −50 mV. The observed effect of pH on the peak behaviour is summarized in Fig. 1.

In the absence of a buffer, the pH can vary during the analysis and the influence of this factor was investigated by substituting 0.1*M* potassium nitrate for the sodium acetate. An indication of any general effects attributable to changes in the nature of the major cation or anion present was sought by observing the Pb and Cu peaks obtained for solutions in which the base electrolyte composition was changed. Electrolytes used in this study included calcium, magnesium, sodium and ammonium chlorides, nitrates and acetates. The results are summarized in Table 1.

In the other part of the investigation, varying amounts of ligands were added to base electrolyte solution (1*M* sodium acetate) of varied pH, containing 100 μg of Pb, Cu or Cd per litre. The ligands used were chloride, ethylenediaminetetra-acetate, nitrilotriacetate, citrate, glycine and 2,2′-bipyridyl, and the ligand to metal-ion ratios were varied from 1:1 to 2, 10 and 100:1. The results from these ligand studies are summarized in Fig. 2 and Tables 2 and 3.

RESULTS AND DISCUSSION

Operational parameters

The evaluation studies confirmed that the influence of experimental parameters such as deposition potential, scan-rate or deposition time followed the pattern already reported in many papers. It was found that up to a certain critical rate, faster stirring, obtained with increased nitrogen flow-rates, enhanced peak heights but at higher rates the peak values remained virtually constant. In the comparison studies, nitrogen flow-rates were kept within this plateau region, and all users of gas stirring are advised to monitor and control this variable carefully.

pH effects

Literature reports on the sensitivity of ASV curves to pH changes are somewhat conflicting. Results obtained[7] with 0.16*M* acetate electrolyte indicate that for copper, cadmium, lead and zinc, the oxidation current peaks are independent of the acidity of the solution up to pH 7. Variation in peak heights was also found[8] to be small over the pH range 3.9–6 for a base solution that was 0.18*M* in acetate and 0.1*M* in sodium chloride. These conclusions contrast with the results of Lewin and Rowell[9] who found that a pH of 5.5 gave an optimum response for Pb, Cd and Cu. In experiments with artificial sea-water,[10] the Cu and Zn

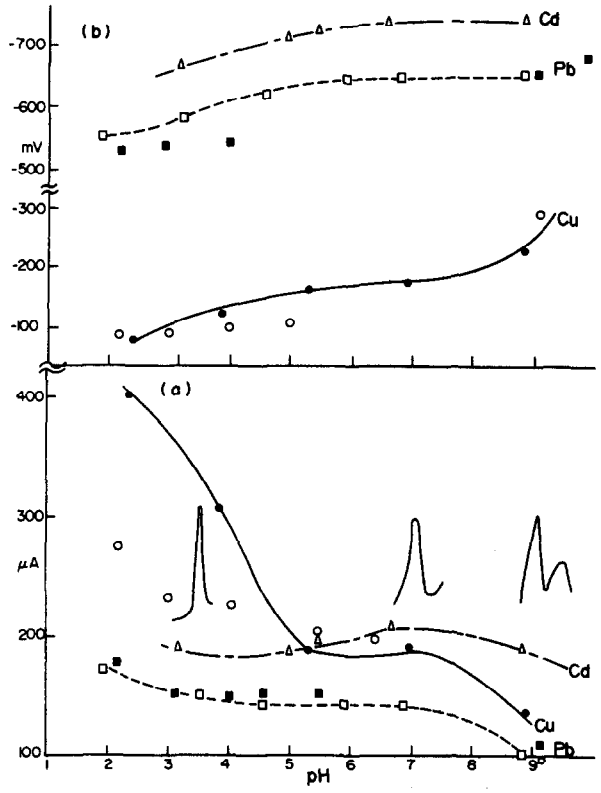

Fig. 1. Effect of pH on ASV peak parameters of Cu and Pb present at 100-μg/l. level. (*a*) Effect on peak current; (*b*) effect on peak potential. Deposition potential −900 mV; deposition time 10 min; stripping scan-rate, 50 mV/sec. □, Pb; △, Cd; and ●, Cu in 1*M* sodium acetate. ■, Pb; ○, Cu in 0.1*M* potassium nitrate. Insets show copper peak shapes (not to scale).

stripping currents have been observed to reach a maximum at a pH of about 6, and with Pb the peak current appeared to be directly proportional to acidity of the solution.

The results of our study, summarized in Fig. 1, show that pH effects can be minimal in the pH 4.5–6.5 range, but outside this region some distinctive changes occur, particularly with copper. Lowering the pH of an acetate buffer solution lowers the conductivity of the solution (through protonation of the anion) and decreases the effective stability of acetato-metal complexes, and the combined effect results in enhancement of copper peaks as the pH decreases. The pH effect is less pronounced in a nitrate base solution, where conductance is increased by the additional protons released in secondary electrolysis reactions (*e.g.*, the pH of potassium nitrate solutions rapidly falls from the initial pH ~ 8 to pH <4.5 after a few stripping cycles). Peaks tend to be sharper in acid media and this leads to greater sensitivity for Cu or Pb determination. Cadmium is said[11] to be best determined at pH >3, presumably because of the more negative reduction potential involved and the lower hydrogen overvoltage on a mercury thin film electrode (MTFE). Hydrogen evolution contributes significantly to the background at the zinc stripping potential (*e.g.*, -1400 mV) and zinc determinations are reported[2] to be strongly affected by pH.

It was observed in the current study that at pH below 5, gas bubbles appear on the mercury electrode during the deposition stage ($H^+ + e \rightleftharpoons \frac{1}{2}H_2$). It might be expected that this competing process would reduce the efficiency of the plating stage for all metal ions, leading to behaviour similar to that noted with cadmium. However, with copper and lead, increasing the acidity enhanced the ASV peak size, and with these systems it must be assumed that the protons or gas bubbles remove surface coatings (*e.g.*, oxides, or hydroxides) or adsorbed species (*e.g.*, acetate) which partially impede electron transfer.

In alkaline media, the peak heights fell (*cf.* Fig. 1) and in the case of copper a small second peak appeared, the peak potential being approximately 100 mV less negative than that for the main copper peak. Sinko and Doležal[7] also observed two copper peaks when examining solutions of pH >7.8, and noted that the sum of the oxidation peak currents was smaller than the oxidation current in an acid or neutral medium. The second peak may be due to the formation of copper(I) hydroxy species at the surface during oxidation of the Cu(Hg) amalgam, or adsorption of copper(II) hydroxy compounds. Zinc in alkaline artificial sea-water, was also observed[10] to yield a small symmetrical peak at a potential 100 mV more negative than the main peak. This second peak was attributed to the adsorption of $ZnCO_3$, or $Zn(OH)_2$, or a mixed zinc hydroxycarbonate on the electrode during oxidation of the Zn(Hg) amalgam.

Though published stability constants or solubility product data[12] can be used to predict the distribution of metal species as a function of pH, the information derived yields no simple explanations for the observed behaviour. This is due in part to the complexity of metal–hydroxide systems. For example, Perrin[13] has postulated that copper forms a series of compounds, of general formula $Cu_n(OH)_{2n-2}(H_2O)_4$ where n increases with pH, and Olin[14] has identified seven different hydroxy species in the lead system, many of them polymeric.

For natural sea-water samples,[10,11,15] the copper, lead and zinc peaks increase in size as the pH is lowered from 8 to 1, while the cadmium response decreases. These pH effects have been interpreted in terms of the metal ions combining with weak acid anions or organic matter to form inert complexes. The increase in peak current at the lower pH is accompanied[10] by an anodic shift in peak potential of *ca.* 30 mV.

Reference to Fig. 1b shows that lowering the pH of an acetate base solution results in a shift of peak potentials to more positive values. Between pH 2 and 5 the relationship between peak potential and pH is approximately linear, and this implies that the conditional potential of the system is pH-dependent. That is, either protons are involved in the redox process or there are changes in the metal-ion/metal half-cell potential, attributable to complex formation. As the pH range of 2–5 corresponds to the transition of the composition of the base electrolyte from acetic acid to predominantly acetate, the conditional potential could well be subject to the influence of acetate coordination to the metal ions. The peak potential was found to change by about 26 mV per pH unit, and for a two-electron transfer process, this implies the involvement of one proton per mole of M^{2+} reduced.

In the pH range 5–9, no significant shifts were noted for the lead and cadmium peaks, but the copper peak moved by about 70 mV. A shift of 35–55 mV has also been observed for zinc peaks when the pH of sea-water samples is reduced from 8.3 to 5.5. This observation was explained[16] in terms of zinc carbonate or zinc hydroxide species, and the same general comment could apply to the copper system.

The relative standard deviation of peak-height determinations is generally about 2–5%, so the varied reports on pH effects cannot necessarily be attributed to experimental error, and more probably reflect secondary reactions such as protonation of electrolyte anions, pH-changes due to electrolysis (most marked in absence of buffers), the dissociation of weak complexes, or differences in electrode nature.

The significance of pH-changes induced by repeated cycles in the same solution was examined by using 0.1*M* potassium nitrate (initially at pH 9.5) as the base solution. After five successive 10-min deposition and rapid stripping cycles, the peak current values for Pb and Cu were observed to change from 25 to 32 μA and from 40 to 90 μA respectively, with concurrent changes in the peak potentials of 26 and 60 mV. After a total of 190 min of ASV cycles, the

Table 1. Effect of supporting electrolyte on peak parameters (deposition at −900 mV for 10 min; stripping scan speed 50 mV/sec

Supporting electrolyte	Ionic strength	Lead		Copper		pH	
		i_p, μA	E_p, mV	i_p, μA	E_p, mV	Initial	Final
NaCl	0.20	165	−487	155	−191	5.7	4.6
$MgCl_2$	0.15	130	−487	139	−167	6.9	5.3
$CaCl_2$	0.15	155	−470	140	−154	6.6	5.6
$Ca(NO_3)_2$	0.15	118	−467	220	−37	5.2	4.1
$CaAc_2$	0.15	96	−499	137	−79	7.3	7.3
$CaAc_2$	0.15	129	−478	158	−66	5.2*	5.2
$NaNO_3$	0.10	129	−482	269	−59	5.6	5.3
NH_4NO_3	0.10	124	−482	265	−59	4.8	4.0
NH_4Cl	0.10	160	−489	142	−194	5.2	4.1
NH_4Ac	0.10	102	−520	98	−147	6.8	6.8
NH_4Ac	0.10	143	−504	209	−59	5.2*	5.2

* Perchloric acid added to reduce pH.
Ac = acetate; i_p = peak current; E_p = peak potential.

solution pH was found to have dropped to 4.7 and the metal-ion peaks had increased in size to 140 (Pb) and 210 (Cu) μA.

The pH changes thus induced complicated attempts to assess the variations which might have been induced by altering the base electrolyte composition. As a compromise it was decided to operate within the plateau region noted on Fig. 1, *i.e.*, between pH 4.5 and 7.

Influence of base electrolyte composition

The range of peak current and peak potential values, obtained with different base electrolyte solutions containing 100-μg/l. levels of Pb or Cu, is shown in Table 1. The values quoted are the mean of eight analyses (each test solution being run twice and then replaced with fresh solution), and peak values have been corrected for any reagent impurities detected in blank runs.

All voltammetric methods need a supporting electrolyte, and the concentration (typically 0.05–0.5M) should be consistent from sample to sample.[2,17] The molarity of our test solutions lies near the lower end of the recommended concentration range but it is considered unlikely that this is the cause of the different responses. For example, Florence[18] found that in lead determinations, with an MTFE, the potassium nitrate concentration could be varied from 0.005 to 1M without affecting the peak current or peak potential. On the other hand, there are problems associated with using very low concentrations of supporting electrolyte,[19] and in differential pulse ASV determinations of Pb and Cd on an MTFE, stripping currents have been observed[20] to diminish greatly at lower supporting electrolyte concentrations, and peak potentials shift to more cathodic values as the solution resistance is decreased.

A perusal of Table 1 indicates that i_p and E_p values may be subject to both cation and anion effects. For example, presence of calcium salts resulted in smaller peaks and more anodic potentials than presence of sodium salts did; ammonium salts affected peak behaviour at higher pH values, *etc.*

The ammonium ion effect was greatest in the copper system, and probably reflects partial conversion of the metal ion into ammine complexes. The distribution of copper ammine complexes is a function of the pH and ammonium ion concentration, and it was calculated that at pH 6.8, with a 0.1M ammonium acetate solution, more of the copper should be present as mono-, bis-, and tris-ammine complexes than as acetato-complexes. At pH 5.2 the last-named predominate, and it can be observed (Table 1) that this change in chemical form results in significant changes in i_p and E_p values. No copper peak was observed by a group[21] who used a base electrolyte which was 0.05M with respect to ammonia and ammonium nitrate (pH 9.3), but another study[25] yielded a peak at −480 mV (*vs.* SCE) with 0.5M ammonia and ammonium nitrate. With these base solutions the copper would be present mainly as the tetra-ammine.

With regard to anion effects, the possible role of acetate ions received comment in the preceding section, and the shift of E_p values for copper to more negative potentials in the presence of chloride is considered in the next.

The effect of chloride ions

It has been previously reported[8] that the presence of chloride has little effect on the i_p values for lead and cadmium, and in fact, for determination of these elements, its addition to the base electrolyte has been recommended,[11] since it appears to sharpen the peak shape. These observations have been confirmed in our study.

The behaviour of copper, however, is markedly influenced by the presence of chloride[8] (*cf.* Table 1), and it has been shown[22] that suppression of copper peaks occurs with chloride concentrations as low as $4 \times 10^{-5}M$. Levels of this order can occur in test solutions through impurities in the base electrolyte used, or through diffusion from cell components.

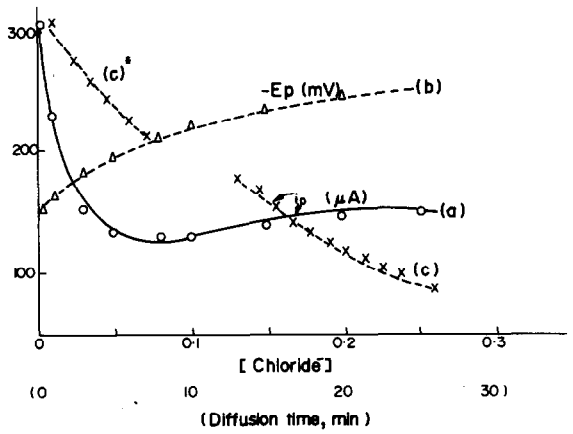

Fig. 2. The effect of chloride concentration on the peak current (*a*), and peak potential (*b*), of copper, present at 100-μg/l. level in 1*M* sodium acetate, pH 5.6, 10 min deposition at −700 mV on an MTFE. Curve (*c*) indicates the peak current suppression induced by chloride ion diffusion from the reference cell into 0.1*M* sodium nitrate solution containing 100 μg/l. Cu. Successive 10-min deposition (−900 mV), and stripping cycles were applied to the same solution; the apparent 5-min break in the curve corresponds in fact to a 60-min deposition period shown in this way for convenience.

The potential influence of diffused chloride on copper peak-height is clearly shown by curve *c* in Fig. 2. In the ESA cells, leached porous-glass (Vycor) plugs provide electrical contact between the test solutions and the 1*M* sodium chloride in which the reference electrode and platinum counter-electrode are immersed. It may be observed that the chloride diffusion rate was sufficient to give a steady decline in signal size, despite the fact that the decreasing pH due to the prolonged electrolysis should have led to increases in signal height. Simultaneously, the peak potentials shifted with time from −30 to −80 mV, in a nearly linear fashion.

It is obvious from these results that, with this type of cell, chloride should be included in the base electrolyte whenever it is planned to determine copper in solutions containing low or variable amounts of chloride. The minimum desirable chloride addition is probably 0.2*M*, since reference to curve *a* in Fig. 2 indicates that the chloride effect levels off beyond this value. Even after suppression of the signal, ASV has sufficient sensitivity to permit determination of μg/l. levels of copper, as shown by the fact that the technique has been successfully applied to sea-water samples.[22]

With increasing chloride additions to acetate base solutions the peaks become increasingly asymmetrical, with the steeper slope occurring on the anodic side, and a slight distortion (shoulder) sometimes appearing on the cathodic side. It is considered[8] that dissolution of copper in the presence of chloride must involve two reactions which are not resolvable at the MTFE, *e.g.*, a one-electron reaction

$$Cu + 2Cl^- \rightarrow CuCl_2^- + e$$

plus some oxidation to copper(II). The slight increase in i_p values observed with $[Cl^-] > 0.1$ (Fig. 2a) could reflect the formation of higher proportions of copper(II) chloro-complexes, *e.g.*,

$$Cu + 3Cl^- \rightarrow CuCl_3^- + 2e;$$

$$Cu + 4Cl^- \rightarrow CuCl_4^{2-} + 2e,$$

under these conditions.

As the Roe and Toni equation[4] for i_p involves n^2, one-electron transitions should give peaks a quarter the size of those for corresponding two-electron processes, hence any change in the degree of dichlorocuprate(I) formation induced by increasing the chloride concentration should lead to peak-height variations.

As shown by curve *b* in Fig. 2, the peak potentials showed a progressive cathodic shift as the chloride concentration was raised. These changes can be attributed to the effect of chloro-complex formation on the conditional electrode potential $E^{o'}$ of the system.

Organic ligand effects

The presence of complexing agents (natural or synthetic) in test solutions can lead to shifts in the dissolution potentials of elements during the stripping process (*cf.* the chloride effect), or a wider spread of the reduction potentials associated with the preliminary electrolysis step. ASV is not capable of distinguishing between free metal ions and labile complexes (*i.e.*, species which dissociate at a rate greater than the rate of plating the free metal), hence any observed changes in ASV signals indicate the presence of non-labile species. The use of such changes to discriminate between chemical forms is complicated by the fact that dissociation of "non-labile" complexes may be induced by shifting the deposition potential to more negative values.[23] When the ligand to metal ratio is only slightly greater than unity the possibility of dissociation of strong complexes cannot be neglected[17] and short deposition times (*e.g.*, <5min) are generally recommended.[2]

It has been stated[24] that i_p measurements permit differentiation between non-labile complexes and "free' ions or labile complexes; E_p values permit differentiation between free metal and complexed metal; the combination of both allows differentiation between free metal and labile metal complexes. In view of the variations which can be introduced by altering pH, deposition potential, and other factors, this statement may need to be qualified significantly. In fact, another investigator has suggested[16] that shifts in E_p may be caused only by inorganic ligands, since no variation in E_p values for zinc was noted when estuarine water samples were treated with organic ligands (*e.g.*, EDTA, adenine) or were irradiated with high-intensity ultraviolet light to break up naturally occurring chelating agents.

To test this contention, studies of Pb, Cu and Cd behaviour in the presence of a series of organic ligands have been made.

Table 2. Effect of varying amounts of EDTA on metal-ion peaks (base electrolyte 1*M* sodium acetate, pH 5.5, 100 μg/l. M^{2+}, stripping scan-rate 50 mV/sec, deposition potential −900 mV (*a*) or −1000 mV (*b*) applied for 10 min)

	Lead[a]		Cadmium[b]		Copper[a]	
EDTA:M^{2+}	i_p, μA	E_p, *mV*	i_p, μA	E_p, *mV*	i_p, μA	E_p, *mV*
0:1	132	−568	200	−726	217	−141
1:1	56	−568	118	−726	105	−142
2:1	12	−568	10	−726	77	−145
10:1	0	—	0	—	116	−148
100:1	0	—	0	—	155	−154

Table 2 summarizes the results obtained when EDTA in varying amounts was added to 1*M* sodium acetate at pH 5.5, containing 100-μg/l. levels of metal ion.

With Cd and Pb, a slight excess of ligand converted the metal ions into non-labile anionic complexes, with a resultant complete loss of signal when a deposition potential of −900 or −1000 mV was used. The copper–EDTA complex is more stable than that formed by the other two metal ions, yet the peaks in this case had i_p and E_p values which were similar to those observed in ammonium acetate or chloride salt solutions (*cf.* Table 1). The magnitude of the copper peak current decreased markedly when more positive deposition potentials were applied, reducing to ~15 μA for −700 mV, and to ~5 μA with plating potentials of −600 or −400 mV. These results imply that the classification of a complex as labile or non-labile may depend on the basic operating conditions initially selected.

The effective stability of EDTA complexes is a function of pH, and when the pH of the copper system was lowered to 2.9, application of a deposition potential of −600 mV yielded a measurable peak (~20 μA). With a 2:1 EDTA:M^{2+} ratio, lowering the pH from 5.5 to 2.9 doubled the size of the small lead peak and the cadmium signal increased from 10 to 70 μA. The behaviour also appears to be a function of the electrode type and total electrolyte system, since E_p (*vs.* SCE) for copper, with a deposition potential of −480 mV and a mercury drop electrode, has been reported[25] to be −200 mV (0.2*M* EDTA); −240 mV (0.2*M* EDTA, pH 7); −340 mV (0.2*M* ammonium carbonate, 0.01% EDTA); and no peak discernible (2*M* sodium acetate/0.1*M* EDTA). With a flow-cell hanging mercury drop electrode and 0.01*M* EDTA, copper peaks appeared[26] at −260 (pH 4.6) and −440 mV (pH 9.5). The peak potentials for lead and cadmium in the same medium were −490 (pH 4.6) or −580 mV (pH 9.5) and −680 (pH 4.6) or −720 mV (pH 9.5) respectively. The values for these two elements in alkaline media are comparable with those quoted in Table 2. Substitution of DCTA for EDTA did not alter the observed peak potential for Pb and Cd, but with copper there was a shift to −150 mV (pH 4.6) and −370 mV (pH 9.5).

The complexes formed by metal ions with nitrilotriacetate (NTA) are less stable than their EDTA counterparts, and this is reflected in the size of peak observed under corresponding experimental conditions. For example, in an acetate solution of pH 5.5, addition of up to 10 moles of NTA per mole of M^{2+} had no effect on the i_p or E_p values of Cd and Pb; a 10:1 ratio halved the "free" copper-ion i_p value. Larger excesses (*e.g.*, 100:1) also caused reductions in the size of Pb and Cd peaks, the magnitude of the effect increasing as the pH was increased from 3 to 9.

Log K_1 values[12] for the NTA complex of Pb, Cd and Cu are around 11, 9 and 13 respectively (*cf. ca.* 17, 16 and 19 for the EDTA species) and thus it can be proposed that even if the effective stability of a complex ion is quite high, its influence on anodic stripping behaviour can depend greatly on the deposition potential and pH.

This generalization was confirmed in studies in which up to a 100-fold excess of citric acid was present. At pH 5.5, the presence of the ligand caused no shift in i_p or E_p for any of the three metal ions examined. At pH 8.9, Pb and Cd behaviour remained unaffected, but copper showed both a shift in E_p and a decrease in i_p with increases in the citrate:Cu ratio above 10:1. Unlike the EDTA system, changing the magnitude of the deposition potential from −500 to −1000 mV had little affect on the copper signal. Further increases to −1300 mV increased the signal to double its smallest size (*i.e.*, from 24 to 48 μA). With a 100:1 citrate:Cu ratio and pH 8.9, E_p shifted by 40 mV and it seems logical to propose that this signal arises from the involvement, in the redox process, of the complex anionic species present in solution at this pH. This conclusion runs counter to the view[25] that only inorganic ligands cause E_p shifts, but is supported by the observed behaviour of copper in the presence of 2,2′-bipyridyl (*cf.* Table 3).

2,2′-Bipyridyl (bipy) differs from the other ligands examined, in that the complexes formed retains a positive charge, and thus could be attracted to the working electrode during the deposition cycle.

The log K value for the lead complex, PbL^{2+}, is ~3 and even with a 100-fold excess of ligand this stability proved to be insufficient to introduce any significant deviations in the stripping signal over the

Table 3. Effect of 2,2'-bipyridyl on copper ASV peaks (base electrolye 1*M* sodium acetate, 100 μg/l. Cu, stripping scan-rate 50 mV/sec, deposition potential −600 mV applied for 10 min, bipy:Cu = 100:1)

	Peak height, *μA*		Peak potential, *mV*	
pH	Cu	Cu + bipy	Cu	Cu + bipy
2.3	398	~200	−75	−152
3.9	306	~120	−110	−205
5.3	194	36, 32	−160	−189, −397
7.0	188	36, 34	−170	−202, −412
8.9	135	39, 39	−226	−209, −415

pH range 2.3–8.9. Cadmium forms a series of complexes (CdL^{2+}, CdL_2^{2+}, CdL_3^{2+}), and in this case the effective stability (and decreased lability) was sufficient for a small (<25%) reduction in i_p to occur at pH values >5.3, with a bipy:Cd ratio of 100:1.

With 100 times more bipyridyl than copper present, there were multiple effects as shown in Table 3. At pH >5, for example, the sharp symmetrical peak obtained in acetate solution was replaced by two distinct but very broad peaks, each having an E_p value different from the value in acetate medium. At pH ~4 the peaks merged, with the more cathodic component being now discernible as a shoulder. At pH 2.3, overlap was almost total (except for a small shoulder on the anodic side), but the total peak current remained only half that observed in the absence of the ligand.

Splitting of the peak was observed only when a large excess of ligand was present. A 10:1 ratio (or less) at pH 5.5 caused no E_p shift in the single peak but the i_p readings were smaller, *e.g.*, 150 μA.

The formation of double peaks could be promoted by adsorption of ligand on the electrode surface, leading to stabilization of both copper(II) [CuL^{2+}, CuL_2^{2+}, CuL_3^{2+}] and copper(I) oxidation products. Log β_2 for the copper(I) species, ML_2^+, is quoted[12] as 14.2 and the common height of the two peaks implies that two successive one-electron transfer processes occur.

Glycine can react with metal ions to form both cationic and anionic species (depending on pH) but the presence of a hundredfold excess of the ligand or pH variations in the range 3–9 produced no significant change in the ASV peaks of Cu, Cd or Pb. The complexes formed are not very stable (*e.g.*, log K_1 is 4–5 for Pb and Cd, and ~8 for Cu) and with an acetate:glycine ratio of about 10^5:1, either acetate coordination predominates or both complexes are very labile.

The addition of thiourea (0.05 or 0.1*M*) to 0.1*M* potassium nitrate medium is reported[25] to shift the copper peak to the region between −510 and −540 mV (*vs.* SCE).

In brief, the presence of organic ligands can result in shifts in E_p and i_p values, but interpretation of ligand effects is complicated by the ill-defined roles of other experimental parameters.

CONCLUSIONS

With adequate control of operating parameters, efficient use of blanks and careful calibration with solutions of similar matrix type, anodic stripping voltammetry provides a sensitive and precise means for determining μg/l. levels of metal ions. This was confirmed by analysing a series of industrial waters and comparing the results with those obtained by carbon-cup or flame atomic-absorption spectrometry, preceded by solvent extraction preconcentration.

However, the studies described in this paper have shown that chemical factors play varying roles in determining peak currents and potentials and before the technique can be confidently applied to direct distinction between chemical forms, a better understanding of complex formation effects would appear to be desirable. Most natural systems tend to contain several components capable of co-ordinating with metal ions, and it remains to be proved whether their behaviour is more complex than that observed in these binary system studies.

In the meantime, classification of the heavy metal content of natural waters into seven groupings is being achieved[27] by using ASV in conjunction with a series of intermediate phase separations.

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INVESTIGATIONS ON THE USEFULNESS OF TIRON IN SEPARATION OF METAL IONS ON THE MACROPOROUS ANION-EXCHANGER AMBERLYST A-26

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Summary—The possibility of application of a sulpho-derivative of an aromatic organic complexing agent—tiron—for separation of cations on the macroporous anion-exchanger Amberlyst A-26 has been investigated. Comparative results obtained with Amberlite IRA 400 have proved the macroporous exchanger to be the more useful. The dependence of retention on pH obtained has been established by the batch method for: Ag, Ni, Co(II), Mn(II), Zn, Cd, Pb, Cr(III), Fe(III), Ga, Al, In, Bi, Ti(IV), V(V). By taking advantage of selectivity differences, the following mixtures have been separated: Al–Ga, Al–Ti(IV), Ti(IV)–Ni, Ni–Fe(III), Ni–Fe(III)–Ti(IV).

Aromatic complexing agents (ACA) containing sulphonic acid groups are particularly useful in separation of metal ions on anion-exchange resins, as shown in our previous papers.[1–6] These compounds display a high affinity for anion-exchangers, as a consequence of their structure, and when retained on the exchange-resin transform it into a selective exchanger, the selectivity depending on the character of the functional analytical groups of the ligand.

Our conclusions concerning the usefulness of sulpho-derivatives of aromatic organic reagents in this context have been confirmed by others.[7–9] So far, however, the usefulness of ACA containing a single benzene ring has not been studied. Our investigations suggest that such compounds have lower affinity for anion-exchangers, hence giving lower selectivity coefficients.[10]

The research presented here concerned the usefulness of the disodium salt of 1,2-dihydroxybenzene-3,5-disulphonic acid (tiron). The reagent has interesting complexing properties and should provide useful separations of those metal ions which differ sufficiently in their affinity for the donor oxygen atoms of the ligand.

Because of the low affinity of tiron for anion-exchangers, macroporous resins were examined. These resins, in view of their large surface, ought to provide a better chance of interaction with the reagent immobilized in the resin phase, and hence a stronger binding of tiron to the exchanger. This should compensate for the absence of a large number of condensed benzene rings in the ligand.

EXPERIMENTAL

Reagents

Ion-exchange resin. Amberlyst A-26 (BDH) was chosen. It is strongly basic, with exchange capacity of 4.1–4.4 meq/g, and particle size 0.4–0.5 mm. Amberlite IRA 400 (Rohm and Hass) was used for comparison; its capacity is 3.1 meq/g.

Solutions of metal ions. Obtained by dissolving appropriate weights of the nitrates in doubly distilled water, and standardized complexometrically. A solution of Ti(IV) was obtained by dissolution of TiO_2 in concentrated sulphuric acid and ammonium sulphate.

Apparatus

Perkin–Elmer atomic-absorption spectrophotometer with HGA-72 graphite furnace. Specol UV-VIS and Specord spectrophotometers. Fraction-collector with time recorder.

The columns were 10 mm bore, fitted with a stopcock.

Procedures

Determination of metal ions. Atomic-absorption spectrometry (AAS) was used, with the methods recommended by Perkin–Elmer (Table 1). Ti(IV) was determined colorimetrically, with tiron as reagent. Silver was determined potentiometrically with an ion-selective electrode.

Determination of exchange capacity for tiron. The static and dynamic methods were used. In the static method, 200 mg of exchange resin in chloride form were shaken with 20 ml of tiron solution of various concentrations. In the dynamic method a known volume of tiron solution of constant concentration was passed through a bed of ion-exchanger of known mass. In both methods the quantity of tiron retained was determined from the difference in concentrations: in the static method the concentrations before and after equilibrium was reached; in the dynamic method the concentrations in the initial solution and the eluate. The tiron concentration was measured spectrophotometrically. Tiron retention was also studied under static conditions as a function of the exchanger form and the pH of the solution.

For comparison the retention of tiron on Amberlite IRA 400 was also studied.

Retention of metal ions on the resin in the presence of tiron. The static method was used with a constant molar ratio of metal ion to tiron [Me]:[tiron] = 1:10, an initial concentration of metal ion of 1.7×10^{-4} *M*, 200 mg of exchanger in chloride form, and 20 ml of solution. The metal ion + tiron solution was added to the resin and the pH value determined. The mixture was shaken for 24 hr and the metal ion concentration was then determined. This period was adequate for reaching equilibrium.

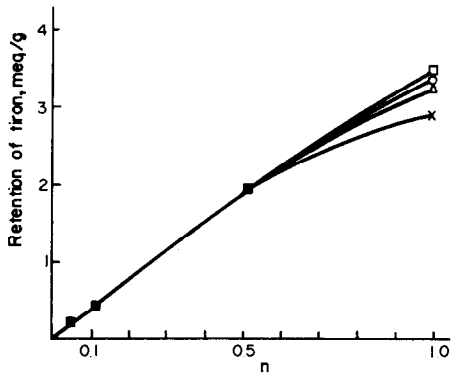

Fig. 1. Effect of tiron concentration on its retention on Amberlyst A-26 ion-exchanger at various pH values. ×, pH 1.0; △, pH 2.0; ⊙, pH 4.0; □, pH 6.0.

In the dynamic method 2 g of the exchange-resin were shaken with 10 ml of 0.128*M* tiron. The resin (with tiron immobilized in the exchanger phase) was placed in a column, and 15 ml of metal ion solution (0.128*M* in tiron and at pH *ca.* 2–3) were passed through the column.

Elution curves were obtained by collecting and analysing fractions.

RESULTS AND DISCUSSION

Retention of tiron

The capacity of Amberlyst A-26 (chloride form) for tiron, determined by the dynamic method, was 4.23 meq/g. The calculation included a correction (5.1%) for tiron retained in the resin pores. Tiron retained in the pores can be washed out with water.

The maximum retention of tiron under static conditions was only 4.1 meq/g. The maximum retention was observed when the ratio (n) of meq of tiron in the initial solution to meq of functional groups in the exchanger was equal to 2. At higher ratios retention decreased.

With rise in n (Fig. 1), pH also had an effect on tiron retention, presumably because of sorption, which is likely to occur at higher tiron concentrations. The effect is decreased by acidification of the solution. Up to $n = 0.46$ the tiron is 100% retained.

Studies were made of retention of tiron on the exchanger (saturation = 31.2% of capacity) on elution with various concentrations of acids. The results are collected in Table 2. There is considerable elution of tiron when the column is washed with 2*M* hydrochloric acid. Elution of tiron depends on the acid used, the sequence of efficiency being $HCl < HNO_3 < HClO_4$, which corresponds to increasing affinities of the mineral acid anions for the exchange resin.

Comparative studies with the strongly basic Amberlite IRA 400 showed that the capacity for tiron, determined under dynamic conditions, also corresponded to the maximum capacity of the exchanger (3.1 meq/g).

Under static conditions, however, maximum retention was only 80% of the total capacity of the resin in chloride form. Under dynamic conditions, even very dilute acids caused elution of tiron.

Retention of metal ions

Retention of metal ions was studied at a 1:10 molar ratio of metal to ligand immobilized in the resin phase, as a function of pH. The results are presented in Fig. 2. No tiron or tiron complexes with metal ions were found in the aqueous phase. It follows that there are marked differences in the metal ion retention.

At the given molar ratio [Me]:[tiron] the following ions are not retained over the pH range from 1 to 6: Ni(II), Co(II), Mn(II), Cr(III). Zn(II) and Cd(II) are feebly retained and there is stronger retention of Ti(IV), In(III), Fe(III), Pb(II), Ga(III), Al(III), V(V). Ag(I), Zr(IV) and Bi(III) are very strongly bound.

On the basis of these static studies it is possible to design separations of mixtures of metal ions. The retention as a function of pH is a convenient parameter for evaluating the selectivity, which arises from differences in the stability of the respective metal ion complexes with tiron.

Table 1. Conditions of determining metal ions by the AAS method

Metal ion	Wavelength *nm*	Band-width *nm*	Volume of sample used for the determination, *µl*
Co(II)	301.8	0.2	20
Cu(II)	325.0	0.7	20
	223.0	0.7	5
Ni(II)	232.5	0.2	20
	346.5	0.7	10
Zn(II)	308.0	0.7	50 or 5
Pb(II)	283.6	0.7	10
Mn(II)	403.3	0.2	10
Al(III)	309.3	0.7	20
	257.0	0.2	10
Ga(III)	287.7	0.7	10
In(III)	304.0	0.7	10
Cr(III)	428.9	0.2	10
Fe(III)	248.3	0.7	5
V(V)	318.3	0.7	10

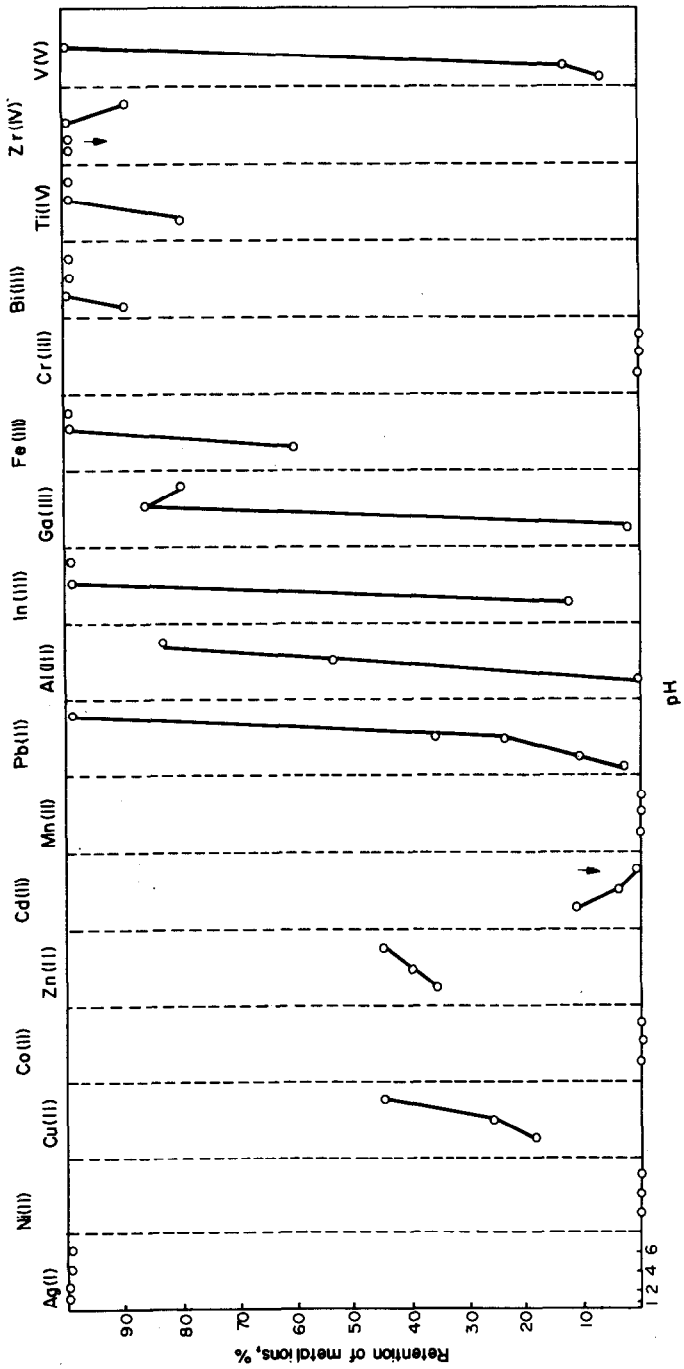

Fig. 2. Dependence of retention of metal ions on Amberlyst A-26 ion-exchanger, in presence of tiron, on pH value.

Table 2. Effect of a mineral acid on tiron retention on Amberlyst A-26 under dynamic conditions

Acid	Concn., *M*	Volume, *ml*	Tiron retention, %
HCl	0.001	120	100
	0.005	60	100
	0.05	190	100
	0.1	200	98.4
	0.5	200	88.7
	2.0	200	35.6
HNO_3	0.1	100	97.5
	0.2	100	93.0
	0.4	100	73.8
$HClO_4$	0.6	100	1.4

Table 3. Literature values of stability constants for metal ion complexes with tiron

Metal ion	log K_1	log K_2	log K_3
Cu(II)	14.5[13]	10.96[13]	
Co(II)	9.49[13]		
Ni(II)	9.96[12]		
Cd(II)	7.69[13]		
Zn(II)	10.41[13]		
Pb(II)	14.77[13]		
Mn(II)	8.6[14]		
Al(III)	16.5[17]	11.98[13]	9.7[17]
	19.02[13]	13.5[17]	
Fe(III)	20.7[12]	15.2[12]	9.9[12]
Ga(III)	19.24[15]		
In(III)	16.36[16]		
H^+	12.7[12]	7.7[12]	

The stability constants do not give an unequivocal guide to the separation efficiency or conditions, however. The results simply show that those metal ions with high affinity for oxygen atoms as donors also have high affinity for tiron.

Comparison of retention results with theoretical values

It seemed of interest to examine whether theoretical calculations could predict the dependence of degree of retention on pH, since this might allow us to draw some conclusions as to the complexation mechanism.

The conditional stability constants were calculated as a function of pH according to Ringbom and Harju.[11] The stability constants used are given in Table 3. It was assumed that within the pH range under study 1:1 complexes are formed (in agreement with the literature data). Ligand-protonation side-reactions were found. Within the pH range 1–6 the effect of OH^- ions can be neglected.

It was also assumed (on the basis of the results for tiron retention) that the ligand is 100% retained in the resin phase, and that only free metal ions are in equilibrium with the resin. This also followed from the spectrophotometric studies. The results obtained are represented in Fig. 3 and show adequate agreement between theoretical and experimental results, except for some divergences in the case of Cu(II).

The results indicate that the assumptions made for the calculations were valid and that metal ion complexation with tiron immobilized in the resin phase occurs in the same way as it does in solution. It may

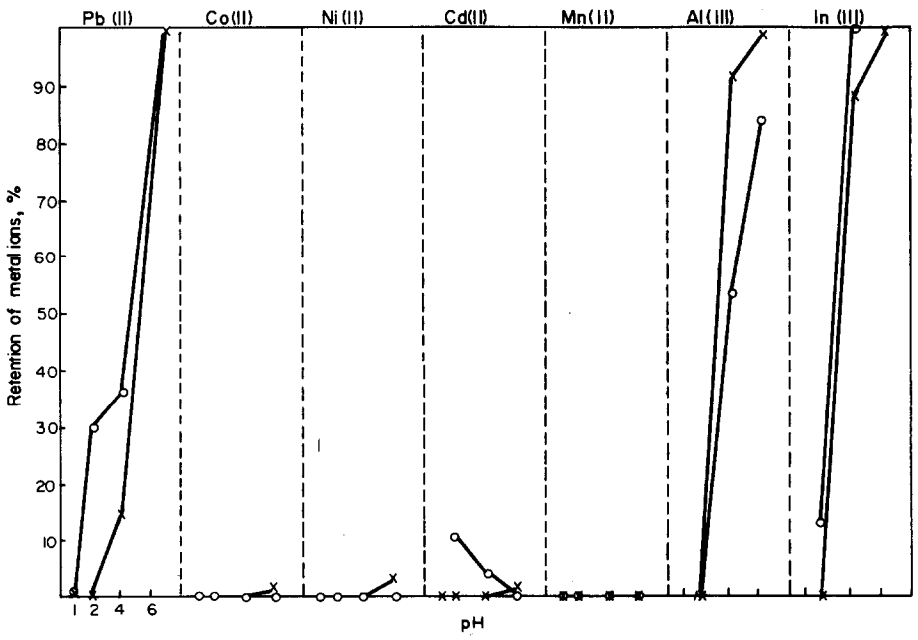

Fig. 3. Comparison of experimental results of metal-ion retention on Amberlyst A-26 ion-exchanger with values obtained theoretically. ⊙, Experimental; ×, theoretical.

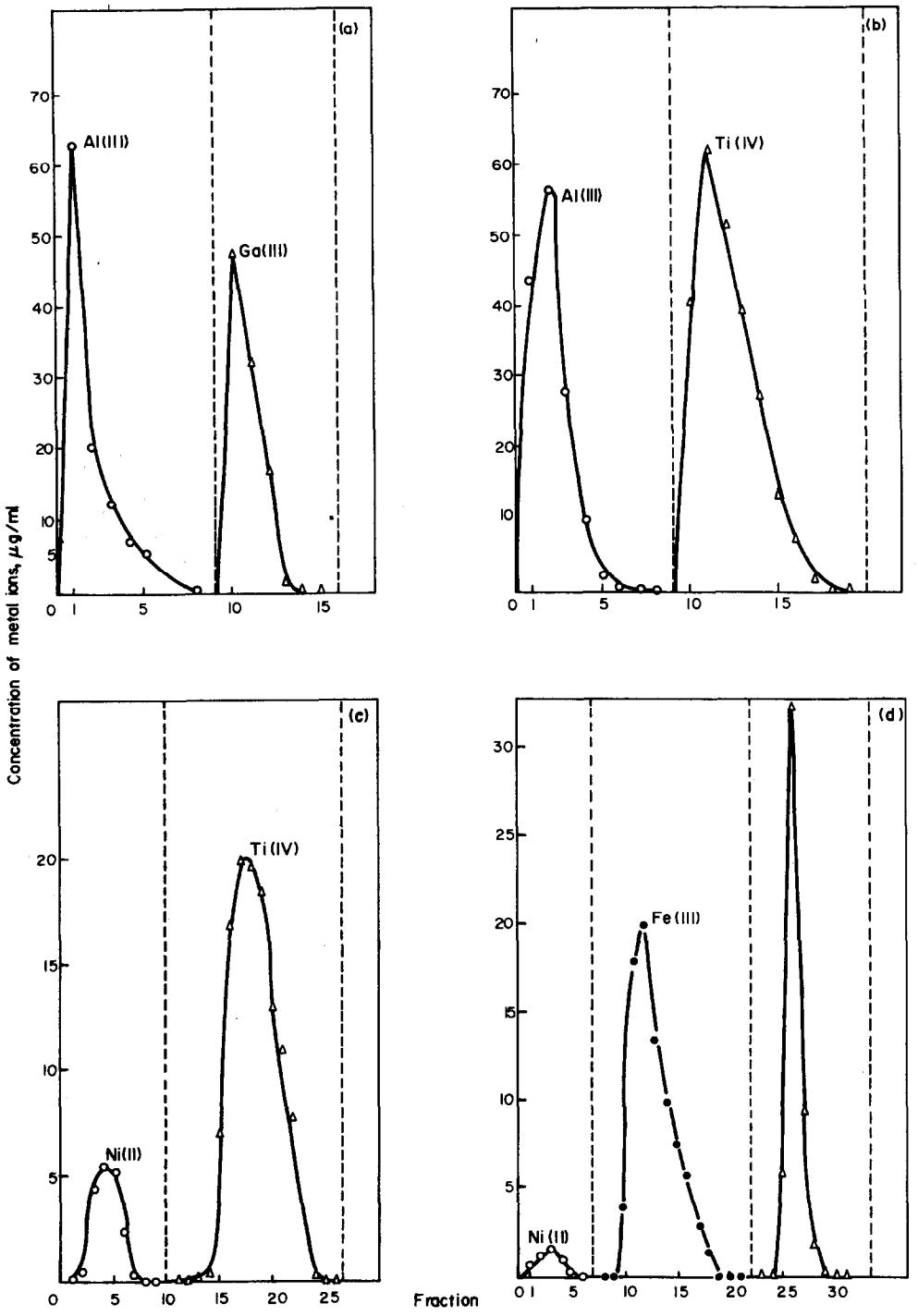

Fig. 4. Elution curves showing the separation of metal ions on Amberlyst A-26 with the eluents indicated in Table 4.

also be assumed that the pH of the solution is the decisive factor in the complexation reaction in the resin phase.

Studies on metal ion separation under dynamic conditions

From the results of the static studies, separations of binary and ternary mixtures of metal ions were predicted. As expected, the metal ions not retained under static conditions by tiron immobilized on the resin could be eluted with water. Highly dilute acids were used to avoid hydrolytic reactions of hydrated metal ions in the eluate.

To elute more strongly bound metal ions, mineral

Table 4. Results of metal separation on Amberlyst A-26 ion-exchanger

Metal ion	No. of detns.	Eluent	Amount of metal, *mg*	
			Added	Found
Al(III)	3	120 ml 0.001*M* HCl	2.30	2.30
Ga(III)		100 ml 0.4*M* HNO_3	2.80	2.80
Al(III)	4	120 ml 0.001*M* HCl	2.32	2.32
Ti(IV)		110 ml 0.6*M* $HClO_4$	5.00	4.98
Ni(II)	5	60 ml 0.005*M* HCl	0.10	0.096
Ti(IV)		100 ml 0.6*M* $HClO_4$	2.52	2.51
Ni(II)	3	60 ml 0.005*M* HCl	0.05	0.05
Ti(IV)		110 ml 0.6*M* $HClO_4$	5.04	4.92
Ni(II)	3	60 ml 0.005*M* HCl	0.05	0.05
Fe(III)		190 ml 0.05*M* HCl	1.00	1.00
Ni(II)	2	60 ml 0.005*M* HCl	0.05	0.05
Fe(III)		190 ml 0.05*M* HCl	1.00	1.00
Ti(IV)		110 ml 0.6*M* $HClO_4$	5.00	4.85

acids of higher concentration were used. The results are collected in Table 4 and the elution curves are presented in Fig. 4.

For comparison, studies were made with Amberlite IRA-400, and Ti was separated from Ni or Cu but separation at higher Ti(IV) excess was difficult. It was found that the chloride form of the resin is useless and therefore the acetate form was used. Tiron was readily eluted when the chloride form was used.

CONCLUSIONS

The results obtained confirm the usefulness of tiron for metal ion separation on the macroporous exchange-resin Amberlyst A-26, and show that Amberlite IRA 400 is less efficient.

In studies by the static method it has been established that even when 45.4% of the resin capacity is occupied by tiron, the latter is still quantitatively retained in the resin phase. In dynamic studies, it has been confirmed that there is strong binding of tiron even when the column is washed with dilute mineral acids. This is important from the practical point of view, because dilute acids are used for selective elution of metal ions.

The dependence of retention of metal ions on the pH at a constant molar ratio of metal to ligand has been shown to be a convenient parameter for prediction of separations. Further studies will include applications to analysis of alloys (*e.g.*, of aluminium).

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STUDY OF THE OXIDATION OF ETHYLENEDIAMINETETRA-ACETIC ACID WITH LEAD DIOXIDE SUSPENSION IN SULPHURIC ACID

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Summary—The stoichiometry of the reaction between lead dioxide suspension and EDTA was studied by derivative polarographic titration and determination of the products. Four moles of Pb(IV) are reduced per mole of EDTA with moderate speed at room temperature in sulphuric acid solutions. Four moles of carbon dioxide and 3 moles of formaldehyde are the products of the oxidation of 1 mole of EDTA. One mole of *N*-hydroxymethylethylenediamine is also thought to be produced. The overall reaction may be written as

$$4Pb(IV) + EDTA + 4H_2O \rightarrow 4Pb(II) + 4CO_2 + 3HCHO + H_2NCH_2CH_2NHCH_2OH + 8H^+.$$

Ethylenediamine is also partly produced if a large excess of lead dioxide is used.

The investigation of the redox reactions of organic sequestering agents such as ethylenediaminetetra-acetic acid (EDTA) with various oxidizing agents has become increasingly important in analytical and environmental chemistry.[1,2] The kinetics and the reaction products of the oxidation of EDTA with Ce(IV)[3] and Mn(III)–EDTA[4] have been reported, but the stoichiometry of the reactions has not been made clear. Another report deals with the oxidative determination of EDTA with potassium permanganate,[5] but says nothing about the kinetics or the products.

Recently, in our laboratory, the redox reaction of lead dioxide suspension, prepared by hydrolysis of lead tetra-acetate, with sodium oxalate in nitric acid was investigated by derivative polarographic titration (conventional potentiometric titration at constant current, the "DPT" method) and determination of the reaction products.[6]

The present work applies the same methods to the redox reaction of lead dioxide suspension with EDTA in sulphuric acid. Four moles of Pb(IV) are reduced per mole of EDTA with moderate speed at room temperature in sulphuric acid media. Four moles of carbon dioxide and three moles of formaldehyde are produced from one mole of EDTA, and one mole of *N*-hydroxymethylethylenediamine is also thought to be produced.

These results are of interest from the viewpoint of heterogeneous reactions in analytical chemistry and also oxidative destruction of polyaminocarboxylic acids in waste-water treatment in environmental chemistry.

EXPERIMENTAL

Reagents and apparatus

A 0.05*M* solution of lead tetra-acetate in glacial acetic acid was standardized by potentiometric titration with sodium oxalate.[7] A 0.02*M* EDTA solution was prepared from the recrystallized dihydrate of the disodium salt. The formaldehyde solution was prepared by diluting commercial formalin and standardized by the sodium sulphite method. The ethylenediamine solution was standardized with hydrochloric acid.[8] Three potentiometers were used to measure the potentials of the anode and cathode *vs.* SCE, and the potential difference between anode and cathode. All titrations were performed at 25 ± 0.2°.

Determination of the reaction stoichiometry

The DPT method was used for determining the reacting ratios, as follows. A 100-ml electrolytic cell fitted with a rubber stopper was used as the reaction vessel, to which 5 ml of 2.5*M* sulphuric acid, 5 ml of 1*M* potassium nitrate, and 35 ml of distilled water were added. The solution was deaerated with nitrogen. Two platinum electrodes, 0.5 mm in diameter and 20 mm long, sealed in glass tubes, were inserted as the anode and cathode. The anode was electrolytically reduced to remove the oxide film on the platinum surface. Then 5.00 ml of 0.05*M* standard lead tetra-acetate solution were added followed by a known amount of standard EDTA solution, nitrogen was passed through the solution for 1 min, and the potential difference produced by the constant current polarization was measured after 1 min more. The waiting time before measurements of potential difference was made longer (3 min) in the neighbourhood of the end-point. The solution was stirred throughout the titration.

Determination of the products

Solutions of lead tetra-acetate and EDTA were mixed in 4:1 molar ratio in 0.25*M* sulphuric acid and allowed to stand for 30 min.

The lead sulphate was filtered off, then dissolved in a small amount of 5*M* ammonium acetate, and the solution was adjusted to pH 4–5 with dilute nitric acid and analysed for lead polarographically.

Particular attention was paid to measuring the amount of carbon dioxide evolved, since contamination with acetic

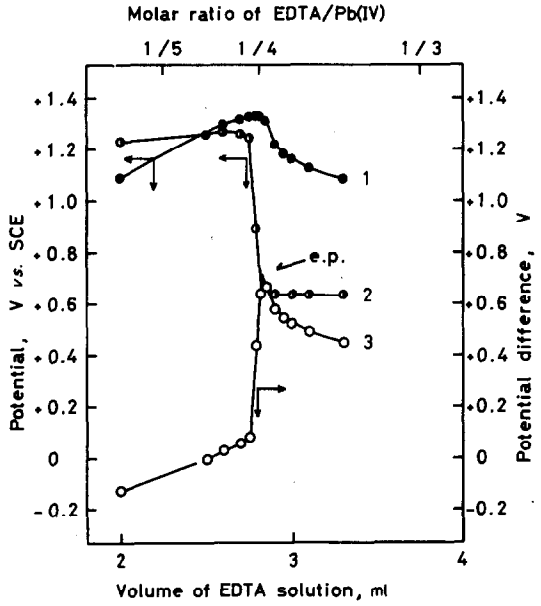


Fig. 1. Titration curve and potential changes of anode and cathode for 5.00 ml of $4.55 \times 10^{-2}M$ lead tetra-acetate hydrolysed in 0.25*M* sulphuric acid and 0.1*M* potassium nitrate, and then titrated with $2.02 \times 10^{-2}M$ EDTA. Current density 19 nA/mm^2: ●: anode potential; ◑: cathode potential; ○: ΔE.

acid was unavoidable. The carbon dioxide evolved was first absorbed in two traps, each containing 50 ml of 0.005*M* barium hydroxide, a stream of nitrogen being passed through the system for 10 hr, as carrier gas. Then 4 ml of conc. nitric acid were added to the traps and the carbon dioxide evolved was transferred into another two traps with a stream of nitrogen. The excess of barium hydroxide in these two traps was titrated with hydrochloric acid. The flow-rate of nitrogen was 10 ml/min in both cases.

Formaldehyde was determined by distillation into a solution of Schiff's reagent or chromotropic acid followed by gravimetric determination with dimedone[9] or titration with iodine.[10]

Ethylenediamine was determined as follows. Lead tetra-acetate and EDTA solutions were mixed in sulphuric acid medium. Lead sulphate and lead dioxide were filtered off and most of the acetic acid was removed by steam distillation. Barium hydroxide crystals were added to remove most of the sulphuric acid and the barium sulphate was filtered off. Iodine and alkali were added to oxidize formaldehyde to formic acid and liberate ethylenediamine from *N*-hydroxymethylethylenediamine. The solution was then evaporated to small volume to remove excess of iodine and raise the efficiency of the subsequent steam-distillation. The solution was placed in a 200-ml Claisen flask, adjusted to pH 13 and steam-distilled. A known volume of hydrochloric acid of known concentration was added to the 400–500 ml of distillate, and the excess of acid was titrated potentiometrically with sodium hydroxide solution.

Table 1. Determination of carbon dioxide

Calcd., *mM*	Found, *mM*	Recovery %	Moles of CO_2 / Moles of EDTA
4.94	4.32	87	3.5
	5.31	107	4.3
	4.47	90	3.6
	4.79	97	3.9
	4.41	89	3.6
	4.59	93	3.7
			Mean 3.8

RESULTS AND DISCUSSION

Determination of reacting ratio

An example of the titration curve obtained by the DPT method is shown in Fig. 1. Curves 1 and 2 show the changes in the potential of the anode and the cathode, respectively. The reduction of lead dioxide and oxidation of OH^- are the electrode reactions before the end-point, at the cathode and the anode, respectively. After the end-point, the reduction of the oxide film on the cathode and oxidation of EDTA are the electrode reactions at the cathode and the anode. Curve 3 is the titration curve giving the changes of potential difference (ΔE) between the anode and the cathode. A peak appears at the end-point, and gives the molar reacting ratio between Pb(IV) and EDTA. The optimum current density was obtained by measuring current–potential curves at both electrodes.

A wide range of current density at the anode is suitable, but a low current density at the cathode

Table 2. Determination of formaldehyde by dimedone method

Moles of Pb(IV) / Moles of EDTA	HCHO, *mmole* Calcd.	HCHO, *mmole* Found*	Moles of HCHO / Moles of EDTA
4	0.121	0.091 ± 0.004	3.0
5.4	0.180	0.141 ± 0.005	3.1
16	0.120	0.103 ± 0.005	3.4
32	0.090	0.077 ± 0.002†	3.4
	0.155	0.180 ± 0.010§	3.5

* Average and deviation are based on three replicates of each of four solutions, except where indicated.

† One solution, three replicates.

§ Three solutions, three replicates of each.

Table 3. Determination of formaldehyde by iodimetry

Moles of Pb(IV)	HCHO, *mmole*		Moles of HCHO
Moles of EDTA	Calcd.	Found*	Moles of EDTA
5.4	0.180	0.170 ± 0.001	3.8
		0.181 ± 0.001	4.0
		0.171 ± 0.002	3.8
		0.177 ± 0.001	3.9
		0.177 ± 0.003	3.9
			Mean 3.9

* Average and deviation are based on three aliquots of the same solution.

(~25 nA/mm^2) is preferable. The titration of 50 ml of $4.55 \times 10^{-3}M$ lead dioxide suspension with $2.02 \times 10^{-2}M$ EDTA at various current densities from 6.3 to 63 nA/mm^2 gave practically identical results, with a 4:1 mole ratio of Pb(IV) to EDTA. A sharp peak on the titration curve was obtained with 19–25 nA/mm^2 current density. However, the titration curves were liable to rise after the end-point when a current density below 19 nA/mm^2 was applied. The reacting ratio was also determined as a function of the concentration of Pb(IV), (1.00 or 2.00 ml of lead tetra-acetate solution) the acidity (0.05 or 0.5*M* sulphuric acid), and temperature (35 or 45°). The 4:1 ratio was found in all cases.

Products

The determination of Pb(II) was tested with a standard lead nitrate solution. The recovery was 99 ± 2%. The determination of Pb(II) in the reaction solution confirmed the 4:1 reacting ratio.

The results for the determination of carbon dioxide are shown in Table 1. The calculated value listed is based on the assumption that all four carboxyl groups of EDTA are decarboxylated. The results indicate that four moles of carbon dioxide are produced per mole of EDTA.

The results for determination of formaldehyde by the dimedone method are shown in Table 2. The effect of other substances present (sulphuric acid, acetic acid, ethylenediamine, lead sulphate and lead dioxide) was studied beforehand, and the recovery of formaldehyde found to be 99 ± 1%. Table 2 indicates that three moles of formaldehyde are produced per mole of EDTA if the ratio Pb(IV):EDTA = 4:1, and more than three if a large excess of lead dioxide is present.

The results for the determination of formaldehyde by iodimetry are also shown in Table 3. The effect of ethylenediamine was studied first. The recovery of formaldehyde was 100 ± 1% in the presence of ethylenediamine in molar ratio between 1:4 and 3:4 to formaldehyde. The results appear to indicate that four moles of formaldehyde are produced per mole of EDTA. However, it has been reported[10] that the *N*-hydroxymethyl compounds of amines can be oxidized by iodine, and the *N*-hydroxymethyl group determined by iodimetry. *N*-Hydroxymethylethylenediamine has not been reported yet in the literature, but it would be formed between ethylenediamine and formaldehyde, and would also be determined by iodimetry. Therefore, it is supposed that three moles of formaldehyde and one mole of *N*-hydroxymethylethylenediamine are produced per mole of EDTA.

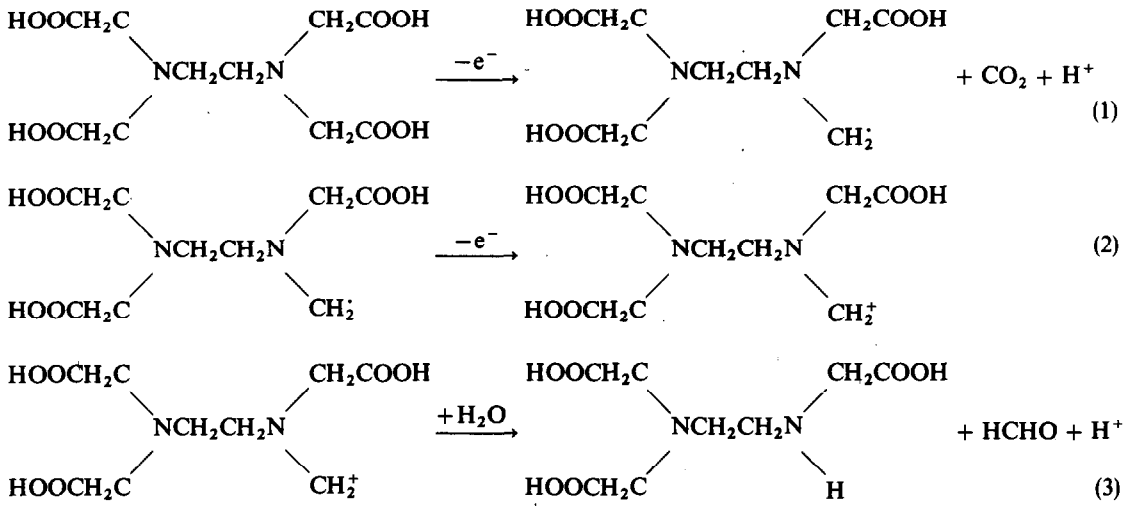
The results for determination of ethylenediamine are shown in Table 4. The calculated value is based on the assumption that one mole of ethylenediamine will be produced per mole of *N*-hydroxymethylethylenediamine. In preliminary experiments on the distillation method for the determination of ethylenediamine, the recovery for the complete procedure was about 72 ± 2%. The results in Table 4 indicate that the value found is about 59% of the calculated value. Taking into account the recovery (72%) in the preliminary experiments, about 82% of the expected amount of ethylenediamine is found. It is therefore postulated that one mole of *N*-hydroxymethylethylenediamine is produced from one mole of EDTA and almost completely recovered as ethylenediamine.

Table 4. Determination of ethylenediamine

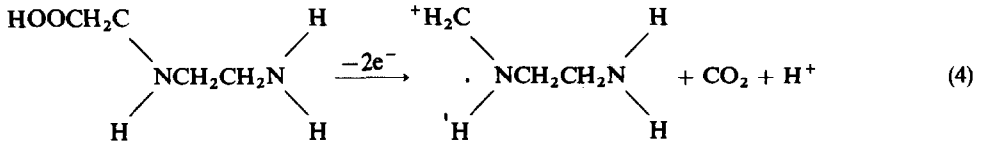
	Ethylenediamine		
EDTA, *mmole*	Calcd., *mmole*	Found, *mmole*	Recovery, %
0.180	0.180	0.109	61
		0.107	59
		0.104	58
		0.116	64
		0.102	57
		0.101	56
		Mean 0.107	59

CONCLUSIONS

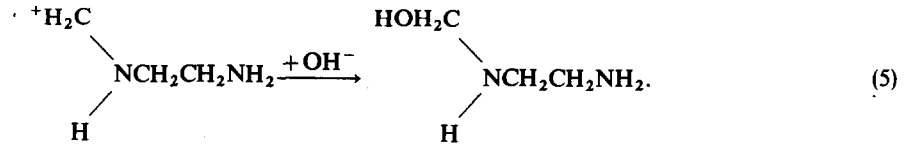
From the results above, the mechanism is suggested to be as follows. EDTA first loses one electron, with decarboxylation, and then a second electron before the detachment of formaldehyde by hydrolysis.



This sequence is repeated in the second and third steps. In the fourth step, it is supposed that after the decarboxylation of ethylenediamine-acetic acid, the hydrolysis does not occur easily,



and *N*-hydroxymethyl-ethylenediamine is produced by addition of OH^- owing to the abnormal Kolbe reaction.[11,12]



Therefore, the overall reaction may be written as

$$4Pb(IV) + EDTA + 4H_2O \xrightarrow{8e^-} 4Pb(II) + 4CO_2 + 3HCHO + H_2NCH_2CH_2NHCH_2OH + 8H^+. \quad (6)$$

In the presence of a large excess of lead dioxide, the detachment of more than three moles of formaldehyde per mole of EDTA proceeds. This is of interest because it may be due to the effect of the adsorptive properties of the surface of lead dioxide.

The reaction is being investigated in media other than sulphuric acid.

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LOGARITHMIC DIAGRAMS IN ACID–BASE TITRATIONS AND ESTIMATION OF TITRATION ERRORS

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Summary—The use of a logarithmic diagram for the estimation of the pH-value at the equivalence point and the titration error when a solution containing one or two acids is titrated with standard alkali is described.

Logarithmic diagrams with a master variable have been applied to acid–base equilibria for many years. They are very useful, since "one picture says more than a thousand equations".[1] A detailed description of the construction of such diagrams has been presented elsewhere.[1–3]

A logarithmic diagram for an acid–base system can be used to determine the concentrations of every species at a particular pH, to find the pH-value in a specified solution, to calculate the titration error when an acid solution is titrated with a base, and to draw the titration curve.

In this article the diagrams are used to derive new equations for the calculation of the pH-value at the equivalence point and the titration error when a solution containing one or two acids is titrated with a solution of a strong base.

THEORY

One acid in the solution

The main reaction when an acid HA is titrated with a standard sodium hydroxide solution is

$$HA + OH^- \rightarrow A^- + H_2O. \quad (1)$$

The acid–base system HA + A^- during the titration can be represented by the logarithmic diagram in Fig. 1. It has been constructed by starting with the equations:

$$[HA] = C_{HA} \Big/ 1 + \frac{1}{(K_{HA}[H_3O^+])} \quad (2)$$

$$[A^-] = C_{HA}/(1 + K_{HA}[H_3O^+]) \quad (3)$$

where C_{HA} is the total concentration of the acid–base system in the solution, and K_{HA} is the stability constant of the acid HA. C_{HA} and K_{HA} are defined by

$$C_{HA} = [HA] + [A^-] \quad (4)$$

$$K_{HA} = [HA]/[H_3O^+][A^-] \quad (5)$$

The rectangular diagram in Fig. 1 has the two axes divided into parts of equal length, the x-axis for pH from 0 to 14 and the y-axis for log C from 0 to −10.

The diagram also has a "system point" (S) with co-ordinates log K_{HA} and log C_{HA}. From this point are drawn lines of slope 0, +1 and −1, which are marked with the symbol of each species beside each linear portion. The linear parts for the species HA

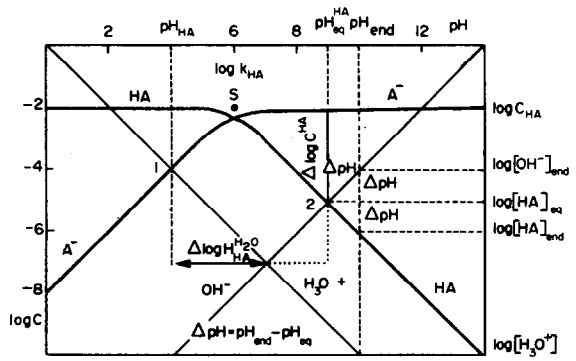

Fig. 1. Logarithmic diagram for the titration of an acid HA with a strong base. The total concentration of the acid during the whole titration is 0.01M. $K_{HA} = 10^6$.

and A^- are joined by short curves passing through a point 0.3 log unit below the system point.

From the upper left-hand corner of the diagram is drawn a straight line of slope -1 (plotting $[H_3O^+]$) and a second straight line of slope $+1$ (plotting $[OH^-]$) from the upper right-hand corner.

At point 1 in the diagram, *i.e.*, at pH_{HA}, the concentrations $[H_3O^+]$ and $[A^-]$ are equal and these are the concentrations in a solution of the pure acid HA. The pH-value of such a solution can of course be calculated from the general equation

$$[H_3O^+]^3 + [H_3O^+]^2/K_{HA} - (C_{HA}/K_{HA} + k_w)[H_3O^+] - k_w/K_{HA} = 0 \quad (6)$$

which under normal conditions is reduced to

$$[H_3O^+] = ((C_{HA} - [H_3O^+])/K_{HA})^{1/2} \approx (C_{HA}/K_{HA})^{1/2} \quad (7)$$

(k_w = ion product of water).

The pH to which the HA-solution should be titrated with a strong base can be read directly from the diagram. At this point [HA] and $[OH^-]$ should be equal and this prevails at point 2 (= pH_{eq}).

Titration error

Every titration is subject to error. A variety of factors (inaccurate recording of volume of titrant consumed, incorrectly calibrated volumetric glassware, inaccurate weights, *etc.*) may contribute to the error. In the following these "physical" errors will not be discussed, but only "chemical" errors that arise from uncertainty associated with the determination of the equivalence point of the titration. The result of a titration will be more or less in error depending on the method used to determine the equivalence point. This point can be read from the logarithmic diagram (point 2 in Fig. 1) or calculated by using the appropriate equations. In practice it is difficult to stop the titration at this value of pH (pH_{eq}), and the titration is terminated at a point in its vicinity. When a visual indicator is employed, it is usually possible to stop the titration at a value of pH that lies within ± 0.3–0.5 pH unit of the estimated (or calculated) value.

As a measure of the error in a titration the difference between the total concentration of strong base added when the end-point is reached (C_{OH}) and the total concentration of the acid can be taken. The percentage error (%e) is obtained when this difference is expressed as a percentage of the true value, that is,

$$\%e = 100(C_{OH} - C_{HA})/C_{HA} \quad (8)$$

Using the rule of electroneutrality the equation can be written

$$\%e = 100([OH^-] - [HA] - [H_3O^+])/C_{HA} \quad (9)$$

All the concentrations in equation (9) can be read from Fig. 1 for every pH-value in the vicinity of the equivalence point. If the diagonal lines representing H_3O^+ and HA are close to each other the concentrations of these two species must be added to give a new line representing the sum $[H_3O^+] + [HA]$. The point of intersection between this line and the line representing OH^- gives the "new" equivalence point, that is, $pH_{eq}^{corr} = pH_{eq}^{HA} + \Delta$. If the vertical distance between the lines for H_3O^+ and HA is bigger than 1.2 scale units, $[H_3O^+]$ can be neglected in comparison with [HA] and $pH_{eq}^{corr} \approx pH_{eq}^{HA}$.* The titration error can then be calculated from

$$\%e = 100([OH^-] - [HA])/C_{HA} \quad (10)$$

If $\Delta pH = pH_{end} - pH_{eq}$ it is seen from Fig. 1 that

$$\log [OH^-]_{end} = \log [HA]_{eq} + \Delta pH \quad (11)$$

$$\log [HA]_{end} = \log [HA]_{eq} - \Delta pH \quad (12)$$

and consequently,

$$\%e = 100([HA]_{eq}/C_{HA})(10^{\Delta pH} - 10^{-\Delta pH}) = 10^{\Delta \log C^{HA}} \cdot \Delta F \cdot 100 \quad (13)$$

where the ratio $[HA]_{eq}/C_{HA}$ is expressed by a distance (= vertical line with arrows) from the equivalence point (point 2 in Fig. 1) to the line $\log C_{HA}$. Values of the factor $\Delta F = (10^{\Delta pH} - 10^{-\Delta pH})$ for different values of ΔpH are shown in Table 1.

In Fig. 1 there is also an arrow line marked $\Delta \log H_{HA}^{H_2O}$, the length of which exactly equals that of the vertical arrow line $\Delta \log C^{HA}$. This means that the ratio $[HA]_{eq}/C_{HA}$ can be expressed by a distance between two pH-values in the diagram, that is, the pH of a solution of the acid HA and the pH of pure water,

$$\log([HA]_{eq}/C_{HA}) = \Delta \log H_{HA}^{H_2O} = pH_{HA} - 7 \quad (14)$$

From Fig. 1 it is also evident that pH_{eq} can be calculated by the aid of $\Delta \log H_{HA}^{H_2O}$, *i.e.*,

$$pH_{eq} = \log K_{HA} - \Delta \log H_{HA}^{H_2O} \quad (15)$$

Summing up the procedure for the calculation of the pH-value at the equivalence point and the titration error, the following equations can be used:

$$pH_{HA} = \tfrac{1}{2}(\log K_{HA} - \log C_{HA}) \quad (16)$$

$$\Delta \log H_{HA}^{H_2O} = pH_{HA} - 7 \quad (14')$$

Table 1. Values of the factor ΔF for different values of ΔpH

ΔpH	ΔF	ΔpH	ΔF	ΔpH	ΔF
0.1	0.46	0.6	3.73	1.2	15.79
0.2	0.95	0.7	4.81	1.4	25.08
0.3	1.49	0.8	6.15	1.6	39.79
0.4	2.11	0.9	7.82	1.8	63.08
0.5	2.85	1.0	9.90	2.0	99.99

* The correction term Δ can be calculated by using the logarithmic diagram. If the vertical distance between the lines representing HA and H_3O^+ (Fig. 1) is d, then $\log [OH^-] = \log [HA] + 2\Delta$ and $\log [H_3O^+] = \log [HA] - d$, that is, $[OH^-] = [HA]10^{2\Delta}$ and $[H_3O^+] = [HA]10^{-d}$. Substitution in the proton condition $[HA] + [H_3O^+] = [OH^-]$ gives $1 + 10^{-d} = 10^{2\Delta}$. If $d \geq 1.2$, then $2\Delta \leq \log 1.063$, or $\Delta \leq 0.013$, which is negligible.

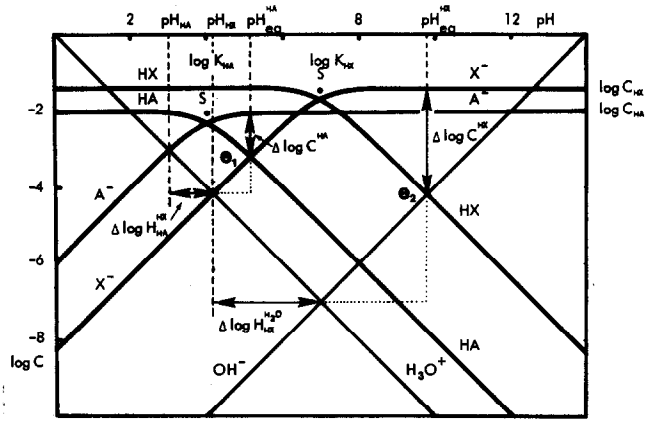

Fig. 2. Logarithmic diagram for the titration of a mixture of two acids, HA and HX, with a strong base. $C_{HA} = 0.01M$ and $C_{HX} = 0.04M$ during the whole titration. $K_{HA} = 10^4$, $K_{HX} = 10^7$.

$$pH_{eq} = \log K_{HA} - \Delta \log H_{HA}^{H_2O} \qquad (15')$$

$$\%e = 10^{\Delta \log H_{HA}^{H_2O}} \cdot \Delta F \cdot 100 \qquad (17)$$

If the acid HA is strong, equation (16) becomes $pH_{HA} = -\log C_{HA}$.

Example 1. A 0.01*M* solution of a weak acid HA is to be titrated with a standard sodium hydroxide solution. Calculate the titration error and the pH at the equivalence point if ΔpH is ±0.4 and $\log K_{HA} = 6$. (See Fig. 1.)

$$pH_{HA} = \tfrac{1}{2}(6 + 2) = 4; \quad \Delta F = 2.11$$

$$\Delta \log H_{HA}^{H_2O} = 4 - 7 = -3$$

$$\%e = \pm 10^{-3} \times 2.11 \times 100 = \pm 0.21$$

$$pH_{eq} = 6 + 3 = 9$$

Example 2. Repeat the calculations in Example 1, assuming that the acid is strong.

$$pH_{HA} = 2$$

$$\Delta \log H_{HA}^{H_2O} = 2 - 7 = -5$$

$$\%e = \pm 10^{-5} \times 2.11 \times 100 = \pm 0.002$$

$$pH_{eq} = 2 + 5 = 7$$

Two acids in the solution

If the solution, in addition to the acid HA, contains another acid HX with the stability constant

$$K_{HX} = [HX]\,[H_3O^+]\,[X^-] \qquad (18)$$

the total acid–base system can be represented by the logarithmic diagram in Fig. 2. The diagram is constructed in the same way as Fig. 1, but now contains two system points and sets of concentration times, one for the acid HA and the other for the acid HX. It is assumed that the two total concentrations are different and that $K_{HX} > K_{HA}$.

When the solution containing both acids is titrated with a strong base, the acid HA will react first. In the diagram the first equivalence point will be found at a point where the reaction (1) has proceeded as far to the right as possible, that is, [HA] is as small as possible, concomitantly with the reaction

$$HX + OH^- \rightarrow X^- + H_2O \qquad (19)$$

having progressed to the smallest possible extent, that is, $[X^-]$ should be as low as possible. These conditions are fulfilled at point e_1 in the diagram, where $pH = pH_{eq}^{HA}$. The corresponding titration error can be determined from the figure by using the equation

$$(\%e)_{HA} = 100\,([X^-]_{end} - [HA]_{end})/C_{HA} \qquad (20)$$

When the titration is terminated within the interval $pH_{eq}^{HA} \pm \Delta pH$ the values of $[X^-]_{end}$ and $[HA]_{end}$ can be read from the diagram.

The titration error can also be calculated by equation (13). The arrow-line $\Delta \log C^{HA}$ is drawn in the diagram.

When the titration is continued to the second equivalence point marked e_2, the pH-value is pH_{eq}^{HX}. The corresponding titration error for the titration of the sum of the two acids can be calculated from the equation

$$(\%e)_\Sigma = 10^{\Delta \log C^{HX}} \cdot \Delta F \cdot [C_{HX}/(C_{HA} + C_{HX})] \cdot 100 \qquad (21)$$

The arrow-line $\Delta \log C^{HX}$ is marked in the diagram. The factor ΔF can be taken from Table 1.

If it is desired to calculate only the titration error in the determination of the second acid HX, it should be kept in mind that an error is made both at the beginning and at the end of the titration. The errors should, of course, be evaluated by statistical methods, but it is usual for one of the errors to be so much larger than the other that the latter can be disregarded.

An idea of the magnitude of the percentage error $(\%e)_{HX}$, can be obtained from

$$(\%e)_{HX} = (1 + r)\,(\%e)_\Sigma + r(\%e)_{HA} \qquad (22)$$

where $r = C_{HA}/C_{HX}$.

In Fig. 2 two horizontal arrow-lines, $\Delta \log H_{HA}^{HX}$ and $\Delta \log H_{HA}^{H_2O}$, have also been drawn. From the logarith-

mic diagram it is evident that these two lines are exactly as long as the arrow-lines marked $\Delta \log C^{HA}$ and $\Delta \log C^{HX}$, respectively. But

$$\Delta \log H_{HA}^{HX} = pH_{HA} - pH_{HX} \tag{23}$$

and

$$\Delta \log H_{HA}^{H_2O} = pH_{HX} - 7 \tag{14}$$

where pH_{HA} and pH_{HX} would be the pH-values for the two acids HA and HX if each were alone in the solution.

By the aid of the two pH-values, *i.e.*,

$$pH_{HA} = \tfrac{1}{2}(\log K_{HA} - \log C_{HA}) \tag{16}$$

$$pH_{HX} = \tfrac{1}{2}(\log K_{HX} - \log C_{HX}) \tag{24}$$

it is possible to calculate the pH-values at the two equivalence points (e_1 and e_2 in Fig. 2) and the corresponding titration errors.

Summing up, we have the following equations.

Titration of HA in the presence of HX

$$pH_{eq}^{HA} = \log K_{HA} - \Delta \log H_{HA}^{HX} \tag{25}$$

$$(\%e)_{HA} = 10^{\Delta \log H_{HA}^{HX}} \cdot \Delta F \cdot 100 \tag{26}$$

Titration of the sum of HA and HX

$$pH_{eq}^{HX} = \log K_{HX} - \Delta \log H_{HX}^{H_2O} \tag{27}$$

$$(\%e)_{\Sigma} = 10^{\Delta \log H_{HX}^{H_2O}} \cdot \Delta F \cdot [C_{HX}/(C_{HA} + C_{HX})] \cdot 100 \tag{28}$$

If the titration error in the determination of HX alone is wanted, equation (22) gives an idea of the magnitude.

Example 3. A solution, which is 0.01*M* in HA and 0.04*M* in HX, is titrated with a solution of a strong base. Calculate the pH-value at the equivalence point and the corresponding titration error when (*a*) the acid HA is titrated, (*b*) the sum of the acids is titrated, (*c*) the acid HX is titrated. The stability constants are: $\log K_{HA} = 4$, $\log K_{HX} = 7$. It is assumed that $\Delta pH = \pm 0.5$ (See Fig. 2).

$$pH_{HA} = \tfrac{1}{2}(4 + 2) = 3; \quad pH_{HX} = \tfrac{1}{2}(7 + 1.4) = 4.2; \quad \Delta F = 2.85$$

(*a*) HA is titrated

$$pH_{eq}^{HA} = 4 + 1.2 = 5.2$$

$$(\%e)_{HA} = \pm 10^{-1.2} \times 2.85 \times 100 = \pm 18$$

(*b*) the sum of HA and HX is titrated

$$pH_{eq}^{HX} = 7 - (4.2 - 7) = 9.8$$

$$(\%e)_{\Sigma} = \pm 10^{-2.8} \times 2.85 \times \tfrac{4}{5} \times 100 = \pm 0.36$$

(*c*) HX is titrated

$$pH_{eq}^{HX} = 9.8$$

$$(\%e)_{HX} = \pm(2 \times 0.36 + 18) = \pm 18.7$$

Example 4. Repeat the calculation in example 3, assuming that the acid HA is a strong acid.

$$pH_{HA} = 2; \quad pH_{HX} = 4.2; \quad \Delta F = 2.85$$

(*a*) HA is titrated

$$pH_{eq}^{HA} = 2 + 2.2 = 4.2 (= pH_{HX})$$

$$(\%e)_{HA} = \pm 10^{-2.2} \times 2.85 \times 100 = \pm 1.8$$

(*b*) the sum of HA and HX is titrated

$$pH_{eq}^{HX} = 9.8$$

$$(\%e)_{\Sigma} = \pm 0.36$$

(*c*) HX is titrated

$$pH_{eq}^{HX} = 9.8$$

$$(\%e)_{HX} = \pm(2 \times 0.36 + 1.8) = \pm 2.5$$

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EXTRACTION–SPECTROPHOTOMETRIC DETERMINATION OF ALUMINIUM IN RIVER WATER WITH PYROCATECHOL VIOLET AND A QUATERNARY AMMONIUM SALT

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Summary—The simple removal of excess of co-extracted reagent in the solvent extraction of anionic metal complexes with a quaternary ammonium salt greatly improves the determination of aluminium with Pyrocatechol Violet (PV) and zephiramine (tetradecyldimethylbenzylammonium chloride). The exchange equilibrium constants for PV reagent and aluminium complex with four univalent anions (halides and nitrate) were determined when chloroform and 1,2-dichloroethane were used as extracting solvents. The constants were compared with those obtained with Pyrogallol Red. The method with PV and chloroform is suitable for the determination of micro-amounts of aluminium in river water. The apparent molar absorptivity of the aluminium complex in chloroform is 8.9×10^4 l mol^{-1} cm^{-1} at 587 nm. The limit of detection and precision achieved with the method are 3 μg l^{-1} and within 4%, respectively. A large excess of reagent can be used, and the ternary complex can be completely extracted over the pH range 5.5–10. Masking agents allow most interferences to be suppressed.

There is a dearth of satisfactory spectrophotometric methods for the determination of micro and trace amounts of aluminium. The most commonly used reagents are aluminon (ammonium aurintri-carboxylate)[1,2] and 8-hydroxyquinoline.[3] They are fairly selective for aluminium, but not sensitive enough for determination of trace amounts of aluminium in natural waters.

Pyrocatechol sulphonphthalein (Pyrocatechol Violet, PV, $C_{19}H_{14}O_7S$) has been used as an analytical reagent for the spectrophotometric determination of aluminium by Anton,[4] Tanaka *et al.*,[5] Chester *et al.*,[6] Shijo[7] and Dougan *et al.*[8] In these methods, however, although the sensitivity could be improved by using a quaternary ammonium salt, the selectivity and the narrow pH range of the complex formation could not be satisfied in the determination of aluminium.

We have already reported an extraction–spectrophotometric determination of trace amounts of iron in waters with Pyrogallol Red (PR) and zephiramine (tetradecyldimethylbenzylammonium chloride)[9] in which the excess of co-extracted reagent was removed from the organic phase by addition of a suitable amount of chloride, resulting in a considerable improvement in sensitivity and selectivity.

In the present work, the extraction of the ternary aluminium–PV–zephiramine complex into organic solvents such as chloroform and 1,2-dichloroethane was examined, and exchange constants for PV and its aluminium complex with various univalent anions (chloride, bromide, nitrate and iodide) were measured and the constants obtained compared with those obtained for the PR systems. A procedure for extraction of the aluminium–PV complex anion with zephiramine with subsequent removal of the excess of co-extracted reagent from the organic phase was also established and applied to the determination of micro and trace amounts of aluminium in river water.

EXPERIMENTAL

Reagent

Pyrocatechol Violet solution, ca. 5×10^{-4}M. Dissolve 96.6 mg of Pyrocatechol Violet in 500 ml of distilled water. Standardize the solution by the mole-ratio method with thorium(IV), which forms a 1:1 complex with PV in aqueous solution.[10] If kept in an amber glass bottle in a refrigerator the solution is stable for at least 2 weeks.

Pyrogallol Red solution, ca. 5×10^{-4}M. Dissolve 100.1 mg of Pyrogallol Red in 500 ml of distilled water and standardize this solution with thorium(IV).[9] The solution is stable for at least a month if kept in a refrigerator.

Standard aluminium solution, ca. 0.1M. Prepare a stock solution by dissolving 9.16 g of aluminium sodium sulphate [$Al_2(SO_4)_3.Na_2SO_4.24H_2O$] in 97 ml of distilled water plus 3 ml of concentrated sulphuric acid. Standardize it by direct titration with EDTA (pH 4: acetate buffer) at 80°, with Chromazurol S as indicator[11] (use a stirrer-hotplate). Prepare the working aluminium solution by accurate dilution.

Aqueous zephiramine solution, 0.01M. Dry zephiramine (tetradecyldimethylbenzylammonium chloride, ZCl) at reduced pressure (about 5 mmHg) and about 50° to constant weight. Dissolve the required amount in distilled water.[12]

Extracting solvent, 0.001M zephiramine in chloroform. Dissolve the required amount of dried zephiramine in chloroform.

Masking agents. Aqueous solutions of thiourea (1*M*), hydroxylammonium sulphate (0.05*M*) and 1,10-phenanthroline (0.01*M*).

Buffer solutions. Sulphuric acid–sodium acetate, potassium dihydrogen phosphate–disodium hydrogen phosphate, ammonia–ammonium sulphate and sodium bicar-

bonate–sodium carbonate solutions. For the determination of exchange constants, 0.05*N* sulphuric acid–sodium acetate (pH 4) and 0.05*M* ammonia–ammonium sulphate (pH 9.5) buffer solutions were used. For the study of analytical conditions, 0.2*N* buffer solutions were used. For the application to the analysis of aluminium in river water, 0.2*M* acetate and 1*M* ammonium buffer solutions were used.

Univalent anion solutions. Sodium chloride, bromide and nitrate and potassium iodide solutions.

General procedure for extraction constants

Transfer a portion of aluminium solution (*ca.* $5 \times 10^{-5}M$) to a stoppered 25-ml test-tube. Add by pipette the PV (*ca.* $5 \times 10^{-4}M$), zephiramine (*ca.* $10^{-3}M$), buffer and univalent anion solution, in that order. Dilute to 5 ml with distilled water and mix thoroughly. Add 5 ml of chloroform and shake for 10 min (to obtain the equilibrium constants, the solution was equilibrated by 30 min of shaking). After phase separation, measure the absorbance of the organic phase in a 1-cm glass or fused quartz cell against a reagent blank.

RESULTS AND DISCUSSION

Determination of the equilibrium constants

To elucidate the extraction mechanism of the aluminium–PV complex, the equilibrium constants for distribution of reagent and complex between chloroform and water were examined. The exchange constant, $K_{A^{n-}}^{nX^-}$, refers to the following reaction:[13]

$$A_{(a)}^{n-} + nZX_{(o)} \rightleftharpoons nX_{(a)}^{-} + Z_nA_{(o)}$$

$$K_{A^{n-}}^{nX^-} = \frac{[X^-]_a^n[Z_nA]_o}{[A^{n-}]_a[ZX]_o^n}$$

or

$$\log([Z_nA]_o/[A^{n-}]_a) = \log D_A = \log K_{A^{n-}}^{nX^-} - n\log([X^-]_a/[ZX]_o)$$

where A^{n-}, Z^+ and X^- are the anion with charge $n-$, the zephiramine cation and the univalent anion (chloride, bromide, nitrate or iodide) respectively and subscripts a and o refer to the aqueous and organic phases respectively. The exchange constants for A^{n-} and X^- were determined by extracting A^{n-} with ZX into an organic solvent from aqueous solutions containing various amounts of X^- and measuring the concentrations of Z_nA in the organic phase.

Exchange constants for PV. At about pH 4, the predominant PV species in the aqueous solution is the H_3R^- ion because the pK_a values of PV (H_4R) are reported as <1, 7.82, 9.76 and 11.7.[14] Therefore, it reacts with ZX to form a 1:1 ion-association complex, which is extracted into chloroform. The exchange constants for H_3R^- and four univalent anions (Cl^-, Br^-, NO_3^- and I^-) were determined according to the general procedure. The plots of $\log([Z.H_3R]_o/[H_3R^-]_a)$ against $\log([X^-]_a/[ZX]_o)$ were all linear with slopes almost -1. The constants calculated are listed in Table 1.

The exchange constants for H_2R^{2-}, HR^{3-} and R^{4-} could not be determined because the pK_a values are too close and the reagent tends to decompose at pH-values above 7.

The exchange constants with 1,2-dichloroethane as organic solvent were determined in the same way. The results are given in Table 1.

The constants for PR (H_4R'; pK_a = 2.56, 6.28, 9.75 and 11.94)[15] in the form $H_3R'^-$ were also determined and are given in Table 1.

Exchange constants for aluminium complex. The aluminium:PV ratio in the complex in aqueous solution and in the complex extracted into chloroform (with 0.001*M* zephiramine) was found to be 1:2 by the mole-ratio (variable PV concentration and $5 \times 10^{-6}M$ aluminium) and continuous-variation (PV plus aluminium concentration $2 \times 10^{-5}M$) methods. The aluminium:zephiramine ratio in the complex extracted into chloroform was found to be 1:3 by the mole-ratio (variable zephiramine concentration with $5 \times 10^{-6}M$ aluminium and $1 \times 10^{-4}M$ PV) and continuous-variation (aluminium plus zephiramine concentration $3 \times 10^{-5}M$ and $1 \times 10^{-4}M$ PV) methods. The extracted species therefore contains aluminium, PV and zephiramine in the proportions 1:2:3.

The exchange constant for the aluminium–PV complex was determined by using a chloroform solution of the aluminium–PV complex from which the excess of reagent had been removed by washing with a buffer at pH 9.5. At this pH, although a little PV reagent decomposes, the aluminium complex in chloroform is stable for at least 24 hr.

The plot of log D_{Al} ($=\log([Z_3Al(HR)_2]_o/[Al(HR)_2^{3-}]_a)$ against $\log([X^-]_a/[ZX]_o)$ for chloride gave a straight line with slope almost -3. The constants obtained for this and the 1,2-dichloroethane and the corresponding Pyrogallol Red systems are given in Table 2.

Table 1. Exchange constants for the univalent anions of the reagents (log $K_{H_3R^-}^{X^-}$) obtained at 25°C

Reagent	Organic solvents	X^-			
		Cl^-	Br^-	NO_3^-	I^-
Pyrocatechol Violet (PV)	Chloroform	2.02 ± 0.02†	0.65 ± 0.05†	0.85 ± 0.02†	−0.48 ± 0.08†
	1,2-Dichloroethane	2.3 ± 0.0$_4$*†	0.9$_1$ ± 0.0$_5$*†	0.6$_2$ ± 0.0$_8$*†	−0.1$_9$ ± 0.0$_5$*†
Pyrogallol Red (PR)	Chloroform	2.70 ± 0.07†	1.35 ± 0.07†	1.59 ± 0.09†	0.34 ± 0.07†
	1,2-Dichloroethane	2.8 ± 0.0$_5$*†	1.4 ± 0.0$_8$*†	1.1 ± 0.0$_7$*†	0.3$_2$ ± 0.0$_4$*†

* A precipitate was formed at the interface between the aqueous and 1,2-dichloroethane phases but reproducible data were obtained.

† The values after the ± signs are the standard deviations.

Table 2. Exchange constants of the complexes and chloride (log $K^{3Cl^-}_{[Al(HR)_2]^{3-}}$) obtained at 25°C

Reagent	Organic solvent	log $K^{3Cl^-}_{[Al(HR)_2]^{3-}}$
Pyrocatechol Violent (PV)	Chloroform	9.51 ± 0.07*
	1,2-Dichloroethane	10.53 ± 0.10*
Pyrogallol Red (PR)	Chloroform	10.24 ± 0.09*
	1,2-Dichloroethane	11.19 ± 0.14*

* The errors after the ± signs are the standard deviations.

Removal of excess of reagent

In the extraction with zephiramine, the excess of reagent is co-extracted with the aluminium complex into the organic phase. Hence, its removal is necessary for accurate determination of the absorbance of the aluminium complex.

The exchange constants for the four extraction systems examined were compared. From Tables 1 and 2, it seems that the difference in extractability between reagent and aluminium complex is largest for the system ternary complex (aluminium–PV–zephiramine) and 1,2-dichloroethane, but that extraction is troublesome because a blue precipitate is produced at the interface. The use of chloroform as solvent eliminates this difficulty, and this system is the best of the four.

Figure 1 shows the degree of extraction of reagent and aluminium–PV complex, calculated by using the exchange constants for chloride listed in Tables 1 and 2. Although the exchange constants for PV in the forms H_2R^{2-}, HR^{3-} and R^{4-} could not be determined, the degree of extraction of reagent at pH 9.5 could be measured. At that pH, the PV reagent was unstable and decomposed slightly, but the aluminium complex with PV was found to be stable for at least 24 hr. The data obtained are also shown in Fig. 1. It is seen that PV is difficult to remove when in the form H_3R^- but is easily removed at pH 9.5 (it is then in the form of a mixture of H_2R^{2-} and HR^{3-}). Therefore the excess of reagent in the organic phase can be removed almost entirely into the aqueous phase at pH 9.5 by adding chloride to the extraction system.

Absorption spectra

The absorption spectra of the aluminium complex with PV and the reagent blank in chloroform at pH 9.5 (ammonia–ammonium sulphate) are shown in Fig. 2. When the excess of co-extracted reagent was removed from the organic phase (curves 1 and 2 in Fig. 2), the absorption maxima at 313 and 587 nm were obtained, with minimum reagent blanks. If the excess of reagent was not removed (curves 3 and 4 in Fig. 2), although absorption maxima were obtained at similar wavelengths, the absorption by the reagent blanks was very high.

In this work, the absorbance at 587 nm was used to determine aluminium because the sensitivity and the reagent blank were much superior to those at 313 nm.

Effect of pH, chloride concentration and time

The effect of pH on the extraction of the aluminium complex and the excess of PV is shown in Fig. 3. The optimum pH range is 5.5–10.0; when the excess of reagent is not removed, the optimum pH range is very narrow, 6.0 ± 0.1. As already said, at pH above 7, a little PV reagent decomposes but the extracted aluminium complex is stable for at least 24 hr, and the excess of reagent is most easily removed when present as H_2R^{2-} and HR^{3-} (or R^{4-}), so the extraction is done at pH about 9.5.

Figure 4 shows the effect of chloride concentration on the extraction of aluminium–PV complex with zephiramine into chloroform at pH 9.5 (ammonia–ammonium sulphate). The optimum concentration of

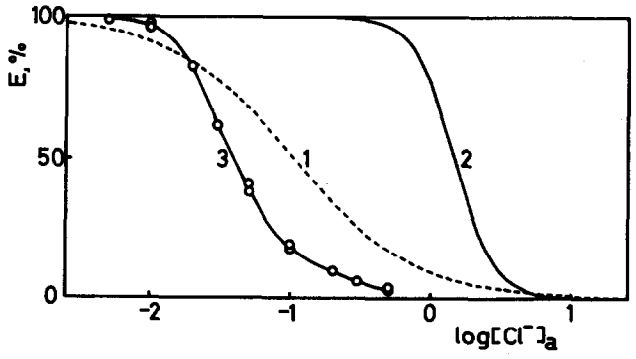

Fig. 1. Degree of extraction of reagent and aluminium complex (into chloroform) plotted against chloride concentration for 0.001*M* zephiramine. 1, PV in the form H_3R^- at pH 4.0; 2, aluminium complex at pH 9.5; 3, PV at pH 9.5.

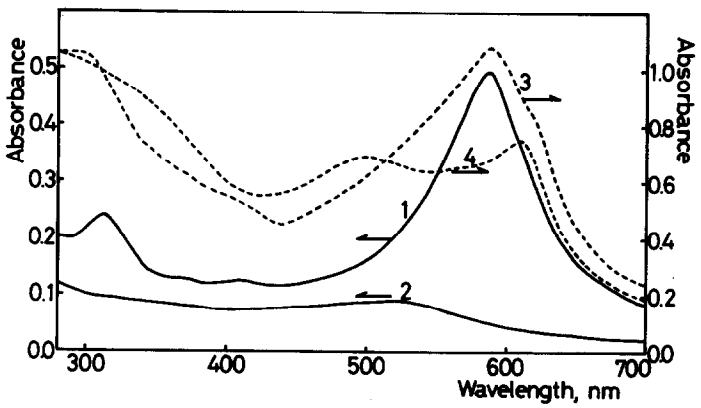

Fig. 2. Absorption spectra in chloroform. 1, Ternary complex with $5 \times 10^{-6}M$ aluminium, $1 \times 10^{-4}M$ PV, $1 \times 10^{-3}M$ zephiramine and $0.5M$ sodium chloride at pH 9.5, measured against chloroform; 2, reagent blank for conditions used for spectrum 1; 3, ternary complex (as for spectrum 1, but without sodium chloride) at pH 9.5; 4, reagent blank for conditions used for spectrum 3.

chloride for the removal of excess of PV from the organic phase is seen to be about $0.5M$.

The time necessary for complete formation of the aluminium complex was examined. When about a 20-fold molar excess of PV was added to $5 \times 10^{-6}M$ aluminium at pH 9.5 (ammonia–ammonium sulphate buffer), reaction was immediate and the time needed for complete extraction of the complex into chloroform was at most 5 min. With the same concentrations of aluminium and reagent but with zephiramine present ($1 \times 10^{-3}M$) complete reaction took 5 min, the shaking time for complete extraction was 10 min and the time needed for complete separation of the two phases was 30 min.

Calibration graph and reagent blank

When the excess of reagent was removed, the absorbance in chloroform at 587 nm was measured in a 1-cm quartz cell against a reagent blank prepared under the same conditions. The calibration graph was a straight line that obeyed Beer's law up to $1 \times 10^{-5}M$ aluminium. The apparent molar absorptivity calculated from the slope of the calibration graph was 8.9×10^4 $1.mole^{-1}.cm^{-1}$ at 587 nm. The reagent blank at 587 nm was 0.052 ± 0.002. These values were reproducible, and the calibration graph and the reagent blank were superior to those obtained when the excess of co-extracted reagent was not removed.

Application to aluminium in river water

Interferences and masking agents. The tolerance limits for metal ions and other ions often present in river water were examined (Table 3). In the general procedure, without masking agents, several metal ions at concentrations of $1 \times 10^{-5}M$ cause errors; with addition of 1 ml of $1M$ thiourea, there are few interferences other than iron(II and III). Iron(II and III) can be masked with hydroxylammonium sulphate and 1,10-phenanthroline. Hence, 0.5 ml of $0.05M$ hydroxylammonium sulphate, 1 ml of $1M$ thiourea and 1 ml of $0.01M$ 1,10-phenanthroline were added, in that order, to mask interfering metal ions. The tolerance limits with masking agents present are also listed

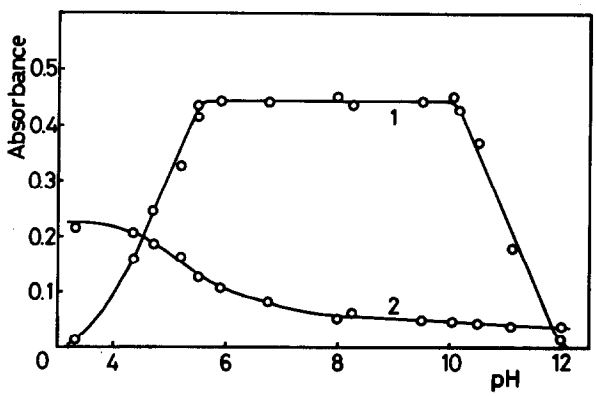

Fig. 3. Effect of pH 1, Ternary complex (except for pH and measurement against a reagent blank, conditions were those used for spectrum 1 of Fig. 2); 2, reagent blank measured against chloroform.

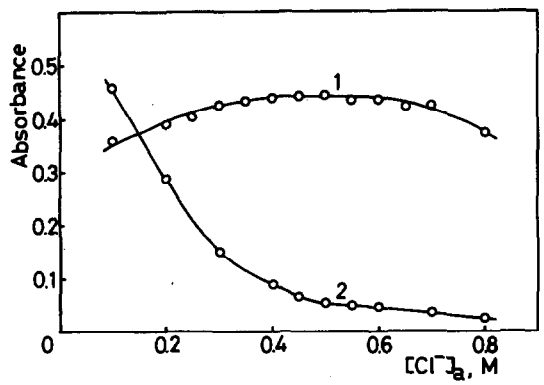

Fig. 4. Effect of chloride concentration. 1, Ternary complex (except for chloride concentration and measurement against a reagent blank, conditions were those used for spectrum 1 of Fig. 2); 2, reagent blank measured against chloroform.

Table 3. Tolerance limits for diverse ions with and without masking agents

Ion	Tolerance limit, *M*
Na^+, K^+, SO_4^{2-}	1*
HCO_3^-	0.02*
Mg^{2+}, Ca^{2+}, $H_2PO_4^-$, SiO_3^{2-}	0.002*
Br^-, NO_3^-	0.001
F^-, I^-, SCN^-	1×10^{-4}
ClO_4^-, dodecylbenzenesulphonate	1×10^{-5}
Cu^+, Ag^+, Hg^{2+}, Cr^{3+}	1×10^{-4}*†
Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, UO_2^{2+}	1×10^{-5}†
Cu^{2+}	1×10^{-4}*‡
Fe^{2+}, Fe^{3+}	1×10^{-5}‡

* Maximum tested.
† Add 1 ml of 1*M* thiourea to the test solution.
‡ Add 0.5 ml of 0.05*M* hydroxylammonium sulphate to the test solution and mix well, then add 1 ml of 1*M* thiourea and 1 ml of 0.01*M* 1,10-phenanthroline.

in Table 3. The tolerance limit is defined as the concentration level at which the interferent causes an error of not more than 4%. Since the concentrations of these ions in river water are generally much smaller than the tolerance limits, they do not interfere in the determination of aluminium by the procedure recommended.

Aluminium in river water

Transfer the acidified (0.5 ml of concentrated sulphuric acid per litre) and filtered (0.45-μm pore size) sample solution (1–5 ml) to a stoppered 25-ml test-tube; add 0.5 ml of 0.05*M* hydroxylammonium sulphate and mix well. Add 1 ml of 1*M* thiourea, 1 ml of 0.01*M* 1,10-phenanthroline and 1.5 ml of 0.2*M* acetate buffer (pH 5.6) and let stand for 1 hr. Add 1 ml of 5×10^{-4}*M* PV solution and dilute to 10 ml with distilled water. Mix thoroughly and let stand for 5 min. Add 5 ml of 0.001*M* zephiramine in chloroform and shake the test-tube for 10 min. After phase separation, discard the aqueous phase. Wash twice with distilled water, discarding the aqueous phase, to remove the aqueous solution completely. Add 5 ml of wash solution (pH 9.5, 1*M* ammonia–ammonium sulphate buffer, 0.5*M* in sodium chloride) and shake for 10 min. Let stand for 30 min and measure the absorbance of the organic phase at 587 nm in a 1-cm quartz (or glass) cell against a reagent blank. Prepare the working curve with standard aluminium solution in the same way.

The analytical results obtained for river, potable and hot-spring waters by the recommended procedure are given in Table 4. The relative standard deviations are less than 4%. The sample volumes were varied

Table 4. Determination of aluminium in river water with Pyrocatechol Violet and zephiramine [sample volume 4 ml except for first result from sample A (2 ml)]

Sample	Sampling date	Aluminium content,** μg/l.	Recovery,† %
Water of Asahi river* A	3 May 1978	99	
		96 ± 3	103
B	3 May 1978	78 ± 3	
C	3 May 1978	142 ± 2	
D	3 May 1978	159 ± 1	99
E	3 May 1978	176 ± 3	
F	3 May 1978	172 ± 3	
G	3 May 1978	123 ± 3	
H	3 May 1978	124 ± 5	96
I	3 May 1978	182 ± 3	
J	3 May 1978	136 ± 2	101
K	4 May 1978	230 ± 8	98
L	4 May 1978	150 ± 2	
Potable water at Miyoshi	4 Sep 1978	99 ± 3	
at Tsushima	8 Sep 1978	137 ± 2	97
Hot-spring water at Miasa	20 Aug 1977	115 ± 4	
at Yubara	3 May 1978	112 ± 1	
at Tunogo	10 Sep 1978	226 ± 3	103

* Symbols A–L denote order of sampling downstream.
† Tested with 0.675 μg of aluminium added.
** The figures after the ± signs are the standard deviations (4 determinations).

Table 5. Comparison of methods for the spectrophotometric determination of aluminium with Pyrocatechol Violet

Reaction medium	Optimum pH range	λ_{max}, nm	ϵ, $l.mole^{-1}.cm^{-1}$	Reagent blank absorbance	Reference
Pyridine–acetic acid–water	5	615	630	0.02	4
Aqueous solution	6.0–6.1	580	6.8×10^4	0.1	5
Aqueous solution	6.1–6.2	585	6.94×10^4	0.06	8
Aqueous solution + cetyltrimethylammonium	9.7–10.2	670	5.3×10^4	0.05	6
Extraction with n-butyl acetate + ethyltridodecylammonium	5.9–6.1	597	8.4×10^4	0.2	7
Extraction with chloroform + zephiramine	5.5–10.0	587	8.9×10^4	0.052 ± 0.002	This work

between 1 and 4 ml, and distilled water was added to each to give a constant volume. For all samples, graphs of amount of aluminium found *vs.* volume of sample taken were linear and could be extrapolated to the same point, which coincided with the point obtained for distilled water. Accordingly, it was concluded that the determination of aluminium in river water by the recommended method was quantitative and distilled water might be used for the reagent blank.

The recovery of aluminium was tested by adding known amounts of aluminium to the sample solutions. The results obtained, also shown in Table 4, showed that aluminium in river water could be extracted quantitatively into chloroform as the ternary aluminium–PV–zephiramine complex.

CONCLUSION

The order of extractability of the ion-association complexes with zephiramine is $[Al(HR)_2]^{3-} > H_3R^- > Cl^- > H_2R^{2-}$, HR^{3-} or R^{4-} in the presence of a suitable amount of chloride (H_4R = PV). The apparent molar absorptivity of the ternary aluminium complex in chloroform is the largest reported for this type of system (Table 5). The optimal pH-range is much wider than that for the other methods (Table 5) because the excess of reagent is removed. The recommended method allows aluminium to be determined in river water with satisfactory results.

The principle of removal of excess of reagent should lead to improved sensitivity and accuracy in many solvent extraction systems. Furthermore, the addition of relatively large amounts of salts reduces the effect of co-extractable anions in sample solutions and causes very effective salting-out, so that phase separation becomes faster.

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DETERMINATION OF CADMIUM AND LEAD IN URINE AND OTHER BIOLOGICAL SAMPLES BY GRAPHITE-FURNACE ATOMIC-ABSORPTION SPECTROMETRY

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Summary—A method for determining cadmium and lead in urine and other biological samples by graphite-furnace atomic-absorption spectrometry is reported. Samples were analysed after wet or dry ashing and without extraction or matrix-modification techniques, in a laminar-flow clean-room; negligible blank contributions were found. Matrix interference effects were observed only for lead and were resolved by the method of standard additions. Five NBS biological reference materials were used as internal quality-control standards. The urinary levels for non-exposed volunteers ranged from 0.16 ± 0.01 to 1.65 ± 0.20 and from 6 ± 1 to 31 ± 6 ng/ml for cadmium and lead, respectively; this corresponds to 0.15 ± 0.02 to 2.01 ± 0.16 and 7 ± 1 to 31 ± 3 μg/day. The average relative standard deviation for 60 urine samples was 10% for cadmium and 13% for lead.

Reliable analytical measurements are very necessary in studies on human health effects of environmental pollutants. When the levels of pollutants are extremely low, such as the trace metal levels of cadmium and lead in normal biological samples, contamination may occur in sampling procedures, in the analytical preparation of samples, from the laboratory environment, and in the measurement of the samples. The objective of this study was to develop the capability to analyse routinely for low levels of cadmium and lead in various biological samples with sufficient accuracy and precision to allow for the independent evaluation of man's exposure to these metals. Our investigation was concerned with the analysis of such samples as urine, diet and faeces, with particular emphasis on the measurement of urine. While the urinary contribution to metabolic balances is not significant for cadmium and lead, urinary excretion represents metabolized material and accurate measurement is required.

Preliminary studies in our general laboratory[1] on determination of trace quantities of these metals in biological samples failed because of high and inconsistent blank levels. To minimize contamination, particularly due to the urban location of our laboratory, the present work was performed in a laminar-flow clean-room laboratory.

The difficulties in the accurate determination of these elements in low level biological materials, such as obtaining a representative sample, contamination by and loss of cadmium and lead, the unavailability of adequate standard reference materials for calibration, and interferences from the high and variable concentration of salts, have already been described.[2–4] Various methods have been reported for minimizing matrix interferences which were observed for atomic absorption. For example, Kubasik and Volosin[5] utilized an extraction procedure before analysis, Ediger *et al.*[6] reduced problems arising from high salt background by adding ammonium nitrate, Hodges[7] used orthophosphoric acid and molybdenum to modify the salt matrix, and Regan and Warren[8] studied the effectiveness of ascorbic acid for reducing interferences.

An intercomparison study was conducted by Kjellstrom *et al.*[9] for assessing the accuracy and precision of the methods used by different laboratories to measure cadmium in biological materials. They found that the results reported for urine at the 1-μg/l. level varied significantly among several laboratories and that no single method could be used as a reference procedure.[9] An extensive intercomparison programme by Lauwerys *et al.*[10] for determination of cadmium and lead in human urine indicated that accurate and precise analytical methods were inadequately developed.

During the past two decades, the values reported for the normal cadmium levels in human urine have differed by a factor of as much as 100. Except for Schroeder and Nason,[11] Tipton *et al.*,[12] and McKenzie and Kay,[13] most investigators have reported normal urinary excretions of less than 2 μg/l. By spectrophotometric measurement Imbus *et al.*[14] obtained a mean of 1.6 μg/l. Katagiri *et al.*[15] reported an average of 1.4 μg/l. for their flame atomic-absorption determinations. Kovats and Bohm[16] and also Elinder *et al.*[17] used extraction procedures and furnace atomic-absorption spectrometry, and obtained values in the range of 0.3–2.8 and 0.3–0.6 μg/l., respectively. Cadmium levels ranging from 0.5 to 1.4 μg/l. of urine were found by Johnson *et al.*[18] who used background-corrected atomic-absorption spectrometry and a micro

sampling technique. Perry *et al.*[19] analysed their samples, with prior wet ashing, by graphite-furnace atomic-absorption spectrometry with background correction and reported the normal urinary cadmium concentration to be 1.2 ± 0.8 μg/l.

The mean values given in the literature for lead in human urine do not vary as widely as those for cadmium. Lead results from an international study begun in 1961 to determine the normal levels in urine by a spectrophotometric method, as reported by Goldwater and Hoover,[20] ranged from 20 to 65 μg/l., with an average of 35. This range was not found to be significantly different from that given in Stopps' review[21] of the normal urinary lead levels reported from 1925 to 1965. More recently, Tada *et al.*[22] reported a mean of 16 μg/l. for lead in urine (by atomic-absorption analysis) and Johnson *et al.*[18] found a range of 19–32 μg/l. (flameless atomic-absorption with a deuterium-arc background corrector).

We report here a simple and direct method for cadmium and lead determination in biological samples by graphite-furnace atomic-absorption spectrometry without extraction or matrix modifications. After wet or dry ashing of the samples, cadmium is measured directly and lead by the method of standard additions.

EXPERIMENTAL

Instrumentation

The analyses were performed with a Perkin-Elmer Model 603 atomic-absorption spectrometer equipped with a graphite furnace (HGA-2100), a deuterium-arc background corrector, and a Model 056 recorder.

Reagents

The water used was demineralized and then distilled. Nitric acid ("Suprapur", 65%, EM Laboratories, Inc., NY) was utilized for wet ashing. Instrument calibration standards were made from 1000-μg/ml cadmium and lead standards in 2% nitric acid (Spex Industries, Inc., NJ). All solutions were diluted to volume in glass, then stored in polyethylene containers. All vessels were cleaned beforehand by soaking in tetrasodium ethylenediaminetetra-acetate solution, thoroughly rinsing, soaking in 50% v/v reagent grade nitric acid, rinsing at least 3 times with demineralized water, then air-drying.

Procedure

Sample collection. Urine samples were collected during working hours for 5 days from 15 non-exposed volunteers from our laboratory in 4 separate periods. The 1st and 2nd collection periods were in consecutive weeks, the 3rd was 1 month later, and the 4th 1 year later. For each period, the urinary excretion was measured and the samples were frozen in the individual's composite collection storage bottle.

Sample preparation. Each composite was thawed and thoroughly mixed, and 1-litre portions were taken for analysis. Each sample was evaporated in a "Vycor" vessel in the presence of high-purity nitric acid, then digested with small increments of acid. Alternatively, the evaporated samples were dry ashed on an electric hot-plate until the residue was completely white. The amount of acid required for sample preparation did not exceed 150 ml. The samples were diluted to 250 ml, a volume which yielded a 4:1 concentration factor and an acidity of 5%.

Weighed portions of the diet, faeces and reference samples were transferred to platinum dishes, dried at 130°, then ashed in a muffle furnace at 430 ± 20° for 24–60 hr. The samples were removed from the furnace and the ash was moistened with a minimal amount of nitric acid. The residue was dried on a hot-plate and the sample ashed again at 430 ± 20° for 12 hr. The ash was dissolved in high purity acid and demineralized-distilled water, then the solution was diluted to an appropriate volume. All sample solutions were stored in polyethylene containers.

Instrumental parameters. Optimum time and temperature settings on the HGA-2100 were found to be as follows: drying, 50–100 sec at 100°; charring, 20 sec at 250°; atomizing, 5 sec at 2100° and 2200° for cadmium and lead respectively. Uncoated graphite tubes were used in the furnace and were purged with argon at a flow-rate of 7.5 ml/min when used in the "interrupt" ("gas-stop") mode. The absorbance peaks were recorded at 228.8 nm for cadmium and at 283.3 or 217.0 nm for lead. Hollow-cathode lamps for cadmium and lead were operated at 6 and 10 mA, respectively.

Measurements. The calibration standards ranged from 5 to 25 pg for cadmium and from 0.05 to 0.25 ng for lead.

Non-specific absorption in the background region was measured for both metals in each sample, since it was necessary to determine whether the capability of the deuterium-arc background corrector was exceeded at the dilutions we measured. The manufacturer warns[23] that a background absorbance of 0.8 or higher may lead to erroneous results, but the background absorbances did not reach this level.

The diluted urine samples were measured for cadmium and lead by injecting 25, 50 or 75 μl into the graphite furnace with Eppendorf μl pipettes and disposable plastic tips. Before injection of the samples, the tips were rinsed at least twice with each of the following: 0.1*M* nitric acid, demineralized-distilled water, and sample solution.

For cadmium, good agreement was found between analyses of the same sample, some of which involved multiple dilutions and various volumes of the same dilution, which suggests that there were no matrix interferences. The method of standard additions gave no improvement. In the direct determination of lead in urine, matrix interferences were found. The absorbance values were severely suppressed, to an extent varying from sample to sample, so aqueous standards could not be used for calibration; the variation between samples prevented the use of matrix-matched calibration standards. The method of standard additions was required to compensate for the matrix effect. As shown in Fig. 1, we found that the slope of the

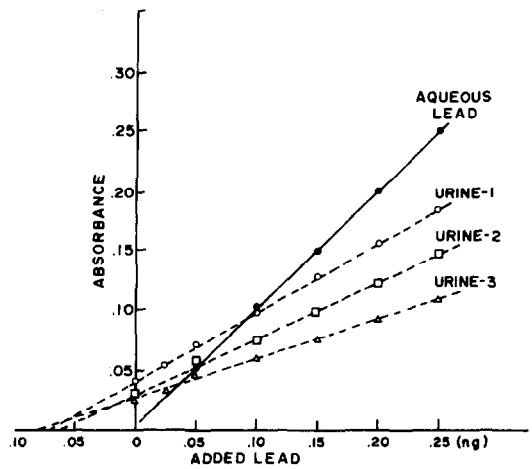

Fig. 1. Calibration curve for aqueous lead standards, and urine standard-addition curves.

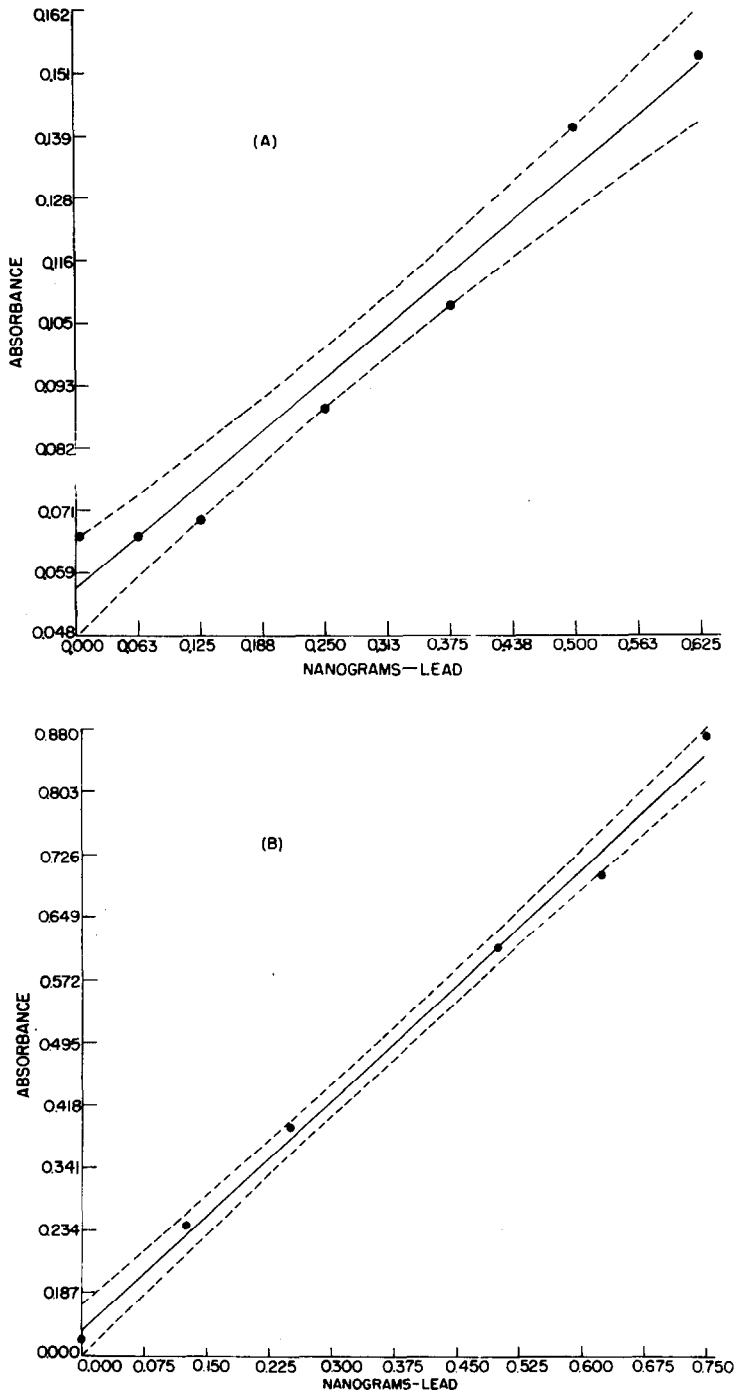

Fig. 2. Graphical display of computer analysis of the experimental values for sample from F-2: (*A*) 10-fold dilution, (*B*) 100-fold dilution.

lead calibration curve obtained with aqueous standards was typically different from the gradients of the standard-addition plots. This effect for lead determination in urine was also observed by Welz.[24]

To gain information on the possible causes of this variation in slope we compared the background measurements, the mineral concentrations of the urine samples and the magnitude of the lead suppression observed. No simple correlation was seen; we found high background-absorbance values to be associated with both low and high total mineral content. Also, we saw no relationship between the degree of suppression and either the sodium or potassium concentrations for the samples.

The initial attempt to use the standard-addition method failed because the severe suppression prevented a linear relationship. As shown in Fig. 2A, the first two additions of lead to the sample, equivalent to one and two times the absorbance value found for the sample alone, showed little or no effect. The solid line is a least-squares fit of a linear equation to the values and the dashed lines are the upper

Table 1. Cadmium and lead results for NBS standard reference materials

	Cd, μg/g		Pb, μg/g	
	EML	*NBS**	*EML*	*NBS*
Bovine liver				
SRM 1577	0.29 ± 0.01†	0.27 ± 0.04	0.45 ± 0.03	0.34 ± 0.08
Orchard leaves				
SRM 1571	0.09 ± 0.01	0.11 ± 0.02	44 ± 2	45 ± 3
Pine needles				
SRM 1575	0.25 ± 0.01	(<0.5)	10.8 ± 0.6	10.8 ± 0.5
Spinach leaves				
SRM 1570	1.46 ± 0.04	(1.5)	1.0 ± 0.1	1.2 ± 0.2
Tomato leaves				
SRM 1573	2.30 ± 0.10	(3.0)	5.5 ± 0.4	6.3 ± 0.3

* Values in parentheses are not certified.
† Standard deviation of replicate measurements.

and lower limits at the 90% confidence level. It is clear that meaningful results could not be obtained. In an effort to overcome this problem we diluted the same sample and measured it at the more sensitive 217.0-nm wavelength. As shown in Fig. 2B, the severe suppression effect was eliminated, enabling us to utilize the method of standard additions.

To check further for both the reproducibility of the measurement and additional matrix effects, multiple volumes of the same sample were analysed. The results for the 25- and 50-μl aliquots were in agreement and the slopes equivalent.

Blank levels

The earlier determination of the blank levels, in our general laboratory, varied from 40 to 65% of a cadmium sample concentration of 110 ng/g and from 1 to 4% of a lead sample concentration of 45 μg/g.[1] The blanks measured for the same reference sample in the laminar-flow clean-room laboratory were 1–1.5% of the sample concentration for cadmium and less than 0.1% of that for lead.

We determined the blank contribution from the nitric acid and the demineralized-distilled water utilized throughout our procedure. The acid contained 0.018 μg of cadmium and 0.48 μg of lead per litre, equivalent to 0.2–1.0% and 0.3–1.2% of the urine levels of cadmium and lead, respectively. The water blanks were lower by a factor of about 10 for both elements. These blank contributions were regarded as negligible with respect to the variance of the results obtained, and no corrections were applied.

Quality control

Since no certified urine standard is currently available for trace metal analysis, we processed and measured five NBS standard reference materials for their cadmium and lead content, as a means of assessing the procedure described above. The results of our measurements are given in Table 1 and are compared with the certified or tentative values listed by NBS. The good agreement between our measurements and the NBS values indicates that the sample preparation methods, analytical procedures, and instrumental techniques are satisfactory.

RESULTS AND DISCUSSION

Shown in Table 2 are the results of determination of cadmium in normal urine samples of the 15 volunteers, 7 females and 8 males. Subjects F-1 and M-1 were in the 20–24 year old age group, F-2 and M-2 in the 25–29 year old group, and so on (one of each sex in each 5-year group), ending with F-7, M-7, and M-8 who were in the same age group of 50–54 years. The cadmium levels ranged from 0.16 ± 0.01 to 1.65 ± 0.20 ng/ml for the 4 collection periods, which began in September 1976. The estimated urinary excretion per day is listed for completeness and is twice the average daily volume collected during each period. We believe it is reasonable to assume that the average amount of urine collected during a daily 8½-hr period closely represents half of the total daily excretion and that our best estimate of each volunteer's daily excretion, with an overall range of 0.63–2.53 l., is as valid as the 1.0–1.5 l./day (for adults) used by some investigators. Our average estimates of daily excretions given in Table 2 were used to calculate the amount of cadmium excreted per day for each volunteer and collection period. The overall excretion levels range from 0.15 ± 0.02 to 2.01 ± 0.16 μg/day.

Table 3 shows that lead levels ranging from 6 ± 1 to 31 ± 6 ng/ml were found for the urine samples. The overall urinary excretion of lead ranged from 7 ± 1 to 31 ± 3 μg/day.

The 15 volunteers were selected in such a way that the sample population would probably be adequate to determine the range of normal urinary excretion levels of cadmium and lead for our urban area. Also we wished to determine whether or not the levels of these metals varied during the 4 collection periods and to what extent they varied among different and apparently healthy individuals, irrespective of the subjects' personal habits, such as diet, smoking, medication, and so forth.

Similar ranges for both metals were found for each of the 4 collection periods; for example, the cadmium urinary excretion levels (μg/day) ranged from 0.32 to 1.95 for period I, from 0.23 to 2.01 for period II, from 0.20 to 1.40 for period III and from 0.15 to 1.21 for period IV. For lead, the ranges for periods I-IV were 13–25, 7–30, 7–23, and 8–31 μg/day. We found no apparent differences among the males and females studied or within the age range of the volunteers. Further interpretation of these values is not within the scope of this paper.

Table 4 shows the initial results of analysis of the levels of cadmium and lead in 6-day metabolism samples obtained through the co-operative efforts of this laboratory and the Metabolic Section of Hines Veterans Hospital in Illinois. The samples are a small part of a more detailed investigation where the sub-

Table 2. Results of cadmium analysis in normal urine samples

Volunteer subject	I Cd, ng/ml	I Excreta* per day, ml	II Cd, ng/ml	II Excreta* per day, ml	III Cd, ng/ml	III Excreta* per day, ml	IV Cd, ng/ml	IV Excreta* per day, ml	I	II	III	IV
									Urinary excretion per day, μg			
F-1	1.61 ± 0.17†	1206	0.23 ± 0.01	1178	0.20 ± 0.02	1304	0.25 ± 0.03	1524	1.94 ± 0.21	0.27 ± 0.01	0.26 ± 0.03	0.37 ± 0.05
F-2	0.84 ± 0.13 0.60 ± 0.09‡	1172	0.48 ± 0.03	992	0.43 ± 0.02	956	0.48 ± 0.04	918	1.16 ± 0.13 0.97 ± 0.12	0.48 ± 0.03	0.41 ± 0.02	0.44 ± 0.04
F-3	0.71 ± 0.13	1632	0.25 ± 0.03	1218	0.16 ± 0.01	1674	0.71 ± 0.09	1074	1.16 ± 0.21	0.30 ± 0.04	0.27 ± 0.02	0.76 ± 0.10
F-4	1.34 ± 0.16	730	0.32 ± 0.02	876	0.28 ± 0.04	734	0.31 ± 0.05	646	0.98 ± 0.12	0.28 ± 0.02	0.21 ± 0.03	0.20 ± 0.03
F-5	1.34 ± 0.08	1102	1.36 ± 0.11	1480	0.70 ± 0.07	1166	0.63 ± 0.09	1470	1.48 ± 0.09	2.01 ± 0.16	0.82 ± 0.08	0.92 ± 0.13
F-6	—	—	0.89 ± 0.10	698	1.00 ± 0.11	642	0.68 ± 0.10	1050	—	0.62 ± 0.07	0.64 ± 0.07	0.71 ± 0.10
F-7	0.88 ± 0.03 0.89 ± 0.08‡	1458	0.92 ± 0.06	1360	1.06 ± 0.05	1352	1.27 ± 0.15	952	1.28 ± 0.01 1.30 ± 0.11	1.25 ± 0.08	1.40 ± 0.07	1.21 ± 0.14
M-1	0.93 ± 0.11	628	0.54 ± 0.03	722	0.28 ± 0.03	716	0.23 ± 0.03	630	0.58 ± 0.07	0.39 ± 0.02	0.20 ± 0.02	0.15 ± 0.02
M-2	1.20 ± 0.20	656	0.28 ± 0.03	834	0.28 ± 0.02	750	0.31 ± 0.06	756	0.79 ± 0.13	0.23 ± 0.03	0.21 ± 0.02	0.24 ± 0.05
M-3	0.22 ± 0.03	1470	0.18 ± 0.02	1434	0.16 ± 0.03	1408	0.27 ± 0.02	904	0.32 ± 0.04	0.26 ± 0.03	0.23 ± 0.04	0.25 ± 0.02
M-4	1.65 ± 0.20	802	0.66 ± 0.10	944	0.49 ± 0.02	866	0.36 ± 0.02	1180	1.32 ± 0.16	0.62 ± 0.09	0.42 ± 0.02	0.42 ± 0.02
M-5	1.35 ± 0.25	774	1.20 ± 0.07	702	0.69 ± 0.05	726	0.46 ± 0.06	628	1.04 ± 0.19	0.84 ± 0.05	0.50 ± 0.03	0.29 ± 0.04
M-6	0.77 ± 0.05	2532	0.69 ± 0.06	2182	0.52 ± 0.05	2052	0.47 ± 0.06	2246	1.95 ± 0.13	1.51 ± 0.13	1.08 ± 0.10	1.05 ± 0.13
M-7	0.77 ± 0.07	2082	0.48 ± 0.05	2064	0.20 ± 0.02	2336	0.25 ± 0.02	2330	1.60 ± 0.15	0.99 ± 0.10	0.47 ± 0.05	0.58 ± 0.05
M-8	0.88 ± 0.03	902	0.74 ± 0.07	826	0.70 ± 0.07	1136	0.50 ± 0.06	1250	0.79 ± 0.03	0.61 ± 0.06	0.80 ± 0.08	0.62 ± 0.08

* Estimated.
† Standard deviation of replicate measurements.
‡ Duplicate sample.

Table 3. Results for lead in normal urine samples

Volunteer subject	Pb, ng/ml I	II	III	IV	Urinary excretion per day, μg I	II	III	IV
F-1	11 ± 3*	7 ± 1	14 ± 1	10 ± 1	13 ± 4	8 ± 1	18 ± 1	15 ± 2
F-2	11 ± 3	12 ± 1	13 ± 2	17 ± 2	13 ± 4	12 ± 1	12 ± 2	16 ± 2
	11 ± 2†				13 ± 2			
F-3	10 ± 2	6 ± 1	9 ± 1	13 ± 1	16 ± 3	7 ± 1	15 ± 2	14 ± 1
F-4	19 ± 3	12 ± 2	10 ± 1	12 ± 1	14 ± 2	11 ± 2	7 ± 1	8 ± 1
F-5	23 ± 3	20 ± 4	19 ± 2	21 ± 2	25 ± 3	30 ± 6	22 ± 2	31 ± 3
F-6	—	24 ± 3	16 ± 3	17 ± 2	—	17 ± 2	10 ± 2	18 ± 2
F-7	17 ± 2	11 ± 1	17 ± 1	18 ± 3	25 ± 3	15 ± 1	23 ± 2	17 ± 3
	14 ± 1†				20 ± 1			
M-1	21 ± 2	31 ± 6	11 ± 1	13 ± 1	13 ± 1	22 ± 4	8 ± 1	8 ± 1
M-2	21 ± 4	22 ± 5	13 ± 2	20 ± 2	14 ± 3	18 ± 4	10 ± 2	15 ± 2
M-3	13 ± 1	18 ± 3	13 ± 1	15 ± 2	19 ± 1	26 ± 4	18 ± 1	14 ± 2
M-4	25 ± 3	21 ± 2	25 ± 1	13 ± 1	20 ± 2	20 ± 2	22 ± 1	15 ± 1
M-5	18 ± 3	17 ± 2	15 ± 1	17 ± 1	14 ± 2	12 ± 1	11 ± 1	11 ± 1
M-6	7 ± 1	9 ± 2	6 ± 1	7 ± 1	18 ± 3	20 ± 4	12 ± 2	16 ± 2
M-7	12 ± 1	11 ± 2	10 ± 2	12 ± 2	25 ± 2	23 ± 4	23 ± 5	28 ± 5
M-8	26 ± 7	19 ± 4	15 ± 2	17 ± 1	23 ± 6	16 ± 3	17 ± 2	21 ± 1
	23 ± 3†				21 ± 3			

* Standard deviation of replicate measurements.
† Duplicate sample.

Table 4. Cadmium and lead in human metabolism samples

	Cd	Pb
	ng/g	*ng/g*
Diet	2.8 ± 0.2*	96 ± 9
	3.1 ± 0.2	90 ± 4
Faeces	14.7 ± 0.5	280 ± 20
	14.2 ± 0.4	290 ± 30
	ng/ml	*ng/ml*
Urine	0.78 ± 0.03	13.2 ± 1.9
	0.61 ± 0.03	14.1 ± 2.7
	0.71 ± 0.04	12.3 ± 2.4

* Standard deviation.

jects are maintained under controlled conditions at different intake levels of calcium. The overall results from the Hines study will be reported independently elsewhere. These urine samples were analysed in triplicate and the diet and faeces in duplicate, to determine the homogeneity of the samples and the precision of the analyses; good agreement was found and the precision of analysis was acceptable.

Although we have used 1-litre samples of urine, this amount is not necessary for the determination of cadmium and lead by graphite-furnace atomic-absorption spectrometry. The large sample was taken here because other trace metal determinations by different methods are under investigation.

The precision for our method ranged between 3 and 19% relative standard deviation (RSD) for cadmium, and between 4 and 27% for lead. The average RSD for all the urine samples was 10% for cadmium and 13% for lead, which reflects the low levels found and both the complexity and variability of the urine matrix.

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SHORT COMMUNICATIONS

DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES BY THIN-LAYER CHROMATOGRAPHY

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Summary—Analysis for 13 common organophosphorus pesticides by thin-layer chromatography is described; 17 solvent systems were examined. With channel thin-layer chromatography, linear calibration graphs were obtained for the range 1–10 μg.

Thin-layer chromatography is frequently used as an alternative to gas chromatography for the determination of organophosphorus pesticides in the study of environmental contamination of soil,[1] air,[2] plant[3,4] and water samples[2,5,6] as well in the control of animal,[7] milk[4] and food products[8–10] and tissues[11] after extraction with n-hexane or acetone.

In the present paper we describe a reliable method for the detection of 13 common organophosphorus pesticides by TLC, with 17 solvent systems, and their semi-quantitative determination by channel thin-layer chromatography.[12,13]

EXPERIMENTAL

Materials

The following organophosphorus pesticides were studied: fenchlorphos, parathion-methyl, parathion, ethion, dimethoate, diazinon, azinphos-methyl, disulfoton, phorate, coumaphos, malathion, azinphos-ethyl, oxydemeton-methyl. Stock solutions (1 mg/ml) were prepared by weighing the pure substances and dissolving them in ethanol, and quantitatively diluted to 0.1 mg/ml for spotting. The same solvent was used for further dilution.

The TLC plates (20 × 20 × 0.025 cm) were coated with Merck F_{254} silica gel.

The silver nitrate reagent used for detection[14] was a 2% solution in acetone–water mixture (3:1 v/v).

Procedure

In the preliminary TLC studies the pesticide solutions (10 μl) were spotted 2 cm from the bottom of the plate by means of a 50-μl "microcap" pipette. After evaporation of the solvent, the plate was developed in a conventional thin-layer tank, in an atmosphere saturated with the solvent, until the solvent front had travelled at least 18 cm. Because of the differences in polarity of the insecticides and their solubilities in water the following solvent systems were used:

(I) n-hexane
(II) n-hexane–benzene (4:1)
(III) n-hexane–benzene (3:1)
(IV) n-hexane–benzene (1:1)
(V) n-hexane–benzene (1:2)
(VI) n-hexane–benzene (1:3)
(VII) benzene
(VIII) n-hexane–benzene–acetonitrile (10:10:1)
(IX) n-hexane–benzene–acetonitrile (10:10:2)
(X) chloroform–benzene (2:1)
(XI) chloroform–benzene (9:1)
(XII) chloroform
(XIII) chloroform–acetone (9:1)
(XIV) chloroform–ethyl acetate–acetonitrile (9:1:1)
(XV) dichloromethane–ethyl acetate (7:3)
(XVI) ethanol
(XVII) acetone–water (1:1).

After development the plate was dried with a cold air-stream, sprayed with the detection reagent and heated for 30 min at 135°. The spots were dark yellow for fenchlorphos and ethion, and yellowish for the others with the exception of oxydemeton-methyl. All the compounds gave dark spots if the plate was sprayed and then irradiated with ultraviolet light (365 nm).

For channel TLC[12,13] the thin-layer plate was divided into narrow development channels 3 mm wide and 13 cm long, by scoring with a stylus and template (Fig. 1) and the solution was applied in the funnel-shaped flares of the channels. The chromatogram was developed until the solvent front had moved 10 cm along the channel. The plate was then treated with detection reagent as above. The spots are rectangular and their area can easily be measured.

RESULTS AND DISCUSSION

Table 1 shows the R_F values of 13 common organophosphorus pesticides in the selected chromatographic systems. None of the solvent systems separates all the pesticides, but systems V–XII provide moderately satisfactory separation. Chloroform–benzene (9:1) is a good solvent for separation of the pesticides into two groups, the first including parathion-methyl, parathion, ethion, disulfoton, phorate and fenchlorphos, which have R_F values >0.8, and the second including dimethoate, diazinon, azinphos-

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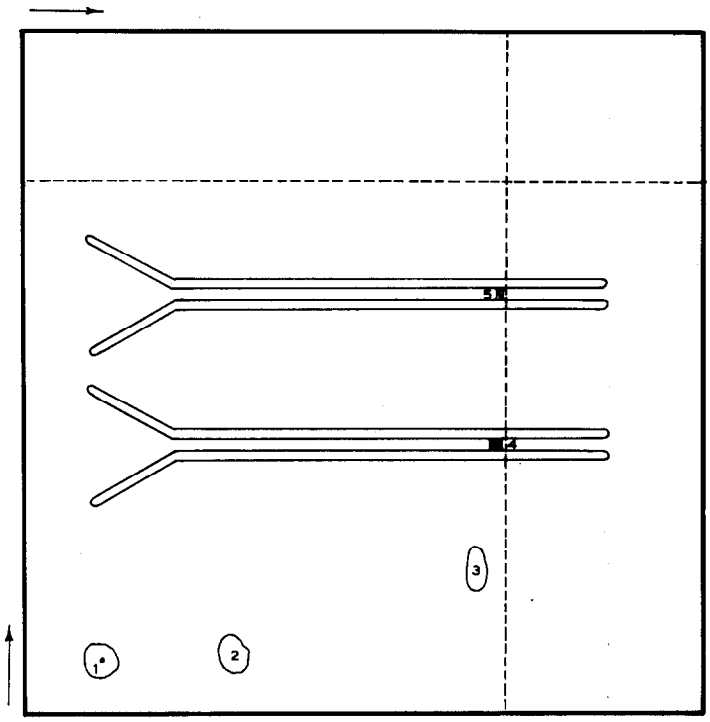

Fig. 1. The arrangement for channel TLC, and its application. Solvents n-hexane–benzene (1:3), dichloromethane–ethyl acetate (7:3). 1, Oxydemeton-methyl; 2, dimethoate; 3, coumaphos, malathion, azinphos-ethyl, azinphos-methyl; 4, parathion; 5, fenchlorphos.

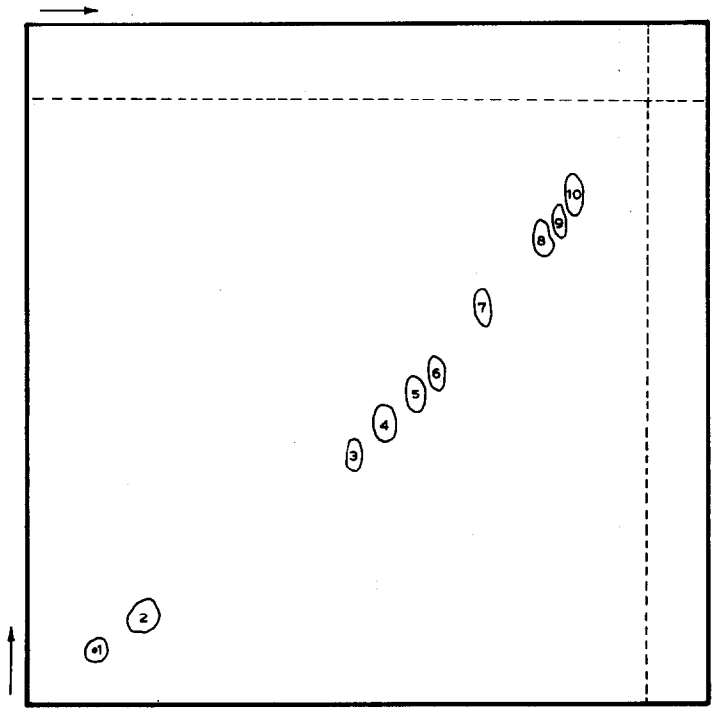

Fig. 2. Solvent chloroform–benzene (9:1). 1, Oxydemeton-methyl; 2, dimethoate; 3, diazinon; 4, azinphos-methyl; 5, azinphos-ethyl; 6, malathion; 7, coumaphos; 8, parathion-methyl; 9, parathion; 10, ethion, disulfoton, phorate, fenchlorphos.

Table 1. R_F-values of organophosphorus insecticides on silica gel 60 F_{254} TLC plates at 18°

Insecticide	100 × R_F*																
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
Fenchlorphos	12	31	35	60	77	81	84	86	82	92	93	94	96	98	93	91	95
Parathion-methyl	0	4	5	16	34	46	61	59	59	80	82	85	96	98	93	91	95
Parathion	4	5	7	21	41	53	66	70	66	83	85	84	96	98	93	91	95
Ethion	0	7	8	26	52	67	75	83	77	88	91	91	91	98	93	91	95
Dimethoate	0	0	0	0	0	0	0	4	9	7	10	9	39	31	37	83	93
Diazinon	0	0	0	0	4	5	9	37	55	35	45	43	93	91	88	91	95
Azinphos-methyl	0	0	0	0	4	5	10	22	37	38	48	49	93	91	88	83	93
Disulfoton	1	9	11	24	44	57	71	82	81	86	91	88	93	98	93	91	95
Phorate	4	14	17	39	60	69	77	85	82	88	91	91	93	98	93	91	95
Coumaphos	0	0	0	0	4	10	21	41	49	68	64	75	94	98	93	91	95
Malathion	0	0	0	0	4	6	20	35	49	49	61	64	88	95	93	91	95
Azinphos-ethyl	0	0	0	0	4	7	15	28	44	59	55	58	83	95	88	91	95
Oxydemeton-methyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	72	92

* The Roman numerals refer to the solvent systems.

methyl, coumaphos, malathion, azinphos-ethyl and oxydemeton-methyl, which have R_F values between 0.0 and 0.65. A second elution at right-angles to the first, with the same solvent system, gives the results represented in Fig. 2.

The first group can also be separated by two-dimensional TLC, first with n-hexane–benzene (1:1) and in the second direction with n-hexane–benzene (1:3). Figure 3 shows the result. The limit of detection is 0.5 μg.

The calibration curves for individual organophosphorus pesticides are constructed for the range 1–10 μg, with dichloromethane–ethyl acetate (7:3) as eluent, or acetone–water (1:1) for oxydemeton-methyl, at 15°. The R_F values are >0.9, and extremely dark and compact rectangular spots are obtained, with area $3h$ mm^2. The spot length (h) is carefully measured under a lens to an accuracy of 0.1 mm. R_F values between 0.8 and 0.9 are the optimum for accurate measurement by this technique.

All the compounds examined gave very similar linear calibration curves, which differed only slightly in slope. As already demonstrated[15] the spot length is a linear function of the mass when the temperature is

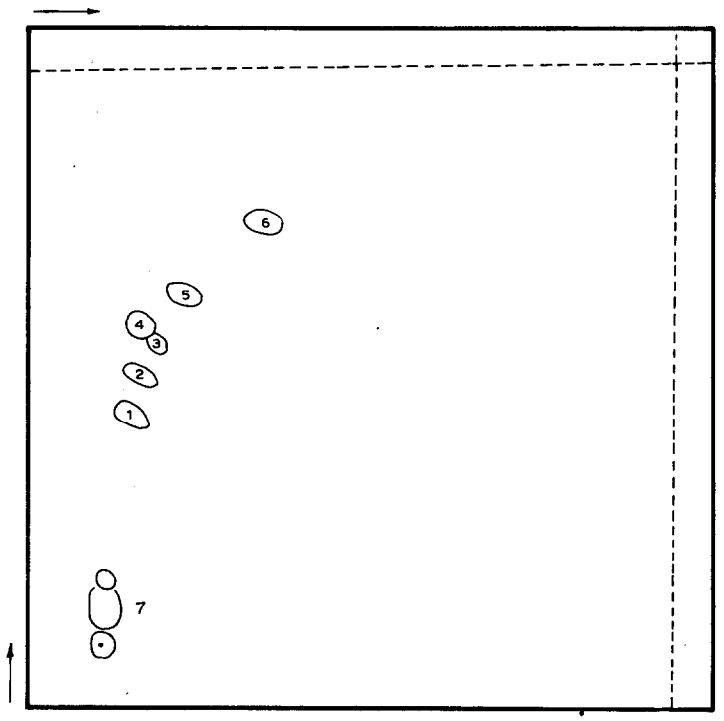

Fig. 3. Solvents n-hexane–benzene (1:1), n-hexane–benzene (1:3). 1, Parathion-methyl; 2, parathion; 3, disulfoton; 4, ethion; 5, phorate; 6, fenchlorphos; 7, oxydemeton-methyl, dimethoate, diazinon, malathion, azinphos-ethyl, azinphos-methyl, fenchlorphos.

kept constant during the development. The reproducibility was established by applying 6 spots, containing 10 μg of each of the same compounds to the same layer and measuring the lengths after solvent development and detection. The relative standard deviation (s_r) was 0.09 mm.

An individual compound in the presence of other pesticides can be semiquantitatively determined by two-dimensional thin-layer chromatography. When the compound of interest is present (established with a chromatogram on a separate plate), the compound is first isolated by use of the most favourable solvent system, then the plate is dried, rotated through 90° and subjected to channel chromatography with a solvent in which the compound has high solubility ($R_F > 0.9$), the channel being drawn so that the spot is in the funnel-shaped flare of the channel.

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INVESTIGATION OF THE EXTRACTION EQUILIBRIUM OF THE VANADIUM–PAR COMPLEX BETWEEN AQUEOUS MEDIUM AND A CHLOROFORM SOLUTION OF A QUATERNARY AMMONIUM CHLORIDE

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Summary—Partition of the VO_2L^- complex (formed from vanadium(V) and PAR [4-(2-pyridylazo)-resorcinol]) between water and a chloroform solution of TOMACl (trioctylmethylammonium chloride) has been studied. The V–PAR–TOMACl complex extracted into the chloroform phase was found to be 1:1:1 in composition. The extraction equilibrium constant is log $K = 4.0 \pm 0.1$.

A frequently used sensitive photometric reagent for vanadium is 4-(2-pyridylazo)resorcinol (PAR). Several authors have studied the extraction of the VO_2L^- vanadium–PAR complex with extractants containing salts of quaternary bases. Tetraphenylphosphonium and tetraphenylarsonium chloride were used by Široki and Djordjevic[1] and the mixed complex was extracted into a chloroform–acetone mixture. Yotsuyanagi *et al.*[2] used the water-soluble tetradecyldimethylbenzylammonium chloride in the presence of DCTA for extraction of the PAR complex.

In the present work trioctylmethylammonium chloride (TOMACl, sparingly soluble in water) is examined.

EXPERIMENTAL

Reagents

Chloroform solution of TOMACl. Prepared and standardized as already described.[3]

Ammonium vanadate solution, 10^{-2}M. Standardized by EDTA.

PAR solution, 10^{-3}M. Prepared as described earlier.[4]

Distribution experiments

A portion of vanadium solution of known pH was mixed with PAR and after 30 min the solution was shaken with TOMACl solution. The optimum contact time was found to be 3 min. The phase-volume ratio (aqueous:organic) was 1:1 or 10:1. The ionic strength was kept constant with potassium or ammonium chloride.

The pH was adjusted with dilute hydrochloric acid or potassium hydroxide solution and the value measured after equilibration was used in the calculations. The vanadium concentration in the aqueous phase at equilibrium was determined spectrophotometrically with PAR at pH 5.5, by measurement at 545 nm against a blank.

From the initial and equilibrium concentrations of vanadium in the aqueous phase the distribution coefficient D was calculated:

$$D = [V']_{org}/[V']_{aq} \quad (1)$$

where $[V']_{org}$ and $[V']_{aq}$ are the analytical concentrations of vanadium in the organic and aqueous phases, respectively.

The distribution was studied as a function of the equilibrium pH (Table 1) and the concentrations of PAR (Table 2), chloride (Table 3) and TOMACl.

Composition of the complex

The mole-ratio method was used; $10^{-5}M$ vanadium solution (0.1M in potassium chloride, pH 5.6) containing various concentrations of PAR was shaken with a large excess of $2 \times 10^{-3}M$ TOMACl (volume ratio 1:1). The absorbance of the organic phase was measured at 560 mm in a 1-cm cell against chloroform solution. The mole-ratio plot showed that a 1:1 vanadium(V)–PAR complex is extracted. Similar experiments showed the vanadium(V)–TOMACl ratio was also 1:1.

DISCUSSION

The species extracted appears to be an ion-pair complex formed by the vanadium–PAR complex and the quaternary ammonium ion:

$$VO_2L^-_{(aq)} + R_4NCl_{(org)} \rightleftharpoons VO_2LR_4N_{(org)} + Cl^-_{(aq)} \quad (2)$$

where L^{2-} denotes the deprotonated complex-forming species of PAR, and R_4NCl the TOMACl. The equilibrium constant is

$$K = \frac{(VO_2LR_4N)_{org}[Cl^-]_{aq}}{[VO_2L^-](R_4NCl)_{org}}. \quad (3)$$

Table 1. Distribution coefficients of vanadium as a function of the equilibrium pH of the aqueous phase (initial concentrations: $[V] = 2 \times 10^{-5}M$, $[KCl] = 0.1M$, $C_{PAR} = 10^{-4}M$, $C_{TOMACl} = 0.002M$); $V_{aq}/V_{org} = 10$)

pH_e	1.86	2.15	2.35	2.75	5.40	6.95	7.10	8.15	8.35	8.80
log D	−0.77	0.52	0.56	0.98	2.24	2.09	2.09	1.09	0.63	0.23

Table 2. Distribution coefficients of vanadium as a function of the PAR concentration (initial concentrations: $[V] = 2 \times 10^{-5}M$; $[NH_4Cl] = 0.1M$; $pH_e = 5.4$; $V_{aq}/V_{org} = 10$)

C_{PAR}, $10^{-4}M$	0.8	1.2	1.6	2.0	4.0	6.0
log D	2.24	2.24	2.17	2.2	1.94	1.75

The distribution coefficient of vanadium can be expressed as

$$D = (VO_2LR_4N)_{org}/[V']_{aq}. \quad (4)$$

Only a fraction of the total amount of the vanadium in the aqueous phase is in the form of VO_2L^-, and can be calculated by means of an α-function:

$$\alpha_{VO_2L^-} = \frac{[V']}{[VO_2L^-]} =$$

$$\frac{[VO_2^+]+[VO_2HL]+[VO_2L^-]+[VO_3^-]+[HVO_4^{2-}]}{[VO_2L^-]} \quad (5)$$

For calculation of $\alpha_{VO_2L^-}$ the following side-reactions and equilibrium constants[5–8] were considered:

$$VO_2^+ + L^{2-} \rightleftharpoons VO_2L^- \qquad K_{VO_2L} = 10^{16.5}$$

$$VO_2L^- + H^+ \rightleftharpoons VO_2HL \qquad K^H_{VO_2HL} = 10^{3.95}$$

$$VO_3^- + H^+ \rightleftharpoons HVO_3 \qquad K^H_{HVO_4} = 10^{3.7}$$

$$HVO_3 + H^+ \rightleftharpoons VO_2^+ + H_2O \qquad K^H_{VO_2} = 10^{3.3}$$

$$HVO_4^{2-} + H^+ \rightleftharpoons VO_3^- + H_2O \qquad K_{HVO_4} = 10^{7.7}.$$

For evaluation of $[VO_2HL]$ and $[VO_2L^-]$, the necessary concentration of free ligand L^{2-} is obtained from the conditional concentration[9] of PAR, [L', in the aqueous phase, and the α-function for the protonation of L^{2-}:

$$\alpha_{L(H)} = \frac{[L']}{[L^{2-}]}$$

$$= \frac{[H_3L^+] + [H_2L] + [HL^-] + [L^{2-}]}{[L^{2-}]}$$

$$= 1 + K_1K_2K_3[H^+]^3 + K_1K_2[H^+]^2 + K_1[H^+] \quad (6)$$

where K_1, K_2, K_3 are the protonation constants of L^{2-} ($\log K_1 = 11.9$; $\log K_2 = 5.6$; $\log K_3 = 3.1$).[10]

[L'] is also given by the mass-balance

$$[L'] = C^o_{Par} - (R_4NVO_2L)V_{org}/v_{aq} \quad (7)$$

where C^o_{PAR} is the original total concentration of PAR

Table 3. Distribution coefficient of vanadium as a function of the chloride ion concentration (initial concentrations: $[V] = 2 \times 10^{-5}M$; $[PAR] = 10^{-4}M$; $V_{aq}/V_{org} = 10$)

	log D		
$[Cl^-]$, M	$pH_e = 5.05$ $C_{TOMACl} = 6 \times 10^{-4}M$	$pH_e = 5.6$ $C_{TOMACl} = 4 \times 10^{-4}M$	$pH_e = 5.6$ $C_{TOMACl} = 2 \times 10^{-4}M$
0.2	1.62	1.23	0.69
0.15	1.72	1.32	0.96
0.1	1.70	1.4	0.99
0.08	1.76		1.16
0.06	1.85		
0.05		1.51	
0.04	1.93		

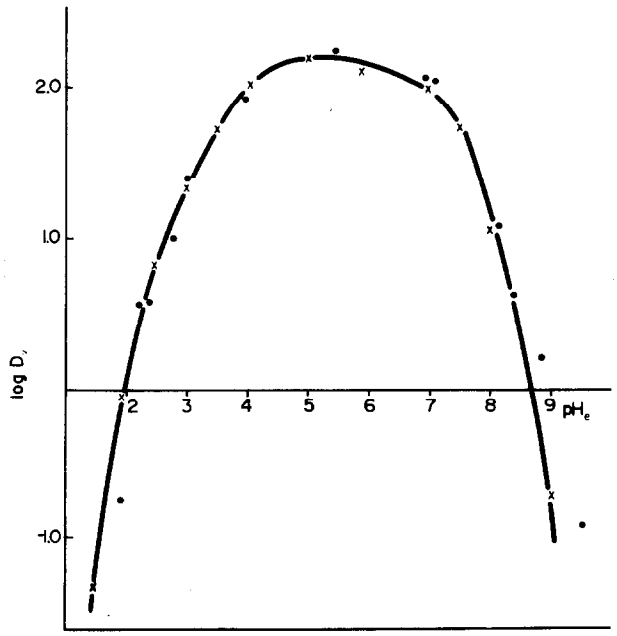

Fig. 1. Distribution coefficient of vanadium as a function of the equilibrium pH of the aqueous phase. $[TOMACl]_{total} = 2 \times 10^{-3}M$, $[KCl] = 0.1M$, $[PAR]_{total} = 10^{-4}M$, $[V]_{total} = 2 \times 10^{-5}M$. × = calculated; ● = measured.

in the aqueous phase, and v_{org} and v_{aq} are the volumes of the organic and aqueous phases respectively.

Equation (5) can then be written as follows:

$$\alpha_{VO_2L^-} = 1 + \frac{\alpha_{L(H)}}{[L']K_{VO_2L}} + K^H_{VO_2HL}[H^+] + \frac{\alpha_{L(H)}}{K^H_{VO_2}K^H_{HVO_3}K_{VO_2L}[L'][H^+]^2} + \frac{\alpha_{L(H)}}{K_{HVO_4}K_{VO_2L}K^H_{HVO_3}K^H_{VO_2}[L'][H^+]^3}. \quad (8)$$

An ion-exchange process takes place between the HL^- form of PAR and the quaternary ammonium chloride. The total concentration of the quaternary ammonium salt in the organic phase can then be expressed as follows:

$$C_{R_4HCl(org)} = (R_4HCl) + (R_4NHL)_{org} + (R_4NVO_2L)_{org}. \quad (9)$$

The ion-exchange process can be considered as a side-reaction of TOMACl:

$$\alpha_{R_4HCl(PAR)} = \frac{(R_4NCl')_{org}}{(R_4NCl)_{org}} = \frac{(R_4NCl)_{org} + (R_4NHL)_{org}}{(R_4NCl)_{org}} = 1 + \frac{K_1K^x[L'][H^+]}{\alpha_{L(H)}[Cl^-]_{aq}} \quad (10)$$

where K^x is the equilibrium constant for exchange of the HL^- form of PAR and the chloride ion[6] ($\log K^x = 2.99 \pm 0.04$) and

$$(R_4NCl')_{org} = C_{R_4HCl(org)} - (R_4NVO_2L)_{org}v_{org}/v_{aq}.$$

From equations (3)–(5) and (10), the equilibrium constant K^e is given by

$$K^e = \frac{D_v[Cl^-]\alpha_{VO_2L^-}\alpha_{R_4NCl}}{C_{R_4NCl(org)}}. \quad (11)$$

The values of $\log K^e$ were obtained by computer program from equations (8), (10) and (11) and the results from 41 different experiments. The most probable value and its standard deviation are $\log K^e = 4.0 \pm 0.1$. To prove the validity of our assumptions and calculations the experimental data obtained and the $\log D_v - pH_e$ relation derived from equation (11) by using the value of $\log K^e = 4.0$ are compared in Fig. 1. As can be seen, the calculated and measured values are in acceptable agreement.

Acknowledgements—Thanks are given to T. Pap for preparing the program for carrying out the calculations. Thanks are also expressed to R.A. Chalmers for valuable consultation and suggestions.

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DICHLORAMINE-B AS REDOX TITRANT IN NON-AQUEOUS OR PARTIALLY AQUEOUS MEDIA

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Summary—Dichloramine-B is proposed as a redox titrant in glacial acetic acid medium. The general conditions for its use and the procedures for estimating hydrazine, ascorbic acid, ferrocyanide, hydroquinone, oxine, antimony(III) and thallium(I) potentiometrically and allyl, crotyl and cinnamyl alcohols by a back-titration procedure are described.

The number of oxidants available for non-aqueous redox titrimetry is limited. Recently, organic haloamines have received considerable attention as redox titrants. Although chloramine-T (CAT) and chloramine-B (CAB) are soluble in water, dichloramine-T (DCT),[1–5] dibromamine-T (DBT)[6,7] and dibromamine-B (DBB)[8] are employed as redox titrants in non-aqueous or partially aqueous media. A recent addition to the group is dichloramine-B (*N,N'*-dichlorobenzenesulphonamide, DCB) which can be used as an oxidimetric titrant in acetic acid medium. Potentiometric determinations of typical reductants such as hydrazine, ascorbic acid, ferrocyanide, hydroquinone, oxine, Sb(III) and Tl(I) and back-titration methods for estimating unsaturated alcohols, such as allyl, crotyl and cinnamic, with it have been developed and are reported in the present communication.

EXPERIMENTAL

Reagents

Dichloramine-B. The methods of Zilberg[9] and Nobel[10] are inconvenient. A simpler method [passing chlorine through an aqueous solution of chloramine-B (CAB)] gives good results and samples of high purity are obtained. CAB was prepared[11] by passing pure chlorine for 1 hr through benzenesulphonamide dissolved in 4*M* sodium hydroxide and heated to 70°. The product was recrystallized from water, 30 g were dissolved in 500 ml of water and pure chlorine was bubbled through the solution for about 2 hr. The white precipitate of DCB formed was filtered off under suction, thoroughly washed with water and dried in a blackened vacuum desiccator. Yield ~100%; m.p. 74°C. The product was stored in brown bottles. The available chlorine was determined by iodometry (found 31.0%; theoretical 31.4%).

DCB was further characterized by infrared and PMR spectroscopy. The infrared spectrum showed two strong bands at 1340 and 1180 cm^{-1}, corresponding to the SO_2 asymmetric and symmetric stretching frequencies respectively. The presence of the N–Cl linkage is shown by the three bands at 538, 563 and 580 cm^{-1}. The ^1H spectrum showed a doublet of a doublet integrating for two protons centered at τ 1.85 which is attributed to the two *ortho* aromatic protons. The two coupling constants for the quartet are 8 and 2 Hz, indicating both 1.2 and 1.3 coupling with the *meta* and *para* protons respectively. The other aromatic proton signals appear as a multiplet at τ 2.1–2.5.

Stock solution of DCB. DCB is only slightly soluble in water (0.125 g/l. at 30°), but fairly soluble in glacial acetic acid (175.2 g/l. at 30°) and other common organic solvents. An approximately 0.1*N* (0.025*M*) solution was prepared by dissolving about 6 g of DCB in 1 litre of glacial acetic acid containing 10% v/v acetic anhydride. Solutions of DCB are light-sensitive and have to be kept in brown bottles, but need daily standardization by addition of potassium iodide solution and titration of the liberated iodine with thiosulphate.

Reductants. Solutions of analytical-reagent quality hydrazine sulphate, ascorbic acid, hydroquinone, potassium ferrocyanide, potassium antimonyl tartarate and thallium(I) sulphate in water and oxine in 50% aqueous acetic acid were prepared and checked by standard methods. Allyl alcohol (Merck) and crotyl alcohol (Fluka) were used without further purification. Cinnamic alcohol (Naarden) was distilled under reduced pressure. The purity of the compounds was checked by phthalation with phthalic anhydride in pyridine.[12] The required quantity of allyl and crotyl alcohol was accurately weighed and dissolved in water; glacial acetic acid was used as solvent for cinnamic alcohol.

Procedures

Direct potentiometric titration was used for all compounds except the alcohols. The latter were estimated by back-titration. For the direct titrations potassium bromide (0.5–1.0 g) was added to the reductants. With oxine and hydrazine, 5–10 ml of glacial acetic acid or of 70% perchloric acid, respectively, were added. In the titration of Tl(I), the white precipitate of TlBr formed on addition of potassium bromide dissolves during the titration as it is converted into $TlBr_3$.

Known volumes of alcohol solution (2–60 mg of the alcohol) were added to a measured excessive volume of oxidant solution (~3.5 mmole of oxidant) in an iodine flask and the mixtures were kept at room temperature with occasional shaking for 10, 45 or 60 min for crotyl, allyl and cinnamic alcohol, respectively. About 10 ml of 20% potassium iodide solution were added and the liberated iodine was titrated with sodium thiosulphate. A blank titration was run with the same volume of DCB solution. Table 1 shows the results obtained when the reaction time was varied, and demonstrates that the times stated are adequate.

Table 1. Extent of oxidation of allyl, crotyl and cinnamyl alcohols with dichloramine-B

Time, *min*	mmole of DCB consumed / mmole of alcohol taken: Allyl alcohol	Crotyl alcohol	Cimmamic alcohol
10	0.6450	0.9995	—
15	—	—	0.7675
20	0.7415	0.9995	—
30	0.8385	0.9995	0.8340
45	0.9995	0.9995	0.9175
60	0.9995	0.9995	1.000
90	0.9995	0.9995	1.000
120	—	—	1.000
150	—	—	1.000
Initial [DCB]/[alcohol]	7	5.5	6.3

Table 2. Potentiometric titrations with dichloramine-B

Reductant	Range studied, *mg*	Maximum error, %
Hydrazine	3–61	0.5
Ascorbic acid	8–88	0.5
Ferrocyanide	8–84	0.8
Hydroquinone	6–90	0.9
Oxine	5–75	0.9
Sb(III)	7–70	0.5
Tl(I)	10–100	0.9

RESULTS AND DISCUSSION

Typical results are presented in Tables 2 and 3. A steady potential was attained almost instantaneously in all the potentiometric titrations. Under the conditions specified, DCB oxidizes hydrazine to nitrogen, ascorbic acid to dehydroascorbic acid, hydroquinone to quinone, ferrocyanide to ferricyanide, Sb(III) to Sb(V), Tl(I) to Tl(III) and brominates oxine. The oxidant undergoes a 2-electron reaction. The amount of alcohol titrated (x mg) was calculated from

$$x = \frac{MN(V_1 - V_2)}{2}$$

where M is the molecular weight of the alcohol, N the normality of the thiosulphate, V_1 is the blank titration (ml) and V_2 the volume of thiosulphate (ml) used to titrate the excess of oxidant.

The potentiometric titrations proceeded smoothly only in presence of bromide. It is likely that bromine, formed as an intermediate product by the oxidation of bromide by DCB, acts as a catalyst. In the case of oxine, bromine produced *in situ* may be looked upon as a reactant since the final product is a bromo-derivative.

The stoichiometry of the oxidation of alcohols with DCB can be represented as:

$$R'CHCH_2OH + \tfrac{1}{2}RNCl_2 \rightarrow R'CHCHO + \tfrac{1}{2}RNH_2 + HCl$$

where R′ is CH_2– for allyl, CH_3CH– for crotyl and C_6H_5CH– for cinnamic alcohol and R = $C_6H_5SO_2$.

The presence of benzenesulphonamide among the reaction products was identified by TLC. A mixture of petroleum ether, chloroform and n-butanol (2:2:1 v/v) was used as the solvent, with iodine as the detection reagent (R_F = 0.88).

The presence of the aldehydes in the reaction mixture was shown by spot-tests,[13] and the aldehydes were also isolated as their 2,4-dinitrophenylhydrazones.[14]

The effect of water on the stoichiometry of oxidation of the alcohols by DCB was studied. While it had little influence on the oxidation of allyl and crotyl alcohol, recoveries of cinnamic alcohol were up to 15% higher when up to 50% water was present in the reaction mixture. The presence of added salts on the rate of oxidation of the alcohols was also investigated. It was found that thiocyanate, Br^- and Cl^-, interfere but PO_4^{3-}, SO_4^{2-}, Ba^{2+}, Zn^{2+} and K^+ do not. Any reductant with E° below that of DCB will interfere. The formal redox potential of the DCB-sulphonamide couple in acetic acid medium was determined by the extrapolation procedure and found to be +1.29 V at room temperature.

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Table 3. Estimation of allyl, crotyl and cinnamic alcohols (mg) with dichloramine-B

Allyl alcohol Taken	Found	Error, %	Crotyl alcohol Taken	Found	Error, %	Cinnamic alcohol Taken	Found	Error, %
2.60	2.61	+0.4	3.37	3.37	0.0	8.74	8.75	+0.1
5.20	5.20	0.0	6.75	6.75	0.0	17.48	17.50	+0.1
15.59	15.58	+0.1	13.50	13.49	−0.1	34.97	35.0	+0.1
25.98	26.20	+0.9	20.25	20.24	−0.1	52.44	52.5	+0.1
41.57	41.9	+0.8	33.75	33.7	−0.1	69.93	70.3	+0.5
51.96	52.2	+0.5	67.50	68.0	+0.7	87.40	86.9	−0.6

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NEW CHROMOGENS OF THE FERROIN TYPE—X

SYNTHESIS AND CHELATION PROPERTIES OF SOME 2-THIAZOLYL- AND 2-PYRIMIDINYLHYDRAZONES

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Summary—Preparation of some new hydrazones which possess the ferroin chromophoric group is described, together with results of spectrophotometric studies of their chelation reactions with iron(II), copper(I), cobalt(II) and nickel(II). Several of the new compounds show promise as highly sensitive chromogenic reagents for simultaneous determinations of these metals.

In search of sensitive chromogenic reagents for iron, copper, cobalt and nickel we have extended our synthetic and analytical studies of hydrazones with ferroin groups to include the newly synthesized compounds reported here. Previous communications in this series have explored various pyridinyl- and pyrazinylhydrazones[1–3] as chromogenic reagents.

The only previously reported 2-thiazolylhydrazones are those of acetone and acetophenone.[4] Only one 2-pyrimidinylhydrazone (that of pyridine-2-aldehyde) has been described in the literature.[5] For this study, therefore, we prepared 2-thiazolyl- and 2-pyrimidinylhydrazones of the following compounds (previously described): quinoline-2-aldehyde,[6] isoquinoline-3-aldehyde,[7] 2-acetylpyridine, 2-benzoylpyridine, di-2-pyridyl ketone, 2-acetylpyrazine,[8] 2-benzoylpyrazine,[9] 2,2′-pyridil, pyrazinylmethyl 2-pyridyl ketone, 2-pyrimidinylmethyl 2-pyridyl ketone,[10] and desoxy-α-pyridoin.[11] The method of Shirakawa *et al.*[12] was followed for the preparation of 2-pyrimidinylhydrazine.[13] A modification of the method of Lee *et al.*[4] was devised for the synthesis of 2-thiazolylhydrazine, which proved easier to carry out, though at the expense of lower yields.

EXPERIMENTAL

Preparation of 2-thiazolylhydrazine

A solution of 10 g of 2-aminothiazole in 80 ml of concentrated hydrochloric acid was diazotized by adding a concentrated aqueous solution of sodium nitrite (7 g) at a temperature between −10° and 0°. To the diazonium solution was then added a solution of 45 g of stannous chloride in 20 ml of concentrated hydrochloric acid (at below 0°). The resulting precipitate was filtered off and added gradually to a dilute sodium hydroxide solution at 0°. After repeated extraction with ether, drying, and removal of the ether, the residue was crystallized twice from benzene. Yield of pure product (m.p. 95°) was 3.5 g (34.5%).

Preparation of 2-pyrimidinyl- and 2-thiazolylhydrazones

A mixture of 0.005 mole each of substituted hydrazine and aldehyde or ketone, 25 ml of absolute ethanol and 1 ml of glacial acetic acid was refluxed for 3 hr. Most of the ethanol was then evaporated, ice water added, and the solution made alkaline with ammonia solution. The precipitate was collected, dried and crystallized from the solvent indicated in Table 1 or Table 2.

Chelation studies

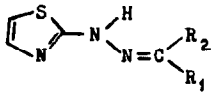
The procedures, reagents, and standard solutions have been described before.[13] Spectra were recorded with a Cary Model 14 spectrophotometer.

RESULTS AND DISCUSSION

Crystallization solvents, melting points, elemental analyses, and structures for the new hydrazones are compiled in Tables 1 and 2. All but one formed coloured chelates with iron(II), copper(I), cobalt(II), and nickel(II) over broad ranges of pH, generally from 3 to 11. Maximum colour formation commonly occurred between pH 5 and 8. Addition of ethanol was necessary to provide solubility for both the chromogen and complex. The iron(II) chelate of VIII lacked adequate solubility even in ethanolic solutions and therefore was extracted from aqueous solution (pH 7) into chloroform for spectrophotometric examination. All other solutions prepared for spectrophotometry were buffered at pH 7 with ammonium acetate and contained ethanol (50% by volume).

Compound XVII failed to form coloured reaction products with iron(II), cobalt(II), and nickel(II) but formed a yellow complex with copper(I). Such behaviour is characteristic of sterically hindered ferroin compounds[15] and suggests that XVII has its phenyl

Table 1. Synthesis and analysis of 2-thiazolyl hydrazones



Compound	R_1	R_2	m.p. °C	Crystn. solvent	Formula	Analysis Calculated, % C	H	N	Found, % C	H	N
I	2-C_5H_4N	H	200	C_2H_5OH	$C_9H_8N_4S$	52.94	3.95	27.44	52.5	4.0	27.5
II	2-Quinolyl	H	222	C_2H_5OH	$C_{13}H_{10}N_4S$	61.41	3.96	22.04	61.1	4.0	22.2
III	3-Isoquinolyl	H	219	C_2H_5OH	$C_{13}H_{10}N_4S$	61.41	3.96	22.04	61.0	3.9	22.1
IV	2-C_5H_4N	CH_3	175	CH_3OH	$C_{10}H_{10}N_4S$	55.04	4.62	25.67	55.1	4.6	26.0
V	2-C_5H_4N	C_6H_5	164	CH_3OH	$C_{15}H_{12}N_4S$	64.28	4.32	19.99	64.2	4.5	20.0
VI	2-C_5H_4N	2-C_5H_4N	135	CH_3OH-H_2O	$C_{14}H_{11}N_5S$	59.78	3.94	24.90	59.9	4.0	25.2
VII	Pyrazinyl	CH_3	210	C_2H_5OH	$C_9H_9N_5S$	49.31	4.14	31.95	49.5	4.4	32.0
VIII	Pyrazinyl	C_6H_5	210 dec.	C_2H_5OH	$C_{14}H_{11}N_5S$	59.78	3.94	24.90	59.6	3.9	24.4
IX	2-C_5H_4N	C_5H_4NCO	154	CH_3OH	$C_{15}H_{11}N_5OS$	58.25	3.58	22.64	57.8	3.5	22.8
X	Pyrazinyl-methyl	2-C_5H_4N	147	CH_3OH	$C_{14}H_{12}N_6S$	56.75	4.08	28.36	56.5	4.0	28.2
XI	2-Pyrimidinyl-methyl	2-C_5H_4N	147	CH_3OH	$C_{14}H_{12}N_6S$	56.75	4.08	28.36	56.4	4.0	28.2
XII	2-Pyridinyl-methyl	2-C_5H_4N	108	Pet. ether	$C_{15}H_{13}N_5S$	61.01	4.44	23.72	61.1	4.2	23.8

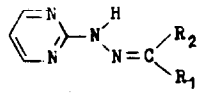
substituent in the R_1 position (not R_2), as shown in Table 2.

The iron(II) chelates exhibit relatively low molar absorptivities (Table 3) and thus afford little advantage in sensitivity for trace iron determinations in comparison to previously recommended chromogens.[16,17] Their ligand:iron ratios (Table 3) indicate that the ligands are terdentate. Presumably all three donor atoms are nitrogen, one each from the three moieties R_1, hydrazonyl, and pyrimidinyl or thiazolyl. For ligands I–VIII and XIII–XX spatial models indicate that terdentate co-ordination leading to bis-chelates of iron(II) is possible only if the ligand has the *anti*-configuration depicted in Tables 1 and 2, *i.e.*, with the R_1 substituent in the *anti*-position relative to the thiazolyl or pyrimidinyl group. For the other ligands no stereochemical assignments are possible solely on the basis of iron(II) chelation stoichiometry, because both their R_1 and R_2 substituents possess groups capable of co-ordination, so that either isomer is capable of terdentate chelation.

Values greater than 2.04, outside the range of probable error, were found for the ligand:iron ratio of some of the hydrazones. Presumably these compounds are slightly impure or contain small amounts of the *syn*-isomer. Ligand:iron ratios for XVII, XVIII and XXI were not determined, owing to solubility limitations or lack of sufficient amounts.

The copper(I), cobalt(II) and nickel(II) chelates exhibited very high molar absorptivities, similar to those of previously studied hydrazone–ferroin compounds. Especially notable are XIV, XV, and XVIII. These are worthy of further attention and will be studied along with other recently described hydra-

Table 2. Synthesis and analysis of 2-pyrimidinyl hydrazones



Compound	R_1	R_2	m.p. °C	Crystn. solvent	Formula	Analysis Calculated, % C	H	N	Found, % C	H	N
XIII	2-C_5H_4N[a]	H	221	CH_3OH	$C_{10}H_9N_5$						
XIV	2-Quinolyl	H	247	Methyl Cellosolve	$C_{14}H_{11}N_5$	67.46	4.45	28.09	67.0	4.5	28.0
XV	3-Isoquinolyl[b]	H	220	CH_3OH	$C_{14}H_{13}N_5O$	62.91	4.90	26.20	63.1	4.9	26.1
XVI	2-C_5H_4N	CH_3	153	Benzene	$C_{11}H_{11}N_5$	61.96	5.20	32.84	62.0	5.1	33.2
XVII	C_6H_5	2-C_5H_4N	195	CH_3OH	$C_{16}H_{14}N_5$	69.80	4.76	25.44	69.5	4.7	25.5
XVIII	2-C_5H_4N	2-C_5H_4N	161	Benzene	$C_{15}H_{12}N_6$	65.21	4.38	30.42	65.3	4.4	30.6
XIX	Pyrazinyl	CH_3	160	CH_3OH	$C_{10}H_{10}N_6$	56.07	4.71	39.23	55.6	4.5	39.1
XX	Pyrazinyl	C_6H_5	226	Benzene	$C_{15}H_{12}N_6$	65.21	4.38	30.42	65.5	4.5	30.0
XXI	2-C_5H_4N	C_5H_4NCO	165	Benzene	$C_{16}H_{12}N_6O$	63.15	3.98	27.62	63.2	4.0	27.4
XXII	Pyrazinyl-methyl	2-C_5H_4N	151	CH_3OH	$C_{15}H_{13}N_7$	61.85	4.50	33.66	61.7	4.5	33.5
XXIII	2-Pyrimidinyl-methyl	2-C_5H_4N	159	CH_3OH	$C_{15}H_{13}N_7$	61.85	4.50	33.66	61.8	4.5	33.6
XXIV	2-Pyridinyl-methyl	2-C_5H_4N	126	CH_3OH·H_2O	$C_{16}H_{14}N_6$	66.19	4.86	28.95	65.8	5.0	28.9

[a] Reference 3. [b] Hydrate.

Table 3. Properties of the iron(II) chelates

Chromogen	Colour	λ, nm	ε, $l.mole^{-1}.cm^{-1}$	L:Fe, mole ratio	Remarks†
I	Brown	580	6.4×10^3	2.18	2,4
II	Orange	637	5.3×10^3	2.01	3,4
		512	1.66×10^4		
III	Brown	556	1.04×10^4	2.26	2,4
IV	Brown	562*	5.2×10^3	2.04	1,4
		488*	8.0×10^3		
V	Green	597	9.2×10^3	2.00	1
VI	Green	600	1.23×10^4	2.00	1
VII	Green	631	5.8×10^3	2.07	2,4
VIII	Brown	644	7.0×10^3		5
IX	Brown	629	1.03×10^4	2.06	2,4
X	Green	620	7.4×10^3	2.10	1,4
XI	Green	595	8.3×10^3	2.21	1
XII	Green	594	7.6×10^3	2.07	2
XIII	Red	550	6.5×10^3	2.17	1,4
XIV	Green	618	6.6×10^3	2.07	2,4
		540	6.0×10^3		
XV	Orange	545	9.2×10^3	2.05	1
		488	1.08×10^4		
XVI	Red	545	6.5×10^3	2.10	1,4
XVII	Colourless	–	–		
XVIII	Red	580	1.07×10^4		
XIX	Green	600	5.4×10^3	2.09	1,4
		425*	1.56×10^4		
XX	Green	618	7.9×10^3	2.06	2
		425*	2.66×10^4		
XXI	Green	613	8.1×10^3	–	4
XXII	Green	556	7.3×10^3	1.97	1,4
XXIII	Green	557	7.3×10^3	2.01	1,4
XXIV	Green	556	7.6×10^3	2.00	1

* Shoulder or at side of band just before reagent blank absorbance becomes appreciable.

† Numbers refer to the following, as indicated: 1, strong complex (absence of curvature in mole-ratio plot); 2, moderately strong complex (slight curvature near equivalence point); 3, weak complex (appreciable curvature); 4, colour faded noticeably after 5 days; 5, spectrum after extraction into chloroform.

Table 4. Properties of the copper(I), cobalt(II), and nickel(II) chelates

	Copper(I)			Cobalt(II)			Nickel(II)		
Ligand	Colour	λ, nm	ε, $l.mole^{-1}.cm^{-1}$	Colour	λ, nm	ε, $l.mole^{-1}.cm^{-1}$	Colour	λ, nm	ε, $l.mole^{-1}.cm^{-1}$
I	Gold	459	1.55×10^4	Orange	479	2.57×10^4	Yellow	446	4.01×10^4
II	Red	486	9.6×10^3	Orange	500	2.70×10^4	Orange	498	4.44×10^4
III	Yellow	441*	1.99×10^4	Gold	462*	3.49×10^4	Yellow	462*	4.00×10^4
IV	Yellow	457	1.51×10^4	Orange	475	2.43×10^4	Yellow	442	3.58×10^4
V	Orange	476	1.70×10^4	Orange red	495	2.67×10^4	Yellow	459	4.04×10^4
VI	Orange	478	1.80×10^4	Orange red	493	2.60×10^4	Gold	460	4.10×10^4
VII	Red	512	1.26×10^4	Magenta	527	1.86×10^4	Orange	495	2.73×10^4
VIII	Red	528	1.35×10^4	Magenta	541	2.18×10^4	Orange	508	3.40×10^4
IX	Orange	425*	1.11×10^4	Orange	525*	1.63×10^4	Orange	525*	2.10×10^4
X	Gold	462	1.65×10^4	Orange	480	2.61×10^4	Yellow	549*	3.98×10^4
XI	Gold	461	1.64×10^4	Orange	479	2.61×10^4	Yellow	447*	3.90×10^4
XII	Gold	462	1.41×10^4	Orange	479	2.53×10^4	Yello..	451	3.94×10^4
XIII	Yellow	425	1.36×10^4	Gold	452	2.58×10^4	Yellow	424	3.83×10^4
XIV	Gold	470	1.81×10^4	Orange	453	2.91×10^4	Gold	467	4.98×10^4
XV	Yellow	430	2.07×10^4	Yellow	448	3.42×10^4	Yellow	415	4.85×10^4
XVI	Yellow	423	6.6×10^3	Gold	452	2.37×10^4	Yellow	419	3.21×10^4
XVII	Yellow	435	1.03×10^4	Colourless	–	–	Colourless	–	–
XVIII	Yellow	451	1.99×10^4	Orange	462	2.98×10^4	Yellow	437	4.52×10^4
XIX	Gold	467	1.26×10^4	Orange	487	2.04×10^4	Yellow	459	3.02×10^4
XX	Orange	441	1.88×10^4	Orange	498	2.24×10^4	Gold	473	3.42×10^4
XXI	Yellow	438*	2.12×10^4	Gold	462*	2.40×10^4	Yellow	445	3.59×10^4
XXII	Yellow	484	1.52×10^4	Gold	452	2.60×10^4	Yellow	429	3.97×10^4
XXIII	Yellow	440	1.95×10^4	Gold	453	2.66×10^4	Yellow	423	3.94×10^4
XXIV	Yellow	442	1.75×10^4	Gold	454	2.47×10^4	Yellow	429	3.76×10^4

* Shoulder or at side of band just before reagent blank absorbance becomes appreciable.

zones[1–3] to develop methods for simultaneous determinations of trace amounts of iron, copper, cobalt and nickel.

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A SPECTROPHOTOMETRIC DETERMINATION OF EUROPIUM IN LANTHANIDE AND OTHER MIXTURES BY USE OF METHYLENE BLUE

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Summary—A spectrophotometric determination of 0.1–1 mg of europium in a lanthanide(III) mixture is described. Europium(III) is selectively reduced in a Jones reductor and the europium(II) allowed to react with a measured excess of Methylene Blue (MB) solution. The dye is reduced to the colourless leuco form according to the equation, MB + 2 Eu(II) → leuco MB + 2 Eu(III) and the excess of MB is determined spectrophotometrically at 664 nm. Optimum conditions are discussed and various applications presented.

Europium(III) is distinguishable, chemically, from other tervalent lanthanide ions by the ease with which it can be reduced to the bivalent state. McCoy[1] demonstrated that the reduction could be carried out selectively for 100–200 mg amounts of europium with a Jones reductor. Foster and Kremers[2] used this technique to reduce 15–60 mg of europium in lanthanide mixtures; the europium(II) solution was run into excess of iron(III) chloride solution and the iron(II) produced was determined by dichromate titration. Methylene Blue (C.I. 52015) readily undergoes reduction to a colourless leuco form, particularly in acid solution.[3] The reaction has been made the basis of titrimetric procedures[4] for titanium(III), tin(II) and molybdenum(III). In the contribution set out here, conditions are described whereby a few tenths of a mg of europium in admixture with yttrium and other lanthanides can be determined by selective reduction to europium(II) in a Jones reductor, followed by reaction with an excess of Methylene Blue, the excess being measured spectrophotometrically.

EXPERIMENTAL

Reagents

Methylene Blue was freed from trimethylthionine by the method of Bergmann and O'Konski.[5] About 0.3 g of the dye in 300 ml of 0.15*M* ammonia solution was extracted repeatedly, with fresh portions of benzene, until no further pink colour was extracted. Then the pH of the aqueous phase was reduced to about 8 and the solution purged of oxygen with a stream of nitrogen, and stored in a polyethylene container. The spectral purity of the Methylene Blue was checked by the method of Bergmann and O'Konski[5] and the concentration determined as described by Ferrey.[6] A portion of this solution was diluted further with water to provide the stock solution for use in europium determination. Five ml of this stock solution, on dilution with water or dilute hydrochloric acid to 250 ml, should give an absorbance of 0.8 at 664 nm with water as reference when measured in cells of optical path-length 10 mm.

Standard europium solution. Europium(III) oxide, (99.9% Eu_2O_3, Rare Earth Products Ltd., Widnes, England) was freshly ignited and 0.232 g of it dissolved in the minimum of concentrated hydrochloric acid and diluted to 100 ml in a standard flask. Dilutions with dilute hydrochloric acid were made as required from this 2.00 mg/ml europium stock solution.

Unless otherwise stated, other chemical substances used were of analytical reagent grade.

A Jones reductor containing 300 g of 20–30 mesh zinc shot was set up according to the procedure given by Belcher and Nutten[7] except that it was conditioned with hydrochloric acid. Absorbances were measured in optical cells of path-length 10 mm, with a Hitachi Perkin–Elmer 139 spectrophotometer.

Procedure

The sample containing between 0.1 and 1 mg of europium in dilute hydrochloric acid is passed through the Jones reductor into a 250-ml standard flask containing 5 ml of Methylene Blue solution, and the reductor washed with about 50 ml of 0.25*M* hydrochloric acid at a flow-rate of 5 ml/min. During the reduction and washing the receiving flask and contents are purged of oxygen with a stream of nitrogen or carbon dioxide. Finally the volume in the flask is made up to 250 ml with water previously purged of oxygen, and the absorbance measured against water at 664 nm. A calibration curve is prepared with the standard europium solutions.

Oxide samples are dissolved directly in hydrochloric acid. Fluorides of lithium, calcium and lanthanum, the last two in as finely divided a form as possible, are dissolved by heating with a 4:1 v/v mixture of concentrated nitric acid and saturated boric acid solution. The europium is co-precipitated with about 200 mg of lanthanum or yttrium by addition of aqueous ammonia. The hydroxide mixture is separated by centrifugation, washed with water, dissolved in hydrochloric acid and applied to the reductor column.

RESULTS AND DISCUSSION

Since the leuco Methylene Blue has no absorbance at 664 nm, if the partially reduced dye solution is measured against water, the calibration curve will have absorbances which decrease with increasing amounts of europium. Subtraction of the absorbance of such solutions from that obtained for the Methylene Blue in the absence of europium will lead to a

calibration curve passing through the origin. Either form of calibration curve, the second also obtainable by direct comparison of the absorbances of the two dye solutions in the spectrophotometer, is linear within experimental error for the specified europium range. Seven measurements on 0.50-mg amounts of europium gave a coefficient of variation of 7% for the measured absorbances (mean values, 0.42 and 0.38 for the first and second type of calibration curve, respectively). This represents a considerably larger uncertainty than that usually obtainable by spectrophotometry. It has its origin in the europium(III) reduction and subsequent reactivity of the europium(II); oxygen from the air and in solution is considered to be largely responsible.

The determination of the Methylene Blue in the original concentrated solution by Ferrey's method[6] enabled the molar absorptivity to be calculated; a value of 8.8×10^4 $l.mole^{-1}.cm^{-1}$ was obtained, in good agreement with the more reliable recorded values.[5] It also made possible confirmation that the dye (MB) was reduced according to the equation

$$MB + 2Eu(II) \rightarrow \text{leuco MB} + 2Eu(III) \qquad (1)$$

within the limits of experimental error. Once formed, the partially reduced dye mixture did not change its absorbance for at least 45 min, provided the solution was previously purged of oxygen and kept in a stoppered container. Methylene Blue dimerizes in solution with a shift in absorption maximum. From the dimerization constant[5] it can be shown that to keep errors from this source acceptable, the measured absorbance at 664 nm should not exceed 0.8. With this restriction the range of europium concentrations which can be determined depends on the degree of dilution of the partially reduced dye solution. However, to retain a satisfactory sensitivity to change in europium content, the range is limited and that given in the procedure is considered to be about optimum.

The method described is subject to interference from any substance which reduces the dye or inhibits the reduction of europium(II). It is intended primarily for application to lanthanide mixtures which if necessary have been obtained by a group separation to remove species which would otherwise interfere with the function of the dye. Lanthanide(III) ions do not interfere in the determination. However, bivalent ytterbium, samarium or neodymium would interfere. Up to 200 mg of each of these three elements in the tervalent state was subjected to the procedure. Only ytterbium at the 200-mg level showed any sign of reducing the Methylene Blue; the reduction corresponded to 0.05 mg of europium and could have been due, at least in part, to europium in the ytterbium sample. Thus large excesses of lanthanides, other than perhaps ytterbium, can be tolerated.

The method has been used to determine europium in a rare-earth europium concentrate (oxide mixture from monazite) obtained from Rare Earth Products Ltd., in gadolinium, terbium and samarium oxides, and in europium-doped crystals of anhydrous lithium, calcium and lanthanum fluorides. Typical results are recorded in Table 1. The europium content of the oxide concentrate was checked by the method of Foster and Kremers[2] and of the oxides of gadolinium and terbium by a fluorimetric method.[8] The fluoride crystals were prepared in the laboratory and the europium content was assumed to be known from the mix and from determinations with known amounts of each constituent in a powder mixture. The samarium oxide was only checked by addition of known amounts of europium to it and measurement of the recovery, but there is no reason to question the result within the limits of error of the method. To conclude, the Jones reductor gives selective and quantitative reduction of a few tenths of a mg of europium in lanthanide(III) mixtures and the resulting europium(II) can be determined indirectly by partial reduction of a known amount of Methylene Blue. The method is reasonably convenient; an analysis takes 15 min from introduction of the sample into the reductor. However, though adequate for many purposes, the precision leaves something to be desired.

Table 1. Some applications of the recommended method to the determination of europium

Substance	Approx. sample size	Europium Found	Europium Expected
Oxide concentrate	10 mg	3.5%	3.61%
Gd_2O_3	1 g	210 ppm	225 ppm
Tb_4O_7	1 g	50 ppm	60 ppm
Sm_2O_3	1 g	240 ppm	—
Li(Eu)F	0.1 g	5.3 mg/g	5.0 mg/g
$Ca(Eu)F_2$	0.2 g	2.4 mg/g	2.5 mg/g
$La(Eu)F_3$	0.3 g	1.2_7 mg/g	1.3_3 mg/g

Gd, Tb and Sm oxides (99.9% purity) from Koch-Light Ltd., Colnbrook, England.

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TITRATION OF PHOSPHONIC ACID DERIVATIVES IN MIXTURES

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Summary—An analytical procedure is described for the determination of the weak acids phosphonomethyliminodiacetic acid and phosphonomethyliminoacetic acid in their mixtures, and the dissociation constants of phosphonomethyliminoacetic acid are reported.

Tetrabutylammonium hydroxide is sufficiently basic in pyridine medium to distinguish successive neutralization steps of polybasic acids. It can be used for the determination of a wide variety of acidic compounds, including weak acids and mixtures of weak acids.

This investigation was intended to solve an industrial problem, determination of the weak acids phosphonomethyliminoacetic acid and phosphonomethyliminodiacetic acid in mixtures of the two. The protonation constants of phosphonomethyliminoacetic acid were also determined.

EXPERIMENTAL

All reagents were of analytical reagent grade purity.

Tetrabutylammonium iodide (TBAI) solution (0.01*M*) in methanol was prepared. Tetrabutylammonium hydroxide (TBAH) solution (0.05*M*) in benzene–methanol (1:1 v/v) was prepared as described by Cundiff and Markunas.[1] A Radelkis OP-204/I universal pH-meter was used, with the cell:

Ag/AgCl	buffer KCl solution	glass membrane	solution investigated	0.01*M* TBAI in methanol	Hg_2Cl_2/Hg
	glass electrode			calomel electrode	

All titrations were done with the same glass–calomel electrode pair. The acids were made up to the same concentration (0.5*M* in water) and a 0.1-ml sample in 50 ml of pyridine was titrated with the TBAH.

Procedure for mixtures

A 10–15 mg sample of the acid mixture is dissolved in not more than 1 ml of water and 50 ml of pyridine and titrated potentiometrically with 0.05*M* TBAH, with use of the glass and modified calomel electrode system described above.

Calculation

Phosphonomethyliminodiacetic acid

$$= \frac{2(v_1 + v_2 - v_3)f}{w} \text{ meq/g}$$

$$\text{Phosphonomethyliminoacetic acid} = \frac{3(v_3 - v_2)f}{w} \text{ meq/g}$$

where v_1 = ml of TBAH required to reach the first inflection, v_2 = ml of TBAH to reach the second, v_3 = ml of TBAH to reach the third, f is the molarity of the TBAH, and w is the sample weight (g).

RESULTS AND DISCUSSION

In Fig. 1, curve 1 shows the titration of phosphonomethyliminodiacetic acid, with two inflections. This acid is tetrabasic ($pK_1 = 2.0$, $pK_2 = 2.25$, $pK_3 = 5.57$, $pK_4 = 10.76$; 0.1*M* potassium chloride, 20°).[2] In pyridine as titration medium, the first two neutralization steps give effectively only a single inflection on the titration curve, and so do the third and fourth. Three inflections are obtained in the titration of phosphonomethyliminoacetic acid, however (curve 2). According to Sprankle *et al.*,[3] this acid is tribasic ($pK_1 = 2.6$, $pK_2 = 5.6$, $pK_3 = 10.6$, aqueous medium; zwitterion equilibrium constant $= 10^{-2}$). Our values (obtained by potentiometric titration and direct algebraic calculation[4]) were $pK_1 = 1.60$, $pK_2 = 5.31$, $pK_3 = 10.15$ (0.1*M* potassium chloride medium, 25°). Hence the titration curve for an equimolar mixture of the two acids gives three inflections, as shown in curve 3.

Both the first and second inflections represent the end-points for two equivalents of phosphonomethyliminodiacetic acid and one equivalent of phosphonomethyliminoacetic acid. The third inflection corre-

Table 1. Potentiometric titration of weak acids

Taken, *mg*		Found, *mg*	
PDA	PA	PDA	PA
1.04	8.15	1.01	8.19
5.20	8.15	5.23	8.17
10.35	8.15	10.40	8.13
10.35	4.07	10.38	4.10
10.35	0.82	10.36	0.86

PDA: phosphonomethyliminodiacetic acid.
PA: phosphonomethyliminoacetic acid.

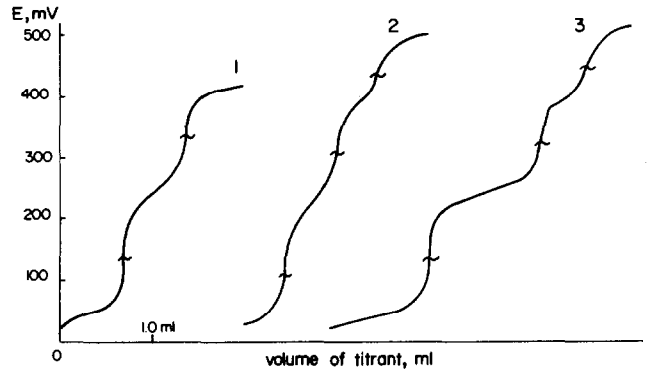

Fig. 1. Titrations curves for: 1—phosphonomethyliminodiacetic acid; 2—phosphonomethyliminoacetic acid; 3—an equimolar mixture of the two.

sponds to the third equivalent of phosphonomethyliminoacetic acid. Whatever the ratio of the two acids in the mixture, there are three sharp inflections. For reasons we cannot explain, in pyridine medium pK_4 for the iminodiacetic acid evidently becomes much lower, whereas pK_3 for the iminomonoacetic acid is relatively unaffected.

Table 1 gives the results obtained for various mixtures of the two acids. Amounts as small as 1.0 mg can be determined with an error of 4–5%. Amounts greater than 3 mg can be determined with an error and precision of 0.5% or better.

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ARSENAZO III AS A SPECTROPHOTOMETRIC REAGENT FOR DETERMINATION OF LEAD

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Summary—Arsenazo III is proposed as a spectrophotometric reagent for the determination of lead. The complex formation begins at pH > 2 and is greatest at pH 4–6. The molar absorptivity of the complex has a mean value of 2.8×10^4 $l.mole^{-1}.cm^{-1}$ at 600 nm and remains nearly constant in the pH range 4–8. The ionic species taking part in the reaction are studied and the equilibrium constants for the different possible reactions are calculated. According to the values obtained, the reaction of $PbOH^+$ with H_5L^{3-} is predominant. The reaction studied is applied for the determination of micro amounts of lead in technical aluminium.

Little information is available on the reaction of Arsenazo III [1,8-dihydroxynaphthalene-3,6-disulphonic acid-2,7 bis(azo-2)-phenylarsonic acid] with lead(II). It has been shown that a blue 1:1 ratio complex is formed in the pH range 4–5 and the sensitivity of the reaction is relatively low ($\epsilon = 1 \times 10^4$ $l.mole^{-1}.cm^{-1}$).[1–5] For that reason a more detailed study of the reaction is of interest, especially with respect to its analytical use. In the present paper a systematic spectrophotometric investigation is made of the complexation of Arsenazo III with lead. The data obtained characterize Arsenazo III as a suitable spectrophotometric reagent for the determination of micro amounts of lead.

EXPERIMENTAL

Reagents

Arsenazo III solution. An aqueous $10^{-4}M$ solution of the reagent was standardized by spectrophotometric titration with thorium nitrate solution at 600 nm and pH 3.

Lead(II) and other metal ion solutions. Solutions, $10^{-2}M$, were prepared from reagent grade substances and standardized complexometrically.[6]

Buffer solutions. Standard potassium hydrogen, phthalate buffer solutions were prepared.[7]

Hexamethyleneammonium hexamethylenedithiocarbamate.[8] A 0.1% solution in chloroform.

Chloroform. Reagent grade, dried and distilled.

Hydroxylamine hydrochloride. An aqueous 30% solution was prepared from reagent grade chemical.

The other solutions were prepared from reagent grade substances.

RESULTS AND DISCUSSION

Spectrophotometric characteristics of the Arsenazo III–Pb(II) complex

The complexation of Arsenazo III with Pb(II) was studied at various pH values and constant reagent concentration (Fig. 1). In acidic medium the complex formation takes place at pH > 2, with maximum absorbance at pH ~ 4. The shape of the spectra remains unchanged up to pH 7, and changes in a slightly alkaline medium, probably because of the appearance of new ionic forms of the metal ion and the reagent. The complex is blue, with two absorption bands, at 605 and 665 nm. The absorption spectrum of Arsenazo III (λ_{max} = 540 nm, Fig. 1, curve 10) is considerably changed as a result of the complex formation.

The composition of the complex was checked by the molar-ratio, Asmus, and Bent and French methods.[9] The results confirmed the 1:1 composition of the complex, in agreement with Savvin.[1,4,5]

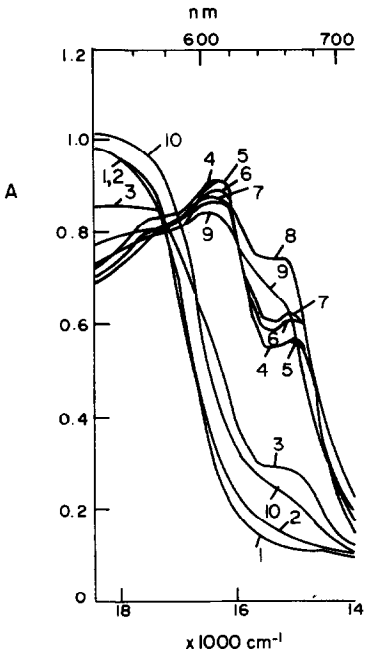

Fig. 1. Absorbance spectra of the Arsenazo III–lead(II) complex at various acidities. $C_M = C_R = 2.04 \times 10^{-5}M$; 2-cm cells *vs.* water; pH (1) 0.83; (2) 1.78; (3) 2.43; (4) 3.30; (5) 4.60; (6) 5.37; (7) 6.75; (8) 8.02; (9) 9.40; (10) spectrum of Arsenazo III at pH 5.35.

Table 1. Molar absorptivity ϵ (10^4 l.mole^{-1}.cm^{-1}) of the lead(II)–Arsenazo III complex at various acidities

pH	3.2	4.1	5.1	6.1	8.0
ϵ	2.30 ± 0.08	2.60 ± 0.08	2.66 ± 0.06	2.88 ± 0.05	2.85 ± 0.05

The molar absorptivity of the complex at $\lambda = 600$ nm was determined in the presence of at least a three-fold excess of Arsenazo III and at various acidities—Table 1.

It is seen from the table that the sensitivity of the reaction remains practically constant over a wide pH range, thus favouring its analytical use. Beer's law is obeyed up to 4.2 ppm. The blue colour of the solutions develops immediately and is stable for more than 24 hr.

Spectrophotometric study of Arsenazo III–lead(II) reaction

The nature of the ionic species taking part in the reaction[10,11] was studied by means of the method based on the relation $A = f(\text{pH})$ for solutions with different reagent concentrations (Fig. 2). The S-shaped curves obtained in this study confirmed the conclusion that the complexation takes place in the pH range 2–4.

The straight part of the S-shaped curves, including the pH range 2.20–3.50, was used for the calculations. In this pH range Arsenazo III exists as the ionic forms H_6L^{2-}, H_5L^{3-}, H_4L^{4-},[12] which react with the different hydroxo-complexes of Pb(II) according to the equation:

$$M(OH)_i^{(z-i)+} + H_mL^{q-} \rightleftharpoons M(OH)_iH_{m-n}L^{(z-i)-(q+n)} + nH^+ \quad (1)$$

where z stands for the charge on the metal ion, i the number of OH^- groups bonded to the metal ion, m the basicity of the anionic form of the reagent with charge q, and n the number of protons split off during the reaction.

The equilibrium constants for these reactions were calculated as described in our previous papers.[12,13] The following equation was used:

$$\log B = \log KC + n\text{pH} \quad (2)$$

where B is a quantity which measures the spectrophotometric effect of competing side-reactions of M and L, K is the equilibrium constant of complex formation and C is the total concentration of the reagent.

$$B = \frac{(A - A_R)(A_0 - A_R)DF}{(A_0 - A)^2}$$

where A is the absorbance of the solution at a given pH, A_R is that of the reagent alone and A_0 is the maximum absorbance, D is a side-reaction coefficient for the protonation of reagent and F a similar coefficient for competitive complexation of the metal ion.[12]

Equation (2) was solved for each ionic form of the reagent with all possible forms of the metal ion—generally 36 equations were solved. The values of the stepwise stability constants of the lead(II) hydroxo-complexes, obtained through interpolation of the data,[14,15] were used in the calculations. All data obtained are summarized in Table 2.

The equilibrium constant K was calculated for the reactions with a whole-number value for the slope of the straight line dependence of log B on pH (Table 3).

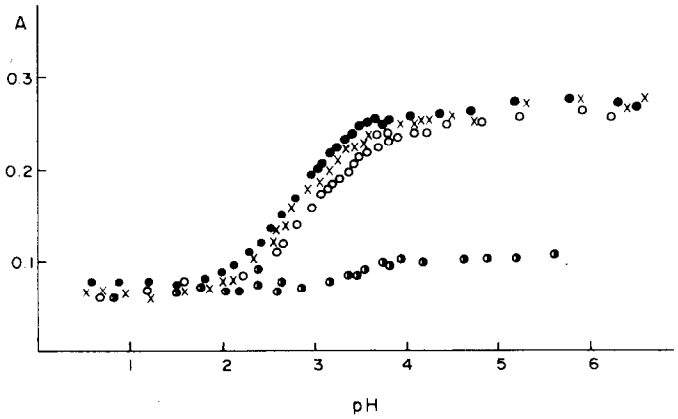

Fig. 2. pH–absorbance plots of Arsenazo III and its complex with lead(III). $C_R = 1.22 \times 10^{-5}M$; ionic strength $0.1M$; $\lambda = 600$ nm; 1-cm cells *vs.* water, pH adjusted with HCl and NaOH. ●—$C_M = 2.45 \times 10^{-5}M$; ×—$C_M = 1.84 \times 10^{-5}M$; ○—$C_M = C_R$; ⊙—Arsenazo III.

Table 2. Slopes (n) for the reactions of Arsenazo III with Pb(II) according to equation (2); $C_R = 1.22 \times 10^{-5} M$

Reacting forms of the reagent	H_6L^{2-}				H_5L^{3-}				H_4L^{4-}			
Reacting forms of the metal ion	Pb^{2+}	$PbOH^+$	$Pb(OH)_2$	$Pb(OH)_3^-$	Pb^{2+}	$PbOH^+$	$Pb(OH)_2$	$Pb(OH)_3^-$	Pb^{2+}	$PbOH^+$	$Pb(OH)_2$	$Pb(OH)_3^-$
$C_M:C_R$	Slope n											
1	2.9	1.9	0.9	0	2.0	1.0	0	−1.0	1.0	0	−1.0	−2.0
1.5	—	—	—	—	—	—	—	—	—	—	—	—
2	3.1	1.8	0.9	0	1.9	0.9	0	−1.1	1.0	0	−1.1	−2.1
	2.7	1.8	0.9	—	1.8	0.8	—	−1.2	0.9	—	−1.2	−2.1

Table 3. Equilibrium constants of the reactions (log K) $\mu = 0.1$

Reacting forms of the reagent	H_6L^{2-}	H_5L^{3-}		H_4L^{4-}
Reacting forms of the metal ion	$PbOH^+$	Pb^{2+}	$PbOH^+$	Pb^{2+}
$C_M:C_R$	log K			
1	5.94 ± 0.03	0.74 ± 0.03	7.23 ± 0.03	3.83 ± 0.04
1.5	6.14 ± 0.06	0.94 ± 0.06	7.43 ± 0.05	3.73 ± 0.06
2	6.13 ± 0.04	0.96 ± 0.04	7.76 ± 0.04	4.03 ± 0.06

Table 4. Interference of some ions in the presence of 54 μg of Pb (λ = 600 nm)

Ion	Amount taken, μg* pH 3	pH 6	Ion	Amount taken, μg* pH 3	pH 6
Ag(I)	650	110	Mn(II)	55	39
Bi(III)	0.2	63	Ni(II)	3×10^3	6
Cd(II)	340	56	Hg(II)	1.2×10^3	200
Cu(II)	6	6	Sn(IV)	1	300
Fe(II)	6	0.6	Ti(IV)	15	15
Fe(III)	0.6	0.6	Zn(II)	32×10^3	65

* The maximum change of the absorbance is ±0.010 in the presence of the quantities of interfering ion shown in the table.

Table 5. Determination of lead in technical aluminium

Lead found, $\bar{x}$, %	N	$\pm S$, %	$\bar{x} \pm \Delta\bar{x}$	$\pm \frac{\Delta\bar{x}}{\bar{x}} \cdot 100\%$
0.008	19	0.002	0.008 ± 0.001	12.5

The values of K thus obtained show that the main reaction is:

$$PbOH^+ + H_5L^{3-} \rightleftharpoons Pb(OH)H_4L^{3-} + H^+ \quad (3)$$

The complex formation in alkaline medium was also studied, but the straight-line portion of the S-shaped curve was very short and should not be used for calculation.

Analytical application of the reaction of Arsenazo III with Pb(II)

The selectivity of the reaction was investigated at different acidities and the data obtained are presented in Table 4.

The results in Table 4 show that the reaction is more selective in more alkaline medium, in fair agreement with previous findings.[13]

Determination of small amounts of lead in technical aluminium used in the food industry

The reaction of lead(II) with Arsenazo III was applied to analysis of technical aluminium intended for production of cooking vessels, which should not contain more than 0.005–0.02% lead. It was established by atomic spectral analysis that the aluminium contained Si 0.18%; Fe 0.32%; Ti 0.019%; Mg 0.016%; Mn 0.02%; Cu 0.005%.

The main problem was the separation of lead from the matrix. The lead was extracted with hexamethyleneammonium hexamethylenedithiocarbamate solution (HMDTC) in chloroform, stripped with 6*M* hydrochloric acid and determined spectrophotometrically.

Procedure

The sample of about 0.5 g of technical aluminium was dissolved in hydrochloric acid (1 + 1) by heating. The solution was boiled for 5 min in the presence of 4 ml of 30% hydroxylamine hydrochloride solution and cooled. After adjustment of the pH to about 0.3 (equivalent to 0.5*M* hydrochloric acid) with sodium hydroxide, lead was extracted by shaking for 2 min with each of four 10-ml portions of 0.1% HMDTC solution in chloroform. The water layer was washed several times with about 1 ml of chloroform. Under these conditions only Cu(II) passes into the organic phase with lead.

From the organic phase Pb(II) was stripped by shaking for 2 min with each of four 10-ml portions of 6*M* hydrochloric acid. Under these conditions Cu(II) remains in the organic phase. The solution was evaporated to dryness. The residue was dissolved in water with heating and placed in 25-ml volumetric flask. Two ml of 0.05% solution of Arsenazo III and 5 ml of phthalate buffer were added, and the absorbance at 600 nm was measured.

The lead content was calculated from a calibration curve plotted for the range 1–5 ml of $1.02 \times 10^{-4}M$ Pb(II). The results obtained are shown in Table 5.

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NEW TURBIDIMETRIC METHOD FOR DETERMINATION OF SULPHATE

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Summary—Sulphate in the range (1–5) × $10^{-5}M$ is determined by addition of 30–40 mesh lead nitrate crystals (0.2 g) to 25 ml of sample solution containing 40–80% ethanol.

Sulphate has been determined by precipitation with organic reagents,[1–6] and by competitive precipitation,[7–9] dye-release,[10–18] turbidimetry[19] and nephelometry.[20–22] The turbidimetric and nephelometric methods are more selective and accurate than the best of the other methods.[19] Nevertheless their applicability is limited, mainly because of the need to stabilize particle growth and obtain a suspension free from impurities.[23,24]

In this communication a new, simple and accurate method is based on the absorbance of a lead sulphate suspension in aqueous ethanol. The method is more sensitive than the barium sulphate method, and more suitable for routine control analysis, as the lead sulphate sols are stable for at least an hour.

EXPERIMENTAL

Reagents

*Standard sulphate solution, 0.005*M. Dissolve exactly 0.3551 g of anhydrous sodium sulphate in distilled water and dilute to 500 ml.

Ethanol, 96%v/v. In the procedure and discussion the volume fraction of ethanol refers to the volume of 96% ethanol added, *not* to volume of absolute (100%) ethanol.

Procedure

Transfer the sample (containing about 0.2 mg of sulphate) into a 100-ml standard flask, dissolve it in 5–10 ml of distilled water, add 50 ml of 96% ethanol and dilute to volume. (The volume of the ethanol may be varied from 40 to 80 ml according to the other components present, but the same concentration must be used for samples and the accompanying standards.) Transfer 25.0-ml aliquots into thoroughly washed and dried 50-ml beakers. Place on a magnetic stirrer (600–800 rpm), add 0.2 g of lead nitrate crystals (30–40 mesh) and stir for 5 min. Determine the absorbance of the suspension at 400 nm, against water as a blank, in 5-cm cells. Convert the absorbance into sulphate contents from the calibration graph.

Calibration graph. Prepare two solutions of 100-ml volume containing 50 ml of 96% ethanol, one with no sulphate (A), and the other with 1 ml of the sodium sulphate stock solution (B). Mix 25, 20, 15, 10, 5 and 0 ml of A with 0, 5, 10, 15, 20 and 25 ml respectively of B. Treat according to the procedure. Draw the calibration curve or find its equation by least-squares.

Effect of reagent concentrations on the lead sulphate turbidity

The stability of colloidal sols of lead sulphate under different experimental conditions was first thoroughly investigated. Crystals of lead nitrate were preferred as precipitant, because they could be added conveniently from a small scoop. This also eliminated the obvious disadvantage of using a reagent solution, which would further dilute the already dilute sulphate solution. The reproducibility also became poorer when solutions of lead nitrate were used. The lead nitrate crystals were dissolved completely within 5 min with the aid of a motor-driven stirrer, giving a concentration of 0.024M. Ten minutes are sufficient for full development of the turbidity, after which the absorbance remains constant for at least an hour. Exact duplication of the rate and manner of mixing, and of standing time, are essential.

Experiments at a fixed sulphate concentration showed that the absorbance was not affected by the ethanol concentration in the range 40–80% v/v, and confirmed that the ethanol and excess of lead nitrate decrease the solubility of the lead sulphate. Use of 50% v/v ethanol still stabilizes the suspension but also permits a reasonably high concentration of inorganic salts. The ethanol concentration can be varied to suit the application (*e.g.*, 75% v/v was used for analysis of ammonium nitrate for trace sulphate).

RESULTS AND DISCUSSION

The procedure can be recommended when sulphate is to be determined in samples not containing elements that form salts sparingly soluble in aqueous ethanol. The method is more sensitive than the other methods and may be used for determination of sulphate impurity in certain reagent-grade chemicals.

The calibration curve, accuracy, precision and sensitivity were thoroughly examined. The absorbance obeys Beer's law in the range (1–5) × $10^{-5}M$ sulphate. From the absorbance A of three parallel determinations at each of five levels of sulphate concentration ($C_{SO_4^{2-}}$,M) the equation of the calibration curve was found to be $A = (0.04 \pm 0.02) + (8.6 \pm 0.8) \times 10^3\ C_{SO_4^{2-}}$; ($P = 95\%$, $f = 13$). The relation was proved linear, (correlation coefficient 0.99) and an F-test [$F = 10.35 > F(95\%, f_1 = 1, f_2 = 13) = 4.67$] showed that the intercept is not zero.[25]

The method was applied to determination of sulphate in ammonium nitrate, in presence of 75% v/v ethanol to decrease the solubility of the lead sulphate. The accuracy and the precision were studied with synthetic samples of sulphate-free ammonium nitrate doped with known amounts of sulphate covering the

Table 1. The test for systematic errors*

SO_4^{2-} taken, $10^{-5}M$	SO_4^{2-} found, $10^{-5}M$	Relative error %
1.0	0.9	−5
2.0	2.0	0
2.5	2.3	−10
3.0	3.0	0
4.0	4.3	+6
5.0	5.1	+1

* Standard deviation $0.28 \times 10^{-5}M$; three individual measurements on separately prepared solutions.

applicable range. The results are given in Table 1. A least-squares analysis[25] showed a linear relation between amount found and amount added, passing through the origin [$F = 0.76$; $F(95\%, f_1 = 1, f_2 = 16) = 2.11$] with a slope equal to unity [$t = 0.73$; $t(95\%, 17) = 2.11$]. This statistical analysis showed the procedure to be free from constant bias and from error increasing with the amount of sulphate.

The method was also applied to a series of ammonium nitrate samples and the results were compared with those of the barium sulphate method (Table 2). A comparison of the standard deviations showed that the new method has superior precision [$F = 4.00 > F(95\%, f_1 = 8, f_2 = 7) = 3.73$]. There was no evidence for bias, since the value of the Darmoi-criterion set equal to 0.1 did not exceed the tabulated value, $R = 3.01$ ($95\%, f_1 = 8, f_2 = 7$).[26]

The results in Table 1 also give a view of the errors of the procedure. They show that the relative error (1–10%) and the precision ($S = 0.28 \times 10^{-5}M$) are satisfactory.[27] It is worth noting that the B.S. method[28] requires the barium sulphate turbidity to be measured within 5 ± 0.5 min; to obtain reasonable results the sulphate concentration should be higher than $5 \times 10^{-5}M$. Under these conditions a relative error of ±10% can be attained. In the Toennis and Bakay method,[21] the stability and reproducibility of the barium sulphate suspensions used were found to be poor at a sulphate level of $(0.7–0.9) \times 10^{-5}M$, which is why a standard addition method was recommended. The ethanol concentration is also limited to a maximum of 30%. According to Coleman *et al.*[24] a relative error of 2% and relative standard deviation of 3.5% for $5 \times 10^{-5}M$ sulphate may be achieved if the mixing of the solutions, the stirring and the measurement of the absorbance at intervals of 10, 20 and 30 sec are performed automatically.

The lower limit of our method is $(5–6) \times 10^{-6}M$ sulphate (0.16–0.19 ppm sulphur), calculated from the calibration curve[25] and from the standard deviation of the blank[28] (10 independent determinations).

Table 2. Comparison of the results obtained by the proposed method and by the $BaSO_4$ method for sulphate in NH_4NO_3

	Proposed method		$BaSO_4$ method	
	A	SO_4^{2-}, %	*A*	SO_4^{2-}, %
	0.155	0.0026	0.165	0.0029
	0.140	0.0022	0.155	0.0028
	0.135	0.0021	0.195	0.0034
	0.130	0.0020	0.180	0.0032
	0.175	0.0030	0.150	0.0026
	0.160	0.0027	0.140	0.0025
	0.165	0.0028	0.110	0.0019
	0.140	0.0022	0.120	0.0039
$\bar{x}$		0.0024		0.0029
s		0.00035		0.00070
$100s/\bar{x}$, %		14.6		24.1

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ROUTINE ACCURATE DETERMINATION OF SILICA IN SILICATE MATERIALS BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY AND SUBSEQUENT COMPUTATION

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Summary—An accurate method for routine determination of silica in silicate materials is proposed. It is based on the decomposition of the silicate materials with $LiCO_3/H_3BO_3$ flux, stabilization of the silica with fluoride, and the use of a suitable calibration, measurement and computation pattern.

In a previous paper,[1] we reported a scheme for the decomposition of silicate materials and determination of major elements by atomic-absorption spectrophotometry (AAS). Silica was determined in solutions stabilized by adding fluoride. When a large amount of the solution is aspirated into the flame, some errors appear, mainly because of variation in sample flow, arising from partial obstruction of the capillary and from fluctuations in the redox properties of the flame. The analysis of deposits inside the capillary has shown that they consist essentially of lithium fluoride and potassium fluoroborate. This has been partially overcome by using a sodium buffer instead of a potassium buffer, because sodium fluoroborate is more soluble. However, formation of LiF is still a source of error in routine work. This and other remaining sources of error may be satisfactorily minimized by using a suitable calibration and measurement pattern, as described below.

EXPERIMENTAL

Apparatus

A double-beam Pye-Unicam SP-1900 atomic-absorption spectrophotometer and an EEL lamp were used. The instrument parameters used were: wavelength 251.6 nm; lamp current, 12 mA; slit-width 0.15 mm; N_2O and acetylene flows 5 and 4.7 l./min respectively; integration time 4 sec. An Ataio-Compucorp Alpha-327 desk calculator was used for programming and computation.

Reagents

The chemicals and solutions used were those specified previously[1] except that the potassium buffer was replaced by a sodium buffer prepared in the same way.

Standard rock samples

The following international standard rock samples were employed: G-2, GSP-1, AGV-1 and BCR-1 from U.S. Geological Survey; GH, GA and DRN from C.R.P.G. (Nancy, France); 269, 375 and 376 from B.C.S. The compositional values used are those referred to by Abbey.[2]

Preparation of the sample solutions

The method proposed in our previous paper[1] was followed, except that the buffer was changed, as already mentioned.

Procedure

Measurement sequence. Standards and samples are measured in the following sequence: H_I–A–H_{II}–B–H_{III}–L–H_{IV}–––H_1–S_1–H_2–S_2–––H_n–S, where H and L are standards having silica contents higher and lower respectively than those expected for the samples (S); A and B are two additional standards with intermediate silica contents. The roman subscripts refer to the standard H introduced before and after any other standard, and the arabic subscript to the same standard introduced before and after samples. This sequence is used in order to refer all the signals to a single standard which is checked during the whole sequence.

Correction of signals. All measurements X are corrected and referred to the H_I signal by use of the expression

$$X_{corr} = \frac{2X H_I}{H_{i-1} + H_i}$$

where X is the signal from any sample or standard; H_{i-1} and H_i are the signals from H obtained immediately before and after X.

Calibration and determination of silica. Calibration is done by fitting the signals for the four standards, by the least-squares method, to the function $y = a_0 + a_1x + a_2x^2$, where y is the percentage of silica in the standard solid sample and x is the corresponding corrected reading. The concentration of silica in a sample is obtained by solving the equation for the corrected signals.

An analytical program for the calculations is easily devised.

RESULTS AND DISCUSSION

At least four standards must be used to obtain the calibration graph. The H and L standards should have silica contents higher and lower than those expected for the samples; the other two, A and B, have a content intermediate between H and L. All calculations involved may easily be carried out with

Table 1. Determination of SiO_2 by AAS in silicate samples

	G-2	GSP-1	BCR-1	GA	376	269§	DRN
Reference values, *%	69.19	67.3	54.85	69.96	67.1	56.7	52.88
Values obtained,† %	69.2	67.2	55.2	69.9	67.0	56.5	53.0
Absolute error, %	0.0	0.1	0.3	0.1	0.1	0.2	0.1

* From Abbey.[2]
† Mean from five determinations.
§ From BCS specification.

a programmable desk-calculator. The silica content in the sample may be obtained with ease by use of the flow-chart.

Determination of silica in standard rocks

The silica content of seven standard rock samples was determined. The four standards used for calibration had the silica contents percentages shown in brackets: GH (75.85), BCS-376 (67.1), AGV-1 (59-72) and PCC-1 (42.15). The instrument was adjusted so that the standard with the highest silica content (GH) gave 1.000 absorbance units in the read-out. The least-squares equation for these four standards was $y = -0.1680 + 0.0603x + 0.00001\ x^2$ with a standard error of 0.15% SiO_2. The minimum difference in SiO_2 content which may be discerned with the function is about 0.09%, so results should be reported to 0.1%. The results obtained are shown in Table 1.

The mean relative error from these seven determinations is 0.23%. The standard deviation of these errors is 0.17%. A possible realistic evaluation of the maximum error in these determinations may be expressed by the formula: maximum error = mean-error + 2*s*. This value is 0.57%. Thus, the result should be within $\pm 0.57\%$ of the true value.

The reproducibility was determined by applying the procedure to ten replicates of G-2 introduced at random in a series of 100 sample solutions. The mean was 69.2% of silica with a relative standard deviation of 0.3%.

The method offers no improvement in precision or accuracy over the manual method, but is time-saving.

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ANALYTICAL DATA

THE PROTONATION AND DIMERIZATION OF 2,4-, 2,5-, 2,6- AND 3,5-DIHYDROXYBENZOIC ACIDS IN 0.5*M* SODIUM PERCHLORATE MEDIUM

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Summary—The protonation equilibria of 2,4-, 2,5-, 2,6- and 3,5-dihydroxybenzoic acids were studied by means of potentiometric titrations at $I = 0.5$ ($NaClO_4$) and 25°. The dimeric species H_6L_2 and H_5L_2 were found to form as well as the monomeric species H_pL in the acidic solutions of 2,4- and 3,5-dihydroxybenzoic acids under the conditions studied. For the other two acids, the protonation scheme can be expressed exclusively in terms of the species H_pL ($p = 1$, 2 or 3).

As a consequence of intermolecular hydrogen bonding, carboxylic acids tend to dimerize in the pure form and in many non-polar organic solvents (*e.g.*, chloroform, carbon tetrachloride and benzene). The association of benzoic and salicylic acids in non-polar solvents has been studied by many investigators. These studies have shown that the dimer of benzoic acid is more stable than that of salicylic acid. In salicylic acid the intramolecular hydrogen bonding between the carboxyl group and the neighbouring hydroxyl group weakens the formation of intermolecular hydrogen bonds.[1–3]

Lee *et al.* have reported the dimerization of salicylic and 3-bromo-5-sulphosalicylic acids in aqueous solution [$I = 3$ ($NaClO_4$) and 25°].[4,5] According to their findings, these acids dimerize, but only slightly and in concentrated solutions.

Salicylic acid and its derivatives are classical reagents in inorganic analytical chemistry, but there are few papers on complex formation with the different hydroxy derivatives of salicylic acid.[6,7] In calculations of the stability constants of the metal complexes of these acids, all the protonation reactions must be known exactly. The aim of the present study is to determine the protonation constants of the four dihydroxy derivatives of benzoic acid, and in particular to find out whether dimeric species are formed in aqueous solutions of these acids.

EXPERIMENTAL

Reagents

All the acids studied were obtained from Fluka AG, and recrystallized from hot water before use.

Apparatus and methods

The apparatus used in the potentiometric titrations was the same as that reported earlier.[7,8] The details of the methods have also been described elsewhere.[7–9]

The concentration ranges of the acids were extended as high as possible in 0.5*M* sodium perchlorate medium. The total concentrations of the acids were varied from 0.001*M* to 0.033*M*, 0.036*M*, 0.055*M* and 0.10*M* for 2,4-, 2,5-, 2,6- and 3,5-dihydroxybenzoic acid, respectively.

Calculations

The constants were calculated as overall stability constants [$\beta_{pr} = K(pH + rL = H_pL_r)$] with the program SCOGS[10] on a Univac 1100/20 computer.

RESULTS

The experimental data obtained potentiometrically were displayed as a plot of $\bar{n}_H$ *vs.* $-\log [H^+]$ for each dihydroxybenzoic acid studied (Fig. 1). As can be seen, $\bar{n}_H$ seems to be a function of $-\log [H^+]$ for the whole pH and C_L region over which the measurements were carried out for 2,5- and 2,6-dihydroxybenzoic acids. In the case of 2,4- and 3,5-dihydroxybenzoic acids, $\bar{n}_H$ also seems to be a function of $-\log [H^+]$, but only in the alkaline pH region. In the acidic pH region, $\bar{n}_H$ is not only a function of $-\log [H^+]$ for these acids, but also depends on the total concentrations, C_L. With decreasing C_L, the $\bar{n}_H$ *vs.* $-\log [H^+]$ curves seem to approach a limiting curve for $C_L < 0.01M$ and $C_L < 0.002M$ for 2,4-dihydroxybenzoic acid and 3,5-dihydroxybenzoic acid, respectively. This indicates the formation of one or more polymeric species when C_L increases above 0.01 and 0.002*M*, respectively.

The computer analyses showed the formation of the two dimeric compounds, H_6L_2 and $H_5L_2^-$, as well as the monomeric H_pL species ($p = 1$, 2 or 3).

The best sets of the overall constants, β_{pr}, obtained with the program SCOGS are given in Table 1. The "best set" of the formation constants is defined as the set which gives the minimum value of the error

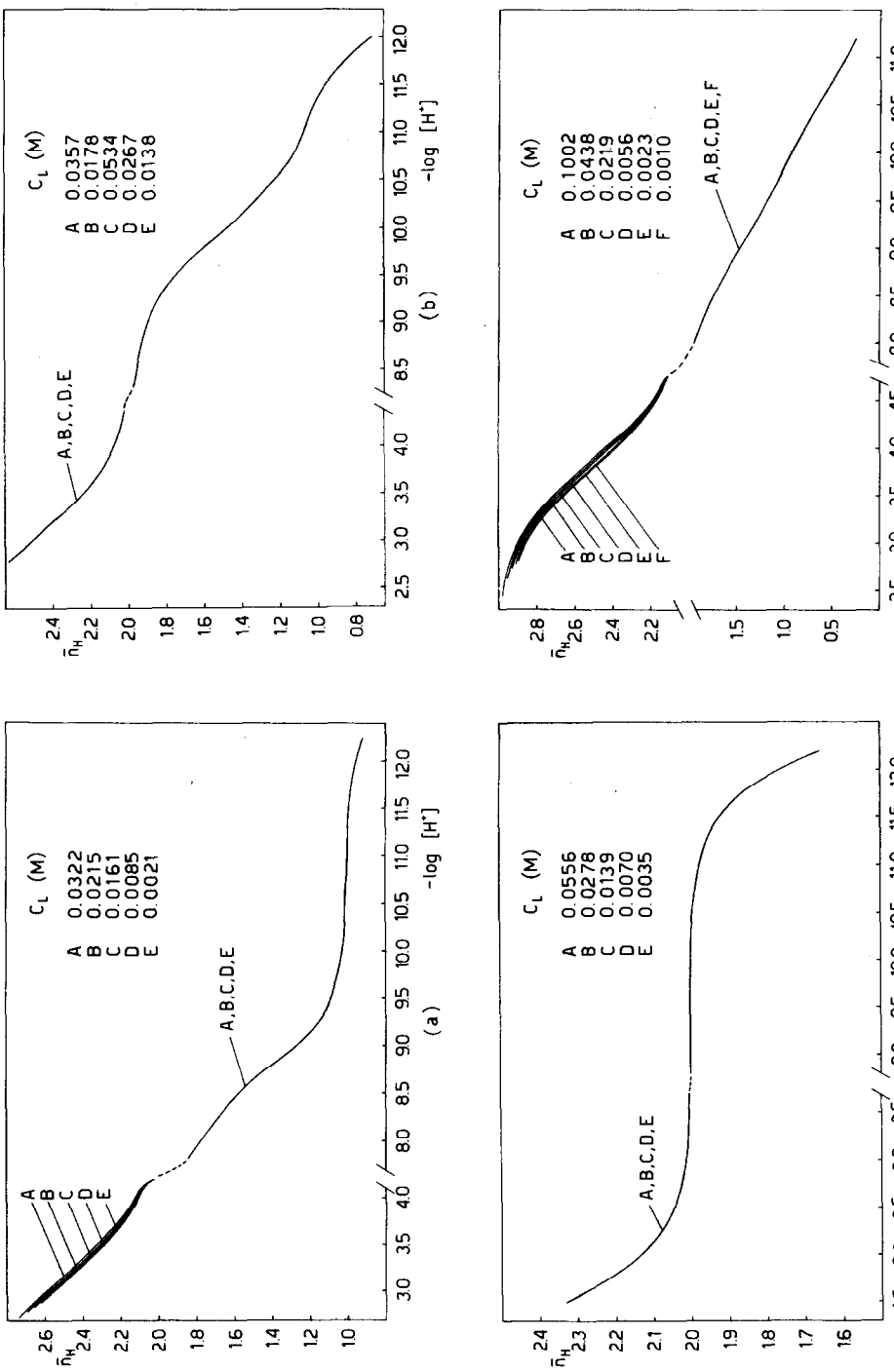

Fig. 1. Experimental $\bar{n}_H$ *vs.* $-\log [H^+]$ curves for a few titrations of 2,4-dihydroxybenzoic (a), 2,5-dihydroxybenzoic (b), 2,6-dihydroxybenzoic (c) and 3,5-dihydroxybenzoic (d) acid with NaOH solution. $\bar{n}_H(\text{exptl}) = \{C_H - ([H^+] - K_w[H^+]^{-1})\}/C_L$.

Table 1. The results obtained in the present work for 2,4-dihydroxybenzoic (1), 2,5-dihydroxybenzoic (2), 2,6-dihydroxybenzoic (3) and 3,5-dihydroxybenzoic (4) acids at $I = 0.5$ ($NaClO_4$) and 25°C; the values in parentheses are the standard deviations

Acid	$\log \beta_{11}$	$\log \beta_{21}$	$\log \beta_{31}$	$\log \beta_{52}$	$\log \beta_{62}$
(1)	13.373 (0.012)	21.928 (0.001)	25.046 (0.002)	47.17 (0.08)	50.556 (0.006)
(2)	12.742 (0.017)	22.737 (0.002)	25.468 (0.002)	—	—
(3)	13.283 (0.015)	25.854 (0.014)	27.050 (0.008)	—	—
(4)	10.736 (0.003)	19.730 (0.003)	23.539 (0.001)	43.520 (0.007)	47.562 (0.002)

squares $U = \Sigma_i$ (titre$_i^{calc}$ − titre$_i^{obs}$).[2] For 2,4-dihydroxybenzoic acid, the standard deviation in titre was 0.011, 0.021 and 0.007 for the models with three parameters (β_{11}, β_{21} and β_{31}), four parameters (β_{11}, β_{21}, β_{31} and β_{62}) and five parameters (β_{11}, β_{21}, β_{31}, β_{62} and β_{52}), respectively, when C_L was 0.0332M and 60 readings were used. The corresponding values for 3,5-dihydroxybenzoic acid were 0.017, 0.089 and 0.007, when C_L was 0.0875M and 60 readings were used. Thus, on statistical grounds, the set with five constants is the best approximation for both these acids.

The protonation constants, K_1, K_2 and K_3, obtained in the present study and those reported in the literature are given in Table 2.

DISCUSSION

The values reported in the literature and those obtained in this work for the logarithms of K_1, K_2 and K_3 are in accord with each other when the differences in ionic strength are taken into account (Table 2).

Because of the *ortho*-effect, the first protonation constant of 2,4-, 2,5- and 2,6-dihydroxybenzoic acids (as well as the second protonation constant of 2,6-dihydroxybenzoic acid) is significantly higher than that of phenol[11] (log $K_1 = 9.79$ at $I = 0.5$ and 25°) and the third protonation constant significantly smaller than the protonation constant of benzoic acid[12] (log $K_1 = 3.96$ at $I = 0.5$ and 25°). In 3,5-dihydroxybenzoic acid there are no intramolecular hydrogen bonds, and the values of the protonation constants of the hydroxyl groups and the carboxyl group are much closer to those for phenol and benzoic acid, respectively. In addition to the *ortho*-effect, there are of course, the inductive effect, the mesomeric effect and the charge effect of the different functional groups, affecting the dissociation of the "acid" protons. The positive mesomeric effect of the hydroxyl group is especially worth noting.

In the solid state and in organic solvents, salicylic acid has been found to exist as the cyclic dimer, H_4L_2, the association taking place through the intermolecular hydrogen bonds formed by the carboxyl groups.[13] As the intramolecular hydrogen bonding is so strong in salicylic acid, Lee *et al.*[4] have suggested that these bonds persist in the dimers H_4L_2 and $H_3L_2^-$. They have further assumed that the neutral dimer of salicylic acid, H_4L_2, would exist in a form similar to that of $H_3L_2^-$ and probably not in the cyclic form occurring in the solid state. According to their assumption, the structure of $H_3L_2^-$ is an open dimer where the association takes place through one hydrogen bond between the carboxyl groups. It is quite reasonable that the intermolecular hydrogen bonds are formed in a similar way for the dimers of dihydroxybenzoic acids as for salicylic acid in aqueous solution. If it is assumed that the reaction $H^+ + 2H_2L^- = H_5L_2^-$ corresponds to the non-chelate reaction and the reaction $2H^+ + 2H_2L^- = H_6L_2$ to the chelate reaction, the constant K_D of the reaction

Table 2. The values of the protonation constants K_1, K_2 and K_3 for 2,4-dihydroxybenzoic (1), 2,5-dihydroxybenzoic (2), 2,6-dihydroxybenzoic (3) and 3,5-dihydroxybenzoic (4) acids reported in the literature and obtained in this study [$K_n = K(H_{n-1}L + H = H_nL)$]

Acid	T, °C	Medium	$\log K_1$	$\log K_2$	$\log K_3$	Refs.
(1)	25	0.5 ($NaClO_4$)	13.37	8.56	3.12	This work
	?	0.1	>13	8.98	3.33	14
	25	0.1 ($NaClO_4$)			3.10	15
	28	0.1 (KNO_3)	10.94	9.35	3.65	16
(2)	25	0.5 ($NaClO_4$)	12.74	10.00	2.73	This work
	?	0.1	>12	10.50	2.97	14
(3)	25	0.5 ($NaClO_4$)	13.28	12.57	1.20	This work
	25	0.1 ($NaClO_4$)			1.08	15
(4)	25	0.5 ($NaClO_4$)	10.74	8.99	3.81	This work
	25	0.2	10.54	9.00	3.84	17

$H_5L_2^- + H_5L_2^- = H_6L_2 + 2H_2L^-$ could be used as a measure of the "chelate effect". The values of $K_D = \beta_{62} \times \beta_{21}^2/\beta_{32}^2$ for the dimers H_6L_2 of 2,4- and 3,5-dihydroxybenzoic acids are 1.18 and 0.96, respectively. Thus, the values of K_D differ slightly from unity in both cases, as noted earlier for salicylic acid,[4] and nothing can be said on the "chelate effect".

From the results obtained it can clearly be seen that the dimerization is diminished by the intramolecular hydrogen bonds. The dimerization is more apparent in 3,5-dihydroxybenzoic acid, where no intramolecular hydrogen bonds are formed, than in the other dihydroxybenzoic acids in which intramolecular hydrogen bonds exist. It can also be noted that the solubility of 3,5-dihydroxybenzoic acid is much higher than that of the other acids studied, owing to the absence of intramolecular hydrogen bonding. Further, it is evident that the dimerization also depends on the acid strength of the carboxyl group. Only in the case of 2,4-dihydroxybenzoic acid were the compounds H_6L_2 and $H_5L_2^-$ detected as minor species of the three hydroxysalicylic acids studied. The value of the protonation constant K_3 decreases in the order 4-hydroxy- > 5-hydroxy- > 6-hydroxysalicylic acid.

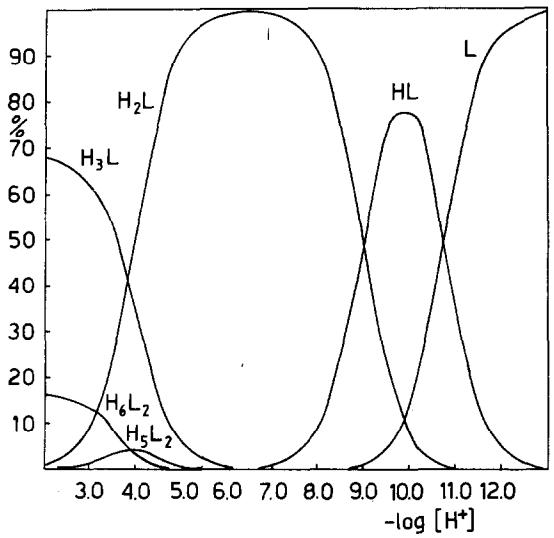

Fig. 2. Distribution of various acid species of 3,5-dihydroxybenzoic acid *vs.* $-\log [H^+]$. $C_L = 0.088M$, $I = 0.5$ ($NaClO_4$) and $T = 25°$.

According to the results of this study, the maximum fractions of the dimers are about 20, 12, 3 and 0.3% for 3,5-dihydroxybenzoic acid at total concentrations of 0.1, 0.05, 0.01 and 0.001*M*, respectively. The corresponding values for 2,4-dihydroxybenzoic acid are 4.5, 2.2, and 0.2% with total concentrations of 0.032, 0.016 and 0.008*M*, respectively. The distribution of various acid species as a function of $-\log [H^+]$ is presented for 3,5-dihydroxybenzoic acid in Fig. 2.

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COMPLEX FORMATION BETWEEN ALUMINIUM AND 3-HYDROXY-7-SULPHO-2-NAPHTHOIC ACID

POTENTIOMETRIC, ABSORPTION-SPECTROPHOTOMETRIC AND SPECTROFLUOROMETRIC STUDIES IN AQUEOUS SOLUTION

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Summary—The formation of chelates between aluminium(III) and 3-hydroxy-7-sulpho-2-naphthoic acid has been studied in aqueous solutions of various ionic strengths, at 298 K. The stability constants of AlL, AlL_2 and AlL_3 and the protonation constants of the ligand, obtained by potentiometry with the glass electrode, are compared with values found by absorption and fluorescence spectrophotometric methods.

The formation of metal chelate complexes by several sulpho-substituted *o*-hydroxynaphthoic acids has been reported earlier in a series of papers (see References). The formation of the beryllium, copper(II) and lanthanide(III) chelates of 3-hydroxy-7-sulpho-2-naphthoic acid have also been studied.[1-3] In the present work the chelation equilibria of 3-hydroxy-7-sulpho-2-naphthoic acid with aluminium ions, and the protonation equilibria of the ligand were studied by different methods.

EXPERIMENTAL

Reagents

The preparation and purification of the monosodium salt of 3-hydroxy-7-sulpho-2-naphthoic acid have been reported earlier.[1]

The aluminium(III) solutions were prepared from aluminium nitrate $Al(NO_3)_3 \cdot 9H_2O$ (Merck).

Apparatus and methods

The potentiometric titrations were carried out with a Radiometer digital titration system DTS 633, consisting of an autoburette ABU 13, pH-meter PHM 64 and digital titrator TTT 61 with an equal-increment accessory constructed in the Department of Chemistry of the University of Oulu. The details of the methods, measurements and experimental conditions, calibrations and electrodes are given elsewhere.[1,2] The total concentration of the ligand was varied between 1.0×10^{-3} and $1.0 \times 10^{-2}M$, and the ratio $C_L:C_{Al}$ between 1 and 10 in the different titrations.

The spectrophotometric titrations were carried out with a Radiometer PHM 64 potentiometer equipped with a Beckman glass electrode and an open-liquid-junction saturated calomel reference electrode system connected by a Masterflex pump and Tygon tubes to a Perkin–Elmer Model 402 spectrophotometer and a digital voltmeter Mk III (Weiss Electronics Ltd.).

The stability constant for the first aluminium complex was resolved by application of a least-squares calculation to the following equation:

$$A = A_{AlL} - \{(A - A_{H_2L})K_1K_2[H^+]^2 + (A - A_{HL})K_1[H^+]\}/[Al]\beta_1 \quad (1)$$

where A is the measured absorbance, A_{AlL}, A_{H_2L} and A_{HL} are the absorbances of the complex and ligand-anion species. K_1, K_2 and β_1 refer to the protonation and metal-complex stability constants. The total concentration of the ligand was $1.95 \times 10^{-4}M$ and the ratio $C_{Al}:C_L$ was between 50 and 80 in these experiments.

The same method was used in fluorescence spectrophotometric measurements with a Farrand Model 801 spectrofluorimeter and a Heath Model EU-20B Servo-Recorder.

The logarithmic values 11.73 and 2.44 were used for the first and second protonation constants K_1 and K_2 of 3-hydroxy-7-sulpho-2-naphthoic acid.[1] The stability constants for the equilibria were computed by means of the program SCOGS,[4] and a UNIVAC 1100/20 computer.

RESULTS AND DISCUSSION

The values obtained potentiometrically for the stability constants for the aluminium chelates of 3-hydroxy-7-sulpho-2-naphthoic acid $\beta_1 = [AlL]/[Al^{3+}][L^{3-}]$, $\beta_2 = [AlL_2^{3-}]/[Al^{3+}][L^{3-}]^2$ and $\beta_3 = [AlL_3^{6-}]/[Al^{3+}][L^{3-}]^3$ are given in Table 1. The titrations reveal the formation of successive chelates as shown in Fig. 1.

The spectrophotometric and spectrofluorometric results are given in Tables 2 and 3. Table 4 shows the data obtained from the fluorescence spectra for the equilibrium constant $K = [H^+]^2[AlL]/[Al^{3+}][H_2L^-]$. The absorption spectra are presented in Figs. 2 and 3, and the fluorescence spectra in Fig. 4.

Table 1. Values of the stability constants of the successive aluminium chelates of 3-hydroxy-7-sulpho-2-naphthoic acid obtained potentiometrically at $I = 0.1$ ($NaClO_4$) and 298 K; standard deviations are given in parenthesis

5 titrations 243 points	$\log \beta_1$	$\log \beta_2$	$\log \beta_3$	$\log k_2$	$\log k_3$
	11.894 (0.003)	21.138 (0.008)	29.06 (0.02)	9.24	7.92

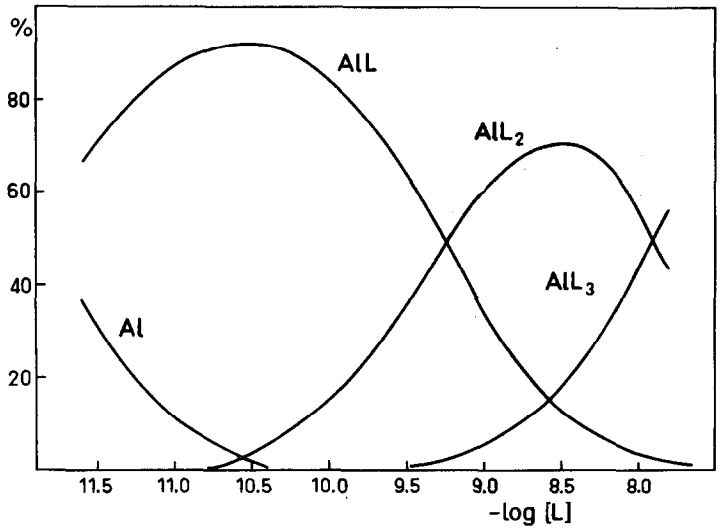

Fig. 1. Distribution of the different aluminium species *vs.* −log[L] in the potentiometric-titration range studied.

Table 2. The spectrophotometric results obtained for the stability constant of the first aluminium complex of 3-hydroxy-7-sulpho-2-naphthoic acid at $I = 0.1$ ($NaClO_4$) and 298 K; $C_L = 1.95 \times 10^{-4} M$

$C_{AL}:C_L$	log β_1					Mean value (s.d.)
	λ = 360 nm	λ = 365 nm	λ = 370 nm	λ = 375 nm	λ = 380 nm	
51.5	11.91	11.94	11.92	11.92	11.95	
67.0	11.93	11.95	11.93	11.94	11.94	
77.3	11.92	11.94	11.95	11.91	11.93	11.932 (0.014)

Table 3. The values obtained spectrofluorometrically for the stability constant of the first aluminium chelate of 3-hydroxy-7-sulpho-2-naphthoic acid at two different ratios of metal to ligand at ionic strength 0.1 (KCl) and 298 K

$C_{AL}:C_L$	log β_1				Mean value (s.d.)
	λ = 445 nm	λ = 455 nm	λ = 465 nm	λ = 475 nm	
16.8	11.30	11.30	11.30	11.30	
48.4	11.33	11.33	11.33	11.34	11.316 (0.018)

Table 4. Evaluation of the equilibrium constant for the formation of the aluminium complex of 3-hydroxy-7-sulpho-2-naphthoic acid in aqueous solution from relative intensities of fluorescence spectra measured at wavelengths in the range 445–475 nm (298 K); $C_L = 5.94 \times 10^{-5} M$, $C_{Al}:C_L = 16.8$, $I = 0.1$ (KCl)

−log [H^+]	445 nm	450 nm	455 nm	460 nm	465 nm	470 nm	475 nm	Mean value
3.137	2.900	2.903	2.892	2.890	2.896	2.893	2.877	2.893
3.208	2.890	2.894	2.885	2.888	2.883	2.892	2.876	2.887
3.291	2.891	2.7895	2.889	2.892	2.891	2.897	2.882	2.891
3.364	2.888	2.888	2.883	2.882	2.877	2.878	2.872	2.881
3.450	2.884	2.883	2.878	2.880	2.877	2.876	2.874	2.879
3.548	2.893	2.899	2.887	2.897	2.897	2.899	2.887	2.894
3.663	2.881	2.883	2.884	2.884	2.888	2.892	2.874	2.884
3.751	2.901	2.907	2.896	2.901	2.902	2.906	2.889	2.900
3.900	2.894	2.892	2.895	2.894	2.893	2.895	2.901	2.895
Mean value	2.891	2.894	2.888	2.890	2.889	2.892	2.881	2.889 (0.009)

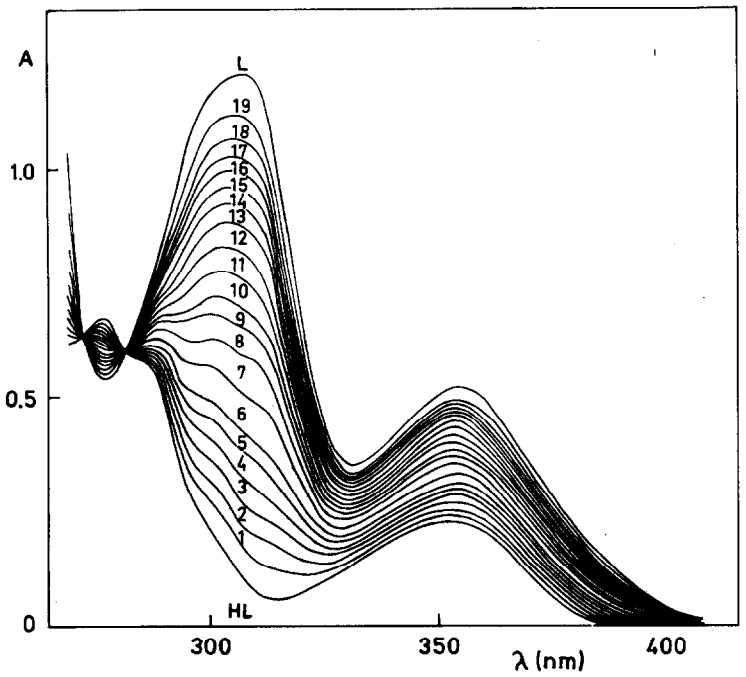

Fig. 2. Absorption spectra of the ligand species HL^{2-} and L^{3-} obtained by spectrophotometric titration. Curves $1 \cdots 19$ refer to solutions with $-\log[H^+] = 10.57 \cdots 12.73$. $C_L = 1.50 \times 10^{-4} M$, $I = 0.216$.

The measurements were made at the peak wavelength range, $\lambda_{max} \pm 25$ nm (Table 6).

Comparison of the stability constant values shows that the log β_1 values at $I = 0.1$ ($NaClO_4$) found potentiometrically and by absorption spectrophotometry—11.89 and 11.93, respectively—to be in relatively good accord, but the corresponding spectrofluorometric value 11.32 (KCl) is somewhat different.

This deviation encouraged us to study also the protonation constants of the ligand by the two spectral methods. The potentiometric values for the protonation constants of 3-hydroxy-7-sulpho-2-naphthoic acid at $I = 0.1$ ($NaClO_4$), log $K_1 = 11.73$ and log $K_2 = 2.44$ have been reported previously.[1]

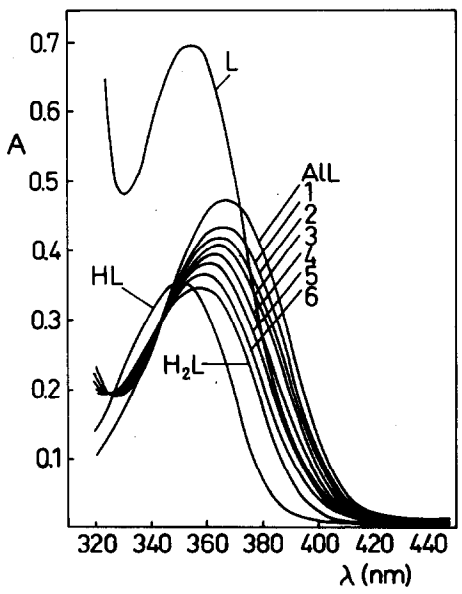

Fig. 3. Absorption spectra of the ligand species and of the formation of the first aluminium chelate AlL. Curves $1 \cdots 6$ refer to solutions with $-\log[H^+] = 2.73 \cdots 1.99$. $C_L = 1.95 \times 10^{-4} M$, $C_{Al} = 2.005 \times 10^{-2} M$, $I = 0.1$.

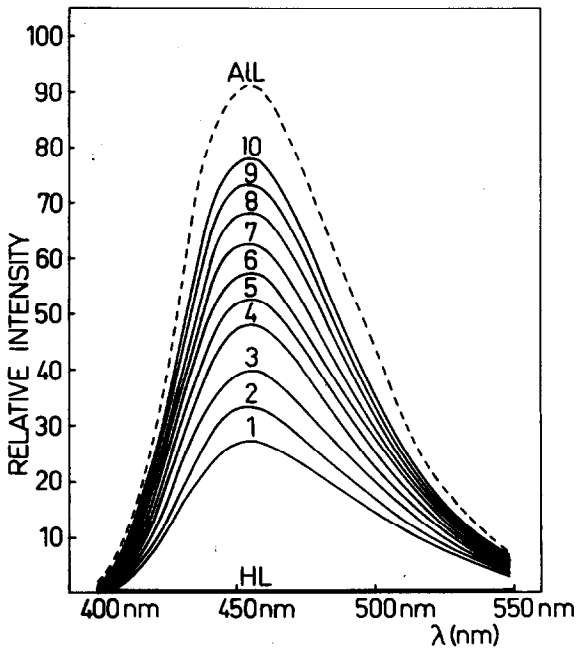

Fig. 4. Fluorescence spectra of the formation of the first aluminium chelate AlL. Curves $1 \cdots 10$ refer to solutions with $-\log[H^+] = 3.00 \cdots 4.06$. Excitation wavelength 365 nm, $C_L = 2.07 \times 10^{-5} M$, $C_{Al} = 1.00 \times 10^{-3} M$, $I = 0.1$.

Table 5. Protonation constants of 3-hydroxy-7-sulpho-2-naphthoic acid calculated from absorption and fluorescence spectra of aqueous solutions of various ionic strengths at 298 K, and the corresponding Debye–Hückel equations evaluated

	I	$\log K_1$ obsd.	$\log K_1$ calcd.
Absorptiometric	0.114	11.89	11.90
	0.216	11.78	11.77
	0.408	11.64	11.65
	1.009	11.50	11.50

$$\log K_1 = 12.53 - 3.054\sqrt{I}/(1 + 1.78\sqrt{I}) + 0.07 \times I$$

Fluorometric	0.006	12.29	12.30
	0.019	12.14	12.16
	0.033	12.07	12.08
	0.055	12.02	11.99
	0.064	11.97	11.97
	0.115	11.86	11.86
	0.215	11.75	11.74
	0.411	11.63	11.64
	0.914	11.61	11.60

$$\log K_1 = 12.50 - 3.054\sqrt{I}/(1 + 1.56\sqrt{I}) + 0.30 \times I$$

	I	$\log K_2$ obsd.	$\log K_2$ calcd.
Absorptiometric	0.008	2.76	2.77
	0.027	2.69	2.67
	0.048	2.61	2.61
	0.068	2.57	2.57

$$\log K_2 = 2.93 - 2.036\sqrt{I}/(1 + 1.96\sqrt{I})$$

Fluorometric	0.056	2.70	2.70
	0.106	2.64	2.64
	0.156	2.61	2.60
	0.305	2.55	2.53
	0.456	2.47	2.50
	0.606	2.48	2.47

$$\log K_2 = 3.01 - 2.036\sqrt{I}/(1 + 2.50\sqrt{I})$$

The values obtained here by absorption spectrophotometry at $I = 0.1$ (KCl) are 11.92 and 2.53 when interpolated by means of the Debye–Hückel equations (Table 5). The corresponding fluorescence spectrophotometric values at $I = 0.1$ (KCl) are 11.88 and 2.65.

Table 6. Spectral data at 298 K

	H_2L^-	HL^{2-}	L^{3-}	AlL
UV absorption				
λ_{max}, *nm*	358	351	355	368
Fluorescence				
λ_{max}, *nm*	—	505	490	455
$\lambda_{excitation}$ *nm*	365	360	365	365

Here too there is some difference between the values obtained by the three experimental methods. The deviation cannot result only from the effect of the inert salt used, for the effect of ionic strength seems to be almost the same, at least at low ionic strengths.

The difference between the values may well be caused by the pH-independent fluorescence intensity of a side-reaction product.

3-Hydroxy-7-sulpho-2-naphthoic acid thus forms strong chelates with aluminium. The chelates are slightly stronger than the aluminium chelates formed by 3-hydroxy-5,7-disulpho-2-naphthoic acid.[5] As a ligand, 3-hydroxy-7-sulpho-2-naphthoic acid is also the weakest acid, with respect to the naphtholic hydroxyl group, in the series of sulpho-substituted 3-hydroxy-2-naphthoic acids studied. The acid strength of 3-hydroxy-7-sulpho-2-naphthoic acid is of the same order as that of 1-hydroxy-4-sulpho-2-naphthoic acid,[6,7] for instance, which again is weaker than 1-hydroxy-4,7-disulpho-2-naphthoic acid.[8]

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DETERMINATION OF MOLYBDENUM IN ORES, IRON AND STEEL BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY AFTER SEPARATION BY α-BENZOINOXIME EXTRACTION OR FURTHER XANTHATE EXTRACTION

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Summary—A simple and moderately rapid method for determining 0.001% or more of molybdenum in ores, iron and steel is described. After sample decomposition, molybdenum is separated from the matrix elements, except tungsten, by chloroform extraction of its α-benzoinoxime complex from a 1.75*M* hydrochloric–0.13*M* tartaric acid medium. Depending on the amount of tungsten present, molybdenum, if necessary, is back-extracted into concentrated ammonia solution and subsequently separated from co-extracted tungsten by chloroform extraction of its xanthate complex from a 1.5*M* hydrochloric–0.13*M* tartaric acid medium. It is ultimately determined by atomic-absorption spectrophotometry, at 313.3 nm, in a 15% v/v hydrochloric acid medium containing 1000 μg/ml of aluminium as the chloride, after evaporation of either extract to dryness with nitric, perchloric and sulphuric acids and dissolution of the salts in dilute ammonia solution.

For use in the Canadian Certified Reference Materials Project and in routine work in the CANMET chemical laboratory, a reasonably simple and reliable atomic-absorption (AAS) method was required for the determination of $\leqslant 200$ μg/g of molybdenum in diverse ores and mill products. Because the determination of molybdenum by AAS is subject to many interferences caused by the formation of refractory (not easily dissociated) compounds in the flame,[1–5] it was considered that a suitable method would involve a relatively selective solvent-extraction preconcentration step.

In recent years, numerous AAS methods based on the separation and preconcentration of molybdenum by extraction of its thiocyanate, 8-hydroxyquinoline, sodium diethyldithiocarbamate, ammonium pyrrolidinedithiocarbamate and dithiol complexes have been reported.[6–8] In these methods, molybdenum is determined by direct aspiration of the organic phase into the flame. A method involving its separation by chloroform extraction of its α-benzoinoxime complex and its ultimate determination in an ammonium chloride–perchloric acid medium has also been reported.[9] Because of the apparent simplicity and relatively high specificity of this extraction procedure,[10–12] and the greater ease of preparation of aqueous calibration solutions, particularly for routine work, the applicability of this separation procedure to the determination of small amounts of molybdenum in ores was investigated.

This paper describes the successful determination of molybdenum in ores, iron and steel, after its separation from the matrix elements, except tungsten, by chloroform extraction of its α-benzoinoxime complex from 1.75*M* hydrochloric acid containing tartaric acid to keep tungsten in solution. Depending on the tungsten content of the sample, molybdenum, if necessary, is subsequently separated from co-extracted tungsten, after back-extraction of both elements into concentrated ammonia solution, by chloroform extraction of its xanthate complex from a 1.5*M* hydrochloric–0.13*M* tartaric acid medium.

EXPERIMENTAL

Apparatus

A Varian Techtron Model AA6 spectrophotometer equipped with a 10-cm laminar-flow air–acetylene burner and a molybdenum hollow-cathode lamp was used for the determination of molybdenum. The following instrumental parameters were employed (Note 1).

Wavelength: 313.3 nm.
Lamp current: 5 mA.
Spectral band-pass: 0.10 nm.
Height of light-path above burner: 8 mm.
Acetylene flowmeter reading: 4.0–4.5 (~3 l./min).
Air flowmeter reading: 6.5 (~13 l./min).
Flame: brightly luminous, fuel-rich.
Aspiration rate: 2 ml/min.

Reagents

Standard molybdenum solution, 1000 μg/ml. Dissolve 1.5000 g of pure molybdenum trioxide in 50 ml of 2% sodium hydroxide solution and dilute the solution to 1 litre with water. Prepare a 100 μg/ml solution by diluting 25 ml of this stock solution to 250 ml with water.

Aluminium, 1% solution. Dissolve 10 g of aluminium metal by heating gently with 400 ml of 50% v/v hydrochloric acid. Cool and, if necessary, filter the solution (Whatman No. 42 paper) into a 1-litre standard flask containing 300 ml of concentrated hydrochloric acid. Dilute the resulting solution to volume with water.

α-Benzoinoxime, 0.2% solution in chloroform.

Potassium ethyl xanthate, 20% solution. Prepare fresh as required.

Ferrous ammonium sulphate, 10% solution. Prepare fresh as required.

Bromine, 20% v/v solution in carbon tetrachloride.

Procedures

Calibration solutions. To eight 100-ml beakers, add, by burette, 1, 3, 5, 10, 15, 20, 25 and 30 ml respectively, of the 100-μg/ml standard molybdenum solution. Add ~10 drops of 50% v/v sulphuric acid to each beaker and evaporate the solutions to dryness. Cool, wash down the sides of the beakers with water and evaporate the solutions to dryness again to ensure the complete removal of sulphuric acid. Add 1 ml of 25% ammonia solution, 1 drop of 0.2% phenolphthalein solution and ~5 ml of water to each beaker and heat gently until the solutions are colourless. Add 10 ml each of concentrated hydrochloric acid and 1% aluminium solution and transfer the resulting solutions to 100-ml standard flasks. Add 10 ml each of concentrated hydrochloric acid and 1% aluminium solution to a ninth flask; this constitutes the zero calibration solution. Dilute each solution to volume with water and mix (Note 2).

Ores. Transfer 0.1–1 g of powdered sample, containing up to ~2.5 mg of molybdenum and 25 mg of tungsten, to a 400-ml Teflon beaker, cover and add 20 ml of 50% nitric acid and 5 ml of 20% bromine solution in carbon tetrachloride (Notes 3 and 4). Allow the solution to stand for ~10 min, then heat gently to remove the bromine and carbon tetrachloride. Add 10 ml of concentrated hydrochloric acid and 20 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, add 5 ml of concentrated hydrofluoric acid and carefully evaporate the solution to ~3 ml. Cool, add 25 ml of water, heat to dissolve the soluble salts, then add 10 ml of bromine water (Note 5) and heat gently to remove the excess of bromine.

Add 10 ml of 20% tartaric acid solution and a small piece of red litmus paper to the resulting solution, then add 50% sodium hydroxide solution, in ~0.5-ml portions, until the solution is alkaline or, depending on the amount of iron present, a mahogany colour. Allow the solution to stand for several min to ensure the complete dissolution of any tungsten trioxide present, then add concentrated hydrochloric acid, dropwise, until the solution is acidic. Add 15 ml in excess and, if necessary, filter the resulting solution (Whatman No. 40 paper) into a 250-ml separatory funnel marked at 100 ml. Wash the beaker and the paper and residue each three times with small portions of water, then discard the paper and residue. Add 5 ml of 10% ferrous ammonium sulphate solution to the funnel, dilute the solution to the mark with water and mix thoroughly. Add 15 ml of 0.2% α-benzoinoxime solution, stopper, shake for 1 min and allow several min for the layers to separate (Note 6).

If tungsten is absent or not more than ~2 mg is present (Note 7), drain the chloroform phase into a 150-ml beaker. Extract the aqueous phase three more times, in a similar manner, with 15-ml portions of the α-benzoinoxime solution. Add 10 ml of 50% v/v nitric acid to the combined extracts and heat in a hot water-bath to remove the chloroform. Add 3 ml of concentrated perchloric acid and 2 ml of 50% sulphuric acid, cover the beaker and heat until the evolution of oxides of nitrogen ceases. Remove the cover and evaporate the solution to dryness. Cool, wash down the sides of the beaker with water and evaporate the solution to dryness again to ensure the complete removal of sulphuric acid (Note 8). Add 1 ml of 25% ammonia solution, 1 drop of 0.2% phenolphthalein solution and ~3 ml of water and heat gently until the solution is colourless. Add sufficient concentrated hydrochloric acid for 1 ml to be present for each 10 ml of final solution, then add the same volume of 1% aluminium solution. Transfer the solution to a standard flask of appropriate size (10–100 ml), dilute to volume with water and mix.

Measure the absorbance of the resulting solution, at 313.3 nm, in a strongly reducing air–acetylene flame (Note 9). Determine the molybdenum content of the solution by relating the resulting value to those obtained concurrently for calibration solutions of slightly higher and lower molybdenum concentrations.

If more than 2 mg of tungsten is present, collect the α-benzoinoxime extracts (Note 10) in a 125-ml separatory funnel (Note 11), then add 10 ml of concentrated ammonia solution, stopper and shake for 4 min. Allow ~5 min for the layers to separate, then drain off and discard the chloroform layer. Add, in succession, 5 ml of 20% tartaric acid solution, 20 ml of water and 15 ml of concentrated hydrochloric acid, blow the resultant ammonium chloride fumes out of the funnel and cool the solution to room temperature. Add 2 ml of freshly prepared 20% potassium ethyl xanthate solution (Note 12), stopper and mix thoroughly. Let the solution stand for ~1 min to allow the formation of the reddish-purple molybdenum xanthate complex, then add 10 ml of chloroform and shake for 1 min. Allow several min for the layers to separate, then drain the chloroform phase into a 150-ml beaker. Extract the aqueous phase three more times, in a similar manner, with 10-, 5- and 5-ml portions of chloroform and 1, 0.5 and 0.5 ml, respectively, of xanthate solution (Note 13). Add 10 ml of 50% nitric acid to the combined extracts and heat in a hot water-bath to remove the chloroform. Add 3 ml of concentrated perchloric acid and 2 ml of 50% sulphuric acid and proceed with the evaporation of the solution to dryness, the dissolution of the salts in 25% ammonia solution and the subsequent determination of molybdenum as described above.

Iron and steel. Transfer 0.1–1 g of sample, containing up to ~2.5 mg of molybdenum and 25 mg of tungsten (Note 4), to a 400-ml beaker, cover and add 10 ml each of concentrated hydrochloric acid and 50% nitric acid. Heat gently until the sample is decomposed, then remove the cover, add 4 drops of concentrated hydrofluoric acid and evaporate the solution to dryness in a hot water-bath. Add 5 ml of concentrated hydrochloric acid and 10 ml of bromine water and evaporate the solution to dryness again to ensure the removal of most of the nitric acid.

If tungsten is known to be absent, add 15 ml of concentrated hydrochloric acid and 10 ml of 20% tartaric acid solution and, if necessary, heat gently to dissolve the salts. If necessary, filter the resulting solution (Whatman No. 40 paper) into a 250-ml separatory funnel marked at 100 ml, add 5 ml of 10% ferrous ammonium sulphate solution, dilute to the mark with water and proceed with the α-benzoinoxime extraction and the subsequent determination of molybdenum as described above.

If tungsten is present, add 5 ml of concentrated hydrochloric acid, ~25 ml of water and 10 ml of 20% tartaric acid solution and, if necessary, heat gently to dissolve the soluble salts. Add 50% sodium hydroxide solution, in ~0.5-ml portions, until the solution is a dark mahogany colour, then allow it to stand for several min to ensure the complete dissolution of tungsten trioxide. Add concentrated hydrochloric acid, dropwise, until the solution is acidic (clear yellow), then add 15 ml in excess. Proceed with the filtration of the solution (if necessary), the α-benzoinoxime extraction and, if necessary, the xanthate extraction (depending on the amount of tungsten present) and

the subsequent determination of molybdenum as described above.

Notes

1. A strongly reducing air–acetylene flame is required to obtain the highest sensitivity for molybdenum. The height at which the beam from the hollow-cathode lamp passes through the flame is also extremely important.[1,5] Consequently, after all other instrumental parameters have been set, the acetylene flow-rate and the height of the light-path above the burner should be adjusted to give maximum absorbance while a solution containing molybdenum is aspirated into the flame.

2. The calibration solutions should be prepared fresh every day because they are not stable on standing. Evaporation of the molybdenum solutions to dryness with sulphuric acid is required for the molybdenum in the resulting calibration solutions to be present in the same form as in the sample solutions obtained after treatment of xanthate extracts with nitric acid, which produces sulphuric acid.

3. The addition of carbon tetrachloride solution of bromine is not necessary if the sample does not contain sulphides.

4. For samples of high molybdenum content, up to 1 g can be taken (tungsten content $\leqslant$25 mg) if the solution ultimately obtained after the addition of 15 ml of concentrated hydrochloric acid is diluted to 100 ml with water. A suitable aliquot of the resultant solution—to which the recommended volume of ferrous ammonium sulphate solution has been added—can subsequently be diluted to ~100 ml in the separatory funnel with 15% hydrochloric acid–2% tartaric acid solution before the α-benzoinoxime extraction step.

5. Bromine water is added to ensure that all of the molybdenum is present in the sexivalent state required for its extraction with α-benzoinoxime.

6. The α-benzoinoxime complexes of molybdenum and tungsten are not appreciably soluble in chloroform. Consequently, if mg-quantities of these elements are present, the chloroform phase will be cloudy or will contain flocculent white material. This does not interfere with the quantitative separation of molybdenum.

7. If the molybdenum content of the sample is so low that the final solution is to be diluted to 10 ml before the determination of molybdenum, the amount of tungsten that is co-extracted at the 2-mg level may interfere by precipitating in the final solution. In that case, it is recommended that the molybdenum should be stripped from the chloroform phase and separated from the co-extracted tungsten by xanthate extraction as described in the subsequent procedure.

8. If the tungsten content of the sample is not known, its presence will be indicated at this point (see also Note 10) by a yellow compound (WO_3) that is insoluble in water. If an appreciable amount is present, add 0.5 ml of concentrated perchloric acid and evaporate the solution to dryness again. Add ~25 ml of water, 5 ml of 20% tartaric acid solution and 1 drop of 0.2% phenolphthalein solution, then make alkaline with 50% sodium hydroxide solution added dropwise. Add concentrated hydrochloric acid, dropwise, until the solution is acidic, then add 6 ml in excess and transfer the solution to a 125-ml separatory funnel marked at 50 ml. Dilute the resultant solution to the mark with water and proceed with the separation of molybdenum by extraction as the xanthate.

9. Scale expansion (~5-fold) is recommended for the determination of approximately 3 μg/ml or less of molybdenum.

10. If the sample contains an appreciable amount of tungsten, the fourth extract will still be cloudy.

11. The separatory funnel should be drained thoroughly after washing, to prevent dilution of the concentrated ammonia solution used for the subsequent back-extraction of molybdenum.

12. The xanthate solution should be added by safety pipette or a graduated or marked medicine dropper, and the extraction should be carried out in a fume hood. Prolonged exposure to xanthate vapour can produce an allergic reaction.

13. Usually a four-stage extraction with a total volume of 4 ml of 20% potassium ethyl xanthate solution is sufficient for the separation of up to 2.5 mg of molybdenum. However, if the aqueous phase is still pink after the fourth addition of xanthate solution, continue the extraction, using 5-ml portions of chloroform and 0.5 ml of xanthate solution until both the aqueous and chloroform phases are colourless.

RESULTS

Calibration solutions

In initial tests, the calibration solutions used for comparison purposes were prepared by direct dilution of appropriate volumes of the standard molybdenum solution and the recommended volumes of concentrated hydrochloric acid and 1% aluminium solution. However, in these tests high results (~2–3 μg/ml at the 25-μg/ml level) were obtained with an air–acetylene flame, when the molybdenum α-benzoinoxime extracts were treated with nitric, perchloric and sulphuric acids, followed by evaporation of the solution to dryness and dissolution of the salts in dilute hydrochloric acid. This was considered to be due to a change in the oxidation state of the molybdenum because a blue compound is produced under these conditions (though only when sulphuric acid is present). Previously, Hutchison,[9] using calibration solutions prepared from ammonium molybdate, obtained low results when aliquots of the standard solutions were evaporated to dryness with perchloric acid and the salts were dissolved in dilute perchloric acid; this was attributed to the formation of molybdic acid. Hutchison found that complete recovery of molybdenum could be obtained if the molybdic acid was subsequently converted into ammonium molybdate by dissolving the salts in dilute ammonia solution and evaporating the resultant solution to dryness. However, high results were still obtained in the present work when this procedure involving evaporation with perchloric acid and subsequent dissolution of the salts in dilute ammonia solution was applied to the α-benzoinoxime extracts. Ultimately it was found that this positive error was due to an increase in the absorbance of molybdenum solutions after evaporation to dryness with sulphuric or perchloric acids and that it can be readily avoided by treating the molybdenum solutions taken for calibration purposes in the same way as the sample solutions. The molybdenum in the resultant calibration solutions will subsequently be present in the same form as in the final sample solutions.

Extraction of the molybdenum(VI) α-benzoinoxime complex

Previous investigators[10,11] showed that the molybdenum(VI) α-benzoinoxime complex can be quantitat-

ively extracted into chloroform from up to approximately 2.3*M* hydrochloric acid, and that the extraction step is reasonably specific when chromium(VI) and vanadium(V) are reduced with ferrous ammonium sulphate and thus prevented from reacting with the reagent.[13] Only niobium, zirconium, tungsten(VI)[12] (and possibly palladium)[13] are partly co-extracted from $\geq 1M$ hydrochloric acid, and the co-extraction of niobium and zirconium can be readily prevented by complexing them with hydrofluoric acid.[12] However, the possible interference of tungsten had to be considered in the present work because it is a common constituent of ores.

Hutchison[9] reported that tungsten does not interfere in the determination of molybdenum by AAS after its separation by α-benzoinoxime extraction. However, tests showed that in an air–acetylene flame 100 μg/ml suppress the absorbance of 20 μg/ml of molybdenum by about 10%. Tungsten also interferes by precipitating in the final dilute acid solution. Tartaric acid cannot be used in this case to keep tungsten in solution because it strongly suppresses the molybdenum absorbance. Tungsten also causes difficulty before the α-benzoinoxime extraction step because of its insolubility in acid media. It has been reported that potassium dihydrogen phosphate complexes tungsten and inhibits the extraction of its α-benzoinoxime complex[14] but this reagent was found to be completely ineffective. Further work showed that up to ~25 mg of tungsten can be kept in solution, in ~1.7*M* hydrochloric acid, with 2 g of tartaric acid and that, under these conditions, up to at least 2.5 mg of molybdenum can be quantitatively extracted as the α-benzoinoxime complex in four successive extractions with 15-ml portions of 0.2% solution of the reagent in chloroform. Approximately 50% of the tungsten initially present in the solution is co-extracted under these conditions. Consequently, a method is required for the subsequent separation of tungsten if more than a few mg are present in the sample taken for analysis.

Separation of molybdenum from tungsten by extraction of its xanthate complex

Previous work by the author[15] showed that molybdenum [after its reduction to molybdenum(V) by xanthate] can be quantitatively extracted into chloroform as the xanthate from 0.1–~2*M* hydrochloric acid. In earlier work the extraction of molybdenum xanthate from 1.5*M* hydrochloric acid containing tartaric acid was used for the separation of molybdenum from tungsten before the spectrophotometric determination of tungsten after its extraction as the thiocyanate–diantipyrylmethane ion-association complex.[16] Consequently, it was considered that this separation procedure could also be used in the present work after back-extraction of the molybdenum and tungsten α-benzoinoxime complexes from the chloroform phase.

Preliminary experiments showed that the molybdenum complex cannot be completely back-extracted from the organic phase by shaking it with 4*M* ammonia solution or 10% sodium hydroxide solution or with 12*M* hydrochloric acid. However, shaking for ~4 min with 15*M* ammonia solution was found to be effective. Under these conditions only negligible amounts of molybdenum (<4 μg at the 2.5-mg level) remain in the organic phase. Subsequent work showed that, after appropriate treatment of the resultant ammoniacal solution, molybdenum can be readily separated from tungsten by extraction as the xanthate from 50 ml of 1.5*M* hydrochloric acid containing ≤1 g of tartaric acid to keep tungsten in solution.

Effect of diverse ions

As mentioned above, only niobium, zirconium, tungsten(VI),[12] and possibly palladium[13] are partly co-extracted as α-benzoinoxime complexes from hydrochloric acid media containing ferrous ammonium sulphate as a reductant for chromium(VI) and vanadium(V). However, tests showed that zirconium is not extracted in the presence of tartaric acid, and niobium forms an insoluble hydrolysis compound during the sample decomposition step. This compound is removed by filtration before the extraction step. The effect of palladium was not considered because more than μg-quantities are not usually present in ores. The amount of tungsten that is co-extracted, at approximately the 2-mg level, will not interfere in the subsequent determination of molybdenum in an air-acetylene flame when aluminium chloride solution[1] is added to the final solution to obviate the interference of tungsten, and if the volume of the solution is ≥25 ml. If the final volume is less, tungsten may precipitate. Because the tungsten compound that is formed on evaporation of the α-benzoinoxime extract to dryness with acids is insoluble in water or dilute acids, dilute ammonia solution is required to dissolve this compound and convert it into ammonium tungstate before the addition of hydrochloric acid and aluminium solution. The excess of ammonia is readily removed by heating the solution.

Applications

To test the reliability of the proposed method, it was applied to the analysis of three CCRMP ores that have been certified for molybdenum and to three CCRMP tungsten ores for which only approximate molybdenum values are available.[17] It was also applied to certified reference iron and steel samples. The results of these analyses are given in Table 1.

DISCUSSION

Table 1 shows that the results obtained for the CCRMP reference ores MP-1, HV-1 and PR-1 and, where applicable, for the National Bureau of Standards (NBS) and British Chemical Standards (BCS)

Table 1. Determination of molybdenum in CCRMP reference ores and in NBS and BCS iron and steel

Sample	Nominal composition, %	Certified value and range, % Mo	Mo found, %: After α-benzoinoxime extraction	Mo found, %: After α-benzoinoxime and xanthate extractions
MP-1 Zinc–tin–copper–lead ore	19.4 Si, 3.6 Al, 5.7 Fe, 3.4 Ca, 11.8 S, 15.9 Zn, 2.4 Sn, 2.1 Cu	0.014 (0.013–0.015)*	0.013†	0.013‡
HV-1 Copper–molybdenum ore	33.9 Si, 6.6 Al, 1.9 Fe, 0.4 S, 1.4 Ca	0.058 (0.056–0.059)*	0.058†	0.056‡
PR-1 Molybdenum ore	39.2 Si, 2.4 Al, 1.2 Fe, 1.4 Ca, 0.8 S	0.59 (0.578–0.610)*	0.589†	0.586‡
CT-1 Tungsten ore	1.0 W, 17.2 Si, 2.9 Al, 8.6 Fe, 12.2 Ca, 2.0 Mg, 0.7 Mn, 8.1 S	(0.03)§	—	0.0008, 0.0008
BH-1 Tungsten ore	0.4 W, 38.0 Si, 3.5 Al, 3.2 Fe, 0.8 S, 0.5 Ca	(0.02)§	0.033	0.033
TLG-1 Tungsten ore	0.1 W, 21.5 Si, 3.0 Al, 17.5 Fe, 16.6 Ca, 2.7 Mg, 1.3 Mn	(<0.01)§	0.0068, 0.0065	—
NBS-3b White iron	0.4 Mn, 1.0 Si, 2.4 C	0.002 (0.001–<0.01)	0.0015, 0.0014	—
NBS-4j Cast iron	3.0 C, 0.8 Mn, 1.3 Si	0.080‖	0.078	—
NBS-12h Basic open-hearth steel	0.8 Mn, 0.2 Si	0.006 (0.005–0.007)	0.0050, 0.0048	—
NBS-33d Nickel steel	3.6 Ni, 0.5 Mn, 0.3 Si	0.246 (0.242–0.249)	0.245	—
NBS-36A Chromium–molybdenum steel	0.4 Si, 2.4 Cr, 0.4 Mn	0.920 (0.91–0.930)	0.918	
NBS-50a Chromium–tungsten–vanadium steel	18.3 W, 3.5 Cr, 1.0 V, 0.5 Si, 0.7 Cu	0.009 (0.005–0.014)	—	0.016, 0.016
NBS-50B Tungsten–chromium–vanadium steel	18.1 W, 0.7 C, 0.3 Si, 4.1 Cr, 1.0 V	0.401 (0.384–0.415)	—	0.401
NBS-111a Nickel–molybdenum steel	0.7 Mn, 0.3 Si, 1.7 Ni	0.222‖	0.227	—
NBS-121B 18 Chromium–11 nickel steel	1.5 Mn, 0.6 Si, 11.2 Ni, 17.7 Cr, 0.4 Ti	0.073 (0.070–0.076)	0.073	—
NBS-123b Niobium-tantalum stabilized stainless steel	0.2 W, 0.8 Nb, 0.2 Ta, 0.5 Si	0.17‖	0.166	0.172
NBS-152 Open-hearth steel	0.5 C, 0.8 Mn, 0.2 Si	0.013‖	0.013	—
NBS-155 Chromium–tungsten steel	0.5 W, 1.2 Mn, 0.3 Si, 0.5 Cr, 0.9 C	0.039 (0.035–0.043)	0.037	0.038
NBS-160a 19 Chromium–14 nickel–3 molybdenum steel	14.1 Ni, 18.8 Cr, 1.6 Mn, 0.6 Si	2.83‖	2.84	—
BCS-219/2 Nickel–chromium molybdenum steel	0.2 W, 2.5 Ni, 0.8 Cr, 0.3 Si, 0.6 Mn	0.43 (0.41–0.45)	0.427	0.425
BCS-273 Mild steel	0.3 W	0.04_5	0.041	0.040

* 95% Confidence limits of the recommended mean value.
† Mean of 3 values.
‡ 25 mg of tungsten added before sample decomposition.
§ C.C.R.M.P. value given for information only (not certified).[17]
‖ N.B.S. provisional result.

iron and steel samples, after separation of molybdenum by α-benzoinoxime extraction alone, are in excellent agreement with the certified values. Similarly, the results obtained for the ores, after the addition of tungsten, and for the NBS and BCS samples containing tungsten, after further separation of molybdenum from co-extracted tungsten by xanthate extraction, are also in good agreement with the certified values and, where applicable, with the values obtained after α-benzoinoxime extraction alone. The results obtained for the three CCRMP tungsten ores, CT-1, BH-1 and TLG-1 are considered to be more reliable than those given for information purposes.[17] The latter are only approximate values obtained in the CANMET chemical laboratory by a spectrophotometric thiocyanate method after the separation of iron by precipitation with sodium hydroxide.

Molybdenum can be separated from tungsten and certain other elements by direct xanthate extraction,[18] but this was not considered in the present work because the extraction step is not sufficiently selective.[15] The co-extraction of iron(III) can be avoided by reducing it with ascorbic acid, and that of copper(II) and vanadium(V) by complexing them with thiourea and potassium hydrogen fluoride, respectively.[18] Tin(II and IV), antimony(III), arsenic(III) and selenium(IV), which are also co-extracted,[15] can be removed by volatilization as the bromides during the sample decomposition step. However, the co-extraction of certain elements that commonly occur in ores, such as cobalt, nickel, silver, bismuth and, particularly, lead cannot be prevented.[15]

Possibly more tungsten can be tolerated during the α-benzoinoxime extraction step if the initial tartaric acid concentration is increased. However, because tartaric acid slightly inhibits the extraction of the molybdenum α-benzoinoxime complex, a more concentrated solution of the reagent in chloroform would be required for the complete extraction of molybdenum. Under these conditions, more tungsten would also be co-extracted. Subsequently, more than 1 g of tartaric acid may be required to keep tungsten in solution before and during the xanthate extraction step. The addition of more is not recommended because too much tartaric acid inhibits the extraction of molybdenum as the xanthate. Up to six extraction stages are required for the complete extraction of 2.5 mg of molybdenum from 50 ml of 1.5–2*M* hydrochloric acid containing 2 g of tartaric acid. The inhibiting effect of tartaric acid is greater at lower hydrochloric acid concentrations.

A hydrochloric acid medium was chosen for the ultimate determination of molybdenum because this acid has very little effect on the absorbance of molybdenum in either an air-acetylene or a nitrous oxide–acetylene flame.[4,5,19] A 15% hydrochloric acid medium was chosen for convenience when working with 10-ml final sample solution volumes. Probably the addition of 1 ml of 50% hydrochloric acid, rather than the concentrated acid, would also be sufficient. However, the concentration of hydrochloric acid in the calibration solutions should be adjusted accordingly. An air–acetylene flame was chosen because it was found to be sufficiently sensitive and because interference effects from other elements, anions and acids are considered to be less in this flame than in the nitrous oxide–acetylene flame.[19,20]

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PRE-CONCENTRATION OF TRACE METALS FROM SEA-WATER FOR DETERMINATION BY GRAPHITE-FURNACE ATOMIC-ABSORPTION SPECTROMETRY*

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Summary—Determination of Cd, Zn, Pb, Cu, Fe, Mn, Co, Cr and Ni in coastal sea-water by graphite-furnace atomic-absorption spectrometry after preconcentration by solvent extraction and use of a chelating ion-exchange resin is described. Following the extraction of the pyrrolidine-*N*-carbodithioate and oxinate complexes into methyl isobutyl ketone, the trace metals are further preconcentrated by back-extraction into 1.5*M* nitric acid. Preconcentration on the chelating resin is effected by a combined column and batch technique, allowing greater preconcentration factors to be obtained. Provided samples are appropriately treated to release non-labile metal species prior to preconcentration, both methods yield comparable analytical results with respect to the mean concentrations determined as well as to mean relative standard deviations. Control and treatment of the analytical blank is also described.

The determination of trace elements, particularly transition metals, in sea-water has received increasing attention in recent years as there is considerable interest not only in their role in the biochemical and geochemical cycles of the oceans, but also in their horizontal, vertical and seasonal variations. In spite of these efforts much of the assimilated data remains unreliable, as a result of difficulties arising from faulty analytical techniques and unsatisfactory sampling and storage procedures, *e.g.*, contamination, loss of constituents, matrix effects, sampling problems and lack of reference standards.

In response to the growing demands of the oceanographic community for improved accuracy and precision of analytical methods and the need for marine reference materials, the National Research Council of Canada in 1976 embarked on the Marine Analytical Chemistry Standards Program with the aim of improving chemical analysis in marine science. As part of this programme, problems associated with the determination of heavy metals in sea-water are currently being examined.

Riley[1] has reviewed analytical methods at present in use for analysis of saline water. Of the instrumental techniques available, neutron activation, isotope-dilution mass-spectrometry, anodic stripping voltammetry and atomic-absorption spectrometry offer the greatest promise as analytical tools for the determination of heavy metals in sea-water. Of these, flame and graphite-furnace atomic-absorption spectrometry are the most extensively employed because of their relative ease of operation, comparatively inexpensive intrumentation, applicability to a large number of elements and low detection limits (for graphite-furnace atomic-absorption).

Several attempts to analyse sea-water directly by graphite-furnace atomic-absorption spectrometry (GFAAS) have been undertaken.[2–8] Direct analysis is highly desirable as it entails minimum sample handling and pretreatment, thereby minimizing the risk of contamination and, more importantly, eliminating the reagent blank common to all chemical pretreatment methods. Although the interference arising from non-specific absorption by light-scattering from co-volatilized salts can be controlled by such techniques as simultaneous background correction, matrix modification,[6] selective volatilization[3] and judicious choice of the thermal pretreatment of the sample after deposition in the atomizer,[9] the sensitivities and detection limits are so seriously impoverished in the presence of the sea-water matrix that, even when metal concentrations are relatively high, direct analysis is limited to a very few elements. As a result of these limitations, a sample preparation technique is usually employed both to preconcentrate the trace elements from the sea-water and to separate them from interfering matrix components. A number of techniques have been used for this purpose, including co-precipitation and co-crystallization,[10,11] chelation and solvent extraction,[12–16] chelating ion-exchange resins[17–22] and electrolytic preconcentration.[23–25] Most separation methods in use, however, are based on the formation of metal–dithiocarbamate complexes and their extraction into an organic solvent. A widespread standard method for this purpose is the ammonium pyrrolidine-*N*-carbodithioate–methyl isobutyl ketone system[12–16] (commonly referred to as

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APDC–MIBK) which allows a multielement analysis of a single extract and may be conveniently used aboard research vessels. Because large-volume extractions are impractical, both from a theoretical and a physical point of view, an upper limit is placed on the sample preconcentration factor which can be achieved with such single-stage separations. For this reason the application of chelating resins to sea-water analysis has become increasingly popular.[17–22] Chelating resins are simple to use and allow much higher preconcentration factors to be attained. Additionally, the sample is not contaminated with heavy-metal impurities from buffers and organic reagents.

The present work was initiated to re-examine the potentialities of, and problems associated with, the APDC–MIBK solvent extraction system for the determination of Zn, Cd, Cu, Co, Cr, Mn, Fe, Ni and Pb in sea-water. These metals were also preconcentrated from water samples by use of the chelating resin Chelex-100. The relative merits of both separation methods have been examined.

EXPERIMENTAL

Apparatus

A Varian Techtron atomic-absorption spectrophotometer model AA-5 fitted with a Perkin-Elmer heated graphite atomizer model 2200 (HGA-2200) and temperature-ramp accessory was used for all trace-metal measurements. The base of the HGA-2200 furnace was modified so that it could be fitted to the optical rail of the spectrophotometer. A simultaneous background correction system similar to that described by Dick *et al.*[26] was used when required. The continuum source was a Hamamatsu deuterium hollow-cathode lamp. Sample solutions were delivered to the furnace in either 10- or 20-μl volumes with a Perkin-Elmer AS-1 auto-sampler, and absorbance peaks were recorded on a fast-response (< 300 msec full scale) Speed Servo II strip-chart recorder (Esterline Corp., Indianapolis, Ind., U.S.A.). The instrumental settings and furnace thermal programmes, optimized for each element, are in general agreement with those suggested by the Perkin-Elmer Corp. and are therefore not reproduced here.

A Beckman Century SS-1 pH-meter with a glass electrode was used for pH measurement.

Polypropylene beakers and borosilicate Squibb-type separatory funnels fitted with Teflon stopcocks and polypropylene tops were used for sample preparations and extractions.

Resin exchange columns were constructed from polypropylene tubing (0.9 and 1.4 cm bore) to which Teflon stopcocks were attached. A 3/16-in. thick polyethylene frit of 35 μm pore-size was fitted into the exchange columns in order to support the resin. To facilitate sample-loading, 100-ml polypropylene funnels were heat-welded onto the top of the columns by means of a soldering gun with a rhodium-plated copper tip. "Separatory-columns" were constructed from globular separatory funnels by fitting a porous frit into the stem of the funnel, as shown in Fig. 1. All sample extracts were stored in screw-capped 30-ml polypropylene bottles.

Reagents

Stock standard solutions (1000 mg/l.) of the elements of interest were prepared by dissolution of the pure metals or their salts. Following dilution with high-purity distilled demineralized water (DIW), their concentrations were verified by standardization against NBS Standard Reference Material 1643 (Trace Elements in Water).

All reagents were purified prior to use. Concentrated nitric, hydrochloric and acetic acids were prepared by sub-boiling distillation in a quartz still from reagent-grade feedstocks.[27,28]

A saturated solution of ammonia (28%) was prepared by bubbling gaseous ammonia (generated by evaporation of liquid ammonia) through a trap containing a basic solution of EDTA prior to passage into chilled high-purity water.[29]

Methyl isobutyl ketone was purified by sub-boiling distillation.

Fresh 5% aqueous solutions of ammonium pyrrolidine-*N*-carbodithioate (APDC, Baker Analyzed) were filtered through a 0.3-μm membrane filter to remove insoluble material and stripped free of metal impurities by repeated extraction with distilled MIBK. In order to stabilize the reagent, the solutions were adjusted to a pH of about 9 with ammonia.[30,31]

8-Hydroxyquinoline (Baker Analyzed) was purified by vacuum sublimation at 120° onto a cold finger (metal oxinate impurities are not volatilized below 160°[32,33]) and 2% solutions of 8-hydroxyquinoline (oxine) in dilute hydrochloric acid were prepared.

A 1*M* ammonium acetate buffer was prepared by combining 77 ml of 28% ammonia solution with 57 ml of glacial acetic acid and diluting to 1 litre. The buffer pH was adjusted to the desired value as required.

A 25% aqueous solution of ammonium nitrate was prepared by the neutralization of high-purity nitric acid with ammonia.

Chelex-100 resin (100–200 and 200–400 mesh, sodium form, Bio-Rad Laboratories, Richmond, CA, U.S.A.) was purified by batch extraction, by washing successively with 5*M* nitric acid, 4*M* hydrochloric acid and DIW. The resin was then converted into the ammonium form by equilibration with 1*M* ammonia and rinsed with DIW.

All reagents were analysed for trace-metal content prior to use. Sub-boiling distilled MIBK was analysed by evaporating a 100-ml aliquot to 5 ml in a polypropylene beaker, adding 1 ml of concentrated nitric acid and completing the evaporation. The residue was dissolved in 2 ml of 0.3*M* nitric acid and analysed by GFAAS. For analysis of the purified APDC a 20-ml aliquot of a 5% solution was

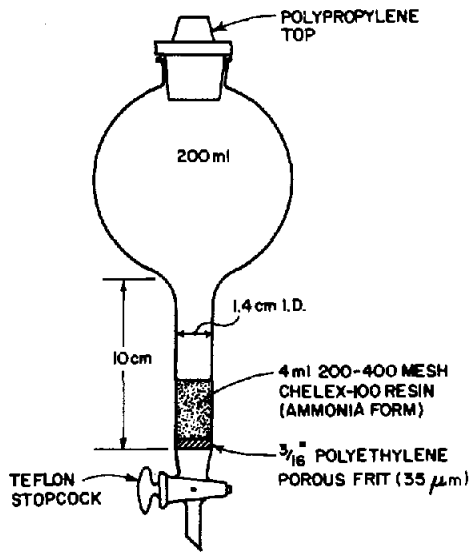

Fig. 1. "Separatory-column" used for Chelex-100 resin preconcentration of trace elements from sea-water.

evaporated to dryness in a Vycor crucible, 1 ml of concentrated nitric acid was added and the evaporation was completed. The residue was then ashed at low temperature in a muffle furnace and the ash taken up in 2 ml of 0.3*M* nitric acid and analysed by GFAAS. Oxine and acetate buffer solutions were analysed directly by GFAAS. DIW and concentrated nitric acid were analysed by spark-source mass-spectrometry.[27,28]

All evaporations were done on a porcelain hot-plate in a "clean" fume-hood.

Coastal sea-water was obtained from the Atlantic Regional Laboratory of the National Research Council of Canada in Halifax, Nova Scotia. The samples had been filtered through a nominally 0.45-μm membrane filter, acidified to pH 1.6 and stored in polyethylene bottles.

Procedures

All sample preparations and analyses were done in a "clean" laboratory equipped with laminar-flow "clean" benches and fume cupboards, providing a class 100 working environment.

All laboratory ware was thoroughly cleaned in nitric acid (1 + 1), rinsed with DIW, further cleaned with hot aqueous 0.05% APDC solution and again rinsed with DIW. Once in use, beakers and separatory funnels were washed with only DIW between runs, as frequent thorough cleaning was found to produce highly variable analytical blanks.

Solvent extractions. Sea-water samples were analysed by the method of standard additions, with successively larger metal spikes added to 100-ml samples of sea-water contained in 250-ml polypropylene beakers, 1.0 ml of 1*N* ammonium acetate buffer, 0.5 ml of 5% APDC solution and 0.5 ml of 2% oxine solution being added to each sample. Two reagent blanks were also prepared, consisting of the added chelating agents and buffer, as well as 15 ml of DIW to act as a physical carrier. The samples and blanks were adjusted to pH 4.0 and heated to 80° on a water-bath for 10 min in order to complex Cr.[31,34,35] The solutions were then transferred to separatory funnels and cooled to room temperature, and 15 ml of MIBK were added to each. Each mixture was shaken vigorously for 3 min and the phases were allowed to separate. After the aqueous phase had been drained from the separatory funnel and its pH adjusted to 9.2 with ammonia, it was recombined with the MIBK phase and the mixture shaken for a further 3 min in order to extract Mn. Following phase separation, the aqueous phase was discarded and the separatory funnel was rinsed with a small volume of DIW to remove the film of sea-water adhering to the glass surface. The metal complexes were then back-extracted by shaking the MIBK phase with 300 μl of concentrated nitric acid for 1 min, then adding 2.7 ml of DIW and shaking for a further 2 min. Following phase separation the 3.0-ml acid layer (~1.5*M* nitric acid) was drained into a 30-ml screw-capped polypropylene bottle. This procedure provided a 33-fold preconcentration of the sample. The MIBK phase was analysed for Co, as this element is not efficiently back-extracted.

Preconcentration on resin. Preconcentration of trace metals with Chelex-100 resin was performed with the aid of the usual resin column and also by the use of the "separatory-column", as described below. Fresh resin was used for each experiment. Operation of the resin columns was as described by Kingston.[36] The columns were slurry-loaded with 4 ml of precleaned NH_4^+-form resin. Two 5-ml portions of 5*M* nitric acid were passed through the resin, followed by two 5-ml portions of 4*M* hydrochloric acid 5-ml of DIW and 5 ml of 2*M* ammonia. The column was then washed with DIW to remove excess of base, and until the effluent pH was 8–9. Samples of 100 ml of sea-water were buffered to pH 5.4 with 4.0 ml of 1*M* ammonium acetate and loaded onto the columns. The effluent flow-rate was adjusted to 0.8 ml/min. After passage of the sample, the column was washed with 5 ml of DIW, four 10-ml portions of pH 5.2 1*M* ammonium acetate buffer and a further 5 ml of DIW. The trace metals were then eluted from the resin with 2.5*M* nitric acid and the eluate was collected in 2-ml fractions for analysis. The resin was cleaned, then regenerated with ammonia, and blanks were run.

For "separatory-column" treatment, the resin and sample were prepared in the same way as above. The buffered sample was added to the separatory-column and the trace metals were extracted by vigorous shaking of the mixture for 3 min. The resin was allowed to settle and the sea-water drained through the resin column at a rate of 1–2 ml/min. The interior of the separatory-column was rinsed with 5 ml of DIW, followed by four 10-ml portions of pH 5.2 1*M* ammonium acetate buffer. The resin column was allowed to drain completely after a final rinse with 5 ml of DIW. The trace metals were then stripped from the resin by vigorously shaking it with 5 ml of 5*M* nitric acid for 3 min. The phases were allowed to separate and the acid extract was drained from the funnel and diluted to 10.0 ml with DIW washings from the column. This eluate was 2.5*M* in nitric acid and was stored in 30-ml screw-capped polypropylene bottles. The resin was then cleaned and regenerated, and analytical blanks were run.

Direct analysis. Coastal sea-water was analysed for Fe and Mn by direct injection of 10-μl volumes into the atomizer. A 5-μl volume of 25% ammonium nitrate solution as matrix modifier[6] was then added and the sample subjected to the following atomization programme: ramp-dry to 110° during 40 sec, hold for 20 sec; ramp-ash to 1000° during 15 sec, hold for 5 sec; atomize by heating at maximum rate to 2700° in 5 sec (using temperature-controlled heating). Nitrogen gas, at a flow-rate of 40 ml/min, was used to purge the interior of the furnace during atomization.

Background correction was used during analysis of the nitric acid back-extracts from the solvent-extraction samples and also during direct analysis of sea-water.

All "standard additions" curves were analysed by regression procedures to obtain the intercepts.

RESULTS AND DISCUSSION

Solvent extraction

The APDC–MIBK system, being relatively unspecific, allows the simultaneous extraction of a large number of transition elements, while selectively rejecting the alkali and alkaline-earth metal ions. However, because of the difficulty encountered in extracting Mn, except when relatively large amounts of APDC are present,[37] as well as the extreme instability of the Mn complex in MIBK,[12,37,38] a double chelate system consisting of APDC and oxine was used, allowing recovery of Mn as the oxinate complex.

Back-extraction. Back-extraction of the trace metals into an acidic aqueous solution has not generally been practised, as objections have been raised with regard to the non-quantitative recovery of metals from the organic layer,[4] as well as the undesirability of increasing the number of steps in the analytical procedure.[39] Although some metals cannot be quantitatively back-extracted, the advantages are considerable. Back-extraction entails little extra sample manipulation and is unlikely to be a source of contamination. One of the shortcomings of the APDC–MIBK extraction system is the instability of the complexes in the organic phase.[7,14] Back-extraction stabilizes the

metals in an acidic solution from which losses are minimal;[14] sample extracts produced in this study suffered no significant change in metal concentration over a period of several weeks. As a consequence of the solubility of MIBK in sea-water (~2%) and its variation with temperature,[12] accurate calculation of the preconcentration factor is difficult. Use of the initial organic:aqueous phase-ratio will always give an underestimate of the preconcentration factor and lead to erroneously high extraction efficiencies if these are mistakenly calculated from the distribution coefficients and *original* phase volumes. Back-extraction of the metal complexes into an accurately known volume of acid eliminates this problem and also allows further preconcentration. A 30–50-fold preconcentration of trace-metals from a 100-ml sample of sea-water, easily achieved after back-extraction, cannot be attained simply with an MIBK extraction, not only because of the unfavourable magnitude of the distribution coefficients of the chelates,[12] but primarily because the solubility of MIBK in sea-water necessitates the use of at least 2 ml of organic phase for 100-ml of aqueous phase. Use of the back-extraction also eliminates problems encountered in pipetting μl volumes of MIBK into the graphite furnace. The low surface tension of MIBK makes sample delivery difficult and the MIBK tends to creep along the length of the furnace tube, severely limiting sample volumes or forcing the analyst to inject the MIBK while the furnace is warm. Also, the analytical response from organometallic compounds is often different from that from inorganic salts,[40] making the determination difficult unless organometallic standards and the same solvent are used. Other problems often encountered when MIBK is used include the variation of analytical response with volume of MIBK injected into the furnace[41] (for the same absolute mass of metal) and possible pre-atomization losses of volatile metal chelates or their decomposition products.[42,43]

Analytical blank. The blank is the most important single sample to be carried through the analytical procedure and it is advantageous to run two blanks simultaneously with the samples. The blank consists of the total of all metals in the reagents added as well as those introduced during sample manipulation and extraction. To ascertain the contribution from each reagent to the analytical blank the concentrations of heavy-metal impurities in all the reagents added were measured (Table 1). Significant amounts of Fe, Cu, Zn and Ni are present in the purified MIBK, possibly as acid-stable volatile organometallic compounds. In contrast to the findings of Stolzberg,[15] the APDC reagent was easily cleaned with MIBK to yield a product having sub-ppM (parts per milliard) concentration levels of heavy metals. Fe, Cd and Zn proved to be major impurities. Significant amounts of Fe, Cu, Cd and Zn remained in the vacuum-sublimed oxine. The acetate buffer contained appreciable amounts of Cd and Zn. No significant impurities were introduced from the DIW or concentrated nitric acid.

Typical analytical blanks obtained by solvent extraction are given in Table 2. Also shown are the calculated blanks based on the data given in Table 1, the volumes of reagents used and the assumption that all reagent impurities are transferred to the blank extract with 100% efficiency. All blanks are presented in terms of the absolute mass of each element. If a 100-ml sample of sea-water is extracted, for example, the total analytical blank for Fe would be 0.31 μg/l.

Table 2. Analytical blanks obtained by solvent extraction

Element	Blank, *ng*	
	Experimental*	Calculated†
Fe	31 ± 4	10
Cu	21 ± 5	11
Pb	7 ± 3	1
Cd	1.1 ± 0.9	0.7
Zn	62 ± 17	3
Ni	<0.08	<8
Mn	<0.006	<1
Co	<0.02	<1
Cr	<0.02	<1

* Average blanks obtained from 22 replicate determinations spanning a period of 12 weeks.
† Absolute blank calculated from total reagent impurities.

Table 1. Trace metals in purified reagents

Element	Concentration, μg/l.					
	MIBK	5% APDC	2% oxine	1*M* ammonium acetate	DIW*	15.8*M* HNO_3
Fe	0.41	2.9	4.1	<0.5	0.005	0.2
Cu	0.55	0.43	2.2	<1	0.007	0.06
Pb	0.04	0.41	<0.5	<0.5	0.002	0.01
Cd	0.02	0.33	0.30	0.10	<0.003	<0.003
Zn	0.13	0.90	0.22	0.40	0.006	0.01
Ni	0.11	0.30	<4	<4	0.002	0.008
Mn	0.004	0.06	<0.3	<0.3	<0.01	0.008
Co	0.05	0.08	<1	<1	<0.01	<0.01
Cr	0.02	0.10	<1	<1	0.002	0.03

* Demineralized water, analysed by spark-source mass-spectrometry.[27,28]

Table 3. Apparent recovery (%) of metal spikes added to sea-water

Element	Method of evaluation*				
	A	*B*	*C*	*D*	*E*
Fe	100	82	67	61	61
Cu	100	100	82	70	70
Ni	—	—	—	157	87
Cd	—	—	—	127	83

* *A*, Calculated from % $R = A_1/(A_1 + A_2)$ where A_1 and A_2 are the signals from the singly and doubly extracted samples respectively.
B, Direct analysis of MIBK extract *vs.* an aqueous standard.
C, Analysis of MIBK extract made up to original volume.
D, MIBK back-extracted with acid, *vs.* an aqueous standard.
E, MIBK back-extracted with acid, *vs.* a matrix-matched standard.

The extraction blanks appear to be satisfactorily low and reproducible. Although the experimental and calculated blanks follow the same general pattern, the experimental blanks exhibit significantly higher levels of Fe, Pb and Zn. High levels of these metals must arise as a result of sample manipulation and solution contact with laboratory wares. It would appear from our experience that the contamination due to leaching from laboratory ware is minimal[27] and that the major proportion of the unaccountable blank arises from external sources such as airborne contamination, both particulate and gaseous (*e.g.*, tetraethyl lead, metal carbonyls).

Extraction efficiency. The efficiency of extraction was evaluated by measuring the recovery of metal spikes added to sea-water samples. A number of methods have been used to calculate the recovery of spikes in solvent-extraction/atomic-absorption procedures[14,16] and the "apparent" recovery may vary for the same extraction, depending on the method of evaluation. In view of this, the apparent recoveries of Fe, Cu, Cd and Ni spikes from coastal sea-water were evaluated by the following methods: *A*, from the ratio of the absorbances for two aliquots, one extracted once and the other twice, it being assumed that the double extraction results in 100% recovery of the spike; *B*, from direct analysis of the MIBK phase, with an aqueous standard for calibration, without taking account of the amount of MIBK (~20%) which dissolves in the sea-water (a 10:1 sea-water:MIBK phase ratio was used); *C*, based on the direct analysis of the MIBK phase, with an aqueous standard, but with the volume of MIBK made up to its original value to correct for the amount dissolved by the sea-water; *D*, based on back-extraction of the metals from the MIBK into a known volume of dilute acid and comparison of the signal from this solution with that from an aqueous standard; *E*, based on back-extraction of the metals from the MIBK into a known volume of dilute acid and comparison of the signal from this solution with that from an aqueous standard which has been matrix-matched to the sample. A comparison of these five methods may be made from the results presented in Table 3. Ni and Cd recoveries were evaluated only by methods *D* and *E*. The data clearly show that the apparent efficiency of extraction varies with the method of calculation. Clearly, evaluation by methods *A*–*D* can be subject to large error. If a portion or all of the added spike has equilibrated with the sample before extraction, evaluation of the recovery by method *A* makes no allowance for any metal lost as non-extractable species. Direct analysis of the MIBK phase, without taking solubility losses into account, leads to high recovery values as a result of the disregarded preconcentration of the sample. Analysis of the MIBK phase is difficult because the analytical response of metal chelates in MIBK may be different from that obtained with aqueous solutions of metal salts.[40] Accurate measurement of the spike recovery would require MIBK solutions of organometallic standards for calibration. Provided the back-extraction is quantitative, calculation of spike recoveries is generally more reliable when the aqueous phase from the stripping is analysed. There are circumstances, however, under which erroneous recovery values are still obtained, as in the case of Ni and Cd. Investigation revealed that the discrepancy was caused by interference from oxine in the back-extract producing an enhancement of the signals. Calibration against a matrix-matched standard eliminated this problem. Because of the difficulty of evaluating the true spike recovery by atomic-absorption techniques, these values have been labelled "apparent" throughout this paper.

The apparent recoveries of spikes added to demineralized water and sea-water are shown in Table 4. The values fluctuate from run to run by about 10%, but are identical within a standard-additions run, as shown by the straight lines obtained. The two values reported for Co and Cr in sea-water are the recoveries of each metal in both the back-extract and the MIBK phase (made up to original volume). Back-extraction is quantitative for the remaining 7 elements. Chro-

Table 4. Apparent recovery of metal spikes added to sea-water

Element	Recovery, %	
	Demineralized water	Sea-water*
Fe	100	76
Cu	100	75
Pb	100	78
Cd	100	83
Zn	90	27
Ni	95	87
Mn	90	89
Co	90_{MIBK}	$12/71_{MIBK}$
Cr	90_{MIBK}	$17/73_{MIBK}$

* Average of 10 replicate determinations on 75-ml aliquots of sea-water preconcentrated by a factor of 25.

Table 5. Precision of solvent extraction method with coastal sea-water

	Sample A			Sample B		
Element	Mean*, μg/l.	SD†	RSD**	Mean, μg/l.	SD	RSD
Fe	1.00	0.12	12	4.87	0.50	10
Cu	0.71	0.09	13	1.20	0.09	8
Pb	0.49	0.08	16	0.39	0.07	18
Cd	0.049	0.006	12	0.098	0.010	10
Zn	4.51	1.16	26	2.63	0.43	16
Ni	0.17	0.01	6	0.49	0.07	14
Mn	1.36	0.28	21	3.71	0.60	16
Co	<0.02	—	—	<0.02	—	—
Cr	0.49	0.24	49	0.28	0.12	43

* Average of 5 replicate determinations on 100-ml aliquots preconcentrated by a factor of 33.
† SD = standard deviation (μg/l.).
**RSD = relative standard deviation (%).

mium must be heated with APDC in order to form the carbamate complex.[31,34,35] No extraction is obtained at room temperature. Extraction of both Cr(III), added as $KCr(SO_4)_2$, and Cr(VI), added as $K_2Cr_2O_7$, was equally efficient, in agreement with results reported by Morris.[43] Co and Cr form extremely non-labile complexes which are relatively inert to attack by even concentrated acid. The MIBK plays a major role in stabilizing the carbamate complex, as Co can be back-extracted quantitatively from an inert solvent such as a freon, *i.e.*, 1,1,2-trichloro-1,2,2-trifluoroethane[14,44] and Cr can be back-extracted with an efficiency of at least 80% from this solvent.[44] Back-extraction of these elements from MIBK is slow, and the efficiency can be increased by shaking the MIBK–acid mixture for a longer period of time.

Although metal spikes can be quantitatively extracted from demineralized water, the recoveries are substantially lower with sea-water. Low spike recoveries from complex matrices are usually attributed to competitive equilibrium reactions binding metal ions into non-extractable forms. In sea-water such losses may include formation of anionic chloro-complexes, non-reversible adsorption on colloidal particles and complexation with naturally occurring ligands to yield non-labile hydrophilic complexes. Despite the fact that spikes can sometimes be quantitatively recovered even from sea-water, it is imperative that the analyst does not lose sight of the fact that added spikes frequently do not equilibrate with the sample[45,46] during the short time required for a typical solvent extraction. The recovery of an ionic spike may bear no relation to the recovery of a metal naturally present in the water, and consequently "well-behaved" standard-additions data may yield completely misleading results.

Precision. The precision of the extraction method was evaluated by analysing coastal sea-water samples and results are presented in Table 5. The data serve to illustrate the precision of the technique as well as the levels of trace metals typically present in coastal sea-water. The precision of determination depends on the element and its concentration. The relative standard deviation is generally lower for higher concentrations of a given metal. Lower RSDs are also obtained for those metals exhibiting low extraction blanks (Table 2). The mean relative standard deviation is 18% (range 6–49%). With the present preconcentration factor (33-fold), Co could not be detected. Subsequent analysis of 800-ml aliquots of sea-water by preconcentration on chelating resin indicated Co levels of approximately 0.02 μg/l. Chromium determinations are the most imprecise, owing to the low natural levels of this element in sea-water, its poor extraction characteristics and poor sensitivity in GFAAS.

Jan and Young[13] have reported that for most metals lower RSD values were obtained for the back-extraction analysis than for direct analysis of the MIBK phase. Such a comparison was not made in this study.

Chelating ion-exchange

Samples of coastal sea-water were also analysed after preconcentration with Chelex-100 resin. Chelex-100, a purified form of Dowex A1 resin, consists of a cross-linked polystyrene matrix with iminodiacetic acid [$-CH_2-N(CH_2COOH)_2$] functional groups. The ability of this resin to retain selectively transition metals from saline media is related to the relative strength of the bonding between the cation and the resin. Alkali and alkaline earth elements do not form chelates with Chelex-100 (except at very high pH)[47,48] whereas many transition metal ions form 1:1 chelate complexes with Chelex-100 with stability constants lower by a factor of 10–100 than those of the corresponding EDTA complexes.[49]

Most laboratories appear to use a chelating resin column in the manner originally described by Riley and Taylor[17] or as recommended by Florence and Batley,[19,50] *i.e.*, sea-water at natural pH (pH 8.1), or buffered in the pH-6 range, is passed through a 6-ml bed of 50–100 mesh Chelex-100 resin (hydrogen or

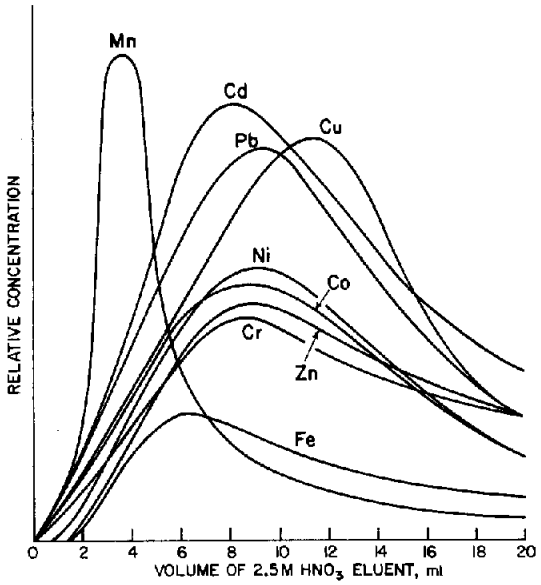

Fig. 2. Elution of trace metals from a 6 × 0.9 cm column of Chelex-100 with 2.5*M* HNO_3 at a flow-rate of 0.8 ml/min.

ammonium form). The transition-metal and the alkali and alkaline-earth metal ions are then eluted with acid and this eluate analysed. Recently, Kingston[36] reported that 1*M* ammonium acetate buffer at pH 5.2 could be used to strip the alkali and alkaline earth metals from the resin before elution of the transition metals, producing a matrix-free eluate, which would permit the utilization of a variety of instrumental techniques otherwise excluded from trace metal analysis of saline samples.

Following Kingston's method,[36] samples were passed through a 3 × 0.9 cm column of 200–400 mesh Chelex-100 resin at a flow-rate of 0.8 ml/min. The sea-water was buffered to pH 5.4 in order to take advantage of the maximum distribution coefficients for trace metals[48,51] and minimum distribution coefficients for Ca and Mg[48] which occur in the pH range 5–6. Despite duplication of all reported experimental variables, however, transition metals could not be eluted from the column in a 7.5-ml volume of 2.5*M* nitric acid, as reported by Kingston.[36] Figure 2 shows the elution curves for the trace metals in 2.5*M* nitric acid. Severe tailing was experienced and the metals could not be recovered with less than 30 ml of acid. This volume could not be substantially reduced even when 5.0*M* nitric acid or *aqua regia* was used for elution. This same difficulty was experienced when 100–200 mesh Chelex-100 resin was used. Use of a wider column (1.4 cm bore) sharpened elution peaks considerably, but quantitative recovery of the metal spikes still required 15–20 ml of acid. Pakalns *et al.*[21] have discussed the problems of recovering trace metals from the resin column and concluded that a minimum of 25 ml of dilute nitric acid is required to elute metals from a 50–100 mesh resin. Although a finer particle size is more efficient for metal removal, elution is very inefficient, even from a 100–200 mesh resin,[21,52] requiring more than 100 ml of acid for quantitative spike recovery.[52] Consequently, only small concentration factors can be achieved unless large sample volumes are used. In addition to this problem, swelling and contraction of the resin (as the counter-ion is changed) create difficulties in maintaining column flow-rates, necessitating frequent operator attention. As a result of these difficulties, column operation of Chelex-100 was abandoned in favour of a "separatory-column" technique (Fig. 1). Separatory-column operation allows any resin particle size to be used without any of the attendant problems associated with column operation. Larger preconcentration factors can be achieved, as metals can be recovered with less than 10 ml of eluent. This is particularly significant when only small volumes of sample are available.

Analytical blanks. Analytical blanks obtained with 200–400 mesh Chelex-100 resin used in a separatory-column are shown in Table 6. With the exception of Fe, blanks are equal to or lower than those obtained by solvent extraction. The blank for zinc is notably lower because of the fewer sample manipulation steps and because the only reagent used for sample work-up is the acetate buffer. With the exception of Fe, the blank can be accounted for solely from the added buffer (Table 1). The small number of sample manipulation steps precludes contamination from other sources. The polyethylene frit used to support the resin was identified as the primary source of the high Fe blanks. Spectrographic analysis (d.c. arc) indicated the presence of about 20 μg of Fe in a typical frit. Acid leaching of 4 ml of Chelex-100 resin (NH_4^+-form) accounted for only 12 ng of the 65-ng Fe blank. The large reservoir of Fe in the frit, 300 times the analytical blank, probably accounts for the persistent leaching of this metal into the acid eluent.

Recovery of spikes. The efficiency of recovery of spikes added to both demineralized water (DIW) and sea-water is shown in Table 7. The values quoted for DIW were obtained without washing the resin with

Table 6. Analytical blanks obtained with Chelex-100

Element	Blank, *ng*	
	Experimental*	Calculated†
Fe	65 ± 6	<22
Cu	14 ± 6	<44
Pb	15 ± 6	<22
Cd	1 ± 1	4
Zn	14 ± 3	17
Ni	<4	<176
Mn	<0.3	<13
Co	<1	<44
Cr	<1	<44

* Average blanks obtained from 24 replicate determinations spanning a period of 8 weeks.

† Absolute blank calculated from total reagent impurities.

Table 7. Apparent recovery of metal spikes added to sea-water

Element	Recovery, %	
	Demineralized water	Sea-water*
Fe	94	57
Cu	100	77
Pb	100	95
Cd	100	89
Zn	100	96
Ni	100	85
Mn	94	44
Co	80	86
Cr	36	8

* Average of 5 replicate determinations on 100-ml aliquots of sea-water preconcentrated by a factor of 10.

pH-5.2 ammonium acetate before stripping the metals with acid. With the exception of Cr, recovery of spikes from DIW was very satisfactory. Whereas Riley and Taylor[17] reported that Cr is very strongly retained by the resin and that no reagent could be found which would elute it completely, in these studies the affinity of the resin for either oxidation state of Cr was low. The resin is not well suited for the determination of Cr under the conditions reported here.

Significantly lower recoveries were obtained for Fe, Cu, Mn and Cr when spikes were added to sea-water. The inorganic matrix is not responsible for this depressive effect;[19,20] low spike recoveries for these elements occur because the acetate wash used to remove Ca and Mg carries with it some of these elements, the greatest loss being for Mn. Although the resin is preferentially selective towards transition metals, the selectivity coefficients for Ca and Mn are almost equal.[53] Washing the resin with pH-5.2 acetate buffer removes 99.9% of the Ca, Mg and K, but a substantial amount of Mn is also lost, as well as some Fe, Cu and Cr. Low recovery of Fe and Mn may also reflect possible losses of these elements by hydrolysis. Adjustment of pH with 1*M* ammonia solution can create high hydroxide ion concentration at the point of mixing of the sea-water and base, leading to hydrolysis of these elements. Relaxation back to equilibrium concentrations at the buffered pH may be very slow compared with the time of the experiment.[54] Such species do not interact with the resin,[21,55] leading to low spike recoveries. Recently, Pakalns *et al.*[21] recommended the addition of sodium tripolyphosphate (20 mg/l.) immediately before pH adjustment, to complex Fe and Mn, thus preventing hydrolysis. Such problems were not encountered with DIW solutions because they did not require the same excessive addition of base as acidified sea-water.

No significant difference in spike recoveries could be observed when 100–200 mesh resin was used. No degradation in performance of the resin was noted after cycling the resin through 10 sample loadings, strippings and regenerations.

Precision. The resin preconcentration technique was evaluated by analysing the coastal sea-water samples, and the results are presented in Table 8. The precision of the determination depended on the element determined and ranged from 6 to 36% relative standard deviation. The mean RSD of 19% is the same as that obtained by solvent extraction preconcentration of this same sample. The large RSDs for Pb and Cr are due to the relatively large and variable Pb blank and the poor recovery and low sensitivity for Cr.

Analytical results. Comparison of the concentration levels found by the two methods (resin preconcentration and solvent extraction preconcentration) (Tables 5 and 8, sample B) indicates that there is generally good agreement. However, results for Cu, Pb and Cd appear to be distinctly lower by the resin preconcentration method than by solvent extraction.

Batley and Florence[46] have shown that a major proportion of "dissolved" Cd, Pb and Cu can be associated with organic and inorganic colloidal species in sea-water. Such species may include hydrous oxides, silicas, clays and sulphides, as well as humic substances and plant and animal detritus. Colloidal particles are not retained by Chelex-100 as the resin pore size is too small to allow the colloids to enter the resin network.[19,46] With the assumption that the low results obtained for these 3 elements could be attributed to this phenomenon, a 450-ml aliquot of the sea-water was acidified to pH 1 with concentrated nitric acid, 500 μl of 30% hydrogen peroxide were added and the solution was irradiated for 24 hr in a quartz cell with a 500-W Hg–Xe ultraviolet source. During this time the sample temperature remained at 70°. With such treatment, large organic molecules, as well as inorganic and organic colloidal material, will decompose, liberating trapped metal ions.[19,55,56] Subsequent analysis of the sample showed a distinct increase in the concentration of Cu, Pb and Cd (Table 8, sample C), bringing the results for these elements into agreement with those obtained by solvent extraction.

The good agreement between the results obtained

Table 8. Chelating ion-exchange preconcentration of sea-water

Element	Concentration, $\mu g/l.$	
	Sample B*	Sample C†
Fe	5.51 ± 0.35	5.22
Cu	0.91 ± 0.06	1.20
Pb	0.19 ± 0.05	0.25
Cd	0.078 ± 0.013	0.100
Zn	3.30 ± 0.58	3.28
Ni	0.48 ± 0.13	0.58
Mn	4.15 ± 0.49	3.77
Co	<0.1	<0.1
Cr	0.33 ± 0.12	0.25

* Average of 5 replicate determinations on 100-ml aliquots of sea-water preconcentrated by a factor of 10.

† Sea-water sample after 24 hr irradiation with a 500-W Hg–Xe lamp at 77°, pH = 1.

by solvent extraction and ion-exchange preconcentration procedures for non-irradiated samples may be due to the low pH at which the sea-water is stored; some of the metals originally trapped in colloidal species may be released on long standing. There is the possibility of metal-bearing colloidal organic species dissolving in MIBK, leading to recovery of this metal fraction in the back-extraction step in the solvent extraction preconcentration.

Direct determination

Of the elements determined in the sample of coastal sea-water, Fe and Mn were chosen for direct determination by GFAAS. Both elements are present in fairly high concentration, exhibit reasonable sensitivity in GFAAS and have appearance temperatures sufficiently high for removal of the more volatile sea-water matrix to be facilitated with the use of high charring temperatures and matrix modification techniques.[6] Peak background absorbances of 0.25, easily handled by the background correction system, were obtained from 10-μl volumes of sea-water. Analysis by the method of standard additions yielded Fe = 5.63 $\pm$ 0.69 μg/l. and Mn = 3.75 $\pm$ 0.40 μg/l., in excellent agreement with the results obtained by analysis after preconcentration by solvent extraction and by Chelex-100 resin.

CONCLUSIONS

Direct analysis of sea-water is preferable to analytical schemes involving chemical separation and preconcentration, but the low concentrations of most elements in many sea-water samples preclude the use of this technique. Preconcentration of trace elements on chelating ion-exchange resin allows rapid processing of large volumes of sea-water and is well suited to speciation studies. Use of a separatory-column technique allows greater sample preconcentration factors to be obtained than are currently possible with conventional column operation. This is particularly advantageous when only small volumes of sea-water are available for analysis. With a minimum of sample handling and no contamination from organic reagents, analytical blanks are much easier to control than those generated by solvent extraction. Chelation and solvent extraction with back-extraction is preferable to resin preconcentration when only small volumes of sea-water are available, as substantial preconcentration can be obtained. Neither technique, however, is well suited to the simultaneous determination of all 9 elements of interest, in a single extract.

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ON THE COMPLEX-FORMATION BETWEEN Cd(II) AND EDTA

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Summary—The complex-formation between cadmium and EDTA has been studied at 25° in 1.0*M* sodium nitrate medium, by measuring with a glass electrode the hydrogen-ion concentrations of a series of solutions containing varying amounts of cadmium and EDTA, and the free concentration of cadmium with a cadmium-amalgam electrode. The experimental data, which were analysed by using the ETITR version of the general error-minimizing computer program LETAGROP, may be explained satisfactorily by assuming the formation of the species CdL, CdHL, CdH_2L and Cd_2L in the pH range 1.8–6.5. The equilibrium constants for the formation of these species are also reported.

The complex-formation between cadmium and EDTA has been studied by a number of workers using different methods, such as potentiometry,[1–3] polarography,[4,5] liquid–liquid extraction[6] and electrophoresis.[7] Some of these methods and the values of the equilibrium constants thus obtained have been reviewed critically by Anderegg.[8] A survey of the literature, however, reveals that no polynuclear Cd–EDTA complexes have been reported so far, although from structural considerations the formation of such species is not unexpected. Indeed, in the electrophoresis study of the complexation of cadmium and zinc with EDTA,[7] the experimental data seem to indicate the formation of a binuclear species when an excess of cadmium is present.

The present work was undertaken when we obtained some results of biochemical significance[9] in our experiments on the influence of different Cd–EDTA complexes on the toxicity and distribution of cadmium in mice. These results gave rise to a suspicion that the equilibrium data so far reported in the literature might be incomplete, thus demanding a thorough investigation of the possibility of formation of a binuclear complex, especially at higher cadmium concentrations. Furthermore, in view of the fact that in chelation therapy EDTA is one of the few complexing agents that are administered to remove the heavy metals from biological tissues,[10] we believe that an equilibrium analysis of solutions, covering a wide range of the total concentrations of metal ion, EDTA and hydrogen ion in order to establish the presence of all probable species, and the equilibrium data on their formation, can provide useful information not only to analytical chemists but also to biologists and ecologists.

EXPERIMENTAL

Reagents

Cadmium nitrate was prepared by dissolving the pure metal in nitric acid, and recrystallized. A solution prepared from the recrystallized salt was standardized with EDTA at pH 10 (ammonia–ammonium nitrate buffer; Eriochrome Black T indicator). Solutions of the disodium or trisodium salt of EDTA were prepared from the free acid by appropriate neutralization, and then standardized with lead nitrate solution at pH 5 (hexamine buffer; Methylthymol Blue indicator). Carbonate-free sodium hydroxide[11] was standardized potentiometrically by the Gran method as modified by Pehrsson *et al.*[12] Sodium nitrate (Merck p.a.) was recrystallized before use. Doubly distilled and freshly boiled water was used to prepare all the solutions.

Apparatus

The titrations were performed in a covered titration vessel of about 200 ml capacity, with five inlets for electrodes, burette, degassing tubes *etc.* In titrations where the amalgam electrode was also used, the titration vessel was replaced by a round-bottomed flask specially designed to hold the amalgam and with provision for insertion of the electrodes, burette and degassing tubes *etc.*

A micro combination glass and silver–silver chloride reference electrode was used. Cadmium amalgam was prepared by electrolysing a cadmium solution over polarographic grade mercury (Merck p.a.), using the arrangement described by Aladjoff[13]. The cadmium concentration in the amalgam was about 0.1% w/w. We chose to work with this concentration because the response has been found to be faster than that obtained with higher concentrations. To check the performance of the electrode during the experiment, the response of two similar electrodes was measured simultaneously. The two readings always agreed within 0.1 mV.

Titrant was added with a pneumatically-operated reagent pipette (AGA, Lidingö, Sweden) that can be adjusted to deliver any volume between 0.1 and 5 ml with a high degree of reproducibility. The e.m.f. values were measured to 0.1 mV with a digital voltmeter.

The temperature of the titration vessel was kept at 25 ± 0.05° by means of a paraffin oil-bath. All measurements were made in a room maintained at approximately 25°.

The solutions were protected from atmospheric carbon dioxide by maintaining a nitrogen atmosphere over them. The nitrogen was taken from a cylinder, passed through "Ascarite" and then saturated with water vapour by bubbling it through 1.0*M* sodium nitrate.

Procedure

In the first series of titrations, with the glass electrode, the cell can be written as:

Reference half-cell	v ml of 1.0*M* $Na(NO_3, OH)$ / V_0 ml of 1.0*M* (Na, Cd, H)(NO_3, L)	Glass electrode

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The hydrogen-ion concentration, $[H^+]$, was calculated from the equation

$$E = E'_{0_E} + Q \log [H^+] + E'_j \qquad (1a)$$

where E'_{0_E} is the standard potential, including the reference electrode potential and the part of the junction potential that is independent of the acidity, and $Q = \ln 10/nF = 59.157/n$ mV at 25°. $E'_j = \Sigma (j_{lmn} c_{lmn})$; see equation (2) for l, m and n. The quantities E'_0 and j_{H^+} in the Nernst relationship (1a) were determined, as a rule, before and after each titration, as described by Pehrsson *et al.*[12] The E'_0 values before and after each titration were constant within 0.2 mV and j_{H^+} was −23 mV.

In the other series of titrations where the cadmium amalgam electrode was also used, the measuring system can be represented as:

Reference|Solution|Cd(Hg) electrode

Reference|Solution|Glass electrode

where the solution had the composition 1.0*M* (Na, Cd, H)(NO_3, L) or 1.0*M* (Na, Cd, H)(NO_3) depending on whether the titrant used was 1.0*M* Na(NO_3, OH) or 1.0*M* Na(NO_3, L).

Here, the hydrogen-ion concentration was calculated as before and the concentration of free cadmium, [Cd(II)] was calculated according to the relationship

$$E = E'_{0_h} + \tfrac{1}{2} Q \log [\mathrm{Cd(II)}] + E'_j \qquad (1b)$$

CALCULATIONS AND RESULTS

The experimental data from five different sets of e.m.f. titrations with glass electrode only, are plotted in Fig. 1 as $Z = (H - h)/C_{Cd}$ as a function of log $[H^+]$, where $h = [H^+]$ denotes the free concentration of hydrogen ions and H is the total concentration of protons added. Figure 2 shows the data from another set of titrations using both the glass and the cadmium-amalgam electrode, the plot being of **F** as a function of log [Cd(II)], where **F** is $(H - h)/C_{Cd}$, log $(C_{Cd}/[\mathrm{Cd(II)}])$ or $(C_{Cd} - [\mathrm{Cd(II)}])/C_L$, and [Cd(II)] denotes the free concentration of cadmium.

We assume that the following equilibrium reactions can take place in the solution in the course of titration: (*a*) protolysis of EDTA; (*b*) hydrolysis of cadmium ions; (*c*) the reactions leading to the formation of various Cd–EDTA species.

The protolysis of EDTA in 1.0*M* sodium nitrate medium at 25° was studied earlier,[14] and the protonation constants were evaluated by using the computer program LETAGROP.[15] Biedermann and Ciavatta[16] made a detailed study of the hydrolysis of Cd(II) in 3*M* sodium perchlorate in the pH range 5.0–7.5 at rather high concentrations (0.1–1.45*M*) of Cd(II). Since we worked with the pH range 1.8–6.5 and the Cd(II) concentrations were much lower (<0.08*M*), we considered it reasonable not to include any hydrolysed Cd(II) species in analysing our data. This assumption was further justified when we made some HALTAFALL[17] calculations, which did not indicate the presence of any hydrolysed species under the experimental conditions used. The following reactions are the most probable between Cd(II), H^+ and EDTA. Charges are omitted for convenience.

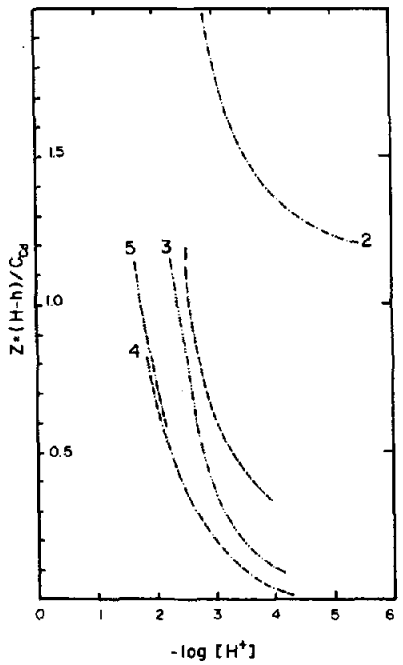

Fig. 1. $Z = (H - h)/C_{Cd}$ as a function of log $[H^+]$. The curves shown represent the titration of solutions of initial composition as follows: 1 (---) $C_H = 6.23_1 \times 10^{-3}M$, $C_{Cd} = 2.71_7 \times 10^{-3}M$, $C_L = 3.11_5 \times 10^{-3}M$, and $V_0 = 80$ ml; 2 (-·-·) $C_H = 1.94_4 \times 10^{-2}M$, $C_{Cd} = 5.97_4 \times 10^{-3}M$, $C_L = 9.97_0 \times 10^{-3}M$, and $V_0 = 75$ ml; 3 (-··-) $C_H = 1.57_4 \times 10^{-2}M$, $C_{Cd} = 7.62_4 \times 10^{-3}M$, $C_L = 7.87_1 \times 10^{-3}M$, and $V_0 = 95$ ml; 4 (-···-) $C_H = 3.98_8 \times 10^{-2}M$, $C_{Cd} = 2.89_7 \times 10^{-2}M$, $C_L = 1.99_4 \times 10^{-2}M$, and $V_0 = 75$ ml; 5 (—0·) $C_H = 4.98_5 \times 10^{-2}M$, $C_{Cd} = 3.62_1 \times 10^{-2}M$, $C_L = 2.49_2 \times 10^{-2}M$, and $V_0 = 80$ ml; against 0.0586*M* sodium hydroxide.

$$\mathrm{Cd} + \mathrm{L} \rightleftharpoons \mathrm{CdL}$$

$$\mathrm{Cd} + \mathrm{H} + \mathrm{L} \rightleftharpoons \mathrm{CdHL}$$

$$\mathrm{Cd} + 2\mathrm{H} + \mathrm{L} \rightleftharpoons \mathrm{CdH_2L}$$

$$\mathrm{Cd} + 3\mathrm{H} + \mathrm{L} \rightleftharpoons \mathrm{CdH_3L}$$

$$2\mathrm{Cd} + \mathrm{L} \rightleftharpoons \mathrm{Cd_2L}$$

where L denotes the fully depronated form of EDTA.

Chemical model

For the reagent components Cd, H and L we can represent the formation of different species by the general expression $(Cd)_l(H)_m(L)_n$, with the equilibrium constants β_{lmn}, given by

$$\beta_{lmn} = [(Cd)_l(H)_m(L)_n][Cd]^{-l}[H]^{-m}[L]^{-n} \qquad (2)$$

None of the models in this work contains more than one mole of L per mole of complex. Consequently, this expression, in the following, will be simplified to

$$\beta_{lm} = [(Cd)_l(H)_m(L)][Cd]^{-l}[H]^{-m}[L]^{-1} \qquad (2a)$$

The following mass-balance equations are valid

$$C_{Cd} = [Cd] + \Sigma l \beta_{lm} [Cd]^l [H]^m [L] \qquad (3)$$

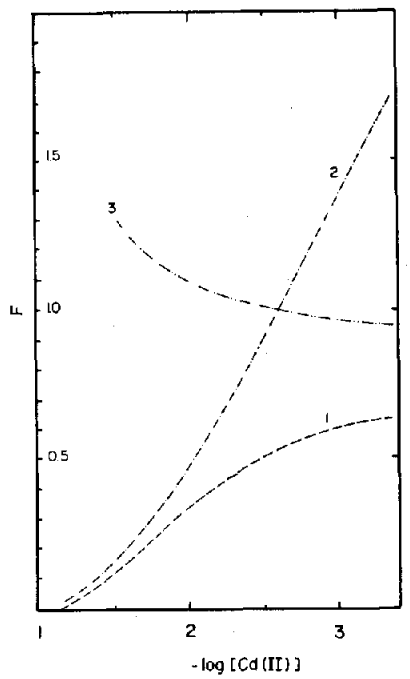

Fig. 2. F as a function of log [Cd(II)], where $F = (H - h)/C_{Cd}$, curve 1 (---); $F = \log (C_{Cd}/[Cd(II)])$, curve 2 (——); $F = (C_{Cd} - [Cd(II)])/C_L$, curve 3 (—·—); [Cd(II)] is the free concentration of cadmium. The data plotted are from the titration of solution of initial composition $C_H = 3.31_7 \times 10^{-7} M$, $C_{Cd} = 7.23_9 \times 10^{-2} M$, and $V_0 = 60$ ml. The composition of the titrant was $C_H = 2.38_0 \times 10^{-2} M$ and $C_L = 3.19_0 \times 10^{-2} M$.

$$C_H = [H] + \Sigma m\beta_{lm}[Cd]^l[H]^m[L] \quad (4)$$

$$C_L = [L] + \Sigma\beta_{lm}[Cd]^l[H]^m[L] \quad (5)$$

where the quantities on the left-hand side of equations (3), (4) and (5) represent the total analytical concentration of cadmium, the total concentration of ionized or potentially ionizable hydrogen and the total concentration of EDTA, respectively.

For potentiometric titrations the following relationship is applicable:

$$C_{H_{exp}} = (C_H^* V_0 - C_{OH}^* v)/(V_0 + v) \quad (6)$$

where

C_H^* = the initial concentration of hydrogen ion (mole/l.) in the titration vessel;
C_{OH}^* = the concentration of sodium hydroxide (mole/l.) in the titrant;
V_0 = the initial volume (litres) of the solution in the titration vessel;
v = the volume (litres) of titrant added.

Computer analysis of the data

For the given values of log [H], C_{Cd} and C_L, and a set of equilibrium constants β_{lmn}, the computer program LETAGROP–ETITR[15] can calculate, for each titration point, the values of [Cd], C_H ($C_{H_{calc}}$) and [L] from (3), (4) and (5), respectively. This is done by using the procedure BDTV,[18] which is also used to calculate C_H ($C_{H_{exp}}$) from (6).

Supplied with the initial estimates of β_{lmn}, the program seeks the "best" values of the set of equilibrium constants, minimizing the error-square sum (*typ 1, val 1*)

$$U = \sum_{1}^{Np} (C_{H_{calc}} - C_{H_{exp}})^2 \quad (7)$$

where Np is the number of experimental points available.

To analyse the experimental data when the concentration of free cadmium, [Cd(II)], was also measured with the cadmium-amalgam electrode, another routine (*typ 3, val 1*) was used. In this case the program calculates the concentration of free cadmium [Cd(II)] not from equation (3), but from the given (V, E) data from the amalgam electrode and minimizes the same error-square sum, equation (7).

In practice the program is used to test various chemical models. The "best" model accepted is the one which gives the minimum error-square sum, U_{min}, and which can explain the given data satisfactorily, within the limits of experimental error. Once the best model has been obtained, the program HALTAFALL[17] can be used to calculate the equilibrium concentrations of different species.

Results

The protonation constants of EDTA that were used are given in Table 1. The sets of experimental data from the glass electrode only and from the glass and amalgam electrodes were analysed separately. Since the formation of the species CdL and CdHL is well established, and the present data show that the average number of Cd(II) ions to each EDTA ion is greater than unity (Fig. 2) indicating the formation of polynuclear species at higher Cd(II) concentrations, we considered it reasonable to test the chemical models given in Table 2. The results of the calculations, summarized in the table, show that model II, in which the formation of the species CdL, CdHL, CdH_2L and Cd_2L is assumed, gives the least error-

Table 1. The equilibrium constants log β_{lm} for the protolysis of EDTA in 1.0M (Na, H) (NO_3, L) medium at 25°, which minimize the error-square sum,

$$U = \sum_{1}^{150} (C_{H_{calc}} - C_{H_{exp}})^2;$$

the limits given correspond approximately[14] to log $[\beta \pm 3\sigma(\beta)]$

Equilibrium reactions	log $[\beta \pm 3\sigma(\beta)]$
$H + L \rightleftharpoons HL$	9.99 ± 0.02
$2H + L \rightleftharpoons H_2L$	16.05 ± 0.02
$3H + L \rightleftharpoons H_3L$	18.52 ± 0.04
$4H + L \rightleftharpoons H_4L$	20.42 ± 0.06

$U_{min} = 11.6$; $\sigma(H) = (U_{min}/Np)^{1/2} = 0.23$.

Table 2. The equilibrium constants log β_{lm} for the formation of species $(Cd)_l(H)_m(L)$ in 1.0M (Na, H) (NO_3, L) at 25°, which minimize the error-square sum

$$U = \sum_{1}^{Np} (C_{H_{calc}} - C_{H_{exp}})^2$$

Model	Equilibrium reactions	Np = 120 Glass electrode only log $[\beta \pm 3\sigma(\beta)]$	U_{min}	σ(H)	Np = 60 Glass + amalgam electrode log $[\beta \pm 3\sigma(\beta)]$	U_{min}	σ(H)
I	$Cd + L \rightleftharpoons CdL$	15.52 ± 0.05	2.37	0.142	15.17 ± 0.17	8.69	0.394
	$Cd + H + L \rightleftharpoons CdHL$	18.05 ± 0.05			17.67 ± 0.14		
	$Cd + 2H + L \rightleftharpoons CdH_2L$	19.63 ± 0.08			18.96 max 19.24*		
II	$Cd + L \rightleftharpoons CdL$	15.56 ± 0.05	1.54	0.115	15.30 ± 0.04	1.86	0.184
	$Cd + H + L \rightleftharpoons CdHL$	18.11 ± 0.05			17.87 ± 0.02		
	$Cd + 2H + L \rightleftharpoons CdH_2L$	19.76 ± 0.09			19.39 ± 0.02		
	$2Cd + L \rightleftharpoons Cd_2L$	16.92 ± 0.22			16.58 ± 0.07		
IIa	$Cd + L \rightleftharpoons CdL$	15.47 ± 0.01	1.57	0.114	15.30 ± 0.14	1.86	0.184
	$Cd + H + L \rightleftharpoons CdHL$	18.01 ± 0.01			17.86 ± 0.21		
	$Cd + 2H + L \rightleftharpoons CdH_2L$	19.63 ± 0.01			19.38 ± 0.13		
	$2Cd + L \rightleftharpoons Cd_2L$	16.54 ± 0.01			16.54 ± 0.21		

The limits given correspond approximately to log $[\beta \pm 3\sigma(\beta)]$.
* When $\sigma(\beta)$ is $> 0.2\beta$, the maximum value (= log $[\beta + 3\sigma(\beta)]$) is given.

square sum in both cases, and thus explains the experimental data satisfactorily. Therefore, it may be accepted as the "best" model.

Figure 3 shows the equilibrium distribution of various species as a function of log $[H^+]$ at two different concentrations of EDTA; Fig. 4 shows the distribution as a function of EDTA concentration at two different pH levels, pH 2 and pH 7.

These calculations are based on the equilibrium constants given in Table 2, model II (glass and amalgam electrodes)

DISCUSSION

The present work shows that at higher concentrations, Cd(II) may also form a binuclear species,

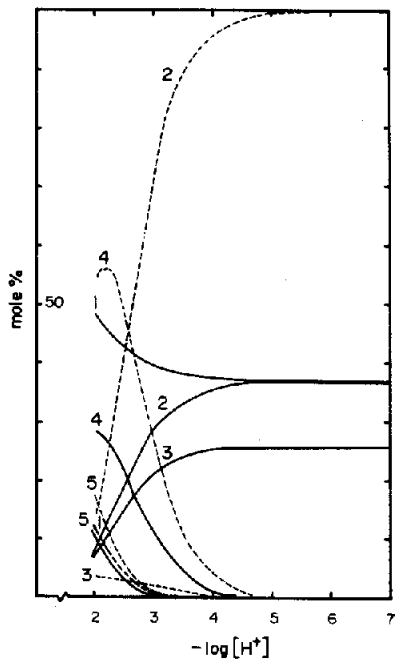

Fig. 3. The equilibrium distribution of various species as a function of log $[H^+]$. (——) $C_{Cd} = 0.05M$, $C_L = 0.025M$; (-----) $C_{Cd} = 0.05M$, $C_L = 0.05M$. (1) Cd(II), (2) CdL, (3) Cd_2L, (4) CdHL and (5) CdH_2L. The calculations are based on the equilibrium constants given in Table 2, model II.

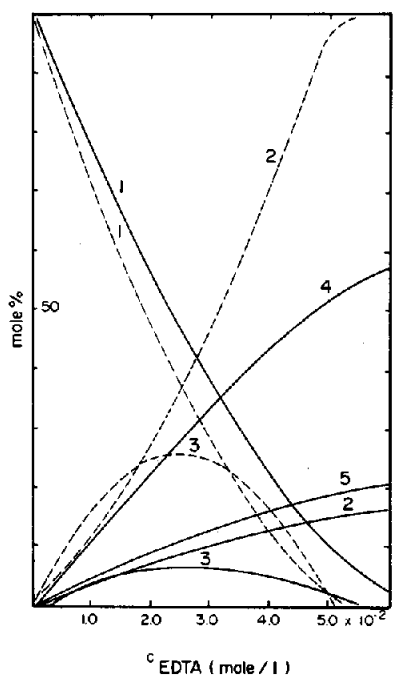

Fig. 4. The equilibrium distribution of various species as a function of the concentration of EDTA at pH = 2 (——) and pH = 7 (-----) (1) Cd(II), (2) CdL, (3) Cd_2L, (4) CdHL and (5) CdH_2L. The calculations are based on the equilibrium constants given in Table 2, model II.

Table 3. The equilibrium constants log K_{lm} for the formation of $(Cd)_l(H)_m(L)$ in different systems

log K_{CdL}	log K_{CdHL}	log K_{CdH_2L}	log K_{CdH_3L}	log K_{Cd_2L}	Ionic medium	Temp., °C	Method	Reference
16.48 ± 0.05	—	—	—	—	0.1*M* KCl	20	GE	1
16.46	2.9	—	—	—	0.1*M* $NaNO_3$	20	GE	2
16.4	—	—	—	—	0.1*M* $NaClO_4$	25	GE	3
16.9 ± 0.1	—	—	—	—	0.1*M* $KClO_4$	20	DISTR	6
15.98	—	—	—	—	0.2*M*?	25	POL	4
16.01	—	—	—	—	0.2–0.4*M* KCl	?	POL	5
16.62 ± 0.05	2.90 ± 0.05	—	—	—	0.1*M* KNO_3	20	(Recommended values)	8
—	2.70	1.79	—	—	1.0*M* KNO_3	25	GE	19
14.25 ± 0.02	3.16 ± 0.02	2.30 ± 0.02	1.57 ± 0.02	—	1.0*M* $NaClO_4$	25	GE + Cd (Hg)	20
15.30 ± 0.04	2.57 ± 0.04	1.52 ± 0.02	—	1.28 ± 0.07	1.0*M* $NaNO_3$	25	GE + Cd(Hg)	This work
15.1 ± 0.1	2.4 ± 0.1	—	—	—	The values in (8) are extrapolated to 1.0*M* ionic strength by the Günthelberg equation*			

* $\log \gamma_i = -Z_i^2 \dfrac{0.5107\sqrt{I}}{1 + 1.5\sqrt{I}}$ where γ_i = activity factor of the ion i, Z_i = the charge of the ion, and I = the ionic strength.

Cd_2L with EDTA, and the mixed polyprotonated species, CdHL and CdH_2L in acid solutions. A triprotonated species, CdH_3L has also been reported,[20] which predominates in the pH range 1–2, but as the working pH in the present work was not sufficiently low, and no reliable values of K_5 and K_6 for EDTA valid for the ionic medium used are available, we did not include this species in our calculations.

As regards the binuclear species, which to our knowledge has not previously been reported, it seems that no studies have been made where an excess of cadmium with respect to EDTA was used, probably to avoid the risk of formation of a solid phase at lower pH, or the precipitation of Cd(II) as hydroxide at higher pH.

The availability of powerful computer programs such as LETAGROP as supplements to the graphical methods (the common procedure for evaluating equilibrium constants in the earlier investigations) has made it possible to take into account most of the species that are likely to form in a solution of suitable composition. This is evident from the fact that both sets of data give comparable results.

The small difference between the two sets of results is not unusual,[21,22] and is probably due to small changes in the activity factors, arising from variations in the ionic medium. Such changes do not influence the selection of the final chemical model but they do influence the values of the equilibrium constants slightly. The difference can be minimized by making corrections for the junction potentials of various chemical species.

As a first approximation some calculations were made utilizing the technique that has been used in this institute.[23] These calculations show that the term $j[Cd^{2+}]$ in equations (1a) and (1b) has the largest effect because of the changing concentration of free cadmium in the course of titration. It may, however, be mentioned that the maximum value of E_j' is very small (0.2 and 0.7 mV for the glass electrode alone and glass and amalgam electrodes respectively) and the present experiments were not designed to obtain a good value for E_j'. Nevertheless, the results given in Table 2, model IIa for the two sets of data seem to be in closer agreement after the correction is applied. We regard the data obtained by using the glass electrode in conjunction with the cadmium amalgam electrode as more reliable and the set of equilibrium constants suggested is that obtained by analysing these data.

Some of the results on the complex-formation between Cd(II) and EDTA reported by different workers are summarized in Table 3. Comparison of the values shows that the results obtained in the present work are in close agreement with those recommended by Anderegg[8] for the formation of CdL and CdHL, allowing for the different ionic medium and temperature. His recommended values extrapolated to 1.0*M* ionic medium by use of the Güntelberg equation are also given in the table. In these calculations the small difference between the activity factors of Na^+ and K^+, and the difference in temperature, were neglected.

EDTA is known to form a weak complex with sodium. This was taken into account in analysing the experimental data, both for evaluating the protonation constants of EDTA[14] and its complex formation with cadmium. A value, log $K = 1.22$, for the formation of the Na–EDTA complex was used in all the calculations.

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STUDIES ON THE EXTRACTION OF PLATINUM METALS WITH TRI-ISO-OCTYLAMINE FROM HYDROCHLORIC AND HYDROBROMIC ACID

SEPARATION AND DETERMINATION OF GOLD, PALLADIUM AND PLATINUM

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Summary—The extraction of Pd(II), Rh(III), Ir(III), Au(III) and Pt(IV) from hydrochloric and hydrobromic acid with 5% tri-iso-octylamine solution in carbon tetrachloride has been studied. The gold extract from hydrochloric acid is yellow and absorbs at 325 nm, the palladium compound is red and absorbs at 290 nm and 467 nm, and the platinum compound is blood-red and shows absorption at 268 nm. The gold, palladium and platinum extracts from hydrobromic acid are crimson, reddish brown and blood-red, with maximum absorption at 260, 345 and 300 nm respectively. Methods have been devised for the separation of gold from platinum and for its determination and also for the simultaneous determination of palladium and platinum.

High molecular-weight amines (HMWA) were first used as extractants by Smith and Page.[1] Various workers[2–5] have used 5% tri-iso-octylamine solution in xylene for the separation of tervalent actinides from lanthanides, and of neptunium from plutonium, americium, curium, uranium, thorium and fission products. Data for extraction of more than thirty elements with this amine have been published[6,7] and recently[8] the complexes formed with molybdenum have been reported. In a previous paper[9] the complexes of palladium(II) and platinum(IV) with tri-iso-octylamine (TIOA) were reported. Binary alloys of gold and palladium find many applications, especially in electroplating, and the analysis of plating baths is important. Hence, the present work deals with the solvent extraction of platinum metals with a view to development of methods for their separation and determination.

There are comparatively few acceptable methods for the determination of gold,[10–16] palladium[17–24] and platinum[15,25,26] in tracer amounts and these usually involve strict pH adjustment in a narrow range. Ascorbic acid[10] and diethyldithiocarbamate[11] have been proposed for the determination of gold and palladium. Recently syn-phenyl pyridyl ketoxime[12] has been used for the simultaneous determination of gold and palladium.

During the study of extraction of noble metals with tri-iso-octylammonium chloride, gold and palladium were found to form yellow and red complexes, with maximum absorption at 325 and 467 nm respectively. The absorption peaks do not overlap and hence provide a quick and new method for the photometric determination of these elements in the presence of each other.

Similarly palladium and platinum form coloured complexes with tri-iso-octylammonium bromide, but the absorption peaks overlap. However, simultaneous equations can be used for determination of both elements in a mixture.

EXPERIMENTAL

Reagents

All the reagents were of analytical grade and used without further purification unless otherwise mentioned.

Palladium stock solution. Palladium sponge (106.4 mg) was dissolved in *aqua regia* and the solution evaporated to dryness. The residue was dissolved in 5 ml of concentrated hydrochloric acid and the solution again heated to dryness. The residue was dissolved in 0.1*M* hydrochloric acid and the solution diluted to give a palladium concentration of 1 mg/ml.

Rhodium stock solution. Rhodium(III) chloride trihydrate (131.7 mg) was dissolved in distilled water and diluted to give a rhodium(III) concentration of 0.5 mg/ml.

Iridium stock solution. Iridium(III) chloride trihydrate (88.2 mg) was dissolved in distilled water and diluted to give an iridium(III) concentration of 0.25 mg/ml.

Gold stock solution, 0.2 mg/ml. Prepared by suitable dilution of $HAuCl_4.4H_2O$.

Platinum stock solution. Platinum oxide (113.5 mg) was dissolved in *aqua regia* and the solution evaporated to dryness. The residue was taken up in *aqua regia*, the solution evaporated to dryness and the salt taken up in 0.1*M* hydrochloric acid and the solution diluted to give a platinum(IV) concentration of 0.5 mg/ml.

Measurement of distribution ratio

Equal volumes (2 ml) of the aqueous and organic phases were shaken mechanically for 2 min at room temperature. Preliminary studies showed that equilibrium was established in this time. The phases were centrifuged and separated. The concentration left in the aqueous phase was

determined by the methods given below, and the concentration in the organic phase was obtained by mass-balance.

Palladium was determined by adjusting the aqueous phase to 4*M* hydrochloric acid concentration and shaking it with 5 ml of 5% TIOA in carbon tetrachloride and measuring the absorbance of the extract at 467 nm against a reagent blank.

Platinum was determined by adjustment to 4*M* hydrobromic acid concentration, shaking with 5 ml of 5% TIOA solution in carbon tetrachloride and measuring the absorbance of the extract at 300 nm against a reagent blank.

Gold was determined by extraction from 2*M* hydrochloric acid with 5 ml of 5% TIOA solution in carbon tetrachloride and measurement of the absorbance of the organic layer at 325 nm against a reagent blank.

Rhodium was determined by heating with 10 ml of 25% stannous chloride solution in concentrated hydrochloric acid for 1 hr in a water-bath, cooling to room temperature, diluting to 50 ml with 2*M* hydrochloric acid and measuring the absorbance at 475 nm against a reagent blank.

Iridium was determined by adjusting to 8*M* hydrobromic acid concentration, heating for 10 min in a water-bath, mixing with 5 ml of 25% stannous chloride dihydrate solution in concentrated hydrobromic acid, cooling in a stream of water exactly 2 min after addition of the reagent, diluting to 25 ml and measuring the absorbance at 402 nm against a reagent blank.

RESULTS AND DISCUSSION

The results given for all the experiments are the means of two runs, and where necessary the experiments were performed in triplicate. The extraction of Pd(II), Rh(III), Au(III) and Pt(IV) with 5% TIOA solution in carbon tetrachloride as a function of acid concentration is shown in Figs. 1 and 2. Palladium is quantitatively extracted from 0.1–6*M* acid but the extraction decreases at higher acid concentration. Gold is extracted over the entire range of acidity tested and platinum behaves almost the same as palladium. The decreased extraction at high acid concentration is presumably due to preferential formation of the hydrohalide of the TIOA.

Rhodium and iridium show negligible extraction at hydrogen-ion concentrations above 1.0 and 4.0*M* respectively. This behaviour does not indicate the absence of anionic chloro-complexes, but is due to the formation of the triply charged hexahalo complexes and the greater difficulty in forming the ion-association complexes with quaternary ammonium ions. The extraction can be formulated as

$$MX^{(n-m)-} + (n-m)\text{TIOAH}^+ \rightleftharpoons MX_n(\text{TIOAH})_{n-m}$$

where m is the oxidation state of the metal ion.

Hence $\log K = \log D - (n-m) \log [\text{TIOA}]$. A plot of $\log D$ *vs.* $\log [\text{TIOA}]$ gave $(n-m) = 1$ for gold and 2 for both palladium and platinum, indicating that the halide complexes are AuX_4^-, PdX_4^{2-} and PtX_6^{2-}.

Quantitative extraction is found to be achieved in 2 min shaking.

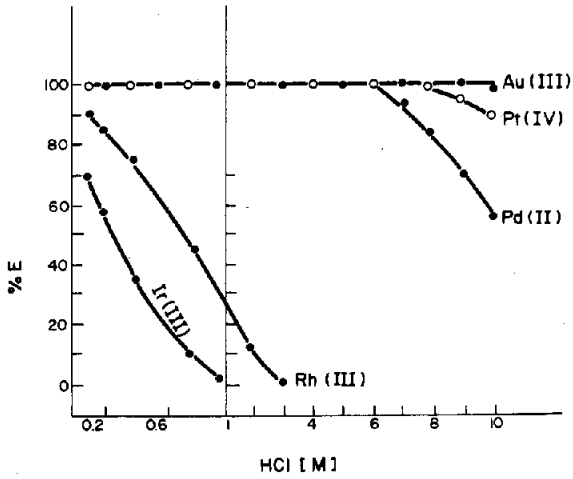

Fig. 1. Extraction of Pd(II), Rh(III), Ir(III), Au(III) and Pt(IV) as a function of HCl concentration.

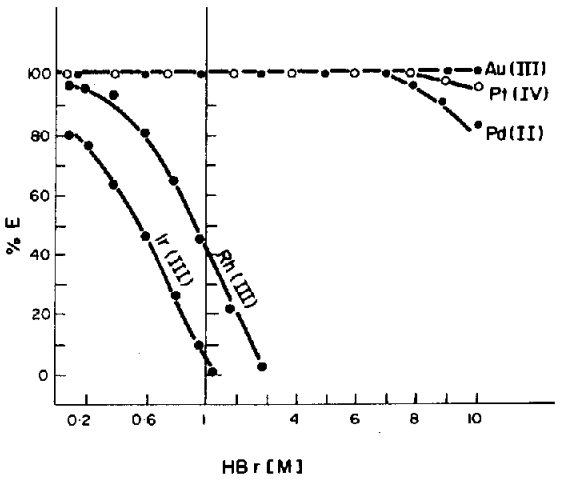

Fig. 2. Extraction of Pd(II), Rh(III), Ir(III), Au(III) and Pt (IV) as a function of HBr concentration.

Solvent

Carbon tetrachloride, chloroform, ethyl acetate and benzene give quantitative extraction of the palladium chloro-complex but xylene, toluene and methyl isobutyl ketone give only 92, 75 and 60% extraction respectively. The gold chloro-complex is quantitatively extracted into carbon tetrachloride, chloroform, methyl isobutyl ketone, ethyl acetate, benzene, toluene and xylene; 75–80% extraction is achieved with amyl alcohol and 50–55% with dichloroethylene. The bromo-complexes of palladium, platinum and gold are quantitatively extracted with carbon tetrachloride, chloroform, benzene, toluene, xylene and methyl isobutyl ketone.

Salting-out agents

The effect of chloride and bromide ions on the extraction of palladium, gold and platinum at constant acidity is shown in Figs. 3 and 4. As the concentration of LiCl or KBr increases, the extraction of platinum decreases, although LiCl and KBr would be expected to act as salting-out agents and enhance

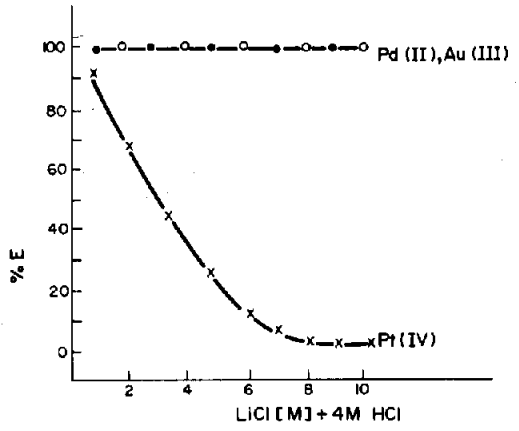

Fig. 3. Extraction of Pd(II), Au(III), and Pt(IV) as a function of LiCl concentration (in 4*M* HCl).

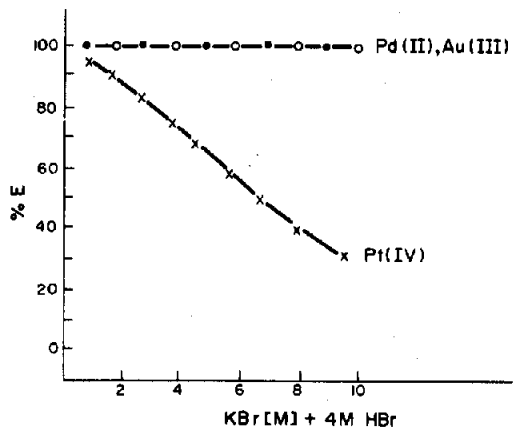

Fig. 4. Extraction of Pd(II), Au(III) and Pt(IV) as a function of KBr concentration (in 4*M* HBr).

extraction. The only explanation that seems plausible is that the increased halide concentration favours competitive formation of the ion-association complex of the tri-iso-octylammonium ion and halide ion, and that the stability constants for the gold and palladium ion-association complexes are sufficiently greater than that of the platinum complex for extraction of these two complexes not to be affected.

Spectral studies

Figure 5 shows the absorption spectra of $(R_3NH)_2PdCl_4$, $(R_3NH)_2PtCl_6$ and $(R_3NH)AuCl_4$ dissolved in carbon tetrachloride and measured against the reagent blank. The palladium compound shows maximum absorption at 290 and 467 nm while gold and platinum absorb at 325 and 268 nm respectively. Beers' law is obeyed in the range 0–2 mg/ml in the solution measured. The apparent molar absorptivities of Pd and Au are 1.4×10^3 and 5.8×10^3 $l.mole^{-1}.cm^{-1}$ respectively. Figure 6 shows the absorption spectra of $(R_3NH)_2PdBr_4$, $(R_3NH)_2PtBr_6$ and $(R_3NH)AuBr_4$, which absorb at 345, 300 and 260 nm respectively. The molar absorptivities for Pd and Pt are 9×10^4 and 4.5×10^4 $l.mole^{-1}.cm^{-1}$ respectively. The properties of the Au, Pd and Pt complexes are shown in Table 1.

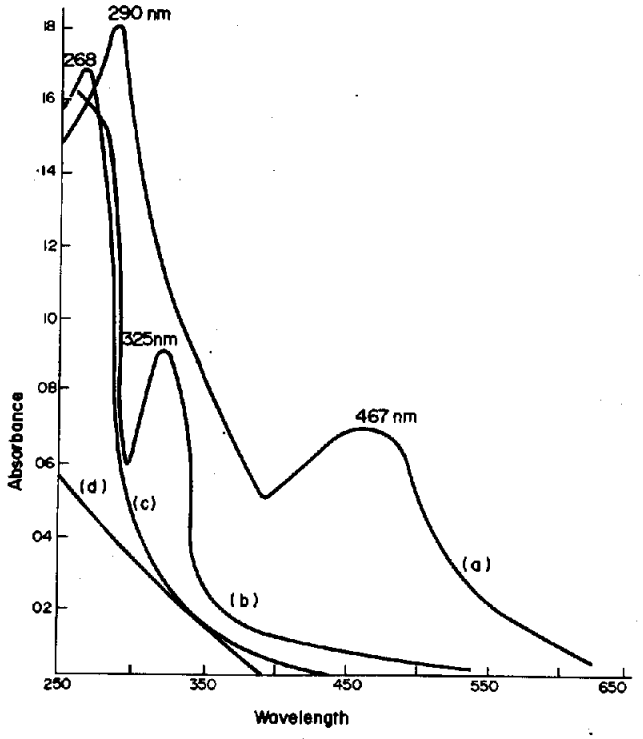

Fig. 5. Variation of absorbance with wavelength for (a) palladium–TIOA–HCl complex (Pd $2.07 \times 10^{-3}M$); (b) gold–TIOA–HCl complex (Au $1.55 \times 10^{-2}M$); (c) platinum–TIOA–HCl complex (Pt $1.5 \times 10^{-3}M$); (d) TIOA/CCl_4 ($7 \times 10^{-2}M$).

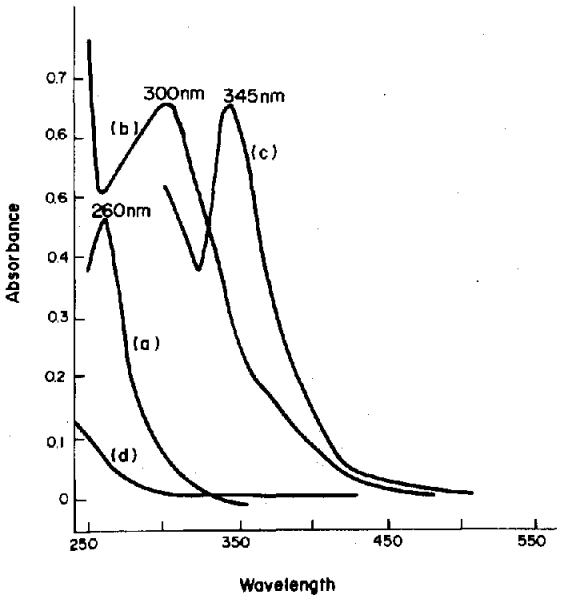

Fig. 6. Variation of absorbance with wavelength for (a) palladium–TIOA–HBr complex (Pd $7.8 \times 10^{-3}M$); (b) gold–TIOA-HBr complex (Au $1.1 \times 10^{-3}M$); (c) platinum–TIOA–HBr complex (Pt $1.4 \times 10^{-2}M$); (d) TIOA/chloroform ($7 \times 10^{-2}M$).

Separation of palladium and platinum

Palladium, gold and platinum are readily extracted with TIOA from a wide range of hydrochloric and hydrobromic acid concentrations but rhodium and iridium are extracted only from 0.1–1.0*M* acid media. This difference in the extractability of the chloro- and bromo-complexes provides a basis for the separation of palladium and platinum from rhodium and iridium. Since palladium, gold and platinum are all extracted quantitatively from hydrochloric acid, their separation from each other poses a problem. However, the extraction of platinum is suppressed to a great extent if the aqueous phase is 6*M* in lithium chloride. Therefore, the extraction of palladium from 4*M* hydrochloric acid/6*M* lithium chloride results in its clean separation from rhodium, iridium and platinum, but gold interferes seriously. The extraction of ruthenium and osmium, if present, can be eliminated by pretreatment of the sample solution with nitric acid and hydrogen peroxide.

Simultaneous determination of gold and platinum or palladium and platinum

Gold, palladium and platinum are extracted quantitatively in one cycle with 5% TIOA in carbon tetrachloride or chloroform. The determination of gold and palladium or palladium and platinum in the same solution is possible because although the absorption peaks overlap, the absorbances are additive and the method of measurement at two wavelengths can be used in combination with the equations

$$A_{300} = \epsilon^{Pt}_{300}[Pt] + \epsilon^{Au}_{300}[Au]$$

$$A_{260} = \epsilon^{Pt}_{260}[Pt] + \epsilon^{Au}_{260}[Au]$$

$$A_{345} = \epsilon^{Pd}_{345}[Pd] + \epsilon^{Pt}_{345}[Pt]$$

$$A_{300} = \epsilon^{Pd}_{300}[Pd] + \epsilon^{Pt}_{300}[Pt]$$

Combination of these gives

$$[Pt] = \frac{A_{300}\epsilon^{Au}_{260} - A_{260}\epsilon^{Au}_{300}}{\epsilon^{Pt}_{300}\epsilon^{Au}_{260} - \epsilon^{Pt}_{260}\epsilon^{Au}_{300}}$$

$$[Au] = \frac{A_{260}\epsilon^{Pt}_{300} - A_{300}\epsilon^{Pt}_{260}}{\epsilon^{Pt}_{300}\epsilon^{Au}_{260} - \epsilon^{Pt}_{260}\epsilon^{Au}_{360}}$$

$$[Pd] = \frac{A_{345}\epsilon^{Pr}_{300} - A_{300}\epsilon^{Pt}_{345}}{\epsilon^{Pd}_{345}\epsilon^{Pt}_{300} - \epsilon^{Pd}_{300}\epsilon^{Pt}_{345}}$$

$$[Pt] = \frac{A_{300}\epsilon^{Pd}_{345} - A_{345}\epsilon^{Pd}_{300}}{\epsilon^{Pd}_{345}\epsilon^{Pd}_{300} - \epsilon^{Pd}_{300}\epsilon^{Pt}_{345}}$$

The molar absorptivities must be measured on the instrument being used.

Procedure for gold and platinum

An aliquot of a solution containing the platinum metals in chloride form is heated repeatedly with nitric acid and hydrogen peroxide to remove osmium and ruthenium, if present, as the volatile oxides. The residue is dissolved in 4*M* hydrobromic acid and extracted with 5% TIOA in chloroform to avoid the extraction of rhodium and iridium. The absorbance of the chloroform extract is measured at 260 and 300 nm against a reagent blank. Results for several synthetic mixtures are summarized in Table 2. The average deviation found was ±0.8% for both Au and Pt.

Table 1. Spectrophotometric properties of gold, palladium and platinum complexes

Complex	λ_{max}, nm	Colour	ϵ, $l.mole^{-1}.cm^{-1}$	ϵ, $l.mole^{-1}.cm^{-1}$		
				260 nm	300 nm	345 nm
$(R_3NH)AuCl_4$	325	Yellow	5.8×10^3	—	—	—
$(R_3NH)_2PdCl_4$	290, 467	Red	1.4×10^3	—	—	—
$(R_3NH)_2PtCl_6$	268	Blood-red	—	—	—	—
$(R_3NH)_2PdBr_4$	345	Reddish brown	9×10^4	—	6.5×10^3	8.2×10^3
$(R_3NH)_2PtBr_6$	300	Blood-red	4.5×10^4	—	4.6×10^3	1.8×10^3
$(R_3NH)AuBr_4$	260	Crimson	—	1.5×10^4	2.2×10^4	—
$(R_3NH)_2PtBr_6$	300	Blood-red	—	3.5×10^4	4.5×10^4	—

Table 2. Analysis of synthetic mixtures of gold and platinum

Au(III), $10^{-4}M$		Pt(IV), $10^{-4}M$		Average deviation, %	
Taken	Found	Taken	Found	Au	Pt
0.255	0.252	0.254	0.250	±0.8	±0.8
0.570	0.564	0.508	0.500	±0.5	±0.8
0.765	0.759	0.762	0.755	±0.4	±0.5
1.020	1.014	1.015	1.00	±0.3	±0.8
1.27	1.126	1.27	1.26	±0.3	±0.4
2.51	2.49	2.50	2.47	±0.6	±0.8
3.35	3.34	3.40	3.36	±0.1	±0.6

Procedure for palladium and platinum

An aliquot of mixture containing palladium and platinum is heated with nitric acid and hydrogen peroxide to remove ruthenium and osmium as their volatile oxides. The residue is dissolved in 4*M* hydrobromic acid and shaken with 5% TIOA in chloroform for 2 min. The organic phase is separated and the absorbance measured at 300 and 345 nm. Results for several synthetic mixtures are summarized in Table 3. The average deviations found were ±0.4% for Pd and ±0.6% for Pt.

Procedure for gold and palladium

A mixture containing up to 50 mg of palladium and 50 mg of gold is adjusted to about 10 ml so that its hydrochloric acid concentration is 0.1–6.0*M*. It is then shaken for 1 min with 10 ml of 5% TIOA in carbon tetrachloride. The organic layer is separated and the absorbance measured at 325 and 467 nm against a reagent blank. Results for some synthetic mixtures are summarized in Table 4. The average deviations for gold and palladium were found to be ±1.6% and ±1.1% respectively.

Determination of palladium in the presence of platinum metals

A portion of palladium chloride solution containing other platinum metals in chloride form is heated to dryness with nitric acid and hydrogen peroxide to remove osmium and ruthenium as the volatile oxides. The residue is dissolved in 5 ml of 5*M* hydrochloric acid and the mixture is shaken with an equal volume of 5% TIOA in carbon tetrachloride for 2 min. The organic layer is separated and the absorbance measured at 467 nm against a reagent blank. The tolerance limit for interference (taken as the amount required to cause a ±2% error in the determination of 100 mg of Pd) is 2 mg of Au, 1 mg of Pt and 50 mg each of Rh, Ir, Os and Ru. Analysis of mixtures which had concentrations of (0.5–5.5) × $10^{-4}M$ Pd and 1.25 × $10^{-4}M$ Pt, Au, Os, Ru, Ir and Rh gave an average deviation of ±0.4%.

Table 3. Analysis of synthetic mixtures of palladium and platinum

Pd(II), $10^{-4}M$		Pt(IV), $10^{-4}M$		Average deviation, %	
Taken	Found	Taken	Found	Pd	Pt
0.566	0.566	0.254	0.254	±0.0	±0.0
1.132	1.12	0.508	0.502	±0.4	±0.6
2.26	2.25	0.762	0.762	±0.2	±0.0
3.40	3.39	1.015	1.01	±0.2	±0.3
4.53	4.52	1.270	1.26	±0.1	±0.4
5.14	5.10	2.38	2.38	±0.4	±0.2
5.52	5.48	2.87	2.86	±0.4	±0.3

Table 4. Analysis of synthetic mixtures of gold and palladium

Au(III), $10^{-6}M$		Pd(II), $10^{-6}M$		Average deviation, %	
Taken	Found	Taken	Found	Au	Pd
0.125	0.124	0.118	0.118	±0.4	±0.0
0.187	0.182	0.236	0.232	±1.6	±0.9
0.250	0.245	0.375	0.368	±1.2	±1.1
0.375	0.370	0.472	0.468	±0.8	±0.4
0.500	0.495	0.615	0.609	±0.6	±0.5
0.750	0.740	0.756	0.745	±0.7	±0.8
1.000	0.990	0.996	0.988	±0.5	±0.4

Effect of other ions

Ions which interfere in the determination of palladium, gold and platinum are thiocyanate, cyanide, sulphide, sulphite, thiosulphate, EDTA, bromide, nitrite and iodide. Iron is extracted under the same conditions but can be masked with potassium dihydrogen phosphate. Many elements such as alkali metals, alkaline earths, Ti, Zr, Hf, W, Nb, Ir, Rh, Cu, Co, Sb, are not extracted or, if extracted, do not interfere photometrically. Silver interferes seriously owing to the precipitation of AgBr. The procedures are very rapid, the results are reproducible and the recovery of all the elements is quantitative.

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AUSTAUSCHER MIT CYCLISCHEN POLYETHERN ALS ANKERGRUPPEN—I

HERSTELLUNG UND CHARAKTERISIERUNG

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Zusammenfassung—Zahlreiche Austauscher mit cyclischen Polyethern als Ankergruppen werden hergestellt une auf ihre Eigenschaften untersucht. 4-Carboxyethyl- und 4-Hydroxypropyl-benzokronenether lassen sich an Kieselgel binden und dienen als stationäre Phasen in der Hochdruckflüssigkeitschromatographie.

Durch Kondensations-, Substitutions- und Copolymerisationsreaktionen mit cyclischen Polyethern unterschiedlicher Struktur und Ringgröße erhält man spezielle Austauscher, die ein bestimmtes Spektrum anorganischer Salze oder organischer Verbindungen zu binden vermögen.[1–10]

In Abb. 1–3 sind die hergestellten Austauscher, über die im folgenden berichtet wird, aufgeführt. Kurzbezeichnungen bedeuten: § = Matrix, B = Benzo, C = Krone, DB = Dibenzo, DN = Dinaphtho, K = Kryptand.

Tabelle 1 zählt die bemerkenswertesten Eigenschaften der neuen Austauscher im Vergleich zu den handelsüblichen auf.

DARSTELLUNG DER AUSTAUSCHER

Monomere Kronenverbindungen sind relativ einfach und mit guten Ausbeuten darzustellen. Einen wesentlich höheren präparativen Aufwand erfordern die bicyclischen Kryptanden. Die Verknüpfung der Ankergruppen mit der Matrix erfolgt vorwiegend über die –C–-Bindung, kann jedoch auch über –C–O–C–, –C–NH–C–, –Si–OH–C– und –Si–C–-Bindungen vorgenommen werden.

Nur O enthaltende Polyether als Ankergruppen

Hergestellt werden Kondensations- und Polymerisationharze mit O-enthaltenden Kronenethern als Ankergruppen. Die Darstellung der Kondensationsharze ist wesentlich einfacher.

Kondensationsharze. Die Kronenether erhält man in allen Fällen analog der Williamsonschen Ethersynthese. Ausgangsprodukte sind Brenzkatechin und Halogenderivate von aliphatischen Ethern bzw. Alkanen. Bei der Darstellung von DB-30-C-10 wird zur Steigerung der Ausbeute je Benzolring eine Phenolgruppe mit 3,4-Dihydro(1-H)-pyran blockiert und vor Bindung der zweiten Etherbrücke wieder mit Salzsäure abgespalten (Abb. 4).

Die Kondensation der Dibenzokronenether mit Formaldehyd geht in reiner Ameisensäure vor sich. Diese wirkt gleichzeitig als gutes Lösungsmittel und als Katalysator (Abb. 5 unten). Die Monobenzokronenether werden zusammen mit einem Hilfsvernetzer in einem Gemisch Ameisensäure/Schwefelsäure kondensiert (Abb. 5 oben).

Polymerisationsharze. Abbildungen 6 und 7 geben die allgemeinen Synthesewege der als Ausgangsverbindungen dienenden kernsubstituierten Mono- und Dibenzo-Kronenether wieder.

Durch Umsetzung von feingemahlenem, chlormethyliertem Polystyrol bzw. durch Copolymerisation mit Divinylbenzol erhält man Austauscher auf Styrolbasis, an denen die Ankergruppen über –C–O–C–, –C–N–C– und –C–C– Bindungen gebunden sind. Abbildung 8 zeigt dies für die Austauscher mit B-15-C-5 bzw. DB-24-C-8 als Ankergruppe.

O und N enthaltende Polyether als Ankergruppen

Als Ankergruppen dienen Aminopolyether (O und N enthaltende Kronenether bzw. Kryptanden) und harnstoffanaloge Verbindungen.

Aminopolyether. (*a*) O und N enthaltende Kronenether werden nach 3 Methoden dargestellt (Abb. 9). Die Synthesen der Austauscher erfolgen analog den in Abb. 5 angegebenen Wegen. Bei der Kondensation mit Formaldehyd wird der sekundäre Stickstoff alkyliert. (*b*) Die Darstellung von Austauschern mit Kryptanden als Ankergruppen erfordert einen großen präparativen Aufwand. Ihnen liegt ein zusammenhängender Syntheseweg zugrunde.[3,4]

Harnstoffanaloge Verbindungen. Die Umsetzung von Benzimidazolon mit Dibromalkylethern bzw. Dibromalkanen führt zu cyclischen Verbindungen mit

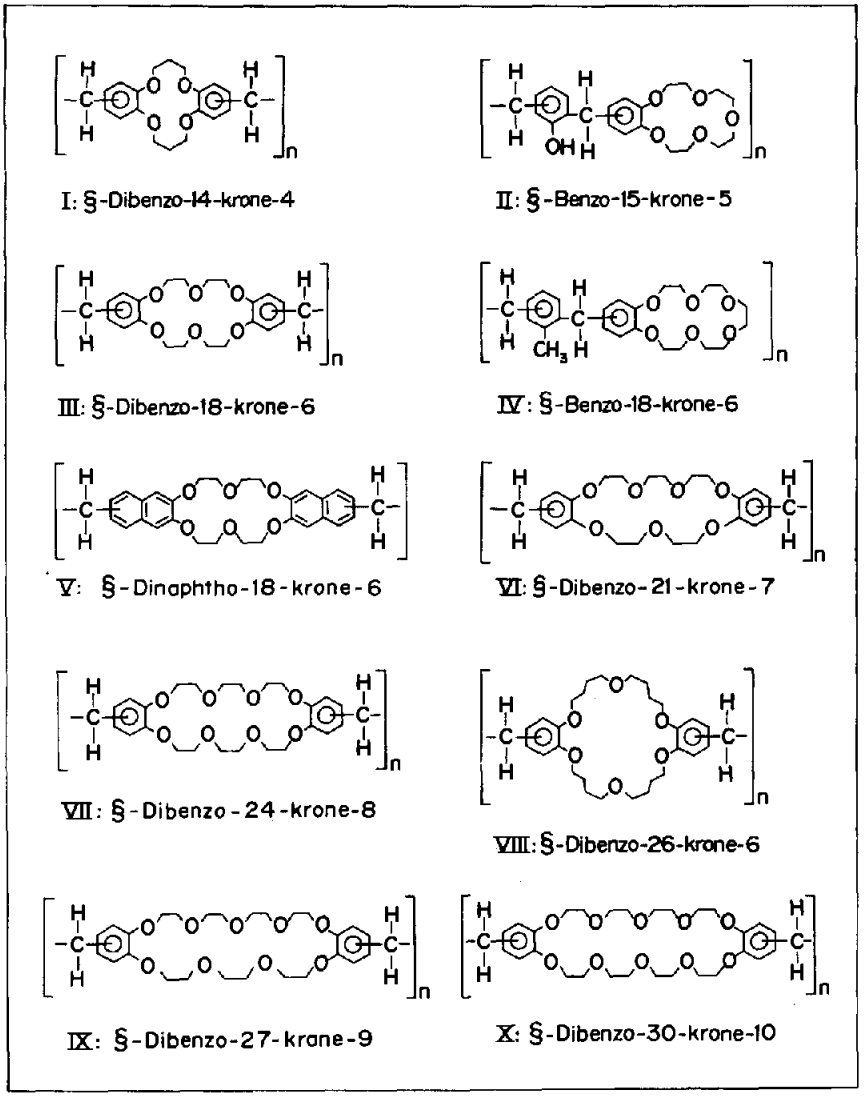


Abb. 1. Kronenether mit O als Heteroatom.

Tabelle 1. Bemerkenswerte Eigenschaften im Vergleich zu handelsüblichen Kationen- und Anionenaustauschern

Hohe Beständigkeit gegen Chemikalien, Temperatur und Radiolyse
Neutralliganden als Ankergruppen
Gleichzeitige Aufnahme von Kationen und Anionen zur Wahrung der Elektroneutralität
Lockerung bzw. Abstreifung der Solvathülle des Kations und Anions
Bindung der Ionen wird in weniger polaren Lösungsmitteln als Wasser, z.B. Methanol, erleichtert
Stabilität der Polyetherkomplexe hängt ab vom Kation, Anion, Lösungsmittel, Größe des Polyetherrings sowie Art und Basizität seiner Heteroatome (O,N,S)
Komplexbildung auch mit Kationen der Alkalielemente
Salzaufnahme steigt bei gleichem Kation in der Reihenfolge der Polarisierbarkeiten der Anionen
Aktivierung von Anionen, auch C-H-acider Verbindungen
Salzaufnahme { nur O als Heteroatom : pH-unabhängig O und N als Heteroatome: pH-abhängig für pH < 3
Elution mit reinen Lösungsmitteln (z.B. Wasser oder Methanol); keine Verunreinigung des Eluats

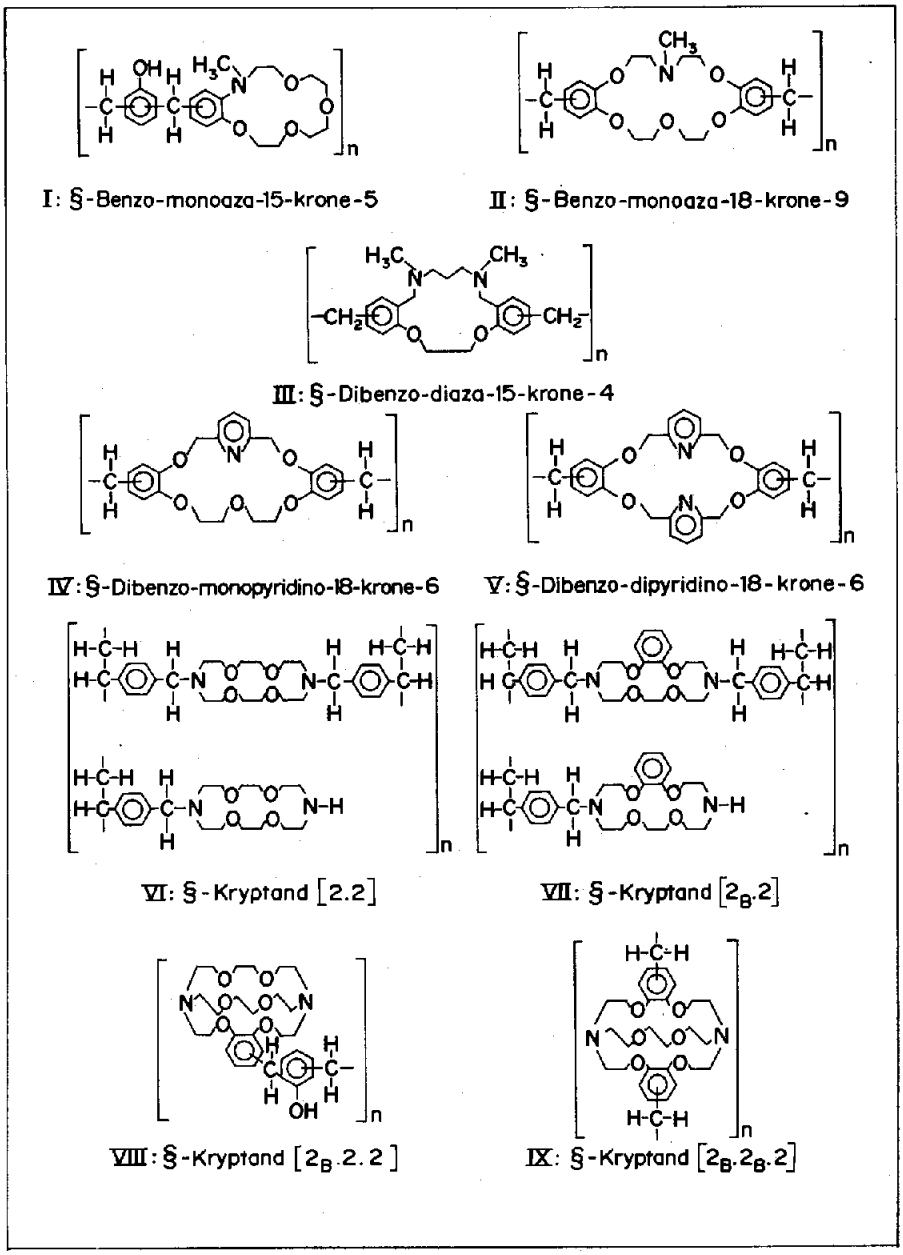


Abb. 2. Kronenether bzw. Kryptanden mit O und N als Heteroatomen.

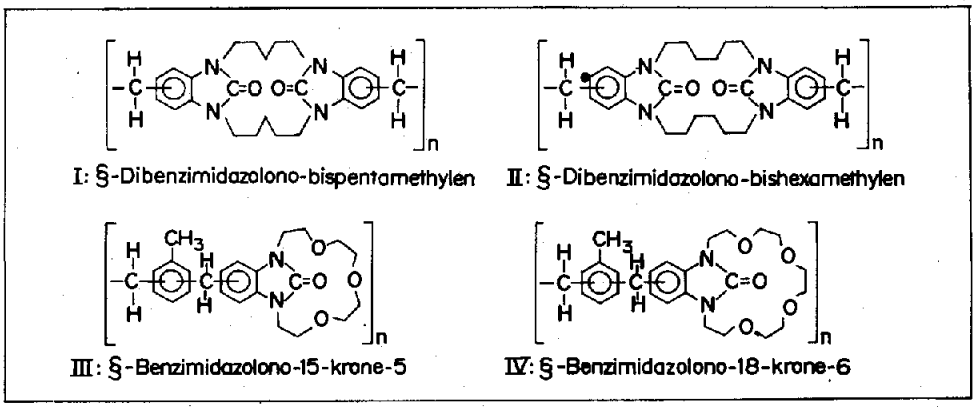


Abb. 3. Harnstoffanaloga mit O und N als Heteroatomen.

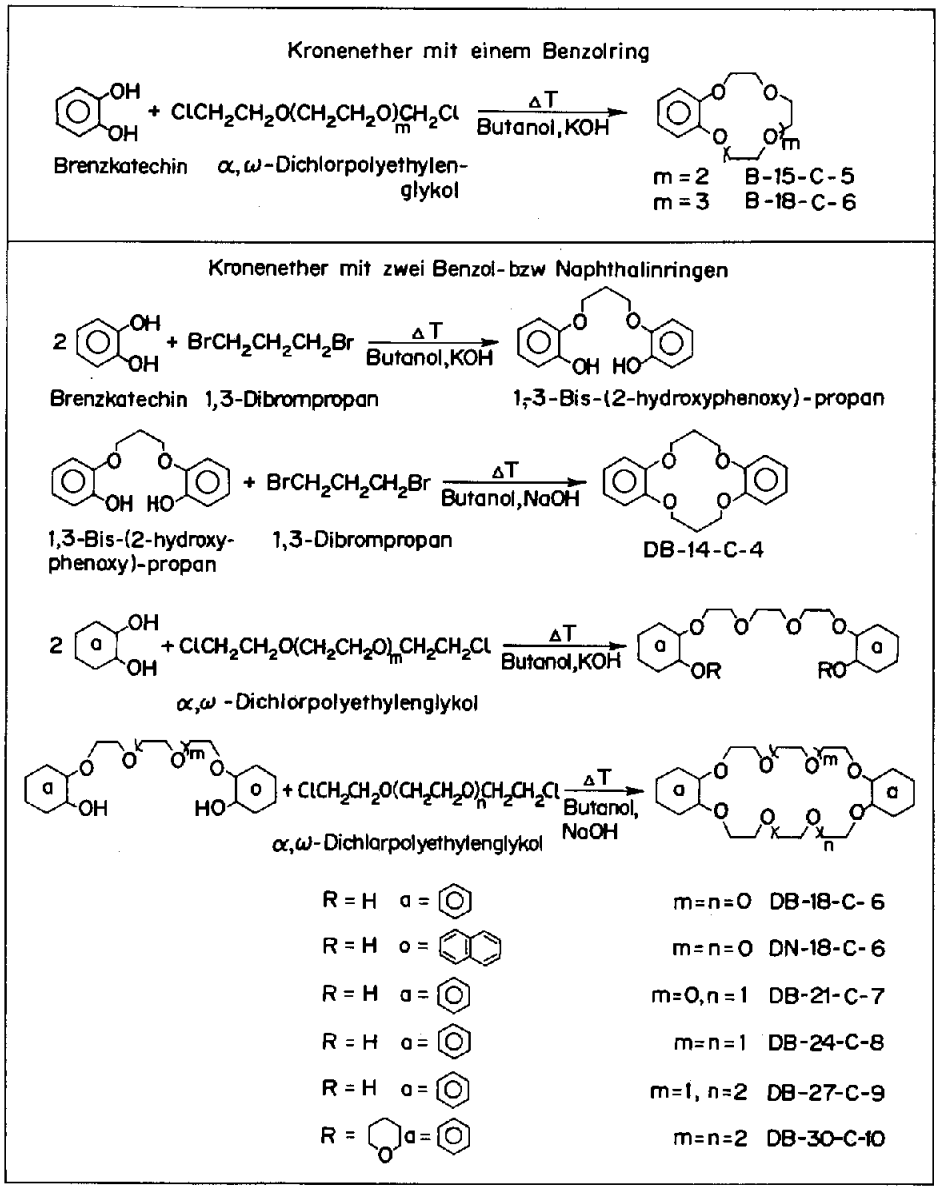


Abb. 4. Kronenether mit O als Heteroatom; Darstellung der Ankergruppen.

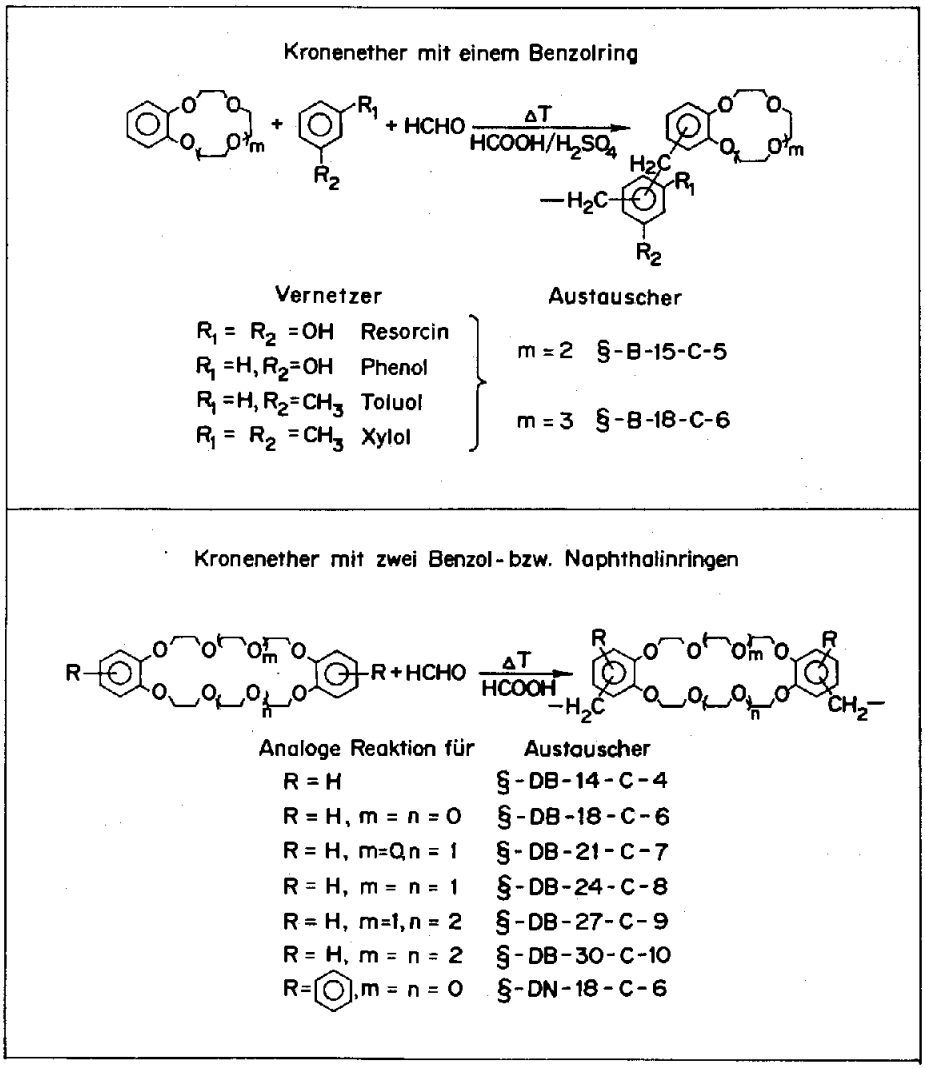


Abb. 5. Kronenether mit O als Heteroatom; Polykondensationsreaktionen zur Darstellung der Austauscher.

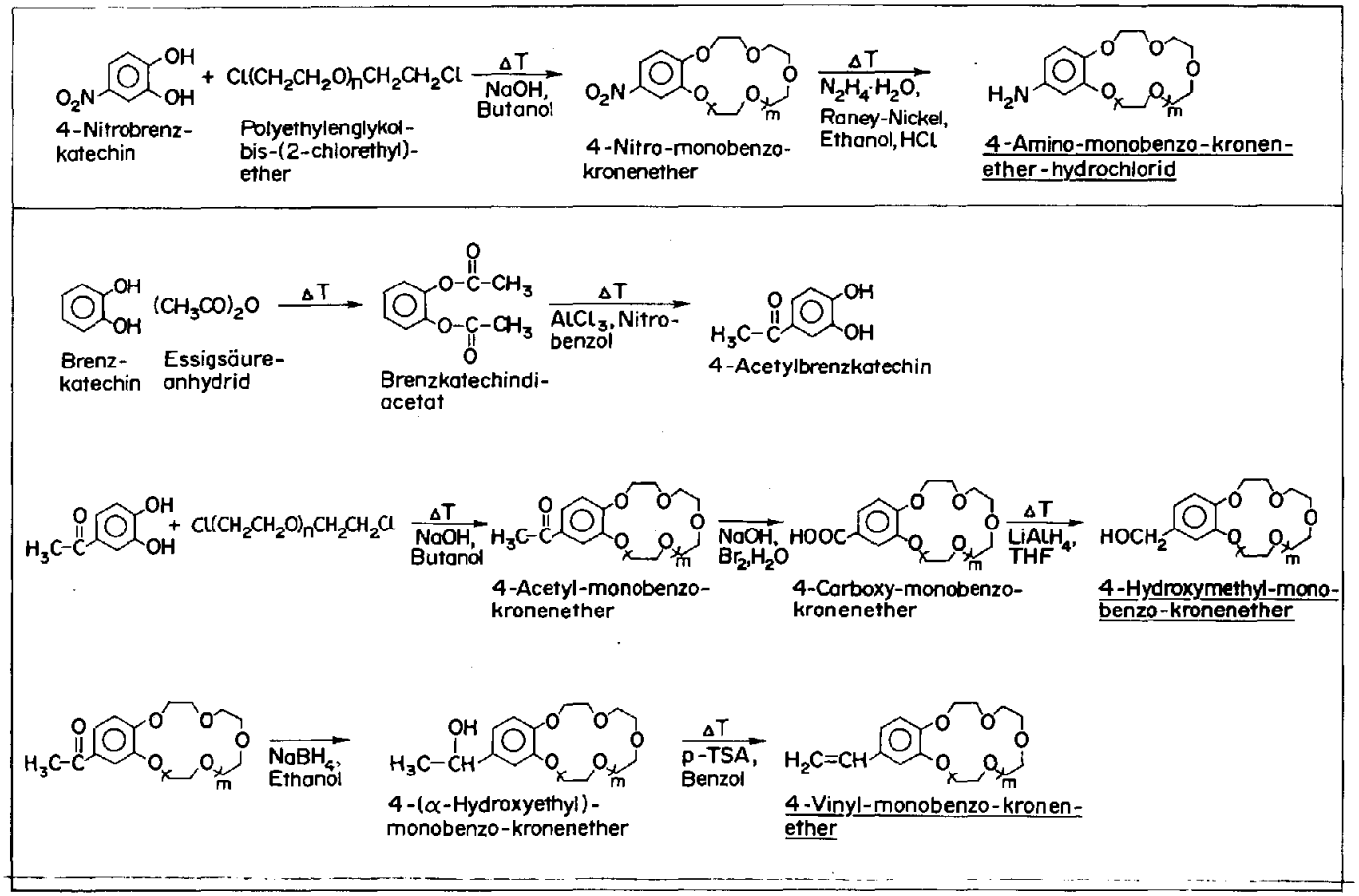


Abb. 6. Kronenether mit O als Heteroatom; Darstellung kernsubstituierter Monobenzo-kronenether.

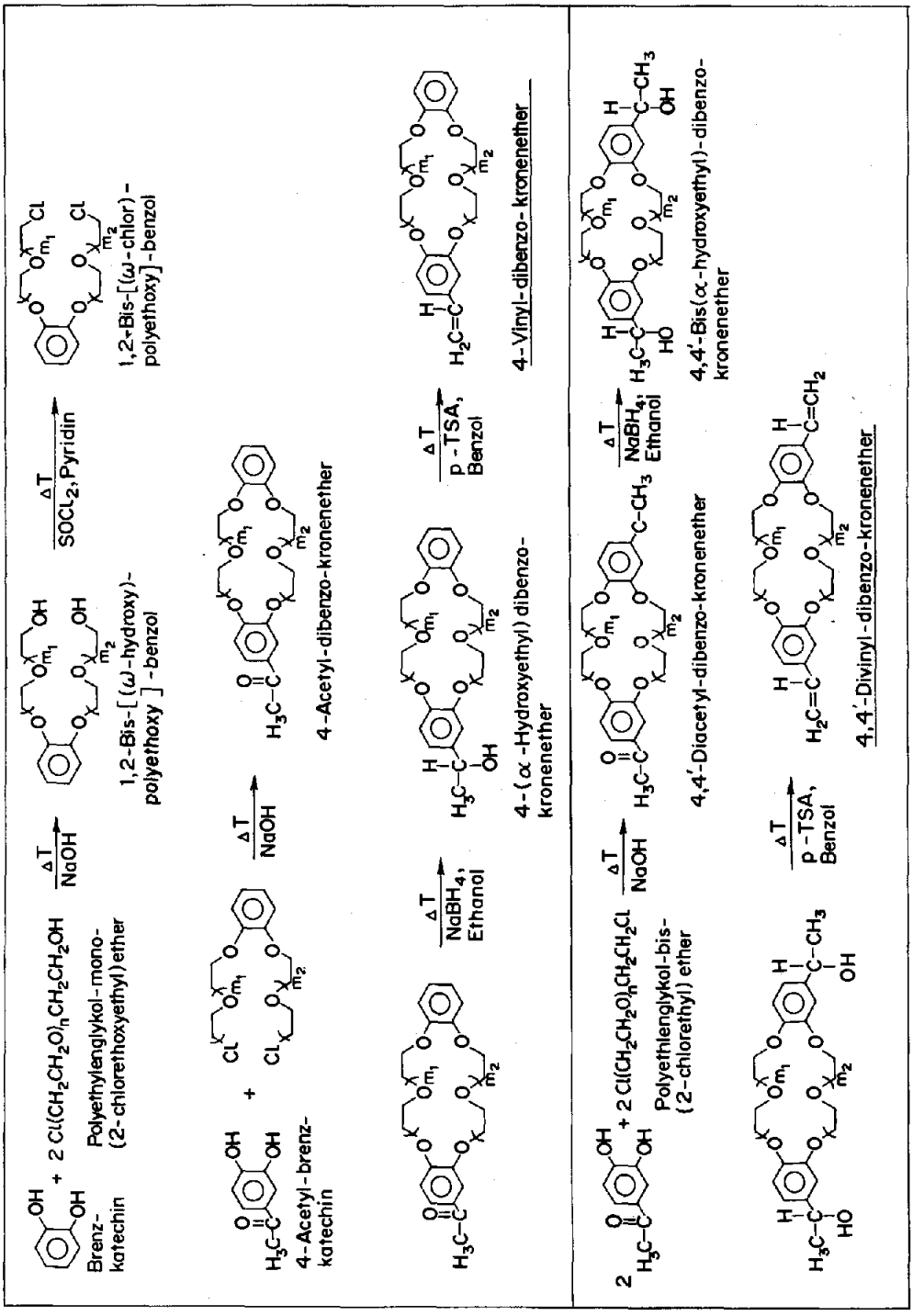


Abb. 7. Kronenether mit O als Heteroatom; Darstellung kernsubstituierter Dibenzo-kronenether.

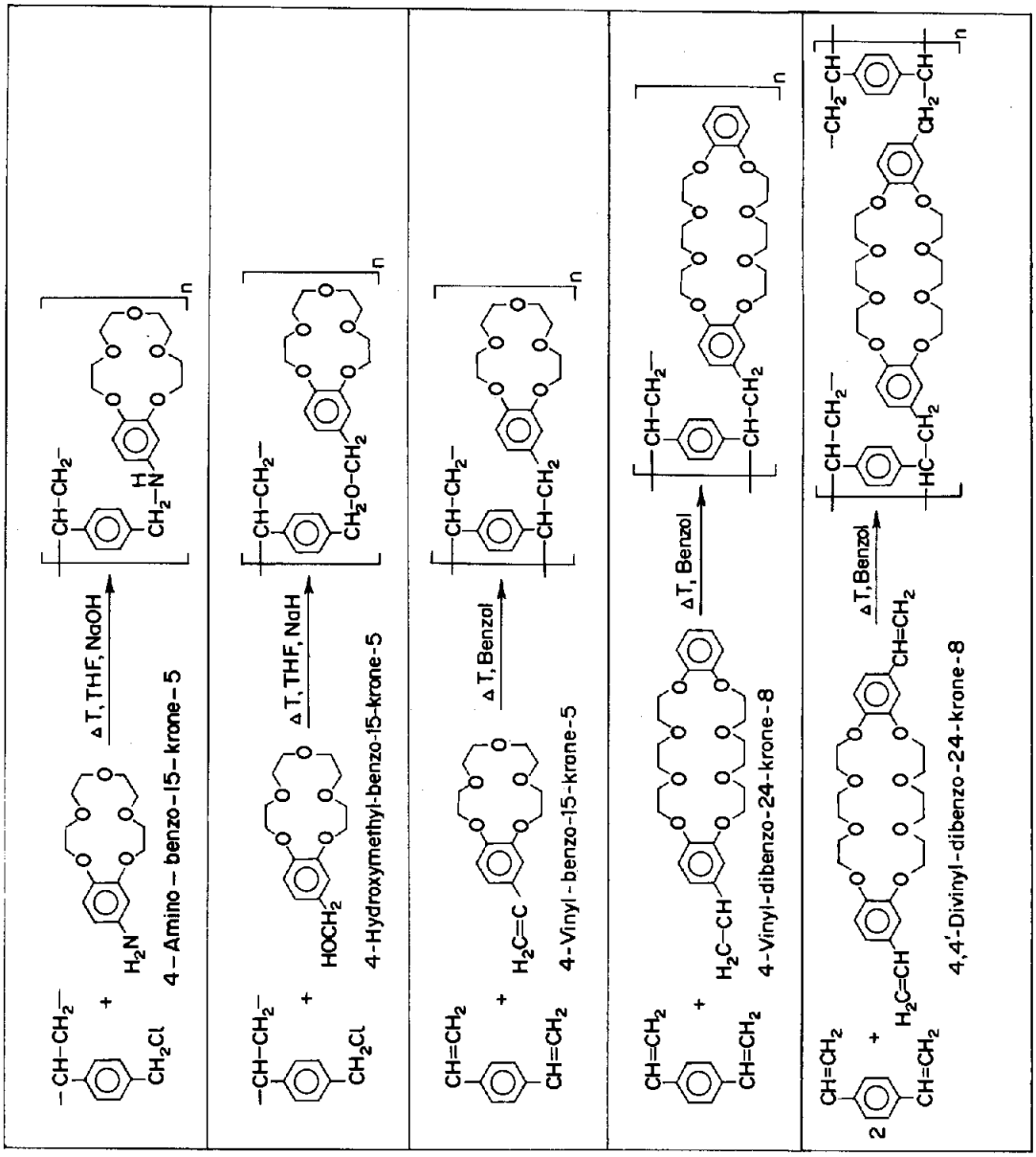


Abb. 8. Kronenether mit O als Heteroatom; Polymerisationsreaktionen zur Darstellung der Austauscher.

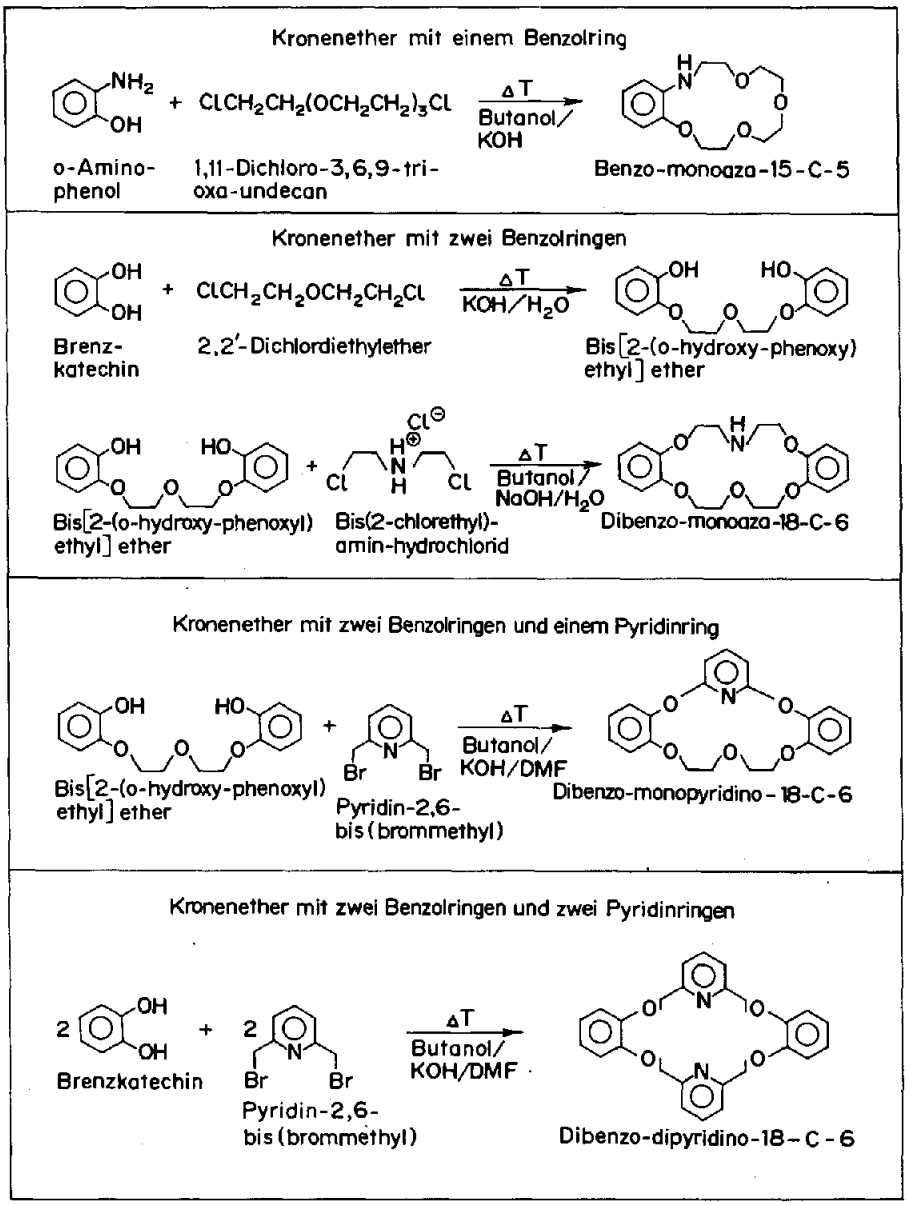


Abb. 9. Kronenether mit O und N als Heteroatomen; Darstellung der Ankergruppen.

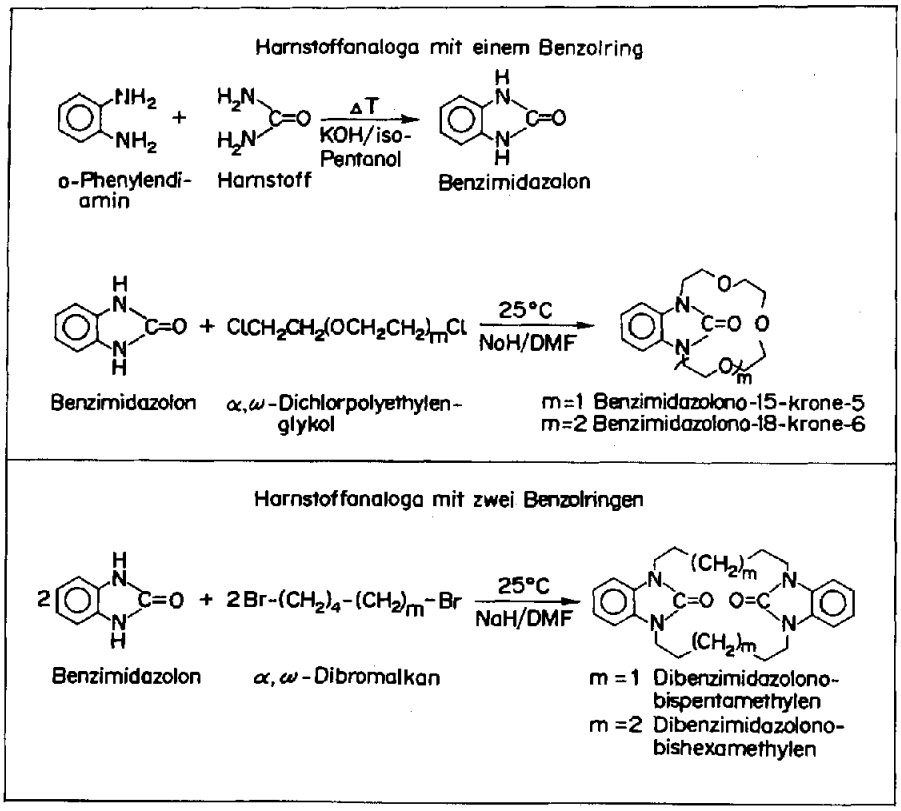


Abb. 10. Harnstoffanaloga mit O und N als Heteroatomen; Darstellung der Ankergruppen.

dreifach substituierten N-Atomen und Carbonyl-Gruppen im Etherring (Abb. 10). Die Synthesen der Austauscher werden auch hier analog den in Abb. 5 angegebenen Wegen vorgenommen.

An Kieselgel gebundene Kronenether

Entsprechende Sorbentien erhält man durch Umsetzung von 4-Hydroxypropylmonobenzokronenethern mit Kieselgel. Sie werden zur Zeit als stationäre Phasen in der Hochdruckflüssigkeitschromatographie erprobt. Ihre Kapazität ist jedoch gering.

Bessere Synthesewege stellen Umsetzungen zwischen 4-Carboxyethylmonobenzokronenethern und Kieselgel dar.

CHARAKTERISIERUNG DER AUSTAUSCHER

Die Austauscher werden eingehend untersucht und charakterisiert durch: Pyrolyse–Gaschromatographie und –Massenspektrometrie (Kennzeichnung der Zusatzkomponenten und Art der Vernetzung), thermische Beständigkeit, Radiolysebeständigkeit, chemische Beständigkeit (Säuren, Basen, Oxidationsmittel), Gleichgewichtsmessungen (maximale Beladungskapazitäten), kinetische Untersuchungen (zeitliche Gleichgewichtseinstellungen, Mechanismus der Kinetik), Selektivität (Verteilungskoeffizienten, Massenverteilungskoeffizienten, Trennfaktoren in Wasser und organischen Lösungsmitteln).

Pyrolyse–Gaschromatographie und –Massenspektrometrie

Über die Pyrolyse–Gaschromatographie und Pyrolyse–Massenspektrometrie von handelsüblichen Ionenaustauschern wurde bereits berichtet.[11–15]

Entsprechende eingehende Untersuchungen an den neu hergestellten Austauschern mit cyclischen Polyethern als Ankergruppen[16,17] ermöglichen Rückschlüsse auf Größe (Anzahl der O-Atome) und Art (O- bzw. O und N als Heteroatome) der Ankergruppe, Matrizes (Kondensations- bzw. Copolymerisationsharz), Vernetzer (Formaldehyd, Toluol, Phenol, Resorcin, Divinylbenzol) und Vernetzungsgrad.

Die Etherbrücken der Ankergruppen werden durch Pyrolyse je nach Anzahl der O-Atome in cyclische Fragmente zerlegt, aus denen sich auf die Ringgröße schließen läßt. Mono- und bicyclische Ankergruppen mit Stickstoff als zusätzlichem Heteroatom spalten den Stickstoff in Form von Alkylaminen und cyclischen N-Verbindungen ab.

Bei den Kondensationsharzen führt die Pyrolyse vorwiegend zur Fragmentierung der Etherbrücken und des Hilfsvernetzers. Das Verhältnis von Kronenverbindung zu eingesetztem Hilfsvernetzer ergibt sich aus den Bandenflächen- bzw. den Peakhöhenverhältnissen der entsprechenden Pyrolyseprodukte. Das

vernetzte aromatische Gerüst bleibt bei den ausschließlich mit Formaldehyd kondensierten Austauschern zum größten Teil als nichtflüchtiger Anteil auf der Pyrolysewendel zurück.

Mit Divinylbenzol vernetzte Copolymere ergeben Pyrolysespektren, die außer den Etherfragmenten zusätzlich typische Bruchstücke der Styrol–Divinylbenzol-Matrix aufweisen. Der Vernetzungsgrad kann bestimmt werden.[12]

EXPERIMENTELLES

Pyrolysiert wird mit einem Curie-Pyrolysator (Fischer). Die Pyrolysedauer beträgt 2 Sekunden.

Pyrolyse–Gaschromatographie

Zur Aufnahme der Pyrolyse–Gaschromatogramme dient der Gaschromatograph F22 (Perkin-Elmer) mit einer 50-m langen Edelstahlkapillare, die mit Silikongummi SE 30 belegt ist. Die Identifizierung der Pyrolysefragmente aus den Gaschromatogrammen geschieht durch Retentionszeitvergleiche mit Testsubstanzen oder durch Massenspektrometrie.

Pyrolyse–Massenspektrometrie

Die Zuordnung der Molekülbanden in den Pyrolyse–Massenspektren ist nur aufgrund der Kenntnisse aus den Pyrolyse–Gaschromatogrammen möglich. Da bei der Pyrolyse–Massenspektrometrie die Pyrolyseprodukte direkt in die Ionenquelle des Massenspektrometers eingeschleust werden, beträgt die Analysendauer 5 sekunden.

Die Pyrolyse–Massenspektrometrie wird in dem GC–MS-System MAT 111 (Varian) durchgeführt. Zur weitgehenden Vermeidung einer Zweitfragmentierung in der Ionenquelle wird das Massenspektrum bei einer Ionisierungsenergie von 16 eV und einem Elektronenstrom von 150 μA aufgenommen. Um reproduzierbare und charakteristische Massenspektren zu erhalten, ist der optimale Zeitpunkt für die Aufnahme der Spektren zu ermitteln. Hierzu wird sofort bei Anstieg des Totalionenstroms eine Serie von Kurzzeit–Massenspektren mit hohem Massendurchlauf aufgenommen. Die Untersuchungen ergeben, daß reproduzierbare und für einen Austauscher charakteristische Massenspektren bei geringem Massendurchlauf erhalten werden.

UNTERSUCHUNGSERGEBNISSE

Monomere

Bei der Pyrolyse wird das Monomer nur zum geringen Teil thermisch gespalten und gelangt gasförmig durch das Einlaßsystem in die Ionenquelle, so daß seine Identifizierung und eine Reinheitskontrolle möglich sind. Im Gegensatz zur Pyrolyse der Austauscher bleiben kaum Zersetzungsprodukte auf der Pyrolysewendel zurück. Es wird hauptsächlich Acetaldehyd abgespalten, so daß man neben dem Molekül Fragmente findet, deren Massen um Vielfache von 44 geringer sind.

Kondensationsharze

Kondensationsharze mit Kronenverbindungen ohne Hilfsvernetzer geben Pyrolyse–Gaschromatogramme, in denen vorwiegend niedrig siedende Substanzen zu erkennen sind. Sie entstehen durch die thermische Fragmentierung der cyclischen Ether. Der Anteil aromatischer Verbindungen ist gering. Die Pyrolysefragmente der Etherbrücken werden mit zunehmender Ringgröße schwerer. Es bilden sich stabile cyclische Ether, wie Methyldioxolan und Dioxan mit ihren verschiedenen Alkylderivaten. Hilfsvernetzer wie Phenol, Xylol bzw. Toluol können deutlich durch ihre Pyrolysebruchstücke identifiziert werden. Bei den mit Phenol vernetzten Austauschern sind die Hauptfragmente Kresol und Dimethylphenol, bei denen mit Toluol vernetzten Toluol und Trimethylbenzol.

Polymerisationsharze

Bei den Austauschern, die durch Substitution an chlormethyliertem Polystryrol erhalten werden, erscheinen die für die Ankergruppe charakteristischen Bruchstücke neben dem ausgeprägten Bandenmuster des vernetzten Polystyrolgerüsts (Abb. 11).

Ein ähnliches Banden- und Peakmuster ergibt sich für die durch Polymerisation von 4-Vinyl-Kronenethern hergestellten Austauscher. Abbildung 12 enthält ein entsprechendes Pyrolyse-Massenspektrum.

Aus der Matrix stammen Methylstyrol (M = 118), Ethylvinylbenzol (M = 132), Divinylbenzol (M = 130).

Beständigkeit, Kapazität, Gleichgewichtseinstellung

Zersetzungstemperaturen, theoretische Kapazitäten, maximale Nutzungskapazitäten und die Zeiten bis zur Gleichgewichtseinstellung für alle Austauscher sind in Tabellen 2 bis 4 zusammengefaßt.

Thermische Beständigkeit. Die thermische Beständigkeit wird mit einem Heizmikroskop (8°/min) und einer Thermowaage (150°/hr) geprüft. Mit zunehmender Ringgröße nimmt die thermische Stabilität ab. Kondensationsharze sind thermisch stabiler als Copolymere.

Gegenüber –C–C–-Bindungen in den aus Divinylbenzol und 4- bzw. 4,4'-substituierten Vinyl-Kronenethern hergestellen Austauschern, bewirkt die Verknüpfung der Ankergruppe mit der Styrolmatrix über –C–NH–C– bzw. –C–O–C–-Bindungen eine Abnahme der thermischen Stabilität.

Chemische Beständigkeit. Die chemische Beständigkeit wird durch Kapazitätsmessungen bestimmt. Kondensationsharze sind gegenüber polaren Lösungsmitteln, wie Aceton, Dichlormethan, Acetonitril, Ethanol, Methanol, Tetrahydrofuran sowie 5*M* Salzsäure, 5*M* Salpetersäure und 5*M* Natronlauge beständig. Auch Oxidationsmittel, wie z.B. verdünnte Kaliumpermanganat- und Wasserstoffperoxid-Lösungen beeinträchtigen die Kapazität nicht. Dagegen nimmt die Kapazität nach Extraktion der Austauscher mit Benzol

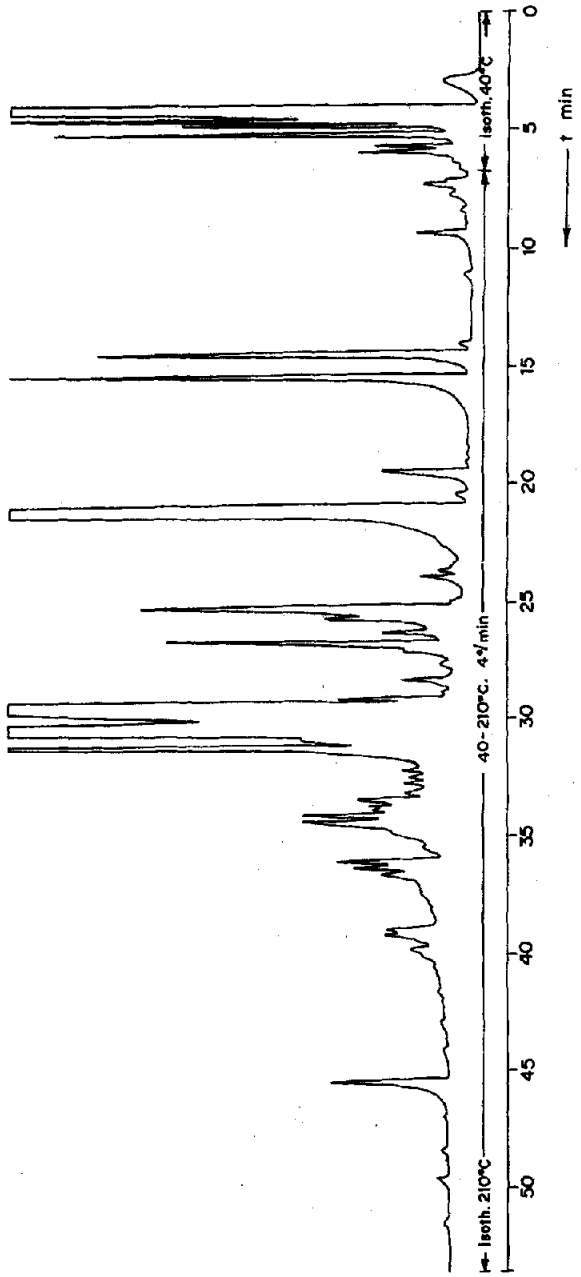


Abb. 11. Pyrolyse–Gaschromatogramm (800°C): Polymerisationsharz aus 4-Hydroxymethy-benzo-15-krone-5 und chlormethyliertem Polystyrol.

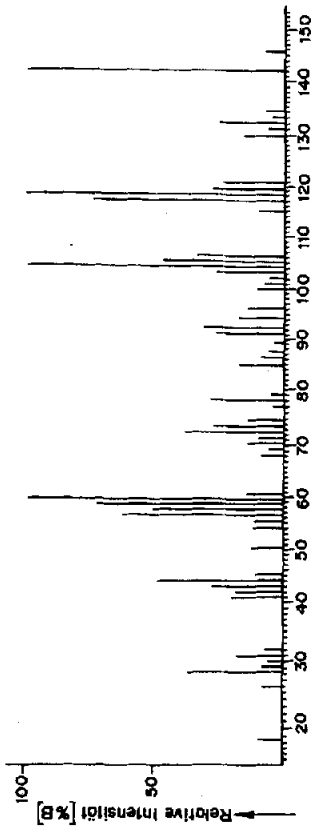


Abb. 12. Pyrolyse–Massenspektren (800°C, 16 eV, 150 μA); Copolymeres aus 4,4'-Divinyldibenzo-24-krone-8 und 13% DVB.

Tabelle 2. Eigenschaften

Ankergruppe	Matrix aus	Zersetzungstemperatur [°C]	theoretische Kapazität [mmol/g AT]	maximale Nutzkapazität für KSCN [mmol/g AT] CH_3OH	H_2O	zeitliche Gleichgewichtseinstellung [min] Korngröße 120µm CH_3OH	H_2O
DB-14-C-4	Formaldehyd/DB-14-C-4	370	2,8	–	–	10	–
B-15-C-5	Formaldehyd/Resorcin/B-15-C-5	230	–	1,8	–	40	–
B-15-C-5	Formaldehyd/Phenol/B-15-C-5	240	2,7	1,9	0,6	15	50
B-15-C-5	Formaldehyd/Toluol/B-15-C-5	270	2,7	1,6	0,6	25	45
B-15-C-5	chlormethyliertem Polystyrol/ 4-Amino-B-15-C-5	190	1,4	0,5	0,3	50	150
B-15-C-5	chlormethyliertem Polystyrol/ 4-Hydroxymethyl-B-15-C-5	190	1,2	0,5	0,3	55	140
B-15-C-5	4-Vinyl-B-15-C-5/Divinylbenzol	210	2,2	0,8	0,4	70	200
B-18-C-6	Formaldehyd/Phenol/B-18-C-6	250	2,4	1,5	0,5	25	55
B-18-C-6	Formaldehyd/Toluol/B-18-C-6	310	2,1	1,3	0,6	20	45
DB-18-C-6	Formaldehyd/DB-18-C-6	290	2,7	2,6	1,5	20	240
DN-18-C-6	Formaldehyd/DN-18-C-6	300	2,2	1,0	0,3	35	90
DB-21-C-7	Formaldehyd/DB-21-C-7	270	2,4	1,5	1,0	15	25

oder Cyclohexanon um 3 bis 8% ab. Austauscher mit Styrolmatrix sowie Copolymerisate aus vinylsubstituierten Kronenethern und Divinylbenzol sind auch gegen Benzol und Cyclohexanon beständig.

Radiolysebeständigkeit. Kondensationsharze sind radiolysebeständiger als Polymerisationsharze. So kann z.B. an dem durch Kondensation hergestellten Austauscher mit DB-24-C-8 als Ankergruppe nach γ-Bestrahlung von 10^9 rad keine Änderung der Kapazität festgestellt werden. Ursache der Stabilität sind wahrscheinlich Hydroxymethyl-Gruppen, die als Radikalfänger dienen.

Kapazität. Die aus Elementaranalysen berechneten theoretischen Kapazitäten sind groß und mit denen handelsüblicher Kationen- und Anionenaustauscher vergleichbar. Die maximalen Beladungskapazitäten hängen von mehreren Parametern ab (Tab. 1). Sie entsprechen bei §-DB-18-C-6, §-K[2.2.], §-K[2_B.2.],

Tabelle 3. Eigenschaften

Ankergruppe	Matrix aus	Zersetzungstemperatur [°C]	theoretische Kapazität [mmol/g AT]	maximale Nutzkapazität für CsSCN [mmol/g AT] CH_3OH	H_2O	zeitliche Gleichgewichtseinstellung [min] Korngröße 120µm CH_3OH	H_2O
DB-24-C-8	Formaldehyd/DB-24-C-8	170	2,2	1,92	0,86	15	260
DB-24-C-8	4-Vinyl-DB-24-C-8/Divinylbenzol	160	1,6	1,32	0,70	40	390
DB-24-C-8	4,4'-Divinyl-DB-24-C-8/ Divinylbenzol	150	1,2	0,84	0,35	30	370
DB-26-C-6	Formaldehyd/DB-26-C-6	290	–	–	–	–	–
DB-27-C-9	Formaldehyd/DB-27-C-9	–	1,9	–	–	–	–
DB-30-C-10	Formaldehyd/DB-30-C-10	230	1,8	–	1,2	–	–
DB-Diaza-15-C-5	Formaldehyd /DB-Diaza-15-C-4	230	2,3	–	–	–	–
B-Monoaza-15-C-5	Formaldehyd/Phenol/ B-Monoaza-15-C-5	230	1,7	–	–	–	–
DB-Monoaza-18-C-6	Formaldehyd/DB-Monoaza-18-C-6	240	1,7	–	–	–	–
DB-Pyridino-18-C-6	Formaldehyd/DB-Pyridino-18-C-6	280	1,9	–	–	–	–

Tabelle 4. Eigenschaften

Ankergruppe	Matrix aus	Zersetzungs-temperatur [°C]	theoretische Kapazität [mmol/g AT]	maximale Nutzkapazität [mmol/g AT] CH_3OH	H_2O	zeitliche Gleichgewichts-einstellung [min] Korngröße ~ 120 µm CH_3OH	H_2O
Kryptand [2 2 2]	chlormethyliertem Polystyrol/K[2.2.2]	230	1,4	1,1 für KSCN	0,3 für KSCN	180	180
Kryptand [2_B 2]	chlormethyliertem Polystyrol/K[2_B 2]	240	1,1	10 für KSCN	0,3 für KSCN	210	210
Kryptand [2_B 2 2]	Formaldehyd/Benzol/K[2_B 2 2]	220	1,3	1,2 für KSCN	0,8 für KSCN	340	150
Kryptand [2_B 2_B 2]	Formaldehyd/K[2_B 2_B 2]	250	1,4	1,3 für KSCN	0,8 für KSCN	180	140
Benzimidazolono-15-C-5	Formaldehyd/Phenol/Benzimid-azolono-15-C-5	290	2,3	0,9 für KSCN	0,6 für KSCN	10	–
Benzimidazolono-15-C-5	Formaldehyd/Toluol/Benzimid-azolono-15-C-5	270	2,2	0,8 für KSCN	0,5 für KSCN	10	–
Benzimidazolono-18-C-6	Formaldehyd/Phenol/Benzimid-azolono-18-C-6	290	2,1	1,5 für KSCN	1,0 für KSCN	10	–
Benzimidazolono-18-C-6	Formaldehyd/Toluol/Benzimid-azolono-18-C-6	300	2,1	1,6 für KSCN	1,3 für KSCN	10	–
Benzimidazolono-bspentamethylen	Formaldehyd/Benzimidazolono-bspentamethylen	270	2,2	1,5 für $Cd(SCN)_4$	1,1 für $Cd(SCN)_2$	10	–
Dibenzimidazolono-bshexamethylen	Formaldehyd/Dibenzimidazolono-bshexamethylen	280	2,2	1,5 für $Cd(SCN)_2$	1,2 für $Cd(SCN)_2$	10	–

§-K[2_B.2.2.] und §-K[2_B.2_B.2.] für Kaliumthiocyanat in Methanol der theoretischen Kapazität, wenn man die Bildung von 1:1-Komplexen annimmt. Die maximalen Beladungskapazitäten liegen in Methanol bei 1–3 mmole/g Austauscher. In Wasser sind sie etwa um die Hälfte geringer. Auch fällt die Beladungskapazität mit steigender Kationenladung.

Bei Austauschern mit nur O als Heteroatom ist die Salzaufnahme vom pH-Wert unabhängig. Bei allen Austauschern mit Stickstoff in der Ankergruppe tritt ab pH <3 Protonierung der N-Atome ein. Eine Komplexbildung mit Salzen findet nur noch in geringem Maße statt (Abb. 13 oben). Aufgrund der gebildeten quartären Ammoniumgruppen liegen dann Anionenaustauscher vor (Abb. 13 unten).

Zeitliche Gleichgewichtseinstellung. Die Gleichgewichtseinstellung erfolgt in Wasser langsamer als in Methanol. Sie wird bei Kondensationsharzen früher

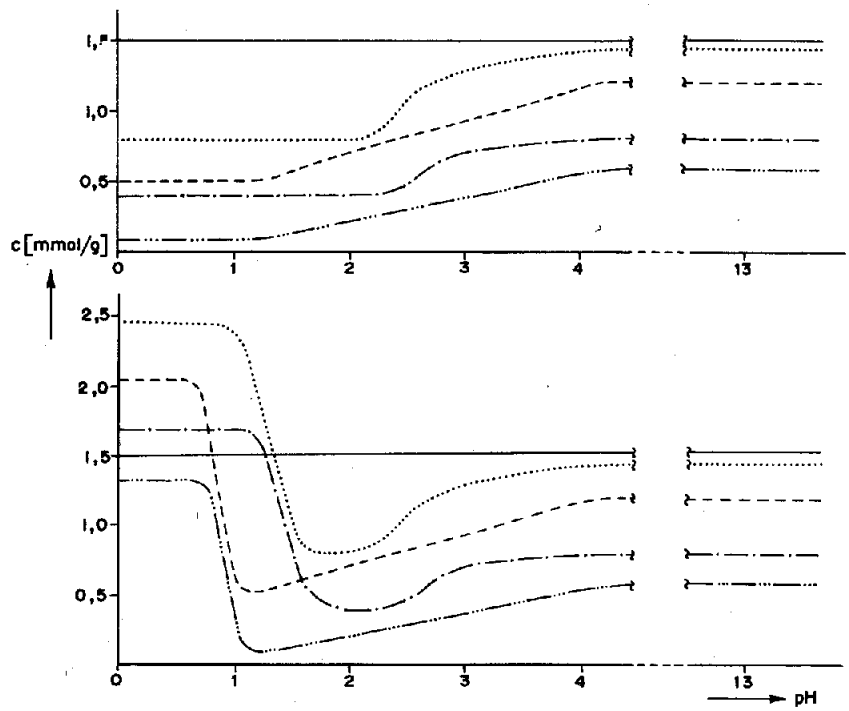
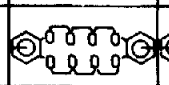
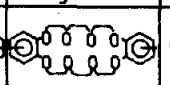


Abb. 13, pH-Abhängigkeit der maximalen Beladungskapazitäten für K^+ (oben) und SCN^- (unten) aus 0.5*M* KSCN-Lösung. —§-DB-18-C-6·····§-DB-monoaza-18-C-6----§-DB-pyridino-18-C-6— §-B-monoazo-15-C-5—§-DB-dipyridino-18-C-6

Tabelle 5. Kinetische Untersuchungen in $1M$ Salpetersaurer/$8 \cdot 10^{-3}M$ Cäsiumnitrat Lösung; Korngröße 3–40 μm

Austauscher	§-(DB-24-C-8)-§	§-(DB-24-C-8)-§ mit 2M HNO_3 vorbehandelt	Lewatit DN	Lewasorb N1	Dowex 50 WX 8
Ankergruppe			$HO_3S\text{-}CH_2$- 	$-SO_3H$	$-SO_3H$
Matrix	Formaldehyd / Ankergruppe	Formaldehyd / Ankergruppe	Phenol / Formaldehyd	Styrol/DVB	Styrol/DVB
Hersteller	Eigenherstellung	Eigenherstellung	Bayer AG	Bayer AG	Dow Chemical Company
Beladungskapazität $\left[\frac{mmol}{g\,AT}\right]$ in HNO_3	0,22	0,22	1,8	2,0	4,0
Anteil der für Cs^+ genutzten Kapazität in %	32,2	29,9	5,6	3,0	3,0
Arbeitsbereich pH	0–14	0–14	5–11	0–14	0–14
thermische Beständigkeit [°C]	170	170	45	100	150
effektiver Diffusionskoeffizient $\left[\frac{cm^2}{s}\right]$	10^{-10}	—	10^{-8} – 10^{-9}	10^{-5}–10^{-6}	10^{-5}–10^{-6}
min bis zu 50% des Gleichgewichtes	7,5	1,5	0,5	0,5	0,5
Mechanismus der Salzaufnahme	Gelkinetik	Mischkinetik	Filmkinetik	Filmkinetik	Filmkinetik

als bei Polymerisationsharzen erreicht. Mit zunehmender Ringgröße der Ankergruppe stellt sich das Gleichgewicht langsamer ein. Temperaturerhöhung beschleunigt nur bei kleineren Ankergruppen, z.B. §-B-15-C-5, geringfügig die Gleichgewichtseinstellung. Bei größeren Etherringen, z.B. DB-24-C-8, wirkt wahrscheinlich die thermische Bewegung der Etherbrücken der Komplexbildung entgegen.

Kinetische Messungen

Kinetische Untersuchungen werden an DB-24-C-8 als Ankergruppe unter Verwendung einer speziellen Apparatur[18] durchgeführt.

Untersuchungsergebnisse mit $1M$-salpetersaurer Cäsiumnitrat-Lösung sind für den durch Kondensation hergestellten Austauscher mit DB-24-C-8 als Ankergruppe und einige handelsübliche Kationenaustauscher in Tabelle 5 zusammengestellt.

Der Diffusionskoeffizient für Cs^+ an §-DB-24-C-8 liegt in der Größenordnung derer an schwachsauren Austauschern mit –COOH-Gruppen und ist um den Faktor 10^3 kleiner als an Dowex 50. Ursache für den niedrigen Stofftransport ist die Gelkinetik. Sie geht in eine Mischkinetik über, wenn durch Vorbehandlung mit $2M$ Salpetersäure zusätzlich hydrophile Carboxylgruppen eingebaut werden. Eine schnellere Gleichgewichtseinstellung ist die Folge. Die Selektivität für Cs^+ bleibt erhalten.

Selektivität

Die Verteilungskoeffizienten sind nur für Kationen gleicher Ladung ein Maß für die Selektivitäten. Eine Trennung ist immer dann möglich, wenn sich der Quotient aus zwei Verteilungskoeffizienten um mindestens 0,2 von 1,0 unterscheidet.

Eine Faustregel gibt eine einfache Entscheidungshilfe bei der Auswahl eines der in Abb. 1–3 aufgeführten Austauscher zur Lösung eines speziellen Trennproblems.

Ein Austauscher bevorzugt das Kation, für das folgende Beziehung am besten erfüllt ist.

$$\frac{\text{Durchmesser des Kations (nach Goldschmidt)}}{\text{Durchmesser des Polyetherrings}} = 0{,}80$$

Verteilungskoeffizienten. Die Verteilungskoeffizienten α (Tabellen 6–8) werden aus Batchversuchen bestimmt. Hierzu schüttelt man 1 g Austauscher mit 20 ml $0{,}05M$ Salzlösung 24 hr und bestimmt dann den Gehalt in der wässrigen Lösung.

$$\alpha = \frac{c_{\text{vorher}} - c_{\text{nachher}}}{c_{\text{nachher}}}$$

Das Harzgerüst, die Art der Verknüpfung und die Substituenten an einem gegebenen Etherring beeinflussen deutlich die Größe der Verteilungskoeffizienten, jedoch nur geringfügig die Selektivität.

Tabelle 6. Verteilungskoeffizienten in Methanol

	§-DB-14-C-4				§-B-15-C-5 Hilfsvernetzer Phenol				§-DB-18-C-6				§-DB-21-C-7				§-DB-24-C-8				§-DB-27-C-9				§-DB-30-C-10			
	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-
Li^+	0,02	0,03	0,02	-	≤0,01	≤0,01	≤0,01	≤0,01	0,2	0,4	0,5	0,5	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	0,05	0,10	0,21	≤0,01	0,06	0,11	0,21
Na^+	0,03	0,05	0,03	0,03	0,73	1,07	1,22	1,69	3,2	4,5	6,6	6,5	0,09	0,26	0,35	0,28	0,13	0,19	0,16	0,27	0,80	1,02	1,12	1,42	0,95	1,10	1,23	1,32
K^+	0,08	0,04	0,08	0,04	1,30	1,56	1,88	2,38	6,5	8,5	13,6	14,1	2,28	2,41	2,66	3,20	0,76	0,80	1,30	1,67	1,91	2,15	2,65	2,88	1,85	2,22	2,83	2,91
Rb^+	0,08	0,04	0,06	-	0,83	1,14	1,32	-	3,4	5,4	7,8	8,7	2,07	3,30	3,61	-	1,32	1,53	1,91	2,16	1,92	2,05	2,49	-	1,83	2,16	2,64	-
Cs^+	0,08	0,05	0,06	-	0,73	0,96	1,13	-	2,1	3,1	4,4	4,8	1,37	2,28	2,42	-	1,46	1,60	2,11	2,42	1,45	1,82	2,13	2,70	1,41	1,70	2,22	2,82
NH_4^+	0,03	0,03	≤0,01	0,03	0,36	0,47	0,54	0,78	-	-	-	-	0,52	0,64	1,12	1,07	0,29	0,28	0,57	0,56	1,80	1,90	1,95	-	1,80	2,00	2,10	-
Mg^{2+}	0,03	0,02	0,02	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,1	≤0,1	0,4	0,3	≤0,01	≤0,01	≤0,01	0,03	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01
Ca^{2+}	0,04	0,03	0,02	0,02	0,09	0,10	0,13	0,21	0,4	0,6	1,3	1,3	≤0,01	≤0,01	≤0,01	0,08	≤0,01	≤0,01	0,03	≤0,01	≤0,01	≤0,01	≤0,01	-	≤0,01	≤0,01	≤0,01	-
Sr^{2+}	0,04	0,03	-	≤0,01	0,23	0,27	-	0,35	0,8	1,6	3,5	3,6	0,06	0,07	-	0,42	≤0,01	0,13	-	0,35	≤0,01	0,05	-	-	0,05	0,07	-	-
Ba^{2+}	0,05	0,03	0,03	0,02	0,31	0,35	0,50	0,47	1,4	2,3	4,6	4,9	0,76	1,24	1,56	0,77	0,16	0,41	0,07	0,36	0,16	0,29	0,39	-	0,61	0,92	1,31	-

Tabelle 7. Verteilungskoeffizienten in Wasser

	§-B-15-C-5 Hilfsvernetzer Phenol				§-DB-18-C-6				§-DB-21-C-7				§-DB-24-C-8				§-DB-27-C-9				§-DB-30-C-10			
	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-	Cl^-	Br^-	I^-	SCN^-
Li^+	≤0,01	≤0,01	≤0,01	≤0,01					≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	0,07	≤0,01	≤0,01	≤0,01	0,05
Na^+	0,06	0,06	0,15	0,17					≤0,01	0,02	0,10	0,13	0,05	0,06	0,06	0,08	≤0,01	≤0,01	≤0,01	0,12	≤0,01	≤0,01	≤0,01	0,10
K^+	0,07	0,10	0,18	0,28	0,10	0,20	0,56	0,92	0,05	0,15	0,19	0,36	0,07	0,08	0,08	0,13	0,05	0,06	0,15	0,22	0,05	0,09	0,15	0,20
Rb^+	0,05	0,09	0,14	-					0,05	0,14	0,18	-	0,18	0,18	0,22	0,25	0,05	0,06	0,16	-	0,05	0,08	0,14	-
Cs^+	0,04	0,06	0,09	-					0,04	0,13	0,16	-	0,20	0,20	0,24	0,29	0,05	0,07	0,09	0,13	0,05	0,07	0,11	0,10
NH_4^+	0,04	0,06	0,08	0,12					0,03	0,09	0,14	0,18	0,05	0,06	0,06	0,08	≤0,01	≤0,01	0,08	-	≤0,01	≤0,01	0,06	-
Mg^{2+}	≤0,01	≤0,01	≤0,01	≤0,01					≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	-	≤0,01	≤0,01	≤0,01	-
Ca^{2+}	≤0,01	≤0,01	0,03	0,05					≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	≤0,01	-	≤0,01	≤0,01	≤0,01	-
Sr^{2+}	0,03	0,05	-	0,05					≤0,01	0,02	-	0,02	≤0,01	≤0,01	-	0,09	≤0,01	≤0,01	-	-	≤0,01	≤0,01	-	-
Ba^{2+}	0,04	0,04	0,07	0,08					0,03	0,05	0,08	0,10	0,02	0,02	0,04	0,14	0,03	0,03	0,07	-	0,02	0,04	0,09	-

Tabelle 8. Verteilungskoeffizienten für Kaliumsalze in Methanol und Wasser

	Methanol									Wasser								
	F^-	Cl^-	Br^-	I^-	SCN^-	OH^-	NO_3^-	SO_4^{2-}	PO_4^{3-}	F^-	Cl^-	Br^-	I^-	SCN^-	OH^-	NO_3^-	SO_4^{2-}	PO_4^{3-}
§-DB-14-C-4	-	0,08	0,04	0,08	0,04	-	-	-	-	-	-	-	-	-	-	-	-	-
§-B-15-C-5 Phenol	1,08	1,30	1,56	1,88	2,38	5,67	1,58	-	0,89	0,05	0,07	0,10	0,18	0,28	0,64	0,11	0,10	0,17
§-DB-18-C-6	5,7	6,5	8,5	13,6	14,6	4,6	13,3	-	7,3	0,05	0,1	0,2	0,56	0,92	0,12	0,27	0,02	0,08
§-DB-21-C-7	1,3	2,28	2,41	2,66	3,20	1,5	11,5	-	2,3	0,02	0,06	0,09	0,37	0,61	0,12	0,15	0,00	0,04
§-DB-24-C-8	0,72	0,76	0,80	1,30	1,67	0,42	0,80	-	0,54		0,07	0,08	0,08	0,13	0,06	0,09	0,04	0,07
§-DB-27-C-9	1,1	1,91	2,15	2,65	2,88	0,65	0,95	-	2,3		0,05	0,06	0,15	0,22	0,08	0,36	0,01	0,03
§-DB-30-C-10	1,2	1,85	2,22	2,83	2,91	0,78	1,10	-	2,2		0,05	0,09	0,15	0,20	0,08	0,50	0,03	0,05

Tabelle 9. Selektivitätsreihen in Methanol

Ankergruppe	DB-14-C-4
Kationen	$K^+, Li^+ > Na^+ > NH_4^+$
Ankergruppe	B-15-C-5
Kationen	$K^+ > Rb^+ > Cs^+, Na^+ > Li^+$
	$Ba^{2+} > Sr^{2+} > Zn^{2+} > Cd^{2+}, Fe^{2+} > Ca^{2+} > Mn^{2+}, Co^{2+}, Ni^{2+} > Mg^{2+}, Hg^{2+}$
Anionen	$SCN^- > I^-, PO_4^{3-} > NO_3^-, Br^- > Cl^- > F^-$
Alkylammoniumsalze	methyl- > ethylsubstituiert
	mono- > di- > tri- > tetrasubstituiert
Ankergruppe	B-18-C-6
Kationen	$K^+ > Rb^+ > Cs^+ > Na^+ > NH_4^+ > Li^+$
	$Ba^{2+} > Sr^{2+} > Ca^{2+} > Cd^{2+} > Zn^{2+}, Fe^{2+}, Cu^{2+} > Co^{2+} > Mg^{2+}, Be^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+}$
Anionen	$OH^- > PO_4^{3-} > NO_3^- > SCN^-, I^- > Br^- > Cl^- > F^-$
Ankergruppe	DB-18-C-6
Kationen	$K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$
	$Ra^{2+} > Ba^{2+} > Eu^{2+} > Zn^{2+}, Sr^{2+} > Cd^{2+} > Ca^{2+} > Fe^{2+} > UO_2^{2+} > Cu^{2+}, Co^{2+} > Ni^{2+} > Hg^{2+}, Mg^{2+}$
	$Fe^{3+} > Cr^{3+} > Pr^{3+} > Ce^{3+} > La^{3+}$
Anionen	$SCN^- > I^- > NO_3^- > Br^- > Cl^- > PO_4^{3-} > OH^- > F^-, SO_4^{2-}$
Alkylammoniumsalze	methyl- > ethyl-, n-propyl- > butylsubstituiert
Ankergruppe	DN-18-C-6
Kationen	$K^+ > Rb^+ > Cs^+ > Na^+ > NH_4^+ > Li^+$
	$Ba^{2+} > Sr^{2+} > Zn^{2+}, Cd^{2+} > Ca^{2+}, Cu^{2+} > Fe^{2+} > Be^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}$

Selektivitätsreihen. Im Gegensatz zu konventionellen stark sauren und stark basischen Austauschern entsprechen die Selektivitätsreihen nicht mehr den lyotropen Reihen von Hofmeister und Schulze-Hardy.[18] Maximale Beladungskapazitäten und Stabilitäten der Polyetherkomplexe laufen parallel. Größenordnungsmäßig gilt die Säure–Base-Theorie von Pearson.

Für Wasser und Methanol als Lösungsmittel gelten im allgemeinen die gleichen Selektivitätsreihen. Sie folgen mit wenigen Ausnahmen denjenigen der monomeren Komplexe. In der Reihe der Anionen steigt die

Tabelle 10. Selektivitätsreihen in Methanol

Ankergruppe:	DB-21-C-7
Kationen:	$Rb^+ > K^+ > Cs^+ > NH_4^+ > Na^+ > Li^+$
	$Ba^{2+} > Sr^{2+} > Ca^{2+}, Mg^{2+}$
Anionen	$SCN^- > I^- > Br^- > Cl^-$
Ankergruppe	DB-24-C-8
Kationen:	$Cs^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > Li^+$
	$Hg^{2+} > Pb^{2+} > Ba^{2+} > Cd^{2+} > Sr^{2+} > Fe^{2+} > Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}$
Anionen:	$SCN^- > I^- > Br^-, NO_3^- > Cl^- > PO_4^{3-}, OH^- > F^-, SO_4^{2-}$
Ankergruppe:	Kryptanden
Kationen:	$K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$
	$Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$
Anionen:	$SCN^-, I^- > Br^-, Cl^-$

Salzaufnahme im allgemeinen in der Reihenfolge ihrer Polarisierbarkeiten. In einigen Fällen hat OH^- eine Sonderstellung. Den Selektivitätsreihen liegen Verteilungskoeffizienten (Tabellen 9–10) bzw. Massenverteilungskoeffizienten (Tabellen 11–13) zugrunde.

Im einzelnen ist folgendes auszuführen.

Ankergruppe DB-14-C-4. In methanolischer Lösung werden nur sehr geringe Mengen an Alkali- und Erdalkalikationen aufgenommen. Geht man zu Dioxan, das eine geringere DK als Methanol besitzt, als Lösungsmittel über, so werden Li^+ und Na^+ in Form von 1:2-Komplexen gebunden. Die aus der Elementaranalyse berechnete Kapazität beträgt 2,8 mmole/g Austauscher, die maximale Beladungskapazität für LiBr in Acetonitril 1,4 mmole/g Austauscher.

Ankergruppen B-15-C-5 und DB-18-C-6. Für Alkali- und Erdalkalikationen ergeben sich in Methanol die gleichen Selektivitätsreihen, was auch aufgrund ihrer in etwa gleichen Ringgrößen zu erwarten ist. Bei B-15-C-5 tritt in Wasser ein Wechsel in der Reihenfolge zwischen Na^+ und Rb^+ und NO_3^- und Br^- auf.

Ankergruppen DB-21-C-7 und DB-24-C-8. Das Maximum der Alkalisalzaufnahme liegt für DB-21-C-7 bei den Rb^+-Salzen. Von DB-24-C-8 mit dem noch etwas größeren Ring werden Cs^+-Salze bevorzugt.

Ankergruppen B-15-C-5 une DB-18-C-6. Für Alkali- und Erdalkalikationen ergeben sich in Methanol die lyse läßt sich eine Kapazität von 2,0 mmole/g Austauscher berechnen. Der Austauscher hat für die Trennung anorganischer Salze keine Bedeutung (Teil II).[19]

Tabelle 11. Selektivitätsreihen in Methanol und Wasser

Ankergruppe	**Benzo-monoaza-15-C-5**
Kationen:	$K^+ > Cd^{2+} > Rb^+ > Cs^+ > Hg^{2+} > Na^+ > NH_4^+ > Ba^{2+}, Sr^{2+}, Ca^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Mg^{2+}, Cr^{3+}, Li^+$ für Chloride in Methanol und Wasser
	$K^+ > Cs^+ > Rb^+ > Cd^{2+} > Na^+ > NH_4^+ > Pb^{2+} > Hg^{2+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Li^+$ für Nitrate in Methanol und Wasser
Anionen:	$SCN^- > I^- > Br^- > NO_3^- > ClO_4^- > Cl^- > ClO_3^- > OH^- > IO_3^- > PO_4^{3-} > F^- > SO_4^{2-}$ für Kaliumsalze in Methanol und Wasser
Ankergruppe:	**Dibenzo-monoaza-18-C-6**
Kationen:	$K^+ > Cd^{2+} > Hg^{2+} > Rb^+ > Cs^+ > Na^+ > NH_4^+ > Ba^{2+} > Sr^{2+} > Ca^{2+} > Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Mg^{2+} > Mn^{2+} > Cr^{3+}, Li^+$ für Chloride in Methanol
	$K^+ > Cs^+ > Rb^+ > Na^+ > Cd^{2+} > Hg^{2+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Cr^{3+}, Li^+$ für Chloride in Wasser
	$K^+ > Pb^{2+} > Rb^+ > Cs^+ > Na^+ > NH_4^+ > Tl^+ > Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Li^+$ für Nitrate in Methanol
	$K^+ > Rb^+ > Cs^+ > Pb^{2+} > Na^+ > NH_4^+ > Tl^+ > Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Li^+$ für Nitrate in Wasser
Anionen:	$SCN^- > I^- > ClO_4^- > Br^- > NO_3^- > Cl^- > BrO_3^- > IO_3^- > PO_4^{3-} > F^- > OH^-$ für Kaliumsalze in Methanol und Wasser
Ankergruppe:	**Dibenzo-dipyridino-18-C-6**
Kationen:	$K^+ > Cd^{2+} > Cs^+ > Rb^+ > Hg^{2+} > Na^+ > NH_4^+ > Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+} > Cr^{3+}, Li^+$ für Chloride in Methanol
	$K^+ > Cs^+ > Rb^+ > Na^+ > NH_4^+ > Cd^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cr^{3+}, Li^+$ für Chloride in Wasser
	$K^+ > Cs^+ > Pb^{2+} > Rb^+ > Na^+ > Hg^{2+} > NH_4^+ > Cu^{2+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Li^+$ für Nitrate in Methanol
Anionen:	$SCN^- > I^- > ClO_4^- > Br^- > NO_3^- > Cl^- > OH^- > BrO_3^- > IO_3^- > F^-, PO_4^{3-}, SO_4^{2-}$ für Kaliumsalze in Methanol und Wasser

Tabelle 12. Selektivitätsreihen in Methanol und Wasser

Ankergruppe:	Dibenzimidazolono-bispentamethylen
Kationen:	$Hg^{2+} > Cd^{2+} > Cs^{+} > Rb^{+} > K^{+} > Mn^{2+} > Ba^{2+} > Sr^{2+}, Ca^{2+} > Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, NH_4^{+} > Li^{+}$ für Chloride in Methanol
	$Pb^{2+} > Tl^{+} > Ba^{2+}, Sr^{2+}, Ca^{2+} > Cs^{+} > Mg^{2+}, Rb^{+}, K^{+} > Cu^{2+}, Na^{+}, NH_4^{+}, Li^{+}$ für Nitrate in Methanol
Anionen	$ClO_4^{-} > SCN^{-} > I^{-} > Br^{-} > Cl^{-} > ClO_3^{-} > OH^{-} > IO_3^{-} > NO_3^{-} > PO_4^{3-}, SO_4^{2-}, F^{-}, BrO_3^{-}$ für Natriumsalze in Methanol
Ankergruppe	Dibenzimidazolono-bishexamethylen
Kationen:	$Cd^{2+} > Ba^{2+} > Mn^{2+} > Sr^{2+}, Ca^{2+} > Cs^{+}, Rb^{+}, K^{+} > Cu^{2+}, Mg^{2+}, Na^{+}, NH_4^{+}, Li^{+}$ für Chloride in Wasser
	$Pb^{2+} > Cs^{+} > Ba^{2+}, Rb^{+}, K^{+} > Ca^{2+}, Mg^{2+}, Na^{+}, Li^{+} > Cu^{2+}, Ag^{+}, Tl^{+}, NH_4^{+}$ für Nitrate in Wasser
Anionen	$ClO_4^{-} > SCN^{-} > I^{-} > Br^{-} > Cl^{-} > ClO_3^{-} > OH^{-} > IO_3^{-} > NO_3^{-} > SO_4^{2-}, PO_4^{3-}, F^{-}, BrO_3^{-}$ für Natriumsalze in Methanol
	$ClO_4^{-} > ClO_3^{-} > BrO_3^{-}, IO_3^{-}, NO_3^{-} > SCN^{-}, I^{-}, Br^{-}, Cl^{-}, F^{-}, OH^{-}, SO_4^{2-}, PO_4^{3-}$ für Natriumsalze in Wasser

Tabelle 13. Selektivitätsreihen in Methanol und Wasser

Ankergruppe	Dibenzimidazolono-bispentamethylen
Kationen:	$Hg^{2+} > Cd^{2+} > Cs^{+} > Rb^{+} > K^{+} > Mn^{2+} > Ba^{2+} > Sr^{2+}, Ca^{2+} > Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, NH_4^{+} > Li^{+}$ für Chloride in Methanol
	$Pb^{2+} > Tl^{+} > Ba^{2+}, Sr^{2+}, Ca^{2+} > Cs^{+} > Mg^{2+}, Rb^{+}, K^{+} > Cu^{2+}, Na^{+}, NH_4^{+}, Li^{+}$ für Nitrate in Methanol
Anionen	$ClO_4^{-} > SCN^{-} > I^{-} > Br^{-} > Cl^{-} > ClO_3^{-} > OH^{-} > IO_3^{-} > NO_3^{-} > PO_4^{3-}, SO_4^{2-}, F^{-}, BrO_3^{-}$ für Natriumsalze in Methanol
Ankergruppe:	Dibenzimidazolono-bishexamethylen
Kationen:	$Hg^{2+} > Cd^{2+} > Ba^{2+} > Mn^{2+} > Sr^{2+}, Ca^{2+} > Cs^{+}, Rb^{+}, K^{+} > Cu^{2+}, Mg^{2+}, Na^{+}, NH_4^{+}, Li^{+}$ für Chloride in Wasser
	$Pb^{2+} > Cs^{+} > Ba^{2+}, Rb^{+}, K^{+} > Ca^{2+}, Mg^{2+}, Na^{+}, Li^{+} > Cu^{2+}, Ag^{+}, Tl^{+}, NH_4^{+}$ für Nitrate in Wasser
Anionen	$ClO_4^{-} > SCN^{-} > I^{-} > Br^{-} > Cl^{-} > ClO_3^{-} > OH^{-} > IO_3^{-} > NO_3^{-} > SO_4^{2-}, PO_4^{3-}, F^{-}, BrO_3^{-}$ für Natriumsalze in Methanol
	$ClO_4^{-} > ClO_3^{-} > BrO_3^{-}, IO_3^{-}, NO_3^{-} > SCN^{-}, I^{-}, Br^{-}, Cl^{-}, F^{-}, OH^{-}, SO_4^{2-}, PO_4^{3-}$ für Natriumsalze in Wasser

Kryptanden. Von den beiden Austauscherarten (Kondensationsharze bzw. Copolymere) haben jeweils die ohne bzw. mit einem Benzolring der Ankergruppe die besseren analytischen Eigenschaften. Unterschiede bei den einzelnen Austauschern treten lediglich in den Aufnahmen von Na^+- und Rb^+-Salzen auf. Die offenen Ringe der Monocyclen begünstigen analog denen der Kronenverbindungen die Aufnahme des leichter polarisierbaren Rb^+-Ions. Demgegenüber findet beim K-[2_B.2.2.] beim Übergang von Methanol zu Wasser als Lösungsmittel eine Inversion Rb^+/Na^+ statt.

Ankergruppen B-monoaza-15-C-5, DB-monoaza-18-C-6 und DB-dipyridino-18-C-6. Da hier die Selektivitäten nach der Größe der Massenverteilungskoeffizienten geordnet sind, enthält jede Reihe Kationen unterschiedlicher Ladung. Die Austauscher sind ausnahmslos kaliumselektiv. Ab pH <3 wirken sie vorwiegend als Anionenaustauscher (Abb. 13).

Ankergruppen Dibenzimidazolono-bispentamethylen bzw. -hexamethylen und Benzimidazolono-15-C-5 bzw. -18-C-6. Auch diese Selektivitätsreihen sind nach der Größe von Massenverteilungskoeffizienten zusammengestellt. Von den Alkali- und Erdalkalikationen nimmt Dibenzimidazolono-bispentamethylen in Methanol Cäsiumchloride bevorzugt vor allen anderen Chloriden und Dibenzimidazolono-bishexamethylen in Wasser Cäsiumnitrat vor allen anderen Nitraten auf. Austauscher mit Benzimidazolono-15-C-5 sind nur zur Trennung organischer Verbindungen geeignet.

Ausblick

Die Anwendungsgebiete der Austauscher mit cyclischen Polyethern sind vielseitig. Sie können in weiten Bereichen der anorganischen und organischen Chemie eingesetzt werden. Auch in der Radiochemie trägt ihre Verwendung zur Lösung spezieller Probleme bei. Hierüber wurde schon in einigen Veröffentlichungen[1–10] berichtet. Eine weitere Veröffentlichung, die sich mit der Anwendung der Austauscher beschäftigt, folgt demnächst in dieser Zeitschrift.[19]

Anerkennungen—Für finanzielle Unterstützung danken wir der Deutschen Forschungsgemeinschaft, Bonn–Bad Godesberg.

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Summary—Numerous exchangers with cyclic polyethers as anchor groups have been prepared and their properties examined. 4-Carboxyethyl- and 4-hydroxypropylbenzo crown ethers were fixed to silica gel and used as stationary phases in high-pressure liquid-chromatography.

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AUSTAUSCHER MIT CYCLISCHEN POLYETHERN ALS ANKERGRUPPEN—II

ANWENDUNGEN

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Zusammenfassung—Austauscher mit cyclischen Polyethern als Ankergruppen besitzen eine große Anwendungsbreite. In der Elutionschromatographie seien u.a. genannt: Kationentrennungen bei gleichen Anionen, Anionentrennungen bei gleichen Kationen, Trennungen nichtsalzartiger organischer Verbindungen, Wasserbestimmungen. Einige monomere Kronenether, insbesondere 4- und 4,4'-alkylsubstituierte Derivate, sind zur Extraktion bzw. die Addukte mit Heteropolysäuren als flüssige Austauscher geeignet. In einigen Fällen lassen sich die Austauscher gut in der Dünnschichtchromatographie und Dünnschichtelektrophorese verwenden. Auch in der präparativen Chemie können die Austauscher vorteilhaft eingesetzt werden. Zu nennen sind: Salzkonversionen zur Reindarstellung schwer zugänglicher Salze, Isolierung bzw. Reinigung organischer Verbindungen, Anionenaktivierung bei organischen Reaktionen.

Über die Herstellung und Charakterisierung von Austauschern mit cyclischen Polyethern als Ankergruppen[1–10] wurde in Teil I[11] berichtet. Im folgenden werden über die genannten Veröffentlichungen hinaus zahlreiche Einsatzmöglichkeiten der Austauscher beschrieben. Eine allgemeine Übersicht über die Arbeitsbereiche für die analytische und die präparative Chemie gibt Tabelle 1.

In den Abbildungen sind die verwendeten Austauscher neben den Diagrammen in Kurzbezeichnungen aufgeführt.

ELUTIONSCHROMATOGRAPHISCHE TRENNUNGEN

Die neuen Austauscher besitzen eine große Anwendungsbreite. An Trennungen seien u.a. genannt: Kationentrennungen bei gleichen Anionen, Anionentrennungen bei gleichen Kationen, Trennungen nichtsalzartiger organischer Verbindungen, Wasserbestimmungen.

Säulenpackungen und Apparaturen

Voraussetzung für gute Trennungen sind, wie bei jeder Säulenchromatographie, sehr sorgfältig gepackte Säulen. Die Kondensate oder Polymerisate werden pulverisiert, naß gesiebt und sedimentiert. Die Säulenfüllung erfolgt mit Hilfe eines Packungstopfes unter Druck. Die Packungseigenschaften lassen sich aus dem Druck am Säulenkopf, den Säulenabmessungen, den Bandenbreiten und den Elutionsvolumina berechnen. Die gepackten Säulen haben im allgemeinen folgende Eigenschaften:

mittlere Teilchengröße:	10 μm
Permeabilität:	$85 \cdot 10^{-15}$ m²
lineare Geschwindigkeit:	0,1 mm/sec
Bodenhöhe:	70 μm für LiCl in Wasser.

Der Aufbau der Versuchsanordnungen ist Abb. 1 zu entnehmen.

Die Packungseigenschaften werden mit einer druckkonstanten Hochdruck-Kolbenmembran-Pumpe (S 4, Orlita) emittelt, Trennungen mit einer flußkonstanten Pumpe (Labordosierpumpe M 20 oder M 80, Kontron) durchgeführt.

Zur Spuren- und Mikroanalyse dienen die Säulen A–D mit Differentialrefraktometern (R 401, Waters oder Multiref 902, Optilab) bzw. einem Bohrloch-Szintillationszähler als Detektoren. Die Nachweis- und Bestimmungsgrenzen liegen in der Größenordnung von μg bis pg. Zu präparativen Zwecken dient Säule E in Verbindung mit einem Durchflußrefraktometer und einem Fraktionssammler.

Anorganische Kationen und Anionen

Von zahlreichen durchgeführten Trennungen werden hier nur einige Beispiele gebracht.

Kationen bei gleichen Anionen

Alkalisalze. Für die Elemente mit niedriger Ordnungszahl (Li^+, Na^+, K^+) nimmt man am besten Austauscher mit kleineren bzw. mittleren Ringgrößen (Ankergruppen: B-15-C-5, DB-18-C-6), für die Elemente höherer Ordnungszahl (K^+, Rb^+, Cs^+) solche mit mittleren bzw. größeren Ringen (Ankergruppen: DB-21-C-7, DB-24-C-8). Die Bindung der Alkalihydroxide an die Austauscher, die für ihren Einsatz als Festbasen wichtig ist, geht aus der Trennung von Natronlauge und Kaliumlauge hervor.[5]

Tabelle 1. Anwendungen der Austauscher mit cyclischen Polyethern als Ankergruppen

Analytische Chemie	Präparative Chemie
Trennung von Kationen (z.B. Alkali- und Erdalkalimetalle, Schwer- und Wertmetalle)	Salzkonversionen (z.B. Iodide, Thiocyanate)
Trennung von Anionen (z.B. Halogenide, Pseudohalogenide)	Reinigung bzw. Isolierung organischer Verbindungen
Trennung nichtsalzartiger organischer Verbindungen	Anionenaktivierung bei organischen Reaktionen (z.B. nukleophile Substitutionen)
Spurenanreicherung (z.B. Radionuklide)	
Bestimmungen von Wasser in anorganischen und organischen Verbindungen	
Säulenchromatographie (Niederdruckflüssigkeitschromatographie, Hochdruckflüssigkeitschromatographie), Dünnschichtchromatographie	
Elektrophorese	

Die Trennung kleiner Mengen Natriumchlorid und Salzsäure innerhalb 1 hr gibt Abb. 2 wieder. Abbildung 3 zeigt das Elutionsdiagramm der schweren Alkalielemente. Die Elution der Chloride mit Wasser erfolgt in 80 min. Beim Vorliegen eines leichter polarisierbaren Anions dauert die Trennung länger.

Die hohe Selektivität der Ankergruppe DB-24-C-8 für Cs^+ gestattet dessen Abtrennung aus einem Gemisch zahlreicher anderer Kationen.[9] Alkylammoniumchloride können sowohl am Austauscher mit B-15-C-5 als auch an dem mit DB-18-C-6 als Ankergruppe getrennt werden.[5,6] In der Reihe strukturisomerer Propyl- und Butylammoniumchloride sind ebenfalls Trennungen durchführbar.[6]

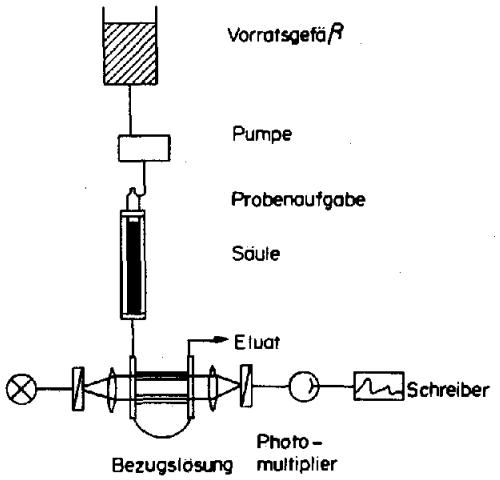

Abb. 1. Versuchsanordnung zur Elutionschromatographie mit Differentialrefraktometer als Detektor. Säulenparameter (Temperatur 25°C):

	A	B	C	D	E
Höhe, *cm*	10	15	20	30	60
Innendurchmesser, *cm*	0,4	0,4	0,4	0,4	2,5
Harzbettvolumen, *ml*	0,67	1,45	1,95	3,20	210
Harzmenge, *g*	0,45	0,80	1,10	1,55	75
Elutionsgeschwindigkeit, *ml/hr*	2–20	2–20	2–20	2–20	20–100
Arbeitsdruck, *bar*	3–15	3–15	3–15	3–20	3–20

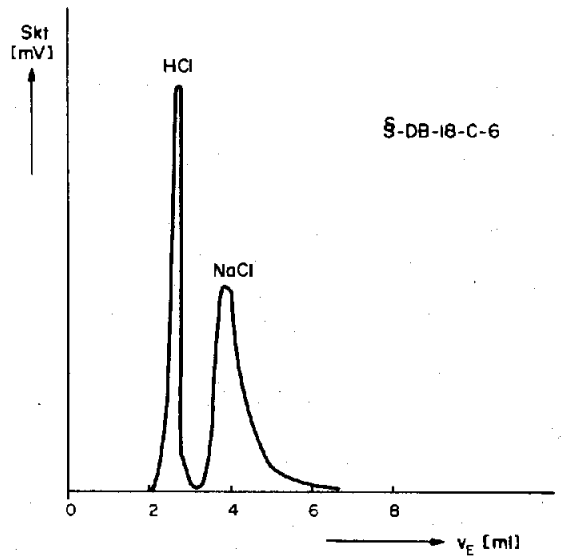

Abb. 2. Trennung von 0,09 mg HCl und 0,3 mg NaCl durch Elution mit Wasser.

Erdalkalisalze. Die Trennung aller Erdalkaliionen kann z.B. an dem Austauscher mit Benzimidazolono-18-C-6 als Ankergruppe durchgeführt werden (Abb. 4).

Ankergruppen mit vergleichbaren Durchmessern, die nur O im Etherring enthalten, binden Ba^{2+} besonders fest, so daß große Elutionsvolumina notwendig sind. Sehr kleine Mengen ^{90}Sr lassen sich von größeren Mengen Ca^{2+} abtrennen, ein Problem, das für die $^{90}Sr^{2+}$-Bestimmung im "fall out" von Bedeutung ist.[6]

An dem Austauscher mit Benzimidazolono-18-C-6 als Ankergruppe ist auch die Trennung Ba^{2+}/Ra^{2+} möglich (Abb. 5). Eine schnelle Elution der von Ba^{2+} befreiten Ra^{2+}-Spuren gelingt am besten mit Wasser (Abb. 5 mitte) bzw. 0,5*M* Salzsäure (Abb. 5 unten). Im letzteren Fall tritt Protonierung der N-Atome ein und es entsteht ein Anionenaustauscher (Teil I,[11] Abb. 13).

Die Abtrennung des Tochternuklids ^{90}Y ($t_{1/2}$ = 64,1 hr) von ^{90}Sr ($t_{1/2}$ = 28,5 hr) gelingt an §-DB-18-C-6.[5]

Übergangsmetallsalze. Die Trennung von $ZnCl_2$, $CdCl_2$, $HgCl_2$ an verschiedenen Austauschern (Abb. 6) ist ein gutes Beispiel für die in Teil I[11] angegebene Auswahlregel. Der Austauscher mit B-15-C-5 als Ankergruppe bindet bevorzugt Zn^{2+}, der mit Dibenzimidazolono-bis-pentamethylen das Cd^{2+} und der mit DB-24-C-8 das Hg^{2+}.

In Wasser oder Methanol lassen sich auch zahlreiche andere Kationen trennen. Bei der Elution mit Methanol erscheint in allen Elutionsdiagrammen eine Wasserbande. Diese kann bei Wasserbestimmungen ausgenutzt werden (siehe unten).

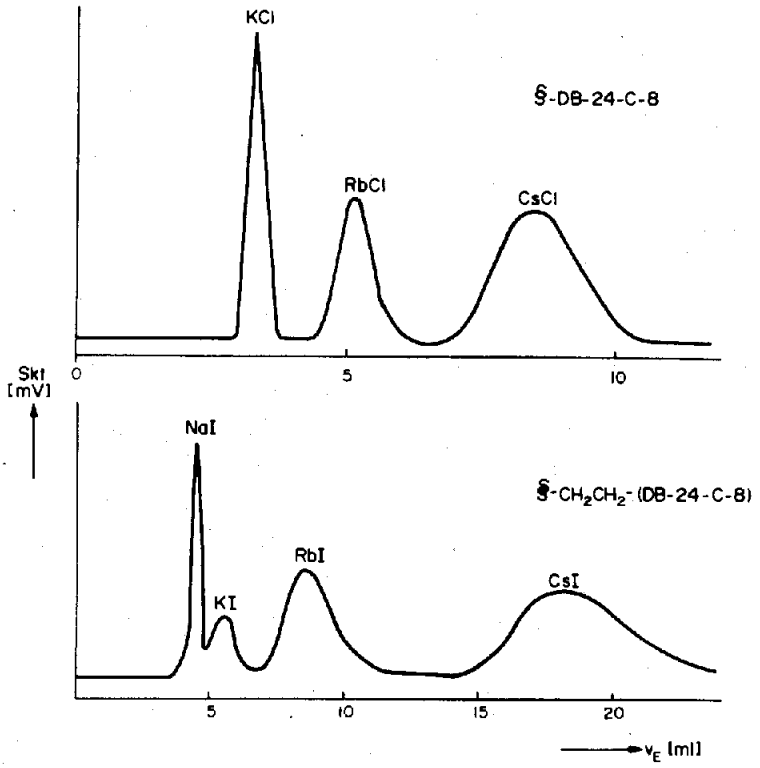

Abb. 3. Trennung von Alkalichloriden bzw. -iodiden durch Elution mit Wasser. Oben: 0,30 mg KCl, 0,42 mg RbCl und 0,54 mg CsCl. Unten: 0,12 mg NaI, 0,08 mg KI, 0,25 mg RbI und 0,36 mg CsI.

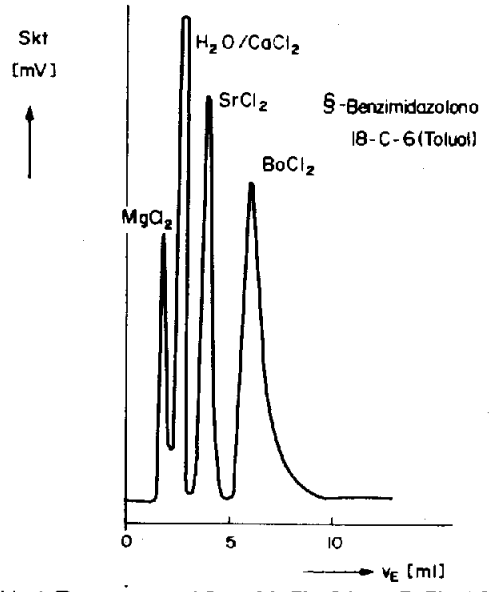

Abb. 4. Trennung von 1,2 mg $MgCl_2$, 0,1 mg $CaCl_2$, 1,3 mg $SrCl_2$ und 1,9 mg $BaCl_2$ durch Elution mit Methanol.

Anionen bei gleichem Kation

Bei gleichem Kation werden Anionen nach ihrer Polarisierbarkeit getrennt. Mit steigender Polarisierbarkeit des Anions vergrößern sich die Elutionsvolumina der Salze. Trennzeit und Trennschärfe hängen auch vom Kation ab. Abbildung 7 zeigt die Trennung der Halogenidanionen. Auch die Trennung mehrfach geladener Anionen ist möglich (Abb. 8 bis 11).

Die Abtrennung von Natriumsulfat-Spuren aus konzentrierten Natriumchlorid-Lösungen gelingt bis zu einem Molverhältnis 1:10000. Abbildung 9 veranschaulicht den SO_4^{2-}-Nachweis in einem Kondensationsprodukt aus *m*-Kresolsulfonsäure und Formaldehyd (mittleres Molekulargewicht 15000).

Die Zuordnung der Elutionsbanden erfolgt nach den Massenverteilungskoeffizienten der zur Synthese des Präparates verwendeten Ausgangssubstanzen. Sulfat kann zusätzlich hiervon im Eluat als Bariumsulfat nachgewiesen werden. Abbildungen 10 und 11 zeigen Trennungen einer Anzahl Natriumsalze.

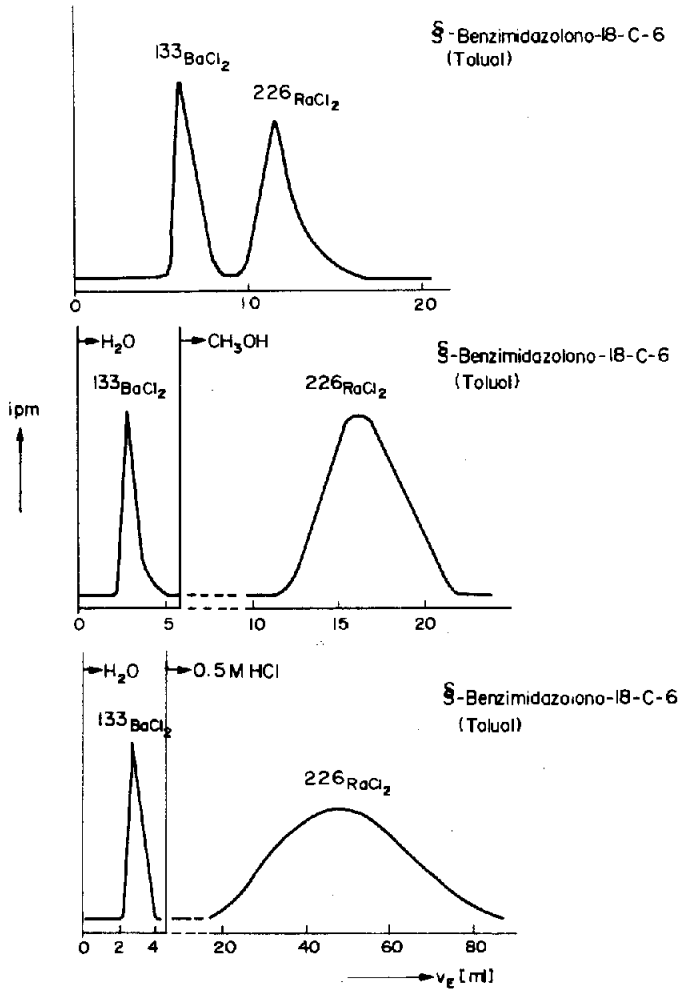

Abb. 5. Trennung von ^{133}Ba-markiertem $BaCl_2$ und ^{226} $RaCl_2$. Oben: 20 ng $BaCl_2$ und 25 ng $RaCl_2$ (Molverhältnis 1:1) durch Elution mit Methanol. Mitte: 4 mg $BaCl_2$ und 0,6 µg $RaCl_2$ (Molverhältnis 10^4:1) durch Elution mit Wasser bzw. Methanol. Unten: 4 mg $BaCl_2$ und 0,6 $RaCl_2$ (Molverhältnis 10^4:1) durch Elution mit Wasser bzw. 0,5*M* HCl.

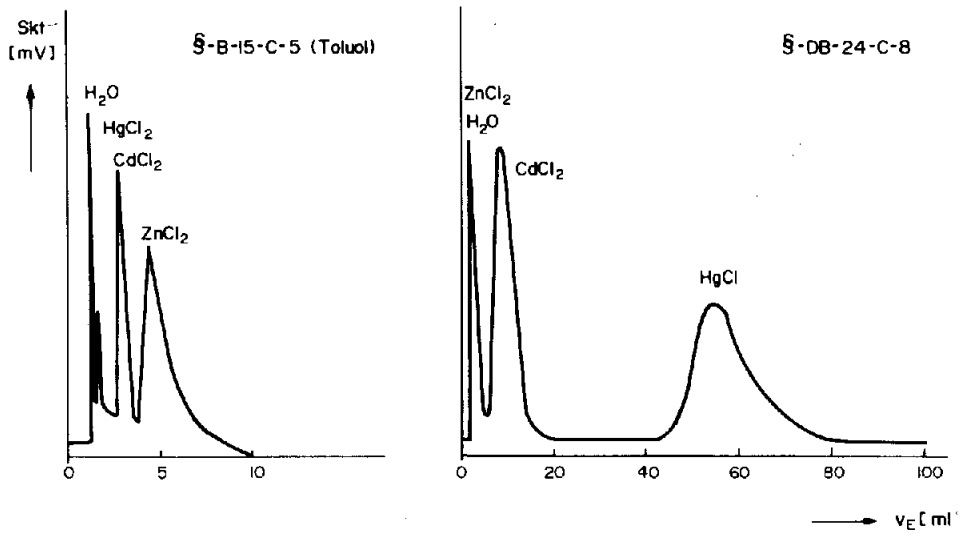

Abb. 6. Trennung von Chloriden der Elemente der 2. Nebengruppe des PSE durch Elution mit Methanol. Links: 0,03 mg $HgCl_2$, 0,41 mg $CdCl_2$ und 0,57 mg $ZnCl_2$. Rechts: 0,02 mg $ZnCl_2$, 0,68 mg $HgCl_2$ und 0,16 mg $CdCl_2$.

Organische Verbindungen

An Austauschern mit makrocyclischen Polyethern als Ankergruppen können auch nichtsalzartige organische Verbindungen getrennt werden. Trennungen, die nur durch die Eigenschaften der Matrix[12] verursacht werden, verlaufen an allen Austauschern gleich gut. Beispiele hierfür sind Trennungen von Maleinsäure/Fumarsäure sowie Alkoholen. Bei anderen Stoffklassen überwiegt der Einfluß der Ankergruppen. Der Mechanismus der Bindung ist in vielen Fällen nicht geklärt.[13,14]

Aromatische Verbindungen. Eine Trennung der Halogenbenzole gelingt gut (Abb. 12).

Heterocyclen. Die Elutionsvolumina der Heterocyclen Furan, Thiophen und Pyrrol steigen in der Reihenfolge O, S, N. Mit steigender Anzahl der Stickstoffatome treten auch stärkere Wechselwirkungen mit den Kronenethern, wie die Trennung Pyridin/Pyrazin/Triazin beweist, auf.[9]

Vitamine. Die Trennung des Vitamin-B-Komplexes ist möglich.[9] Sie dient u.a. zur Klärung der Frage, welche Mikroorganismen ein spezielles Vitamin auf einem Nährboden erzeugen bzw. verbrauchen. Zur photometrischen Bestimmung kann natürliches Vitamin C von störenden Kohlenhydraten abgetrennt werden (Abb. 13).

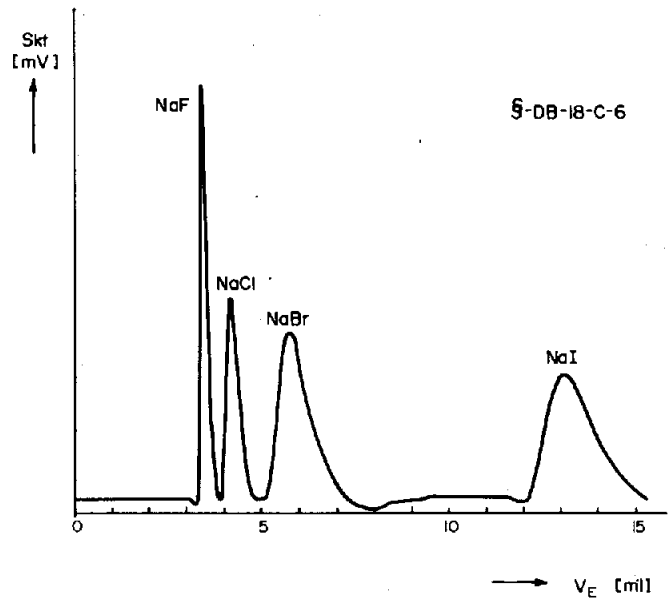

Abb. 7. Trennung von 0,42 mg NaF, 0,35 mg NaCl, 0,62 mg NaBr und 2,25 mg NaI durch Elution mit Wasser.

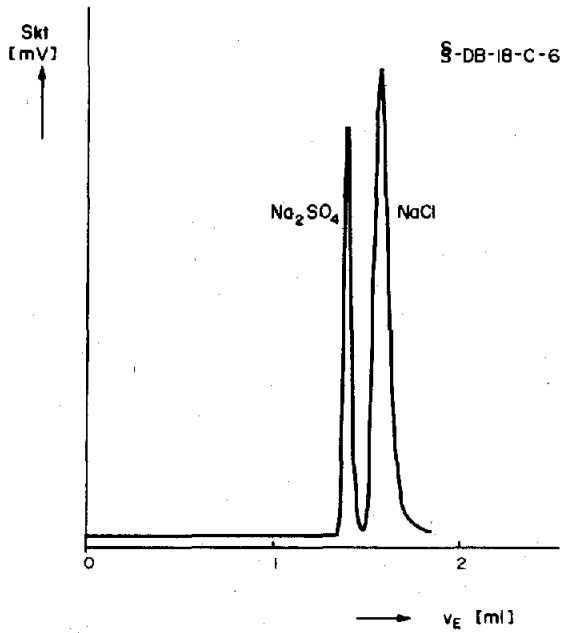

Abb. 8. Trennung von 0,43 mg Na_2SO_4 und 0,58 mg NaCl durch Elution mit Wasser.

Wasserbestimmungen

Als Beispiel für die quantitative Anwendung der Elutions-Chromatographie an Austauschern mit cyclischen Polyethern als Ankergruppen dient die Bestimmung von Wasser in Methanol als Lösungsmittel.

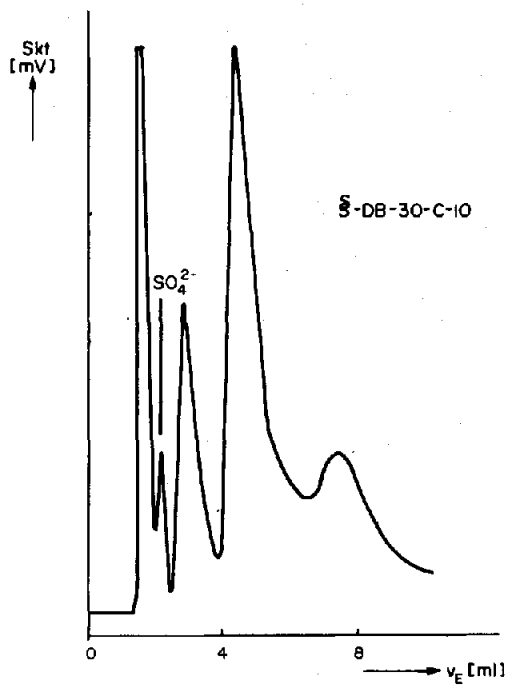

Abb. 9. Elution von 5 µl der Lösung eines linearen polymeren Kondensationsproduktes aus *m*-Kresolsulfonsäure und Formaldehyd (mittleres Molekulargewicht $15 \cdot 10^3$) mit Wasser.

Somit lassen sich Kristall- und Anlagerungswasser in Salzen und organischen Verbindungen bestimmen.[5,6,8–10]

Abbildung 14 gibt Elutionskurven einiger Salze mit unterschiedlichem Wassergehalt wieder. Die Versuche zeigen darüber hinaus, daß das Solvat- und Kristallwasser in den meisten Salzen bei der Komplexbildung quantitativ abgestreift wird.

FLÜSSIGE AUSTAUSCHER

Das Extraktionsvermögen monomerer und linearer polymerer Kronenether[15] sowie Heteropolysäuren wird durch niedrige Extraktionskoeffizienten der Kronenether einerseits und die geringe Löslichkeit der Heteropolysäuren andererseits begrenzt.

Verwendet man zur Extraktion dagegen definierte Addukte aus Kronenethern und Heteropolysäuren, diesen flüssigen Austauschern eine erhebliche Steigerung der Extraktionskoeffizienten.

Ein interessantes Beispiel ist der Vergleich der Extraktionseigenschaften von den Addukten aus Hexawolframatokieselsäure (HSiW) und Dibenzo-24-Extraktionseigenschaften von den Addukten aus Hexawolframatokieselsäure (HSiW) und Dibenzo-24-krone-8 bzw. Bis(nonylbenzo)-24-krone-8 als Extraktionsmittel für die Cs^+-Extraktion (Abb. 15). DB-24-C-8/HSiW zeigt lediglich eine Addition der Extraktionsfähigkeit von DB-24-C-8 und HSiW. Für BNB-24-C-8/HSiW ergibt sich dagegen ein positiver Synergismus für eine Adduktzusammensetzung von 4:1. Ursache für das unterschiedliche Verhalten sind vermutlich sterische Effekte, die durch die Nonylreste verursacht werden. Das Addukt eignet sich zur selektiven Cs^+-Extraktion aus mittelaktiver Waste-Lösung.[9]

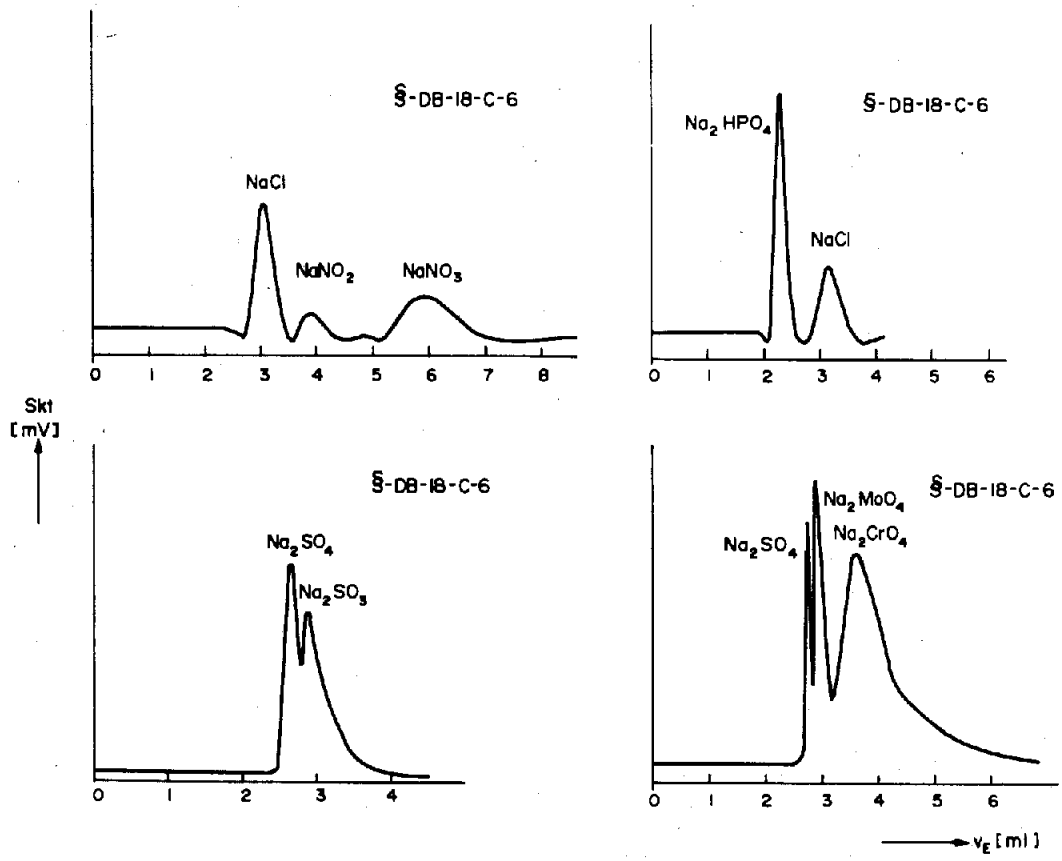

Abb. 10. Trennung von Natriumsalzen durch Elution mit Wasser. Oben links: 0,35 mg NaCl, 0,41 mg $NaNO_2$ und 0,51 mg $NaNO_3$. Oben rechts: 0,57 mg Na_2HPO_4 und 0,35 mg NaCl. Unten links: 0,17 mg Na_2SO_4 und 0,25 mg Na_2SO_3. Unten rechts: 0,43 mg Na_2SO_4, 0,62 mg Na_2MoO_4 und 0,49 mg Na_2CrO_4.

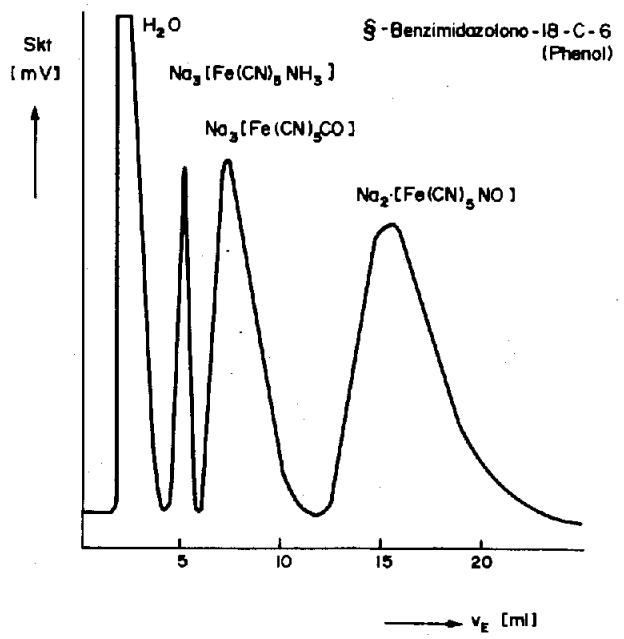

Abb. 11. Trennung von Komplexsalzen durch Elution mit Methanol: 0,6 mg $Na_3[Fe(CN)_5NH_3]$, 0,9 mg $Na_3[Fe(CN)_5CO]$ und 1,3 mg $Na_2[Fe(CN)_5NO]$.

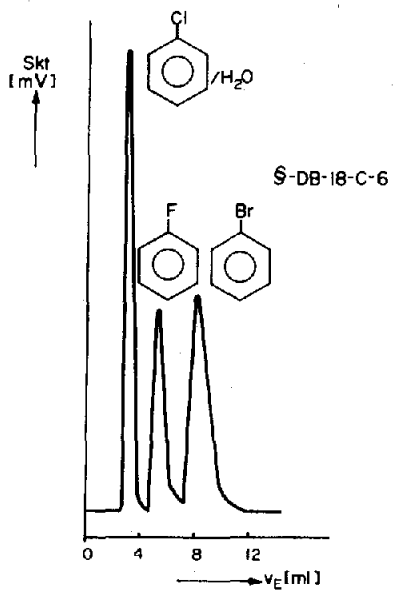

Abb. 12. Trennung von 1,0 mg Fluorbenzol, 2,0 mg Chlorbenzol und 1,0 mg Brombenzol durch Elution mit Methanol.

Den vierstufigen Syntheseweg zur Darstellung von Bis(nonylbenzo)-24-krone-8 enthält Abb. 16.

Mit dieser Reaktionsfolge lassen sich auch andere 4-bzw. 4,4'-alkylsubstituierte Kronenether darstellen. Sie können zur selektiven Extraktion von Kationen eingesetzt werden.

DÜNNSCHICHTCHROMATOGRAPHIE UND DÜNNSCHICHTELEKTROPHORESE

Die Austauscher sind auch als Adsorbentien in der Dünnschichtchromatographie und Dünnschichtelektrophorese einsetzbar. Hierzu werden Polyethylenterephthalat-Folien mit Suspensionen aus pulverisierten Austauschern und Polyvinylalkohol beschichtet. Die Austauscherschichten sind sehr abriebfest und bruchsicher. Verwendbar sind zahlreiche organische Laufmittel, ausgenommen Chloroform, Dichlormethan und Dioxan. Auch die geringe hellgelbe Eigenfärbung der Schichten stört nicht, da sie durch Übersprühen mit einer Suspension aus Kieselgel G überdeckt werden kann.[16]

Der in Tabelle 2 enthaltenen Elementkombination liegt der Qualitative Trennungsgang[17] zugrunde.

Ein Vergleich der durch Dünnschichtelektrophorese auf der Austauscherschicht erhaltenen Ergebnisse mit Trennungen durch Papierelektrophorese ergibt verschiedene Reihenfolgen der R_B-Werte (Tabelle 3).

Dadurch können bestimmte neutrale Aminosäure, wie z.B. Isoleucin, Valin, Serin und *o*-Aminobuttersäure, die auf Papier selbst bei Anwendung höherer Feldstärke und längerer Elektrophoresezeiten überlappende Zonen bilden, auf den Austauscherschichten getrennt werden.

Die Trennung der Konservierungsmittel Benzoesäure, Salicylsäure und Sorbinsäure durch Dünnschichtchromatographie auf der Austauscherschicht gelingt gut. Die Austauscherschicht ist hier den bisher verwendeten Adsorbentien überlegen (Tabelle 4).

PRÄPARATIVE CHEMIE

Die Einsatzmöglichkeiten der neuen Austauscher in der präparativen Chemie sind in Tabelle 1 enthalten.

Salzkonversionen

Schwerzugängliche oder wenig beständige anorganische Salze lassen sich durch Salzkonversion sowohl nach dem Säulen- als auch nach dem Batchverfahren darstellen. Abbildung 17 veranschaulicht die säulenchromatographische Herstellung von RbSCN. LiSCN und RbCl werden zu LiCl und RbSCN umgesetzt.

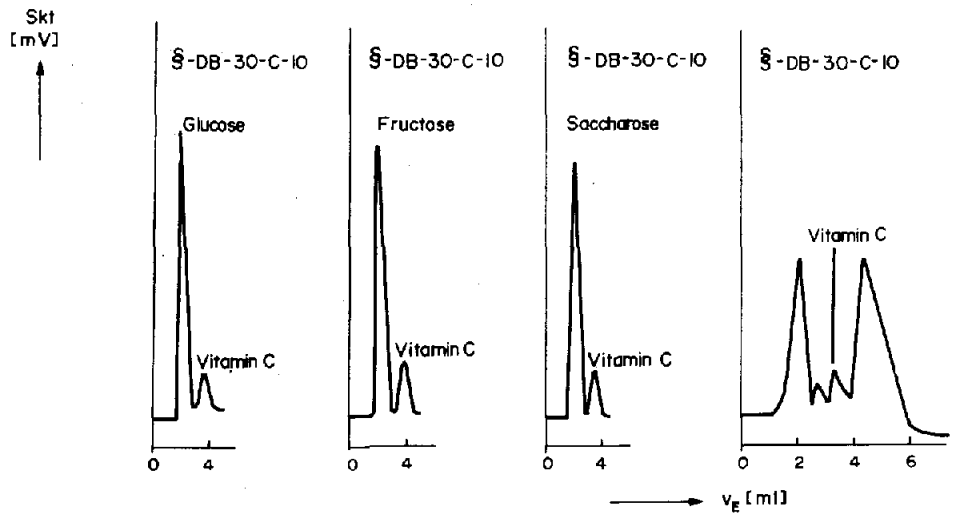

Abb. 13. Trennung von Vitamin C und Kohlenhydraten bzw. Abtrennung von Vitamin C aus dem Saft einer Zitrone durch Elution mit Wasser: 0,05 mg Vitamin C, 0,02 mg Kohlenhydrat, 1 ml Saft.

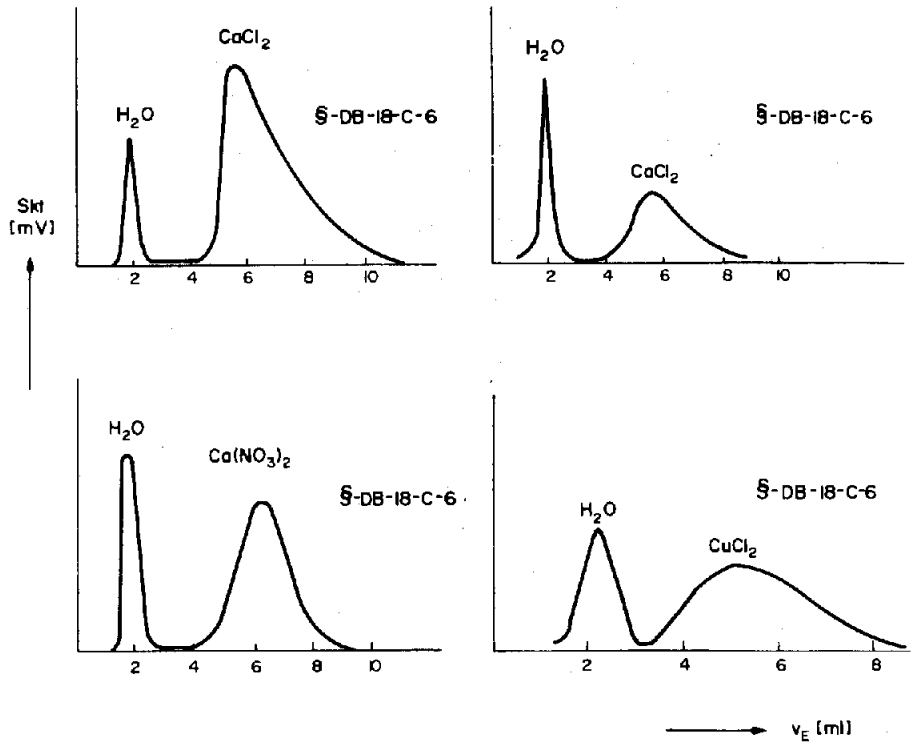

Abb. 14. Wasserbestimmung durch Elution mit Methanol. Oben links: 12,9 μg $CaCl_2 \cdot 2H_2O$; rechts: 6,4 μg $CaCl_2 \cdot 6H_2O$. Unten links: 9,1 μg $Ca(NO_3)_2 \cdot 4H_2O$; rechts: 168 μg $CuCl_2 \cdot 2H_2O$.

Den großen Elutionsvolumina und entsprechend langen Trennzeiten der Säulenversuche stehen die kleinen Volumina und kürzeren Darstellungszeiten der Batchversuche gegenüber (Tabelle 5).

Das Säulenverfahren ermöglicht Umsetzungen mit 100% iger Ausbeute. Diese wird beim Batchverfahren nicht erzielt. Das letztere erfordert jedoch kaum präparativen Aufwand. Nach Beendigung des entsprechenden Schüttelversuches können die chromatographisch und spektroskopisch reinen Salze mit wenig Lösungsmittel vom Austauscher ausgewaschen werden (Apparatur nach Soxhlet).

Auf diese Art können leicht g-Mengen schwer zugänglicher Thiocyanate und Iodide der Alkali- und Erdalkalielemente in wäßriger Lösung hergestellt werden.

Ein weiteres interessantes Beispiel für den Einsatz der Methode ist die Darstellung von Natriumhexa-

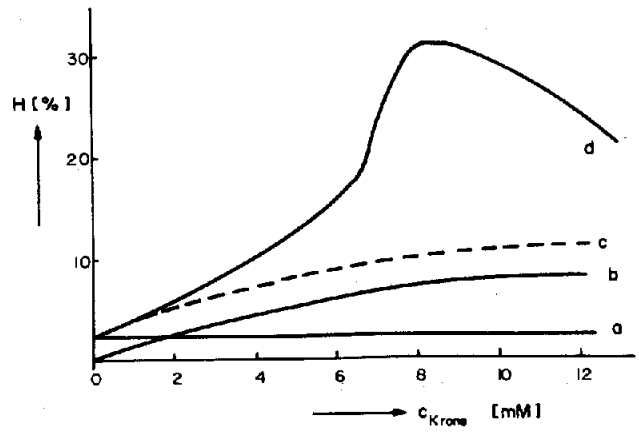

Abb. 15. Cs^+-Extraktion aus "simulierter" mittelaktiver Waste-Lösung mit Kronenether/HSiW-Gemischen in Nitrobenzol. a, 2 mmole HSiW; b, BNB-24-C-8 bzw. DB-24-C-8; c, DB-24-C-8/HSiW; d, BNB-24-C-8/HSiW.

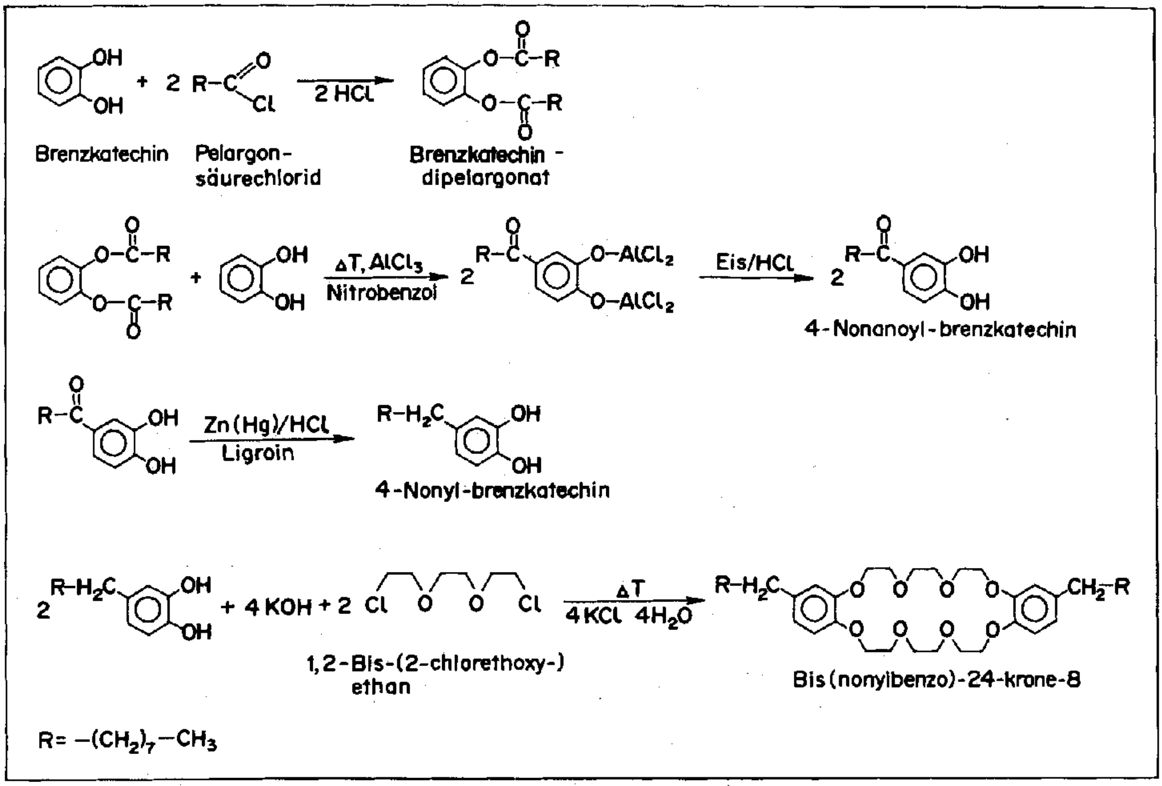

Abb. 16. Darstellung von Bis(nonylbenzol)-24-krone-8.

Tabelle 2. Anwendung in der Dünnschichtchromatographie und Dünnschichtelektrophorese

Stoffgruppe	Dünnschichtchromatographie §-DB-18-C-6 (R_f-Werte)	Laufmittel	Dünnschichtelektrophorese §-DB-18-C-6 (R_B-Werte)	Elektrophoresebedingungen
Ammoniumcarbonat-Gruppe mit Mg^{2+}	unbehandelte Schicht $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$	Ethanol / Wasser (10 : 1,5 v/v)	$Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$	Grundelektrolyt 1M Zitronensäure in Ethanol/Wasser (1:1 v/v) Feldstärke: 40 V/cm Stromstärke: 0,5–0,8 mA
^{90}Strontium-^{90}Yttrium	HCl-behandelte Schicht $^{90}Y^{3+} > {}^{90}Sr^{2+}$	Methanol / Wasser (90 : 10 v/v)	——	
Ammoniumsulfid-Urotropin-Gruppe (Schulanalyse)	unbehandelte Schicht $Cr^{3+} > Mn^{2+} > Co^{2+}, Ni^{2+} > Al^{3+} > Zn^{2+} > Fe^{3+}$ HCl-behandelte Schicht: $Al^{3+} > Cr^{3+}, Mn^{2+} > Co^{2+}, Ni^{2+} > Zn^{2+} > Fe^{3+}$	Methanol / 12,5 M HCl (1 Tropfen HCl auf 10 ml Methanol)	$Mn^{2+} > Co^{2+} > Zn^{2+} > Ni^{2+} > Al^{3+} > Fe^{3+}$	
Schwefelwasserstoff-Gruppe, Kupfer-Gruppe	unbehandelte Schicht $Cu^{2+} > Hg^{2+} > Bi^{3+} > Cd^{2+}, Pb^{2+}$		$Cd^{2+} > Pb^{2+} > Cu^{2+} > Hg^{2+}, Bi^{3+}$	

Tabelle 3. Elektrophorese von neutralen Aminosäuren

Literatur	18	19	20, 21	eigene Ergebnisse
Trägermaterial	Papier	Papier	Papier	§-DB-18-C-6
Grundelektrolyt	Pyridinacetatpuffer	0,75 M Ameisensäure/ 1M Essigsäure (1; 1)	0,6 M Ameisensäure u. 2 M Essigsäure	1,09 M Ameisensäure u 2,6 M Essigsäure
pH-Wert	3,6	2,25	1,9	1,64
Elektrophoresefeldstärke und -dauer	100 V/cm 180 min	55 V/cm 150 min	60 V/cm 200 min	40 V/cm 60 min
R_B-Werte				
Gly	133	143	140	97
αABS	107		106	135
Ser	68	102	102	156
Val	100	100	100	100
Ileu		97	94	33
Pro	51	81		135
Phe	79	79		20
Tyr	79	70	67	135
Try	88	59		3

thiocyanatoferrat(III) (Abb. 18). Auf anderem Weg ist dieses Komplexsalz nur mit sehr viel größerem Aufwand zu erhalten.[24]

Reinigung organischer Verbindungen

Reinigung von Penicillinen. Bei der Synthese von zwei Penicillinen liegen Gemische mit dem Ausgangsprodukt 6-Aminopenicillansäure (6-APS) vor. Die beiden neu hergestellten Penicilline lassen sich wegen der Instabilität gegenüber Säuren und Basen nicht an herkömmlichen Trägermaterialien, wie Al_2O_3, SiO_2 usw. chromatographisch reinigen. Eine Umkristallisation entfällt wegen ihrer geringen thermischen Beständigkeit. An §-DB-30-C-10 gelingt jedoch die Isolierung der Penicilline aus dem jeweiligen Gemisch (Abb. 19 links und rechts).[25]

Hydrolyseempfindliche Substanzen sind auch in wasserfreien Lösungsmitteln trennbar.

Isolierung der Hauptcardeolide aus Digitalis Purpurea Folium. Hauptcardeolide des roten Fingerhutes sind die Steroide Purpureaglykoside A und B. Sie spalten leicht Glucose ab, so daß sie zur Anwendung immer frisch isoliert werden müssen. Außer geringen Mengen dieser Cardeolide (0,1–0,5%) finden sich in den Blättern weitere Glykoside sowie Digitonin, Flavone, Carbonsäuren und Fette.

Beide Cardeolide lassen sich mit dem Austauscher DB-18-C-6 als Ankergruppe (Säule E) in g-Mengen von den Ballaststoffen abtrennen. Die Dünnschichtchromatogramme[26] des Extraktes sowie der isolierten Fraktionen der reinen Herzglykoside zeigt Abb. 20.

Tabelle 4. Dünnschichtchromatographie von Benzoesäure/Salicylsäure/Sorbinsäure auf verschiedenen Trennschichten

Literatur	22	22	22	23	eigene Ergebnisse
Schichtmaterial	Cellulose	Kieselgel/Kieselgur	Kieselgel/Kieselgur	Kieselgel	§-DB-18-C-6
Laufmittel	n-Butanol/NH_3/ H_2O (70:20:10 v/v)	Hexan/Eisessig (96:4 v/v)	Petrolether/Ether/ Eisessig (80:20:1 v/v)	$CHCl_3$/Eisessig (90:10 v/v)	Methanol
Entwicklung	5-6 h	20 cm in 1,5 h, evtl. Mehrfachentwicklung	3-fache Entwicklung		8-10 cm in 1 h
R_B-Werte					
Salicylsäure	1,00	1,00	1,00	1,00	1,00
Sorbinsäure	1,07	1,28	0,91	1,11	1,83
Benzoesäure	0,91	1,54	1,11	1,08	1,40

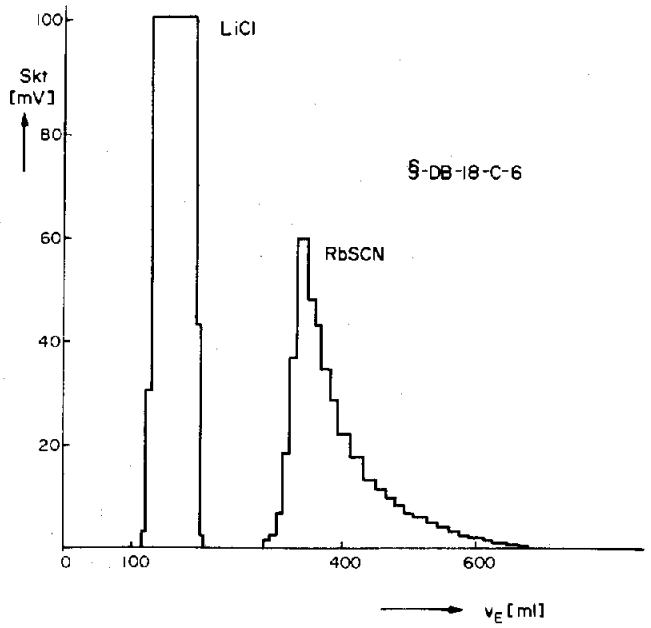

Abb. 17. Salzkonversion (LiSCN + RbCl → LiCl + RbSCN) von 0,520 g LiSCN und 0,975 g RbCl durch Elution mit Wasser.

Anionenaktivierung bei organischen Reaktionen

Durch Anionenaktivierung mit Hilfe von cyclischen Polyethern kann eine Vielzahl organischer Synthesen in wasserfreien Lösungsmitteln durchgeführt und verbessert werden.[9] Dies ist besonders wichtig, wenn Wasser den Reaktionsablauf stört.

Ein Vorteil der Austauscher gegenüber der Verwendung monomerer cyclischer Polyether liegt in ihrer leichten Abtrennbarkeit, Rückgewinnung durch Filtration und erneuter Verwendung. Es tritt keine Verunreinigung des Lösungsmittels ein. Von Nachteil ist die längere Reaktionszeit. Nach beendeter Reaktion wird der Austauscher abfiltriert und das Lösungsmittel unter Vakuum abdestilliert.

Der Einsatz der beladenen Austauscher begünstigt besonders nukleophile Substitutionen.

Ein interessantes Beispiel ist die Umsetzung von α-*p*-Dibromacetophenon mit Kaliumacetat zu *p*-Bromphenacylessigsäureester.[9] Die gebildeten *o*-Bromphenacylfettsäureester dienen zur quantitativen Bestimmung von Fettsäuren mit Hilfe des Ultraviolett-Detektors. Die saubere Abtrennung des Kronen ethers aus dem Reaktionsgemisch ist deshalb insbesondere hier von Wichtigkeit.

Eine Übersicht über weitere nucleophile Substitutionsreaktionen gibt Tabelle 6. Die Verwendung des Austauschers zur Anionenaktivierung führt zu besseren Ergebnissen als die Verwendung von Aliquat

Tabelle 5. Herstellung von Alkali- und Erdalkalithiocyanaten durch Salzkonversion

Umsetzung	Elutions-volumen ml	isoliertes Volumen ml	isolierte Mengen g	Zeit h	Kapazitäts-ausnutzung %	Ausbeute %
§-DB-18-C-6 Säulenverfahren (Säule E, 75 g Austauscher)						
$LiSCN + RbCl \rightarrow LiCl + \underline{RbSCN}$	640	400	1,155	6,4	10	100
$LiSCN + CsCl \rightarrow LiCl + \underline{CsSCN}$	480	230	1,528	4,8	10	100
§-DB-18-C-6 Satzverfahren (4g Austauscher)						
$2LiSCN + SrCl_2 \rightarrow 2LiCl + \underline{Sr(SCN)_2}$		50	0,098	5	4,6	8
$2LiSCN + BaCl_2 \rightarrow 2LiCl + \underline{Ba(SCN)_2}$		50	0,213	5	7,7	14

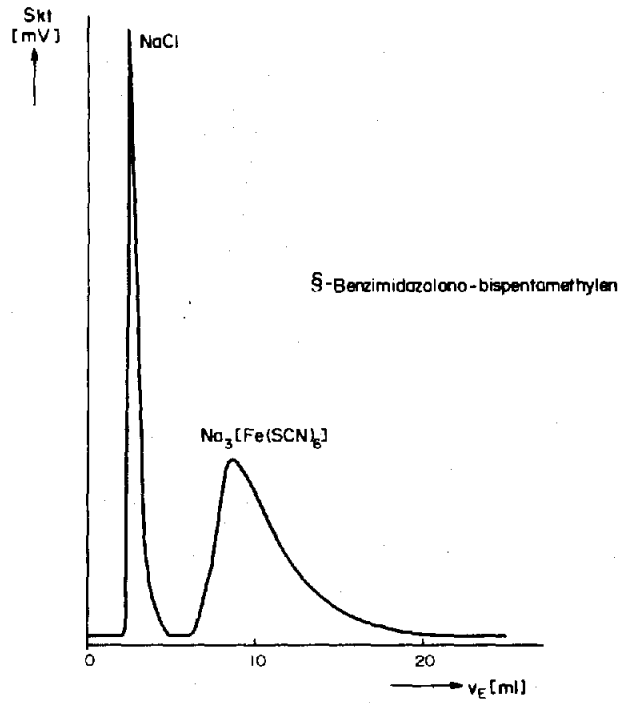

Abb. 18. Salzkonversion ($6NaSCN + FeCl_3 \rightarrow 3NaCl + Na_3[Fe(SCN)_6]$) von 0,98 mg NaSCN und 0,33 mg $FeCl_3$ durch Elution mit Wasser.

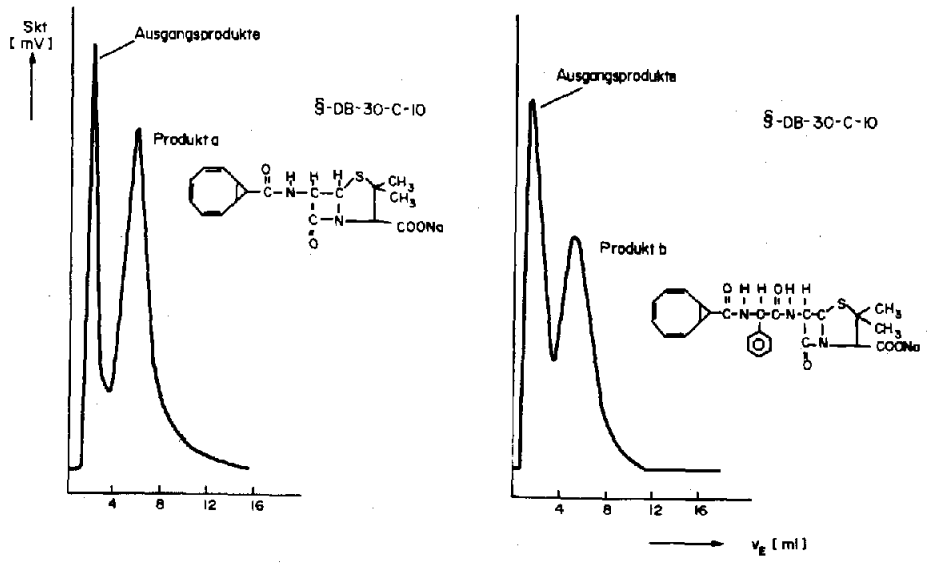

Abb. 19. Auftrennung ungereinigter Penicilline [20 mg Gemisch mit Produkt a (links), bzw. Produkt b (rechts)] durch Elution mit Wasser.

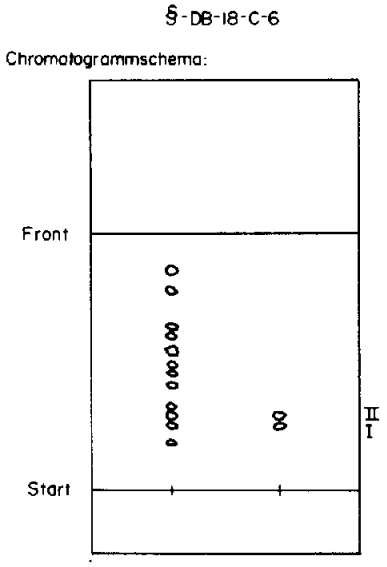

Abb. 20. Dünnschichtchromatogramme. Links: Extrakt von Digitalis Purpureae Folium; rechts: abgetrennte Fraktion. R_f-Werte: I. 0,25 (Purpureaglycosid B); II. 0,29 (Purpureaglycosid A).

336^{27} (Handelsname des technischen, nicht ganz einheitlichen Methyltrioctylammoniumchlorids). Eine Ausnahme ist die Umsetzung mit Kaliumfluorid.

AUSBLICK

Das vorliegende Arbeitsgebiet ist noch stark ausbaufähig. Hier wurden teilweise nur Zwischenergebnisse mitgeteilt. Eine vollständige Bearbeitung vieler Probleme steht noch an. Von großem Interesse ist u.a. die Erzielung schneller Trennungen mit an Kieselgel gebundenen cyclischen Polyethern, der Einsatz der festen und flüssigen Austauscher zur Entfernung von $^{134/137}Cs^+$ aus mittelaktiven radioaktiven Abfallösungen und die Verwendung der Austauscher zur Anionenaktivierung unter besonderer Berücksichtigung von Ringöffnungspolymerisationen.

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Tabelle 6. Nucleophile Substitutionsreaktionen mit aktivierten Anionen:
$RX + M^+Y^- \rightarrow RY + M^+X^-$

			$R\atop R'$>N^+-CH_3Cl^- (Aliquat 336)		§-DB-18-C-6			
Lösungsmittel	RX	MY	t[h]	Umsatz [%]	MY	t[h]	Umsatz [%] mit AT	Umsatz [%] ohne AT
CH_3CN	$C_6H_5CH_2Cl$	KF	25	30	KF	25	0	0
CH_3CN	$C_6H_5CH_2Cl$	KBr	5	39	KBr	5	54,8	42,0
CH_3CN	$C_6H_5CH_2Cl$	KI	-	-	KI	0,25 0,5	98 100	75 95
CH_3CN	$C_6H_5CH_2Cl$	NaCN	10	88	KCN	2	86	51,6
CH_3CN	$C_6H_5CH_2Cl$	$NaCH_3COO$	4,5	100	KCH_3COO	1 2	98 100	23 45,7
CH_3CN	$C_4H_9OSO_2CH_3$	$NaCH_3COO$	5	61	KCH_3COO	5 2	100 97	42,6
CH_2Cl_2	$C_4H_9OSO_2CH_3$	KNO_2	30	32	KNO_2	30	42	6

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Summary—Exchangers with cyclic polyethers as anchor groups have a large range of applications such as separations of cations with a common anion, of anions with a common cation, and of neutral organic compounds, and the determination of water by elution chromatography. Some crown ether monomers, especially 4- and 4,4′-alkyl-substituted benzo-derivatives are suitable for extractions and their adducts with heteropoly acids are used as liquid ion-exchangers. The exchangers are also applied in thin-layer chromatography and thin-layer electrophoresis. Furthermore the exchangers are successfully used in preparative chemistry, *e.g.*, in salt conversions in order to isolate salts which are difficult to prepare by other means, in isolation and purification of organic compounds, and for anion activation in organic reactions.

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HOW TO WRITE A PAPER ON A NEW ANALYTICAL METHOD BASED ON ION-EXCHANGE OR ION-EXCHANGE CHROMATOGRAPHY

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Summary—Recommendations are given concerning the development of a new analytical method using ion-exchange or ion-exchange chromatography. The main points concerning the title, introduction, experimental and discussion sections of any publication arising from the new method are listed.

In an analytical determination, separation steps still play a very important role. In spite of the many efforts to simplify analytical procedures in the last few decades, by use of ion-selective electrodes or highly specific chemical reaction conditions for the determination of a single component in presence of many others, separations are inevitably necessary if mixtures of components of very similar behaviour are to be analysed or the component which is to be determined is present in very low amount compared to the matrix elements.

Among the methods used for separation of ionic or non-ionic components in the liquid phase, ion-exchange procedures play a prominent part. Many papers are published dealing with ion-exchange, the use of different types of ion-exchanger to improve analytical methods, or procedures for analysis of complex samples by ion-exchange chromatography.

In each of Walton's biennial *Analytical Chemistry* review articles on ion-exchange and chromatography[1–4] about 500 papers were selected from the thousands published, preference being given to those presenting new materials or methods. Papers on routine methods or of marginal interest were omitted. From the representative tables in these review articles and the references, however, it appears that more than 50% of the selected papers were reports on analytical applications. It therefore seems likely that as much as 90% of the total number of papers in the two-year period would be on new or known analytical methods using ion-exchange or ion-exchange chromatography.

Because such a large and increasing number of papers is published throughout the world on ion-exchange methods, and in many cases it is rather difficult to recognize a new principle or an advantage of the method recommended, it seems advisable to give some directives concerning the essential requirements for a paper describing a new method or procedure using ion-exchange.

The aim of the present paper is to give a summary of the most important points, and in this way to give help to authors preparing papers, and also to the editors and referees of the periodicals, who have to judge the manuscripts. It is hoped that consideration of the points presented here will lead to an improvement in the preparation of papers and a decrease in the redundancy of the papers published.

TITLE

Since literature searches and the processing of published papers are done these days more and more by computers, and it is primarily the titles that are processed directly, it is very important that the title should give the essence of the paper unambiguously, and while short, should still include all the necessary words for adequate computer classification.

For example, in the following three titles three very different topics—waste-water analysis, a new separation method and a new ion-exchanger—are emphasized. The titles contain the most important words necessary for conveying tentative but full information to the reader.

(*a*) Separation and determination of vanadium and iron in waste-waters of power plants, by use of ion-exchange chromatography.

(*b*) Rapid and selective ion-exchange chromatographic separation of vanadium and iron with PAR as complex-forming agent.

(*c*) A new type of hydrophilic-matrix ion-exchanger used for chromatographic separation of vanadium and iron.

INTRODUCTION AND GENERAL REMARKS

In the introduction of the paper it is usual to give a brief survey of the literature, and an explanation of the problem to be investigated and solved. At the same time it is very important to state clearly whether the ion-exchange method presented serves to improve an existing analytical method, or is of direct use for analysis of certain substances, or is used as a model for a new separation principle.

It is necessary to give the aim of the ion-exchange operation used: separation of interfering substances, purification, enrichment, salt splitting, decreasing the amount of matrix components, separation of multi-component mixtures, improvement of sensitivity or selectivity of a detection step, introduction of a new ion-exchanger, *etc.*

The ion-exchange procedures themselves, irrespective of the aim of the work, can be characterized according to the following classification.[5]

(1) Ion-exchange procedures based on total exchange. In this group belong all the simple procedures where ions are exchanged quantitatively, for enrichment of trace amounts of metal or other ions or non-ionic organic substances, for separation of interfering ions or elements, or simply to change the ionic composition of an electrolyte or to convert a salt into an acid or *vice versa*. Column techniques are mainly used, but batch methods may also be employed.

(2) Separations of components of similar behaviour by ion-exchange chromatography. Although in some cases it is difficult to distinguish between the simple ion-exchange procedures mentioned above and chromatography, the following considerations may apply as a rule. In the procedures mentioned the exchange of certain ions is complete (or at least is expected to be complete) and if separations are made the separation factors are high (usually greater than 100). Ion-exchange chromatography serves for separation of ions or molecules of similar behaviour and the separation factors are usually low.

The techniques used are mainly column chromatography but chromatography on thin-layers or paper impregnated with ion-exchangers is also employed.

(3) Ion-exchangers used as carriers. Ion-exchangers loaded with certain reagent ions or enzymes *etc.* can be used as stable reagents. There are also other methods in which the ion-exchanger acts as a carrier on which the reaction takes place.

There are many other type of applications of ion-exchangers but the most frequently and widely used belong to the three groups above.

Although the requirements for description of new methods differ for these three classes, there are some main points which are very similar and in general important for consideration.

Ion-exchange procedures provide only a means of separation, and for application to determination always need subsequent measurement steps. It is important that these steps should be in full accordance with the conditions and the quality (precision, accuracy and rate) of the ion-exchange operation. If there are divergences in performance between the ion-exchange and the instrumental steps, the complete procedure will give less reliable results.

It is advisable to characterize the ion-exchange process itself, giving an explanation of the ion-exchange reaction or (in the case of chromatography) stating the fundamental principle controlling the distribution of the components between the two phases (ion-exchange, ligand-exchange, ion-exclusion, ion-pair formation *etc.*).

Ion-exchange processes can be described by chemical reactions. Many equilibrium constants of ion-exchange reactions are known, and available in the literature. There are also excellent collections of complex-formation and protonation constants. By use of these data taken from the literature, preliminary informatory calculations can be done before experimentation is begun, and the suitable reagents and conditions (concentration, pH) *etc.* can be predicted. In this way much superfluous and tedious experimental work can be avoided. For planning inorganic and organic ion-exchange separations see references 9 and 10.

In the experimental part of the paper it is usual to give first a brief description of the reagents and instruments used.

Since the reproducibility of simple ion-exchange methods and especially of chromatographic separations depends to a high extent on the properties of the ion-exchanger, full characterization of the exchanger is very important. It should include the following points.

(*a*) The full commercial name, designation and source of the ion-exchanger used. If the exchanger is not commercially available, a detailed description of its preparation should be given.

(*b*) Classification according to type (cation, anion, chelate resin exchangers, *etc.*).

(*c*) Description of the physical and chemical structure of the ion-exchanger. If a solid conventional exchanger is used it is important to give the chemical composition of the matrix (styrene–divinylbenzene co-polymer, cellulose, dextran or silica-based, inorganic, *etc.*).

(*d*) Type of the matrix (macroporous, gel porous surface or porous layer *etc.*).

(*e*) Characterization of the cross-linking of the resin (the nominal DVB content of a styrene–divinylbenzene co-polymer resin, *etc.*) if a resin is used.

(*f*) Name of the functional groups (sulphonic acid, carboxylic acid, quaternary ammonium, iminodiacetic acid *etc.*).

(*g*) Form of the ion-exchanger particles (beads or granules).

(*h*) Particle size of the air-dried ion-exchanger. Preferably the upper and lower size limits should be given and not just the mean diameter.

(*i*) Capacity of the ion-exchanger, in equivalents per litre of swollen bed or in equivalents per kg of dry (or air-dried) substance. It is also advisable to give the exact description of the method used for the determination of the capacity, or at any rate a reference to its source.

In listing the apparatus used a full description should be given of the column if column operation is used (commercially or specially made; glass, plastic or metal; water-jacket; dimensions *etc.*). If it is not of the usual design a figure is also recommended.

Rigorous description of the auxiliary equipment is most important if high-performance separations are made. The resolving power of the column, the clear-cut separation of the components and the reproducibility of the separations depend not only on the nature of the column packing and on the chemical and physical conditions chosen, but also on the equipment used. The form of the peaks of the separated components also depends on the nature of the inner surface of the column, on the mode of injection of the sample, on the pulsation of the pump, on the dead volume of the detector used, *etc.*

In the case of simple ion-exchange operation the form and size of the ion-exchange column may also play an important role. The bread-through capacity of the column depends not only on the flow-rate, temperature and composition of the solution, but also on the column form.

Since the compositions of the sample solutions investigated may vary between wide limits, it is very important to suggest experimental conditions such that samples of widely different composition can be analysed with confidence, *i.e.*, conditions that will always give quantitative separations. The parameters suggested must, of course, be based on results of model experiments.

The exact description of the pretreatment of the ion-exchanger of the preparation of the ion-exchange column is important not only in separation by high-performance chromatography, but in all cases where ion-exchangers or columns are used, because the operational parameters may be influenced by the pretreatment of the resin. The diffusion rate of the ions in the resin phase depends on the form and swelling of the ion-exchanger. The use of incompletely conditioned or swollen resin may cause erroneous results.

If column operation is used the preparation and characterization of the column should be described accordingly, in the following terms.

(*a*) Preliminary treatment of the ion-exchanger (swelling, washing, treatment with acid or alkali, details of temperature and time).

(*b*) Preparation of the column (prepacked or not, method of filling). If a solution and not a pure solvent was used in the preparation, the composition of the solution is also necessary.

(*c*) Size of the column. The length and diameter of the settled ion-exchange bed is to be given.

If an ion-exchange thin-layer or paper or a batch method is used, then the preliminary treatment must be given in detail.

In the development of a new ion-exchange procedure all the factors which may influence the separations or detections wanted must be considered. Since the composition of the sample and of the eluent, the pretreatment of the resin or column, the mode of introduction of the sample, the temperature, flow-rate, contact time *etc.* may all influence the results, they must be optimized. Increasing the temperature will lead to faster establishment of the local equilibria, but may also lead to decreased selectivity. In column operations a high flow-rate shortens the separation time but increasing the flow-rate also usually decreases the column efficiency. The efficiency of regeneration of the ion-exchanger or ion-exchange column is sometimes a complicated function of composition, temperature and volume of the regenerants.

Before the description of the ion-exchange procedure, a detailed description of the preparation and treatment of the sample to be investigated is necessary (sample size, dissolution procedure, permitted volume of the solution prepared, adjusted final volume *etc.*).

If a column method is used the description of the ion-exchange procedure should include the following

(*a*) The preparation of the eluent.

(*b*) The pretreatment of the column.

(*c*) The mode of introduction of the sample into the column and the volume of sample to be introduced (lower and upper limits).

(*d*) All the important conditions of the process (temperature, pressure, flow-rate *etc.*), composition of the liquids used in the main steps and in washing or rinsing steps completing the main procedure. In the case of gradient elution the exact form and parameters of the gradient should be given.

(*e*) The method of determination after the ion-exchange or chromatography. If a selective sorption or elution procedure was used, the volume of the collected effluent and the mode of detection or determination of the separated individual components should be listed.

(*f*) The evaluation of the measurements, standardization of the method used, and the standard deviation and possible errors of the determinations.

If a batch method is used, it is very important to give data on the resin:liquid ratio, the mode and duration of mixing, the temperature used and the removal of the ion-exchanger, rinsing *etc.* Because batch ion-exchange is never complete (but in certain cases can be accepted as practieally quantitative) the permitted limits of the resin:sample solution ratio should be clearly given.

If the substance in question is to be sorbed or removed by ion-exchange quantitatively from a solution (either a column or a batch method is used) then it must be verified by the results of model experiments that the adsorption of the ions or molecules of the sample was quantitative under the conditions given. The interferences of other substances possibly present should also be examined and given. Suggestions as to the preliminary separation or masking of interfering ions and molecules are required.

Because interferences are generally more serious in the batch method than in column operation (since interferences can be overcome here by the use of longer columns) the amounts of foreign ions tolerated must be given correctly.

To confirm the validity of the method developed, the results of model experiments with known volumes

of solutions of different compositions (containing various commonly associated components) should be given and the amounts of foreign species tolerated should be tabulated.

The regeneration procedure, including the preparation, volume, flow-rate and temperature of the solutions used, and the method for assessing its completeness, should be given. If the ion-exchanger can become decomposed or spoiled during the exchange process or regeneration step then the extent of the loss of capacity should also be mentioned.

DISCUSSIONS AND CONCLUSIONS

The results obtained by the complete ion-exchange analytical method should be compared with those of a reference method or other methods used for similar purposes, and the advantages of the new method emphasized. The comparison should include precision, accuracy, speed, concentration range, and economics. The lower and upper limits of the amount of substance which can be determined with suitable accuracy should be specified. For chromatographic separations the comparison should also cover the resolution, limits of detection of the components, long-term stability of the system, ease of separation, repeatability of retention data and determinations, *etc.*

Any basic new principle or idea making the new method superior to earlier techniques or procedures should be clearly stated.

If there are points which need verification (*e.g.*, whether different column packing methods yield more efficient columns, higher selectivity *etc.*) these should be proved by experiments comparing the new results with those obtained by other techniques or methods.

It should be kept in mind that the height of one theoretical plate is not itself an absolute measure of the value of the column, since it depends on the nature of the components in question and also on the actual conditions of the separation.

If the main ion-exchange reaction is unusual in nature and cannot be explained in simple terms because of such factors as additional chemical reactions (complex formation, *etc.*) and the quantitativeness of the reaction is important for complete ion-exchange, then the reactions should be classified and the selected conditions verified by experimental results.

TERMINOLOGY

In choosing the terms used in the experimental and discussion sections of the paper, attention should be paid to the IUPAC recommendations for presentation of results,[6] and on nomenclature in ion-exchange[7] and chromatography.[8]

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ELECTROCHEMICAL DETECTORS FOR FLOWING LIQUID SYSTEMS

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Summary—A review of the theory, applications, and advantages and disadvantages of the major types of electrochemical detectors in flowing systems is presented in this report.

One of the most sensitive methods of analytical chemistry is voltammetry (or polarography, when a dropping mercury electrode is the working or indicator electrode). This technique is essentially based on the recording of current–voltage curves produced from the electrolysis of an oxidizable or reducible substance, which is present in the solution being analysed, at the surface of the working electrode. With the voltammetric or polarographic technique, both quantitative and qualitative data are provided from a single experiment. Quantitative data are obtained from the height of the polarographic wave (the "diffusion current" or "limiting current"), which is proportional to the bulk concentration of the electroactive material. For qualitative identification, the potential at which the reduction or oxidation wave has reached half the total wave-height—the half-wave potential, $E_{1/2}$—is characteristic for each substance in a given electrolyte solution and with a given electrode system (working, reference, and, in some cases, auxiliary electrode).

Classical polarography as described above, introduced by Heyrovský[1] in 1922, requires the use of static sample solutions because of the effect of stirring or solution flow on the limiting current. At potentials at which electrolysis of the sample substance occurs, the concentration of the substance at the electrode surface approaches zero. Current flow is sustained and increased by fresh sample substance being transported from the bulk of the solution by some mass-transfer process to the electrode surface. As the applied potential is increased beyond the half-wave potential, the rate of mass-transfer becomes current-limiting. The mass-transfer processes include diffusion (movement in response to the concentration gradient formed between the electrode surface and the bulk of the solution when electrolysis is initiated), migration (caused by the influence of the electrical charge at the electrode on charged electroactive species), and convection (caused by solution agitation, such as stirring or flowing). In a static system containing an excess of charged, non-electroactive (in the region of interest) supporting electrolyte, convection and migration are negligible, and diffusion alone controls mass-transfer.

Under these classical polarographic conditions, the familiar Ilkovič equation governs the relationship between limiting current and bulk concentration: $i_l = 708nD^{1/2}m^{2/3}t^{1/6}C$, where i_l = limiting current, n = number of electrons involved in the electrode reaction, D = diffusion coefficient of the electroactive species, m = mass of mercury flowing per unit time, t = drop time, and C = bulk sample concentration. With working electrodes other than dropping mercury (voltammetry), the maximum or peak current is given by the Randles-Sevcik equation: $i_p = kn^{3/2}AD^{1/2}V^{1/2}C$, where i_p is the peak current, k is the Randles-Sevcik constant (=269 according to the calculations of Nicholson and Shain),[2] A is the electrode surface area, V is the potential-sweep rate, and the other terms are as already defined. Detailed theoretical treatments of polarography/voltammetry can be found in any number of standard electrochemical texts as well as in an excellent review article by Adams.[3] The theory of voltammetry is treated in a most rigorous manner by Nicholson and Shain.[2]

Electroanalysis in a flowing system generally entails the application to the electrodes of a fixed potential at or near the potential which yields the limiting current in a static system of similar composition. After a constant background current (caused by reduction or oxidation of other species in the electrolyte solution and by other factors related to the electrode system or the instrument) is established, the current caused by the reduction or oxidation of the substance of interest as it flows past the working electrode can be recorded as a function of time.

Under such forced convection conditions, both diffusion and convection contribute to the limiting current. This makes the solution of the corresponding equations more complex. Derivation of the equations for convective voltammetry requires a hydrodynamic treatment which leads to the concept of a thin diffusion boundary layer close to the electrode surface. The liquid-flow velocity within this diffusion boundary layer is not zero except at the electrode surface. Viscosity changes in this layer must be taken into account. Any transition from pure diffusion to convection must take place continuously. The diffusion

boundary layer has no exactly defined thickness, but is simply a depth which is defined as the region within which the maximum change in sample concentration occurs.[4] By using solution hydrodynamics,[5] it can be shown that, when the applied potential causes the concentration at the electrode surface to equal zero, the limiting current is given by $i_l = nFADC/\delta_N$, where F is the Faraday, δ_N is the thickness of the diffusion boundary layer, and the other terms are as already defined. The quantity δ_N can be defined in terms of such parameters as D, the diffusion coefficient, v, the kinematic viscosity of the solution, L, the characteristic length or geometry of the electrode surface along which the liquid flows, and U, the solution velocity.

With so many variables involved, it is difficult to deduce the current–concentration relationship from the fundamental parameters. Important information, however, can be obtained from the equation. First, the limiting current equations for most convection electrodes will be functions of the general form $i_l = f(C,U,L,D,v)$. Secondly, the equation indicates that any means of reducing the value of δ_N will increase i_l. Empirical relationships between δ_N and the solution flow-rate can be expressed as $\delta_N = B/U^a$, where B is a constant for a given set of conditions and the exponent, a, depends on the nature of the convection and the boundary conditions used for solving the equation of convective–diffusional mass transfer.[4,6] Thus, if the flow-rate is increased, the quantity δ_N is decreased and i_l is increased. It is evident, therefore, that with precise control of experimental conditions (flow-rate, electrode geometry, *etc.*) the proportionality of limiting current to concentration will hold and quantitative analytical results can be obtained from flowing systems.

Because of the requirement of a static system in classical polarography and the theoretical complexities and experimental difficulties arising from measurements in flowing systems, applications of electrochemical detection to flowing systems were quite slow to be developed. In spite of these difficulties, such applications did arise in response to two different, yet related, needs. First, the routine polarographic procedure itself—sample preparation, clean-up, dilution, oxygen removal, addition of supporting electrolyte and maxima suppressors—was time-consuming. To circumvent these difficulties, various continuously-monitoring or automated electrochemical systems were developed. Secondly, continuous analysis of flowing systems, such as column effluents and process streams, was recognized to be more advantageous than batch analysis of collected fractions. With continuous analysis, analytical results were immediately available, and collection and handling of multiple sample fractions were eliminated.[7] If the stream was deaerated before reaching the electrode, the polarographic/voltammetric technique—with its high sensitivity and selectivity (offered by the judicious choice of applied potential)—was a logical candidate for continuous analysis of flowing systems.

The earliest studies of continuously-recorded polarographic measurements tended to be empirical in nature and were limited to applications such as the determination of oxygen content of lake water,[8] activated sludge,[9,10] and water used in metabolic studies.[11] The first use of electroanalysis in a forced-convection flowing stream was reported in 1947 by Müller.[12] With a platinum microelectrode sealed in the constricted portion of a glass tube, the limiting current was found to be a linear function of the bulk concentration of an electroactive species in the fluid stream and of the logarithm of the flow-rate. Even at this early date, Müller suggested the possibility of inserting such an electrode into the bloodstream for *in vivo* bioavailability studies. The use of a dropping mercury electrode (DME) for the continuous monitoring of chromatographic column effluents containing proteins was briefly reported by Drake.[13] The technique of polarographic detection in liquid chromatography was extensively developed, however, by Kemula,[14] who called the method chromatopolarography.

In the years immediately following the work of Kemula, several designs and applications of DME flow-through cells with fixed applied potential were reported. Wilson and Smith[15] measured the effect of flow-rate on limiting current in cells with the direction of flow horizontal, vertically downward, and vertically upward relative to the drop direction of the DME. After determining that vertically downward flow resulted in the least deviation of the limiting current from the value obtained under static conditions, these authors measured dissolved sulphur dioxide continuously in plant streams. Rebertus *et al.*[16] described a sensitive and continuous polarographic method for monitoring the concentration of various metal ions eluted from an ion-exchange column. Blaedel and Todd utilized polarographic detection following ion-exchange chromatography, as a means of directly detecting metal ions and organic acids[7] and indirectly detecting amino-acids, after on-stream conversion of the amino-acids into copper–amino-acid chelates and then into copper–EDTA chelates, which were the species detected.[17] During this time period (approximately the decade of the 1950s), other investigators reported designs for DME flow-through cells[18–20] with applications including the determination of metal ions,[21–23] amino-acids,[24] alkaloids[25] and phosphate insecticides containing a nitro group.[26] In many of these papers it was reported that the limiting current increased with increasing flow-rate (as predicted by hydrodynamic theory), but the effect was not large for moderate flow-rates. A typical polarographic flow-through cell is shown in Fig. 1.[23]

While a considerable amount of progress was being made in research laboratories in the use of electrochemical flow-cells, the technique did not receive general acceptance, and interest in and practical usage of

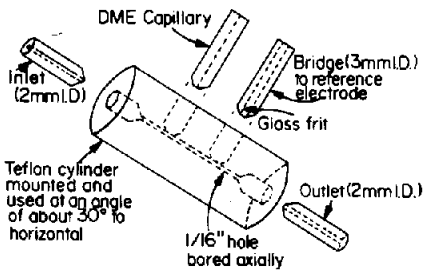

Fig. 1. Polarographic flow cell designed by Blaedel and Strohl.[23] (Reproduced with permission from *Analytical Chemistry*, 1964, **36**, 445. Copyright by the American Chemical Society.)

the technique declined for a number of reasons. One of the primary reasons was the fact that, while "homemade" detectors were being used successfully, commercial instrumentation of this type was unavailable. Since many laboratories were unwilling or unable to build their own equipment, use of the technique did not spread as it should have. In addition, the inefficiencies of classical liquid chromatography, the major application for electrochemical flow-through detectors, resulted in wide peaks and only moderate sensitivity (often milligram limits of detection).[27] Efficient electrochemical flow-through cells were difficult to design, especially in regard to the very small dead volumes needed for greater sensitivity; the geometry of prototype detectors was difficult to reproduce exactly; the detectors were not easy to handle, with the need for very careful control of experimental parameters such as flow-rate and electrode surface preparation; the requirement of an electrolyte dissolved in the solution analysed restricted the choice of solvents to essentially aqueous media. The DME itself presented a number of problems: the dropping mercury caused recorder oscillations or noise; the need to remove "spent" mercury drops from the flow stream added to design complexities; the DME had a limited anodic potential range; oxygen had to be removed to prevent interference with reduction processes of interest. In the few instances where solid electrodes were utilized, non-reproducibility of the electrode surface, caused by surface oxide formation and adsorption of reaction products, was a major drawback. Thus, when other analytical methods which were less prone to difficulties or were more sensitive than liquid chromatography with electrochemical detection became commercially available, these methods (such as atomic-absorption spectroscopy for metals, and gas chromatography) became preferred for many applications.

Beginning approximately a decade ago, high-performance liquid chromatography (HPLC) has experienced a phenomenal resurgence of interest owing to considerable advances in technique, mainly in column technology, resulting in extremely sharp elution bands and therefore excellent resolution. After much initial effort was unsuccessfully directed to developing a sensitive universal detector for HPLC, research became focused on the development of more selective detectors.[28] The major limitation has been the lack of a detector sensitive enough for HPLC to compete with gas chromatography. Modern electrochemical techniques possess considerable sensitivity, but suffer from poor resolution of components with similar half-wave potentials in a mixture. Hence combining the resolution of HPLC with the sensitivity of electrochemical detection yields a powerful analytical technique.

The characteristics of an ideal detector for HPLC include the following:[6] high sensitivity, low limit of detection, large linear dynamic range, continuous operation, low internal volume, and independence of column parameters such as rate of flow of the eluent. Under the proper conditions, electrochemical detectors can satisfy these criteria to a large extent. To date, the three detectors for HPLC which have achieved the most widespread popularity are light-absorption, fluorescence, and electrochemical. Electrochemical detection has been found to have a number of advantages, primarily sensitivity, selectivity and economy.[29] The advantages of electrochemical detection can be summarized as follows.[28,30]

1. Sensitivity. The detection method is generally sensitive down to the nanogram level, and in some cases even to the picogram level, depending on the compound characteristics, chromatographic retention time, and applied potential. In applicable systems, the detection limit is typically lower (by as much as a factor of 1000) than that of commercially available ultraviolet detectors.

2. Selectivity. Electrochemical detection is selective to species containing an electrochemically oxidizable or reducible moiety. While a vast number of organic compounds yield absorption spectra in the ultraviolet or visible region, most organic compounds are not electroactive in an easily accessible potential range. Thus, electrochemical detection is ideal for trace analysis in complicated matrices (*e.g.*, body fluids). Fortunately, a large number of biochemicals, pharmaceuticals, food additives, pesticide residues, industrial antioxidants, plant phenolics, and other compounds of bioanalytical and commercial interest are electroactive and amenable to electrochemical detection.[29] In addition to the selectivity resulting from the electroactivity requirement, the potential applied to the detector can be adjusted to discriminate between two or more incompletely separated electroactive compounds with different oxidation or reduction potentials. This type of selectivity was well illustrated by Buchta and Papa.[31] The same degree of selectivity is rarely possible with an ultraviolet detector since the absorption peaks of organic compounds are broad and usually less sensitive to change in substituent than electrochemical response is.[29]

3. Wide linear range. Typical linear-response ranges cover 4–5 orders of magnitude of concentration.

4. Electrochemical detection is easily adapted to automatic operation and data-acquisition.

5. Low dead volumes. Modern electrochemical detector cells have internal volumes of a few microlitres or less, giving negligible hydrodynamic broadening of chromatographic zones.

6. No sample preparation. Derivatives normally do not have to be prepared as they do for GC work and for fluorescence detection.

7. Low cost. Direct conversion of chemical signal into an electrical signal, with no intermediate optical carriers, results in inexpensive, simple, and reliable electronic instrumentation.

The recent introduction of commercially available electrochemical flow-through detectors has significantly increased activity in this area. While most applications of this detection technique have been in the field of HPLC, applications to other flowing systems, such as process stream and dissolution rate studies, have been reported.

As mentioned earlier, electrochemical detection in flowing systems does have characteristic problems. The dependence of current flow per unit concentration on flow-rate, solution pH and ionic strength, cell geometry, condition of the electrode surface, and, in the case of HPLC, volume injected, requires careful control of experimental parameters. The reduction of dissolved oxygen interferes with cathodic measurements and necessitates deaeration of the solvent. Metal-ion contaminants and other electroactive impurities in the electrolyte or carrier solvent increase the observed background current and hence decrease the sensitivity. Thus, the purity of electrolytes and solvents is critical, and contact of the flow-stream with metal apparatus, from which metal contaminants can be leached, should be minimized. A major drawback of electrochemical detection is the need for an electrically conductive sample solvent, limiting its applications to aqueous systems containing inorganic salts or acids or to mixtures of water with water-miscible organic solvents. Because electrochemical response is so dependent on the overall solution characteristics, gradient elution (the stepwise changing of mobile phase concentration or composition to obtain more rapid chromatographic separation) cannot be used with electrochemical detectors.[32]

While the choice of reference electrode (usually saturated calomel or silver/silver chloride) and, if desired, auxiliary electrode (usually a noble metal such as platinum) is generally not critical to detector performance, the choice of working electrode material is. Although most of the early work in this technique involved the DME, solid electrodes (including various forms of carbon, platinum, gold, and mercury films on these materials) have been extensively studied and successfully applied in recent years. Each type of electrode material has both advantages and disadvantages.

The DME has an extensive cathodic range of polarization owing to the large over-potential for the evolution of hydrogen, enabling it to be used at potentials even more negative than -2 V, depending on the supporting electrolyte used. This characteristic makes mercury most desirable for electro-reductions. Additionally the DME provides a constantly renewed electrode surface, essentially eliminating the problem of electrode surface contamination. Unfortunately, the disadvantages of a DME are many. The usable anodic potential range is limited by the oxidation of the mercury metal at approximately $+0.2$ V (*vs.* the saturated calomel reference electrode). This precludes the use of the DME for most electro-oxidizable compounds. Other disadvantages of DME flow-through detectors are the current oscillation over the lifetime of the drop, necessitating some form of damping, the need to remove oxygen from the supporting electrolyte solution, the awkward mechanical problem of designing a cell through which both the flowing solution and the mercury drops must pass, and the effect of flow-rate on the drop-time (caused by turbulence in the vicinity of the drop when stream velocity is high). The use of a mercury pool in place of the DME does not satisfactorily eliminate these problems. The motion caused in the pool by the flowing stream causes an irregular limiting current and a large charging-current background.[6]

While solid electrodes may be used to analyse for easily reducible compounds, they are most advantageous for the study of oxidation processes because of their wide anodic (positive) polarization range and low residual current within this range. Generally greater sensitivity, simpler cell design, and lower noise levels than with a DME add to the attractiveness of solid electrodes. The major problem with solid electrodes is non-reproducibility of the electrode surface, primarily caused by adsorption and surface oxide formation. Thus, cleaning of the solid electrode, or surface renewal, becomes a major consideration.

The most widely employed solid electrode flow-cells over the past decade utilized carbon-paste working electrodes. The carbon-paste electrode, which is made from spectral-grade graphite and organic solvents immiscible with water (such as silicone oil), has the advantages that it can be made quickly and that its residual current is lower than that of many other types of solid electrode.[33] Carbon electrodes, especially carbon-paste, tend to be more stable and show memory effects less frequently than noble metal electrodes.[33,34] When a carbon-paste electrode does become surface-contaminated, renewal of the surface is generally quite easy; the surface of the electrode is simply wiped off with a tissue and a fresh layer of carbon paste is smoothed back on the surface. Carbon paste has a fair potential range which can be adjusted by using different waxes or oils in the paste.[35] The major disadvantage of carbon-paste electrodes in flowing systems is that the carrier solvent must be water or an aqueous solution of a polar organic solvent (*e.g.*,[30] a maximum of 20% ethanol in water has been suggested). Organic solvents tend to strip away the impregnating liquid from the carbon, rapidly causing deterioration of the electrode. Thus,

analysis with carbon-paste electrodes is restricted to compounds soluble in predominantly aqueous media. High cathodic residual currents make carbon-paste electrodes unsuitable for the detection of most reducible compounds. Furthermore, different formulations of carbon paste exhibit variations in sensitivity, and incorrect electrode preparation results in spurious signals because of flaking of the paste and projection of the paste into the solution cavity.[36] Finally, carbon-paste electrodes are rather slow to attain a constant background current in flowing systems; for the background current to equilibrate requires from several minutes for changes in flow-rate to several hours for daily start-up at high sensitivity settings.[32,37]

For use with non-aqueous solvents, glassy-carbon electrodes have been quite successful. Glassy carbon is an impermeable, electrically conductive material resistant to chemical effects and can be used directly as an electrode.[33] Since it is obtained as a smoothly polished solid rod, it is more durable than carbon paste. Glassy carbon has a wider useful potential range than carbon paste (from -1.3 to $+1.5$ V *vs.* an Ag/AgCl reference electrode has been reported[38]) so both oxidizable and reducible species can be electrolysed. Glassy-carbon electrodes exhibit low residual current and respond considerably more rapidly to solvent changes and daily start-up. Surface contamination, however, is more significant with glassy carbon than with carbon paste; surface cleaning is required more frequently and is more complicated with glassy carbon than with carbon paste. Cells for use in non-aqueous solvents, including glassy-carbon and noble-metal detectors, tend to be significantly more expensive than carbon-paste cells.[35]

Pyrolytic graphite[36] and silicone rubber-based carbon electrodes[33,39] are similar to glassy carbon in ruggedness, applicability to non-aqueous systems, low residual current, and large anodic as well as cathodic potential ranges. These electrodes also suffer from surface adsorption.

Platinum electrodes have been used to a limited extent in flow-through cells, mainly for inorganic ions.[6,40–42] Platinum is not attractive for general organic use in aqueous solutions since surface films of oxide readily form during use, yielding large residual currents and affecting the electrode reactions. These problems are less severe in non-aqueous solvents. Gold,[42] silver[40] and cadmium[43] electrodes have been utilized in flow-through cells to a lesser extent. Mercury-plated solid electrodes offer the wide cathodic potential range of mercury and the design and noise advantages of solid electrodes. While mercury-film electrodes have successfully been used for reduction processes,[31] preparation and maintenance of a uniform electrode surface is quite cumbersome.

Currently, there are four general types of electrochemical detection methods for HPLC eluents and other flowing systems. These are polarography, amperometry, coulometry, and conductivity. To date, amperometric detectors have been by far the most popular, while conductimetric detectors are generally considered the least useful. The first three detection methods listed generally encompass the following principles: if the working electrode is maintained at any fixed potential (relative to a reference electrode) at or near the limiting-current plateau for the compound of interest (as determined from current–potential recordings for a static sample solution), the background current will remain constant as long as the solution velocity and the composition of the supporting electrolyte remain constant; as the electroactive compound of interest flows past the electrode, electrolysis occurs and the resulting current (additional to background current) is proportional to the concentration of the electroactive species. The merits and applications of each type of electrochemical detector will be discussed separately.

Polarographic detectors

Employing the DME as the working electrode, polarographic flow-through detectors electrolyse only a very small portion of the available electroactive species. The resulting current flow, however, is still proportional to the bulk concentration (see earlier discussion of hydrodynamic voltammetry theory). In spite of the narrow anodic potential range and other disadvantages of a DME flow-cell (listed above), applications of such detectors continue to be reported. HPLC with polarographic detection has been used for the separation and determination of a number of important classes of compound, including pesticides,[44–46] vitamins and analgesics.[46] Wasa and Musha[47] determined nitropyridine derivatives by HPLC using horizontal and vertical types of DME.

A recently introduced commercially available polarographic detector for HPLC is illustrated in Fig. 2.[48] A delivery tip (A) directs the eluate (B) through the supporting electrolyte (C) toward a mercury drop electrode (D) where electrolysis of sample occurs. Once every second a fresh mercury drop of constant area is presented to the eluate. This rapid introduction of a "fully-grown" drop minimizes oscillations caused by drop growth. Premature dislodgement of drops caused by solution flow is effectively eliminated in this detector. The effective dead volume of the cell is less than 1 μl and the limit of detection

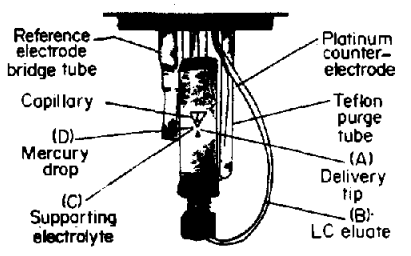

Fig. 2. PAR™ Model 310 polarographic flow-through detector.[48] (By courtesy of Princeton Applied Research Inc.)

claimed for many compounds is in the lower nanogram-region.

Polarographic flow-through detectors have been combined with automated analysis systems for the measurement of metal ions[49,50] and pharmaceutical products.[51,52] Cullen *et al.*[53] used an automated technique with polarographic detection to monitor the dissolution of tablet and capsule formulations of the drug lorazepam, a 1,4-benzodiazepine.

Voltammetric analysis is well suited for drug dissolution measurements since the technique has a wide linear dynamic range and is specific for the compound of interest in the presence of excipients. The DME has the additional advantage of a constantly renewable surface, free from the build-up of interferences during dissolution. Hackman and Brooks[54] measured the dissolution rate of the drugs chlordiazepoxide, ornidazole, trimethoprim and isoniazid, by both non-invasive (electrodes placed directly in the dissolution flask) and invasive (use of a flow-through cell) polarographic techniques. While both methods yielded essentially the same values, the dissolution curves obtained by the invasive method were virtually noise-free and easier to interpret.

Amperometric detectors

The operation of an amperometric detector is similar to that of a polarographic detector in that only a small portion of the available electroactive species (often 1–10%) is electrolysed in the cell. Since solid or stationary working electrodes are used, amperometric detectors enjoy the already listed advantages over polarographic detectors, especially greater applicability to electro-oxidation reactions, simplicity of design, and generally greater sensitivity. Of the four general types of electrochemical detector, amperometric detectors are the simplest in design and easiest to construct, and therefore least expensive. In addition, they allow more rapid material through-put than coulometric detectors (see below). Drawbacks of amperometric detectors are that changes in flow-rate or temperature will affect the observed current, and comparison with standards is required to determine sample concentration. The latter is hardly a drawback, however, for HPLC detection, where sample preparation and injection are normally the primary sources of error and relative measurements (internal or external standards) predominate.[29]

Although amperometric detectors are generally more sensitive than polarographic or conductimetric detectors, there are conflicting opinions about their sensitivity relative to that of coulometric detectors. Some investigators have maintained that coulometric detectors give greater sensitivity, since more of the available electroactive material is electrolysed (100%, by definition).[6,55] Others[29] claim that, although less material is actually reacted, amperometric detectors give greater sensitivity. This is explained as follows: as more electrode surface area is added downstream to improve efficiency in a coulometric detector, each increment of surface area contributes proportionately less to the total amount of material converted but approximately equally to the background current due to solvent component electrolysis. Thus, this greater increase in background current relative to that in sample electrolysis current results in an overall decrease in sensitivity. Buchta and Papa[31] seem to agree with the latter view, but take a more "middle-ground" approach. They state that, unless the signal-to-noise ratio increases when the effective area or length of the working electrode is increased, no major difference is expected in the limit of detection obtained with the two types of detectors.

Amperometric detectors have been by far the most utilized electrochemical flow-through detectors in recent years. The heart of most amperometric detectors is either a thin-layer cell or a tubular electrode. Thin-layer cells are more popular in practice, owing to the ease of achieving a small volume (less than 1 μl) and to the variety of electrode materials that can be used.[29] Thin-layer cells have been constructed with the stream flowing parallel to the electrode embedded in the channel wall or with the stream directed perpendicular to the electrode surface followed by radial dispersion, the so-called wall-jet detector.

A commercially available design of the former type of thin-layer cell, introduced by Kissinger and co-workers, is shown in Fig. 3.[30] It has been applied by Kissinger and co-workers to the determination of catecholamines and their metabolites,[27,56–65] uric acid,[27,66–68] ascorbic acid,[27,69] acetaminophen,[70] *p*-aminohippuric acid,[71] and phenolic compounds[72–74] in biological fluids, pharmaceutical preparations, tissues, plant matter, and foodstuffs. Detector selectivity was demonstrated by the determination of traces of *p*-aminophenol impurity in acetaminophen dosage forms.[70] Using the same detector design, Adams and his group have also studied catechol-

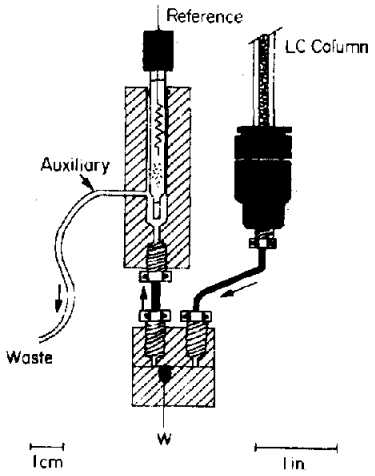

Fig. 3. Thin-layer amperometric detector for HPLC. W = carbon-paste or glassy-carbon working electrode with embedded metallic pin for electrical contact.[30] (By courtesy of Bioanalytical Systems Inc.)

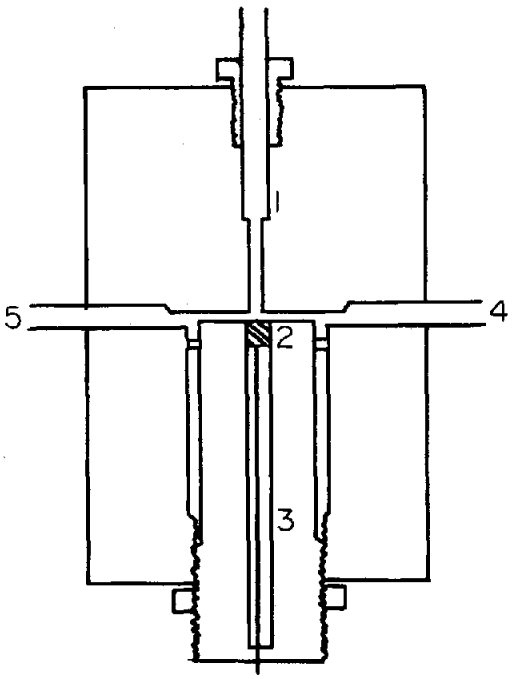

Fig. 4. Schematic diagram of wall-jet detector.[38] 1, Inlet nozzle; 2, glassy-carbon disc working electrode; 3, adjustable working electrode body; 4, reference electrode; 5, counter-electrode exit. (Reproduced from the *Journal of Chromatographic Science* by permission of the copyright holders, Preston Publications Inc.)

amines,[75] especially in brain tissues,[76,77] as well as ascorbate in brain tissue[78] and aromatic amine carcinogens.[79] Application of this commercial detector is becoming routine, with other investigators utilizing it for the determination of catecholamines and their metabolites,[80–83] isomeric hydroxyanilines,[84] halogenated anilines,[32] the pesticide 2-phenylphenol,[85] and the pharmaceuticals procarbazine,[37] β-cetotetrine[86] and 8-hydroxycarteolol.[87] Nanogram and in many cases picogram limits of detection are routinely reported.

Wall-jet amperometric detectors, in which the stream flows from a nozzle perpendicularly onto the electrode surface (usually carbon), have several useful characteristics.[38]

1. High sensitivity. Rapid convective mass-transfer, caused by the perpendicular impact between solution and electrode, gives very high sensitivity. Picogram limits of detection have been reported.[38,96,97]

2. Variable cell volume. In typical designs, the distance between the nozzle tip and the electrode surface is readily adjusted.

3. Freedom from surface adsorption. The washing effect of the rapidly incoming solution "cleans" adsorbed species from the electrode surface.

An amperometric detector of wall-jet design is commercially available.[28] The design by Fleet and Little[38] is shown in Fig. 4.

Tubular electrodes are very easily incorporated into flowing systems. They do suffer, however, from the difficulty of constructing the very small volume cells required and polishing and cleaning the inner working surface of the electrode to minimize adsorption effects. Blaedel and co-workers have utilized tubular platinum electrodes[98–100] to determine inorganic ions in flow systems. Tubular carbon electrodes have been used to determine ascorbic acid,[101] methyldopa,[102] and total cholesterol in serum.[103]

Blank[88] has described an amperometric detector which contains two carbon-paste working electrodes in the thin-layer channel. The two electrodes are maintained at different potentials and the two resulting current outputs are fed to a two-pen recorder. For compounds that overlap chromatographically, but have different electrolysis potentials, the system provides selective detection. It can also provide two simultaneous determinations of a single species, giving a saving in time for routine duplicate analysis. Eggli and Asper[89] have designed an electrochemical double cell in which a column electrode of amalgamated silver powder is used to reduce cystine quantitatively to cysteine, which is detected amperometrically at a mercury pool electrode. Lemar and Porthault[90] attacked the problem of the unsuitability of electrochemical detection for normal phase chromatography by designing a cell with provision for mixing a polar non-aqueous electrolyte solution with the non-polar column effluent upstream from a glassy-carbon electrode. A differential amperometric detector (two identical detectors coupled by a differential amplifier) was designed by Brunt and Bruins[91] to measure the difference in current between a mobile-phase stream with sample and one without sample. This effectively overcomes the problem of the high background current (caused by electrode potential, pH of eluate, and electroactive impurities in the eluate) sometimes encountered with electrochemical detection.

Although constant-potential amperometry is generally sufficient for most applications, potential-pulse experiments have been reported.[42,92] Such techniques offer the advantages of decreased dependence of the measured current on flow-rate (the short potential-pulse duration minimizes the development of a diffusion layer at the electrode and the dependence of the thickness of this layer on flow-rate) and increased electrode stability (since the potential is held at the initial non-reacting potential for most of the experimental time, surface oxidation or adsorption is minimized and any contamination which does occur can often be removed by the return to the initial potential after the pulse). Differential pulse methods can improve the selectivity of electrochemical detectors for compounds which react at a higher potential than other unseparated components (not possible in constant-potential work).[29,92] Because of the significantly poorer sensitivity and more complex experimental requirements, however, pulse methods have generally been avoided.[29]

Various other amperometric flow-through detectors have been reported, utilizing working electrodes of

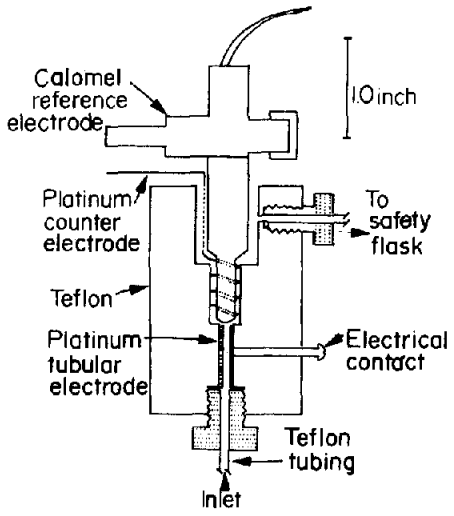

Fig. 5. Coulometric detector designed by Johnson and Larochelle.[6] (Reproduced by permission of the copyright holders, Pergamon Press, Ltd.)

glassy carbon,[93] silicone rubber-based carbon,[34,39,94] a mercury pool,[95] and mercury-coated platinum.[31,47] Pungor and co-workers[34,94] reported the use of their detector with HPLC, for dissolution rate studies, and for *in vivo* drug measurements.

Coulometric detectors

The design of coulometric flow-through detectors requires electrodes with large surface areas combined with small cell volumes in order to achieve or at least approach the desired 100% electrolysis of the electroactive species present (the basic requirement of coulometry). Coulometric detection offers the advantage that variations in flow-rate, temperature or electrode area do not affect detector response (current) as long as 100% efficiency is maintained. Calibration with standards is not required (except for checks on detector efficiency), since the amount of sample compound can be obtained directly from Faraday's law $Q = nFN$, where Q is the measured number of coulombs, n is the number of electrons involved in the reaction, F is the Faraday, and N is the number of moles of sample converted into product. While sensitivity is often in the same general region as that of amperometric detectors (see above), coulometric detectors have the following disadvantages: complicated cell designs (100% efficiency is difficult to achieve in flowing systems); strict potential control over the entire large electrode area is difficult to maintain, resulting in decreased detector selectivity for overlapping peaks; generally high background currents; electrode surface contamination more critical, since coulometry is an absolute technique.[31,35]

Johnson and co-workers have successfully utilized tubular platinum[6,41] and tubular cadmium electrodes[43] as coulometric detectors for various inorganic species. Figure 5 illustrates the coulometric detector designed by Johnson and Larochelle.[6] Electrodes of carbon, silver, or platinum gauze were used by Takata *et al.*[40,104] for the coulometric determination of heavy metal ions, halogens, amino-acids and carboxylic acids in flowing systems at levels from 5×10^{-7} to 5×10^{-10} mole of material. Poppe and co-workers have used large planar glassy-carbon electrodes to determine coulometrically the drugs perphenazine and fluphenazine in blood[55,105] at concentrations of 1–20 ng/ml. Other investigators have utilized coulometric flow-through detectors with working electrodes made of platinum tubes,[106] thin plates of carbon,[107] and columns filled with granular amalgamated nickel[108] or granular glassy carbon.[109]

Conductimetric detectors

Since conductivity is a universal property of ionic species in solution and shows a simple dependence on species concentration,[110] conductimetric detection has been studied as a means of monitoring ionic species in flowing streams, such as ion-exchange column effluents or process streams. A typical cell for conductivity measurement consists of a tube made of an insulator in which are embedded electrodes made of a noble metal or graphite. The cell is fitted directly into the stream to be monitored. Generally, a constant alternating voltage is applied to the electrodes, and the resulting current is measured. The conductivity is then deduced by knowledge of the cell constant (dependent on the geometry of the cell) and Ohm's law, since the current flowing is proportional to sample conductivity.[111]

Conductivity detectors are cheap, simple to instal and operate, and can have low dead volume (a few μl). They are potentially quite sensitive when the species of interest is the major ionic species present and has a changing concentration. In most cases, however, the conductivity from the species of interest is essentially "swamped" by that from the electrolyte and/or impurities in the solution stream as well as from the aqueous solvent itself.

Measurement of conductivity as a method for the detection of solutes in other than trace amounts was first utilized in 1951 by James *et al.*[112] Duhne and Sanchez de Ita[113] determined sodium nitrate at the μmole level, using small silver-tube electrodes. Inorganic ions, amino-acids, proteins and methylglucosides in column effluents were detected conductimetrically by Jackson,[114] using thin aluminium sheet electrodes wrapped around the column. His detector, however, had a large dead volume and suffered from excessive noise. Conductimetric detectors with platinum electrodes have been utilized for the determination of inorganic ions in aqueous solution[115] and sulphur in organic solvents.[116] Pecsok and Saunders[115] found that the detector response was non-linear over the concentration range studied, because 90–95% of the total conductivity was due to impurities and the water itself. Small *et al.*[110] minimized this background conductivity problem by using a combination of resins which removed the background electrolyte

ions, leaving only the species of interest as the major conducting species in the effluent.

This paper attempts to present a comprehensive yet brief review of the theory, operation and applications of the basic types of electrochemical flow-through detector for liquid systems. To keep the length within reasonable limits, however, a number of related techniques have been omitted, including rotating electrodes (stirred solutions), ion-selective electrodes in flowing streams, and electrochemical detectors for gas chromatography (*e.g.*, reference 117).

In addition, several recent highly-specialized applications of electrochemical detectors can only be briefly mentioned. The use of *in vivo* voltammetric techniques is being actively pursued by Adams' group, which has implanted carbon microelectrodes in appropriate fluid streams of the small animal body to determine drugs injected into brain tissue[118] and metabolites in cerebrospinal fluid.[119,120] Several researchers have successfully combined anodic stripping voltammetry with flow-through electrochemical cells (*e.g.*, reference 121), while others have used such cells for electrosynthesis (*e.g.*, reference 122). Interest has recently been taken in use of amperometric flow-through detectors for determining redox enzyme activity, substrates, and co-factors. The efforts being made in these applications are rather well described in a review article by Heineman and Kissinger.[123]

During the last decade, electrochemical flow-through detectors have gone from being almost completely ignored to being used routinely in many laboratories. As the applications of HPLC continue to increase, so should interest in selective and sensitive HPLC detectors, including the electrochemical types. Growing interest in drug dissolution rates and process stream monitoring will expand the areas of application for electrochemical flow-through detectors. The recent introduction of low-cost, commercially available detectors is certain to accelerate the use of this technique.

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RADIOTRACER STUDY OF THE DISSOLUTION OF AMALGAMS

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Summary—The kinetics of dissolution of lead, thallium and zinc amalgams in contact with acid solutions containing dissolved oxygen were studied. The results were interpreted on the basis of the Wagner theory of the additivity of electrochemical part-processes. It was found that the rate of dissolution may be described by the differential equation for zero-order reactions. The numerical values of the rate constants of the dissolution were determined and compared with the values calculated on the basis of the limiting diffusion current of the oxygen reduction. The results confirm that the diffusion of the oxygen depolarizer is the rate-determining part-process.

The dissolution of amalgams is worthy of attention for various reasons: (*i*) preparative chemical and radiochemical problems may be solved by using differences in the dissolution behaviour of various amalgams, (*ii*) conclusions can be drawn regarding the factors affecting the kinetics and mechanism of reduction processes with amalgams, and (*iii*) the dissolution of amalgams has a significant influence on electrolysis at a mercury cathode, on amalgam exchange processes, and on cementation with amalgams. These methods have been used widely in analytical and radioanalytical chemistry for the separation and purification of metals, and for the separation and decontamination of radioactive elements.[1–6]

The present paper reports the results of investigations on the dissolution of dilute lead, thallium and zinc amalgams in acid solutions containing dissolved oxygen.

EXPERIMENTAL

Materials

The isotopes ^{204}Tl and ^{65}Zn were obtained from the Radiochemical Centre (Amersham, England). The decay isotope ^{212}Pb was separated from a ^{228}Th solution. The radiochemical purity of the isotopes was checked radioanalytically. All chemicals used were the purest available. Clean distilled mercury was used.

Procedure

The amalgams, labelled with a suitable radioactive indicator, were prepared by electrolysis at a mercury cathode. The schematic diagram of the apparatus for the study of the preparation and dissolution of amalgams is presented in Fig. 1. Figure 2 shows data relating to the rate of dissolution of lead from an amalgam (labelled with ^{212}Pb) in 0.1*M* acetic acid saturated with gaseous oxygen. The amount of lead dissolved was determined from the increase in the radioactivity of the solution, an aliquot of the solution (0.1 ml) being separated after a predetermined time and its radioactivity determined with a γ-scintillation counter. Radioactive measurements were always made about 7 hr after the end of the experiments, to allow equilibrium to be reached between the ^{212}Pb and its decay products. The amount of lead dissolved is expressed as a percentage (y) of the total amount of lead in the whole system. Amalgamated lead does not dissolve in oxygen-free 0.1*M* acetic acid.

DISCUSSION

The anodic half-reaction is the oxidation of the amalgamated metal:

$$M(Hg) \rightarrow M^{n+} + ne^{-}. \quad (1)$$

The cathodic half-reaction is the reduction of the dissolved oxygen, which takes place in two steps accord-

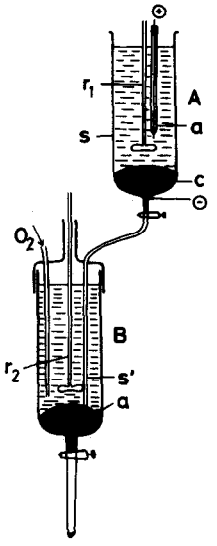

Fig. 1. Schematic diagram of the apparatus for the study of the dissolution of amalgams. (A) Electrolysis cell: (c) mercury cathode, (a) platinum wire anode, (s) solution of the metal ion to be electrolysed, labelled with a suitable radioactive indicator. (B) Corrosion cell: (a) amalgam, (s') 0.1*M* acid solution containing dissolved oxygen, (r_1, r_2) rotating stirrers.

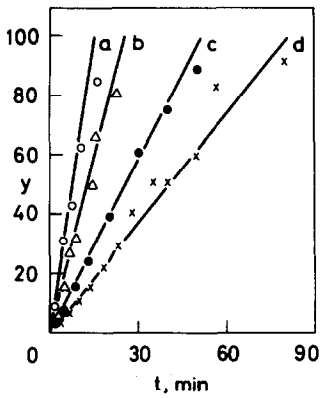

Fig. 2. Rate of dissolution of lead amalgam. Volume of amalgam 2 ml. Volume of solution 20 ml. Area of amalgam in contact with the solution 5.3 cm^2. Temperature 20°. Amalgam concentration (g/ml): (a) 0.0026, (b) 0.0052, (c) 0.0104, (d) 0.0156.

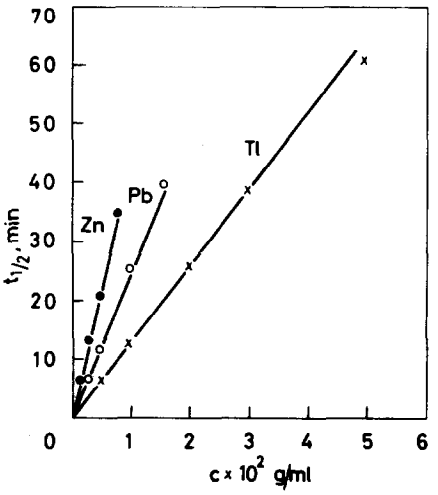

Fig. 3. Half-period of the dissolution process as a function of the initial concentration of the amalgam.

ing to the equations:[7]

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \qquad (2)$$

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O. \qquad (3)$$

As a result of these electrochemical half-reactions a mixed potential (E_k) develops on the amalgam–solution interface, at which the anodic current density (i_a) for metal dissolution is equal to the cathodic current density (i_c) for the reduction of oxygen:[8]

$$i_a = i_c \qquad (4)$$

or

$$\left(\frac{dy_{Pb}}{dt}\right)_{E_k} = \left(\frac{dm_{O_2}}{dt}\right)_{E_k} \qquad (4')$$

where y_{Pb} is the fraction of the lead in the amalgam that is dissolved in time t and m_{O_2} is the amount of oxygen reduced in the same time.

It can be seen from the data of Fig. 2 that the rate of dissolution as a function of time may be described by the differential equation for zero-order reactions:

$$\left(\frac{dy_{Pb}}{dt}\right)_{E_k} = k_1. \qquad (5)$$

Thus the following correlation exists between the specific rate constant and the half-life for the reaction:

$$k_1 = \frac{c_0}{2t_{1/2}} \qquad (6)$$

where c_0 is the initial concentration of the amalgam.

The half-period for the dissolution process as a function of the initial concentration of lead in the amalgam is shown in Fig. 3.

Similar studies were made of the kinetics of the dissolution of thallium amalgam labelled with ^{204}Tl, and zinc amalgam labelled with ^{65}Zn, in 0.1M sulphuric acid saturated with gaseous oxygen. The corresponding data are also presented in Fig. 3. The values of the rate constants ($\mu eq \cdot cm^{-2} \cdot sec^{-1}$) determined from the slopes are:

$$k_{Pb} = 1.21 \times 10^{-2}$$

$$k_{Tl} = 1.26 \times 10^{-2}$$

$$k_{Zn} = 2.22 \times 10^{-2}.$$

These values agree relatively well with the values of the rate constants calculated from the limiting diffusion current for the reduction of oxygen. The limiting diffusion current can be given by

$$i_{d,h} = \frac{nFDc_{O_2}}{\delta} \qquad (7)$$

or

$$\left(\frac{dy_{O_2}}{dt}\right)_{E_k} = \frac{Dc_{O_2}}{\delta} = k_2 \qquad (7')$$

where D is the diffusion constant of the dissolved oxygen, δ is the thickness of the Nernst adsorption

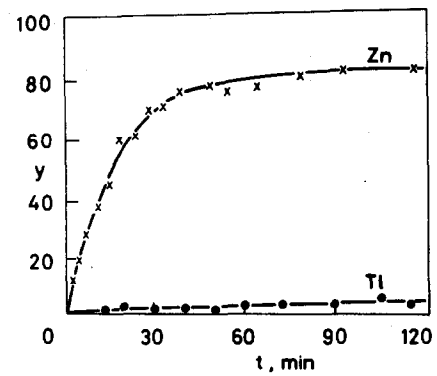

Fig. 4. Dissolution of zinc and thallium amalgams in oxygen-free 0.1N sulphuric acid contained hydrogen peroxide. Volume of solution 20 ml. Concentration of hydrogen peroxide 0.005M. Volume of amalgam 2 ml. Initial concentration of amalgam: Zn 3.25 mg/ml; Tl 2.04 mg/ml. Temperature 20°.

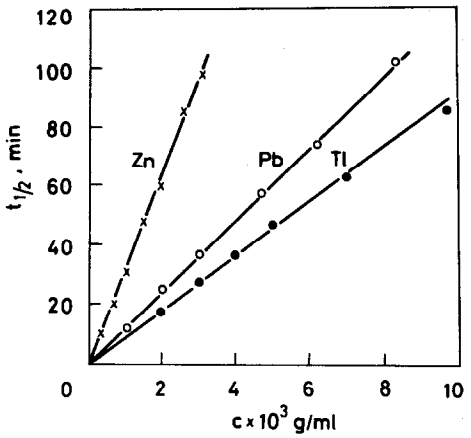

Fig. 5. Dissolution of amalgams in contact with 0.1*M* acid saturated with air. Half-period of the process as a function of the initial concentration of the amalgam.

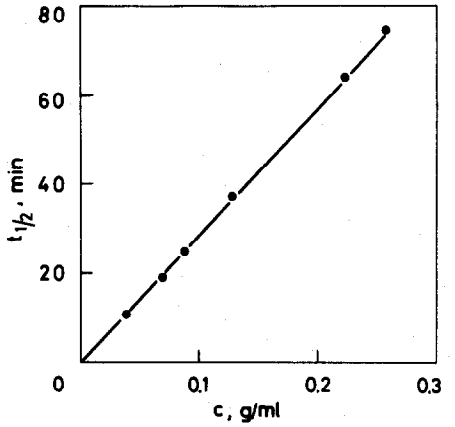

Fig. 6. Variation of the half-period of the dissolution process in 0.1*N* sulphuric acid saturated with chlorine, as a function of the initial concentration of zinc amalgam.

layer, and c_{O_2} is the concentration of dissolved oxygen in the solution, which remained constant since the oxygen reduced at the amalgam–acid interface was replaced by dissolution of an equivalent amount of gas, gaseous oxygen being bubbled through the vigorously stirred solution during the whole dissolution process.

From equation (7′) the rate constant for the cathodic part-process can be calculated, giving $k_2 = 5.7 \times 10^{-9}$ mole.cm^{-2}.sec^{-1} when the following values[9,10] are substituted: $D = 1.86 \times 10^{-5}$ cm^2/sec, $c_{O_2} = 1.45$ μmole/ml, $\delta = 5.0 \times 10^{-3}$ cm. The value for δ is that for a vigorously stirred solution.

It appears from a comparison of the rate constants for the anodic and cathodic part-processes that the reduction of 1 mole of oxygen results in the dissolution of 2 equivalents of lead or thallium, or 4 of zinc.

These results suggest that the reduction of oxygen during the dissolution of zinc from its amalgam proceeds according to the equation

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

whereas the equation

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$$

applies to the dissolution of lead and thallium from their amalgams. The intermediate product, hydrogen peroxide, is thermodynamically unstable (the normal oxidation–reduction potential for the O_2/H_2O_2 system is $E^0 = +0.68$ V, whereas that for the H_2O_2/H_2O system is $E^0 = +1.77$ V) but is stabilized kinetically for mechanistic reasons. It can be identified as a product of oxygen reductions only in those cases where cathodic reduction at the electrode involves a high activation energy. Hydrogen peroxide is also produced as an intermediate in the dissolution of zinc amalgam, but it is further reduced according to the equation

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O.$$

This is confirmed by experiments in which amalgamated zinc was dissolved in an oxygen-free acid solution containing hydrogen peroxide (Fig. 4). The reduction of hydrogen peroxide by thallium amalgam, however, has a high activation energy, as a result of which the metal virtually does not dissolve in an oxygen-free solution contained hydrogen peroxide.

Similar results were obtained when the kinetics of the dissolution of lead, thallium and zinc amalgams in contact with 0.1*M* acid saturated with air instead of with oxygen were investigated (Fig. 5). The corresponding rate constants (μeq.cm^{-2}.sec^{-1}) are:

$$k'_{Pb} = 2.1 \times 10^{-3}$$

$$k'_{Tl} = 1.7 \times 10^{-3}$$

$$k'_{Zn} = 3.5 \times 10^{-3}.$$

From equation (7′) the value of the rate constant of the cathodic part-process is $k'_2 = 9.1 \times 10^{-10}$ mole.cm^{-2}.sec^{-1} ($c_{O_2} = 2.5 \times 10^{-7}$ mole/ml).

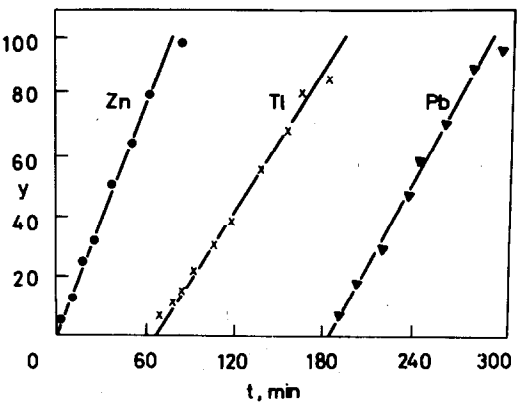

Fig. 7. Rate of dissolution of zinc–thallium–lead mixed amalgam. Concentrations Zn 1.0 mg/ml, Tl 6.1 mg/ml, Pb 3.2 mg/ml. Acetic acid, 0.1*M*, saturated with air.

A similar study was made of the kinetics of the dissolution of zinc amalgam in contact with $1N$ sulphuric acid saturated with gaseous chlorine. The value of the rate constant determined from the data in Fig. 6 is $k_1 = 0.34\ \mu\text{eq} \cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$. From equation (7') the rate of the cathodic part-process for reduction of chlorine can be calculated to be $k_2 = 1.8 \times 10^{-7}$ mole $\cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$ from the data[11] $D_{Cl_2} = 1.41 \times 10^{-5}$ cm^2/sec, $c_{Cl_2} = 65\ \mu$mole/ml, $\delta = 5 \times 10^{-3}$ cm.

The kinetics of the dissolution of zinc–thallium–lead mixed amalgams in contact with $0.1M$ acetic acid saturated with air were also investigated. It can be seen from the data of Fig. 7 that the dissolution of a less noble metal is practically complete before that of the next more noble metal begins. The concentration ratio of the ions in solution during the dissolution of mixed amalgams can be taken into account in accordance with the galvanic principles referring to mixed electrodes.

The results obtained may be of use in preparative and analytical chemistry.

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LINEAR TITRATION PLOTS WITH ION-SELECTIVE ELECTRODES*

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Summary—A computational method for simultaneous determination of the equivalence volume and the Nernstian slope of an ion-selective electrode is described. Treatment of data from linear titration and standard addition procedures is simplified by the use of equal additions of standard solution. Equivalence volumes and concentrations were determined with errors of 0.5–2% by the proposed method.

Ion-selective electrodes provide a convenient and fast means for the determination of activities of free ions. The analysis may be performed by direct measurement of a potential: the activity or concentration of the ion is then determined by reference to a calibration curve or to the Nernst equation if the standard potential and the slope of the electrode are known. The standard addition method is frequently used when a background effect must be eliminated, but when accurate results are required, titrations should be used. The use of titrations is restricted, however, by the shortage of suitable reagents. However, many metal ions do form stable complexes with several ligands, so titrimetric analysis is possible in the concentration range 10^{-1}–$10^{-4}M$. The inflexion point of the titration curve is usually taken as the equivalence point, but the use of linear titration plots (so-called Gran plots) has increased considerably during the last fifteen years.[1,2] Such plots have also been used in procedures involving ion-selective electrodes. The commonly used standard subtraction method is actually a modification of the linear-titration-plot method.

Calculation of the points which give the linear titration plot requires the use of the Nernst equation. The value of the standard potential of the cell has no effect on the result, so an arbitrary value may be used to give convenient numerical values. However, the Nernstian slope of the electrode must be known quite precisely, and this is a major drawback of the method. Usually the points are calculated on the basis of the theoretical Nernstian slope or with a value determined previously. The necessity for use of a correct value for the slope is often overlooked, despite the comments of several authors, in connection with the standard addition technique.[3–6]

Electrodes seldom show the theoretical Nernstian slope, and observed values change with time and use. This study aimed to devise a method in which the influence of the slope of the ion-selective electrode could be taken into account when linear titration plots were used.

THEORY

Consider a substance M titrated with L according to the reaction:

$$M + L \rightarrow ML. \quad (1)$$

The concentration of M is measured with an electrode sensitive to M:

$$E = \underbrace{E_0 + E_j - E_{ref} + S\log f_M}_{E_0'} + S\log[M] \quad (2)$$

where E is the potential of the cell; E_0 is the standard potential of the electrode; E_j is the liquid junction potential; E_{ref} is the potential of the reference electrode; S is the Nernstian slope of the electrode and f_M is the activity coefficient of the ion M. When the temperature and ionic strength are kept constant, E_j, S and f_M' are constant and E_0' is then the practical standard potential of the cell. If the equilibrium constant of reaction (1) is large enough for the reaction to be regarded as complete, the following equation can be derived:[7]

$$V_e - V = \frac{V_0 + V}{C_L} 10^{(E - E_0')/S} \quad (3)$$

where V_e is the equivalence volume; V_0 is the initial volume of solution; V is the volume of titrant added and C_L is its concentration.

Equation (3) is valid for a cation: for anions the signs of E and E_0' are negative. Because there are constant terms in equation (3), it can be written in the form

$$V_e - V = k(V_0 + V)10^{E/S} \quad (4)$$

where k is a constant. Plotting $(V_0 + V)10^{E/S}$ as a function of V results in a straight line intercepting the V-axis at the point V_e, provided E_0' and S do not change during the titration. The slope of the straight line, its linearity and intercept with the V-axis depend

* This paper was given in part at Euroanalysis III, Dublin, 1978.

on the value of S used in the calculations. When a value of S not valid for the particular electrode is used, a spurious value for the equivalence volume may be obtained. This is illustrated in Figs. 1(*a*) and (*b*) with artificial data. Potentials are first calculated from equation (3) with $E_0' = 200$ mV, $V_0 = 100$ ml, $V_e = 10.00$ ml, $C_L = 0.1M$ and in (*a*) $S = 29.58$ mV and in (*b*) $S = 59.16$ mV. Different values of S are then assumed and the lines calculated according to equation (4). The results are given in detail in Table 1.

As can be seen in Figs 1(*a*) and (*b*) and in Table 1 the value of V_e is critically dependent on the value taken for S. This is more evident with an electrode responding to bivalent ions than to univalent ions. A change of 2% in S will result in a change of about 1% in V_e for bivalent ions and about 0.5% for univalent ions. Also, if an incorrect value is used for S the plot will not be linear. This cannot be seen in Fig. 1, however, because of the small scale used. The displacement of V_e, the intercept, with different values of S, depends also on the E_0'-value of the cell.

The effect of the value of S on V_e can be dealt with by determining both simultaneously. Equation (4) is valid at every titration point:

$$V_1, E_1: \quad V_e - V_1 = k(V_0 + V_1)10^{E_1/S} \tag{5}$$

$$V_2, E_2: \quad V_e - V_2 = k(V_0 + V_2)10^{E_2/S} \tag{6}$$

$$V_3, E_3: \quad V_e - V_3 = k(V_0 + V_3)10^{E_3/S}. \tag{7}$$

Subtraction of equation (6) from equation (5) gives:

$$V_2 - V_1 = k[(V_0 + V_1)10^{E_1/S} - (V_0 + V_2)10^{E_2/S}] \tag{8}$$

and (6)–(7) gives:

$$V_3 - V_2 = k[(V_0 + V_2)10^{E_2/S} - (V_0 + V_3)10^{E_3/S}]. \tag{9}$$

Division of equation (8) by equation (9) gives:

$$\frac{V_2 - V_1}{V_3 - V_2} = \frac{(V_0 + V_1)10^{E_1 S} - (V_0 + V_2)10^{E_2/S}}{(V_0 + V_2)10^{E_2/S} - (V_0 + V_3)10^{E_3/S}}. \tag{10}$$

If the titration is performed with equal additions of titrant, *i.e.*, $V_2 - V_1 = V_3 - V_2$, equation (10) can be reduced to:

$$(V_0 + V_1)10^{E_1 S} - 2(V_0 + V_2)10^{E_2/S} + (V_0 + V_3)10^{E_3/S} = 0 \tag{11}$$

Table 1. The equivalence volume, V_e, determined by the linear plot method [equation (4)] with different values of S: the theoretical value of V_e is 10.00 ml

Bivalent ion			Univalent ion		
S, mV	V_e, ml	Error, %	S, mV	V_e, ml	Error, %
26	10.44	+4.4	56	10.18	+1.8
28	10.18	+1.8	58	10.06	+0.6
30	9.96	−0.4	60	9.96	−0.4
32	9.76	−2.4	62	9.86	−1.4

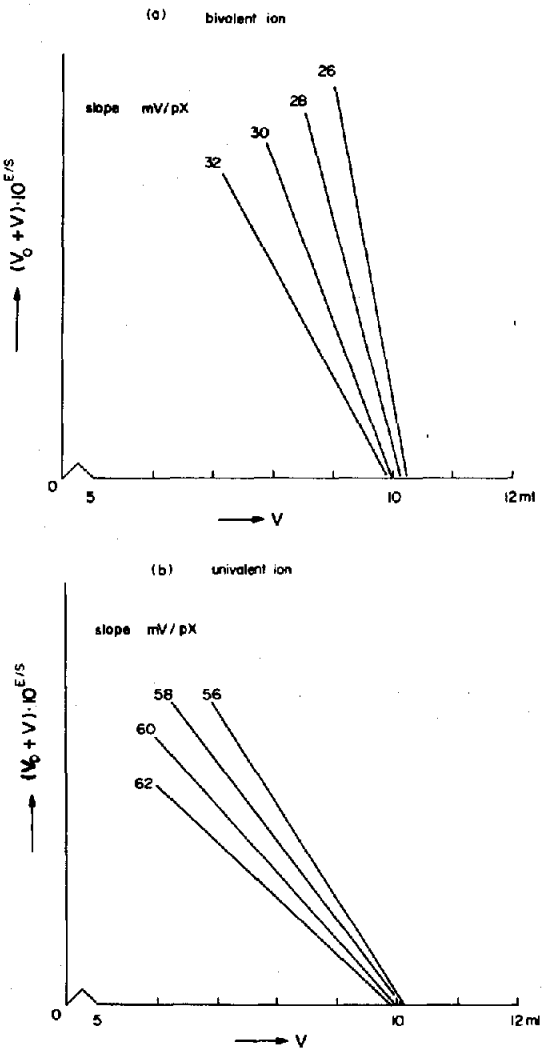

Fig. 1. Effect of the value of S on the linear titration plots obtained by use of equation (4). (*a*) bivalent ion, (*b*) univalent ion.

or, expressed in a general form when more than three points are used, as is common in titrations:

$$(V_0 + V_1)10^{E_1/S} - 2[(V_0 + V_2)10^{E_2/S} - (V_0 + V_3)10^{E_3 S} + \ldots\ldots - \ldots + (V_0 + V_{n-1})10^{E_{n-1} S}] + (V_0 + V_n)10^{E_n S} = 0 \tag{12}$$

where positive and negative terms alternate in the quantity in the square brackets, and n is the number of titration points and should be an odd number. Equation (12) can be solved numerically by giving different values to S: the correct value satisfies the equation. For anions the sign of E in equation (12) should be negative. The minimum value of n is 3 but it is advisable to use more points because otherwise the experimental error in E_2 has a large effect on the result because of the factor 2 in equation (12). The linear titration plot can then be obtained by use of

equation (4), and the value of S which satisfies equation (12).

E'_0 can also be determined from the slope of the linear titration plot (SLP):

$$E'_0 = S \log\left(\frac{-SLP}{C_L}\right) \qquad (13)$$

The method proposed can be applied to the standard addition procedure, where the result is again critically dependent on the value of S. The left-hand side of equation (4) becomes $(V_e + V)$ and the straight line intercepts the V-axis at the point $(-V_e)$. The value of S is determined simultaneously and if E'_0 is required it can be calculated from equation (13) by changing the sign of SLP.

EXPERIMENTAL

Apparatus

Potentials were measured with an Orion 801 potentiometer with a resolution of ±0.1 mV. An Orion double-junction reference electrode and Metrohm Ag/AgCl and saturated-calomel electrodes were used as reference electrodes. The indicator electrodes were Metrohm glass, fluoride and Ag/S electrodes, copper and lead Selectrodes[8,10] and a chalcocite[9] copper electrode. Coated-wire electrodes were made by electrolysing the salt onto the wire and then polishing it with a soft tissue. All measurements were made at 25 ± 0.1° and the solutions were mixed with a magnetic stirrer.

Reagents

All chemicals were Merck *p.a.* grade. Constant ionic strength was maintained with 0.1 M potassium nitrate. For determination of fluoride the ionic medium was 1 M TISAB.

RESULTS AND DISCUSSION

The advantage of the method proposed in this paper is that S is determined at the same time as V_e, as well as E'_0 if required. The method presupposes that S remains constant over the concentration range covered by the titration—normally one order of magnitude. If the electrode does not show Nernstian response, *i.e.*, it does not follow equation (2), this method gives erroneous results because it is very sensitive to any deviations from equation (2). Also, potentials should be measured to 0.1 mV. It is important that the different volume increments give potential differences of several mV; otherwise errors in measurement of V and especially E have too great an effect on the result. Calculations are most conveniently done by using a programmable calculator.

It has been shown theoretically that in the standard subtraction and addition methods the relative standard deviation due to uncertainty in S decreases when the amount of standard substance added increases.[11] When the ratio of added substance to analyte, in the standard subtraction method, increases to greater than 0.5, the relative standard deviation becomes very

Table 2. Results of analyses

Electrode	Titrations V_e, ml: Calc.	Found	S, mV/pX	E'_0, mV	Reference electrode
		Cu^{2+} with EDTA			
Selectrode[8]	7.26	7.28	31.19	373.7	(Ag/Ag/Cl)
	5.19	5.16	29.79	383.0	(Ag/AgCl)
Chalcolite[9]	5.74	5.75	29.34	227.6	(Orion d.j.)
Cu wire coated with CuS	7.26	7.30	32.84	116.9	(Ag/AgCl)
		Pb^{2+} with EDTA			
Selectrode[10]	5.04	5.07	21.88	75.0	(Hg_2Cl_2/Hg)
		Ag^+ with I^-			
Metrohm Ag/S	12.58	12.59	55.71	543.9	(Orion d.j.)
Ag wire coated with Ag_2S	12.58	12.56	53.24	530.2	(Orion d.j.)
		HCl with NaOH			
Glass electrode (Metrohm)	5.00	5.00	59.28	462.8	(Orion d.j.)
	Standard addition method Concentration, M:				
Fluoride (Metrohm)	2.63×10^{-4}	2.58×10^{-4}	56.19	−269.5	(Orion d.j.)
Copper (Chalcolite)	4.2×10^{-5}	4.4×10^{-5}	30.32	230.2	(Orion d.j.)

low. The same is true here: the points from the last 20% of the titration curve give the most accurate values of V_e. In this region small additions of the standard solution give large changes in E and it is important that the ΔV values are accurate and reproducible. Points close to the equivalence point should not be used because many electrodes do not show Nernstian response here.

In the standard addition method the relative standard deviation due to inaccuracy in S is quite large even when the standard to analyte ratio is 10 or more.[11] This indicates the need for an accurate value of S. Also, the additions of standard should give distinct potential changes as discussed above.

The method has been tested in several titrations and in the standard addition technique, with different ion-selective electrodes and home-made coated wires. The results are summarized in Table 2.

The value for the slope S given in Table 2 is slightly dependent on the points chosen for the evaluation. The slight change in S does not, however, have any great effect on the simultaneous determination of V_e. The values of S given in Table 2 are obtained with one set of points on the titration curve. When other points on the same curve were used, differences of up to 0.5 mV/pX in S could be obtained, but the change in V_e is only of the order of 0.1%. Thus the values of S and E'_0 in Table 2 are practical rather than absolute.

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A COMPLEXOMETRIC METHOD FOR THE DETERMINATION OF SMALL AMOUNTS OF AN ALKALINE-EARTH METAL IN MIXTURE WITH OTHER METALS

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Summary—A discussion of the conditions for the complexometric determination of the minor component in a mixture of two metal ions is presented. It is based on expressions of the Ringbom type for the titration error. These are derived by the use of logarithmic diagrams. The results have been used to devise an analytical procedure for the determination of the alkaline earth metals in the presence of a large excess of nickel or zinc.

In a study of mixed crystal formation in the magnesium–nickel hydroxide system it became necessary to develop a procedure for the determination of small amounts of magnesium in the presence of large and varying amounts of nickel. The procedure is based on a back-titration and displacement titration carried out in sequence. A direct titration did not work well, partly because of difficulties in masking large quantities of nickel and partly on account of trouble with blocked indicators.

Mannema and den Boef[1–3] have presented an exhaustive analysis of the feasibility of various types of titrations. It is based on algebraic expressions for the titration curve. Since their treatment is rather involved, an alternative approach will be presented first as a background to the proposed method.

THEORY

In this section the conditions for the determination of the minor component in a mixture of two metal ions will be examined. The theory will be based on expressions of the type used by Ringbom[4] for the titration error. These expressions will be derived by the use of logarithmic diagrams.[5] The usual notation will be employed. Since all stability constants and concentrations are conditional, unprimed symbols will be used, however.

The two metal ions in the mixture are denoted by M and N and the stability constants are assumed to be in the order $K_{NY} > K_{MY}$. The titration error[4] for the direct titration of the sum is then

$$C_M^{\frac{1}{2}} K_{MY}^{-\frac{1}{2}} (10^{\Delta} - 10^{-\Delta}),$$

where Δ in this case is the difference between log [M] at the equivalence point and at the end-point. The corresponding error in the stepwise titration of N is

$$C_N^{\frac{1}{2}} (1 + K_{MY} C_M)^{\frac{1}{2}} K_{NY}^{-\frac{1}{2}} (10^{\Delta} - 10^{-\Delta}).$$

For a derivation of this expression see Appendix 1. N can often be determined with good accuracy even if C_M is considerably greater than C_N. For example, with $\Delta = 0.3$ and $C_M = 10C_N$, the relative error is $<1\%$ for $K_{NY}/K_{MY} > 10^6$ and $K_{NY}C_N > 10^5$.

In the reverse case, with C_M substantially smaller than C_N, the analysis for M is likely to fail, since the error in the determination of C_N will greatly affect the result for C_M obtained as the difference $(C_M + C_N) - C_N$. If, however, the metals were present as MY and NY in the sample a displacement titration of MY by a third metal, T, would give C_M unaffected by the error in C_N. From these considerations the following analytical procedure suggests itself.

1. $M + N (\gg M) + Y \rightarrow MY + NY + Y$ (slight excess).
2. $MY + NY + Y$ (slight excess) $\xrightarrow[\text{with T}]{\text{Back-titration}} MY + NY + TY.$
3. $MY + NY + (TY) \xrightarrow[\text{with T}]{\text{Displacement titration}} M + NY + TY.$

In order to secure proper conditions pH adjustments may be necessary between the steps.

The accuracy of the determination of C_M will be affected by the amount of Y added in excess and the titration errors in steps 2 and 3. Obviously the excess of Y should be as small as possible. The titration error, F, for the back-titration is derived in Appendix 2. The result is

$$F = (C_M K_{MY}^{-1} + C_N K_{NY}^{-1} + C_T K_{TY}^{-1})^{\frac{1}{2}} (10^{\Delta} - 10^{-\Delta}). \quad (1)$$

The titration error in the back-titration of Y will thus be determined by the metal having the greatest ratio C_X/K_{XY}, (X = M, N or T). It does not matter

whether X is present in the sample originally or added as the titrant. With $\Delta = 0.3$ the relative error in measuring C_Y (excess) will be $< 1\%$ for $K_{XY}C_X > 10^5$ if C_Y (excess) is $\sim C_X$. In the proposed procedure it is, however, the absolute rather than the relative error which is of importance for the accuracy of determination of C_M.

The titration error for the displacement titration in step 3 in the analytical scheme is

$$F = (C_N K_{NY}^{-1} + C_T K_{TY}^{-1})^{\frac{1}{2}} \times (1 + K_{MY}C_M)^{\frac{1}{2}}(10^{\Delta} - 10^{-\Delta}) \quad (2)$$

according to Appendix 3. The error will again be largely determined by the metal with the smallest value of K_{XY}, (X = N, T) whether it is the titrant or not. The relative error will be less than 1% for $\Delta = 0.3$ and $C_X/C_M = 10$ if $K_{XY}/K_{MY} > 10^6$ and $K_{XY}C_M > 10^6$.

With common complexing agents rather few combinations of metals can be found which satisfy the conditions required by equations (1) and (2). They are, however, fulfilled for a large number of metals (N) when M is an alkaline earth metal and Y is EDTA, if step 2 is performed in alkaline medium and step 3 in acid solution.

EXPERIMENTAL

In the tests of the procedure M has generally been magnesium and N has been nickel or zinc. Copper has been used as the titrant. Van der Meer, den Boef and van der Linden[6,7] have successfully used this titrant in displacement and back-titrations, with a copper-selective electrode. Since this electrode must be conditioned for several hours between titrations,[7] the HgY/Hg electrode was chosen instead, despite its non-Nernstian response in alkaline medium.[8]

MgY has the smallest of the stability constants concerned and from equation (1) it is seen that the pH for the back-titration should be chosen so that K_{MgY} attains its largest value. This occurs in the pH range 10–12. As indicated by equation (2) the displacement titration should be carried out in a pH range where K_{MgY} is small and K_{NiY} (or K_{ZnY}) and K_{CuY} are large. This condition is fulfilled around pH = 5 and step 3 was carried out in an acetate buffer at this pH.

The effect of the pH used in the back-titration of Y, on the results of magnesium, has been investigated. The excess of EDTA was about 40% and the results are presented in Table 1. The choice of pH does not appear to be critical. At pH = 10 the potential jump was rather poor and at pH = 12 the results seem to be low. Titrations carried out in the presence of nickel or zinc gave similar results. Table 2 contains data from a series with $C_{Ni}/C_{Mg} \sim 10$. Under the same conditions the errors for barium or strontium were slightly larger than those for magnesium. From the results of these experiments pH = 10.8 was chosen for the back-titration. K_{MgY} has its maximum value at this pH and phenol was used as buffer reagent.

Table 1. Effect of pH in the back-titration of Y on the recovery of magnesium: 54.8 μmole of Mg in a volume of 20 ml in each determination

pH	10.0	10.5	10.8	11.0	11.5	12.0
Absolute error, μmole	−0.1	−0.2	−0.1	−0.2	−0.4	−0.6

Table 2. Effect of pH on the determination of magnesium in the presence of nickel: the sample contained 53.6 μmole of Mg and 512 μmole of Ni in 20 ml; pH_1 and pH_2 refer to the back-titration and displacement titration, respectively

pH_1	pH_2	Absolute error, μmole
10.5	5.0	−0.3
10.8	4.5	−0.2
10.8	5.0	−0.2
11.5	5.0	+0.5
12.0	5.0	−0.4

The results from analyses of samples with varying C_{Ni}/C_{Mg} ratios are shown in Table 3. They indicate that even with ratios of the order 100:1 accurate results can be obtained.

The shape of the titration curves is shown in Fig. 1. In the back-titration a symmetric titration curve is obtained since HgY is stable and the MY–M couple acts as a buffer for Y. In the replacement titration HgY is not stable after the equivalence point and the titration curve is asymmetric. If the volume at the largest potential change is taken as the equivalence volume, an error of about 0.005 ml is introduced (see *Procedure*). For unknown reasons the two potential breaks are considerably smaller than calculated.

The procedure has also been tested on a number of combinations of magnesium with other metal ions. The results will not be reproduced here since they were all predictable from the appearances of the logarithmic diagrams.

Procedure

Make a preliminary determination of the total metal content on an aliquot of the sample. Then to a second aliquot add an almost equivalent amount of EDTA, 2 mmole of phenol, 3 drops of $1 \times 10^{-3}M$ HgY and make up with water to *ca.* 20 ml. Adjust the pH to 10.8 with 1*M* sodium hydroxide. Slowly add EDTA until the HgY electrode indicates a potential jump, and then add a slight excess of the reagent. Back-titrate the excess of EDTA with copper(II) solution until the equivalence point is passed by 0.1 ml. Lower the pH to 5.0 ± 0.2 with acid and 3 ml of 1.0*M* acetate buffer (pH 4.8). Continue the titration with copper until the second potential break is passed by 0.1 ml.

Apparatus

The titration curves were recorded with a Radiometer titration outfit (REA160/TTT60/ABU13/PHM64) in combination with a temperature-controlled titration vessel. The equipment was supplied with a sensing unit in order to avoid overtitration. An amalgamated silver wire was used as the indicator electrode and placed 0.3 cm from the delivery tip of the burette. A fibre-tipped mercury(I) sulphate electrode, K601, was used as reference electrode in the

Table 3. Determination of magnesium in the presence of 512 μmole of nickel

Mg added, μmole	Mg found, μmole	Absolute mean error, μmole	Number of titrations
107.7	107.6	−0.1	3
54.8	54.6	−0.2	2
21.5	21.6	+0.1	2
11.0	11.0	0	2
1.08	1.08	0	2

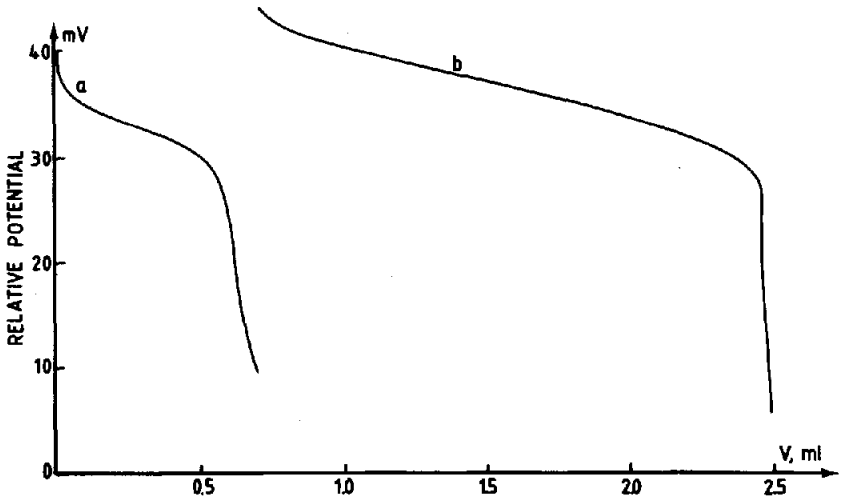

Fig. 1. (*a*) Back-titration and (*b*) displacement titration of 49.1 μmole of Mg in the presence of 565 μmole of Zn with 0.02674*M* $CuSO_4$. Starting volume = 26.7 ml and C_Y (excess) = 16.25 μmole. The 1.840 ml read from the diagram is equivalent to 49.2 μmole of Mg.

determination of magnesium. In the determination of strontium and barium a saturated calomel electrode, K401, was used to avoid interference from leaking sulphate ions.

Reagents

All reagents were "Specpure" or equivalent purity, or were redistilled or recrystallized.

APPENDIX 1

The titration error at the first equivalence point in the stepwise titration of M and N with Y

The titration error, F, in the determination of N is, by definition,

$$F = |C_N - C_Y|. \tag{1.1}$$

In the following, the absolute-magnitude notation will be dropped. By insertion of the expressions for the total concentrations

$$C_N = [N] + [NY]; \quad C_Y = [NY] + [MY] + [Y] \tag{1.2}$$

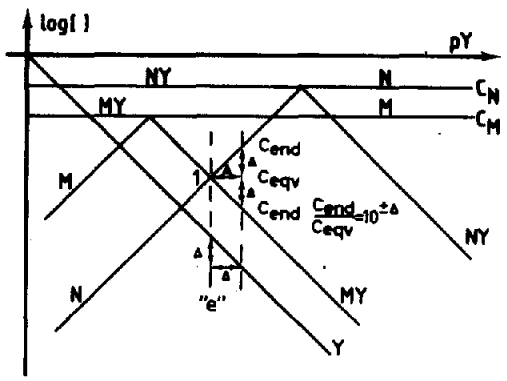

Fig. A1. Logarithmic diagram showing the concentrations at the equivalence point "1" and at the end-point for the titration of N in the presence of M. The distance between the end-point and the equivalence point along the pY-axis is Δ.

equation (1.3) is obtained:

$$F = [N] - [MY] - [Y]. \tag{1.3}$$

At the equivalence point $F = 0$ and thus

$$[N]_e = [MY]_e + [Y]_e. \tag{1.4}$$

This condition is fulfilled at point "1" in the logarithmic diagram (Fig. A1). The difference between pY at the equivalence point and the end-point is denoted by Δ. Then the concentrations at the end-point can be written (see Fig. A1)

$$[N] = [N]_e 10^{\Delta}, \quad [MY] = [MY]_e 10^{-\Delta}, \quad [Y] = [Y]_e 10^{-\Delta}$$

and the error

$$F = [N]_e 10^{\Delta} - ([MY]_e + [Y]_e)10^{-\Delta}.$$

Inserting equation (1.4) yields

$$F = ([MY]_e + [Y]_e)(10^{\Delta} - 10^{-\Delta}) = [N]_e(10^{\Delta} - 10^{-\Delta}) \tag{1.5}$$

This result can be generalized. The titration error will always be of the form

$$F = \Sigma[A_i] - \Sigma[B_j] \tag{1.6}$$

and at the equivalence point ($F = 0$)

$$\Sigma[A_i]_e = \Sigma[B_j]_e. \tag{1.7}$$

In the logarithmic diagram the species A_i are represented by lines with the same slope, either $+1$ or -1. Species B_j are also represented by lines of equal slope but with opposite sign to those representing A_i. Hence from equation (1.6):

$$\begin{aligned} F &= \Sigma[A_i]_e 10^{\Delta} - \Sigma[B_j]_e 10^{-\Delta} \\ &= \Sigma[A_i]_e(10^{\Delta} - 10^{-\Delta}) \\ &= \Sigma[B_j]_e(10^{\Delta} - 10^{-\Delta}). \end{aligned} \tag{1.8}$$

If K_{MY} and K_{NY} are not too close (if this is not the case the titration will fail anyway) then $[M] \sim C_M$ and $[NY] \sim C_N$ and

$$K_{MY} = \frac{[MY]}{C_M[Y]}; \quad K_{NY} = \frac{C_N}{[N][Y]}. \tag{1.9}$$

Equations (1.9), (1.4) and (1.8) yield

$$F = C_N^{\frac{1}{2}} K_{NY}^{-\frac{1}{2}} (1 + K_{MY} C_M)^{\frac{1}{2}} (10^{\Delta} - 10^{-\Delta}). \tag{1.10}$$

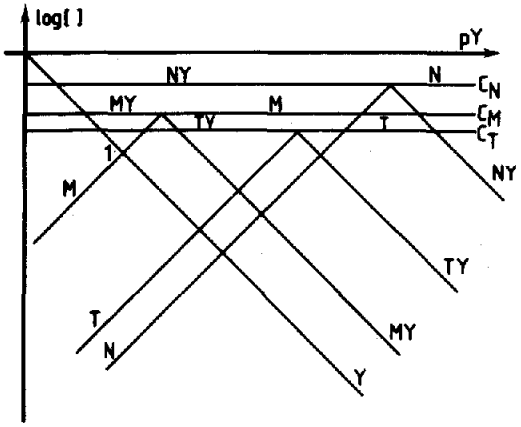

Fig. A2. Logarithmic diagram for the back-titration of the excess of Y with T in the presence of M and N. The equivalence point is situated at the point marked "1".

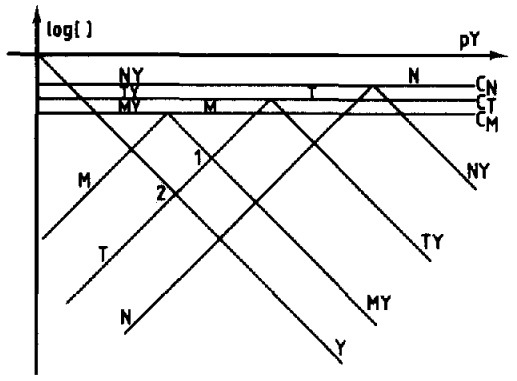

Fig. A3. Logarithmic diagram for the displacement titration of MY by T. The equivalence point is situated between the points marked "1" and "2", depending on the value of K_{MY}.

APPENDIX 2

The titration error in the back-titration of the excess of Y

The titrant is denoted by T. The titration error is

$$F = C_T - C_Y(\text{excess}) = C_T - (C_Y - C_N - C_M). \quad (2.1)$$

On introduction of the expressions for the total concentration, F is found to be

$$F = [T] + [N] + [M] - [Y]. \quad (2.2)$$

The equivalence point ($F = 0$) corresponds to point "1" in Fig. A2.

According to equation (1.8), F can be written

$$F = [Y]_e(10^{\Delta} - 10^{-\Delta}). \quad (2.3)$$

As can be seen from Fig. A2, $C_M \sim [MY]$, $C_N \sim [NY]$ and $C_T \sim [TY]$ at the equivalence point. $[Y]_e$ is found from $[Y]_e = [T]_e + [N]_e + [M]_e$ and the equilibrium expressions $K_{MY} = C_M[M]^{-1}[Y]^{-1}$ *etc.* to be

$$[Y]_e = (C_M K_{MY}^{-\frac{1}{2}} + C_N K_{NY}^{-\frac{1}{2}} + C_T K_{TY}^{-\frac{1}{2}})^{\frac{1}{2}} \quad (2.4)$$

which on insertion into equation (2.3) yields equation (1) in the main text.

APPENDIX 3

The titration error in the displacement titration of M

The titration error in the displacement titration is

$$\begin{aligned} F &= [C_T - C_Y(\text{excess})] - C_M \\ &= C_T - C_Y - C_N - C_M) - C_M \\ &= C_T - C_Y - C_N \end{aligned} \quad (3.1)$$

which upon insertion of the expressions for the total concentrations can be written

$$F = [T] + [N] - [Y] - [MY]. \quad (3.2)$$

Under the conditions depicted in Fig. A3 the equivalence point is at the position marked "1". If K_{MY} is very small it will be situated at point "2" instead. From the diagram it is seen that $[M] \sim C_M$, $[NY] \sim C_N$ and $[TY] \sim C_T$. According to equation (1.8), F can be written

$$\begin{aligned} F &= ([Y]_e + [MY]_e)(10^{\Delta} - 10^{-\Delta}) \\ &= [Y]_e(1 + K_{MY}C_M)(10^{\Delta} - 10^{-\Delta}). \end{aligned} \quad (3.3)$$

$[Y]_e$ is found from the equilibrium expressions and equation (3.2), with $F = 0$, to be

$$[Y]_e = (C_N K_{NY}^{-1} + C_T K_{TY}^{-1})^{\frac{1}{2}}(1 + K_{MY}C_M)^{-\frac{1}{2}} \quad (3.4)$$

Equations (3.3) and (3.4) yield equation (2) in the main text.

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FLOW-INJECTION ANALYSIS OF TRACES OF LEAD AND CADMIUM BY SOLVENT EXTRACTION WITH DITHIZONE

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Summary—An automated solvent extraction method for determination of lead and cadmium down to the 50 ng/ml level has been developed. Optimum conditions for selective and sensitive determination were designed and verified by using the flow-injection scanning method.

The reasons for wanting to control pollution of our environment are well founded and known. Of the variety of species spread as a result of human activities the heavy metals are amongst the most dangerous because their toxicity is beyond dispute, and their non-degradability accounts for their long-lasting effects. Though mercury contamination was the first to attract public concern, the emphasis has recently shifted towards other heavy metals, notably cadmium and lead. The reason is that the sources of mercury pollution are relatively few, well known and therefore easy to control, while lead is being uncontrollably spread over large areas by cars powered by leaded petrol. Also lead and cadmium are still used as components of glazes on household containers and utensils.

Selective determination of traces of cadmium and lead at the ppm level and below is most frequently performed, after suitable preconcentration, by atomic-absorption spectrophotometry. If, however, a large number of samples has to be analysed, the preconcentration becomes too labour- and time-consuming, and also involves a risk of cross-contamination. That is why electrochemical methods, based on the formation of amalgams, are gaining increasing importance, being inherently more sensitive. Of these, anodic stripping voltammetry is a well-known and powerful technique, capable of determination down to the ppM (parts per milliard) level. Most recently, a new and ingenious method, called potentiometric stripping analysis, was developed by Jagner[1,2] and will undoubtedly become very important in trace metal analysis in the immediate future. Thus it might seem that there is not much point in developing yet another method, unless it could yield a significantly higher sampling frequency and a pronounced simplicity of operation. With this in mind, attention was focused on automation of a spectrophotometric procedure, not least because spectrophotometric methods, and instruments, are still the most commonly used in analytical laboratories. The classical dithizone method was selected because it might allow the development of a procedure sensitive enough for analysis of most environmentally relevant materials.

Since its introduction by Fischer[3] more than 50 years ago, dithizone has found, owing to its high sensitivity, a large number of applications in trace analysis for metals. With a number of metals it forms intensely coloured extractable chelates, the stability of which is pH-dependent:

$$M^{n+} + n(HA)_0 \rightleftharpoons (MA_n)_0 + nH^+$$

$$K = \frac{[MA_n]_0 a_H^n}{[M^{n+}][HA]_0^n}$$

where K is the extraction constant, $[M^{n+}]$ is the concentration of the metal ion, $[HA]_0$ that of the reagent in the organic phase, $[MA_n]_0$ the concentration of the extracted metal chelate and a_H the hydrogen-ion activity, all concentrations being those after equilibration through shaking has been completed. Thus, the higher the K value, the lower the concentrations of metal ion that can be recovered, or, the more acidic the aqueous phase can be with the extraction still quantitative. The theory of metal chelate solvent extraction is well described in the book by Starý,[4] which gives extraction constants for many chelate metal systems and details of procedures for selective extraction of a number of metals. Besides the K value, the $pH_{\frac{1}{2}}$ value for a certain reagent concentration and aqueous phase composition may often serve as a useful guide for practical analysis, this being the pH at which 50% of the metal is extracted. Within the last 10 years, however, the solvent extraction of and spectrophotometric determination of heavy metal chelates has been gradually replaced by atomic-absorption spectrophotometry, mainly because of the labour-consuming operations of measuring, shaking and separating the organic and aqueous phases, and the relatively large reagent consumption. However, the advent of automated extraction, based on the principle of flow-injection analysis (FIA), eliminates these drawbacks and

increases the speed of analysis so much that the analytical read-out for an extracted sample is available in less than 30 sec after sample injection.

It was Karlberg *et al.* in Sweden[5,6] and Bergamin *et al.* in Brazil[7] who independently realized the great potentialities of extracting various species automatically by the FIA technique.[8,9] The flow-injection system used in the present work (Fig. 1) is basically the same as that described by Karlberg.[5] The sample to be extracted is injected into an aqueous carrier stream, to which the organic phase is continuously added at *a*; after passage through the extraction coil *b*, made of Teflon tubing (0.8 mm bore), the organic phase is separated at point *c* and carried through the flow-cell for the continuous spectrophotometric measurement. The truly ingenious method of dispersing the organic phase is described in the original work[5] which also contains the constructional details for the separator (*c*). More recently, a continuous filter, furnished with a hydrophobic phase-separation paper, has been used.[10]

It might appear that the development of a method for determination of lead and cadmium would be a straightforward adaptation of the manual procedure to the FIA extraction system. However, all the data in the literature are equilibrium data, obtained by shaking the organic and aqueous phases until equilibrium has been reached. Also, whenever a masking agent is used in a manual method it is added to the sample solution well in advance of extraction, so that the complexation in the aqueous phase has sufficient time to occur. In the FIA system all the reactions have to take place nearly simultaneously, in no less than 15–20 sec. Thus, although the equilibrium extraction data can serve as a basis for selecting the composition of the aqueous phase, the final choice has to be made from experiments performed under exactly the same dynamic conditions as those to be used for the analysis.

Flow-injection scanning is a new technique used for finding the optimum conditions (such as pH, type of masking agent, reagent concentrations) for a given determination performed in the chosen manifold. The system depicted in Fig. 2 shows the original manifold (Fig. 1) supplemented with an autoburette (ABU) and a pH-meter–potentiostat (pH) which controls the pH gradient formed in the gradient vessel (G) so that the pH of the aqueous phase (AQ) is continuously altered. The pH-meter output and the spectrophotometer output are simultaneously recorded by means of an *X–Y* recorder. Thus by repeated injection of a sample of the same composition, a pH profile may be obtained, indicating $pH_{\frac{1}{2}}$, maximum extraction and the influence of metal ion hydrolysis. Such a scanning procedure is performed either in the absence of complexing agents (by starting in a medium of nitric acid and adding strong base), or by using a solution of phosphoric, acetic or citric acid or other complexing agents. In the present work attention was focused on the extraction of lead, cadmium and zinc, because their dithizonates are extracted within approximately the same pH-region and have similar colours.

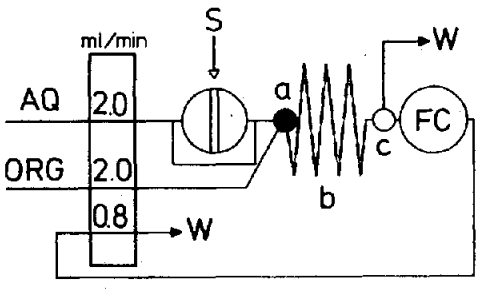

Fig. 1. FIA manifold for the determination of lead and cadmium by solvent extraction with dithizone in CCl_4. Sample volume 100 μl; S, injection port furnished with a by-pass; *b*, mixing coil (length 1 m, bore 0.8 mm); *a* and *c*, mixing and separating elements, respectively; FC, flow-cell; W, waste.

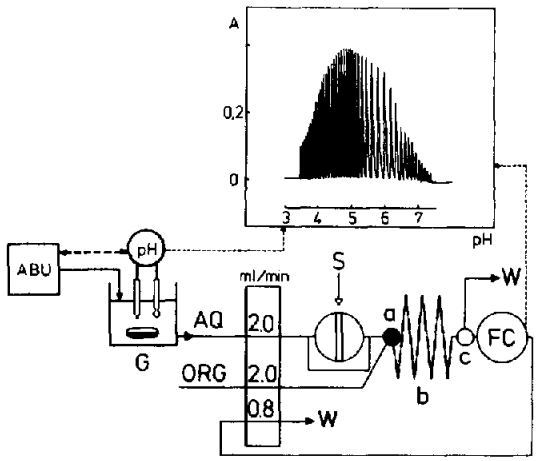

Fig. 2. The FIA scanning method as illustrated by the solvent extraction of lead by $10^{-4}M$ dithizone solution in carbon tetrachloride, with measurement in a spectrophotometric flow cell at 520 nm. The pH in the gradient vessel G, originally containing $10^{-3}M$ HNO_3, is gradually changed by adding 0.1*M* NaOH from an autoburette (ABU). Thus an aqueous carrier stream (AQ) of gradually changing pH (controlled by a potentiostatically-controlled pH-electrode) is pumped to the sampling valve (S) where a 30-μl injection of $10^{-4}M$ metal salt solution is made at regular intervals. After extraction with organic phase (ORG) and phase separation (point c) the absorbance (A) of the extracted metal dithizonate is continuously registered in the flow-cell (FC). The pH measured by the glass electrode and the absorbance measured by the spectrophotometer are simultaneously fed to an *X–Y* recorder, the output of which (in this case for lead) is shown at the top of the figure. It takes less than 25 min to make the 45 injections shown and investigate the pH-range between 3.5 and 7.5.

EXPERIMENTAL

Instruments

The manifold (Fig. 1) was made from plastic toy components (Lego, Billund; Denmark) and Teflon tubing (0.8 mm bore) except for the pump tubes, which were Tygon for

the aqueous solution (AQ) and Technicon rubber tubes for the organic solutions (ORG and W).

The sample volume (100 μl for determination of Cd and Pb and 30 μl for the FIA scanning method) was injected—by means of a precisely machined rotary valve, similar to that described previously[9]—into the aqueous stream, which was propelled by an Ismatec peristaltic pump, Model 13 GJ-4, operated at speed 10.

The aqueous and organic streams were mixed at point *a*, by a modified standard A8 T-connector (part No. 116-0232B, Technicon, U.S.A.).[5] The organic stream was led into the platinum capillary, and the aqueous stream entered through the glass capillary. The stream then passed through the reaction coil (*b*), which was 1 m long. The separating element (*c*) was a DO H-connector (part No. 116-0203-00, Technicon, U.S.A.), into which was inserted a thin band of Whatman phase-separating paper, facilitating the division of the stream.

By differential pumping, all the aqueous phase and excess of organic phase was forced upwards to waste. Only organic phase was carried into the flow-cell (Hellma, Germany, type OS 178.12, volume 18 μl, light-path 10 mm), and measured by a Corning colorimeter, Model 254, connected to a Servograph REC 61 recorder furnished with an REA 110 500-mV unit (Radiometer A/S, Denmark).

The FIA scanning (Fig. 2) was performed by means of a system consisting of an autoburette ABU 13, pH-meter 26, glass electrode G202 B, calomel electrode K401 (all Radiometer A/S, Denmark), a scanning titrator (home-made), and a magnetic stirrer No. 34522 (CENCO, The Netherlands). The pH-meter and spectrophotometer outputs were simultaneously registered by means of an X–Y recorder (Watanabe, Japan).

Reagents

All chemicals were of analytical reagent grade, except the acetic acid, which was laboratory grade.

The organic phase was a solution of dithizone in carbon tetrachloride (25 mg/l.).

The aqueous phase was 0.01M disodium hydrogen phosphate dihydrate (pH = 9) for the determination of cadmium, and 0.01M diammonium hydrogen citrate (pH adjusted to 8.0 with means of 0.1M sodium hydroxide) for the determination of lead.

Sample preparation

Pottery glaze. The glaze was leached with a 1:1 mixture of 0.01M acetic acid and 0.001M nitric acid poured into the specimen to within 1 cm of the top. The leaching was allowed to take place for 90 min at 100°.[11] To avoid interference of zinc and cadmium in the determination of lead, 1 ml of 0.1M potassium ferrocyanide was added per 100 ml of leach solution, after leaching was complete. After addition of 1 ml of 0.1M sodium hydroxide per 100 ml of leach solution, the samples were injected into the FIA system.

Petrol. For the determination of lead, 400 μl of petrol were added to a solution consisting of 0.5 ml of 0.1M iodine and 50 ml of 0.01M potassium bromide, and the mixture was heated to 80° in a water-bath for 2 min to decompose the tetraethyl lead. After cooling, 100 μl of this solution were injected and measured in the FIA system.

Standard solutions were prepared from lead nitrate, lead acetate, cadmium sulphate and zinc acetate, by appropriate weighing and dilution.

DEVELOPMENT OF THE METHODS

The result of a flow-injection scan for lead, zinc and cadmium is summarized in Fig. 3a. The measurements were performed by starting with 0.001M nitric acid, to which 0.1M sodium hydroxide was added in the generator vessel G (Fig. 2), under potentiostatic control. A solution of dithizone in carbon tetrachloride ($10^{-4}M$) was used as the organic phase (ORG) and 30-μl samples of $10^{-4}M$ metal nitrate solution were injected repeatedly by the valve S at approximately 45-sec intervals (owing to the dispersion of the aqueous sample zone between points S and *a*, the dithizone was in 10-fold molar ratio to the metal ion at the point of contact between the organic and aqueous phases). After the extraction, the phases were separated and the absorbance of the organic phase was measured at 520 nm, and the scans were replotted as per cent extraction, as shown in Fig. 3a. It can be seen that in the absence of complexing agent zinc would interfere in the determination of both lead and cadmium. Further, the $pH_{\frac{1}{2}}$ values obtained by FIA scanning are higher than those listed for equilibrium measurements (see Discussion). When the same experiments were repeated by starting with 0.001M phosphoric acid (Fig. 3b) the influence of formation of phosphate complexes could be seen on the extraction curve for zinc, where formation of zinc phosphate

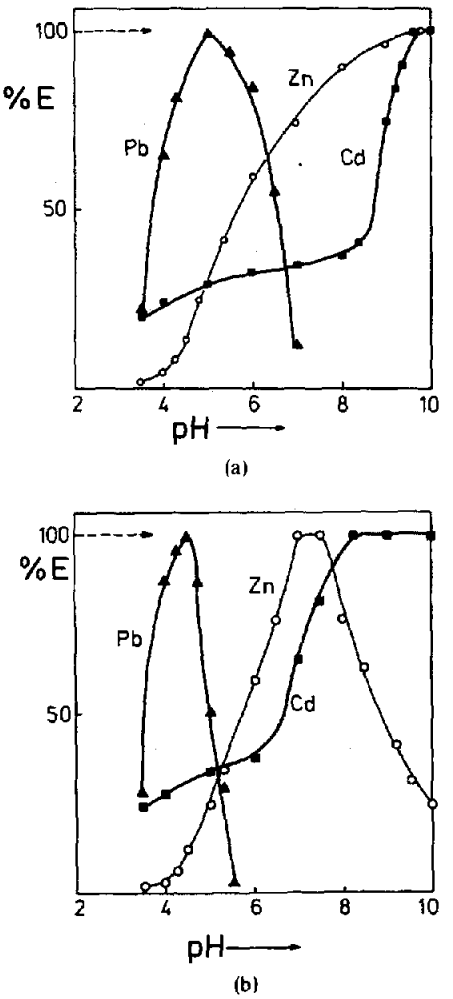

Fig. 3. Results of the flow-injection scans for lead, zinc and cadmium, obtained by starting with (a) $10^{-3}M$ HNO_3; (b) $10^{-3}M$ H_3PO_4. %E denotes per cent extraction. The concentration of each metal species was 10 ppm.

caused decrease of the extraction of zinc dithizonate above pH 8, thus making the extraction of cadmium selective at pH >10. Also, formation of lead phosphate depressed the extraction of lead dithizonate at pH >5, which narrows even more the pH-range in which lead can be extracted (compare Figs. 3a and 3b). Thus at this stage the conditions for selective extraction of cadmium from a phosphate solution at pH 10 had been ascertained, but the determination of lead could not be performed, owing to the interference of zinc and the narrow pH-range for extraction.

For this pH-range to be extended, the lead should be present as a soluble complex of medium stability which would prevent the formation of lead hydroxide, but not the formation and extraction of lead dithizonate. Acetate is suitable for this purpose, but the interference of zinc still remains a problem. Of the various masking agents known,[12,13] none proved effective for suppressing the extraction of zinc, probably because of the non-equilibrium conditions in the FIA system. Even cyanide was not very effective, unless used in high concentrations (>1%), which are not very pleasant to handle. Finally, the use of potassium ferrocyanide, which forms an insoluble zinc compound (log K_{SO} − 15.4)[14] was tried and met with success (Fig. 4). This allowed the use of diammonium hydrogen citrate in the carrier stream for the selective determination of lead, and of disodium hydrogen phosphate for the determination of cadmium.

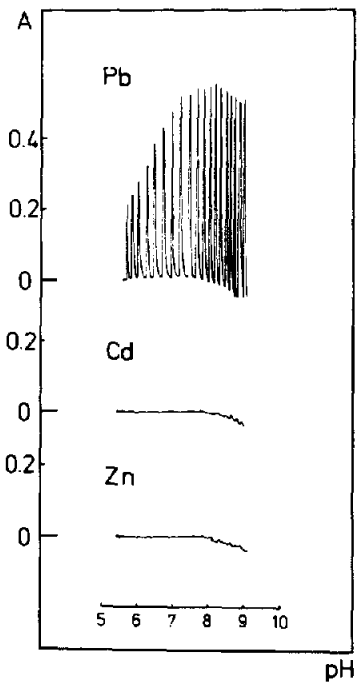

Fig. 4. Result of the flow-injection scans of lead, cadmium and zinc, with $K_4[Fe(CN)_6]$ as masking agent: 1 ml of 0.1*M* $K_4[Fe(CN)_6]$ was added to 100 ml of 10-ppm metal solution. The aqueous phase was 0.01*M* diammonium hydrogen citrate (pH = 5.4), and the organic phase a 25-mg/l. solution of dithizone in carbon tetrachloride. The sample volume was 100 µl.

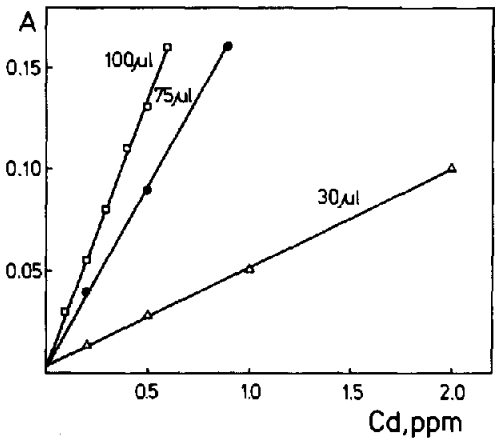

Fig. 5. Influence of sample volume on the signal, in the extraction of cadmium. The aqueous phase was 0.01*M* disodium hydrogen phosphate (pH = 8), the organic phase was that used for Fig. 4.

As one objective of the work was to find conditions for high sensitivity measurements, the influence of sample volume and phase-volume ratio on the signal (peak-height) was studied. The manifold in Fig. 1 was used for this purpose and the extraction of cadmium was used to find the conditions for maximum sensitivity. As expected from the theory of FIA, the peak-height increases with increasing sample volume (Fig. 5) and increasing pumping rate of the aqueous phase (Fig. 6). Both effects are due to the fact that the metal to be measured becomes preconcentrated into a smaller volume of organic phase. A limitation to the degree of preconcentration obtainable in this way is the minimum sampling frequency that is acceptable. A suitable compromise was thought to be 90 samples per hour, which allowed determination of cadmium

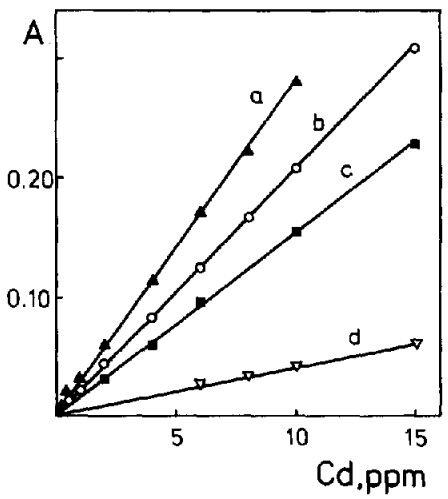

Fig. 6. Influence of the pumping rate of the organic phase on the degree of extraction of cadmium. The organic and aqueous phases were those used for Fig. 5. Injected sample volume 100 µl. The pumping rates of the aqueous phase were: a, 2.3 ml/min; b, 2.0 ml/min; c, 1.5 ml/min; d, 1.0 ml/min, while that of the organic phase was 2.0 ml/min.

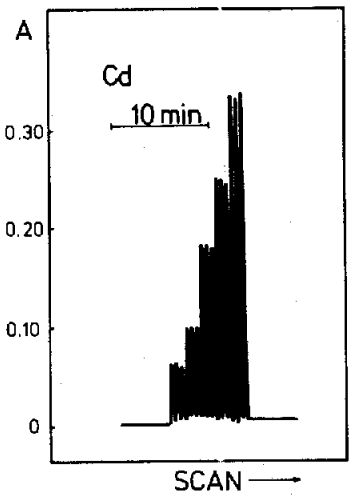

Fig. 7. Recorder output for a series of cadmium standard solutions. All samples (volume 100 μl) were injected in triplicate. The concentrations of Cd are, from left to right: 0.1, 0.2, 0.3, 0.4 and 0.5 ppm.

down to the 100 ppM level (Fig. 7) (sample volume 100 μl, pumping rate 2 ml/min for both phases). Practically the same conditions were found for the determination of lead.

Lead was determined in glaze and petrol to check the applicability of the new method to samples of both very low and comparatively high lead content. The analysis of glaze was based on a standard procedure[11] based on leaching pottery glaze with acetic acid. The leaching procedure was, however, slightly modified by replacing some of the acetic acid with nitric acid. Doing this retained the acidity needed for efficient leaching (pH 3.5) but avoided the use of too high an acetate concentration (1*M*) which would later prevent complete extraction of lead. The solutions obtained by leaching of different samples of pottery for 90 min were injected—after neutralization and addition of ferrocyanide—into the FIA system depicted in Fig. 1. A record of two calibration series (a and c), encompassing a range of 200–1000 ppM of lead, is shown in Fig. 8, together with a series (b) of 11 samples leached from different glaze materials. The results are summarized in Table 1. Only two items (4 and 7) exceeded the maximum acceptable level for lead in glaze.

For determination of lead in petrol, the Schöniger flask was first tried for sample destruction. Owing to the high sensitivity of the present method, it would have been sufficient to burn as little as 50 μl of sample in oxygen and directly inject the absorption solution (identical in composition to that used for leaching the glaze) into the FIA system. However, practically no lead was recovered, probably because the tetraethyl lead evaporated together with the petrol, before the sample—placed on a strip of filter paper—was transferred into the flask and combusted. Therefore, a wet decomposition procedure, developed by Henderson and Snyder,[15] was tried and found successful. It is based on mixing 400 μl of sample with 50 ml of an aqueous solution of iodine and potassium bromide. This mixture mixture can be directly injected into the FIA extraction system (manifold in Fig. 1), precalibrated in the usual manner. Figure 9 shows the record for a series of standards from 0.8 to 4.0 ppm lead and two samples of commercial leaded petrol (standard brand 93 octane and super brand 99 octane), which we found to contain 375 and 403 mg of lead per litre, respectively (according to the manufacturer's information the 93 and 99 octane brands contain on average 370 and 380 mg/l, respectively, with a range of 350–390 mg/l. in both cases). The very fast and simple decomposition procedure is suitable for measurement of the total lead content and also, with modifications, of various organic-lead compounds.[15]

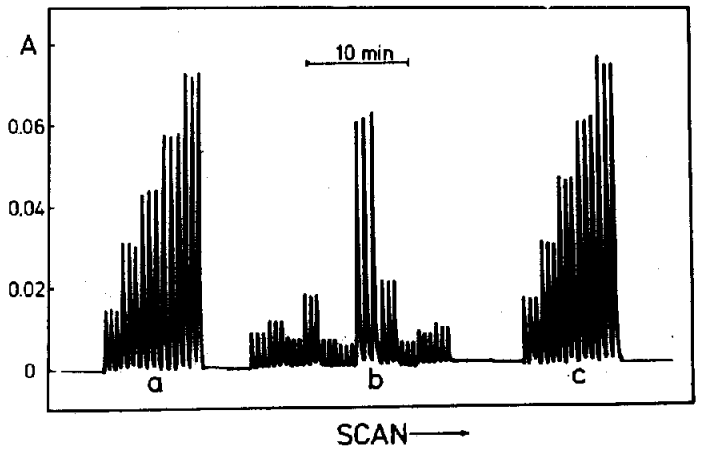

Fig. 8. Determination of lead in pottery glaze samples by FIA solvent extraction with dithizone. From left to right: (a) lead standards, concentration range 0.2–1.0 ppm; (b) samples of pottery, 1–11 (from left to right, cf. Table 1); (c) the same series of lead standards as in (a). All samples were injected in triplicate after addition of masking agent ($K_4[Fe(CN)_6]$). Sample volume 100 μl.

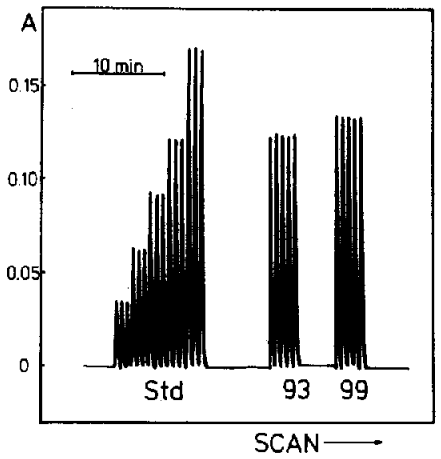

Fig. 9. Determination of lead in petrol by FIA solvent extraction with dithizone. From left to right: a series of standards containing 0.8, 1.6, 2.0, 3.0 and 4.0 ppm Pb; two samples of commercially available leaded petrol (standard brand 93 octane and super brand 99 octane, respectively). All standards were injected in triplicate, the samples were injected in quintuplicate. Sample volume 100 μl.

DISCUSSION

Though this method for lead and cadmium is sufficiently sensitive to allow analysis of most environmentally interesting samples, it may be useful to discuss the ways in which its sensitivity might be further increased. The simplest method would be to increase the gain of the electronic amplifier. Judging from Figs. 7 and 8 this approach would not lead very far, though it would allow doubling the sensitivity without noticeable decrease of the reproducibility because of baseline variations. Next, the optical path of the flow-cell could be doubled to 20 mm. Though this would decrease the sampling frequency only very slightly, to some 80 samples per hour, it would increase the price of the instrumentation, as simple spectrophotometers (such as Corning models 254 or 252) cannot accommodate the thicker flow-through cell. Thus, to analyse samples containing less than, say 50 ppM of lead or cadmium, a preconcentration of the heavy metal would become a necessity. Though this could be done by extraction with dithizone solution trapped in polyurethane foam,[16] followed by Schöniger-flask decomposition of the column material, this approach would have the inherent disadvantages of all preconcentration methods, and therefore a more sensitive method, such as potentiometric stripping analysis[1,2] would be preferable.

The FIA scanning method proved an invaluable practical tool in finding the optimum composition of the carrier stream. Comparison of the $pH_{\frac{1}{2}}$ values (and the K-values computed from them, see Table 2) with the equilibrium data listed in the literature shows the FIA scanning method consistently yields lower values. This is due to two factors. First, under the dynamic conditions used, extraction and complexation equilibria are not necessarily reached. Next, more notably, the pH recorded in the scan does not truly reflect the equilibrium pH, but rather the pH in the generator vessel, which in the absence of buffering agents (such as in the scan in Fig. 3a), is certainly altered by protons released from the dithizone by the metal ion during equilibration in the extraction coil.

Table 1. Determination of lead in pottery glaze samples (ppm of Pb in the leaching solution, and mg of Pb per m^2 of glazed surface)

Sample	Origin (country)	Colour of the glaze	Pb	
			mg/l.	*mg/m²*
1 Mug	England	White	1.02	0.21
2 Bowl	Spain	Yellow-green	1.40	0.22
3 Mug	England	White	0.70	0.16
4 Bowl	Spain	Yellow	2.50	0.48
5 Mug	England	White	0.80	0.14
6 Bowl	Spain	Brown	0.50	0.13
7 Mug	Spain	Yellow	8.50	2.60
8 Ash tray	Denmark	Yellow	3.00	—
9 Mug	England	Brown	0.70	0.14
10 Mug	England	Orange	1.05	0.20
11 Ash tray	Denmark	Blue	1.20	—

Table 2. Extraction of lead with dithizone in carbon tetrachloride: log K values obtained by solvent extraction by FIA and by classical equilibrium procedures

Medium	$pH_{\frac{1}{2}}$ (FIA)	log K (from $pH_{\frac{1}{2}}$)	log K (calc. FIA)	log K (Starý[4])	log K (Irving[17])
Citrate ($10^{-2}M$)	6.1	−4.2	−4.6	0.44	−2.86*, −3.53†

* Citrate medium.
† Citrate + cyanide medium.

Though a flow-through pH electrode could be inserted after the extraction coil, the measurement of pH in the presence of organic phase would be erratic, and use of the more complex instrumental arrangement would not be justified, bearing in mind that the object is to find the optimum composition of the *carrier stream* for performing an actual analysis.

Conclusion

The automated solvent extraction method based on the flow-injection principle allows rapid and simple determination of lead and cadmium down to the 50-ppM level, with use of about 1 ml of organic extractant per analysis, with analytical read-out available 20 sec after sample injection. The instruments used are much cheaper than an atomic-absorption spectrophotometer. The flow-injection scanning method, used for finding the optimum composition of the carrier stream, has been found extremely valuable and it is believed that its use will greatly assist the development of many other types of FIA procedures.

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PRECISION AND LINEARITY OF DETERMINATIONS AT HIGH CONCENTRATIONS IN ATOMIC-ABSORPTION SPECTROMETRY WITH HORIZONTAL ROTATION OF THE BURNER

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Summary—The variation in precision of analytical measurements and linearity of analytical calibration curves resulting from a reduction of flame path-length by rotation of the burner head in flame atomic-absorption spectrometry is described. The precision is found to be approximately equivalent to that with the normal burner slot alignment, and the linear range is found to be improved in some cases. The application of burner rotation to determination of zinc in Standard Reference Material 1648—Urban Particulate Matter, is shown to provide precision and accuracy typical of flame atomic-absorption analysis.

Several approaches are employed for the determination of high concentrations of elements by atomic-absorption spectrometry (AAS). The most straightforward is dilution of the sample to give a concentration in the linear range of the analytical curve. This may be time-consuming, however, and although usually very little precision is lost when accurate volumetric glassware is employed,[1] gross dilution errors and contamination may occur, which may be difficult to track down.

Three methods are available to avoid dilution. First, a less sensitive absorption line may be employed,[2] such as the use of the sodium 330.3-nm line for determination of high sodium concentrations;[3] this is only sensible, however, when the concentration of the sample falls in the linear region of the calibration curve. Furthermore, the intensity of the source emission line corresponding to such a less sensitive transition may be considerably less than that of the line corresponding to the most sensitive absorption line, thus resulting in greater instrumental noise and poorer signal-to-noise ratio (SNR).

Secondly, electronic "curve correction" may be used in conjunction with measurement on the non-linear portion of the analytical calibration curve, but problems occur when the shape of the curve (*i.e.*, the sensitivity) changes because of changes in the source line-width or in the ratio of the intensity of the analyte line to that of non-absorbable or less absorbable lines present within the spectral bandpass. Furthermore, precision is generally worse when "curve correction" is used than when measurement is restricted to the linear portion of the calibration curve, since the sensitivity is decreased on the curved portion and the noise sources that are limiting factors at high concentrations remain relatively constant as the concentration increases.

The third approach is to reduce the effective flame path-length by turning the burner head relative to the optical path, when this can be done without blocking the reference beam. This can result in a sensitivity change of approximately an order of magnitude[1] or more with no loss of linearity. However, a common belief is that analytical precision is reduced by this method, since the turbulent outer edges of the flame form a larger fraction of the cell length, and clogging of the burner slot in the presence of complex matrices would be expected to have a more severe effect than when the burner is aligned normally.

The purpose of this study is to evaluate the variation of analytical precision resulting from rotation of the burner slot. Copper and zinc were used as model elements, with two different atomic absorption spectrometers. As an example of a practical analysis by this method zinc was determined in Standard Reference Material SRM-1648, Urban Particulate Matter and the precision was found to be that typically expected from normal flame AAS.

EXPERIMENTAL

Instrumentation

Measurements were performed on a Varian Techtron AA-175 and a Pye Unicam SP-10 atomic-absorption spectrometer. Both instruments have single-beam optical systems. Direct absorbance read-out was used on the AA-175, and an external integrating digital voltmeter for transmission measurements on the SP-10. The instrumental parameters are listed in Table 1.

Standard preparation

Standards were prepared by serial dilution of 10-mg/ml stock solutions, prepared as described by Dean and Rains.[4]

Preparation and analysis of SRM-1648 (Urban Particulate Matter)

Approximately 200 mg of sample is transferred to a covered Teflon beaker and digested for 1 hr with 10 ml of nitric acid purified by sub-boiling distillation.[5] The sample

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Table 1. Instrumental parameters

	Cu (AA-175)	Cu (SP-10)	Zinc (SP-10)
Wavelength *nm*	324.7	324.7	213.9
Flame	Stoichiometric air–acetylene		
Burner head	10-cm slot burner		
Observation area	1 cm above the burner head		
Burner orientation to optical path	0°, 90°	0°, 45°	0°, 45°
Lamp current, *mA*	4	8	5
Integration time, *sec*	3	1	1
Spectral bandpass (or slit-width)	0.5 nm	(150 μm)	(150 μm)

is cooled, 5 ml of hydrofluoric acid and 10 ml of perchloric acid are added, and the digestion mixture is refluxed for 4 hr. The cover is then removed and the solution is evaporated to dryness. A mixture of 2 ml of nitric acid, 2 ml of hydrochloric acid and 30 ml of demineralized water is added and the solution is heated at low temperature until all solids have dissolved. The solution is then transferred to a 100-ml standard flask and diluted to the mark, and analysed with a stoichiometric air–acetylene flame, the zinc 213.9-nm resonance line being used, and the single-slot burner head at 45° to the optical path. Standard addition is employed to check for chemical interferences (none was found).

Precision measurements

The precision and noise sources were evaluated by methods similar to those described by Bower and Ingle[6] and by Liddell.[7] Sixteen replicate measurements of absorbance (AA-175). or transmission (SP-10) were used to calculate the relative standard deviations of the signals used in construction of precision plots.

RESULTS AND DISCUSSION

There are several published works concerning noise sources and the precision of atomic-absorption measurements, both at very low absorbances and in the optimum range for analysis.[6–19] Bower and Ingle[8] and Liddell[7] have reported that at the detection limit, the dominant noise source for copper determination is source flicker- and shot-noise and for zinc determination is flame transmission flicker. We observed these same source-noise limitations in the case of copper whether the burner was parallel to the optical axis or not. For zinc, transmission flicker was the main noise limitation with the burner parallel to the light-path but with the burner head at 45° to the optical path, flame transmission flicker was no longer a dominant noise source since the flame path-length was considerably decreased; source-related noises then became dominant.

In the optimum analysis range for both elements ($0.2 < A < 1.0$) analyte absorption flicker was the limiting noise source, as reported by Bower and Ingle.[8]

When the AA-175 was used for copper determination, placing the burner slot at 90° to the optical path reduced the response by a factor of 20 (from an absorbance of 0.026 per ppm to 0.0013/ppm) and made the detection limit poorer (raising it from 0.05 ppm to 1.1 ppm; a factor of 22), which is what would be expected if source-related noises limited the analytical precision at low absorbances.

With the SP-10 for copper determination and the burner slot at 45° to the optical path, the response decreased by a factor of 25 (from 0.0263/ppm to 0.00105/ppm) and the detection limit was raised from 0.05 ppm to 1.2 ppm (a factor of 24) results similar to those with the AA-175.

With the SP-10 used for zinc, and the burner slot at 45° to the optical path, the response decreased by a factor of 24 (from 0.117/ppm to 0.0049/ppm) but the detection limit was increased by a factor of only 13 (from 0.03 ppm to 0.4 ppm). This more favourable effect on the raising of the detection limit (compared with that for copper) is a result of minimization of the flame transmission-flicker noise, by the reduction in the absorption path-length of the flame on rotation of the burner slot. Thus, the effect of burner rotation on the range covered by the calibration curve is less for zinc than for copper.

In Fig. 1, analytical calibration curves for copper (taken on the AA-175) are shown. Linearity extends to higher absorbances when the burner slot is at 90° to the optical path than when it is parallel to it. There are several possible explanations for this.

(*i*) If resonance broadening were significant in flames and the source line-width were comparable to the absorption line-width, the absorption line-width would increase at higher concentrations, which would increase linearity since the ratio of absorption line-width to emission line-width would increase. However, resonance interactions have been shown not to be a significant source of broadening in flames.[20]

(*ii*) If the analyte species diffused laterally (*i.e.*, at right angles to the slot) in the flame at high concentrations—with the burner slot parallel to the optical path—into areas of the flame not traversed by the light beam, a curvature of the analytical calibration curve towards the concentration axis would occur. There would not be a significant effect with the burner slot turned at an angle to the optical path. However, lateral diffusion processes are not dependent on analyte concentration and so this effect is unlikely.[21]

(*iii*) The most plausible explanation is the inhomogeneity of the absorption with respect to the focused source radiation.[22] Near the ends of the burner slot

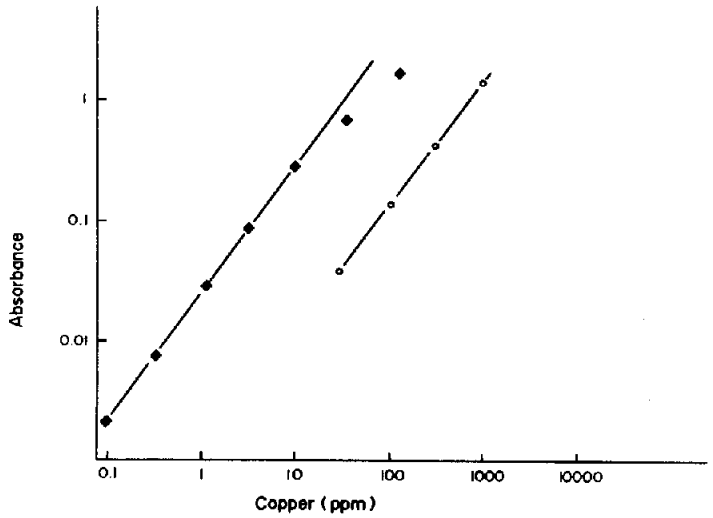

Fig. 1. Analytical calibration curves for copper (measured on the AA-175), illustrating the increase in linear dynamic range on rotation of the burner slot. —■—, burner slot parallel to optical path; —○— burner slot at 90° to optical path.

some source radiation may miss the flame completely, while some analyte atoms may escape the radiation in the flame centre where the beam is focused. Thus the effective absorption by the analyte species varies along the path-length. The result is a deviation from Beer's law analogous to that caused by the presence of another weakly absorbing line in the spectral bandpass. The effect is not as significant with the burner slot turned to the optical path, since the entire source beam passes through the flame. It should be noted that the improvement in linear dynamic range will only occur when the Beer's law deviation induced by inhomogeneous absorption is significant compared to any deviations caused by source line-broadening or less absorbing or non-absorbing radiation falling within the monochromator spectral bandpass.

In Fig. 2, similar precision is found with the burner head parallel or at 90° to the optical axis. However, for both instruments it is apparent that at an absorbance of about 0.1 (*i.e.*, at approximately 10 ppm with the burner slot parallel and 300 ppm with the burner slot at 90° to the axis), the precision is poorer by a factor of about 2 in the latter case, though from the relative sensitivities it would be expected to be the same. This may be caused by an increased flicker in the analyte absorption, caused by the relatively greater variations in path-length when the burner slot is not parallel to the optical axis. Since at higher concentrations the precision with the burner slot turned becomes equivalent to the best precision with the burner slot parallel to the optical path, this flicker effect must decrease with increasing concentration.

In Fig. 3, the effect of rotating the burner head on precision of zinc determinations is shown. The precision curves for both burner orientations appear very similar, the precision being slightly better with the

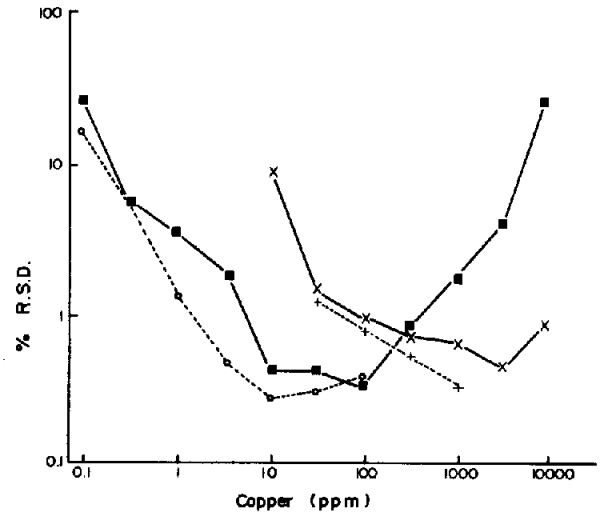

Fig. 2. Precision curves for copper. —■—, SP-10 (burner slot parallel); —×—, SP-10 (burner slot at 45°); —○—. AA-175 (burner slot parallel); —+—, AA-175 (burner slot at 90°).

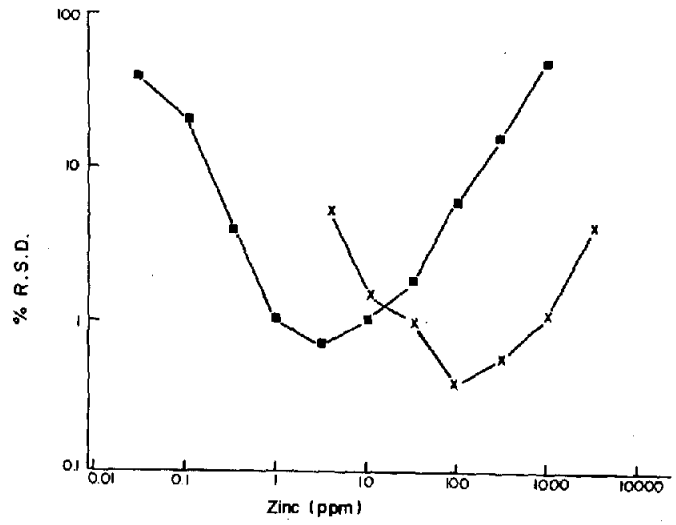

Fig. 3. Precision curves for zinc. —■—, SP-10 (burner slot parallel); — × —, SP-10 (burner slot at 45°).

burner head at 90° to the optical path. The improved precision at lower concentration is due to the minimization of flame transmission flicker as a dominant noise source and the improved precision at high concentration appears to be a result of the reduction in flame background emission, which has been shown to be a dominant noise source at high absorbances in the determination of zinc.[8]

In Table 2, the application of burner-head rotation in the determination of zinc at relatively high concentrations (10 ppm) in SRM-1648 (Urban Particulate Matter) is demonstrated. The analytical precision of 0.7% relative standard deviation is typical of high-precision atomic-absorption analysis.

CONCLUSIONS

Although in some cases burner rotation may cause minor loss of precision, in other cases no loss or even a gain in precision may occur. Depending on the source-radiation path through the flame and the magnitude of the other sources of Beer's law deviations, rotation of the burner slot may increase the linear dynamic range of analysis at the upper end of the analytical curve. If flame transmission flicker noise is a limiting factor, rotating the burner head may also increase the linear concentration range at the lower end of the analytical curve.

Table 2. Determination of zinc in SRM-1648* with burner slot at 45° to optical path

Sample†	Zinc, *mg/g*
1	4.73
2	4.67
3	4.75
4	4.76
5	4.77
6	4.76
7	4.73
Average =	4.74
Standard deviation =	0.03
Relative standard deviation =	0.7%

* Certified value, Zn = 4.76 ± 0.14 mg/g.

† Sample dried at 105° in an air-oven for 16 hr, then stored in a desiccator.

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DETERMINATION OF THALLIUM AND LEAD IN CADMIUM SALTS BY ANODIC STRIPPING VOLTAMMETRY WITH ADDITION OF SURFACTANTS TO SUPPRESS THE CADMIUM PEAKS

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Summary—Conditions have been found which make possible the determination of thallium and/or lead in cadmium and its salts without preliminary separation. The electrochemical activity of the cadmium, which usually interferes in the determination of thallium, is inhibited by the addition of 0.01% of polyethylene glycol of M.W. 4000. Thallium is determined by electrolysis at −0.74 V *vs.* SCE, in 0.1*M* EDTA solution: $10^{-8}M$ thallium can be determined in the presence of 0.1*M* cadmium, while copper and lead at $10^{-2}M$ and $10^{-5}M$ respectively do not interfere. Lead is determined in 0.1*M* acetic acid containing 0.1% cetyltrimethylammonium bromide (CTAB). The addition of CTAB shifts the cadmium peak, as well as the optimum deposition potential for cadmium, to more negative values, making it possible to determine lead in the presence of cadmium as long as the deposition potential lies in the range between −0.50 and −0.56 V *vs.* SCE. Lead can be determined in the presence of ten times as much thallium.

The anodic stripping voltammetric determination of impurities in the presence of a large excess of matrix element is very difficult or even impossible when the matrix element is reduced, or oxidized, at potentials close to the potentials of the impurities. In such cases, prior separation is necessary, which results in poorer precision and accuracy, and prolongs the determination. Anodic stripping voltammetry is therefore not as useful as some other methods for such combinations of trace and matrix elements.

The aim of our investigation was to eliminate the influence of cadmium as the matrix element undergoing reduction and oxidation at potentials close to those of the impurities determined, in this case thallium and lead, by introducing a selective inhibitor which changes the conditions for reduction and oxidation of cadmium.

The influence of surface-active substances on anodic stripping voltammetric determinations has been known almost since the hanging mercury drop electrode came into use.[1] Systematic investigations in this field were undertaken by Kemula and Głodowski.[2] They suggested a possible analytical application of the effect for the determination of cadmium in the presence of indium by addition of gelatin. Neeb and Kiehnast[3] added 3,4-dichlorobenzyltriphenylphosphonium chloride as an inhibitor, in the determination of thallium in the presence of a large amount of bismuth, and Triton X-100 for determination of thallium in the presence of copper, bismuth and antimony. Investigations of the influence of a surface-active substance on anodic stripping determinations were carried out by Batley and Florence[4,5] and recently also by Lukaszewski *et al.*[6,7] However, none of these papers dealt with the determination of impurities in the presence of the matrix. Other papers dealing with the analytical use of inhibition of electrochemical activity have appeared in the fields of d.c. polarography[8–12] and a.c. polarography.[13–15] These papers suggested the possibility of determining ions with relatively low electrode potentials, mainly thallium(I) and lead in the presence of a considerable excess of ions with high electrode potentials, such as copper, bismuth and cadmium. In the majority of cases the supporting electrolyte contained strong complexing agents.

Addition of a surfactant in anodic stripping voltammetry usually results in one of the following effects: (*a*) partial or complete suppression of the peak, (*b*) change in the peak potential, (*c*) change in the dependence of peak-height on the deposition potential. In some cases the peak-height may increase instead of being suppressed.[6] In our work we have tried to make use of these effects for determination of traces of thallium and lead in cadmium.

The stripping peaks for thallium and lead are at potentials close to that of cadmium, the difference being sufficient for the determination of thallium or lead in the presence of a small amount of cadmium. However, if cadmium is the matrix element, and thus present in relatively very large amounts, it is necessary to remove it before the determination. Hence we have attempted to suppress the electrochemical activity of the cadmium selectively by adding a surfactant to the system, making possible the determination of thallium or lead without the separation step.

The determination of thallium in the presence of cadmium was carried out in 0.1*M* EDTA medium with polyethylene glycol of M.W. 4000 as surfactant. The influence of this surfactant on the anodic stripping peak of cadmium has been examined in detail previously.[6,7] This study suggests the possibility of completely suppressing the cadmium peak.

The determination of lead in the presence of cadmium was performed in 0.1*M* aqueous acetic acid with 0.1% cetyltrimethylammonium bromide (CTAB) as surfactant. The choice of surfactant and its concentration was based on experiments carried out in another study.[16]

EXPERIMENTAL

Apparatus

An LP-7 polarograph (Laboratorni Pristroje Co.) with voltage scan-rate of 400 mV/min was used for the thallium determinations, and an OH-102 polarograph (Radelkis) with voltage scan-rate of 250 mV/min for the lead determinations. The controlled-temperature Kemula electrode equipment was manufactured by Radiometer.

Reagents

Polyethylene glycol (PEG) of M.W. 4000 (Carl Roth), analytical grade EDTA, cadmium nitrate, acetate and sulphate, semiconductor grade acetic acid, nitric acid and ammonia, spectral grade cadmium metal and sulphate, and reagent grade CTAB were used.

The water was doubly distilled in a Heraeus quartz still. The standard solutions of thallium(I), lead and cadmium were prepared by dissolving the metals in nitric acid and then diluting as required.

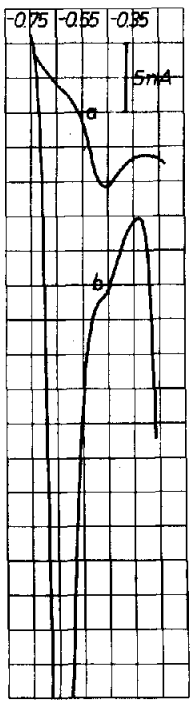

Fig. 1. Peaks for thallium ($1 \times 10^{-8}M$) in the presence of cadmium ($5 \times 10^{-2}M$) in 0.1*M* EDTA (*a*), before and (*b*), after addition of PEG 4000. Deposition potential −0.75 V *vs.* SCE, electrolysis time 10 min.

Table 1. Accuracy and precision of the determination of thallium(I) in the presence of cadmium and PEG 4000

Series	Number of tests	Added Cd, *M*	Added Tl(I), *nM*	Found Tl(I), *nM*	Std. devn., *nM*
I	8	0.1	10	10.0	2.0
II	8	0.1	60	60.5	4.9

Table 2. Thallium content of cadmium salts

Cadmium salt	Grade	Sample wt., *g*	Thallium content, *ppm*
$Cd(NO_3)_2 \cdot 4H_2O$	A.R.	0.772	0.37
$Cd(CH_3COO)_2 \cdot 2H_2O$	A.R.	0.0666	3.85
$CdSO_4 \cdot 8/3H_2O$	A.R.	0.0064	22.4
$CdSO_4 \cdot 8/3H_2O$	Spectral	0.320	0.04

The solutions were deaerated by passage of purified nitrogen, and were brought to 25 ± 1° before measurement.

Determination of thallium in the presence of a large amount of cadmium

The medium used was 0.1*M* EDTA. As EDTA complexes copper, lead and cadmium, the deposition step for these metals must be at a more negative potential than would otherwise be needed to get a satisfactory stripping peak. As the behaviour of thallium is not affected by EDTA, it can still be deposited at the same potential as when EDTA is not present, and in the presence of reasonable amounts of lead, copper and cadmium. However, when very large amounts of these metals are present, as for example when they constitute the sample matrix, they are deposited to some extent along with the thallium, causing an interference, as shown in Fig. 1a for measurements on

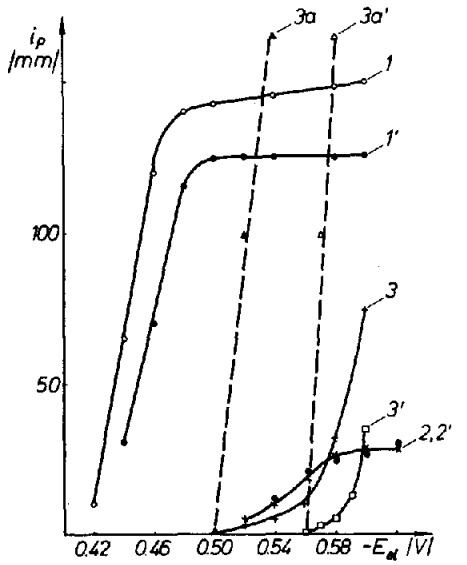

Fig. 2. Dependence of the peak-height on the deposition potential for lead (1, 1'), thallium (2, 2) and cadmium (3, 3', 3a, 3a') measured on 0.1*M* acetic acid. Concentration of Pb and Tl $1 \times 10^{-6}M$, Cd $1 \times 10^{-3}M$. 1, 2, 3, 3*a*—without CTAB; 1', 2', 3', 3*a*'—with 0.1% CTAB.

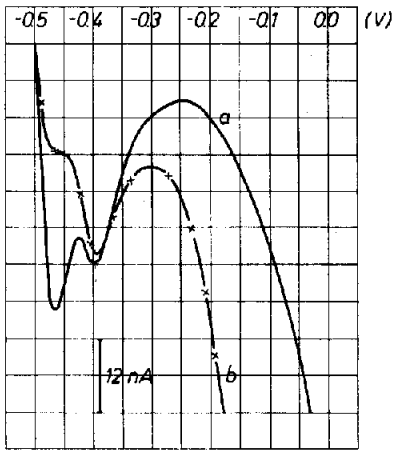

Fig. 3. Peaks for lead ($1 \times 10^{-7}M$) in $1 \times 10^{-3}M$ cadmium solution $0.1M$ acetic acid. Concentration of CTAB: *a*, 0; *b*, 0.1%. Deposition potential −0.52 V *vs.* SCE, electrolysis time 5 min.

$5 \times 10^{-2}M$ cadmium solutions. Lead and copper, at concentrations of $10^{-5}M$ and $10^{-2}M$ respectively, do not interfere with the thallium determination. Addition of 0.01% of PEG, M.W. 4000, suppresses the cadmium peak to such an extent that thallium can be determined in the presence of a very large excess of cadmium (Fig. 1b). The highest permissible concentration of cadmium is $0.1M$ (0.28 g of sample in 25 ml of solution, which represents a 1:1 ratio of cadmium to EDTA. If more than the stoichiometric amount of cadmium is present, the PEG is not capable of suppressing the interfering peak. The addition of PEG does not increase substantially the maximum permissible amounts of lead and copper.

The precision of the determination was investigated for thallium concentrations of $6 \times 10^{-8}M$ and $1 \times 10^{-8}M$ in the presence of $0.1M$ cadmium. Metallic cadmium of spectral grade was dissolved in nitric acid and found to be free from thallium. The concentration of PEG in the final solutions was 0.01%. The deposition was carried out at −0.75 V *vs.* SCE for 10 min. The results, presented in Table 1, suggest that the proposed method is free of any systematic error. When the thallium concentration is $6 \times 10^{-8}M$ the precision is typical for trace anodic stripping voltammetry, but it worsens at lower concentrations ($1 \times 10^{-8}M$).

Thallium was determined in a range of cadmium salts by the proposed procedure. Smaller samples were taken for samples showing relatively high contamination. The results are shown in Table 2.

Procedure. Weigh out not more than 0.28 g of cadmium and dissolve it in 2–3 ml of concentrated nitric acid, then evaporate the excess of acid. Dissolve the remaining moist salts in a small amount of water and add 10 ml of $0.25M$ EDTA and 0.25 ml of 1% polyethylene glycol (M.W. 4000) solution. Adjust the pH to 4.6–4.8, transfer to a 25-ml standard flask and make up to the mark with water. Transfer 20 ml of the solution into the electrochemical cell and pass purified nitrogen through it for 15 min. Stop the flow of nitrogen, start the stirrer, and electrolyse for 10 min at −0.75 V *vs.* SCE. Switch off the stirrer, and record the stripping voltammogram after a rest period of 1 min. The measurement cycle should be carrier out three times in all, and the results compared with the calibration curve.

A sample of a cadmium salt taken for analysis should not contain more than 0.28 g of cadmium. Dissolve the salt in water as for the "moist salts" mentioned above, and follow the remainder of the procedure.

Determination of lead in the presence of a large amount of cadmium

Up to now, none of the published procedures has allowed selective suppression of the cadmium peak without seriously affecting the conditions for the lead determination. The presence of thallium is an additional complication, because its peak appears near to that of lead. When 0.1% of cetyltrimethylammonium bromide (CTAB) is

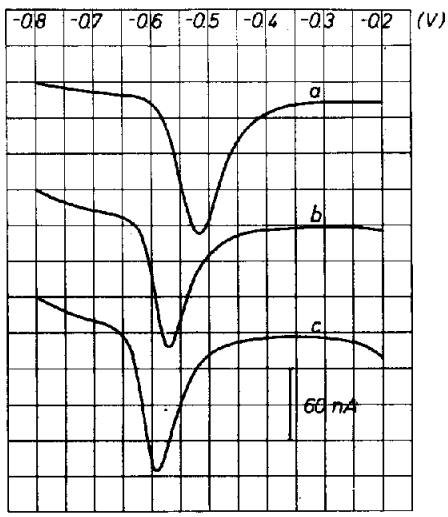

Fig. 4. Peaks for cadmium ($1 \times 10^{-6}M$) in $0.1M$ acetic acid in the presence of CTAB. Concentration of CTAB: *a*, 0; *b*, 0.01%; *c*, 0.1%. Deposition potential −0.8 V *vs.* SCE, deposition time 2 min.

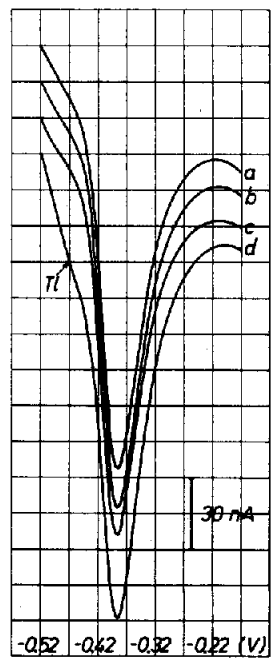

Fig. 5. Peaks of lead ($1 \times 10^{-6}M$) in $0.1M$ acetic acid containing 0.1% CTAB, in the presence of thallium. Concentration of thallium: *a*, 0; *b*, $1 \times 10^{-6}M$; *c*, $1 \times 10^{-5}M$; *d*, $2 \times 10^{-5}M$. Deposition potential −0.52 V *vs.* SCE, deposition time 2 min.

Table 3. Lead content of cadmium salts

Cadmium salt	Sample wt., *mg*	Number of tests	Average concentration of lead, *nM*	Std. dev. *nM*	Lead content, *ppm*
$Cd(CH_3COO)_2 \cdot 2H_2O$	20	3	10.9	1	2.8
	250	7	122	10	2.5
$Cd(NO_3)_2 \cdot 4H_2O$	20	3	15.3	0.6	3.7
	200	7	135	10	3.5

added to a 0.1*M* acetic acid solution containing cadmium, the stripping peak potential and the minimum deposition potential shift to more negative values. In the case of lead these shifts are insignificant, allowing small amounts of lead to be determined in the presence of a large amount of cadmium.

In 0.1*M* acetic acid without CTAB the lead, thallium and cadmium peaks appear at −0.39, −0.45 and −0.52 V *vs.* SCE respectively. The variation of peak-height with deposition potential for these elements is shown in Fig. 2 (curves 1, 2, 3 and 3a). Cadmium was studied at higher concentration than the other elements ($1 \times 10^{-3}M$), *i.e.*, under conditions corresponding to a sample with cadmium as the matrix element. As the measured currents for cadmium were quite large, these measurements were made at a sensitivity 0.03 times that for the other elements. Curve 3a shows the results for cadmium measured at the same apparatus sensitivity as the other elements. According to Fig. 3a, the determination of lead in the presence of a large amount of cadmium would require a deposition potential of -0.49 ± 0.01 V *vs.* SCE.

The addition of 0.1% of CTAB to the solution results in a negative shift of the cadmium deposition potential by more than 50 mV (Fig. 2, curves 3′ and 3*a*′) and the stripping peak itself shifts by a similar amount (Fig. 4). The corresponding shift for lead is about 20 mV, while for thallium there is no change at all (Fig. 2, curves 1′ and 2′). The presence of CTAB thus extends the range of deposition potentials at which lead can be determined selectively in the presence of a large amount of cadmium to about 50 mV, between −0.50 and about −0.56 V *vs.* SCE. The improvement in the lead determination after addition of 0.1% of CTAB can be seen in Fig. 3*b*.

Electrolysis at −0.52 *V vs.* SCE does not prevent the simultaneous deposition of some thallium (Fig. 2). However, for a given concentration, thallium will give a significantly smaller peak than lead will when the deposition potential is −0.52 V, and only if there is more than twenty times as much thallium as lead will it interfere (Fig. 5). The procedure was used to determine lead in two analytical-grade cadmium salts, $Cd(CH_3COO)_2 \cdot 2H_2O$ and $Cd(NO_3)_2 \cdot 4H_2O$. Measurements were made on two considerably different weighed portions of each salt according to the procedure described below. The results are collected in Table 3. The precision of the method is sufficient for this type of determination.

Procedure. Dissolve 250 mg of cadmium salt in a few ml of 0.1*M* acetic acid, transfer to a 25-ml standard flask and make up to the mark with 0.1*M* acetic acid. Transfer 20 ml of the solution into the electrochemical cell and pass purified nitrogen through it for 15 min. Stop the flow of nitrogen, add 2.5 ml of 1% CTAB solution, and electrolyse at −0.52 V *vs.* SCE for 5 or 10 min (depending on the lead content in the sample). Switch off the stirrer, adjust the potential to −0.50 V *vs.* SCE and after a 1-min rest period record the stripping voltammogram from −0.50 V to 0.0 V. The measurement cycle should be carried out three times in all in the same solution but with a new mercury drop each time. The measurements for the calibration curve are made under the same conditions as the determinations.

DISCUSSION

The methods described permit determination of traces of thallium and lead in the presence of cadmium as matrix element without the need for a preliminary separation. Anodic stripping voltammetry is shown to compare well with other methods of trace analysis in terms of speed, cost, precision and accuracy (separation steps always lead to increased errors). It is worth mentioning that not only total suppression of a peak, but also the shift of the peak potential and of the optimum deposition potential in the presence of an inhibitor can be utilized, provided the effects are examined in some detail first.

Thallium can be determined in cadmium as matrix element in solutions containing PEG of M.W. 4000, in the presence of $1 \times 10^{-5}M$ lead, *i.e.*, at concentrations which are not in practice likely to be exceeded with materials of high purity. Lead can be determined in the presence of ten times as much thallium in a separate determination after the addition of CTAB. A larger amount of thallium deforms the lead peak and the determination cannot be performed (Fig. 5). In the majority of cases this performance will be adequate, as the amount of lead is usually greater than that of thallium, but this limitation could be a disadvantage in some cases. The other drawback is the necessity for carrying out separate determinations of thallium and of lead.

Lead can be determined in the presence of cadmium without a surface-active inhibitor being present, but as the range for the optimum deposition potential is only 20 mV the potential can easily fall outside these limits. Addition of CTAB does not complicate the analytical procedure at all, but it does extend the optimum range for the deposition potential to more than 50 mV, thus significantly increasing the probability of remaining within it.

Acknowledgement—The authors are grateful to Professor Wiktor Kemula for helpful discussion.

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THE USE OF APPROXIMATION FORMULAE IN CALCULATIONS OF ACID–BASE EQUILIBRIA—II*

SALTS OF MONO- AND DIPROTIC ACIDS

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Summary—The pH of solutions of salts of mono- and diprotic acids is calculated by use of approximation formulae and the theoretically exact equations. The regions for useful application of the approximation formulae (error <0.02 pH) have been identified. For salts of monoprotic acids, areas are symmetrically equal to those of the acids. For salts of diprotic acids the ranges generally depend on K_2/K_1.

The previous paper compared the solutions of the theoretically exact equations and the approximation formulae for the pH of solutions of mono- and diprotic acids, and identified the regions in which the approximation formulae give the correct pH (within ± 0.02).[1] This paper deals with pure solutions of single salts of mono- and diprotic acids. These salts are produced at the equivalence points in acid–base titrations, so the regions identified in this paper can be used for the calculation of titration curves.

THEORY

Salts of weak monoprotic acids

For the salt NaA of an acid with dissociation constant K_A, with analytical concentration C_S, material balance gives

$$C_S = [Na^+] = [A^-] + [HA] \quad (1)$$

and charge balance gives

$$[H^+] + [Na^+] = [OH^-] + [A^-]. \quad (2)$$

These equations give

$$[H^+] = K_A \frac{[OH^-] - [H^+]}{C_S - [OH^-] + [H^+]} \quad (3)$$

which yields the exact equation[2]

$$[H^+]^3 + (K_A + C_S)[H^+]^2 - K_w[H^+] - K_AK_w = 0. \quad (4)$$

Since the solution will be basic, the term $[H^+]$ on the right of equation (3) can be neglected, to provide the approximate equation

$$C_S[H^+]^2 - K_w[H^+] - K_AK_w = 0 \quad (5)$$

and the solution

$$[H^+] = \frac{K_w + \sqrt{K_w^2 + 4K_AK_wC_S}}{2C_S}. \quad (6)$$

Ignoring the second term in equation (5) provides the simplest approximation formula:

$$[H^+] = \sqrt{\frac{K_AK_w}{C_S}}. \quad (7)$$

Salts of weak monoprotic bases

For the salt BHCl of a base with dissociation constant K_B, material balance gives

$$C_S = [Cl^-] = [BH^+] + [B] \quad (8)$$

and charge balance gives

$$[H^+] + [BH^+] = [OH^-] + [Cl^-]. \quad (9)$$

These equations give

$$[OH^-] = K_B \frac{[H^+] - [OH^-]}{C_S - [H^+] + [OH^-]} \quad (10)$$

which yields the exact equation

$$[OH^-]^3 + (K_B + C_S)[OH^-]^2 - K_w[OH^-] - K_BK_w = 0. \quad (11)$$

Since the solution will be acid, the term $[OH^-]$ on the right of equation (10) can be neglected, to give the approximate equation

$$C_S[OH^-]^2 - K_w[OH^-] - K_BK_w = 0 \quad (12)$$

and the solution

$$[OH^-] = \frac{K_w + \sqrt{K_w^2 + 4K_BK_wC_S}}{2C_S}. \quad (13)$$

Ignoring the second term in equation (12) provides the simplest approximation formula:

$$[OH^-] = \sqrt{\frac{K_BK_w}{C_S}}. \quad (14)$$

Salts of a weak monoprotic acid and a weak monoprotic base

For the salt BHA, material balance gives

$$C_S = [BH^+] + [B] = [A^-] + [HA] \quad (15)$$

* Part I—*Talanta*, 1979, **26**, 605.

and charge balance gives

$$[H^+] + [BH^+] = [OH^-] + [A^-]. \quad (16)$$

These equations give

$$[H^+] + \frac{K_B C_S}{[OH^-] + K_B} = [OH^-] + \frac{K_A C_S}{[H^+] + K_A} \quad (17)$$

which yields the exact equation

$$K_B[H^+]^4 + \{K_B(K_A + C_S) + K_w\}[H^+]^3 + K_w(K_A - K_B)[H^+]^2 - K_w\{K_A(K_B + C_S) + K_w\}[H^+] - K_A K_w^2 = 0. \quad (18)$$

When the solution is acid, the term $[OH^-]$ on the right of equation (17) is ignored, which gives the approximate equation

$$K_B[H^+]^3 + \{K_B(K_A + C_S) + K_w\}[H^+]^2 + K_A K_w[H^+] - K_A K_w C_S = 0. \quad (19)$$

When the solution is basic, ignoring the term $[H^+]$ on the left of equation (17) gives the approximate equation

$$K_A[OH^-]^3 + \{K_A(K_B + C_S) + K_w\}[OH^-]^2 + K_B K_w[OH^-] - K_B K_w C_S = 0. \quad (20)$$

When the second terms of both sides in equation (17) are transposed,

$$\frac{[H^+]^2 + K_A[H^+] - K_A C_S}{[H^+] + K_A} = \frac{[OH^-]^2 + K_B[OH^-] - K_B C_S}{[OH^-] + K_B} \quad (21)$$

and the terms $[H^+]^2$ and $[OH^-]^2$ are neglected, the approximate equation

$$K_B(K_A + C_S)[H^+]^2 + K_w(K_A - K_B)[H^+] - K_A K_w(K_B + C_S) = 0 \quad (22)$$

is obtained, the solution being

$$[H^+] = \frac{-K_w(K_A - K_B) + \sqrt{K_w^2(K_A - K_B)^2 + 4K_A K_B K_w(K_A + C_S)(K_B + C_S)}}{2K_B(K_A + C_S)}. \quad (23)$$

When the terms K_A and K_B are almost equal, omission of the second term in equation (22) yields

$$[H^+] = \sqrt{\frac{K_A K_w(K_B + C_S)}{K_B(K_A + C_S)}}. \quad (24)$$

When K_A and K_B are much smaller than C_S, equation (24) reduces to

$$[H^+] = \sqrt{\frac{K_A K_w}{K_B}}. \quad (25)$$

"Acid" salts of weak diprotic acids

For the salt NaHA of an acid with successive dissociation constants K_1 and K_2, material balance gives

$$C_S = [Na^+] = [H_2A] + [HA^-] + [A^{2-}] = [HA^-]\left\{\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]}\right\} \quad (26)$$

and charge balance gives

$$[H^+] + [Na^+] = [OH^-] + [HA^-] + 2[A^{2-}] = [OH^-] + [HA^-]\left\{1 + \frac{2K_2}{[H^+]}\right\}. \quad (27)$$

These equations give the exact equation[2]

$$[H^+]^4 + (K_1 + C_S)[H^+]^3 + (K_1K_2 - K_w)[H^+]^2 - K_1(K_2C_S + K_w)[H^+] - K_1K_2K_w = 0. \quad (28)$$

When the solution is acid, ignoring the term $[OH^-]$ in equation (27) provides the approximate equation

$$[H^+]^3 + (K_1 + C_S)[H^+]^2 + K_1K_2[H^+] - K_1K_2C_S = 0. \quad (29)$$

When the solution is basic, if the term $[H^+]$ on the left of equation (27) is ignored, the approximate equation

$$C_S[H^+]^3 - K_w[H^+]^2 - K_1(K_2C_S + K_w)[H^+] - K_1K_2K_w = 0 \quad (30)$$

is obtained. Subtraction of equation (26) from (27) gives

$$[H^+] = [OH^-] + [HA^-]\left\{\frac{K_2}{[H^+]} - \frac{[H^+]}{K_1}\right\}$$

which leads to

$$[H^+] = \sqrt{\frac{K_1(K_2[HA^-] + K_w)}{K_1 + [HA^-]}}. \quad (31)$$

Replacement of the term $[HA^-]$ in equation (31) by C_S provides

$$[H^+] = \sqrt{\frac{K_1(K_2C_S + K_w)}{K_1 + C_S}}. \quad (32)$$

When K_1 is smaller than C_S and K_w is neglected, equation (32) reduces to

$$[H^+] = \sqrt{K_1K_2}. \quad (33)$$

Salts of weak diprotic acids

For the salt Na_2A, material balance gives

$$2C_S = [Na^+] = 2\{[H_2A] + [HA^-] + [A^{2-}]\} \quad (34)$$

and charge balance gives

$$[H^+] + 2C_S = [OH^-] + [HA^-] + 2[A^{2-}]. \quad (35)$$

These equations give the exact equation[2]

$$[H^+]^4 + (K_1 + 2C_S)[H^+]^3 + \{K_1(K_2 + C_S) - K_w\}[H^+]^2 - K_1K_w[H^+] - K_1K_2K_w = 0. \quad (36)$$

Since the solution will be basic, the term $[H^+]$ in equation (35) can be ignored, giving the approximate equation

$$2C_S[H^+]^3 + (K_1C_S - K_w)[H^+]^2 - K_1K_w[H^+] - K_1K_2K_w = 0. \quad (37)$$

Omission of the terms $[H_2A]$ in equation (34) and $[H^+]$ in equation (35) yields the approximate expression

$$C_S[H^+]^2 - K_w[H^+] - K_2K_w = 0 \quad (38)$$

and the solution

$$[H^+] = \frac{K_w + \sqrt{K_w^2 + 4K_2K_wC_S}}{2C_S}. \quad (39)$$

Ignoring the second term in equation (38) provides the simplest approximation formula:

$$[H^+] = \sqrt{\frac{K_2K_w}{C_S}}. \quad (40)$$

Addition of the hydroxide-ion contribution from the first dissociation step modifies equations (39) and (40), in terms of $[OH^-]$, to give

$$[OH^-] = \frac{-K_w + \sqrt{K_w^2 + 4K_2K_wC_S}}{2K_2} + n\frac{K_w}{K_1} \quad (41)$$

$$[OH^-] = \sqrt{\frac{K_wC_S}{K_2}} + n\frac{K_w}{K_1} \quad (42)$$

where n is a positive integer.*

Calculations

Calculations were first done by the same method as described in Part I.[1] Then two calculators were used, the first programmed to solve the approximation formula for pH and the other set to see whether a given pH satisfied the exact equation. The pH-value obtained from the first at a certain concentration was put into the second, and if correct would produce a result of zero. If the result was positive, 0.02 was added to the pH value put in, or if it was negative, 0.02 was subtracted from the value, and this procedure was repeated until the sign of the result changed; in this way the correct solution to the exact equation could be obtained within ±0.02 pH unit. The same evaluation was applied for other concentrations and for various values of the constants. Thus the regions in which the approximation formulae give the pH correctly within ±0.02 were delineated correct to one decimal place with respect to both the pK and $-\log C_S$ values.

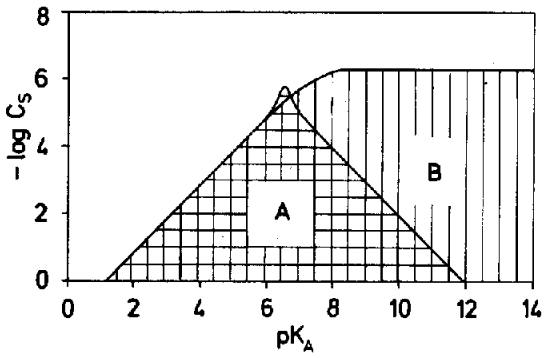

Fig. 1. The range of application of equation (7) (area A, horizontal hatching) and equation (6) (area B, vertical hatching).

RESULTS AND DISCUSSION

Salts of weak monoprotic acids

Figure 1 shows the conditions for which equations (6) and (7) give results differing by ≤0.02 pH unit from those obtained from equation (4). Area (A) applies to the range for use of equation (7) and area (B) to that for equation (6). Area (B) increases with increasing pK_A and the boundary reaches a plateau at $pK_A \geq 8$.

Salts of weak monoprotic bases

The considerations above apply equally to salts of weak monoprotic bases if K_A is replaced by K_B in Fig. 1. Area (A) applies to the range for use of equation (14) and area (B) to that for equation (13), because the term $[H^+]$ in equations (3)–(7) is replaced by $[OH^-]$ in equations (10)–(14), and K_A by K_B.

Salts of a weak monoprotic acid and a weak monoprotic base

Figure 2 shows the range of applicability of equation (25) if $K_B/K_A \leq 10^{-1}$ [to give results within 0.02 pH unit of the value given by equation (18)]. The range depends on K_B/K_A. As this ratio decreases, the range becomes smaller and shifts to the left. If $K_A/K_B \leq 10^{-1}$, Fig. 2 still applies if K_A is replaced by K_B in the abscissa.

* The dissociation and hydrolysis reactions of Na_2A are

$$Na_2A \rightarrow 2Na^+ + A^{2-} \quad (i)$$

$$A^{2-} + H_2O \rightleftharpoons HA^- + OH^- \quad (ii)$$

$$HA^- + H_2O \rightleftharpoons H_2A + OH^-. \quad (iii)$$

The contribution of $[OH^-]$ from reaction (*iii*) is approximately given by $[H_2A]$. The equilibrium equation of reaction (*iii*) can be written as

$$\frac{K_w}{K_1} = \frac{[H_2A][OH^-]}{[HA^-]}. \quad (iv)$$

From reaction (*ii*), $[HA^-] \sim [OH^-]$, so $[H_2A] \sim \frac{K_w}{K_1} \sim$ the $[OH^-]$ contribution from reaction (*iii*). The factor n provides a simple means of assessing the effect of a fairly wide variation in the contribution of this correction term.

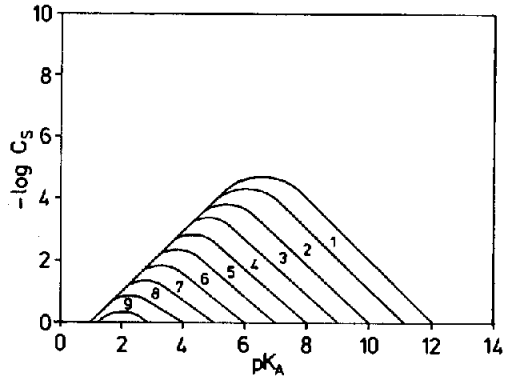

Fig. 2. The range of application of equation (25). Numbers indicate $-\log(K_B/K_A)$. The range covers the area bounded by the curve to the right of the number.

As shown in Fig. 3, the range of application of equation (24) becomes smaller as K_B/K_A decreases. Figure 4 shows that equation (23) gives a similar result.

As shown in Fig. 5, the range of application of equation (19) spreads as K_B/K_A decreases, becoming the same as area (B) in Fig. 1 of Part I[1] when K_B/K_A becomes 10^{-8}. This indicates that the salts effectively behave as monoprotic acids if $K_B/K_A \leqslant 10^{-8}$. These

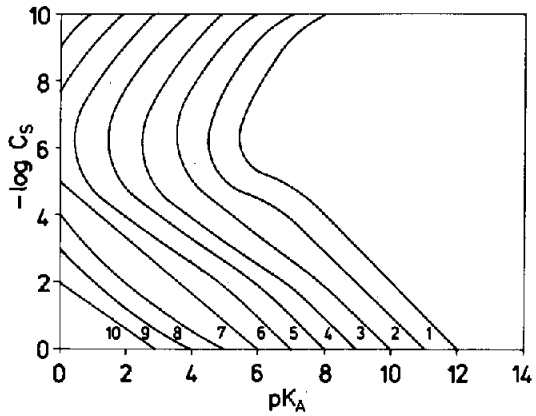

Fig. 3. The range of application of equation (24). Designation as in Fig. 2.

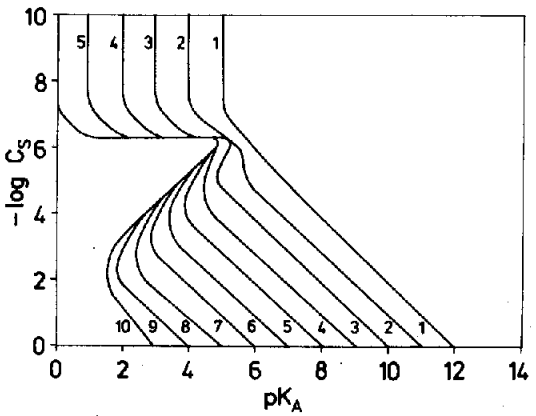

Fig. 4. The range of application of equation (23). Designation as in Fig. 2.

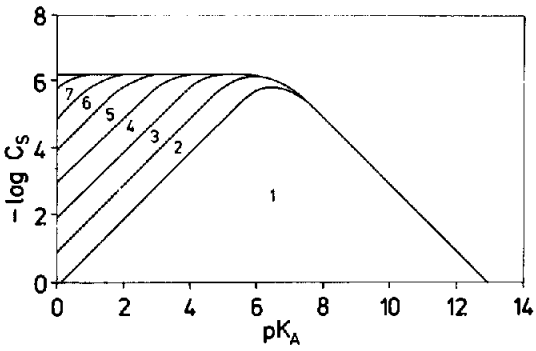

Fig. 5. The range of application of equation (19).

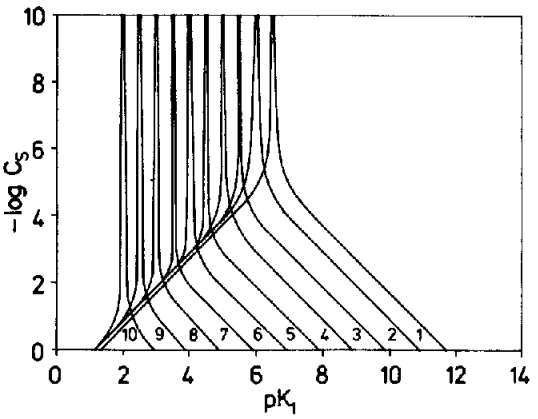

Fig. 6. The range of application of equation (33). Numbers indicate $-\log(K_2/K_1)$. The ranges are the funnel shapes bounded by the line to the right of the number, and the corresponding image.

considerations apply equally to equation (20) if K_A is replaced by K_B in Fig. 5.

"Acid" salts of weak diprotic acids

Figure 6 shows that the range of application of equation (33) becomes smaller and shifts to the left as K_2/K_1 decreases. On the other hand, the range for

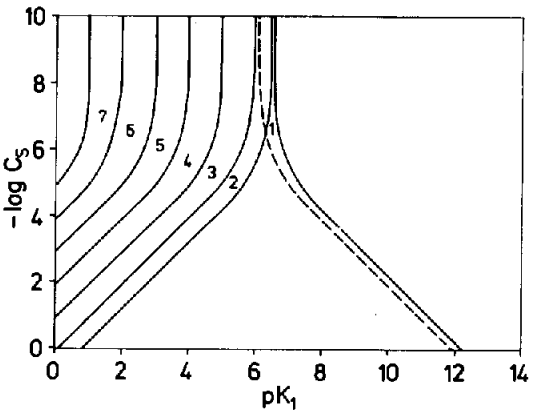

Fig. 7. The range of application of equation (32). The numbers indicate $-\log(K_2/K_1)$. The range for $K_2/K_1 = 0.1$ is given by the funnel shape symmetric with respect to the number 1. For other ratios the range is bounded by the line to the left of the number and by the broken line on the right.

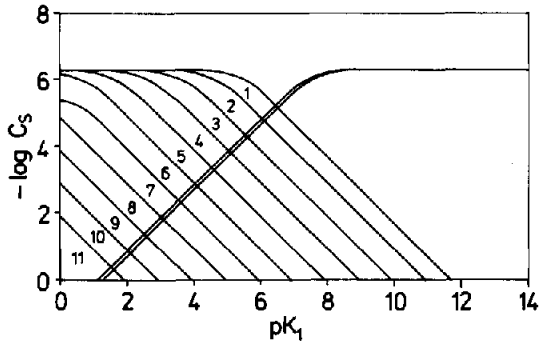

Fig. 8. The range of application of equation (29) lies under the convex lines to the right of the number which corresponds to $-\log(K_2/K_1)$. The range of application of equation (30) lies under the upper of the two right-hand lines for $K_2/K_1 = 0.1$ and under the lower line for other ratios.

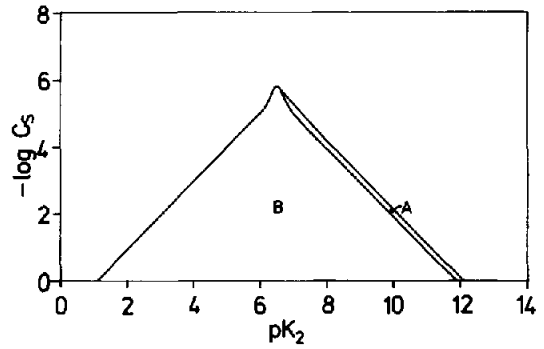

Fig. 9. The range of application of equation (40).

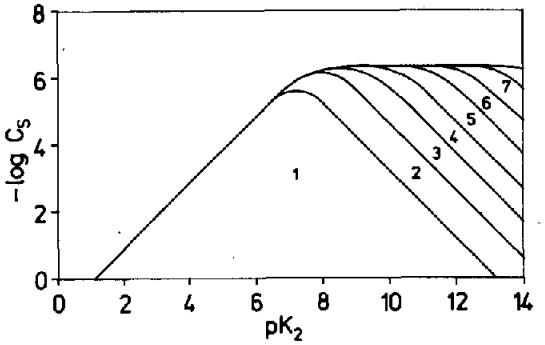

Fig. 10. The range of application of equation (39). The numbers are $-\log(K_2/K_1)$ and the range is bounded by the curve to the right of the number.

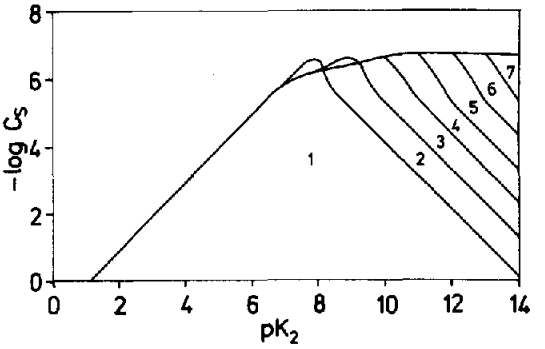

Fig. 11. The range of application of equation (41) when $n = 1$. Designation as for Fig. 10.

use of equation (32) spreads out as K_2/K_1 decreases, as shown in Fig. 7. On the left-hand side of Fig. 8 is shown the range of application of equation (29), which decreases as K_2/K_1 decreases. The range for use of equation (30) is described on the right-hand side of Fig. 8. The range is not affected by K_2/K_1 when the ratio is 10^{-2} or less.

Salts of weak diprotic acids

Figure 9 shows the range of application of equation (40). The range depends on K_2/K_1. When this ratio is 10^{-1} the range covers both areas (A) and (B), but if this ratio is less than 10^{-2} only area (B) applies and is the same as area (A) in Fig. 1, derived from equation (7). As shown in Fig. 10, the range of application of equation (39) spreads out as K_2/K_1 decreases, becoming the same as area (B) in Fig. 1 when K_2/K_1 becomes 10^{-8}. This indicates that salts of diprotic acids effectively behave as those of monoprotic acids if $K_2/K_1 \leqslant 10^{-8}$.

When $n = 1$, the range of application of equation (42) is described by area (B) in Fig. 9, regardless of K_2/K_1. When $n = 2$, the same range as for $n = 1$ applies if $K_2/K_1 \leqslant 10^{-2}$, but the range becomes slightly narrower at the right-hand side of area (B) if $K_2/K_1 = 10^{-1}$. Figure 11 shows that when $n = 1$, equation (41) gives a slightly wider range than equation (39).

When $n = 2$, the range for use of equation (41) is almost the same as the areas in Fig. 10 for a given ratio.

The range of application of equation (37) is not affected by the ratio K_2/K_1 and is the same as the total area delineated in Fig. 10.

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THE USE OF APPROXIMATION FORMULAE IN CALCULATIONS OF ACID–BASE EQUILIBRIA—III*

MIXTURES OF MONO- OR DIPROTIC ACIDS AND THEIR SALTS

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Summary—The pH of mixtures of mono- or diprotic acids and their salts is calculated by use of approximation formulae and the theoretically exact equations. The regions for useful application of the approximation formulae (error <0.02 pH) have been identified. The regions become narrower as the concentrations of the mixtures decrease. In diprotic buffers most ranges may be calculated by the quadratic approximation formulae if K_2/K_1 is less than 10^{-4}.

The previous papers in this series compared the solutions of the theoretically exact equations and the approximation formulae for the pH of solutions of acids[1] and salts,[2] and identified the regions in which the approximation formulae give the pH correctly within ±0.02. This paper deals with mixtures of mono- or diprotic acids and their salts. These mixtures are widely used as buffer solutions. In the earlier papers[1,2] the concentrations of the acids and salts were plotted on a logarithmic scale. However, buffer solutions are made up from approximately equimolar concentrations of an acid and its conjugate base, so here the concentrations are plotted on a decimal scale. For monoprotic acid buffers, mixtures of an acid or base and its salts are treated, whereas three couples are considered for diprotic acid buffers: H_2A–NaHA, H_2A–Na_2A and NaHA–Na_2A. These systems appear during acid–base titrations.

The calculations were done in the same way as in Part II.[2]

THEORY

Mixtures of a weak monoprotic acid and its salt

For a mixture with initial concentration of acid C_A and that of salt C_S, material balance gives

$$C_A + C_S = [HA] + [A^-] \quad (1)$$

and charge balance gives

$$[H^+] + [Na^+] = [H^+] + C_S = [OH^-] + [A^-]. \quad (2)$$

These equations give

$$[H^+] = K_A \frac{C_A - [H^+] + [OH^-]}{C_S + [H^+] - [OH^-]} \quad (3)$$

which yields the exact equation[3]

$$[H^+]^3 + (K_A + C_S)[H^+]^2 - (K_A C_A + K_w)[H^+] - K_A K_w = 0. \quad (4)$$

When the solution is acid, the term $[OH^-]$ in equation (3) is ignored. This provides the approximate solution

$$[H^+] = \frac{-(K_A + C_S) + \sqrt{(K_A + C_S)^2 + 4K_A C_A}}{2}. \quad (5)$$

When the solution is basic, the term $[H^+]$ on the right of equation (3) is ignored. This provides the approximate solution

$$[H^+] = \frac{K_A C_A + K_w + \sqrt{(K_A C_A + K_w)^2 + 4K_A K_w C_S}}{2C_S}. \quad (6)$$

Ignoring both the terms $[H^+]$ and $[OH^-]$ on the right of equation (3) provides the simplest approximation formula, known as the Henderson equation:

$$[H^+] = K_A \frac{C_A}{C_S}. \quad (7)$$

Mixtures of a weak monoprotic base and its salt

For a mixture with initial concentration of base C_B and that of salt C_S, material balance gives

$$C_B + C_S = [B] + [BH^+] \quad (8)$$

and charge balance gives

$$[H^+] + [BH^+] = [OH^-] + [Cl^-] = [OH^-] + C_S. \quad (9)$$

These equations give

$$[OH^-] = K_B \frac{C_B - [OH^-] + [H^+]}{C_S + [OH^-] - [H^+]} \quad (10)$$

* Part II—*Talanta*, 1980, **27**, 187

which yields the exact equation

$$[OH^-]^3 + (K_B + C_S)[OH^-]^2 - (K_B C_B + K_w)[OH^-] - K_B K_w = 0. \quad (11)$$

When the solution is acid, the terms $[OH^-]$ on the right of equation (10) are ignored. This provides the approximate solution

$$[OH^-] = \frac{K_B C_B + K_w + \sqrt{(K_B C_B + K_w)^2 + 4K_B K_w C_S}}{2C_S}. \quad (12)$$

When the solution is basic, the terms $[H^+]$ in equation (10) are ignored. This provides the approximate solution

$$[OH^-] = \frac{-(K_B + C_S) + \sqrt{(K_B + C_S)^2 + 4K_B C_B}}{2}. \quad (13)$$

Ignoring both the terms $[H^+]$ and $[OH^-]$ on the right of equation (10) provides the simplest approximation formula:

$$[OH^-] = K_B \frac{C_B}{C_S}. \quad (14)$$

Mixtures of H_2A and $NaHA$

For the mixture with initial concentration of acid C_A and that of the "acid" salt C_H, material balance gives

$$C_A + C_H = [H_2A] + [HA^-] + [A^{2-}] = [HA^-]\left\{\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]}\right\} \quad (15)$$

and charge balance gives

$$[H^+] + [Na^+] = [H^+] + C_H = [OH^-] + [HA^-] + 2[A^{2-}] = [OH^-] + [HA^-]\left\{1 + \frac{2K_2}{[H^+]}\right\}. \quad (16)$$

These equations give the exact equation[3]

$$[H^+]^4 + (K_1 + C_H)[H^+]^3 + \{K_1(K_2 - C_A) - K_w\}[H^+]^2 - K_1\{K_2(2C_A + C_H) + K_w\}[H^+] - K_1 K_2 K_w = 0. \quad (17)$$

When the solution is acid, the term $[OH^-]$ in equation (16) is ignored. This provides the approximate equation

$$[H^+]^3 + (K_1 + C_H)[H^+]^2 + K_1(K_2 - C_A)[H^+] - K_1 K_2(2C_A + C_H) = 0. \quad (18)$$

Ignoring the terms $[A^{2-}]$ in equations (15) and (16) further provides the approximate expression

$$[H^+] = K_1 \frac{C_A - [H^+]}{C_H + [H^+]} \quad (19)$$

and the solution

$$[H^+] = \frac{-(K_1 + C_H) + \sqrt{(K_1 + C_H)^2 + 4K_1 C_A}}{2}. \quad (20)$$

Addition of the hydrogen-ion contribution from the second dissociation step modifies equation (20) to

$$[H^+] = \frac{-(K_1 + C_H) + \sqrt{(K_1 + C_H)^2 + 4K_1 C_A + nK_2}}{2} \quad (21)$$

where n is a positive integer.

When the solution is basic, the term $[H^+]$ on the left of equation (16) is ignored. This provides the approximate equation

$$C_H[H^+]^3 - (K_1 C_A + K_w)[H^+]^2 - K_1\{K_2(2C_A + C_H) + K_w\}[H^+] - K_1 K_2 K_w = 0. \quad (22)$$

Ignoring the term $[H_2A]$ in equation (15) further provides the approximate expression

$$[H^+] = K_1 \frac{C_A + [OH^-]}{C_H - [OH^-]} \quad (23)$$

and the solution

$$[H^+] = \frac{K_1 C_A + K_w + \sqrt{(K_1 C_A + K_w)^2 + 4K_1 K_w C_H}}{2C_H}. \quad (24)$$

When the term $[H^+]$ on the right of equation (19) or $[OH^-]$ in equation (23) is ignored, both reduce to the simplest approximation formula:

$$[H^+] = K_1 \frac{C_A}{C_H}. \quad (25)$$

Mixtures of H_2A and Na_2A

For the mixture with initial concentration of salt C_S, material balance gives

$$C_A + C_S = [H_2A] + [HA^-] + [A^{2-}] \quad (26)$$

and charge balance gives

$$[H^+] + [Na^+] = [H^+] + 2C_S = [OH^-] + [HA^-] + 2[A^{2-}]. \quad (27)$$

These equations give the exact equation

$$[H^+]^4 + (K_1 + 2C_S)[H^+]^3 + \{K_1(K_2 + C_S - C_A) - K_w\}[H^+]^2 - K_1(2K_2 C_A + K_w)[H^+] - K_1 K_2 K_w = 0. \quad (28)$$

Similar approximations to those described above give the cubic approximate equations, but they are not presented here.

When the solution is acid, ignoring the terms $[OH^-]$ in equation (27) and $[A^{2-}]$ in equations (26)

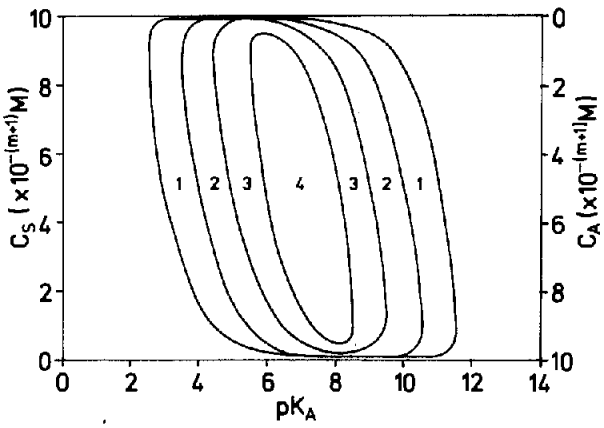

Fig. 1. The range of application of equation (7). Numbers and m indicate $-\log(C_A + C_S)$.

and (27) provides the approximate expression

$$[H^+] = K_1 \frac{C_A - C_S - [H^+]}{2C_S + [H^+]} \quad (29)$$

and the solution

$$[H^+] = \frac{-(K_1 + 2C_S)}{2} + \frac{\sqrt{(K_1 + 2C_S)^2 + 4K_1(C_A - C_S)}}{2}. \quad (30)$$

When the solution is basic, omission of the terms $[H^+]$ in equation (27) and of $[H_2A]$ in equation (26) yields the approximate expression

$$[H^+] = K_2 \frac{2C_A + [OH^-]}{C_S - C_A - [OH^-]} \quad (31)$$

and the solution

$$[H^+] = \frac{2K_2C_A + K_w}{2(C_S - C_A)} + \frac{\sqrt{(2K_2C_A + K_w)^2 + 4K_2K_w(C_S - C_A)}}{2(C_S - C_A)}. \quad (32)$$

Mixtures of NaHA and Na_2A

Material balance gives

$$C_H + C_S = [H_2A] + [HA^-] + [A^{2-}] \quad (33)$$

and charge balance gives

$$[H^+] + [Na^+] = [H^+] + C_H + 2C_S = [OH^-] + [HA^-] + 2[A^{2-}]. \quad (34)$$

These equations give the exact equation[3]

$$[H^+]^4 + (K_1 + C_H - 2C_S)[H^+]^3 + \{K_1(K_2 + C_S) - K_w\}[H^+]^2 - K_1(K_2C_H + K_w)[H^+] - K_1K_2K_w = 0. \quad (35)$$

Since the solution will be basic, the terms $[H_2A]$ in equation (33) and $[H^+]$ in equation (34) are ignored. This provides the approximate expression

$$[H^+] = K_2 \frac{C_H + [OH^-]}{C_S - [OH^-]} \quad (36)$$

and the solution

$$[H^+] = \frac{K_2C_H + K_w + \sqrt{(K_2C_H + K_w)^2 + 4K_2K_wC_S}}{2C_S}. \quad (37)$$

Addition of the hydroxide-ion contribution from the first dissociation step modifies equation (37) to[2]

$$[OH^-] = \frac{-(K_2C_H + K_w)}{2K_2} + \frac{\sqrt{(K_2C_H + K_w)^2 + 4K_2K_wC_S}}{2K_2} + n\frac{K_w}{K_1}. \quad (38)$$

where the last term is derived as described in Part II.[2]

RESULTS AND DISCUSSION

Mixtures of a weak Monoprotic acid and its salt

Figure 1 shows the range of applicability of equation (7) [to give results within 0.02 pH unit of the value given by equation (4)]. The ranges become narrower as the combined concentration $(C_A + C_S)$ decreases.

Figure 2 shows the conditions for which equations (5) and (6) give results differing by $\leqslant$0.02 pH unit from those obtained from equation (4). The range decreases as the combined concentration decreases. When the concentration is 10^{-1}–$10^{-4}M$, the range of application of equation (5) lies to the left of the convex line for a given concentration in Fig. 2, and the range for use of equation (6) lies to the right of the corresponding concave line. Both equations (5) and (6) can be applied in the region between the convex and concave lines for a given concentration. When the total concentration is $10^{-5}M$ and $10^{-6}M$, the ranges are separated as shown in Figs. 3 and 4.

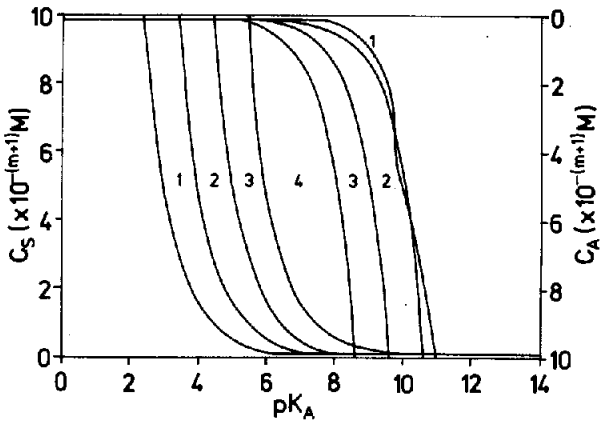

Fig. 2. The range of application of equations (5) and (6) (see text for details).

Mixtures of a weak monoprotic base and its salt

The considerations above apply likewise to these mixtures. The range for use of equation (14) corresponds to that for (7), of (12) to (6), and (13) to (5), in Figs. 1–4, if C_A is replaced by C_B and pK_A by pK_B.

Mixtures of H_2A and $NaHA$

Figure 5 shows the range of application of equation (25) when the combined concentration ($C_A + C_H$) is $10^{-1}M$. The range spreads out as K_2/K_1 decreases, becoming the same as area 1 in Fig. 1 when K_2/K_1

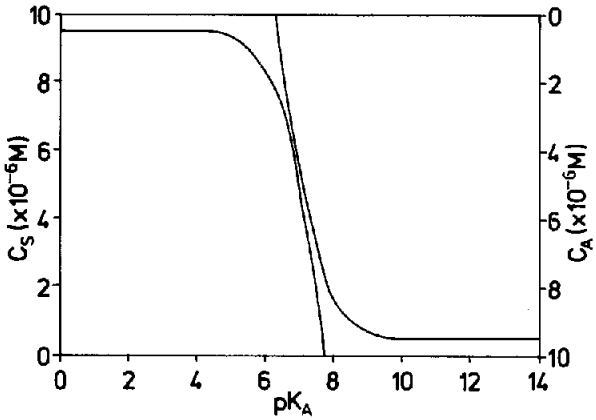

Fig. 3. The range of application of equation (5) on the left, and of equation (6) on the right, when $C_A + C_S = 10^{-5}M$.

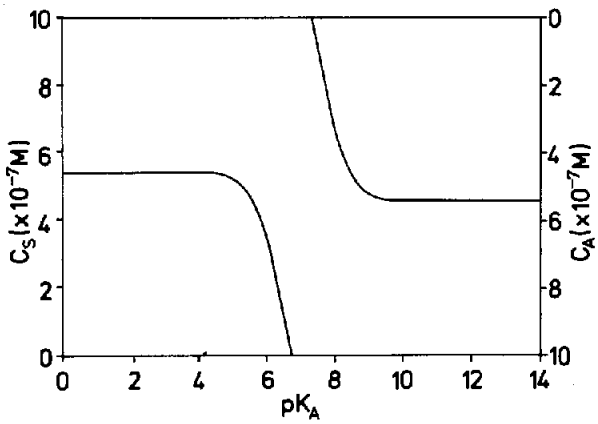

Fig. 4. The range of application of equation (5) on the left, and of equation (6) on the right, when $C_A + C_S = 10^{-6}M$.

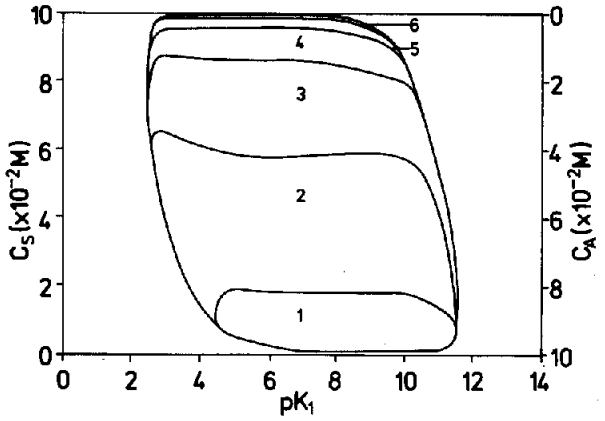

Fig. 5. The range of application of equation (25) when $C_A + C_H = 10^{-1}M$. Numbers indicate $-\log(K_2/K_1)$.

becomes 10^{-6}. When the concentration is less than $10^{-2}M$, the range will become narrower, as can be seen from Fig. 1.

Figure 6 shows the range for use of equations (20) and (21) when the concentration is $10^{-1}M$. The range spreads out as K_2/K_1 decreases. When $n = 1$ and $n = 2$ in equation (21), each area is modified by the addition of nK_2 for a given ratio of K_2/K_1 up to 10^{-3}. When $n = 3$, the range becomes much broader, but gives results more than 0.02 pH in error at some places. When the concentration is $10^{-2}M$ and $10^{-3}M$, the range of application of equations (20) and (21) becomes narrower, as shown in Figs. 7 and 8.

Figure 9 shows the range of application of equation

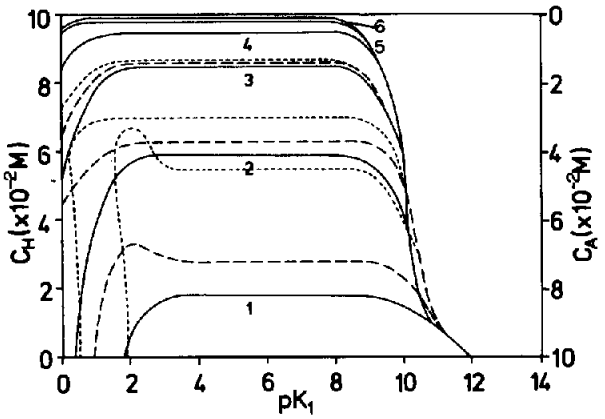

Fig. 6. The range of application of equation (20) (——), and equation (21) with $n = 1$ (– – –) and $n = 2$ (·····) when $C_A + C_H = 10^{-1}M$. Numbers indicate $-\log(K_2/K_1)$.

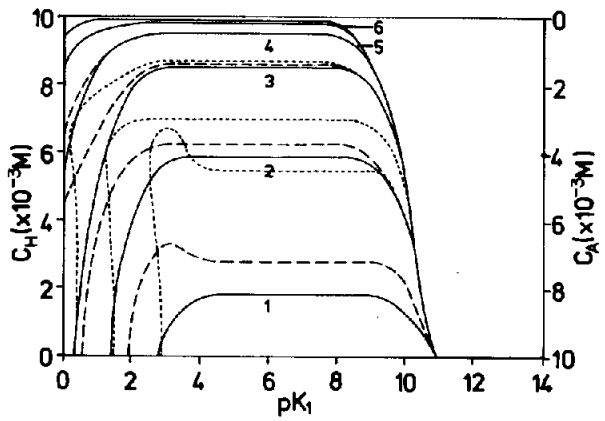

Fig. 7. The range of application of equations (20) and (21) when $C_A + C_H = 10^{-2}M$.

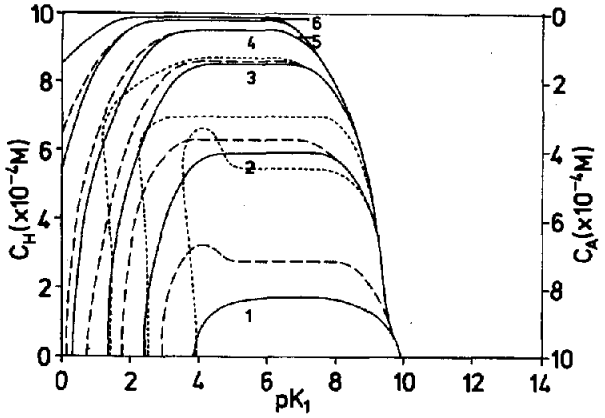

Fig. 8. The range of application of equations (20) and (21) when $C_A + C_H = 10^{-3}M$.

(24) when the concentration is $10^{-1}M$. The range spreads out as K_2/K_1 decreases but equation (24) cannot be used when $C_H = 0$.

As shown in Fig. 10, the range for use of equation (18) is very broad, but iterated computation is required to obtain the result.

Mixtures of H_2A and Na_2A

Hereafter only the ranges for use of the quadratic approximation formulae are identified, because the formulae can be solved directly and the ranges are relatively broad, whereas the ranges for use of the simplest approximation formulae are narrow and the cubic approximation equations cannot be solved directly.

Figure 11 shows the range of application of equations (30) and (32) when the combined concentration ($C_A + C_S$) is $10^{-1}M$. The range for use of equation (30) lies under the convex lines for a given ratio of K_2/K_1 and the range of equation (32) is above the

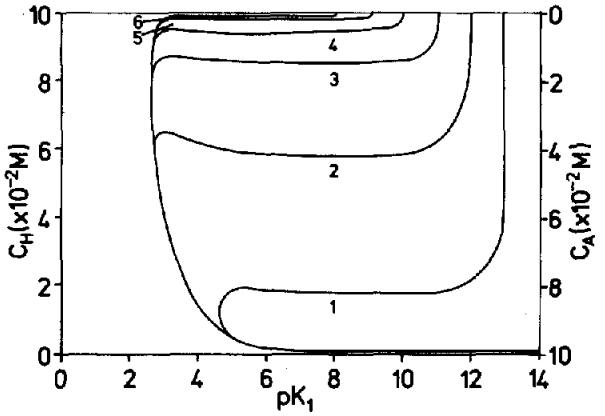

Fig. 9. The range of application of equation (24) when $C_A + C_H = 10^{-1}M$.

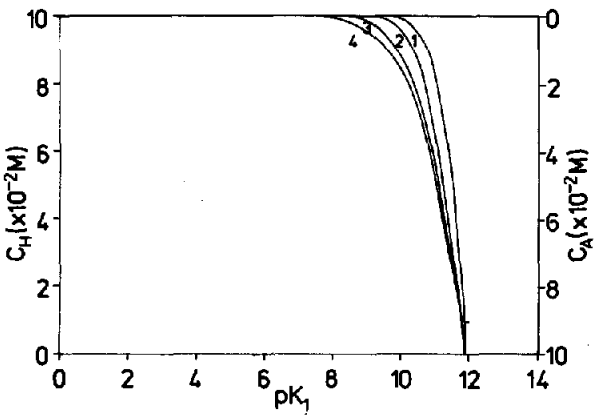

Fig. 10. The range of application of equation (18) lies under the convex lines when $C_A + C_S = 10^{-1}M$. Numbers indicate $-\log(K_2/K_1)$.

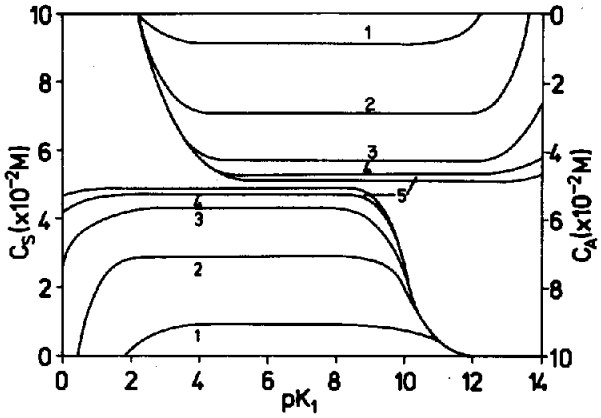

Fig. 11. The range of application of equation (30) lies under the convex lines and that of equation (32) above the concave lines, when $C_A + C_S = 10^{-1}M$.

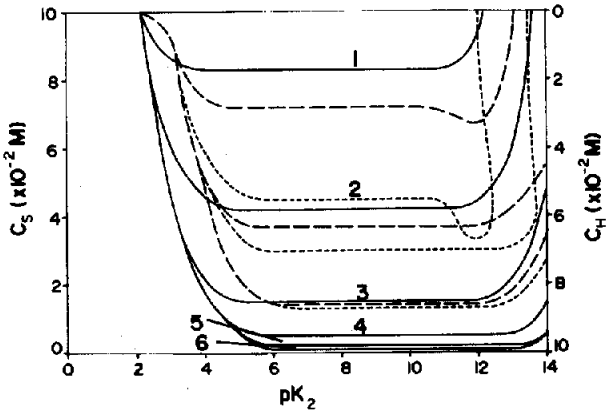

Fig. 12. The range of application of equation (37) (——), and equation (38) for $n = 1$ (– – –), and $n = 2$ (- - - - -) when $C_H + C_S = 10^{-1}M$.

concave lines. Both ranges spread out as K_2/K_1 decreases. When $C_S = C_A$, equations (30) and (32) cannot be solved. When the concentration is less than $10^{-2}M$, the range will become narrower, as can be seen from Figs. 7 and 8.

Mixtures of NaHA and Na_2A

Figure 12 shows the range of application of equations (37) and (38) when the combined concentration ($C_H + C_S$) is $10^{-1}M$. The range spreads out as K_2/K_1 decreases and is modified by nK_w/K_1, with $n = 1$ and $n = 2$ in equation (38), for a given ratio of K_2/K_1 up to 10^{-3}. When the concentration is less than $10^{-2}M$, the range will become narrower and will be shown in Part IV of this series.

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SEPARATION OF INORGANIC BROMIDES BY ADSORPTION GAS CHROMATOGRAPHY

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Summary—Gas chromatography has been used to separate the volatile bromides of Zr, Nb, Mo, Tc, In, Sn, Sb, Bi, Te and I. SiO_2, NaBr, KBr and CsBr have been used as stationary phases and Br_2/N_2 and $Br_2/BBr_3/N_2$ as mobile phases. Temperature-programmed as well as isothermal gas chromatographic separations have been carried out.

Gas chromatography has been used in inorganic trace analysis for preconcentrating or separating species which cannot be measured directly because of matrix effects or mutual interference of trace components.

It is usually necessary in inorganic gas chromatography to form compounds which are sufficiently volatile to be transported in the gas phase. In Table 1 a comparison of the gas chromatography of inorganic and organic compounds is made. For inorganic compounds high temperatures up to 1000° are used (not necessary for chelates; the term "inorganic gas chromatography" does not cover the separation of organometallic compounds). In inorganic gas chromatography chemisorption takes place and reactions with mobile phases have to be taken into account. In many cases transport mechanisms contribute to the separation and therefore the presence of reactive gases in the mobile phase may be necessary, although this may complicate the interpretation of the results. Owing to the high temperatures used and the presence of reactive carrier gases, detectors have to be placed outside the column. It is preferable to use selective detectors and in the present study we have used radioactivity detectors and labelled compounds. This investigation is part of an extensive study of the gas chromatography of inorganic compounds, including chlorides,[1–5] oxides,[6,7] $AlCl_3$-complexes[8] and bromides.[9]

EXPERIMENTAL

The apparatus consisted of a heated quartz column, filled with quartz granules (particle size: 315–630 μm) and has been described elsewhere.[5] The temperature profile in the oven was improved by means of additional heating coils at the column ends and is shown in Fig. 1. The temperature peak at the column end does not greatly influence the retention times but a temperature decrease over a greater length of the column (broken line) would make the retention times appear longer than they really are.

The carrier gas was nitrogen loaded with brominating agents, either bromine or a mixture of bromine and boron tribromide, the partial pressures being regulated by their vapour pressures and the temperature. The volume flow-rate was 5–30 ml/min and the partial pressure of the reactive gases in the carrier gas was 60 mmHg for bromine and 4 mmHg for boron tribromide.

The stationary phase was quartz granules either alone or coated with alkali metal bromide. The packed column was heated at slightly above the melting point of the bromide for about 1 hr before use. The nuclides of the elements investigated were produced by irradiation of 93% enriched uranium-235 with thermal neutrons, or by high-energy proton-induced fission of uranium-238. The radioactivity was measured by γ-spectrometry.

Table 1. 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	Reactions with the analyte and the stationary phase
Detector	Often non-selective	Selective and placed outside the column
Identification	Often retention time	often by spectroscopic data

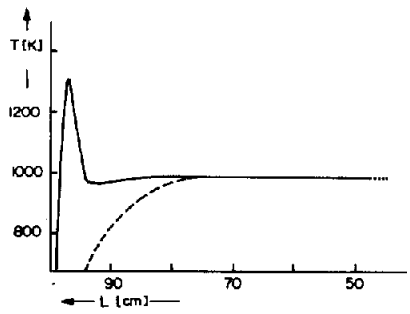

Fig. 1. Temperature profile at the end of the chromatographic oven.

RESULTS AND DISCUSSION

Brominating agents have a strong influence on the gas chromatographic behaviour of the compounds investigated. With nitrogen loaded with bromine as the mobile phase, some elements (zirconium, niobium) could not be eluted from the column. Following a suggestion of Zvara *et al.*[10] we added to the mobile phase a small amount of boron tribromide, and obtained very different results (Fig. 2), there being three main differences: (*i*) the elution of the elements Zr, Nb, Mo and In when boron tribromide is added to the mobile phase, (*ii*) the lower volatility of Tc when BBr_3 is present and (*iii*) the higher volatility of Te when BBr_3 is present.

These effects can be interpreted if the properties of boron tribromide are taken into consideration. Lapport *et al.*[11] have shown that gaseous bromide does not convert certain metal oxides into their bromides, but boron tribromide is reactive enough to do so. This was important in our experiments, where very low concentrations of the elements were used. In this case even small amounts of oxygen-containing contaminants (*e.g.*, the oxide surface of the uranium metal nuclide source or water impurities in the mobile phase) can cause the formation of oxybromides or oxides. Boron tribromide reacts with these compounds, however, favouring the formation of pure bromides. It also removes water, which might be introduced into the system by the inert gas (nitrogen) or released by the column material (quartz), and thus further reaction of the compounds formed can be avoided.

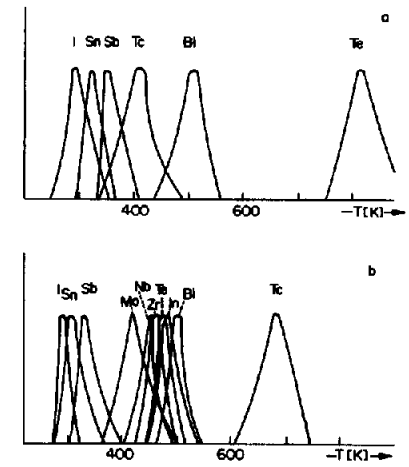

Fig. 2. Temperature-programmed separations on quartz granules. Brominating agents (a) bromine; (b) a mixture of bromine and boron tribromide.

As the method of detection does not provide information on the chemical nature of the compounds eluted, these were identified from literature data. Technetium is the only element that shows higher volatility when bromine is used as halogenating agent. This can be explained as due to the formation of the volatile oxybromide $TcOBr_3$ with Br_2, and of the less volatile $TcBr_4$ when BBr_3 is added to the carrier gas. A list of the compounds present in the gas phase is given in Table 2. The reason for the low volatility of tellurium, zirconium, niobium, molybdenum and indium in the absence of BBr_3 can be attributed to the formation of either oxygen-containing compounds, or compounds with the element in a lower oxidation state (*e.g.*, InBr or $InBr_3$). The use of BBr_3 was advantageous for the volatilization of numerous elements, but the separation was not satisfactory. As a result the stationary phase was changed to quartz granules coated with alkali metal bromide. It was discovered previously,[4,12] that the use of alkali metal chlorides as stationary phases influenced the separations because of the formation of anionic complexes of the general formula:

$$n\mathrm{Me^{I}X} + \mathrm{Me^{m}X_{m}} \rightleftharpoons \mathrm{Me^{I}_{n}Me^{m}X_{n+m}}$$

where Me^{I} represents an alkali metal ion, X a halide ion and Me^{m} a metal ion with charge $m+$.

Similar effects are expected for the experiments done under brominating conditions. Results of temperature-programmed chromatograms with alkali metal bromides as stationary phases are shown in Fig. 3. There is a dependence of the retention temperatures on the size of the alkali metal ion, which reflects the differing stability constants of the complexes. It should be emphasized that the aim of these

Table 2. Separated compounds and their boiling points[14]

Element investigated	Expected compound	b.p., *K*
Zr	$ZrBr_4$	630 subl.
Nb	$NbBr_5$	635
Mo	$MoBr_4$	—
Tc	$TcBr_4$	Unstable
	$TcOBr_3$	Subl.?
In	$InBr_3$	Subl.[15]
	InBr	935 subl.
Sn	$SnBr_4$	478
	$SnBr_2$	893
Sb	$SbBr_3$	288
Bi	$BiBr_3$	726
Te	$TeBr_4$	Decomp. > 553
	$TeOBr_4$	—
I	IBr	389

Table 3. Resolution for different element pairs

Stationary phase	Resolution for element pairs Nb/Zr	Nb/Te	Nb/Mo	Sb/Nb	Te/Zr	Te/Bi
SiO_2	0.58	0.33	0.27	0.81	0.11	0.49
NaBr	0.82	0.58	0.32	0.63	0.39	0.61
KBr	2.66	0.77	2.15	0.39	2.13	0.65
CsBr	—	1.19	—	0.27	—	—

gas chromatographic separations is not the complete separation that is necessary in organic gas chromatography, where the retention time is measured with a non-selective detector. When a selective detector is used, the separation is satisfactory if no interelement interferences occur.

By comparing the gas chromatograms of Figs. 2 and 3 it can be seen that the system $N_2/Br_2/BBr_3$–KBr gives the optimum separation. This is useful when the aim is to isolate all the compounds present. However, it is more interesting to know which system offers the optimum conditions for the separation of one element from a group, or for a separation of two elements from each other. In order

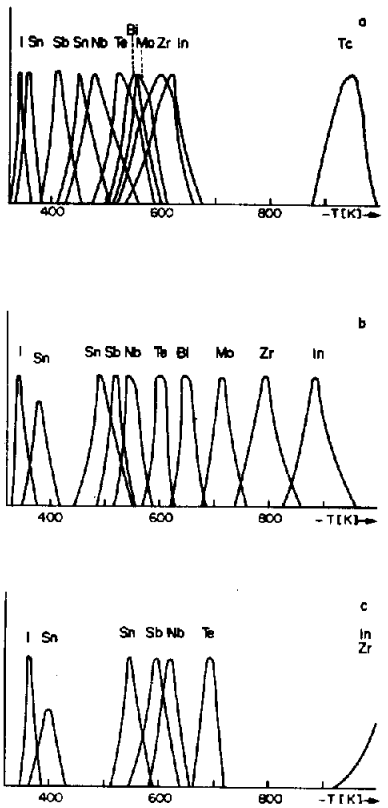

Fig. 3. Temperature-programmed separations with a mixture of bromine and boron tribromide. Stationary phases (a) NaBr; (b) KBr; (c) CsBr.

* Obtained by drawing the tangents to the inflection points on the sides of the peaks, producing them to the base-line, and measuring the distances between the intersections. Strictly speaking, the equation is applicable only to Gaussian peaks.

to make quantitative statements about the separations we calculated the chromatographic resolution of pairs of elements on different stationary phases. Table 3 gives the values of the resolution obtained by temperature-programmed gas chromatography on quartz and various alkali metal bromides. These values are calculated from the equation:

$$R = \frac{2T}{w_1 + w_2}$$

where R is the resolution, T the distance between two maxima, and w_1, w_2 are the peak widths* at the base-line, so that $R = 1.5$ corresponds to complete separation of the elements. In most cases the resolution improves with change in stationary phase in the sequence $SiO_2 < NaBr < KBr < CsBr$. However, this is not true for antimony and niobium. The increase in thermal stability of the adsorbed state is higher for antimony than for niobium, so the chromatographic peaks are not so well separated. A separation can be optimized by looking for a pair of elements with the highest retention value, $r_{2,1}$, defined as the ratio of the retention times:

$$r_{2,1} = t_{r_2}/t_{r_1}$$
$$= \exp[(\Delta S_2 - \Delta S_1)/R - (\Delta H_2 - \Delta H_1)/RT].$$

The retention values for $NbBr_5$ and $TeBr_4$ are plotted as a function of the temperature and the stationary phase in Fig. 4. The retention value is higher at lower temperatures, but at low temperatures the peaks become broader because of the longer separation time. The optimum separation is obtained on CsBr at a temperature of 400°.

Temperature-programming is advantageous when species of very different volatilities are to be separated. For the separation of compounds of comparable

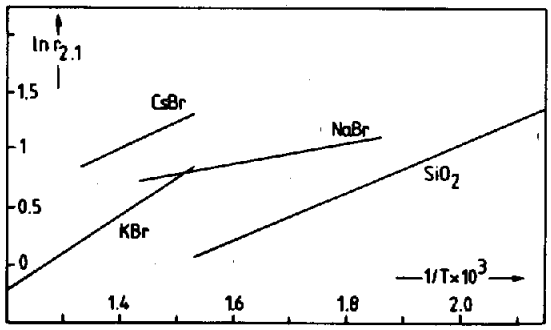

Fig. 4. Retention values for $NbBr_5/TeBr_4$ on different stationary phases as a function of the inverse of the temperature.

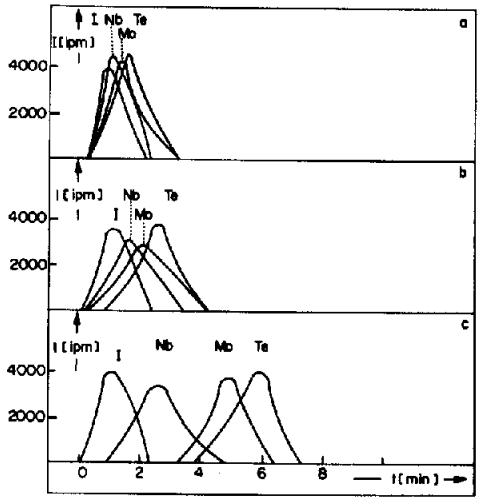

Fig. 5. Isothermal gas chromatographic separations on quartz with Br_2/BBr_3 as brominating agents in the mobile phase. (a) at 583 K; (b) at 528 K; (c) at 478 K.

volatility it is more convenient to use isothermal gas chromatography. If an appropriate temperature is chosen, the resolution obtained in isothermal experiments is better than that achieved by temperature-programming.

Figure 5 shows the chromatograms obtained by using quartz as the stationary phase and nitrogen, bromine and boron tribromide mixtures as the mobile phase, at different temperatures. The resolution achieved by temperature-programming for the compounds of Te and Nb on quartz was $R < 0.5$. The resolution obtained in the isothermal experiment at 478 K (*i.e.*, a temperature between those corresponding to appearance of the two peaks in the temperature-programmed separation) was $R = 1.0$, which means practically complete separation of the components (about 2% cross-contamination). An advantage of the isothermal method is speed, because the cooling time is shorter.

A comparison of these results with those of earlier experiments under chlorinating conditions reveals only small differences in behaviour between chlorides and bromides. Bromides are generally more volatile than chlorides, but an additional factor is the presence of boron tribromide in the mobile phase. Boron tribromide may form anionic complexes similar to the chloride complexes of the type $Me^{I}BCl_4$.[13]

Although the bromide complexes should have a lower thermal stability, an excess of boron tribromide can have a stabilizing effect on the transported compounds. Another difference from the chloride studies is that the niobium product is more volatile than that of tellurium, which may be explained by the composition of the mobile phase. Finally, there are some differences in the behaviour of molybdenum and antimony. Under chlorinating conditions these elements are partly converted into the pentachlorides, while under brominating conditions they form species of lower oxidation state which are less volatile.

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SHORT COMMUNICATIONS

LIMITS OF DETECTION AND SELECTIVITY COEFFICIENTS OF A PVC-BASED ANION-SELECTIVE ELECTRODE

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Summary—A PVC-based anion-selective electrode was constructed with use of Capriquat as ion-exchanger. Experimental data were in good agreement with $E = E_0 - (RT/F)\ln(C + \sqrt{C^2 + A_x})/2$. The parameter A_x governs the limit of detection. It was experimentally shown that A_x was related to the selectivity coefficient K_{xy} by $K_{xy} = A_y/A_x$.

Ion-selective electrodes respond selectively to a certain ion, and the relationship between the potential of the electrode and the concentration of the ion usually follows the Nernst equation.[1] In dilute solution, however, the response deviates from the ideal expected from the Nernst equation: the slope of the plot of potential *vs.* log concentration becomes smaller with decrease in concentration of the ion, and finally the potential stays constant below a certain concentration, which is regarded as the limit of detection. For solid-state ion-selective electrodes, the limit of detection is considered to depend ultimately on the solubility product of the membrane sensor material.[2–6] On the other hand, for a liquid membrane or a polymer-based electrode, the limit of detection is thought to be governed by leakage of ion-exchanger. However, few quantitative studies concerning the limit of detection have been reported.[7–9]

In a previous paper,[10] we derived a theoretical equation describing the relationship between the concentration of the anion in the test solutions (hereafter denoted by C) and the electrode potential, by taking the leakage of ion-exchanger into consideration. The equation is

$$E = E_0 - (RT/F)\ln(C + \sqrt{C^2 + A_x})/2 \qquad (1)$$

where E_0 is a constant depending only on the concentration of the internal reference solution, A_x is a parameter governing the limit of detection, and R, T and F have their usual thermodynamic significance. The value of A_x depends on the species of anion, x.

When $C^2 \gg A_x$, equation (1) can be simplified to give the familiar Nernst equation, whereas under the condition that $C^2 \ll A_x$, it tends asymptotically to a constant value which depends only on A_x. Thus, the parameter A_x is related to the limit of detection. Moreover, the value of A_x is closely correlated with the selectivity coefficient as will be described later.

Earlier,[10] we demonstrated that equation (1) held well for the potential of a liquid-membrane electrode which was constructed by dissolving Crystal Violet in nitrobenzene. The present paper shows that equation (1) is also applicable to a poly(vinyl chloride) membrane electrode and discusses the relationship between the limit of detection and the selectivity coefficient.

EXPERIMENTAL

Preparation of PVC-based membrane electrode

The preparation of the PVC membrane was essentially the same as that employed by previous investigators.[11–13] Capriquat (Dojindo Laboratories, Kumamoto, Japan), employed as the positively charged ion-exchanger, was used as delivered, and contained mainly trioctylmethylammonium chloride, with a few per cent of decyl derivatives as impurity.[14] After addition of 0.15 ml of $10^{-2}M$ Capriquat solution in tetrahydrofuran (THF) to 10 ml of THF containing 0.4 g of poly(vinyl chloride) (PVC) and 1.5 ml of dioctyl phthalate (as plasticizer), the THF was slowly evaporated at room temperature from the mixture placed in a flat Petri-dish (60 cm^2 in area). The membrane thus obtained was transparent and 0.1–0.2 mm thick. A piece of the membrane was glued to a PVC tube with THF. Solutions of various sodium salts ($10^{-2}M$) were placed inside the tube as the internal reference solution. Before measurement of emf, the electrode was soaked overnight in a $10^{-2}M$ solution of the anion of the reference salt.

Measurements of emf

The potential difference between the test solution and the internal reference solution was measured with an electrometer (Takeda Riken Co., Tokyo, Type TR-8651) through a pair of calomel electrodes. Leakage of potassium chloride from the calomel electrodes was minimized by use

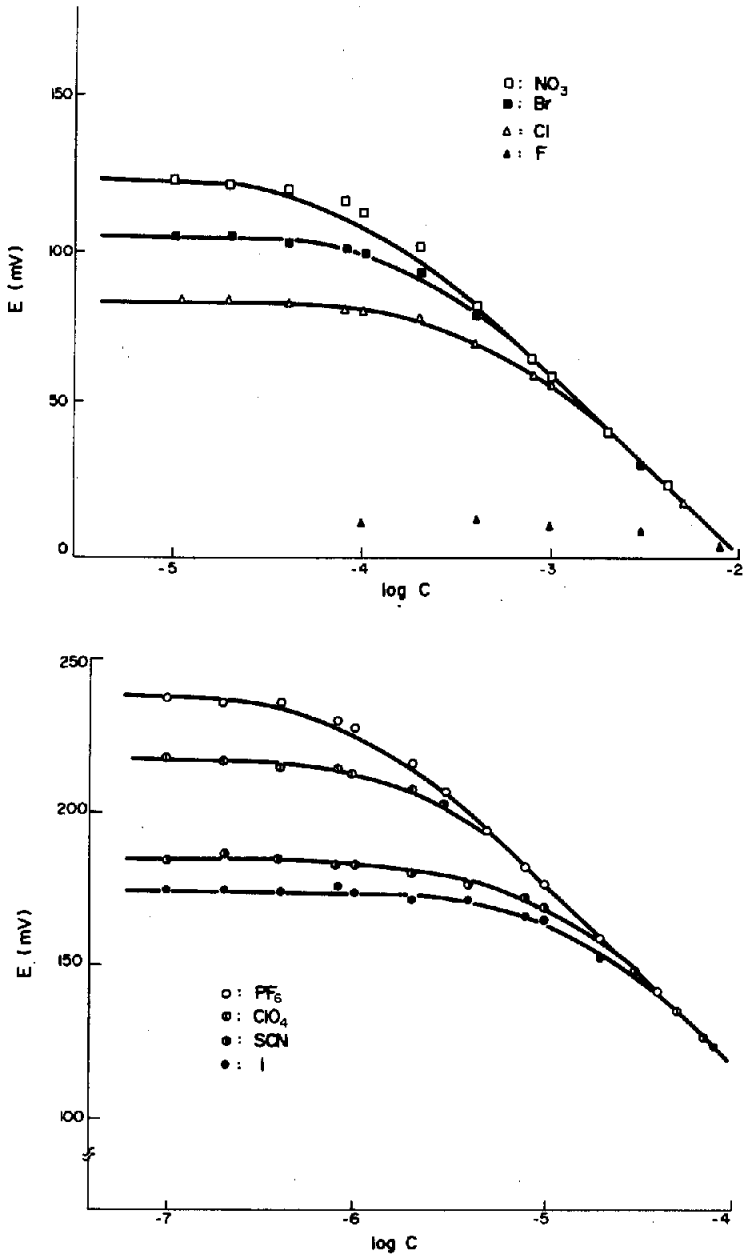

Fig. 1. Plots of potential difference *vs.* log concentration of various anions for a given membrane (measured against $10^{-2}M$ reference solution in a concentration cell). Solid lines represent the curves calculated by using equation (1) and the values of A_x listed in Table 1.

of double-junction electrodes. The electrometer was connected to a pen-recorder to confirm that the observed emf remained constant. The temperature of the test solution was kept at 25.0° by circulating round the vessel water from a thermostat. The test solution was stirred magnetically.

Determination of selectivity coefficient

The selectivity coefficient, K_{xy}, was determined by means of the bi-ionic method,[16,17] where the membrane separates two electrolyte solutions of the same concentration but containing different anion species, x and y. The potential difference between these two solutions, E_k is expressed by the equation:[10,18]

$$E_k = (RT/F) \ln K_{xy}$$

It has been pointed out that the selectivity coefficient K_{xy} determined by this method is equal to that determined by the other methods only when sufficiently concentrated electrolyte solutions are used for measurements of E_k.[16,17] Therefore, $0.1M$ solutions were used. By the method proposed by Srinivasan and Rechnitz,[15] the selectivity coefficient for perchlorate with respect to nitrate, K_{NO_3,ClO_4} was found to be 4.5×10^2 for the present membrane. As described later, the value obtained by the bi-ionic method was 4.1×10^2, in good agreement.

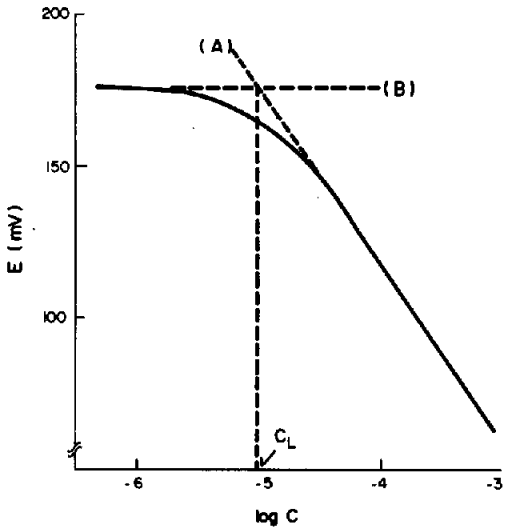

Fig. 2. Schematic illustration of equation (1) and determination of C_L, for $A_x = 4 \times 10^{-10}$ mole^2/l^2.

RESULTS AND DISCUSSION

In Fig. 1, the membrane potentials observed are plotted as a function of the concentration for various anions in the test solution. As described above, the membrane potential was measured under the condition that the PVC-membrane separates two solutions containing the same anion, *i.e.*, in a concentration cell. From the figure, we conclude that the limits of detection for various anions decreased in the order $F^- < Cl^- < Br^- < NO_3^- < I^- < SCN^- < ClO_4^- < PF_6^-$. This sequence is commonly found for the selectivity coefficients of anion-selective electrodes.[19]

Figure 2 shows the curve calculated for equation (1) with A_x assumed to be 4×10^{-10} mole^2/l^2, indicating that the potential changes in accordance with the Nernst equation in the relatively concentrated region and begins to level off from the Nernst equation in the dilute region. Thus, in the concentrated and dilute regions, the curve tends asymptotically to the two straight lines described by the Nernst equation and by a constant independent of the concentration, respectively, as shown by broken lines in Fig. 2. The first line is denoted by (A) and expressed by $E = E_0 - (RT/F) \ln C$, and the second is denoted by (B) and is given by $E = E_0 - (RT/F) \ln (\sqrt{A_x}/2)$. According to this equation, the asymptotic value of the electrode potential decreases by 8.9 mV when the value of A_x is doubled. Denoting the intercept of (A) and (B) by C_L as in Fig. 2, we obtain the relation $C_L = \sqrt{A_x}/2$. Thus the value of A_x can be evaluated from C_L as illustrated in Fig. 2. The values of A_x thus obtained are listed in Table 1. The solid lines in Fig. 1 represent the curves calculated by using the values of A_x listed in Table 1, indicating that equation (1) agrees adequately with the experimental points. It may be noted that if the relative concentration C/C_L ($= 2C/\sqrt{A_x}$) is greater than 2, equation (1) can practically be approximated

Table 1. A_x and $K_{NO_3,x}$ for various anions

Anion	A_x, mole^2/l^2	$K_{NO_3,x}$
F^-	—	7×10^{-3}
Cl^-	6.0×10^{-7}	3.4×10^{-2}
Br^-	1.0×10^{-7}	2.3×10^{-1}
NO_3^-	2.3×10^{-8}	—
I^-	4.8×10^{-10}	2.9×10
SCN^-	2.2×10^{-10}	1.1×10^2
ClO_4^-	1.6×10^{-11}	4.1×10^2
PF_6^-	3.2×10^{-12}	7.0×10^3

by the Nernst equation, while equation (1) deviates from the Nernst equation by 17.8 mV when $C/C_L = 1/2$. Also, the electrode responds to more dilute solution when the value of C_L, and hence of A_x, becomes smaller.

If the ion-exchanger dissociates nearly completely in the membrane phase, the parameter A_x is given by[10]

$$A_x \cong 4\sigma^2/b_x \qquad (2)$$

where σ is the concentration of the ion-exchanger in the membrane and b_x is defined by the equation:

$$RT \ln b_x = (\mu_x^\circ + \mu_x^\circ) - (\mu_s^{\circ,m} + \mu_x^{\circ,m}) \qquad (3)$$

where μ_i° and $\mu_i^{\circ,m}$ (i = x,s) stand for the standard chemical potentials of the anion (x) and of the ion-exchanger (s) in the aqueous and membrane phases, respectively. The selectivity coefficient, K_{xy}, is expressed by equation (4) when the ion-exchanger dissociates nearly completely:

$$K_{xy} = (u_y b_y)/(u_x b_x) \qquad (4)$$

where u_i stands for the mobility of anion i in the membrane phase. It has been shown that the selectivity coefficient is determined mainly by the value of

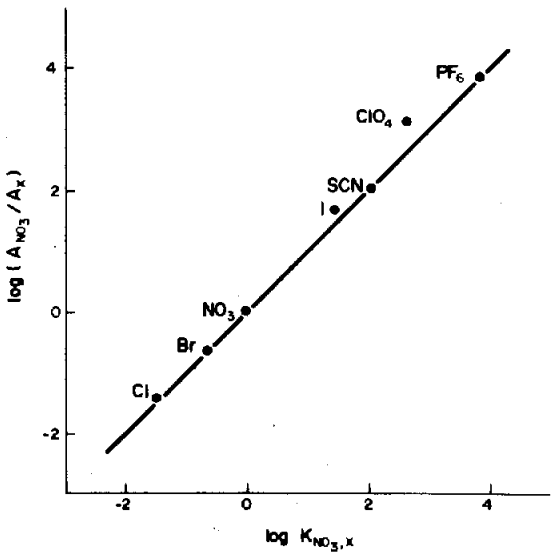

Fig. 3. Relationship between A_x and selectivity coefficient, K_{xy}.

b_y/b_x.[20] In other words, the mobilities of various anions do not vary significantly from one to another.[21] Therefore, from equations (2) and (4), we obtain equation (5)

$$K_{xy} \simeq A_x/A_y \quad (5)$$

The values of $K_{NO_3,x}$ for various anions x are listed in Table 1. Equation (5) indicates that the plot of log $K_{NO_3,x}$ *vs.* log(A_{NO_3}/A_x) gives a straight line with unit slope and passing through the origin. This relation is shown in Fig. 3, indicating that equation (5) holds for all anion species examined.

In conclusion, the membrane potential of a PVC-based membrane electrode is described quantitatively by equation (1), where A_x determines the limit of detection of the electrode. The value of A_x is related to the selectivity coefficient as shown in equation (5). The selectivity coefficient obtained followed a sequence common to anion-selective electrodes. This sequence is closely correlated with the hydration energy of the anions, thereby indicating that the aqueous solvation energies play a predominant role in determining the selectivity and limit of detection of PVC-based membrane electrodes.

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SOLVENT EXTRACTION SEPARATION OF ZIRCONIUM FROM MALONATE SOLUTION WITH HIGH MOLECULAR-WEIGHT AMINES

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Summary—Zirconium is quantitatively extracted with 4% Amberlite LA-1 or LA-2 in xylene, from 0.01 *M* malonic acid medium at pH 3.0 and stripped from the organic phase with 2*M* hydrochloric acid, then determined spectrophotometrically at 665 nm as its complex with Arsenazo III. Zirconium is separated from various other elements by selective extraction and stripping. The method has been applied to the analysis of zircon.

Zirconium has been extracted from chloride media with various high molecular-weight amines such as tri-iso-octylamine (TIOA),[1] methyldi-n-octylamine (MDOA),[2] trioctylamine (TOA)[3] and tribenzylamine (TBA).[4] Such extractions have been used for its separation from various alloys[5] and hafnium.[6] Zirconium has been extracted from sulphate media with 5% MDOA in xylene or TIOA in kerosene.[7] Amberlite LA-1 or LA-2 in chloroform[8] or benzene[9] has also been used for the extraction of the sulphato complex. Zirconium has been extracted from perchlorate and nitrate media with disubstituted amines.[10] However, extraction from aqueous media containing organic acids has not been widely studied, the only investigation so far being of the use of oxalic acid media and extraction with TIOA.[11,12]

This paper presents a systematic investigation of the extraction of zirconium from malonic acid media with long-chain amines such as Amberlite LA-1 and LA-2, Aliquat 336 S, Alamine 336 S, Primene JM-T and TIOA. The method is used for determination of zirconium in zircon.

EXPERIMENTAL

Reagents

Stock zirconium solution was prepared by dissolving ~1 g $Zr(NO_3)_4.5H_2O$ in 25 ml of hot conc. nitric acid and diluting to 250 ml with demineralized water. The solution was standardized by EDTA titration[13] and a 25-μg/ml zirconium solution prepared by appropriate dilution.

Amberlite LA-1, Amberlite LA-2, Primene JM-T, Aliquat 336 S, Alamine 336 S and TIOA were used without further purification, and with appropriate diluents. The liquid anion-exchangers were converted into the malonate form as described earlier.[14,15]

Procedure

A solution containing 25 μg of zirconium was mixed with 5 ml of 0.02*M* malonic acid, adjusted to pH 3.0 with 0.01*M* sodium hydroxide or malonic acid and made up to 10 ml. The solution was transferred into a separating funnel and shaken with 10 ml of extractant (*e.g.*, 4% Amberlite LA-1 in xylene) for 5 min on a wrist-action flask-shaker. The aqueous phase was discarded. The organic phase was shaken with 10 ml of 2*M* hydrochloric acid to strip the zirconium. The acid layer was withdrawn, mixed with 2 ml of 0.1% Arsenazo III solution and 3 ml of freshly prepared 1% gelatin solution, and made up to 25 ml with 2*M* hydrochloric acid. It was allowed to stand for about an hour to develop the colour. The absorbance of the green complex was measured at 665 nm against a reagent blank. The amount of zirconium was computed from a calibration curve.[13]

RESULTS AND DISCUSSION

Extraction as a function of pH

The pH ranges for the extraction of zirconium were ascertained by carrying out extractions between pH 1.0 and 7.0 with 4% solutions of the various liquid anion-exchangers (Fig. 1). The optimum pH for quantitative extraction was 2.25–5.5 with Primene JM-T, 2.0–5.0 with Amberlite LA-1 or LA-2, and 2.5–5.25 with Aliquat 336 S. The extraction was not quantitative with TIOA or Alamine 336 S. With Primene JM-T and Aliquat 336 S, although the extraction was quantitative, emulsification was a serious problem, and could not be circumvented by addition of alcohols such as octanol or decanol. However, the emulsion could be eliminated by centrifuging at about 4000 rpm.

Effect of various diluents

Solutions of 4% Amberlite LA-1 in various diluents such as benzene, toluene, xylene, hexane, cyclohexane, carbon tetrachloride, chloroform and kerosene were tested. The phase-volume ratio was 1:1, as otherwise an emulsion was formed. Xylene was found to be the most effective diluent (Table 1). Benzene, toluene, carbon tetrachloride and chloroform caused either turbidity or emulsion. Hexane, cyclohexane and kerosene were inefficient as diluents. The extraction (at pH 3.0) was complete in 5 min and stripping in 2 min.

Malonic acid and Amberlite LA-1 concentrations

Table 2 shows that extraction is quantitative at malonic acid concentration $>7 \times 10^{-4}M$. Hence,

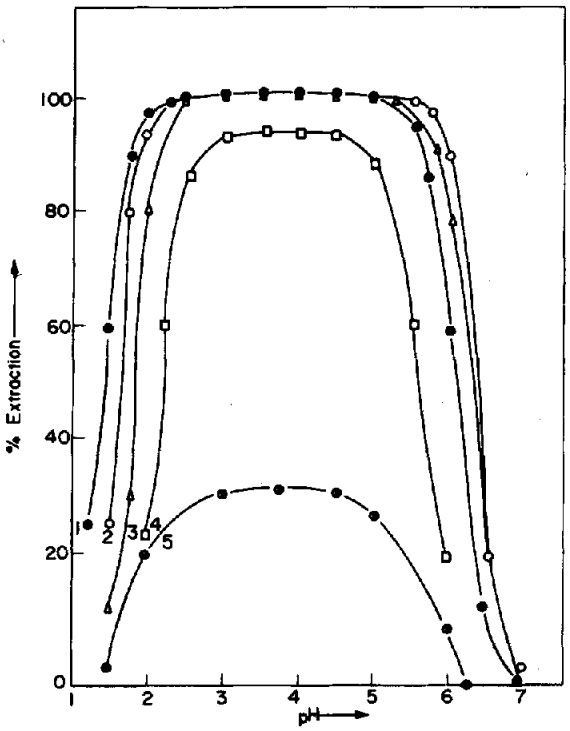

Fig. 1. Extraction of Zr from malonic acid solution by various 4% amine solutions in xylene. (1) Amberlite LA-1; (2) Primene JM-T; (3) Aliquat 336S; (4) Alamine 336S; (5) Tri-iso-octylamine.

0.01*M* concentration of malonic acid is recommended.

It was similarly found that 0.06*M* Amberlite LA-1 in xylene gives complete extraction (Table 3), and a 4% solution is recommended.

Different liquid anion-exchangers

Zirconium was extracted with the various liquid anion-exchangers, with benzene, xylene, chloroform, hexane and kerosene as the diluents. The extraction was practically quantitative with Amberlite LA-1 and LA-2, Aliquat 336 S and Primene JM-T in benzene or xylene, but incomplete with these exchangers in other diluents. Alamine 336 S and TIOA were poor extractants in all the diluents. Hence Amberlite LA-1 was chosen for further studies.

Stripping agents

After extraction of the zirconium, it was stripped with 10 ml of reagents of various concentrations

Table 1. Effect of various diluents on extraction with Amberlite LA-1

Diluent	Extraction, %
Benzene	99.5
Toluene	99.0
Xylene	99.6
Chloroform	92.0
Carbon tetrachloride	75.0
Hexane	66.0
Cyclohexane	60.0
Kerosene	77.4

Table 2. Effect of malonic acid concentration

Malonic acid, $10^{-4}M$	Extraction, %
1.0	60.0
2.0	87.9
3.0	95.0
4.0	97.5
5.0	98.6
6.0	99.0
7.0	99.6
8.0	99.6

[sodium hydroxide, ammonia and sodium carbonate solutions (0.1–2*M*), hydrochloric acid, nitric acid, sulphuric acid and hydrobromic acid (0.1–4*M*), and lithium sulphate (0.5–2*M*)]. Stripping was complete with any of the acids at concentrations $>1M$. The alkalis were unsuitable as they not only accelerated the hydrolysis of zirconium but also promoted emulsion formation. Stripping was incomplete with lithium sulphate or sodium carbonate. Lower concentrations of sulphuric acid ($<0.5M$) and higher concentrations of hydrochloric acid ($>6M$) were not suitable, because of anionic complex formation and retention by the liquid anion-exchanger. Thus for practical purposes, 2*M* hydrochloric acid was thought most suitable as the stripping agent.

Metal ion concentration

Zirconium was extracted quantitatively in the concentration range of 0.005–2.0 mg in 10 ml in a single extraction with 4% Amberlite LA-1 in xylene, the average recovery being 99.6%, relative standard deviation 2%. It is possible to extract higher concentrations of zirconium quantitatively with a larger volume and higher concentration of Amberlite LA-1.

Nature of species extracted

This is thought to be an ion-association complex $[R_2NH_2^+]_2.[ZrO\ (malonate)_2^{2-}]$. This formulation is supported by graphical evidence, the slopes of plots of log *D* *vs.* log amine concentration at a fixed malonic acid concentration and of log *D* *vs.* log malonic acid concentration at fixed amine concentration being 1.85 and 2.3 respectively.

Table 3. Effect of Amberlite LA-1 concentration

Amberlite LA-1, $10^{-2}M$	Extraction %
0.5	29.0
1.0	60.0
1.5	78.0
2.0	87.5
2.5	90.3
3.0	93.4
4.0	96.7
6.0	99.6
8.0	99.6
10.0	99.6

Table 4. Effect of various ions on extraction of 25 μg of Zr

Foreign ion	Tolerance limit, *mg*	Foreign ion	Tolerance limit, *mg*
Ag^{+}	1.0	Pr^{3+}	0.24
Tl^{+}	0.50	Sm^{3+}	0.20
Tl^{3+}	0.50	Gd^{3+}	0.25
In^{3+}	0.26	Dy^{3+}	0.20
Ga^{3+}	0.25	Be^{2+}	1.0
Cu^{2+}	0.95	Mn^{2+}	0.25
Cd^{2+}	0.52	Co^{2+}	0.63
Hg^{2+}	0.20	Ni^{2+}	0.48
As^{3+}	0.50	Mg^{2+}	1.50
Sb^{3+}	0.40	Ca^{2+}	1.50
Bi^{3+}	0.25	Sr^{2+}	1.50
Pt^{4+}	0.25	Ba^{2}	0.55
Fe^{2+}	0.48	Li^{+}	5.0
Fe^{3+}	0.28	Na^{+}	5.0
Cr^{3+}	0.25	K^{+}	5.0
Al^{3+}	0.60	Rb^{+}	2.0
Ti^{4+}	0.12	Cs^{+}	2.1
Sn^{4+}	0.40	SeO_3^{2-}	0.18
Th^{4+}	0.13	TeO_3^{2-}	0.15
U^{6+}	0.25	WO_4^{2-}	0.20
Y^{3+}	0.50	$B_4O_7^{2-}$	0.40
La^{3+}	0.20	$Mo_7O_{24}^{6-}$	0.10
Ce^{3+}	0.20	SiO_3^{2-}	0.25
Nd^{3+}	0.20		

Separation from other metal ions

Zirconium was extracted in the presence of various other ions (Table 4). The tolerance limit was set as the amount of foreign ion required to cause ±2% error in the recovery of zirconium. Alkali and alkaline earth metal ions, thallium(I), iron(II), silver, arsenic(III), yttrium, tin(IV) and all lanthanides except lanthanum, cerium(III), praseodymium and neodymium, were not extracted along with zirconium because they do not form a malonato-complex at pH 3.0. Hence zirconium was separated from these metals.

Metals forming weaker complexes with malonic acid, such as zinc, cadmium, nickel, copper, cobalt, aluminium, lanthanum, praseodymium and neodymium, were easily scrubbed from the organic phase with water before stripping of zirconium with 2*M* hydrochloric acid.

Gallium(III), bismuth(III), iron(III), uranium(VI) and mercury(II) form strong complexes with malonic acid and are extracted along with zirconium. However, zirconium can be stripped selectively with 5*M* hydrochloric acid, the chloro-complexes of the other species being retained by the exchanger.[15] The other metals could then be stripped with 1*M* sodium hydroxide.

Zirconium can be separated from scandium and cerium by stripping in sulphate media. After extraction of these metals along with zirconium, cerium is first stripped with 0.05*M* sulphuric acid followed by scandium with 0.5*M* sulphuric acid and finally zirconium with 2*M* hydrochloric acid.

Zirconium and indium are separated by stripping the zirconium with 4*M* nitric acid followed by stripping indium with 2*M* hydrochloric acid. Zirconium is separated from thorium by stripping the former with 6*M* nitric acid followed by stripping the latter with 2*M* hydrochloric acid.

Titanium and zirconium are separated by first stripping titanium with 8*M* hydrochloric acid followed by stripping zirconium with 2*M* hydrochloric acid.

Zirconium is separated from selenite, tellurite, tungstate, borate and silicate by selective stripping of zirconium with 2*M* hydrochloric acid followed by stripping of all oxy-anions with 1*M* sodium hydroxide.

Analysis of zircon for zirconium

About 0.3 g of zircon was fused with 4 g of borax in a platinum crucible. After cooling, the mass was lixiviated with 2*M* hydrochloric acid and diluted to 250 ml in a standard flask.[16] Then 25 ml of this stock solution were mixed with 25 ml of concentrated hydrochloric acid, and the solution was boiled to remove silicon as silica gel. The filtrate was diluted accurately to 100 ml with demineralized water. A 5-ml aliquot was taken and the zirconium extracted as already described. Aluminium and some of the lanthanides were stripped from the organic phase with water, then scandium and cerium (and other lanthanides) were stripped with 0.5*M* sulphuric acid, followed by stripping of titanium with 8*M* hydrochloric acid, and zirconium with 5*M* hydrochloric acid. Iron was stripped with 6*M* nitric acid and finally uranium with 1*M* sodium hydroxide. All these metals were then determined by standard procedures. The results (with actual amount of element present given in parentheses) were ZrO_2 64.9% (64.7%), TiO_2 0.50% (0.55%), Fe_2O_3 0.04% (0.06%), Al_2O_3 3.0% (2.9%), U_3O_8 0.034% (0.032%).

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COMPLEXOMETRIC DETERMINATION OF MAGNESIUM IN NODULAR CAST IRON AND ALLOYED CAST IRON ROLL SAMPLES

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Summary—A complexometric method for the determination of magnesium in nodular cast iron, alloyed cast iron and roll samples has been developed. The bulk of the iron is removed by ether extraction and the phosphate as zirconium phosphate. The other elements are removed by extraction with dithiocarbamate into chloroform. Magnesium is then titrated with EDTA at pH 10, with Eriochrome Black T as indicator. Calcium interferes, but is very rarely present in such cast iron samples.

Magnesium is added to certain cast irons and alloyed cast irons to prevent the formation of flake graphite. Although some other innoculants such as Ce, Li, Y, Sr, Ba have also been reported to give the spheroidal graphite structure, the role of magnesium in producing spheroidal graphite is well known and its content in nodular cast iron generally varies from 0.005 to 0.2%. Several methods have been suggested for determining magnesium in cast iron.

Sajó and Repas[1] have used EDTA after removal of iron by mercury cathode electrolysis. Green[2] has suggested the zinc oxide separation of iron and manganese, and treatment of the filtrate with sodium cyanide and Tiron as masking agents. Reichert[3] has also suggested the EDTA method. Although the bulk of the iron can be removed by mercury cathode electrolysis and zinc oxide separation, solvent extraction methods are much preferred nowadays since they are less time-consuming and cumbersome.

In the complexometric method for the determination of magnesium, phosphorus interferes seriously because magnesium phosphate precipitates at pH 10. Collier[4] has studied the effect of phosphorus in the determination of calcium and magnesium by the EDTA method and suggested converting the phosphate ion into phosphomolybdate and extracting this with n-butanol–chloroform mixture. Brunisholz[5] has used an ion-exchange method to separate phosphate.

The British Standard[6] and BCIRA[7] methods suggest extracting the bulk of the iron with isobutyl acetate and the other interfering elements with cupferron–chloroform and diethyldithiocarbamate chloroform before EDTA titration, but say nothing about phosphorus.

A method has now been developed in which the bulk of the iron is removed by extraction with diethyl ether and the phosphate precipitated as zirconium phosphate, at the same time the excess of zirconium, along with chromium and the last traces of iron *etc.*, being removed by precipitation with hexamine. Manganese, nickel, copper and molybdenum are removed by extraction with sodium diethyldithiocarbamate and chloroform, and magnesium is then determined with EDTA.

EXPERIMENTAL

Procedure

Weigh 5 g of sample into a 400-ml beaker, add 50–60 ml of hydrochloric acid (1 + 1) and cover immediately with a watch-glass. When the reaction ceases, oxidize the solution by dropwise addition of nitric acid, evaporate to dryness, add 30 ml of hydrochloric acid (1 + 1) and boil for 1–2 min, filter through a paper-pulp pad, and wash the pad several times with hot dilute hydrochloric acid (1 + 1) and finally 3 or 4 times with hot distilled water.

Evaporate the filtrate to 15–20 ml. Transfer the solution into a 250-ml separating funnel, washing the beaker with 15 ml of concentrated hydrochloric acid. Wash the beaker with 35 ml of diethyl ether, transferring the ether into the separating funnel. Shake the funnel, releasing the pressure from time to time, run the aqueous phase into another separating funnel and extract it with a further 35 ml of diethyl ether.

Transfer the aqueous phase to a 400-ml beaker and warm on a water-bath to remove ether. Add 1% zirconium nitrate [$ZrO(NO_3)_2.5H_2O$] solution (1 ml per 2 mg of phosphorus present, plus 2 ml excess; the phosphorus content must be known). Dilute to about 200 ml and add ammonia solution (1 + 1) dropwise till the pH is 5 (use indicator paper). Add 25–30 ml of 30% hexamine solution, heat to boiling and keep at low heat for 10 min. Filter off on Whatman 41 paper and wash with hot 1% hexamine solution several times.

Evaporate the filtrate to about 30 ml, transfer the solution into a 250-ml separating funnel and dilute to about 100 ml. Add 20 ml of 20% sodium diethyldithiocarbamate solution, shake well for 1 min, add 20 ml of chloroform and shake thoroughly. Allow the layers to separate and discard the chloroform layer. Repeat the extraction, with a further 10 ml of dithiocarbamate solution and 20 ml of chloroform each time, until the chloroform layer is perfectly colourless.

Transfer the aqueous layer into a 250-ml conical beaker, evaporate the solution to about 30 ml, cool thoroughly, add a pinch of ascorbic acid and hydroxylamine hydrochloride, 5 ml of 15% triethanolamine solution, 25–30 ml of ammonia-ammonium chloride buffer (pH 10) and 5 ml of 5% potassium cyanide solution, and titrate with 0.01*M* EDTA, to the blue colour of Eriochrome Black T as indicator.

Table 1. Determination of magnesium in synthetic samples

	Mg added, *mg*	Mg found, *mg*
Phosphorus not removed	1.00	0.55
	2.00	1.20
	3.00	Slight turbidity
	4.00	White precipitation
	5.00	Titration impossible
Phosphorus removed	1.00	1.04
	2.00	1.95
	3.00	2.82
	4.00	4.11
	5.00	5.10

Use analytical-grade reagents and run a blank along with the sample.

1 ml of 0.01*M* EDTA = 0.000243 g of Mg.

RESULTS AND DISCUSSION

Magnesium was determined in 5-g samples of magnesium-free cast iron to which 1–5 mg of magnesium had been added (Table 1). The composition of the iron was C 3.46%, Si 1.62%, P 0.18%, Cr 0.90%, Mo 0.27%, Ni 1.30%, Mn 0.55%.

Typical results for reference samples are shown in Table 2 and compared with those obtained by atomic-absorption spectrophotometry. The phosphorus content in pig iron and cast iron may be as high as 1.5% but for the production of malleable and spheroidal cast iron it should be low, and is generally 0.02–0.15%. For the determination of magnesium in such samples the interference due to phosphorus must be eliminated. Even in the atomic-absorption method there is some phosphorus interference, which is generally overcome by addition of a large amount of strontium to the test solution.

Dodson *et al.*[10] studied the effect of phosphate in the ether extraction of ferric iron but observed that the phosphate was only partially extracted along with the iron.

In the present method phosphate was removed by addition of about 15 mg of zirconium for every 10 mg of phosphorus, plus a small excess for complete precipitation. Hexamine buffers the solution at about pH 6–6.5 and should be added in large excess to precipitate chromium and remaining iron completely. In the BCIRA method[7] chromium is removed as chromyl chloride, which is difficult to achieve completely, and any residual chromium(VI) would interfere in the final titration.

The titration end-point may be improved by the addition of a small amount of magnesium-EDTA solution to the test solution.

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Table 2. Determination of magnesium in alloyed cast iron

Sample	General composition %	Mg, % Certified value	By atomic absorption	By our method
Euro-Standard 481–1 Bureau of Analysed Samples	C–3.91, Si–2.29, P–0.019, Cu–0.15, Cr–0.063, Mo–0.011, Ni–1.19	0.051	—	0.052 0.054
Works samples				
1	C–3.58, Si–1.59, P–0.158, Cr–0.89, Mo–0.20, Ni–1.26, Mn–0.48		0.053	0.049 0.051
2	C–2.78, Si–1.85, P–0.079, Cr–0.30, Mo–0.30, Ni–2.90, Mn–0.55		0.042	0.039 0.040
3	C–3.25, Si–1.60, P–0.19, Cr–0.31, Mo–0.28, Ni–2.95 Mn–0.50		0.01	0.007 0.008

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EXPERIMENTAL PARAMETERS OF VANADIUM DETERMINATION BY ATOMIC-ABSORPTION SPECTROSCOPY WITH GRAPHITE-FURNACE ATOMIZATION

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Summary—It was found that impregnation of a graphite cuvette (HGA-72) with salts of elements which form stable carbides (Ta, Si, Nb, Zr, W, La) decreases the absorbance signal for vanadium. The slope of the atomization curves indicates that formation of vanadium atoms is inhibited, probably by formation of a ternary compound between the impregnating element, vanadium and graphite. On the contrary bigger signals and better repeatability of results may be achieved when the cuvette is coated with pyrolytic graphite and methane is added to the sheath gas. The presence of methane increases the atomization efficiency and compensates for the disadvantageous influence of any air present in the sheath gas.

The advantage of the electrothermal atomization of technique of atomic-absorption spectroscopy in determination of metals in low concentrations and small samples has stimulated investigations on determination of vanadium by this technique in various materials such as sea-water,[1] air,[2] biological samples,[3] steel[4] and most commonly in petrochemicals.[5–8] The authors of these papers have pointed out numerous effects which influence the absorbance of vanadium. It is mentioned that formation of vanadium carbides in graphite cuvettes[9] makes the detection limit higher and that this harmful effect can be diminished by covering the graphite surface with pyrolytic carbon.[10] It is also suggested that a similar effect may be achieved by impregnating the graphite atomizer with other carbide-forming elements.[11] Such a procedure has been used by Ortner and Kantuscher[12] for determination of silicon and by Runnels *et al.*[13] for beryllium, manganese, chromium and aluminium. Nevertheless there are lacking more systematic studies of the atomization parameters in vanadium determination.

In this work the effect of modification of the graphite cuvette surface and composition of the gas atmosphere on vanadium determination have been investigated.

EXPERIMENTAL

Apparatus

Perkin–Elmer model 300 atomic-absorption spectrophotometer with HGA 72 graphite atomizer and Hitachi Perkin–Elmer model 159 recorder. Perkin–Elmer hollow-cathode lamp.

Reagents and solutions

Vanadium standard stock solution (1 mg/ml) was prepared by dissolving the appropriate amount of ammonium metavanadate in dilute ammonia, acidifying with conc. nitric acid and diluting to volume. Working solutions were obtained by dilution with 0.01*M* hydrochloric acid.

Sodium tungstate, sodium silicate, zirconium nitrate and lanthanum chloride solutions were prepared, containing 1% of the element of interest. Niobium chloride solution (1% Nb) was prepared as a colloidal suspension. Tantalum powder (1 g) was dissolved in 5 ml of hydrofluoric acid, 10 ml of conc. hydrochloric acid and 10 ml of 30% hydrogen peroxide, then 5 ml of conc. sulphuric acid were added, and the solution was evaporated to a small volume and diluted to 100 ml.

Measurements

Standard measurements were made on 50-μl samples containing 1 ppm of vanadium, dried at 110° for 75 sec, ashed for 20 sec at 1700°, and atomized during 30 sec at 2660°.

Atomization curves for 100 ng of vanadium were recorded over the temperature range from 1750° to 2660° (rate of heating 2400°/min°).

The absorbance was measured at 318.4 nm, with a 0.7 nm band-pass, at a lamp current of 30 mA. The rate of argon flow was 1.5 l./min and other gases were mixed with argon by means of a peristaltic pump. In the background correction the absorbance of a blank was taken into account.

Cuvettes were impregnated by injection of solutions which contained 1–5 mg of the element, drying at 100° and then heating to 2660° at 1200°/min. In the case of tantalum the procedure followed that of Ortner and Kantuscher,[12] *i.e.*, the cuvette was soaked in tantalum solution for 24 hr, dried for 12 hr at 120° and finally heated to 2660°.

RESULTS AND DISCUSSION

Impregnated cuvettes

Following the investigations of other authors[12,13] the effect of Si, Zr, Ta, W, Nb and La on determination of vanadium was investigated. As shown in Table 1, the absorbance of vanadium was seriously depressed by the presence of lanthanum or tungsten, and this may be attributed to mechanical losses on

Table 1. Effect of various elements on absorbance of 50 ng of vanadium in 0.02*M* HCl

	—	W	Si	La	Zr	Ta	Nb
Absorbance with 1 mg of element added to the sample	0.40	0.12		0.045			
Absorbance with cuvette impregnated with element indicated		0.15	0.38	0.38	0.28	0.31	0.32

the particles of tungsten and lanthanum salts. Such an effect was not observed when the cuvette was impregnated with these (and the other salts investigated) before the sample was injected. Nevertheless, in the case of some cuvettes the absorbance was considerably lowered by impregnation of the atomizer with tungsten whereas impregnation with lanthanum or silicon caused little lowering of the signal (Table 1). It seems more probable that such behaviour is due to formation of three-component systems containing carbon and two other elements (one of them vanadium) than to modification of the graphite structure.[11] The formation of V–Ti–C, V–Nb–C, V–Ta–C and V–Hf–C systems has been mentioned in the literature;[14] change in the surface of the cuvette would be expected to increase the absorbance rather than decrease it. Similar conclusions may be reached from study of the atomization curves (Fig. 1). Comparison of the atomization rate in untreated and impregnated cuvettes indicates decrease of the peak value and of area under the curve, and delay of the appearance maximum when the impregnated cuvettes are used. For untreated cuvettes the maximum appears in the range 2600–2620°, whereas impregnation shifts it to 2660° *i.e.*, to the highest temperature obtained with our instrument.

The effect of pyrolytic graphite

Decomposition of methane at *ca.* 2200° produces pyrolytic graphite on the atomizer surface, and this advantageously influences the peak height and area without, however, changing the temperature of occurrence of the peak.

The effect of the presence of methane in the argon sheath-gas was observed by Kantor *et al.*[15] who noted that reaction of methane with water vapour formed in the furnace extends the life-time of the cuvettes and compensates for the presence of oxidants. This is important in the case of vanadium, for which a rather high atomization temperature is needed. The absorbance of vanadium in the presence of methane (Figs. 2 and 3) added at a flow-rate between 1.2 and 5.4 ml/min is larger than that with pure argon. Methane influences the atomization effi-

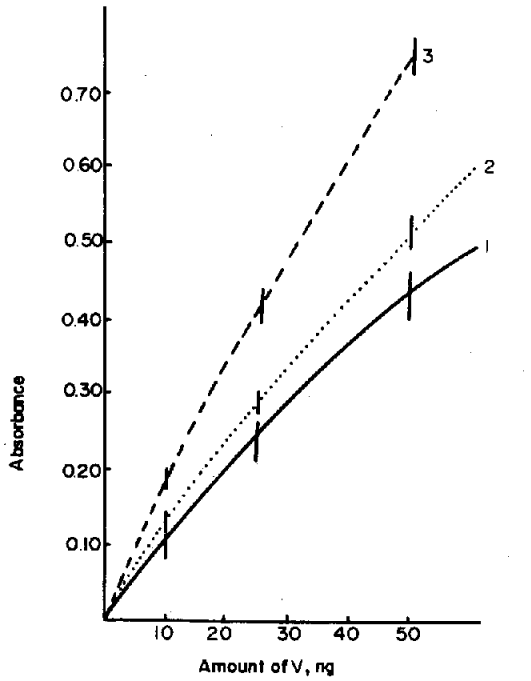

Fig. 2. Calibration curves for vanadium. 1—Untreated cuvette; 2—cuvette covered with pyrolytic graphite; 3—covered with pyrolytic graphite, with methane flow (5.4 ml/min) during atomization.

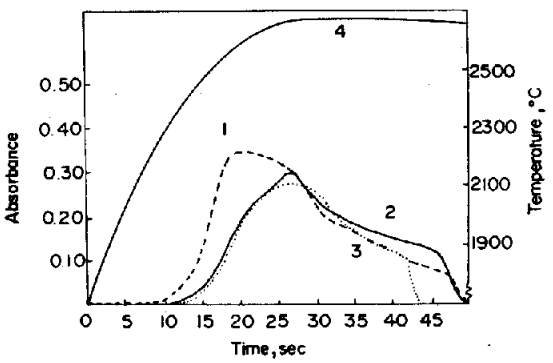

Fig. 1. Atomization curves (temperature increase 2400°/min) for 100 ng of V. 1—Untreated cuvette; 2—impregnated with 1 mg of W; 3—impregnated with 1 mg of Ta; 4—temperature change during atomization.

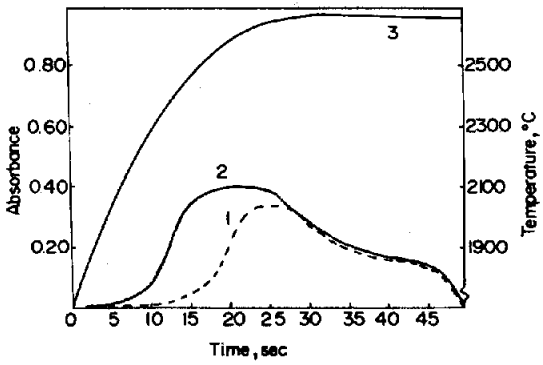

Fig. 3. Atomization curves for 100 ng of V in cuvettes covered with pyrolytic graphite. 1—Pure argon flow; 2—argon with addition of methane (5.4 ml/min); 3—temperature change during atomization.

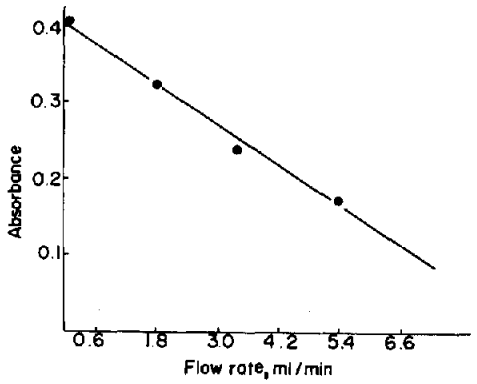

Fig. 4. The effect of air added to argon, on the absorbance of 50 ng of V in 0.01*M* HCl.

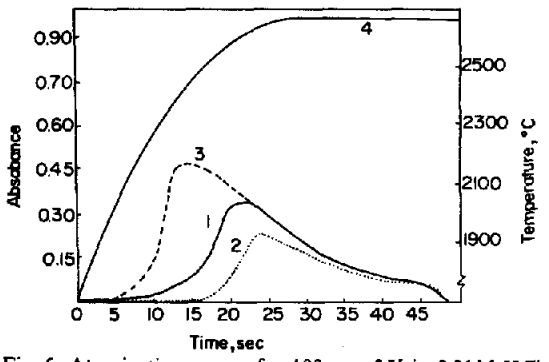

Fig. 5. Atomization curves for 100 ng of V in 0.01*M* HCl in cuvettes covered with pyrolytic graphite. 1—argon gas; 2—argon + air (2.7 ml/min); 3—argon + air (2.7 ml/min) + methane (5.4 ml/min).

ciency, and the peak occurs earlier, at 2600°. On the basis of the mechanism postulated by Sturgeon and Chakrabarti[11] it may be supposed that dissociation of gaseous vanadium oxide is shifted towards formation of free metal atoms by the reaction promoted between oxygen and methane:

$$VO \rightarrow V + O \quad (1)$$

$$O + CH_4 \rightarrow 2H_2 + CO \quad (2)$$

The effect of air in the sheath gas

Small quantities of air may occur in the argon as an impurity or because of diffusion from the surroundings. Therefore the effect of the presence of air was tested by mixing small amounts with the argon with the aid of a peristaltic pump. Increasing the amount of air decreases the absorbance of vanadium (Fig. 4). When air is present in the argon the atomization curve shows a decrease in both peak height and area (Fig. 5). Also, maximal absorbance occurs at higher temperature, which may be explained by the unfavourable shift of the equilibrium of reaction (1). To compensate for this, methane was also added to the argon. Under these conditions the maximum of the absorbance appears at 2420°, which is about 200° lower than without methane added. The height of the peak and its area increase, favourably influencing the results. This indicates that atomization in these conditions is faster and more efficient. Such an effect can be explained neither by a change of atomization mechanism nor by a change in graphite structure on the atomizer surface. The most probable explanation seems to be the increase of the atomization rate through the additional exothermal process between methane and oxygen, which raises the vapour temperature in the cuvette. This is similar to the effect of hydrogen, which increases the temperature by about 100° at 1700°.

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COMPLEXOMETRIC PHOTOMETRIC TITRATIONS WITHOUT AN INDICATOR

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Summary—Calculations of titration curves in advance with the aid of conditional constants saves some experimental work. The limits of a conventional titration curve are determined, the titration of lead with EDTA being used as an example.

When the curve of absorbance (A) *vs.* pH is plotted for a 1:1 chelate ML which can be measured spectrophotometrically, every point on the curve is determined by the following equations:

$$A = \epsilon'_{ML}[ML'] + \epsilon'_{L}[L'] + \epsilon'_{M}[M'] \quad (1)$$

$$K'_{ML} = \frac{K_{ML}\alpha_{ML}}{\alpha_M\alpha_L} = \frac{[ML']}{[M'][L']} \quad (2)$$

where ϵ' is the molar absorptivity of the species indicated, conditional concentrations are indicated by primes, and the side-reaction coefficients α are defined by

$$\alpha_M = 1 + \sum [OH]^n K_{M(OH)_n} \quad (3)$$

$$\alpha_L = 1 + \sum [H]^n K_{H_nL} \quad (4)$$

$$\alpha_{ML} = 1 + \sum [H]^n K_{MH_nL} + \sum [OH]^n K_{M(OH)_nL} \quad (5)$$

$$K_{MH_nL} = \frac{[MH_nL]}{[ML][H]^n} \quad (6)$$

$$K_{M(OH)_nL} = \frac{[M(OH)_nL]}{[ML][OH]^n} \quad (7)$$

$$K_{MOH} = \frac{[MOH]}{[M][OH]} \quad (8)$$

$$K_{H_nL} = \frac{[H_nL]}{[H]^n[L]}. \quad (9)$$

For simplicity, charges are omitted, and the path-length is taken as 1 cm. Strictly speaking, in equation (1) the general term $\epsilon'_x[x']$ means $\Sigma\epsilon_{x_i}[x_i]$ where x_i refers to the ith individual species contributing to the conditional concentration.

The equations assume, of course, that no side-reactions need to be taken into account other than the ones used to calculate the conditional constants as above.

If symbols in square brackets represent molar concentrations in solution and C the total added concentrations,

$$[M'] = C_M - [ML'] \quad (10)$$

$$[L'] = C_L - [ML']. \quad (11)$$

Equations (10) and (11) combined with equation (1) give

$$A = \epsilon'_{ML}[ML'] + \epsilon'_{L}(C_L - [ML']) + \epsilon'_{M}(C_M - [ML']) \quad (12)$$

and

$$[ML'] = \frac{A - (\epsilon'_L C_L + \epsilon'_M C_M)}{\epsilon'_{ML} - \epsilon'_L - \epsilon'_M}. \quad (13)$$

Equations (10) and (11) combined with equation (2) give

$$K'_{ML} = \frac{[ML']}{(C_M - [ML'])(C_L - [ML'])}. \quad (14)$$

By using equations (13) and (14), it is an easy matter to calculate [ML'] and so K'_{ML} from the molar absorptivities and total concentrations. Molar absorptivities can be calculated from the curve.

Similar expressions have been reported by Martell and Calvin,[1] Karadakov and Venkova[2] and McBryde.[3]

Once the conditional stability constant is thus known, the photometric titration curve for any given pH can be calculated in advance, thus saving a lot of unnecessary investigational work. As an example of this the titration of lead with EDTA will be discussed.

This titration has been mentioned by several authors, most of whom refer to the original paper by Wilhite *et al.*[4] The system Pb–EDTA affords, however, some interesting points that may have escaped these authors and it has therefore been chosen as a basis for the following discussion.

EXPERIMENTAL

A Hitachi–Perkin–Elmer 100-60 Spectrophotometer was adapted, with a loose cover having a 2-cm circular hole to admit the tubes connected to the flow-through cuvette. The

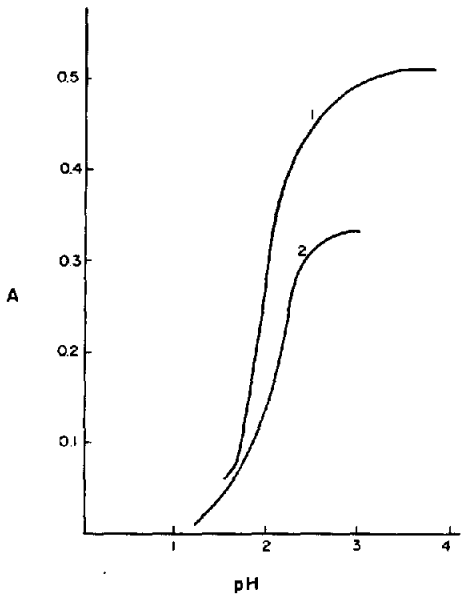

Fig. 1. The absorbance of the Pb–EDTA complex as a function of pH. 1 – $C_M = 10^{-4.17} M$. 2 $C_M = 10^{-4.40} M$.

hole was covered with aluminium-based paper during the titrations. Absorbances were measured in a 1-cm flow-through cuvette, with addition and mixing of solutions and measurements of pH being done in the reaction beaker outside the photometer. No buffer was used—the pH was adjusted with fairly concentrated sodium hydroxide solution and/or perchloric acid so that the total volume of the solution was not appreciably changed. The solution was mixed by a magnetic stirrer and sufficient time was allowed to elapse after each addition, since the reaction is slightly slow. The pH was measured with a Metrohm Herisau E-532 digital pH-meter and a combination electrode.

EDTA solutions were prepared by diluting standard solutions made by dissolving Merck *pro analysi* Titriplex III in doubly distilled water. The lead perchlorate solutions were made by evaporating lead nitrate solutions to fumes with perchloric acid and diluting to known volumes with doubly distilled water and perchloric acid.

RESULTS AND DISCUSSION

Determination of the conditional constant

The absorbance of equimolar mixtures of lead and EDTA was determined for two concentrations and various acidities. The results are shown in Fig. 1.

By the method described above, $\log K'$ was determined at different pH values and was used to obtain $\log K$ (Table 1) from

$$\log K = \log K' + \log \alpha_{Y(H)} \qquad (15)$$

since α_M and α_{ML} will be 1 at these acidities.

Similar calculations for the curve $C = 10^{-4.40} M$ gives $\log K = 18.4 \pm 0.6$, which shows that accurate values for the stability constant can be determined only if the jump in the curve is sufficiently high.

To calculate the values in Table 1, ϵ'_{Pb} was assumed to be zero. ϵ'_{PbEDTA} was calculated to be 7.35×10^3 l.mole^{-1}.cm^{-1} from the maximum absorbance values of the curve and ϵ'_L was shown to be

Table 1. Calculation of $\log K_{PbEDTA}$ from the curve for $C = 10^{-4.17} M$

pH	A^*	$\log K'$	$\log \alpha_Y$	$\log K$
2.69	0.475	6.52	11.46	(17.98)
2.40	0.443	6.03	12.29	18.32
2.22	0.392	5.41	12.86	18.27
2.06	0.334	4.96	13.41	18.37
2.03	0.321	4.88	13.51	18.39
1.97	0.261	4.53	13.73	18.26
1.84	0.171	4.07	14.22	18.29
1.73	0.106	3.71	14.65	18.36
1.58	0.064	3.40	15.27	(18.67)
		Mean		18.32 ± 0.05 [~24°]
		Anderegg[5]		18.3 ± 0.2 [25°]

* A corrected for dilution by acid [see equation (17)].

negligible (~70 l.mole^{-1}.cm^{-1}) in a separate determination. In this connection it may be mentioned that it proved extremely difficult to achieve consistent values for the molar absorptivities. The exact placing of the cuvette, the exact composition of the solution and many other factors influence the absorbance. This difficulty emphasizes the advantages of photometric titrations over single absorbance measurements, in that the exact values of the molar absorptivities are not so critical. Even interfering complexing agents can be tolerated as long as the conditional constant stays reasonably high.

Calculation of the theoretical titration curve

The theoretical titration curve can be calculated in advance, if the values of the different molar absorptivities, *i.e.*, ϵ'_{ML}, ϵ'_M and ϵ'_L, and the conditional constant of the metal–ligand complex, K'_{ML}, are known. From equations (2), (10) and (11) an expression for [ML'] can be derived:

$$[ML'] = \frac{1}{2}\left[\left(C_L + C_M + \frac{1}{K'} \pm \sqrt{\left(C_L + C_M + \frac{1}{K'}\right)^2 - 4C_M C_L}\right)\right]. \qquad (16)$$

This value for [ML'], combined with calculated values for [M'] and [L'] [equations (10) and (11)], gives the absorbance at every point of the titration curve (equation (1)], when a correction is applied for dilution during the titration:

$$A_{exp} = A_{calc}\left(\frac{V_0}{V + V_0}\right) \qquad (17)$$

where V_0 = initial volume and V = volume of titrant added.

Alternatively, the experimental value can be correspondingly corrected to obtain A_{calc}.

Curves for different acidities (different values of the conditional constant) and different concentrations of

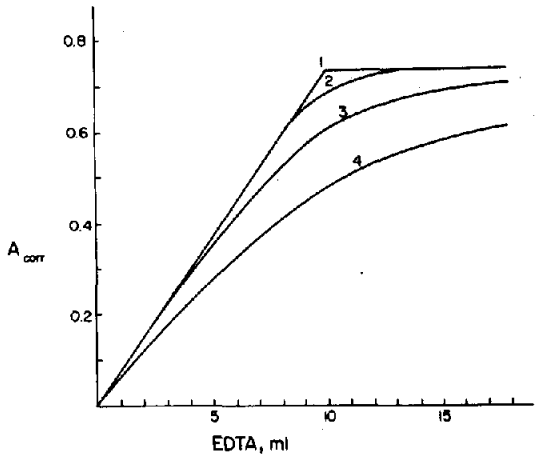

Fig. 2. The absorbance of $10^{-4}M$ lead solutions titrated with $10^{-3}M$ EDTA at different acidities. 1—pH = 3.0, 2—pH = 2.5, 3—pH = 2.2, 4—pH = 2.0.

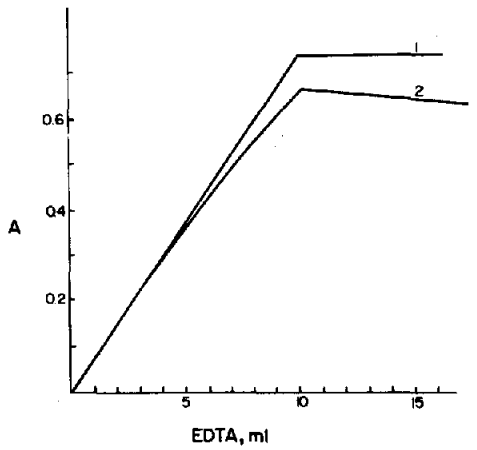

Fig. 4. The absorbance of a $10^{-4}M$ lead solution at pH ≥ 3 titrated with $10^{-3}M$ EDTA. 1—corrected curve, 2—uncorrected curve.

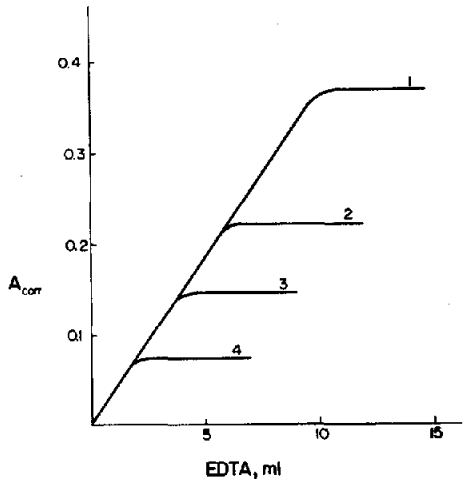

Fig. 3. The absorbance of lead solutions of pH 3.0 titrated with $10^{-3}M$ EDTA. 1—$C_M = 5 \times 10^{-5}M$, 2—$3 \times 10^{-5}M$, 3—$2 \times 10^{-5}M$, 4—$10^{-5}M$.

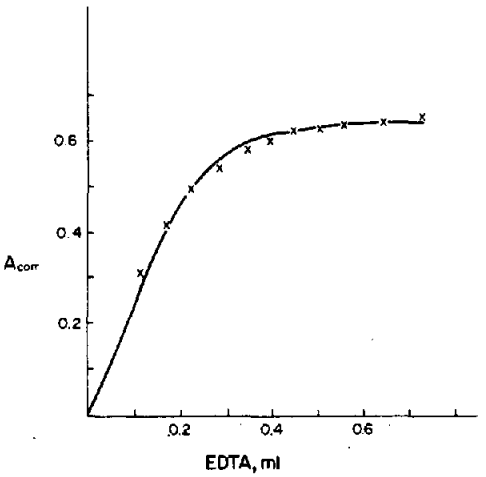

Fig. 5. The experimental curve (crosses) for titration of a $9 \times 10^{-5}M$ lead solution at pH 2.18 with $0.035M$ EDTA, compared with the theoretical curve.

titrand in the titration of lead with EDTA are shown in Figs. 2 and 3.

From Fig. 2 it is apparent that lead cannot be titrated accurately with EDTA at pH values lower than about 3 and then only if the concentration of the titrand is at least $10^{-5}M$ (Fig. 3).

In comparing calculated curves with experimental ones, equation (17) should always be borne in mind: *i.e.*, experimental absorbance values should be corrected for the dilution of the solution before any comparison is made. Figure 4 shows the effect of dilution on an experimental curve. The first part of the curve is a distinctly curved line, which causes an error in the determination, whereas the corrected curve shows two straight-line portions which can be extrapolated to intersect at the equivalence point.

Figure 5 compares a calculated curve with an experimental one determined with the same instrumentation as the curves in Fig. 1.

Some possibilities for utilizing curved lines will be discussed later.

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ANNOTATIONS

AUTOMATED GRAVIMETRIC TITRATIONS

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Summary—Gravimetric titrations are performed by an automated system consisting entirely of readily available components, such as a digital electronic top-loading balance, a pH-meter, a titration controller, a titration valve assembly, and related accessories. Substantial increases in accuracy and speed result, along with improved data-handling capability and increased system flexibility.

Titration is a form of quantitative analysis in which a reagent of known concentration is matched, under equilibrium conditions, against the species to be determined. The amount of the titrant used to reach the real, or derived, equivalence point is readily translated into the value sought. Since titrations are generally performed with solutions and volumes are readily and inexpensively measured, the term "volumetric" has long been regarded as synonymous with "titrimetric". This is particularly inappropriate, because titrations of the highest accuracy have always been performed with weight burettes. Besides having the advantage of the inherently greater accuracy in determining mass instead of volume, the measurement is not subject to errors related to the temperature expansion coefficient of the titrant. It also simplifies the preparation of the solutions. The concentration of the titrant is simply defined in terms of milliequivalents (or other mass units) per kilogram, instead of milliequivalents per litre.

The advantages of gravimetric (weight) titrations have long been acknowledged, with recent work stressing the availability of new equipment.[1,2] In particular, the widely available digital electronic top-loading balances and their associated data-handling and data-acquisition accessories make gravimetric titrations most attractive, particularly where partial or full automation is desired. Of primary importance here is the capability of these balances for fast electronic taring of the load, which performs the equivalent of the refilling and zeroing of conventional and piston burettes. Further, since their operation is basically force-sensing, there is no significant movement of the pan during operation. This makes it possible to tare not only the titrant-containing bottle on the pan but also, significantly, the attached tubing through which the titrant is delivered to the titration vessel. The balance, after the initial taring, indicates directly the amount of titrant used in the titration. Additional features include stabilization-sensing circuitry which ensures that only true values are acquired, and provision for an analogue output which can be used with an X–Y recorder for drawing titration curves, or for pH-stat applications. The generally available digital output is readily used with compatible calculators/printers for hard copy in any desired units. It can also be used as input to computers when more sophisticated data-handling is required. Overall, these balances make excellent modular components for a variety of titration systems. Since a balance is generally required anyway for the weighing of samples, and a sample and a titrant bottle are interchangeable on the pan, the balance can do double duty, with significant economic advantage. The great flexibility inherent in this approach is another advantage.

Semi-automatic or automatic titration systems can readily be assembled on a modular basis, with the LUFT® Master Controller capable of providing all the needed control and sequencing functions. The Controller is compatible with a wide variety of commonly used measuring equipment such as pH/mV-meters, conductivity meters, colorimeters, turbidimeters, amperometric and Karl Fischer inputs, *etc.* The value of the variable measured in the titration vessel is fed through the recorder output of the meter to the Controller which, in a feed-back loop, brings the variable quickly and without overshoot to a predetermined value corresponding to the end-point. It does so by means of a dual, parallel, automatic solenoid valve assembly (LUFT® A-GT) which controls the flow of the titrant from the bottle on the balance pan to the titration vessel. All wetted surfaces of the unit are made of either glass or Teflon®, and the flow-rates through the parallel channels are set by adjustment of integral stopcocks.

The discontinuous point-to-point algorithm[3] used by the Controller has been subjected to theoretical[4] and practical[5–7] investigations, and provides a mini-

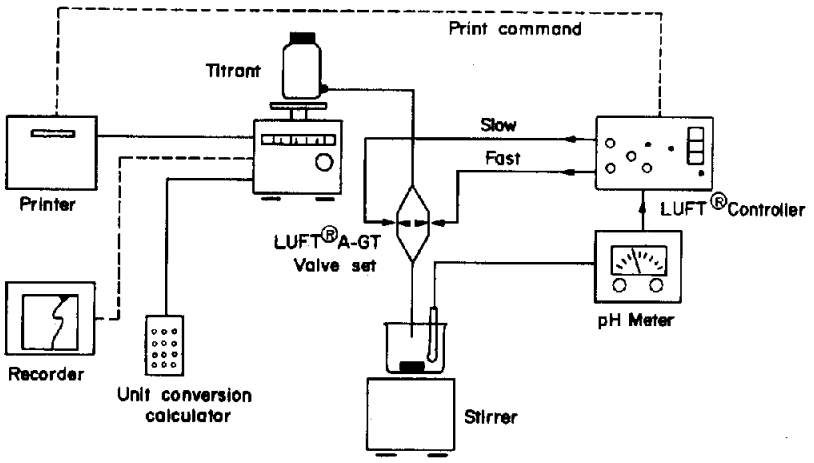

Fig. 1. Typical arrangement for automated gravimetric titration.

mum-time, no-overshoot action in linear and non-linear systems alike. It does this by an initially high flow-rate which brings the variable (*e.g.*, pH) rapidly to the knee of the titration curve, and then automatically switches to an adjustable low flow-rate for continuing the titration to the preset end-point value. At the end-point an adjustable time-delay circuit takes over to ensure that the solution has reached equilibrium, based on the constancy of the variable over a specific time-period. When equilibrium is confirmed, the Controller automatically terminates titration control and activates separate outputs which are readily used to activate a print-out, transfer data to a computer, take a new sample, reprogramme the Controller for a new end-point, or initiate the next step in a sequential operation. The action of the Controller can be easily interrupted (stepped) during the titration to allow for special system dynamics. The Controller is well suited for automation of a variety of titration procedures, including back-titrations and successive titrations in the same solution.

The basic system shown (Fig. 1) is capable of completing a typical acid–base titration in 10–20 sec. Overall cycle times are also much faster than usual, since no time is required to refill a burette; the electronic tare performs the equivalent function faster and more reliably. The end-point is reached with a precision of about 0.01 pH units, and many balances can be read to 1 mg—an order of magnitude improvement over the readability of most burettes. Other configurations can be used when titration curves have to be recorded, or more complex procedures or calculations are called for.

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THE PURIFICATION OF WATER FOR INORGANIC ULTRATRACE ANALYSIS

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Summary—Various methods of preparing high-purity water are compared in terms of the cadmium, lead and copper contents of the product. The best system of those tested is the Millipore Milli-Q system, but it is strongly recommended that it should be fed with distilled (*not* demineralized) water.

In ultratrace analysis for heavy metals the use of water of high purity is a necessity. For electrochemical methods in particular, the water should be free from metals, as well as from organic impurities which may be adsorbed on the surface of the electrodes. In contrast, methods such as atomic-absorption spectrometry are not similarly influenced by traces of organic material.

There are many ways of purifying water on the laboratory scale; in this work the different methods are discussed, and the water qualities obtained are characterized by analysis for copper, lead and cadmium with differential pulse anodic stripping voltammetry.

EXPERIMENTAL

Water purification systems

The central demineralization system consisted of a scavenger (anionic) ion-exchange resin and a mixed bed (anionic and cationic) resin. PVC pipes were used throughout the system.

For normal distillation a Fisons water still with 4 l./hr capacity was used. The apparatus, which was made from Pyrex glass, was fed with demineralized water.

A Heraeus-Schott Bi 18 still with 1.8 l./hr capacity was used for double distillation in quartz. The still was fed with tap water instead of demineralized water, to avoid contamination by organic material from the ion-exchange resins.

The optimal water purification system consisted of a Fi-stream 4 l./hr water still (Fisons Scientific Apparatus), which was fed with centrally demineralized water, and a Milli-Q water system (Millipore); the complete set-up is shown in Fig. 1. In some preliminary work the Fi-stream unit was replaced by a special prefilter (Millipore MF-Lifegard cartridge) which removed most of the organic material in the demineralized feed water, before this entered the Milli-Q system.

Measurements

The water samples were analysed for copper, lead and cadmium by differential pulse anodic stripping voltammetry. A 0.1 M acetic acid–sodium acetate buffer (pH 4.7) served as supporting electrolyte; only Suprapur (Merck) chemicals were used. Details of the analytical procedure are given elsewhere.[1,2]

RESULTS AND DISCUSSION

The concentrations of copper, lead and cadmium in the different water samples are given in Table 1.

The results for the centrally demineralized water are given in the first column; obviously the central demineralization system removes heavy metals very efficiently, and the water quality obtained may be quite satisfactory for many purposes. However, the water contains relatively large amounts of organic material from the ion-exchange resins.

The values given in the next column in Table 1 indicate that very little is gained by using a simple

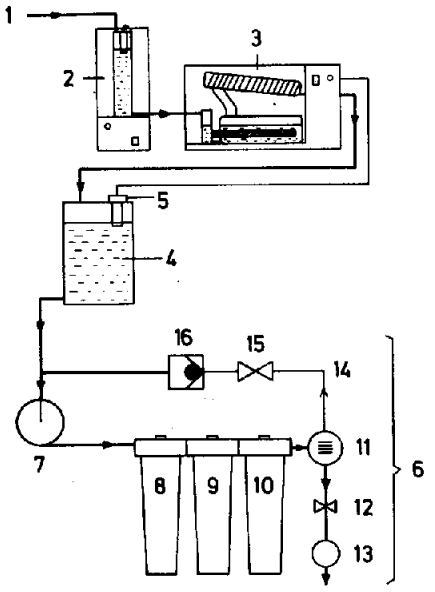

Fig. 1. Recommended water purification system. 1, Inlet for centrally demineralized water; 2, flow controller (only necessary for centrally demineralized water); 3, Fi-stream water still; 4, water reservoir, polyethylene tank; 5, pressure switch; 6, Millipore Mille-Q system; 7, pump; 8, activated carbon cartridge; 9, 10, ion-exchange cartridges; 11, resistivity meter; 12, ball valve; 13, membrane filter 0.22 μm; 14, recirculation path; 15, flow controller; 16, check valve.

Table 1. Concentration (μg/l.) of copper, lead and cadmium in water purified by different methods

Metal	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

distillation of the centrally demineralized water. However, the distilled water presumably contains less organic material. The metal concentrations would possibly have been lower if the water still had been cleaned thoroughly at very frequent intervals, although some metals might have been carried over with traces of the organic resin material.

A significant improvement in purity is obtained when the water is doubly distilled in quartz, as shown in Table 1 and Fig. 2c. It is here worth mentioning

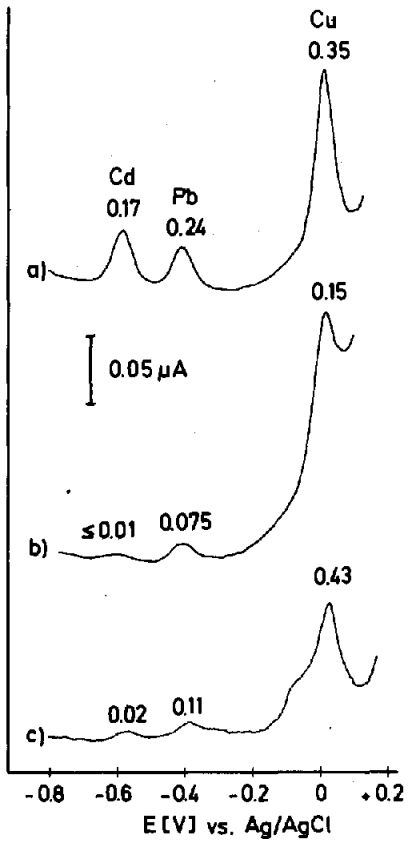

Fig. 2. Anodic stripping voltammograms of different water samples; a 0.1 *M* acetate buffer was used as supporting electrolyte. The numbers above the signals represent the concentrations in μg/l. (*a*) Water from the Milli-Q system, illustrating the insufficiency of the normal cleaning procedure, after break-down of the system owing to the use of centrally demineralized feed water; electrolysis time 5 min. (*b*) Water from the Milli-Q system after optimal performance had been restored; electrolysis time 15 min. (*c*) Doubly quartz-distilled water; electrolysis time 10 min.

that the apparatus was fed with tap water, to minimize the amount of organic material within the system. The main disadvantage of the double distillation procedure is the relatively low production capacity of the system (*ca.* 2 l./hr).

The lowest concentrations of copper, lead and cadmium were obtained with the Milli-Q system, as shown in the last column in Table 1 and in Fig.2b. The real concentrations in the water sample are probably even lower than the values given in the Table, because these also include trace metals from the acetate buffer used. The Milli-Q system has a very high draw-off rate (1.5 l./min) which will meet the requirements of most laboratories.

It is highly advisable to use a water still unit (see Fig. 1) to provide the feed water for the Milli-Q system. Above all, water from a central demineralization source should never be used to feed the Milli-Q system. Contrary to the information given by the manufacturer,[3] that suspended matter, gelatinous materials and slime from centrally demineralized water can be removed by a special MF-Lifegard cartridge, we found that this cartridge only delayed for a short while the complete break-down of the whole system, when demineralized feed water was used. Furthermore, after such a break-down it proved difficult to restore the optimal purification efficiency of the Milli-Q equipment. A normal cleaning of the housing bowls followed by sterilization with sodium hypochlorite[3] and replacement of the cartridge elements proved insufficient, as illustrated in Fig. 2a. The concentrations found were considerably higher than those claimed by the manufacturer.[4]

However, an additional cleaning of all parts of the system, including all the tubing, with a neutral detergent (Extran MA 02, Merck), followed by a recirculation of the detergent solution and repeated rinsing with distilled water, finally restored the expected high performance of the Milli-Q system.[4]

The use of a modern automatic water still for providing feed water eliminated the above-mentioned problems. The cost of such an apparatus is quickly repaid by the increased lifetime of the rather expensive cartridge elements of the Milli-Q system. The water still is fed with either tap water, or water from a central demineralization source.

Finally, it should be mentioned that there is no point in obtaining water of high purity, if not all the other precautions necessary in ultra trace analysis are strictly adhered to. Generally, the purified water

should be used as soon as possible, to avoid any contamination during storage.

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DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN INDUSTRIAL WASTE WATER AT THE ng/ml. LEVEL

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Summary—A combination of column, thin-layer and capillary gas chromatography was investigated as a quantitative method for determination of polycyclic aromatic hydrocarbons in industrial waste water at the 10–100 ng/ml level. The method gives 90–95% overall recovery. Analytical results for polycyclic aromatic hydrocarbons in waste water from a Norwegian aluminium plant are presented.

Because of their carcinogenic nature and great stability in natural biological systems, polycyclic aromatic hydrocarbons have attracted increasing attention during the last few years. Polycyclic aromatic hydrocarbons (PAHs) occur naturally in crude oil and certain marine sediments.[1] They also form important constituents of several kinds of pitch. Small amounts of PAHs are found in cigarette smoke, automobile exhaust gases, smoked foods and in certain industrial effluent gases and waters.

Hellmann[2] has reported a simplified routine determination of PAHs in waste water by means of thin-layer chromatography and fluorimetry, starting with extraction into carbon tetrachloride. This is often the preferred method when individual components are not to be determined. A gas-chromatographic method using a short, packed column was described by Fryćka.[3] This method is capable of separating critical pairs of PAHs, *e.g.*, benzo[*a*]pyrene and benzo[*e*]pyrene, in the course of a few minutes. The use of glass capillary gas chromatography for determination of PAHs in cigarette smoke was first reported by Grimmer.[4] For several types of sample, the initial separation of PAHs from water is still accomplished by slightly modified versions of this method,[4] *viz.* extraction into cyclohexane, washing the cyclohexane extract with dimethylformamide, and gas-chromatographic determination of the individual components. One or two internal standards are used in quantitative determinations. Glass capillary columns with 80,000–120,000 theoretical plates are required.

Owing to their very low solubility in water, PAHs occurring in industrial waste water are largely adsorbed on suspended solids. Analyses performed in our laboratory indicate that waste water from the aluminium industry has a PAH content of 10–150 μg/l. after filtering through a Micropore filter.

The quantitative removal of organic pollutants from water is usually achieved by extraction into an organic solvent. This process is, however, time-consuming and often necessitates the use of large volumes of solvent. In order to circumvent these drawbacks, adsorption on a solid phase is often used. Musty and Nickless[5] reported the use of Amberlite XAD ion-exchangers for sorption of chlorinated insecticides and polychlorinated biphenyls (PCBs) from water. Gesser *et al.*[6] used porous polyurethane resin as a sorbent for PCBs. The use of Tenax (a newly developed porous polymer based on 2,6-diphenylphenylene oxide), as a promising sorbent for both PCBs and PAHs was first described by Leoni *et al.* in 1975.[7]

In the present work we report a series of experiments, using Tenax for the removal of PAHs from standard water solutions. The method is applied to waste water samples from a Norwegian aluminium plant. A detailed analytical procedure is described for the routine determination of PAHs in industrial waste water. A combination of column, thin-layer and gas-chromatographic techniques is used.

EXPERIMENTAL

Standard solutions

Pure PAHs were initially dissolved in acetone. Stock solutions were prepared by dropwise addition of 10 ml of these solutions to 10 litres of vigorously stirred water. In order to ensure complete dissolution, the stirring was continued overnight. The composition of the standard water solutions is shown in Table 1.

Table 1. Composition of water stock solution

Standard solution	Concentration, μg/l.
Anthracene	11
Pyrene	32
Chrysene	43
3-Methylcholanthrene	12
Total PAH	98

Reagents and apparatus

Chromatographic column (Quickfit CR 13/30) (Fig. 1); Perkin–Elmer F33 gas chromatograph with PEP 2 electronic integrator system; Merck DC Alufolien, Kieselgel 60 F254 thin-layer plates, 20 × 20 cm. Tenax GC 60/80 mesh.

All solvents were Merck, analytical grade.

Procedure

Water samples are passed through the Tenax column at a rate of about 5 ml/min. Residual water is removed from the Tenax by passing nitrogen gas through the column. The Tenax material is then transferred to a Soxhlet apparatus. PAH and other organic compounds are extracted by reflux for 4 hr with 35 ml of acetone. The Tenax can then be dried and reused. Preliminary experiments indicate that the extraction time can be reduced to 10–15 min by the use of ultrasonic extraction. It is not known, however, whether the Tenax material can then be reused. Residual water is removed from the acetone extract by passing the solution through a small column of anhydrous sodium sulphate and washing with 5 ml of acetone. The extract is evaporated on a water-bath at 50° in a stream of nitrogen to a volume of approximately 0.5 ml. The residual solution is transferred to a thin-layer plate. The thin-layer plates are developed in a 4:1 v/v mixture of hexane and benzene. The time required for the development is 30–35 min. A single development of the TLC plate is sufficient for separating PAH from other compounds such as paraffins, naphthenes, acids and phenols. The spots are located visually under a UV-lamp and the areas containing aromatic compounds are marked. The appropriate portions of the thin-layer are scraped into a glass flask. PAHs are extracted by vigorous shaking for 2 hr with 5 ml of chloroform. Extraction of PAHs from the thin-layer material is a critical step in the procedure. (Extraction from laboratory prepared plates was easier than from commercial plates, *e.g.*, Alufolien, Merck.) Extraction into approximately 1 ml of chloroform by treatment in an ultrasonic bath for about 10 min was shown to be the best procedure. The internal standard is added to the chloroform in advance so that the samples can be analysed directly after centrifugation. After filtration or centrifugation, the solution may be used for spectrophotometric determination of total PAHs and gas-chromatographic determination of individual PAH components.

The conditions for the gas chromatography are as follows.

Column: glass capillary, 25 m × 0.28 mm coated with SE-30, detection by flame ionization detector.
Carrier gas: helium at a flow-rate of 2 ml/min.
Injector temperature: 300°.
Detector temperature: 300°.
Volume injected. 1 μl.
Column temperature: initially 100°, held for 4 min before programme starts.
Programming: 3°/min.
Final temperature: 260°.

RESULTS AND DISCUSSION

Extraction and thin-layer chromatography

The separation of PAHs from water on the Tenax column is the first and the most critical step in the analytical procedure. Unsatisfactory results are often due to neglect of details at this step. The most important operating parameters are the height of the Tenax column and the flow-rate of the water sample through the column. In this work, the height of the column was decreased from 30 to 10 cm without any appreciable decrease in PAH recovery. The optimum flow-rate was found to be 8–10 ml/min, for a Tenax column of 10 cm height and 13 mm diameter. This should be contrasted with earlier reports[7] where a flow-rate of 50 ml/min was used. In our work a flow-rate of 50 ml/min would correspond to a significant leakage in the Tenax column. The concentration of PAHs in the standard water solution and in water which had been passed through the Tenax column was checked by means of liquid–liquid extraction.

The PAH values obtained by gas chromatography are presented in Table 2. As indicated, excellent agreement is obtained at the 100-ng/ml level between the present method and the much more time-consuming liquid–liquid extraction technique. Table 2 shows the overall recovery of PAHs from water at the 100- and the 10-ng/ml levels. At the 100-ng/ml level the recovery is 90–95%. At the 10-ng/ml level the recovery decreases to 70–90% and the spread of the analytical results increases. At or below the 10-ng/ml level, the amount of PAHs recovered may be increased by using a larger volume of water, for instance 5 or 10 litres.

Unused Tenax may contain small amounts of low molecular-weight components which can interfere with PAH components of the group chrysene, benzofluoranthene, benzo[*e*]pyrene, on a thin-layer chromatographic plate. It is therefore recommended that unused Tenax be extracted with acetone overnight. As shown in Fig. 2, the acetone extract of unused Tenax

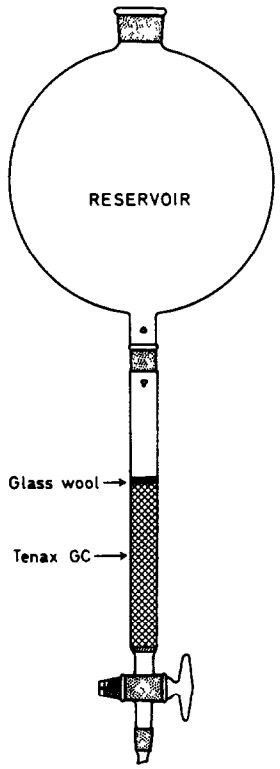

Fig. 1. Sorption column with Tenax bed.

Table 2. PAH yield from water samples

Hydrocarbon	Amount added μg/l.	Liquid–liquid extraction* μg/l.	Present method, undiluted sample μg/l.	±s.d.†	Present method, 10 times diluted sample μg/l.	±s.d.
Anthracene	11.0	10.3	10.2	0.5	0.8	0.1
Pyrene	32.0	30.8	30.2	0.7	2.6	0.1
Chrysene	43.0	41.3	40.3	0.6	3.2	0.3
3-Methylcholanthrene	12.0	10.9	11.0	0.3	0.9	0.1
Total	98.0	93.3	91.7		7.5	
Total yield, %		95	94		77	

* Glass capillary column GC method.
† All standard deviations (μg/l.) are based on 4 parallel results.

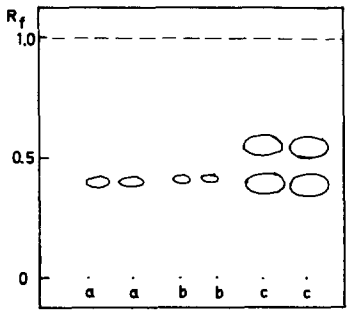

Fig. 2. TLC of unused Tenax extracted with acetone: a, b, two different Tenax batches (unused) extracted with acetone; c, acetone PAH solution, 25 μg of PAH per spot.

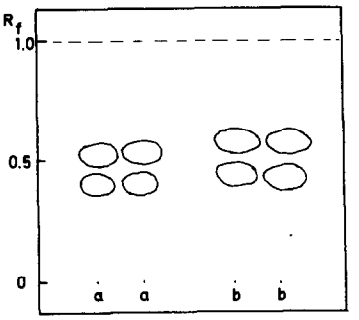

Fig. 3. TLC of PAH on Kieselgel 60 F254 (Merck): a, sorbed on Tenax and extracted into acetone (initial concentration corresponds to 25 μg of PAH per spot); b, initial acetone solution 25 μg of PAH per spot.

Table 3. Typical results for waste water from an aluminium plant

Substance	Sample 1 Run 1, μg/l.	Sample 1 Run 2, μg/l.	Sample 2 Run 1*, μg/l.	Sample 2 Run 2, μg/l.
Biphenyl	3.2	3.6	<1	<1
Fluorene/fluorenone	6.3	8.4	2.9	3.2
Phenanthrene	16.9	23.1	14.2	14.0
Anthracene	2.8	2.8	1.1	1.2
Fluoranthene	20.8	18.9	10.8	12.4
Pyrene	15.3	12.7	5.6	6.0
Benzo[*a*]fluorene	3.2	3.4	1.6	1.5
Benzo[*b*]fluorene	2.8	3.0	1.3	1.3
Benzdiphenylensulphide	3.4	3.5	2.0	1.7
Benzo[*a*]anthracene	2.5	2.8	5.6	5.5
Chrysene/triphenylene	5.8	6.0	15.6	16.0
Benzo[*b,k*]fluoranthene	6.8	6.8	38.1	38.0
Benzo[*e*]pyrene	2.6	2.7	16.2	16.4
Benzo[*a*]pyrene	1.3	1.5	7.0	7.4
Dibenzoanthracene	3.4	4.3	8.2	8.0
Anthanthrene	<1	<1	3.2	3.2
Coronene	<1	<1	1.9	2.0
Dibenzopyrene	<1	<1	4.0	4.3

* The chromatogram for this run is shown in Fig. 4.

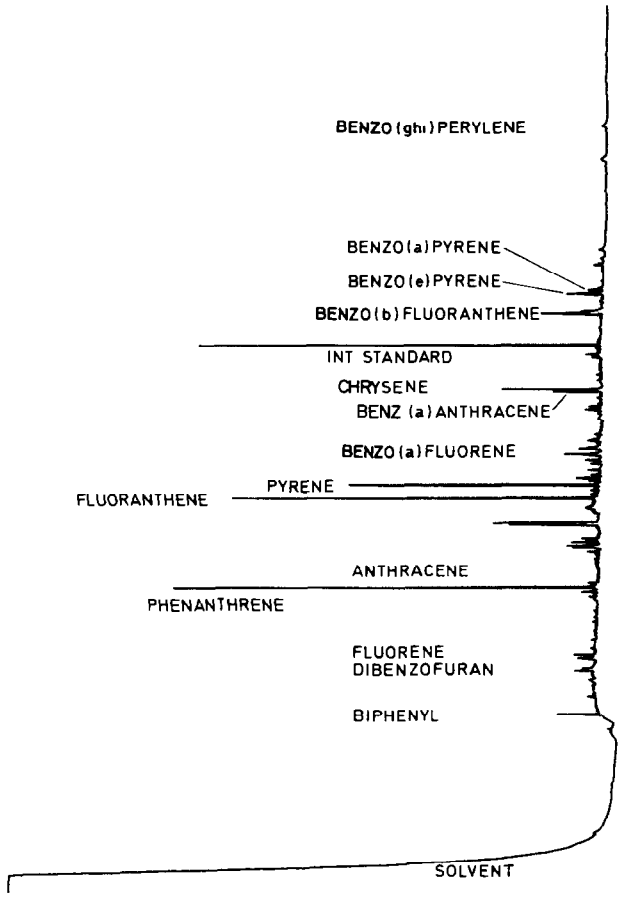

Fig. 4. Typical chromatogram of industrial sample with standard added.

gives rise to fluorescent spots on the TLC plate, with a retention factor of about 0.4. Since several PAH components have about this retention factor, these artifacts will interfere in the TLC procedure. Judging from their intensity, this interference will be small at the 100-ng/ml level, but may become dominant at lower concentrations. These artifacts will also interfere in the gas-chromatographic analysis.

When analysing industrial waste water, it is advisable to protect the Tenax column against irreversible contamination. This can be achieved by using a short Celite 545 prefilter.

The TLC plate must be preconditioned in chloroform before use. This preconditioning is particularly important when the PAH-level is very low, 10 ng/ml or less. One-dimensional TLC will give a good separation of semipolar PAH components from non-polar paraffins and naphthenes which will follow the solvent front, and from the more polar acids and phenols which will remain at the bottom of the TLC plate. Figure 3 shows a thin-layer chromatogram of four PAH components. Under ultraviolet light, the chromatographic spots may serve as a visual indication of the amount of PAH. In Fig. 3 it is noticeable that the total area of the chromatographic spots on the left-hand side (a) of the TLC plate is less than the area to the right (b). Both sets of chromatographic spots correspond to the same initial amount of PAH (50 μg). The spots to the left, however, correspond to PAH components which have passed through the analytical procedure and thus have suffered a loss of about 10%. This loss is visible on the TLC plate.

In Table 3 and Fig. 4 we show results obtained for waste water samples from a Norwegian aluminium plant. Parallel determinations were carried out on samples of 500 ml each. The standard deviation for the series is approximately 1 μg/l.

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QUANTITATIVE SEPARATION OF GALLIUM FROM ZINC, COPPER, INDIUM, IRON(III) AND OTHER ELEMENTS BY CATION-EXCHANGE CHROMATOGRAPHY IN HYDROBROMIC ACID–ACETONE MEDIUM

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Summary—Gallium can be separated from Zn, Cu(II), In, Cd, Pb(II), Bi(III), Au(III), Pt(IV), Pd(II), Tl(III), Sn(IV) and Fe(III) by elution of these elements with 0.50*M* hydrobromic acid in 80% acetone medium, from a column of AG50W-X4 cation-exchange resin. Gallium is retained and can be eluted with 3*M* hydrochloric acid. Separations are sharp and quantitative except for iron(III) which shows extensive tailing. With 0.20*M* hydrobromic acid in 80% acetone as eluting agent, all the species above except iron(III) and copper(II) can be separated from gallium with very large separation factors. Only a 1-g resin column and small elution volumes are required to separate trace amounts and up to 0.5 mmole of gallium from more than 1 g of zinc or the other elements. Hg(II), Rh(III), Ir(IV), Se(IV), Ge(IV), As(III) and Sb(III) have not been investigated, but should be separated together with zinc according to their known distribution coefficients. Relevant elution curves, results for the analysis of synthetic mixtures and for amounts of some elements remaining in the gallium fraction are presented.

Probably the first method using ion-exchange chromatography for a selective and generally applicable separation of gallium from other elements is that described by Kraus *et al.*, utilizing selective adsorption of the chloride complexes of the elements on anion-exchange resins.[1] Iron(III), uranium(VI) and antimony(V) are among the few elements which accompany gallium. To separate the iron, reduction to the bivalent state (which is not sorbed) has been recommended. Titanium(III),[2] metallic iron[3] and a silver reductor[4] have been suggested as reducing agents. Ascorbic acid, which is one of the most generally used reducing agents for iron(III), does not seem to be quantitative in the presence of both a high concentration of hydrochloric acid and an ion-exchange resin which preferentially sorbs the higher oxidation state of iron or its complexes. According to our own experiments a small fraction of the iron is always retained by the resin. Korkisch *et al.*[5] have shown that iron(III) and gallium can be separated by anion-exchange of the thiocyanate complexes. The separation factor is quite small and only microgram amounts of gallium can be separated from a maximum of 1.25 mg of iron, while recoveries are only between 96 and 98%. An anion-exchange separation of gallium from indium and aluminium in hydrochloric acid–2-methoxyethanol and hydrochloric acid–acetone mixtures has been described by Korkisch *et al.*[6] Iron(III) accompanies gallium and the behaviour of most other elements has not been investigated.

The fact that gallium forms considerably more stable complexes than zinc with organic ligands such as tartrate, oxalate, sulphosalicylate and EDTA can be utilized to effect anion- as well as cation-exchange separations.[7] Many other univalent and bivalent ions are separated together with zinc, but tervalent ions such as indium and iron(III) will accompany gallium. Furthermore, the final determination step often requires the destruction of the organic reagent. This can be quite tedious. In ammonium carbonate solutions containing excess of ammonia, gallium forms anionic carbonate complexes, while zinc, copper and nickel form cationic ammine complexes. This has been utilized for the development of cation- and of anion-exchange separations.[8]

Unfortunately, many elements are insoluble or have limited solubility in ammonium carbonate solutions. This limits the general applicability of the method very considerably. Instead of ammonium carbonate, a sodium hydroxide–ammonia mixture can be used, leading to the formation of anionic hydroxide complexes of gallium which pass through a cation-exchanger column, while the ammine complexes of nickel, cobalt, zinc and copper are retained.[9] Again, the method has limitations because many elements are insoluble, and aluminium is among the elements which accompany gallium.

Nelson *et al.*[10] have used anion-exchange in hydrochloric–hydrofluoric acid mixtures for the separation of gallium from zinc, arsenic and germanium. The presence of hydrofluoric acid makes this approach less attractive and various elements, among them iron(III), will accompany gallium.

In order to separate gallium from iron(III), Blasius *et al.*[11] have sorbed gallium and iron(III) from oxalate solution at pH 4 on an anion-exchanger and then eluted gallium preferentially with 1*M* sodium hydroxide. This approach can also separate gallium from

many other elements, but not from aluminium, and the sodium and oxalate in the eluate make it unattractive for further work. A cation-exchange procedure to separate gallium from As(III), Sb(III), Ge, In, Cu(II), Zn, Pb(II) and iron(II) has been described by Klement *et al.*[12] The separation factors for the separation from Cu(II), Zn(II) and Fe(II) are rather small with a value of about 2, and large columns (100 cm long) and elution volumes (2 litres) have to be used for the quantitative separation of larger amounts of these species. Iron(III) cannot be separated satisfactorily.

Only the first of the methods[1–4] described above is of wide general applicability. Available information on distribution coefficients[13] indicates that by use of cation-exchange in hydrochloric acid–acetone mixtures, very much larger separation factors (than in aqueous hydrochloric acid) can be obtained for separation of gallium from many elements. The separation from iron(III), however, is still rather poor. Hydrobromic acid and its mixtures with acetone do not seem to have been investigated as possible eluents for the selective separation of gallium. An investigation of available data on cation-exchange distribution coefficients[14] reveals that separation factors for the separation of gallium from many critical elements, such as zinc, indium, cadmium and tin, are extremely large in this system and can reach values of more than 1000 in 0.2*M* hydrobromic acid in 80% acetone medium. Even the Ga–Fe(III) pair, which is difficult to resolve in hydrochloric acid, has a reasonable separation factor of about 10 in the 0.5*M* hydrobromic acid–80% acetone medium. The separation of gallium from a wide variety of other elements in this system has therefore been investigated in more detail. Because gallium and especially iron(III) show considerable tailing with 8% cross-linked resin,[14] a 4% cross-linked resin was used for the investigation. This paper describes the separation from those elements which are less strongly retained than gallium.

EXPERIMENTAL

Reagents and apparatus

Gallium chloride puriss. prepared from 99.99% pure gallium was obtained from Fluka AG, Switzerland. All other reagents used were of analytical-reagent grade. Water was distilled and then passed through an Elgastat demineralizer before use. The resin was the AG50W-X4 sulphonated polystyrene cation-exchanger of 200–400 mesh particle size, marketed by BIO-RAD Laboratories, Richmond, California. Borosilicate glass tubes of 20 or 10 mm bore, with fused-in glass sinters of No. 2 porosity, and a burette tap at the bottom and a B19 or a B14 ground-glass joint at the top, were used as columns. In the description of eluting agents, "0.5*M* hydrobromic acid in 80% acetone" means a 4:1 v/v mixture of acetone and 2.5*M* hydrobromic acid, any volume change on mixing being disregarded.

A Perkin–Elmer 303 and a Varian–Techtron AA-5 were used for atomic-absorption and a Zeiss PMQII for molecular-absorption measurements.

Elution curves

Elution with 0.5M hydrobromic acid in 80% acetone. An ion-exchange column containing 65 ml (15 g dry weight) of AG50W-X4 resin (200–400 mesh) was equilibrated by passage of about 100 ml of 0.2*M* nitric acid in 65% acetone. The resin column was 18 cm in length and 2.15 cm in diameter before equilibration. A solution containing about 1 mmole each of zinc and gallium in about 25 ml of 0.2*M* nitric acid in 65% acetone was passed through the column, and washed onto the resin with the same reagent. The zinc was then eluted with 0.50*M* hydrobromic acid in 80% acetone at a flow-rate of 2.5 ± 0.5 ml/min. Elution was continued until 500 ml of the eluting agent had been passed through the column. The gallium was then eluted with 3.0*M* hydrochloric acid, at the same flow-rate. Fractions (25 ml) were taken with an automatic fraction collector from the beginning of the zinc elution step. After evaporation the dryness the residue was dissolved in 10 ml of 0.1*M* hydrochloric acid. The zinc and gallium in the fractions were determined by atomic-absorption spectrometry, with an air–acetylene flame and the 213.9 and 287.4

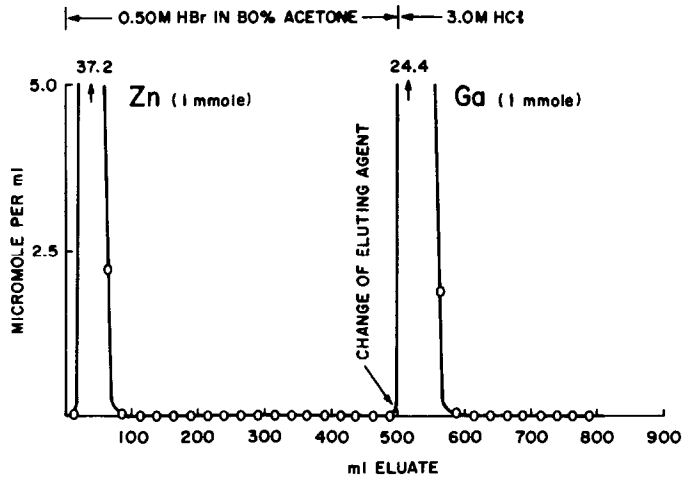

Fig. 1. Elution curve for Zn–Ga. Column of 65 ml (15 g) of AG50W-X4 resin, 200–400 mesh (180 × 21.5 mm). Flow-rate 2.5 ± 0.5 ml/min.

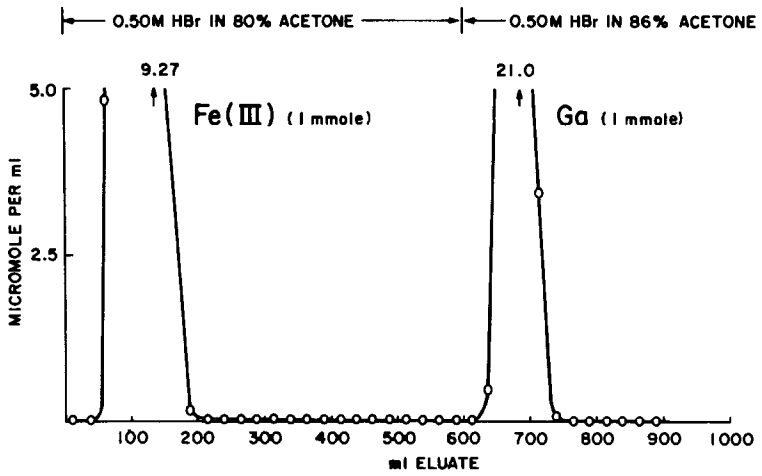

Fig. 2. Elution curve for Fe(III)-Ga. Column conditions as for Fig. 1.

nm lines, respectively, with appropriate dilution when required. The experimental curve is shown in Fig. 1.

Figure 2 shows an elution curve for the iron(III)–gallium pair (1 mmole of each) under the same experimental conditions, but the gallium being eluted with 0.50*M* hydrobromic acid in 80% acetone instead of 3.0*M* hydrochloric acid. In another experiment elution with 0.50*M* hydrobromic acid in 80% acetone was continued until gallium appeared in the eluate. Gallium was first found in the eluate after 700 ml of the eluting agent had been passed through the column. When a column containing 43 ml (10 g) of the same resin was used and 1 mmole of each element was present, gallium first appeared at an elution volume of 325 ml. The experimental curve is illustrated in Fig. 3.

Copper(II) shows a relatively wide elution peak very similar to that of iron(III) but with less tailing. In, Cd and Bi(III) show very narrow peaks similar to that of zinc. When a large amount of lead(II) is present, a precipitate of lead bromide is formed on the column in the border region between the nitric acid and hydrobromic acid reagents. In spite of this the precipitate very soon redissolves and the elution peak for 1 mmole of lead(II) is only very slightly wider than that for zinc (Fig. 1). Au(III), Pt(IV), Pd(II), Tl(III) and Sn(IV) are sorbed from solutions containing halide but appear in the eluate virtually at the solvent front. Their peaks are also quite narrow and similar to that of zinc.

*Elution with 0.50*M *hydrobromic acid in 65% acetone and 0.20*M *hydrobromic acid in 80% acetone.* An ion-exchange column containing 4.3 ml (1 g dry weight) of AG50W-X4 resin (200–400 mesh) was equilibrated by passage of about 10 ml of 0.5*M* hydrobromic acid in 65% acetone. The resin column was 5.5 cm in length and 1.0 cm in diameter before equilibration. A solution containing about 20 mmole of zinc and 0.2 mmole of gallium in about 200 ml of 0.5*M* hydrobromic acid in 65% acetone was passed through the column and the elements were washed onto the resin and zinc was eluted with small portions of the same reagent, at a flow-rate of about 1.0 ± 0.3 ml/min. The elution was continued until a volume of 200 ml had been passed. Fractions (10 ml) were taken with an automatic fraction collector from the beginning of the sorption step. Gallium was then eluted with 3.0*M* hydrochloric acid at a flow-rate of 1.5 ± 0.3 ml/min. The fractions were evaporated to dryness and each residue was dissolved in 10 ml of 0.1*M* hydrochloric acid. Zinc and gallium in the fractions were determined by atomic-absorption spectrometry as described above. The experimental elution curve is shown in Fig. 4. In another experiment the elution with 0.5*M* hydrobromic acid was continued until gallium appeared in the eluate. The first traces were detected (~0.5 ppm) after 500 ml of eluting agent had been passed through. The experiment was repeated on the same column but with 0.20*M* hydrobromic acid in 80% acetone for column equilibration.

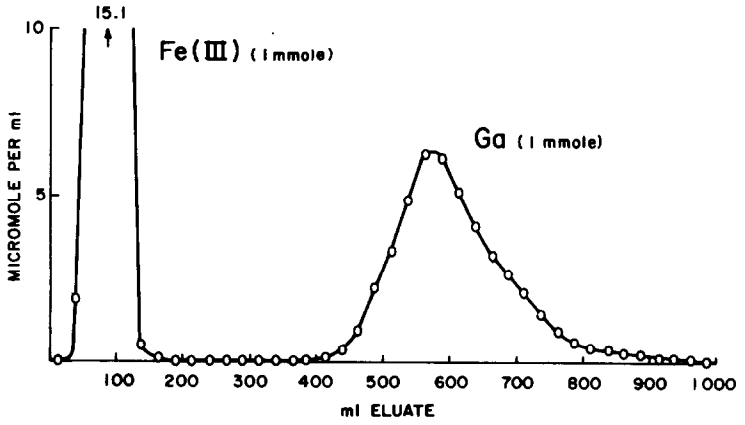

Fig. 3. Elution curve for Fe(III)–Ga with 0.50*M* HBr in 80% acetone. Column of 43 ml (10 g) of AG50W-X4 resin, 200–400 mesh (210 × 13 mm). Flow-rate 2.5 ± 0.5 ml/min.

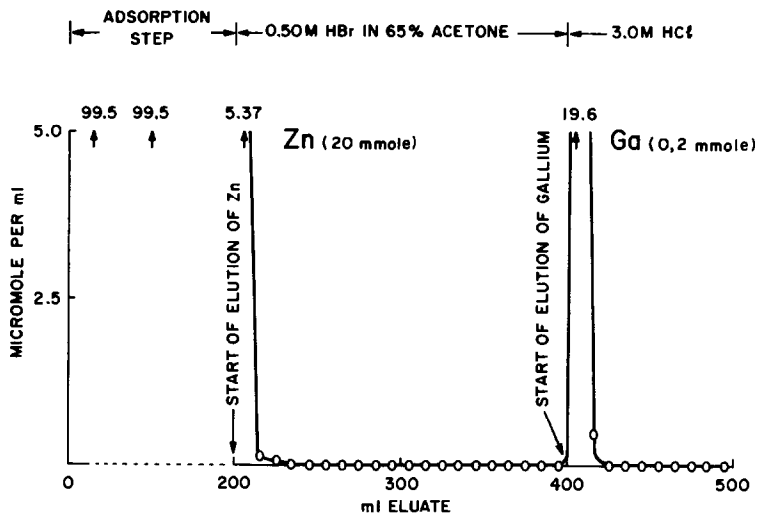

Fig. 4. Elution curve for Zn–Ga. Column of 4.3 ml (1 g) of AG50W-X4 resin, 200–400 mesh (55 × 10 mm). Flow-rate 1.0 ± 0.3 ml/min for 0.5*M* HBr in 65% acetone and 1.5 ± 0.3 ml/min for 3.0*M* HCl.

No gallium (<0.1 ppm) appeared in the first 1000 ml of eluate, but zinc showed slightly more tailing. On the other hand, a faster gravity flow could be maintained (2.0 ± 0.3 ml/min) because of the lower viscosity of the solution.

Quantitative separations of binary mixtures

Separations with 0.50M hydrobromic acid in 80% acetone. Aliquots of standard solutions of gallium and one other element in dilute nitric acid were measured out and mixed with enough acetone and nitric acid to make the final concentrations of these about 65% and 0.3*M* respectively in a volume of 25 ml. Three standards each of gallium and of the other element were measured out for comparison. The synthetic mixtures were passed through columns containing 65 ml of AG50W-X4 ion-exchange resin (200–400 mesh). The columns were 20.5 cm long and 2.0 cm in diameter and had been equilibrated by passage of about 100 ml of 0.3*M* nitric acid in 65% acetone. Complete transfer to the resin was effected by washing with the same reagent. Zn, Cu(II), Fe(III), In, Cd, Pb(II) and Bi(III) were then eluted with 350 ml of 0.50*M* hydrobromic acid in 80% acetone and the eluate was collected for determination. Au(III), Pt(IV), Pd(II), Tl(III) and Sn(IV) were sorbed from 25 ml of 0.5*M* hydrobromic acid in 65% acetone and the eluate was collected for determination from the beginning of the sorption step. Acetone was removed from the column by passage of about 100 ml of 0.3*M* nitric acid and gallium was then eluted with 250 ml of 3.0*M* hydrochloric acid. A flow-rate of about 3.0 ± 0.5 ml/min was used throughout.

The excess of acid and the acetone were removed from the eluates by evaporation and the organic matter in the eluates was destroyed by a sulphuric–nitric acid treatment (small amounts of other elements) or an ion-exchange step (large amounts of other elements), when required. Tartaric acid was added to solutions containing tin(IV), before evaporation, to prevent loss of tin. The amounts of the elements in the samples and standards were then

Table 1. Quantitative separation of synthetic mixtures* (elution with 0.50*M* HBr in 80% acetone)

Taken, *mg*			Found, *mg*	
Ga	Other element		Ga	Other element
72.14	Zn	66.91	72.14 ± 0.05	66.93 ± 0.04
72.14	Zn	0.0335	72.13 ± 0.04	0.0336 ± 0.0005
0.361	Zn	133.8	0.360 ± 0.003	133.8 ± 0.1
72.14	Cu(II)	67.32	72.13 ± 0.05	67.31 ± 0.04
72.14	Cu(II)	0.0673	72.14 ± 0.02	0.0673 ± 0.0003
0.361	Cu(III)	134.6	0.361 ± 0.003	134.7 ± 0.1
72.14	Fe(III)	56.08	72.15 ± 0.04	55.80 ± 0.09
72.14	Fe(III)	0.561	72.16 ± 0.04	0.550 ± 0.005
0.721	Fe(III)	112.2	0.719 ± 0.004	112.2 ± 0.1
72.14	In	115.7	72.14 ± 0.04	115.6 ± 0.1
71.14	Cd	112.9	72.13 ± 0.03	112.9 ± 0.1
72.14	Pb(II)	205.1	72.15 ± 0.05	205.1 ± 0.1
72.14	Bi(III)	206.4	72.14 ± 0.05	206.3 ± 0.2
72.14	Au(III)	193.8	72.15 ± 0.03	193.8 ± 0.2
72.14	Pt(IV)	194.6	72.13 ± 0.04	194.5 ± 0.2
72.14	Pd(II)	107.1	72.14 ± 0.04	107.2 ± 0.1
72.14	Tl(III)	203.3	72.16 ± 0.04	203.2 ± 0.2
72.14	Sn(IV)	117.3	72.14 ± 0.03	117.4 ± 0.3

* Average of 3 determinations.

Table 2. Quantitative separation of synthetic mixtures* (elution with 0.20*M* HBr in 80% acetone)

Taken, *mg*			Found, *mg*	
Ga	Other element		Ga	Other element
14.43	Zn	~1340	14.43 ± 0.02	not determined
0.361	Zn	~1340	0.360 ± 0.003	not determined
0.0361	Zn	~1340	0.0362 ± 0.0006†	not determined
0.361	In	~1160	0.361 ± 0.004	not determined
0.361	Cd	~1130	0.361 ± 0.003	not determined
0.361	Bi	~1030	0.360 ± 0.004	not determined

* Average of 3 determinations.
† Spectrophotometry as complex with Methylthymol Blue.

determined. Duplicate blanks on all the reagents used were run through the whole procedure and the results corrected accordingly. In addition, the residual amounts of some significant elements remaining in the gallium fractions were determined by atomic-absorption spectrometry. Results for the separations are presented in Table 1, residual amounts of some elements in the gallium fractions are shown in Table 3 and the analytical methods used are summarized in Table 4.

*Separation with 0.20*M *hydrobromic acid in 80% acetone.* Aliquots of standard solutions of gallium and of one other element in hydrobromic acid were measured out, mixed and evaporated to dryness. Each residue was dissolved in 50 ml of 0.20*M* hydrobromic acid in 80% acetone and the solution passed through an ion-exchange column containing 4.3 ml of AG50W-X4 ion-exchange resin (200–400 mesh). The resin columns were 5.5 cm in length and 1.0 cm in diameter and had been equilibrated by passage of about 10 ml of 0.20*M* hydrobromic acid in 80% acetone. The solution was washed onto the resin with small portions of the same reagent (about 20 ml) and the other element was then eluted with another 60 ml of this reagent. Acetone was removed from the column by passage of about 20 ml of 0.2*M* hydrochloric acid. Finally, gallium was eluted with 40 ml of 3.0*M* hydrochloric acid. The eluate containing gallium was collected, evaporated to dryness and the gallium determined. Duplicate blanks on all reagents used were run simultaneously and the results corrected accordingly. The results of the determinations are presented in Table 2.

Table 3. Amount of other element found in gallium fraction

Other element	Taken, *mg*	Found in gallium fraction, *μg*	Average, %
Fe(III)	56.08	293 ± 84*	0.52
Fe(III)	0.561	10.2 ± 3.3*	1.82
Fe(III)	112.2	380 ± 27*	0.34
Fe(III)	nil (blank)	13.7 ± 0.8	—
Zn	66.91	<0.5*	<0.001
Zn	nil (blank)	3.7 ± 0.5	—
Cu(II)	67.32	3.1 ± 0.6*	0.005
Cu(II)	nil (blank)	<0.5	—

* Blank subtracted.

DISCUSSION

The method described provides a useful means for the quantitative separation of gallium from Zn, In, Cd, Pb(II), Bi(III), Cu(II), Au(III), Pt(IV), Pd(II), Tl(III), Sn(IV) and (incompletely) Fe(III). All these species can be eluted from a column containing 65 ml of AG50W-X4 resin of 200–400 mesh particle size with 0.50*M* hydrobromic acid in 80% acetone as eluting agent, while gallium is retained. Separation is sharp and quantitative for all elements except iron(III), which shows extensive tailing and cannot be recovered quantitatively (≥99.9%) with reasonable elution volumes. With 350 ml of eluting agent, from about 0.5% of the iron (1 mmole present) to about 2% (microgram amounts taken) was found in the gallium fraction, compared with 0.005% for copper (Table 3), which has about the same distribution coefficient. The tailing of iron(III) is worse when the solution is evaporated to incipient dryness with hydrobromic acid and the wet salts are dissolved in the eluting agent before the solution is passed through the column. Up to 30% of the iron(III) can be retained under these conditions, apparently as a separate peak. A speculative explanation is that cationic mixed-ligand and maybe even bridged bromide complexes

Table 4. Analytical methods used

Element	Method
Ga, Fe(III), Cu(II), In	Complexometrically with DCTA; back-titration with $ZnSO_4$ at pH 5.5 with Xylenol Orange as indicator. Small amounts of Ga, Fe(III) and Cu(II) by atomic-absorption spectrometry.
Zn, Pb(II)	Complexometrically with EDTA at pH 5.5, with Xylenol Orange as indicator. Small amounts of Zn by atomic-absorption spectrometry.
Cd	Complexometrically with EDTA in slight excess of ammonia; Methylthymol Blue as indicator.
Bi	Complexometrically with EDTA in dilute acid solution (pH ~ 1) with Xylenol Orange as indicator.
Tl(III)	Complexometrically with EDTA in the presence of tartrate at pH 9; Methylthymol Blue as indicator.
Au(III), Pt(IV)	Gravimetrically as the metal.
Pd(II)	Gravimetrically as dimethylglyoximate.
Sn(IV)	Gravimetrically as SnO_2 after precipitation with tannic acid from neutral tartrate solution.

with slow ligand-exchange rates could be formed during the pretreatment and on the column.

Other species forming bromide complexes such as Hg(II), Rh(III), Ir(IV), Se(IV), Ge(IV), As(III) and Sb(III), have not been investigated in detail, but should be separated easily, together with Zn, according to their known distribution coefficients.

Gallium is only moderately strongly retained from 0.50*M* hydrobromic acid in 80% acetone. The distribution coefficient is about 65 and the separation factor has a value of about 8 for the separation from iron(III) and copper(II) (the most critical elements). A 65-ml (15-g) column was therefore used for the separation of mmole amounts. When a 43-ml (10-g) column was used, the first traces of gallium appeared in the eluate at a volume of about 325 ml when 1 mmole of gallium was present, and the separation was marginal (Fig. 3). With the 65-ml column the corresponding volume is about 700 ml.

When iron and copper are present only in minor or trace species and their separation is of lesser importance, zinc, indium and the other elements can be sorbed from and eluted with 0.20*M* hydrobromic acid in 80% acetone. In this case gallium is very strongly sorbed and separation factors for the Ga–Zn, Ga–In and Ga–Cd pairs, with values of more than 3000, are the largest known for these separations. They are considerably larger than the separation factors for cation-exchange in hydrochloric acid–acetone mixtures. The fact that gallium is retained while the other elements pass through is an advantage when traces of gallium have to be separated from large amounts of metals such as zinc. With anion-exchange in hydrochloric acid[1] zinc is retained preferentially.

From 36.1 μg to 14.43 mg of gallium have been separated from more than 1 g of zinc on a 4.3-ml (1-g) resin column by elution of zinc with 0.20*M* hydrobromic acid in 80% acetone (Table 2). Only small elution volumes are required (60 ml), more than 99.9% of the zinc having been eluted after the first 30 ml. The elution of zinc is even sharper with 0.50*M* hydrobromic acid in 65% acetone, but the higher viscosity of this eluting agent somewhat reduces the flow-rate and gallium is less strongly retained. Smaller amounts of gallium, even in the submicrogram range, may also be separated. A 1-g column can retain at least 0.5 and probably even 1 mmole of gallium, as indicated by the fact that no gallium appeared in the eluate after 1000 ml of eluting agent had been passed through. In, Cd, Bi(III), Au(III), Pt(IV), Pd(II), Tl(III) and Sn(IV) are separated as easily as zinc. Large amounts of lead(II) are more easily separated by sorption from 0.50*M* hydrobromic acid in 65% acetone, because lead is more soluble in this reagent.

Some species, such as Co(II), Ni(II), Mn(II), U(VI), Al, Be, Mg and Ca are retained together with gallium. If the gallium is eluted preferentially with 0.20*M* hydrochloric acid in about 85% acetone,[15] or with 0.50*M* hydrobromic acid in 80% acetone,[16] all these elements should still be retained. This aspect of a possible selective separation of gallium from almost all other elements has yet to be investigated.

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DETERMINATION OF SILVER IN COPPER AND LEAD METALS AND ALLOYS AND IN ZINC AND SELENIUM BY ATOMIC-ABSORPTION SPECTROMETRY AFTER SEPARATION BY EXTRACTION OF THE TRI-*n*-OCTYLMETHYLAMMONIUM–SILVER BROMIDE COMPLEX

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Summary—A simple and sensitive combined solvent extraction and atomic-absorption spectrometric method has been developed for the determination of silver in copper and lead metals and alloys and in zinc and selenium. Optimal conditions have been established for the extraction and determination of silver. Silver is extracted as the tri-*n*-octylmethylammonium–silver bromide complex and determined by atomic-absorption spectrometry by spraying the extract directly into the flame. As little as 0.2 μg of silver in a sample can be determined.

The determination of silver in copper, lead, zinc, selenium, blister copper, copper alloy or lead alloy is often needed for their metallurgical evaluation. For the determination of traces of silver in these materials, spectrophotometric methods using diphenylthiocarbazone (dithizone),[1–4] *p*-dimethylaminobenzylidenerhodanine[5–10] and copper diethyldithiocarbamate[5,11,12] have been widely employed. However, the dithizone and *p*-dimethylaminobenzylidenerhodanine methods usually require complicated procedures because of low selectivity of the reagents, and are tedious and time-consuming; in addition, the sensitivity of the latter type of method is relatively low. The copper diethyldithiocarbamate method has higher selectivity but is not sufficiently sensitive for the determination of silver around the 1 ppm level.

Solvent extraction combined with atomic-absorption spectrometry is generally suitable for a sensitive, simple and rapid determination of trace quantities of silver; the sensitivity is enhanced by extraction and concentration of silver into an organic solvent and spraying the extract directly into the flame, and a relatively simple and rapid determination becomes possible because no highly selective extraction of silver is usually needed for its atomic-absorption determination. A dithizone[13] or diethyldithiocarbamate[14] extraction combined with atomic-absorption spectrometry has been successfully used for the determination of traces of silver in copper[13,14] or copper alloy.[14]

Several investigations on the extraction behaviour of silver from various media with high molecular-weight amines and ammonium salts, such as di-*n*-butylamine,[15] *n*-dodecyl(trialkylmethyl)amine (Amberlite LA-1),[16] tri-*iso*-octylamine,[17] tri-*n*-dodecylamine,[18,19] tri-*n*-hexylamine,[20] tetra-*n*-hexylammonium chloride[20] and iodide,[21] or tri-*n*-alkylmethylammonium nitrite (Aliquat 336),[22] have indicated that these reagents are effective extractants for silver. A tri-*n*-octylamine extraction and atomic-absorption spectrometry were effectively used for the determination of traces of silver in uranium.[23] These previous investigations suggest that this combination of methods should be suitable for the simple and sensitive determination of traces of silver in various materials. In the present work, the extraction of silver with tri-*n*-octylamine (TOA), tri-*n*-octylmethylammonium chloride [TOMA(Cl)] and tri-*n*-octylmethylammonium bromide [TOMA(Br)] was investigated and the extraction with TOMA(Br) was applied to the determination of traces of silver in copper, lead, zinc, selenium, blister copper, copper alloy and lead alloy. The proposed method involves the separation of silver from the major constituents by solvent extraction with an *n*-butyl acetate solution of TOMA(Br) in the presence of hydrobromic acid and the direct spraying of the extract into the flame. The optimal conditions for the extraction of silver and interferences from various acids and elements are discussed.

EXPERIMENTAL

Apparatus

A Hitachi 208 atomic-absorption spectrophotometer with a three-slot 10-cm long burner, a Hitachi 056 recorder and a Hitachi HLA-3 hollow-cathode lamp were used with the following operating conditions: wavelength 328.1 nm, slit-width 0.18 mm (entrance) and 1.0 mm (exit), burner height position 2, lamp current 10 mA, air flow-rate 14 l./min, acetylene flow-rate 1 l./min.

Reagents

Hydrobromic acid, 0.2 and 3M.

Nitric acid, 7 and 9M.

Tartaric acid solution, 25%.

TOA solution. Dilute 6 ml of TOA to 200 ml with *n*-butyl acetate.

TOMA(Cl) solution. Dilute 6 ml of TOMA(Cl) to 200 ml with *n*-butyl acetate.

TOMA(Br) solution. Dilute 6 ml of TOMA(Cl) to 20 ml with *n*-butyl acetate. Transfer this solution to a separatory funnel and shake with two 40-ml portions of 3*M* hydrobromic acid for 10 min each time (discard the aqueous layers). Dilute the organic layer to 200 ml with *n*-butyl acetate.

Standard silver solution. Dissolve 0.500 g of silver metal in 20 ml of nitric acid (1 + 1) and make up to 500 ml with water. Dilute the solution to the desired concentration immediately before use.

Preparation of calibration curve

Transfer portions of standard silver solution (containing up to 4 μg of Ag) to 200-ml separatory funnels. To each solution and a blank (water), add 1 ml of 7*M* nitric acid and 10.0 ml of 3*M* hydrobromic acid, and dilute to 150 ml with water. Shake vigorously with 10.0 ml of the TOMA(Br) solution for 5 min. Discard the aqueous layer. Shake the organic layer with 50 ml of 0.2*M* hydrobromic acid for 3 min. Discard the aqueous layer. Spray the organic layer into the flame and measure the relationship between the atomic-absorption signals and the amounts of silver.

Procedures for the analysis of samples

Selenium. Decompose 1–5 g of sample with 50 ml of 9*M* nitric acid and evaporate to dryness on a steam-bath. Add 2.5 ml of 7*M* nitric acid and 50 ml of water, and heat gently to dissolve the salts. Transfer the solution to a 200-ml separatory funnel. Add 10.0 ml of 3*M* hydrobromic acid, dilute to 150 ml with water, and proceed as for preparation of the calibration curve.

Copper, lead and zinc metals, and copper alloy. Decompose 0.1–1 g of sample with 10 ml of 7*M* nitric acid (for copper, zinc and copper alloy) or with 10 ml of water and 10 ml of 7*M* nitric acid (for lead). Evaporate to dryness (or to a syrup) on a steam-bath. Add 1 ml of 7*M* nitric acid and 50 ml of water, and heat gently to dissolve the salts. Transfer the solution with about 70 ml of water to a 200-ml separatory funnel. Add 10.0 ml (for copper, zinc and copper alloy) or 2.5 ml (for lead) of 3*M* hydrobromic acid and make up to 150 ml with water. Then complete the detemination of silver as for selenium samples.

Blister copper. Decompose 0.1 g of sample with 10 ml of 7*M* nitric acid and evaporate to dryness (or to a syrup) on a steam-bath. Add 1 ml of 7*M* nitric acid and 50 ml of water, and heat gently to dissolve the salts. Transfer the solution to a 100-ml standard flask and dilute to the mark with water. Transfer an aliquot (1–4 μg of Ag) of the solution to a 200-ml separatory funnel. Add 10.0 ml of 3*M* hydrobromic acid and dilute to 150 ml with water. Complete the determination as for selenium samples.

Lead–antimony alloy. Decompose 0.1–1 g of sample with 20 ml of tartaric acid solution and 5 ml of 7*M* nitric acid; heat gently to assist the decomposition. Transfer the solution to a 100-ml standard flask and dilute to the mark with water. Transfer an aliquot (1–4 μg of Ag) of the solution to a 200-ml separatory funnel. Add 2.5 ml of 3*M* hydrobromic acid and make up to 150 ml with water. Complete the determination as described above.

RESULTS AND DISCUSSION

Extraction of silver chloride and bromide TOA and TOMA ion-association complexes

The extraction behaviour of silver with TOA and TOMA(Cl) from hydrochloric acid solution, and with TOA and TOMA(Br) from hydrobromic acid solution was investigated by using the procedure for preparation of the calibration curve but without washing the organic layer with the 50 ml of 0.2*M* hydrobromic acid.

Silver was found to be best extracted with TOA from 0.02–0.3*M* hydrochloric acid or 0.02–0.5*M* hyd-

Table 1. Effects of hydrochloric acid, hydrobromic acid, TOA and TOMA concentrations on the extraction of silver

HCl or HBr concentration, *M*	TOA or TOMA concentration, % *v/v*	Extraction system* and scale reading			
		TOA–HCl	TOA–HBr	TOMA(Cl)–HCl	TOMA(Br)–HBr
0.02†	3.0	41.2	41.5	42.0	42.0
0.05†	3.0	40.9	41.7	42.2	42.2
0.10†	3.0	41.0	41.4	42.0	42.2
0.20	3.0	41.0	41.5	42.0	42.0
0.30	3.0	41.0	41.5	42.0	42.0
0.50	3.0	38.3	41.5	41.8	42.1
1.0	3.0	31.6	40.2	39.6	42.0
1.5	3.0	23.6	31.4	34.6	38.9
2.0	3.0	17.6	22.9	29.5	33.7
3.0	3.0	10.2	11.6	18.7	19 0
0.20	0.2	17.9	32.0	42.9	43 7
0.20	1.0	37.5	41.5	42 9	43.7
0.20	2.0	40.8	41.5	42.5	42.9
0.20	3.0	41.0	41.5	42.0	42.0
0.20	4.0	41.0	41.5	41.1	42.0
0.20	5.0	41.0	40.7	39.3	42.0
0.20	7.0	41.0	39.3	38 4	41.1
0.20	10	39.3	37.4	35.7	38.6

* Silver taken 2.0 μg.

† Five ml of 3*M* sulphuric acid were added to the aqueous phase to avoid formation of an emulsion or a turbidity.

Table 2. Effects of other acids on the extraction of silver

[Acid], *M*	Extraction system* and scale reading TOA–HCl	TOA–HBr	TOMA(Cl)–HCl	TOMA(Br)–HBr
No addition	41.0	41.5	42.0	42.0
H_2SO_4—1.0	41.0	41.7	42.3	42.1
2.0	41.3	41.4	42.2	41.7
4.0	40.6	41.2	41.4	42.2
HNO_3—0.01	41.3	41.5	42.0	41.9
0.03	36.7	41.5	41.2	41.7
0.05	33.6	41.5	40.2	42.3
0.10	25.7	41.7	37.6	41.6
0.20	18.3	41.4	31.6	42.1
0.30	13.3	40.5	26.9	42.3
0.50	9.3	38.7	18.6	40.2
1.0	3.8	32.5	6.9	36.1
1.5	2.1	23.7	2.8	26.6
2.0	1.4	2.7	0.9	17.8
$HClO_4$—0.01	16.7	39.1	4.8	39.9
0.10	0.6	12.9	0.3	13.8
0.20	0.0	7.0	0.0	7.8
HCl—1.0		41.5		42.0
2.0		38.9		42.0
3.0		35.0		38.5
4.0		29.6		34.5
5.0		21.9		23.2
6.0		13.7		14.1

* Silver taken 2.0 μg; HCl or HBr concentration 0.2*M*; TOA or TOMA concentration in *n*-butyl acetate 3% (v/v).

robromic acid, with TOMA(Cl) from 0.02–0.5*M* hydrochloric acid, and with TOMA(Br) from 0.02–1.0*M* hydrobromic acid (Table 1). The optimal acid concentration ranges in the bromide extraction systems were wider than those in the corresponding chloride extraction systems. Differences in the atomic-absorption signals among the four extraction systems at the respective optimal hydrochloric and hydrobromic acid concentrations were practically negligible.

The concentrations of TOA, TOMA(Cl) and TOMA(Br) in *n*-butyl acetate were varied. Maximal absorption readings for the TOA–HCl and TOA-HBr systems were obtained with 2–7% and 1–4% v/v TOA, respectively (Table 1); the decreases in signal with lower and higher TOA concentrations are probably due to a decrease in extraction of silver with decrease in the TOA concentration and a decrease in aspiration rate of the extract into the flame with an increase in viscosity of the solvent, respectively. In the TOMA(Cl)–HCl and TOMA(Br)–HBr systems, the absorption signals decreased gradually with increasing TOMA(Cl) and TOMA(Br) concentrations (Table 1); this decrease may also be due to the effect of viscosity on the aspiration rate.

The shaking time for the extraction was varied from 1 to 10 min; the signals were independent of the shaking time in all the extraction systems.

Effects of other acids on the extraction of the silver chloride and bromide TOA and TOMA complexes

The effects of sulphuric, nitric, perchloric and hydrochloric acids on the extraction of silver were investigated (Table 2); the experimental procedure was as above. The interfering effect of nitric acid in the four extraction systems decreased in the order TOA–HCl > TOMA(Cl)–HCl ≫ TOA–HBr > TOMA(Br)–HBr. Perchloric acid interfered even at a concentration as low as 0.01*M*. Tartaric acid up to at least 4% in the aqueous phase was found not to interfere.

On the basis of these results, the TOMA(Br)–HBr system with 0.2*M* hydrobromic acid, 3% TOMA(Br) in *n*-butyl acetate and 5-min extraction, was adopted for the determination of silver in the materials mentioned.

Effects of various elements

Effects of various elements on the extraction and determination of silver by use of the TOMA(Br)–HBr system (under the conditions just mentioned) were investigated.

There was no interference from 1 mg of Pd(II), Pt(IV); 5 mg of Al, Be, Ce(IV), Cr(III), Hg(II), Mg, Ti(IV), Tl(III), V(V), Zr; 10 mg of Au(III), In; 50 mg of As(V), Bi, Ca, Co, Mn(II), Sb(III); 100 mg of As(III), Fe(III), Sn(IV), Te(IV); 1 g of Zn; 5 g of Ni.

Cadmium up to 10 mg and thallium(I) up to 1 mg did not interfere, but larger amounts caused negative errors. Amounts of 5 mg of molybdenum(VI) caused slightly positive error.

Five grams of copper, selenium(IV), or 1 g of lead tended to cause slightly negative error, but this tendency disappeared if the organic layer was washed with 50 ml of 0.2*M* hydrobromic acid for 3 min; in the presence of 1 g of lead, the extraction from 0.05*M* hydrobromic acid was adopted because a lead bro-

Table 3. Analytical results for various samples

Sample	Sample taken, *g*	Ag added *μg*	Total Ag found, *μg*	Ag content in the sample, *ppm*	Average *ppm*
Selenium metal	5.0	0	2.00	0.40	
	5.0	0	1.90	0.38	
	5.0	2.0*	3.85	0.37	0.38
	5.0	2.0*	3.80	0.36	
Copper metal	0.20	0	1.84	9.2	
(electrolytic)	0.20	0	1.90	9.5	
	0.20	1.0*	2.80	9.0	9.1
	0.20	1.0*	2.77	8.9	
Copper metal	0.50	0	2.85	5.7	
(electrolytic)	0.50	0	2.94	5.9	
	0.50	1.0*	3.82	5.6	5.8
	0.50	1.0*	3.91	5.8	
Copper metal	0.20	0	2.32	12	
(tough pitch)	0.20	0	2.37	12	
	0.20	1.0*	3.45	12	12
	0.20	1.0*	3.35	12	
Lead metal	1 0	0	1.10	1.1	
	1.0	0	1.20	1.2	
	1.0	2.0*	3.15	1.2	1.1
	1.0	2.0*	3.10	1.1	
Lead metal	0.5	0	1.70	3.4	
	0.5	0	1.60	3.2	
	0.5	1.0*	2.68	3.4	3.3
	0.5	1.0*	2.65	3.3	
Zinc metal	1.0	0	1.70	1.7	
	1.0	0	1.75	1.8	
	1.0	2.0*	3.80	1.8	1.7
	1.0	2.0*	3.60	1.6	
Zinc metal	1.0	0	2.93	2.9	
	1.0	0	2.84	2.8	
	1.0	1.0*	3.86	2.9	2.9
	1.0	1.0*	3.90	2.9	
Blister copper	0.10	0	7.17	72	
	0.10	0	7.27	73	
	0.10	10*	17.18	72	72
	0.10	10*	17.22	72	
Brass	0.10	0	1.47	15	
(60% Cu–40% Zn)	0.10	0	1.41	14	
	0.10	2.0*	3.47	15	15
	0.10	2.0*	3.45	15	
Nickel silver	0.1	0	0.96	9.6	
(Cu 55.1%, Ni 18.2%,	0.1	0	0.98	9.8	
Mn 0.3%, Zn balance)	0.1	1.0*	1.93	9.3	9.6
	0.1	1.0*	1.97	9.7	
Lead–0.7% antimony	0.5	0	3.60	7.2	
alloy	0.5	0	3.60	7.2	
	0.5	4.0*	7.72	7.4	7.3
	0.5	4.0*	7.75	7.5	
Lead–1.0% antimony	0.1	0	3.17	32	
alloy	0.1	0	3.22	32	
	0.1	4.0*	7.49	35	33
	0.1	4.0*	7.42	34	

* Silver, in the form of aliquots of standard silver solution, was added to the solid samples before the decomposition.

mide precipitate was formed when the hydrobromic acid concentration was 0.2*M*.

Applications

Silver in copper, lead, zinc, selenium, blister copper, copper alloy and lead alloy was determined by the proposed method (Table 3); the amounts of silver added to samples before the decomposition were recovered quantitatively. As little as 0.2 μg of silver in a sample or in an aliquot of the sample solution could be determined. The proposed method is simple and sensitive.

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DOSAGE DES VITAMINES LIPO ET HYDROSOLUBLES DANS LES PREPARATIONS POLYVITAMINEES PAR CHROMATOGRAPHIE LIQUIDE HAUTE PERFORMANCE

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Résumé—La séparation, l'identification et le dosage des vitamines lipo et hydrosolubles sont réalisés par chromatographie de partage à polarité de phases inversée sur support de microsilice greffée en C 18. Les vitamines hydrosolubles sont directement séparées avec la phase mobile 1% acide acétique/acétonitrile (90:10 v/v) et révélées à l'aide d'un détecteur ultraviolet à l'exception de l'acide pantothénique. L'efficacité de la séparation des vitamines liposolubles et la précision de leur dosage sont liées aux conditions opératoires. La composition des excipients et tous les constituants des préparations pharmaceutiques (solutés aqueux, huileux, dispersions, émulsions) gouvernent le choix des solvants d'extraction et de la préparation de la prise d'essai à injecter; en fonction des constituants de la formule, la polarité de la phase mobile (acétonitrile/eau 95:5 v/v) peut être modifiée. Les conditions opératoires sont précisées et le mémoire décrit des exemples d'application de l'HPLC au dosage des vitamines hydrosolubles (B.1, B.2, C, PP. B.6) et liposolubles (insaponifiables d'huiles, vitamine A et esters, cholé et ergocalciférol, tocophérol et acétate) dans des préparations multivitaminées (solutés, suspensions, sirops, excipients gras, *etc.*).

Le dosage des vitamines dans les mélanges polyvitaminés présente de nombreuses difficultés en raison de la complexité et de la variété des mélanges comme de la diversité des diverses matières premières à base de vitamines liposolubles; ainsi la vitamine A est commercialisée sous formes libre ou enrobées hydrodispersibles, à l'état d'alcool ou de ses divers esters, comme à l'état d'insaponifiables d'huiles de foies de poissons. Cette diversité se retrouve également dans la présentation galénique des mélanges polyvitaminés préparés avec des excipients aussi différents que ceux utilisés pour la fabrication de comprimés, de capsules à excipient gras ou de solution aqueuse renfermant des agents tensioactifs assurant une bonne dispersion des vitamines liposolubles sans oublier la présence d'antioxydants et éventuellement d'édulcorants, ou d'aromatisants qui compliquent les problèmes analytiques d'identification et de dosage.

Les méthodes biologiques ou physicochimiques classiques sont le plus souvent d'application très longue et délicate mais permettent par des techniques souvent spécifiques un titrage individuel de chaque vitamine.[1,2]

La chromatographie liquide haute performance (HPLC) même avec un appareillage simple, c'est-à-dire en utilisant une seule pompe et un détecteur par absorbance ultra-violette à longueur d'onde fixe, permet d'effectuer le dosage de l'ensemble des vitamines, des mélanges polyvitaminés en deux séquences—le premier temps assure le dosage des vitamines hydrosolubles (à l'exception du panthénol et des pantothénates non révélés en ultra-violette) et le deuxième temps assure le dosage des vitamines liposolubles.

La chromatographie liquide haute performance a déjà donné lieu à un très grand nombre d'investigations dans le domaine des vitamines, par exemple citations 3 à 13. Toutefois la plupart des mémoires se rapportant à l'analyse de solutions vitaminées mettent en évidence l'efficacité du procédé; l'application à des formes pharmaceutiques n'est pas en général immédiate et implique une mise au point préalable des techniques d'extraction des vitamines dispersées dans des excipients variés. C'est à cette préoccupation que répond le présent mémoire qui propose une méthode aussi simple et rapide que possible de préparation de l'échantillon à chromatographier. On ne trouvera pas dans cet exposé de développement théorique concernant l'HPLC mais des indications pratiques directement ou aisément transposables à des cas particuliers.

Le procédé HPLC proposé a été unifié dans toute la mesure du possible bien que s'adressant à des préparations galéniques et à des formes de vitamines très diverses. Dans tous les cas, le procédé de séparation en phase inverse a été utilisé sur une même colonne de 30 cm renfermant comme phase stationnaire une microsilice portant une phase greffée en C 18 (colonne micro Bondapak—Waters). La phase mobile choisie pour le dosage des vitamines hydrosolubles est identique pour toutes les formes galéniques expérimentées; elle est constituée par un mélange de sol-

vants renfermant un contre ion qui assure une meilleure séparation des pics et des résultats quantitatifs plus fidèles.[14]

La phase mobile assurant la séparation des vitamines liposolubles renferme également les mêmes constituants, quelle que soit la forme galénique ou les différentes vitamines liposolubles considérées. Seule une légère modification des proportions du mélange des solvants a été nécessaire dans le cas particulier d'un sirop avec aromatisant afin d'obtenir une bonne séparation des vitamines.

La détection a toujours été effectuée par spectrophotométrie d'absorption à longueur d'onde fixe de 280 mm.

Le choix des sensibilités est évidemment fonction de la concentration, de la longueur d'onde du maximum d'absorption et de l'extinction spécifique de chaque vitamine sous sa forme considérée.

REMARQUES PRELIMINAIRES

Les conditions opératoires sont données à titre indicatif et des modifications doivent être apportées en fonction de la nature des vitamines et des diverses formes pharmaceutiques examinées.

Pour chaque formulation polyvitaminée, il est indispensable d'effectuer une mise au point préalable permettant le meilleur choix des conditions opératoires. Cette mise au point doit commencer par l'étude de chaque vitamine individuellement et de chaque composant de l'excipient absorbant dans l'ultraviolet, afin d'éviter qu'un pic parasite puisse avoir un temps de rétention identique à celui d'un pic caractéristique de vitamine à doser. Ainsi dans les formulations étudiées, il convient de tenir compte du cas particulier de l'aromatisant dans le sirop, du petit pic supplémentaire imputable à la riboflavine base observé dans les préparations renfermant de la riboflavine phosphate de sodium ainsi que des nombreux pics parasites observés dans l'insaponifiable d'huile de foie de poissons. Toutes les mises au point ont été effectuées sur des préparations polyvitaminées reconstituées puis appliquées à des formulations du commerce.

PARTIE EXPERIMENTALE

Matériel

Chromatographe Waters comportant une pompe 6000 A haute pression à débit constant et un injecteur universel U 6K sans septum pour micro et macro injection en débit continu (Waters).

Détecteur M 440 par absorbance ultra-violette utilisé avec une longueur d'onde fixe de 280 nm (Waters).

Colonne microsilice portant une phase greffée en C 18 pour chromatographie liquide en phase inverse—colonne de 30 cm micro Bondapak en C 18 (Waters).

Réactifs

Sulfate de sodium anhydre.
Solution aqueuse à 1% d'acide acétique.
Acétonitrile (qualité HPLC).
Acide heptane sulfonique 1 en solution (Pic B.7 Waters).
Trichloréthylène, n hexane, chloroforme.
Acide aminobenzoïque (étalon interne).
Ménadione (étalon interne).

Mode opératoire

Pendant toutes les manipulations de solutions vitaminées, il convient de travailler en lumière atténuée ou sous lumière inactinique. La préparation de l'échantillon à injecter est différente selon la forme galénique et pour les vitamines hydrosolubles peut aller de la simple dilution du soluté à une extraction à chaud en milieu acétique avec élimination de l'excipient gras par un solvant organique non miscible. Le mode de préparation dépend également de la nature des vitamines utilisées; ainsi la riboflavine phosphate de sodium se dissout beaucoup plus facilement dans l'eau que la riboflavine base.

Dans le cas des vitamines liposolubles dissoutes dans un excipient gras (soluté huileux ou capsules) la préparation de la solution à injecter s'effectue aisément par simple dilution dans un solvant organique renfermant l'étalon interne. En revanche, la préparation de l'échantillon à injecter est beaucoup plus longue lorsque les vitamines liposolubles sont dispersées dans un excipient aqueux ou sont présentées sous une forme enrobée; une extraction par solvants successifs, l'évaporation et la dissolution de l'extrait dans la solution hexanique d'étalon interne impliquent les conditions opératoires rigoureuses.

Divers essais ont conduit à utiliser la ménadione qui appartient au groupe des vitamines, comme étalon interne, utilisé en général à la concentration de 2 mg par ml. Dans les conditions opératoires choisies, le temps de rétention de cet étalon est voisin du temps de rétention des différentes formes de vitamine A expérimentées: insaponifiable d'huile de foie de poisson, acétate, propionate, il est très différent du temps de rétention du palmitate de vitamine A, qui, de plus, présente une absorbance spécifique beaucoup plus faible. De ce fait, la concentration en étalon interne doit être adaptée à chaque cas particulier; elle est augmenté (de l'ordre de 4 mg/ml dans l'hexane) avec les solutés huileux renfermant une forte proportion de vitamine A, insaponifiable ou esters (à l'exception du palmitate) par rapport à la vitamine E, inversement elle est diminuée lorsque la vitamine A est présente sous la forme de palmitate.

Préparation des solutions à examiner

(*a*) *Vitamines hydrosolubles*

Formes galéniques liquides. Diluer les solutés buvables ou injectables et les sirops avec une solution aqueuse d'acide *p*-aminobenzoïque utilisé comme étalon interne de façon à obtenir une solution renfermant par ml des teneurs de l'ordre de:

Vitamine B.1—chlorhydrate de thiamine: 0,08 mg; Vitamine B.6—chlorhydrate de pyridoxine: 0,08 mg; Vitamine PP—amide nicotinique: 0,4 mg; Vitamine C—acide ascorbique: 2,0 mg; Vitamine B.2—riboflavine phosphate de sodium exprimé en riboflavine base: 0,06 mg; Etalon interne—acide *p*-aminobenzoïque: 0,05 mg.

Filtrer la solution diluée sur filtre Millipore de 0,45 μm avant injection.

Formes capsules. Homogénéiser le contenu de plusieurs capsules et effectuer une prise d'essai correspondant à environ:

Vitamine B.1: 4 mg; Vitamine B.6: 4 mg; Vitamine PP: 20 mg; Vitamine C: 100 mg; Vitamine B.2 (riboflavine base): 3 mg.

Mettre la prise d'essai en suspension dans 20 ml d'acide acétique à 10% v/v et chauffer au bain-marie à 80° en agitant pendant 5 à 6 mn. Après refroidissement, extraire les excipients gras par 5 ml de trichloréthylène et receuillir la phase aqueuse dans une fiole jaugée de 50 ml renfermant 2 ml de solution aqueuse à 1,25 mg par ml d'acide *p*-aminobenzoïque utilisé comme étalon interne. Compléter à

50 ml avec de l'eau et filtrer sur filtre Millipore de 0,45 µm avant d'injecter.

La solution à injecter renferme par ml sensiblement les mêmes quantités de vitamines et étalon interne que la solution à injecter obtenue par dilution des solutés.

Forme comprimés. Réduire en poudre fine une dizaine de comprimés et prélever une prise d'essai renfermant sensiblement la même quantité de vitamines que la prise d'essai utilisée pour les capsules.

Epuiser la poudre de cette prise d'essai par 20 ml d'acide acétique à 10% v/v chauffée à 80°. Continuer l'épuisement de la poudre avec de l'eau et transférer les solutions extractives dans une fiole jaugée de 50 ml renfermant 2 ml de solution aqueuse à 1,25 mg par ml d'acide *p*-aminobenzoïque utilisé comme étalon interne. Compléter à 50 ml avec de l'eau. Centrifuger puis filtrer la phase surnageante sur filtre Millipore de 0,45 µm avant d'injecter.

Une seconde extraction, sur une prise d'essai sensiblement identique, conduite dans les mêmes conditions, mais uniquement avec de l'eau préalablement bouillie et refroidie à température ambiante, assure après injection un titrage plus correct de la vitamine C quand les comprimés renferment des oligo-éléments.

(*b*) *Vitamines liposolubles*

Dilution directe. Soluté huileux renfermant une forte quantité de vitamine A (propionate) et une faible quantité de vitamine E. Diluer le soluté avec une solution hexanique de ménadione renfermant 4 mg par ml, de façon à obtenir une solution renfermant par ml des teneurs de l'ordre de:

5000 U.I.: propionate de vitamine A; 750 U.I.: cholécalciférol; 0,5 mg: acétate de tocophérol; 4 mg: ménadione (étalon interne).

Filtrer la solution diluée sur filtre Millipore de 0,45 µm avant injection.

Capsules avec excipient gras renfermant la vitamine A à l'état d'ester palmitique. Homogénéiser le contenu de plusieurs capsules et effectuer une prise d'essai correspondant à environ

10000 U.I. de palmitate de vitamine A; 2000 U.I. d'ergo ou cholécalciférol; 4 mg d'α-tocophérol.

Déliter la prise d'essai dans 10 ml de solution hexanique de ménadione renfermant 0,1 mg par ml et après agitation vigoureuse, centrifuger. Filtrer la phase surnageante sur filtre Millipore de 0,45 µm avant injection.

La solution injectée renferme par ml des teneurs de l'ordre de:

1000 U.I. de palmitate de vitamine A; 200 U.I. d'ergo ou cholécalciférol; 0,4 mg d'α-tocophérol; 0,1 mg de ménadione (étalon interne).

Extraction a partir de formes liquides. Les vitamines liposolubles sont dispersées dans un excipient aqueux et dans les formulations expérimentées, la vitamine A est sous forme d'insaponifiable d'huile de foie de poisson ou sous forme de palmitate de vitamine A.

Diluer extemporanément les solutés buvable ou injectable, ou les sirops avec de l'eau, de façon à obtenir une solution renfermant par ml environ:

500 U.I. de vitamine A; 100 U.I. de vitamine D; 0,2 mg de vitamine E.

Introduire 2 ml de la solution diluée dans une ampoule à décantation et extraire à trois reprises avec 10 ml de chloroforme, puis une fois avec 10 ml de n hexane. Les phases organiques sont jointes après filtration à travers un petit coton supportant 1 g environ de sulfate de sodium anhydre et évaporées sous vide à température inférieure à 40°. Refroidir immédiatement le résidu dans un bain d'eau glacée. Introduire 1 ml de solution hexanique de ménadione à 2 mg par ml dans le cas ou le soluté examiné renferme de l'insaponifiable d'huile de foie de poisson ou à 0,1 mg par ml dans le cas ou le soluté renferme du palmitate de vitamine A.

Après dissolution rapide du résidu, filtrer sur filtre Millipore de 0,45 µm, recueillir le filtrat dans un tube plongé dans un bain d'eau glacée et injecter immédiatement.

La solution injectée renferme par ml des teneurs de l'ordre de:

1000 U.I. de vitamine A (insaponifiable ou palmitate); 200 U.I. de vitamine D_2 ou D_3; 0,4 mg de vitamine E (α-tocophérol ou acétate de tocophérol); 0,1 ou 2 mg de ménadione.

Extraction a partir de comprimés ou de dragées. Les comprimés utilisés pour la mise au point de la technique renferment de l'acétate de vitamine A et la vitamine D_3, cholécalciférol, sous une forme enrobée hydrodispersible.

Prélever un nombre de comprimés correspondant à une prise d'essai de l'ordre de:

11000 U.I. de vitamine A; 2000 U.I. de vitamine D_3; 4 mg de vitamine E.

Débarrasser éventuellement les dragées de leur couche externe puis les réduire en poudre fine; ajouter 5 ml d'eau avant de placer la suspension aqueuse obtenue au bain-marie à 65° pendant 10 mn, en agitant fréquemment.

Introduire la suspension aqueuse dans une ampoule à décantation et l'extraire à deux reprises avec 15 ml de chloroforme. Transférer ensuite la suspension aqueuse dans un pot à centrifuger; extraire par agitation vigoureuse avec 10 ml de n hexane puis centrifuger pour rompre l'émulsion. Joindre toutes les solutions extractives organiques après filtration à travers un petit coton supportant 1 g de sulfate de sodium anhydre et évaporer sous vide à température inférieure à 40°. Refroidir immédiatement le résidu dans un bain d'eau glacée, puis le dissoudre dans 10 ml de solution de ménadione à 2 mg par ml. Filtrer sur filtre Millipore de 0,45 µm en recueillant le filtrat dans un tube plongé dans un bain d'eau glacée. Injecter immédiatement.

La solution injectée renferme par ml des teneurs de l'ordre de:

1100 U.I. de vitamine A; 200 U.I. de vitamine D; 0,4 mg de vitamine E; 2 mg de ménadione.

Préparations des solutions témoins

(*a*) *Vitamines hydrosolubles*

Formes galéniques liquides. Préparer extemporanément trois échantillons reconstitués renfermant respectivement 80–100 et 120% des quantités théoriques des vitamines hydrosolubles du soluté ou du sirop en essai. Dissoudre les vitamines dans un excipient de composition identique à celui de l'échantillon.

Les trois échantillons témoins sont traités parallèlement, dans les mêmes conditions que le soluté examiné (dilution, addition de l'étalon interne d'acide *p*-aminobenzoïque).

Formes capsules et formes comprimés. Dans la mesure où les excipients des capsules et des comprimés n'interfèrent pas, trois solutions témoins sont préparées par dissolution des vitamines hydrosolubles dans une solution aqueuse d'acide acétique à 4% (v/v).

Les trois solutions prêtes pour l'injection renferment respectivement 80–100 et 120% de la quantité théorique de chaque vitamine hydrosoluble présente dans la solution extractive prête à l'injection, et la même concentration de l'étalon interne que la solution extractive (0,05 mg d'acide *p*-aminobenzoïque par ml).

(*b*) *Vitamines liposolubles*

Les solutions témoins sont préparées selon des modalités différentes en fonction des formes galéniques examinées. Elles sont obtenues par simple dissolution des vitamines liposolubles dans l'hexane dans le cas des solutés huileux, capsules et comprimés. Dans le cas de dispersions aqueuses de polyvitamines, les solutions témoins sont préparées dans des conditions définies.

Dans tous les cas, chaque solution renferme le même composé vitaminé que celui figurant dans la formule.

Solutions directes dans l'hexane. Préparer extemporanément trois solutions de vitamines liposolubles dans n hex-

Tableau 1.

	Soluté huileux	Capsules	Comprimés
Vitamine A propionate, *U.I./ml*	5000		
Vitamine A acétate, *U.I./ml*			1100
Vitamine A palmitate, *U.I./ml*		1000	
Vitamine D_2 ou D_3, *U.I./ml*	750	200	200
Vitamine E (α tocophérol), *mg/ml*		0,4	0,4
Vitamine E (acétate d'α tocophérol), *mg/ml*	0,5		
Etalon interne (ménadione), *mg/ml*	4	0,1	2

ane renfermant respectivement 80–100 et 120% de la quantité théorique de chaque vitamine présente dans la solution extractive prête pour l'injection. La concentration de l'étalon interne de ménadione est identique dans les trois solutions témoins et dans la solution à examiner. L'ordre de grandeur de la concentration de chaque vitamine dans la solution témoin à 100% est donné dans Tableau 1.

Dispersions aqueuses. Lorsque les vitamines liposolubles se trouvent dispersées dans un excipient aqueux (solutés buvables, injectables, sirops), il est nécessaire de procéder différemment.

Préparer trois échantillons reconstitués renfermant respectivement 80–100 et 120% de la quantité théorique de chaque vitamine liposoluble présente dans la formule; les autres composants de la formule (vitamines hydrosolubles et excipients) en quantités correspondant à celles de la formule.

Les trois échantillons témoins sont traités parallèlement dans les mêmes conditions, que le soluté examiné (dilution, extraction, addition de l'étalon interne).

Chromatographie liquide haute performance

Solvants d'élution. Pour les vitamines hydrosolubles la phase mobile commune pour toutes les formes galéniques expérimentées est un mélange de solution aqueuse à 1% (v/v) d'acide acétique et d'acétonitrile (89:11 v/v). Additionner ce mélange de 1,5% (v/v) de Pic B. 7, agiter et attendre 5 à 10 mn avant de filtrer.

Pour les vitamines liposolubles la phase mobile commune pour toutes les formes galéniques expérimentées sauf la forme sirop est acétonitrile/eau (95:5 v/v).

Pour la forme sirop expérimentée, un des aromatisants du sirop se révèlant avec un temps de rétention identique à celui de l'étalon interne, la phase mobile est acétonitrile/eau (92:8 v/v).

Toutes phases mobiles sont filtrées sur filtre Millipore de 0,45 μm et dégazer dans un bain à ultra-sons.

Volume injecté, débit et temps de rétention. Pour vitamines hydrosolubles, injecter successivement 12 μl de chaque solution témoin et de chaque solution en essai. Débit et temps de rétention sont différents selon que les formes galéniques et les solutions témoins renferment de la riboflavine phosphate de sodium ou de la riboflavine base. Les autres vitamines hydrosolubles sont sous une forme identique dans toutes les préparations expérimentées. Les débits et temps de rétention sont donnés à titre indicatif ci-dessous (Tableau 2).

Pour vitamines liposolubles la nature et la concentration des vitamines et de l'étalon interne dans les solutions injectées déterminent le choix des conditions opératoires qui sont donc différentes selon la forme galénique soumise au contrôle. Les volumes injectés, débits et temps de rétention sont donnés à titre indicatif (Tableau 3).

Tableau 2.

	Solutes buvables injectables, sirops	Capsules, comprimés
	Vitamines	*Vitamines*
Débit, ml/mn	1 C, B_2 (phosphate)	1,2 C, PP, E.I. B_6, B_2 (base)
	PP, E.I., B_6	
	5 B_1	4,2 B_1
Temps de rétention, mn		
Vitamine C	3	2,5
Vitamine B_2 (phosphate)	3,5	—
Vitamine PP	6	5,5
Etalon interne (E.I.)	8,5	7
Vitamine B_6	11	10
Vitamine B_2 (base)	—	15
Vitamine B_1	19	24

Atténuation du détecteur et calcul. Dans tous les cas, l'absorbance est déterminée à 280 nm. Les atténuations du détecteur sont réglées de façon à obtenir des pics d'une hauteur suffisante pour permettre une bonne interprétation quantitative.

Tableau 3.

	Soluté huileux	*Capsules*	*Comprimés*	*Solutés aqueux*		*Sirop*
Volumes injectés, µl	12	20	15	18		18
Débit, ml/mn						
	2	2 *Vitamines* E.I., D et E	2 *Vitamines* E.I., A (acétate)	2 *Vitamines* E.I., D E	2 *Vitamines* E.I., A (insaponifiable	1 *Vitamines* aromatisant
		5 A (palmitate)	1 D, E	5 A (palmitate)		2 E.I., A (insaponifiable) D, E
Temps de retention, mn						
Aromatisant	—	—	—	—	—	3
Etalon interne (E.I.)	1,5	1,5	1,5	1,5	1,5	3,5
Insaponifiable de vitamine A		—			2,5	3,5
Propionate de vitamine A	3,5	—	—	—	—	—
Acétate de vit. A	—	—	3,5	—	—	—
Cholé- ou ergocalciférol	8	8	13	8	8	13,5
α Tocophérol	—	10	17,5	—	10	16
Acétate d'α tocophérol	13	—	—	13	—	—
Palmitate de vit. A	—	21	—	24	—	—

Atténuation du détecteur et calcul

Dans tous les cas, l'absorbance est déterminée à 280 nm. Les atténuations du détecteur sont réglées de façon à obtenir des pics d'une hauteur suffisante pour permettre une bonne interprétation quantitative.

Calculer le rapport de la surface de chaque pic correspondant à chaque vitamine et de la surface du pic de l'étalon interne. Tracer la droite étalon correspondant à chaque vitamine et se reporter à ce graphique pour déterminer la teneur en la vitamine considérée de l'échantillon en essai.

RESULTATS

Vitamines hydrosolubles

La mise au point de la méthode a été effectuée sur des solutions témoins des diverses vitamines hydrosolubles. La seule difficulté rencontrée pour le dosage de ces vitamines réside dans le choix des conditions opératoires de la HPLC proprement dite, assurant des temps de rétention bien distincts pour chaque vitamine et une bonne reproductivité quantitative.

Une série d'essais a été effectuée sur chacune des solutions témoins de vitamine à des concentrations variées afin de vérifier les limites dans lesquelles l'absorbance mesurée est proportionnelle à la concentration. Les essais ont montré que pour une prise d'essai donnée soumise au dosage l'absorbance mesurée est proportionnelle à la concentration dans des limites de $\pm 20\%$ de cette teneur.

Ainsi qu'il a été précisé chaque formulation doit faire l'objet d'une adaptation particulière dépendant de la nature de chaque vitamine et de la forme pharmaceutique considérée.

A titre indicatif, deux chromatogrammes de vitamines hydrosolubles sont reproduits: chromatogramme d'un extrait de capsules polyvitaminées renfermant de la riboflavine base (Fig. 1, I), et chromatogramme d'un soluté polyvitaminé renfermant la vitamine B.2 sous forme riboflavine phosphate de sodium. Dans ce cas, un petit pic supplémentaire de riboflavine base venant de l'hydrolyse partielle de l'ester est observé (Fig. 1, II).

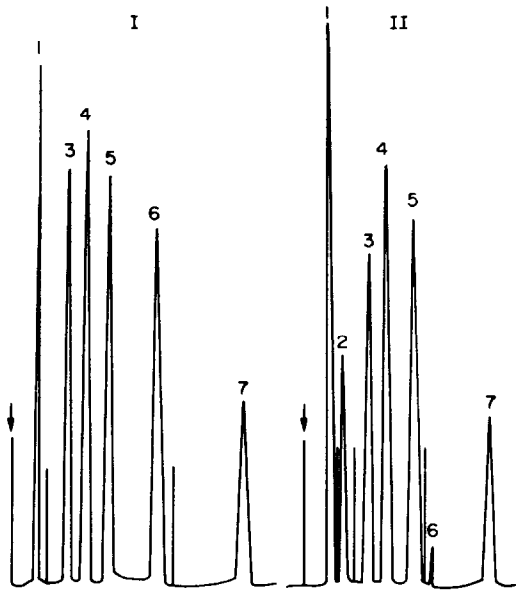

Fig. 1. Chromatogrammes de I, capsules polyvitamines; II soluté polyvitaminé: 1—vitamine C; 2—riboflavine phosphate de sodium; 3—amide nicotinique; 4—etalon interne; 5—chlorhydrate de pyridoxine; 6—riboflavine base; 7—chlorhydrate de thiamine.

La mise au point effectuée sur des préparations polyvitaminées reconstituées a permis de s'assurer de la bonne reproductibilité des résultats; les quantités des vitamines introduites sont retrouvées avec une dispersion de l'ordre de 5%, à la condition de toujours injecter des solutions préparées extemporanément.

La technique a été appliquée à des formulations polyvitaminées du commerce. L'examen de quelques résultats figurant à titre indicatif (Tableau 4) fait apparaître que l'on peut doser les vitamines avec une précision convenable.

Des essais comparatifs ont montré que l'HPLC conduit à des résultats sensiblement identiques à ceux obtenus par les méthodes colorimétriques ou fluorimétriques de dosage des vitamines hydrosolubles, tels

Tableau 4.

	Vitamine C, *mg*		Vitamine PP, *mg*		Vitamine B.*1*, *mg*		Vitamine B.*6*, *mg*		Vitamine B.*2*, *mg* (exprimée en base)	
	Théorie	*Trouvé*	*Théorie*	*Trouvé*	*Théorie*	*Trouvé*	*Théorie*	*Trouvé*	*Théorie*	*Trouvé*
Soluté buvable										
Formule I	50		10		2		2		1,5	
Echantillon 1		48		10,1		1,9		2,1		1,5
Echantillon 2		50		10,3		1,95		2,1		1,55
Echantillon 3		49		10,1		1,75		1,95		1,55
Soluté buvable										
Formule II	50	47	10	10,4	2	1,95	2	2,05	1,5	1,55
Soluté injectable	50		10		2		2		1,5	
Echantillon 1		50		10,4		1,9		2,05		1,55
Echantillon 2		49		10		1,9		2		1,45
Sirop	50	47	10	10	2	1,95	2	2,05	1,5	1,55
Capsules, formule I	55	53	10	9,8	2	2	2	2,05	1,5	1,50
Capsules, formule II	55	52	10	9,7	2	2,05	2	2	1,5	1,45
Comprimés dragéifiés	60	56	10	10,3	2	2,05	2	2	2	2

que les dosages de la vitamine B.1 par fluorimétrie du thiochrome, de l'acide nicotinique par la réaction au bromure de cyanogène, de la vitamine C par le dichlorophénol indophénol et de la vitamine B.6 par le 2-6 dichloroquinonechlorimide.

La précision de la méthode et la dispersion des résultats sont identiques; l'avantage de l'HPLC réside dans la plus grande rapidité de la technique qui permet d'arriver au résultat à partir d'une seule prise d'essai au lieu de recourir à une méthode particulière pour chaque vitamine.

De même, la chromatographie haute performance permet de suivre la stabilité des vitamines hydrosolubles dans les préparations polyvitaminées. C'est ainsi que l'on a pu observer dans un échantillon par HPLC (Tableau 4) comme par fluorimétrie du thiochrome une chute de la vitamine B.1 au cours de la conservation.

On notera que les résultats mentionnés dans le Tableau 4 ont été calculés en tenant compte de l'étalon interne et en se référant à une droite d'étalonnage. Si l'on effectue le titrage directement sans étalon interne et par rapport à un seul témoin, la précision est nettement moins bonne et permet seulement de vérifier avec une large approximation, la présence des vitamines dans le rapport prévu dans la formule. Le procédé n'est pas applicable au dosage de l'acide pantothénique non décelé dans les conditions de révélation en l'ultraviolet. La détection et le dosage après dérivatisation feront l'objet d'un développement particulier.[15]

Vitamines liposolubles

La mise au point du dosage des vitamines liposolubles par HPLC dans les formulations polyvitaminées a exigé deux séries d'essais préliminaires.

Une première série effectuée sur des vitamines isolées dissoutes en solvant organique a permis de choisir les conditions opératoires de l'HPLC. Ces conditions définies se sont révélées directement applicables aux formes galéniques à excipient gras.

La difficulté de la mise au point réside essentiellement dans la présence de pics parasites venant soit des excipients, soit des vitamines, elles-mêmes et qui ne doivent en aucun cas interférer avec les pics caractéristiques des vitamines à doser. Les formules renfermant de la vitamine A naturelle = insaponifiable d'huile de foie de poisson, ont dû être particulièrement étudiées sur ce point.

La deuxième série d'essais à eu pour objet de choisir le meilleur solvant d'extraction permettant un dosage correct des vitamines liposolubles dispersées dans un milieu aqueux. Il s'est avéré que le meilleur solvant d'extraction de la vitamine A n'était pas celui qui convenait le mieux pour les autres vitamines E et D. Ce sont des extractions successives avec des solvants différents qui ont permis de retrouver quantitativement l'ensemble des vitamines dispersées en milieu aqueux. De nombreux essais effectués sur des solutions témoins de vitamines préparées par dissolution directe ou après extraction d'un milieu aqueux ont montré que pour une prise d'essai donnée soumise au

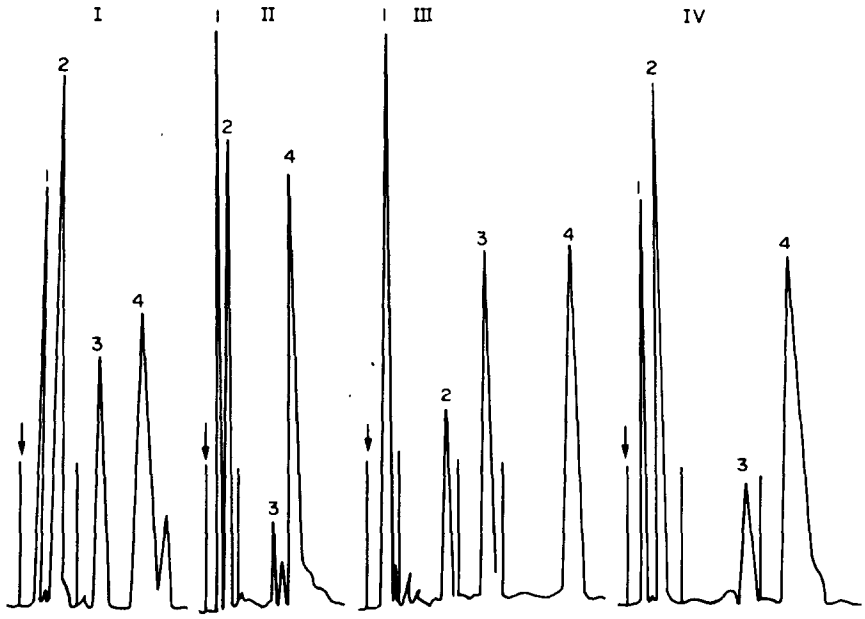

Fig. 2. I, Soluté huileux: 1—étalon interne; 2—propionate de vitamine A; 3—cholécalciférol; 4—acétate d'α-tocophérol. II, Soluté aqueux formule I: 1—étalon interne; 2—insaponifiable d'huile de foie de poisson; 3—ergocalciférol; 4—α tocophérol. III, Soluté aqueux formule II: 1—étalon interne; 2—cholécalciférol, 3—acétate de tocophérol; 4—palmitate de vitamine A. IV, Comprimés: 1—étalon interne; 2—acétate de vitamine A (forme enrobée); 3—cholécalciférol (forme enrobée); 4—α-tocophérol.

dosage, l'absorbance mesurée est proportionnelle à la concentration dans des limites de ±20% de cette teneur.

Comme il a déjà été observé pour le dosage des vitamines hydrosolubles, le dosage des vitamines liposolubles dans toute préparation polyvitaminée exige une mise au point préalable par paliers successifs = choix des conditions opératoires de la chromatographie s'adaptant le mieux aux vitamines considérées, puis choix du meilleur procédé d'extraction.

Quatre chromatogrammes reproduits à titre indicatif, montrent une séparation satisfaisante des vitamines liposolubles quelle que soit la forme sous laquelle les vitamines A, E ou D ont été utilisées dans les formulations.

Ainsi le chromatogramme d'un soluté huileux (Fig. 2, I) renfermant du propionate de vitamine A associé à la vitamine D.3 et à l'acétate de tocophérol proportionnellement en petite quantité, permet par la hauteur relative des pics observés, une interprétation quantitative satisfaisante.

Sur le chromatogramme d'une solution extractive d'un soluté aqueux (Fig. 2, II) renfermant de l'insaponifiable de vitamine A, de la vitamine D.2 et de l'α tocophérol, il est observé un pic parasite imputable à une fraction de l'insaponifiable de vitamine A entre les pics de la vitamine D.2 et de l'α tocophérol; le choix du solvant et du débit permet parfaitement de séparer ces trois pics.

Il est en général procédé à une seconde injection avec un volume plus important de cette solution extractive pour obtenir un pic de vitamine D.2 plus grand et permettant une meilleure interprétation quantitative mais avec cette seconde injection, le pic de l'α tocophérol sort du graphique et n'est plus interprétable par des moyens manuels.

L'injection d'une solution extractive d'un autre soluté aqueux renfermant du palmitate de vitamine A, de vitamine D.3 et de l'acétate de tocophérol permet d'obtenir un chromatogramme (Fig. 2, III) tout à fait différent du précédent sur lequel, le temps de rétention de l'acétate de tocophérol apparait plus grand que le temps de rétention de l'α tocophérol, et la vitamine A se trouve reportée en fin de chromatogramme.

Le chromatogramme d'une solution extractive de

Tableau 5.

	Vitamine A, *U.I.*		Vitamine E, *mg*		Vitamine D, *U.I.*	
	Théorie	*Trouvé*	*Théorie*	*Trouvé*	*Théorie*	*Trouvé*
Soluté huileux injectable (Vit. A propionate, tocophérol acétate, vitamine D.3)	5.10^5		50		$7{,}5.10^4$	
Echantillon 1		$5{,}1.10^5$		49		$7{,}35.10^4$
Echantillon 2 (*)		$4{,}7.10^5$		47		$6{,}95.10^4$
Soluté aqueux injectable (Vit. A insaponifiable, α tocophérol, vitamine D.2)	5.10^3		2		1000	
Echantillon 1		$4{,}7.10^3$		2,05		1020
Echantillon 2		$4{,}8.10^3$		2,00		990
Soluté aqueux buvable, formule I (Vit. A insaponifiable, α tocopherol, vitamine D.2)	5.10^3		2		1000	
Echantillon 1		$4{,}9.10^3$		2,00		960
Echantillon 2		$4{,}8.10^3$		2,05		980
Echantillon 3 (*)		$4{,}1.10^3$		1,80		890
Soluté aqueux buvable, formule II (Vit. A palmitate, tocopherol acétate, vitamine D.3)	5.10^3		2		1000	
Echantillon 1		$5{,}05.10^3$		1,95		960
Echantillon 2 (*)		$4{,}4.10^3$		1,90		900
Sirop (Vitamine A insaponifiable, α tocophérol, Vitamine D.3	5.10^3	$5{,}0.10^3$	2	2,10	1000	970
Capsules, formule I (Palmitate de vitamine A, α tocophérol, vitamine D.2)	5.10^3		2,1		1000	
Echantillon 1		$4{,}85.10^3$		2,15		990
Echantillon 2 (*)		$4{,}5.10^3$		1,85		920
Capsules, formule II (Palmitate de vitamine A, α tocophérol, vitamine D.3)	$5{,}5.10^3$	$5{,}3.10^3$	2,1	2	1000	960
Comprimés dragéifiés (Acétate de vitamine A enrobé, vitamine D.3 enrobée, α tocophérol)	$5{,}5.10^3$		2,2		1000	
Echantillon 1		$5{,}3.10^3$		2,0		990
Echantillon 2 (*)		$4{,}8.10^3$		1,75		910

* Echantillons ayant dépassé la date de péremption.

comprimés (Fig. 2, IV) renfermant des vitamines enrobées = acétate de vitamine A et cholécalciférol ainsi que l'α tocophérol montre ici encore une bonne extraction des vitamines et une séparation des pics satisfaisante.

La méthode a été appliquée à diverses formes galéniques polyvitaminées du commerce. D'après nos essais, l'injection de plusieurs prises d'essai d'une même solution extractive montre que les résultats sont reproductibles avec une dispersion de l'ordre de 5%.

Pour un même échantillon, les résultats des dosages de la vitamine A, par HPLC recoupent ceux des méthodes traditionnelles: spectrophotométrie dans l'ultraviolet, colorimétrie dans visible selon la réaction de Carr et Price; cependant, la rapidité d'exécution et la précision de la HPLC sont supérieures. Les méthodes classiques de dosage des vitamines Det E impliquent une saponification, une extraction et une séparation par chromatographie sur couches minces rendant illusoire la précision du résultat qui n'est obtenu qu'avec une très large approximation.

De plus, l'HPLC permet de suivre la stabilité des vitamines liposolubles dans les préparations polyvitaminées avec plus de rigueur.

Les résultats obtenus (Tableau 5) sur des formes commerciales de fabrication récente demeurent compris dans une limite qui en général n'excède pas 10% et demeure compatible avec la qualité pharmaceutique; des résultats obtenus sur quelques lots ayant dépassé la date de péremption révèle la chute de la teneur en vitamines.

On ne saurait trop insisté sur le respect des conditions opératoires; en particulier, la précision des résultats est liée à l'emploi d'un étalon interne et à l'appréciation des concentrations en se référant à un diagramme d'étalonnage.

Par ailleurs, les préparations polyvitaminées renferment des additifs antioxydants tels que le butylhydroxytoluène et le butylhydroxyanisole. Dans les conditions opératoires décrites pour le dosage des vitamines, les antioxydants ne peuvent être dosés. Leur détection et leur dosage dans les matières premières à base de vitamines liposolubles et les formes galéniques polyvitaminées, impliquent des conditions particulières qui font l'objet d'un autre mémoire.[16] Dans l'état actuel des méthodes de la chimie analytique organique, l'HPLC se révèle comme un procédé de choix pour le dosage des polyvitamines.

Le présent mémoire propose essentiellement des techniques qui après une extraction relativement aisée autorise la détection et le dosage des vitamines hydro et liposolubles dans les formes pharmaceutiques par HPLC. Les performances du procédé peuvent être améliorées par l'emploi d'autres détecteurs ou en procédant par gradient d'élution. Les résultats enregistrés améliorent le contrôle de qualité des préparations polyvitaminées et les techniques mises au point sur des formes galéniques sont d'ores et déjà susceptibles de transposition à d'autres formulations.

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Summary—The separation, identification and determination of the fat- and water-soluble vitamins are realized by partition chromatography with a reversed-phase system made by bonding a C_{18} group to silica. The water-soluble vitamins are directly separated with the mobile phase 1% acetic acid/acetonitrile (89:11 v/v) and are revealed by an ultraviolet detector, except for pantothenic acid. The separation efficiency and precision of determination of the fat-soluble vitamins depend on the operational conditions. The composition of the excipients and all the constituents of pharmaceuticals (aqueous and oil solutions, injections, dispersions, emulsions) determine the choice of the extraction solvents and the preparation of the solution to be injected; the polarity of the mobile phase (acetonitrile/water 95:5 v/v) can be changed, and the choice depends on the components to be separated. The experimental conditions are specified and some examples are given of application of HPLC to determination of water-soluble vitamins (B1, B2, C, PP, B6) and fat-soluble vitamins (non-saponifiable oils, vitamin A and its esters, cholecalciferol, ergocalciferol, and tocopherol and its acetate) in multivitamin formulations (solutions, suspensions, syrups, fatty excipients *etc.*).

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LIQUID-MEMBRANE DODECYLBENZENESULPHONATE ION-SELECTIVE ELECTRODE EMPLOYING VICTORIA BLUE AS THE COUNTER-ION

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Summary—The dodecylbenzenesulphonate anion forms an ion-pair with the Victoria Blue cation and this is easily extracted into nitrobenzene. It was found that the calibration curve of the dodecylbenzenesulphonate ion-selective electrode based on this system shows good linearity in the range 10^{-3}–$10^{-7}M$. Selectivity coefficients were evaluated for some anions. The results indicated a poor tolerance towards dodecyl sulphate, perchlorate and periodate. Dodecyl sulphate, dodecylbenzenesulphonate and several kinds of synthetic anionic detergents have been successfully titrated potentiometrically with a solution of Zephiramine (benzyldimethyltetradecylammonium chloride) by using the electrode as an indicator electrode.

In recent years, many surfactant ion-selective electrodes sensitive to dodecyl sulphate,[1–5] octyl sulphate,[3] dodecylbenzenesulphonate,[3,6,7] and *p*-toluenesulphonate[3,8,9] have been reported. Usually the potential of a surfactant ion-selective electrode is neither stable nor reproducible, because of the strong surface activity of the ion being determined, so in practice it is not suitable for the determination of the surfactant ion concentration directly from a calibration curve. We have already reported a liquid-membrane type surfactant ion-selective electrode[5,6] constructed by solidifying the nitrobenzene extract of the ion-pair formed by the Zephiramine cation (benzyldimethyltetradecylammonium, $Zeph^+$) and dodecylbenzenesulphonate (DBS^-) anion onto a platinum wire electrode by the addition of naphthalene. This electrode shows high sensitivity for both DBS^- and $Zeph^+$ but unfortunately the electrode must be freshly remade after each series of measurements. In this paper, the behaviour of this electrode, exhibiting a high sensitivity and performance, and its application to the potentiometric titration of alkyl sulphate, alkylbenzenesulphonate and some synthetic detergents, are reported. Several kinds of detergents were successfully titrated either manually or automatically, and the results compared with the familiar Epton method.

EXPERIMENTAL

Apparatus

All the potential measurements were made with a Corning pH-meter, model 110. A Corning double-junction Ag|AgCl electrode, No. 476067, or calomel electrode, No. 476109, was used as the reference electrode. Some potentiometric titrations were made with a Hiranuma recording autotitrator, RAT-11.

Reagents

A 10m*M* sodium dodecylbenzenesulphonate stock solution was prepared by dissolving 3.485 g of the reagent in 1 litre of demineralized water. Victoria Blue, *ca.* 60% pure, was obtained from Tokyo Kasei Kogyo Co. and used without further purification. Zephiramine was supplied by the Dojindo laboratory and a 10m*M* stock solution was prepared by dissolving 3.690 g of reagent in 1 litre of water. The stock solutions of 1m*M* Victoria Blue and 10m*M* Zephiramine were standardized by potentiometric titration with silver nitrate, a Hiranuma recording titrator being used.

Preparation of liquid ion-exchange membrane

The liquid ion-exchange membrane was prepared by extraction of the ion-association complex of DBS^- and VB^+. To a 10-ml portion of 10m*M* DBS^- solution in a separatory funnel were added 15 ml of 1m*M* VB^+ and 10 ml of nitrobenzene (NB), then the mixture was shaken for 15 min. The organic phase was separated and freed from water by addition of anhydrous sodium sulphate. The potential of the DBS^- ion-selective electrode in the presence of foreign anions is given by the Nikolskii–Eisenman equation:[10]

$$E = E^\circ - \frac{2.303RT}{F} \log(a_{DBS} + \Sigma K^{Pot}_{DBS^-,J} a_j^{1/z_j}) \quad (1)$$

where E° is a constant, a_{DBS^-} and a_j are the activities of the principal ion and the jth interfering ion, $K^{Pot}_{DBS^-,J}$ is the selectivity coefficient and z_j the absolute value of the charge on the interfering ion. The composition of the cell including the ion-selective electrode is as follows:

Ag|AgCl internal reference electrode	Internal ref. soln., 1m*M* DBS^- · 1m*M* NaCl	Liquid ion-exchanger, 1m*M* VB^+–DBS^- in NB	Sample soln., containing DBS^-	Ag|AgCl or SCE

Ion-selective electrode

The cell assembly used in this study was similar to that described elsewhere.[11,12] The slope of the calibration curve and the range of linear Nernstian response of the electrode did not change significantly with time for more than two weeks. The potential change produced by change in concentration is rapidly attained, 95% of the steady-state value being reached in 0.5 sec or less.

RESULTS AND DISCUSSION

Selection of organic solvent

Five organic solvents, chloroform, *o*-nitrotoluene, 1,2-dichloroethane, benzyl alcohol and nitrobenzene were used for the preparation of the ion-exchange membrane. Figure 1 shows the calibration curves for the ion-selective electrodes made with the five solvents. Only the nitrobenzene extract gave a good calibration curve.

Selection of ion-pair concentration

Calibration curves for the DBS^- ion-selective electrode with varying VB^+–DBS^- ion-pair concentrations are given in Fig. 2. The electrode constructed with 1m*M* ion-pair concentration was found to be the best because the slope of the calibration curve had almost the Nernstian value and also the stability of the potential was best. The observed potential drift for 10^{-4} and $10^{-5}M$ sample solutions was only 3–4 mV during 20 min. The linear Nernstian response range for DBS^- was from 10^{-3} to $10^{-7}M$.

Selection of internal reference electrode

Platinized platinum, silver–silver chloride, platinum, and silver electrodes were tested as the internal reference electrode. As shown in Fig. 3, the best linear response was given by the silver–silver chloride electrode.

Electrode response as a function of pH

The pH of a $10^{-4}M$ DBS^- solution was adjusted with 0.1*M* hydrochloric acid or sodium hydroxide. The total ionic strength was kept at 0.1*M* with sodium chloride. As shown in Fig. 4, the potential of the ion-selective electrode is almost constant over the pH range 2–11.

Selectivity coefficient

The selectivity coefficients were evaluated by the mixed-solution method.[13] Ionic concentrations were converted into activities by using Kielland's table of single-ion activities.[14] The results are summarized in Table 1. The electrode shows good selectivity for DBS^- relative to most common inorganic ions; in particular, dihydrogen phosphate, which is often added to commercially available synthetic detergents, does not interfere. The electrode has, however, no selectivity for DBS^- relative to dodecyl sulphate.

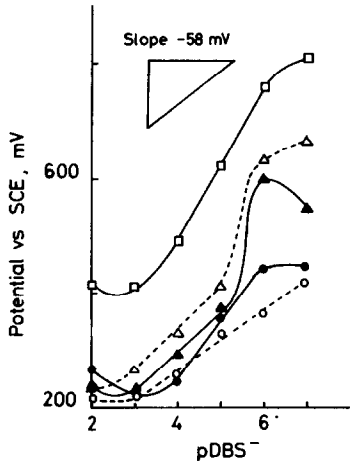

Fig. 1. Effect of solvents: —□— chloroform, ---△--- *o*-nitrotoluene, —▲— 1,2-dichloroethane, —●— benzyl alcohol, ---○--- nitrobenzene. Ion-pair concentration: $10^{-4}M$. Internal reference solution: $10^{-2}M$ DBS^-. Internal reference electrode: Ag|AgCl electrode.

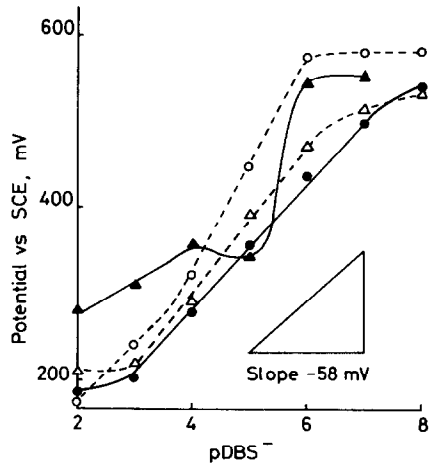

Fig. 2. Effect of DBS^-–VB^+ ion-pair concentration in the ion-exchange membrane: —▲— $10^{-5}M$, ---△--- $10^{-4}M$, —●— $10^{-3}M$, ---○--- $10^{-2}M$. Internal reference solution: $10^{-3}M$ NaDBS in $10^{-3}M$ NaCl. Temperature 20.0°C.

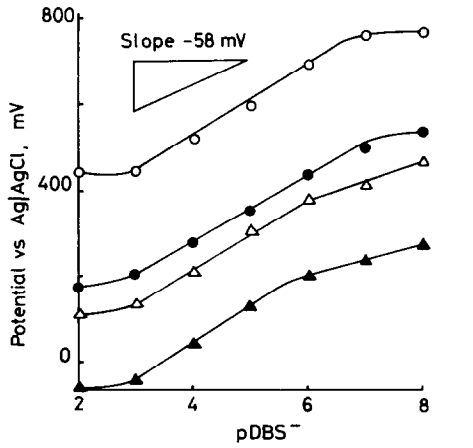

Fig. 3. Selection of internal reference electrode: —○— Pt–Pt, —●— Ag|AgCl, —△— Pt, —▲— Ag. Ion-pair concentration: $10^{-3}M$. Temperature 20.0°C.

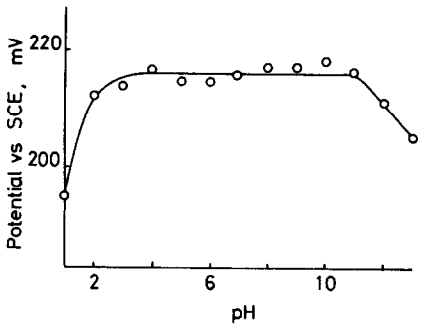

Fig. 4. Electrode response as a function of pH: Sample solution: $10^{-4}M$ NaDBS containing sodium chloride, hydrochloric acid and/or sodium hydroxide. Ionic strength: 0.1 *M*. Temperature 20.0°C.

Potentiometric titration

The electrode is not suitable for direct potentiometry, because the potential is not stable enough. The electrode has, however, been successfully applied to potentiometric titration. Figure 5 shows typical curves for titration of dodecylbenzenesulphonate with

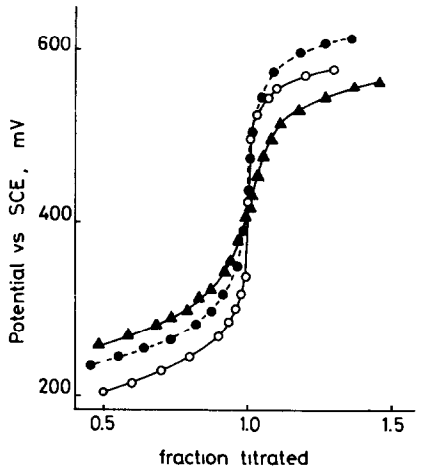

Fig. 5. Potentiometric titration of 10 ml of NaDBS solution with standard $Zeph^+Cl^-$ solution: —○— *ca.* $10^{-2}M$ NaDBS titrated with $9.12 \times 10^{-3}M$ $Zeph^+Cl^-$, —●— *ca.* $10^{-3}M$ NaDBS titrated with $9.10 \times 10^{-4}M$ $Zeph^+Cl^-$, —▲— *ca.* $10^{-4}M$ NaDBS titrated with $9.10 \times 10^{-5}M$ $Zeph^+Cl^-$.

Table 1. Selectivity coefficients for the DBS^- ion-selective electrode

Anion	$-\log K^{Pot}_{DBS^-,j}$
Cl^-	5.7
SO_4^{2-}	5.6
Br^-	4.8
BO_2^-	4.8
$H_2PO_4^-$	4.8
NO_3^-	4.6
CH_3COO^-	4.4
HCO_3^-	4.4
ClO_3^-	4.1
I^-	3.7
SCN^-	2.4
OH^-	2.3
IO_4^-	1.0
ClO_4^-	0.6
$C_{12}H_{25}SO_4^-$	0.05

Zephiramine of varying concentrations. Even in the titration of $10^{-4}M$ DBS^- with the same concentration of Zephiramine, an adequate potential jump was observed at the end-point. Though many quaternary onium cations can be used as the titrant, we prefer Zephiramine because of its high purity and

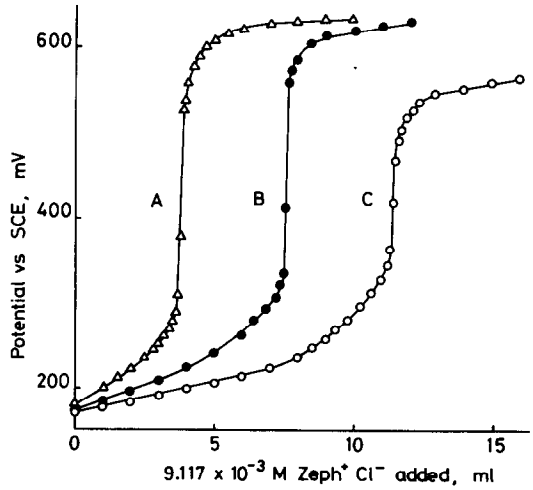

Fig. 6. Potentiometric titration of synthetic detergent: "Family" diluted 50-fold; A: 5 ml; B: 10 ml; C: 15 ml.

Table 2. Comparison of the determination of anionic detergents

Anionic surface-active substance	Mean and 95% confidence limits of $1.826 \times 10^{-3}M$ $Zeph^+Cl^-$ required*, *ml* Present method	Epton's method	Relative difference %
Sodium dodecyl benzenesulphonate	5.04 ± 0.01	5.01 ± 0.01	0.6
Sodium dodecyl sulphate	5.44 ± 0.04	5.41 ± 0.02	0.6
"Family"†	4.75 ± 0.00	4.74 ± 0.01	0.2
"Coop K Soft"‡	6.08 ± 0.01	5.94 ± 0.01	2.4
Shampoo§	5.64 ± 0.00	5.57 ± 0.01	1.3

* Five-ml portions of sample solution were titrated.
† Kao Soap Co.; 200-fold dilution.
‡ Minasama Soap Co.; 100-fold dilution.
§ Tamanohada Soap Co.; 100-fold dilution.

cheapness. After the end-point the electrode responds to Zephiramine, so there is sharp indication of the end-point. As shown in Fig. 6, one of the kitchen cleaning materials, "Family" (Kao Soap Co.), was successfully titrated with Zephiramine solution. The main component of "Family" is sodium polyoxyethylene alkyl ether sulphate. Dodecylbenzenesulphonate and several synthetic detergents were titrated by the present method and the results were compared with those obtained by Epton's method.[15–17]

Table 2 shows that the method based on use of the electrode gives results within 2.5% of those obtained by using Epton's method.[15–17]

Thus, the determinations of DBS^- in turbid, coloured or fluorescent samples are feasible.

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A SEMI-AUTOMATED SINGLE-CELL GRADIENT-TITRATION SYSTEM, USING ION-SELECTIVE ELECTRODES AS END-POINT SENSORS

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Summary—A novel system for semi-automated potentiometric analysis by gradient titration has been devised. In this technique both the reagent gradient generation and the titration are carried out in the sample container. The determination of chloride ion, with two ion-selective electrodes used simultaneously as an end-point sensor, is chosen to illustrate the technique.

Potentiometry with ion-selective electrodes is currently finding increasing application in the field of continuous analysis.[1,2] In most on-line monitoring applications of these devices the direct potentiometric approach is employed, where the measured potential is related to the concentration of the determinand ion, by means of a previously prepared calibration curve or the injection of suitable standards. The precision attainable by direct potentiometry is seriously limited by the logarithmic potential–activity relationship inherent in the Nernst equation. A 1-mV error in potential for a univalent electrode gives an error of 4% in the measured activity. This limitation is overcome when ion-selective electrodes are used as end-point sensors in titrations, but at the expense of the convenience of direct potentiometry.

The usual method of potentiometric end-point indication is to follow the change in activity of either the reagent added or the sample, with a selective electrode and a constant-potential reference electrode. The latter is usually a reversible electrode of the second kind, such as calomel or silver/silver chloride, supported in a tube containing a salt solution. Electrical contact between the reference half-cell and the solution is made by various means, and errors often result from variations in liquid-junction potential caused by a flow of reference electrolyte into the sample. In addition to altering the ionic strength, this electrolyte may act as an interferent. Porous junctions are also liable to blockage, especially in streams containing particulate matter. The reference electrode may, however, be another membrane electrode, the membranes being selective toward the same ion, as in null-point potentiometry,[3] or different ions. In the latter case, a fixed activity of one of the ions makes the respective electrode act as a constant-potential (reference) electrode, and the cell has no liquid junction. The fluoride electrode has been used as a reference electrode,[4] and with suitable instrumentation[5] both glass pH and glass Na^+ electrodes can be used as reference electrodes for direct potentiometry and potentiometric titrations. A new variant of differential potentiometric titration at zero current, with two membrane-electrodes, has been presented for the titration of cyanide with silver nitrate solution.[6] This system has been adapted to provide the end-point sensor for the titration of chloride ions.

The technique of gradient titration[7] has been developed by Fleet and co-workers[2,8–11] as an approach to the automation of ion-selective electrode potentiometry, which combines the convenience of direct potentiometry with the precision of a titration process. In the simplest approach a constant flow of sample is mixed with a stream of reagent, the concentration of which is increased to form a gradient. The gradient can be generated either by a two-cup mixing technique[12,13] or by coulometric generation.[11] The simplest means of gradient production is shown in Fig. 1. Two vessels (I and II) initially contain solutions A and B at concentrations C_A, C_{B_0}. Solution A is pumped from I to II and then the mixture out from II. The concentration (C_B) in II at any instant can be expressed as follows:[13]

$$C_B = C_A + \left(\frac{C_{B_0} - C_A}{V_{B_0}}\right)[(F_A - F_B)t + V_B]^{(F_A/F_B - F_A)} \quad (1)$$

where F_A = flow-rate from I to II, F_B = flow-rate from II, V_{B_0} = initial volume in II and V_B = volume in II at time t.

If $F_B = 2F_A$, equation (1) reduces to:

$$C_B = C_A + \left[\frac{C_{B_0} - C_A}{V_{B_0}}\right](V_B - F_A t). \quad (2)$$

* Present address: SGS Kenya Ltd, P.O. Box 90264, Mombasa, Kenya.

† Present address: HSA Reactors Ltd, 17-44 Fasken Drive, Rexdale, Ontario, Canada.

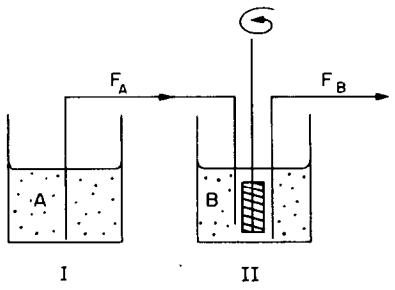

Fig. 1. Experimental arrangement for gradient production.

When A is water, $C_A = 0$, and the concentration of B is then a direct linear function of time.

If, however, vessels I and II contain different solutions, A and B, then their concentration profiles *in vessel II* will show a decreasing gradient of B and an increasing gradient of species A. When vessel I contains a titrant and vessel II the sample solution, the titration equivalence-point will be reached at the time x when the stoichiometric concentration ratio is obtained (Fig. 2a). With a fixed reagent concentration (C_A) any concentration of sample (C_{B_0}) may be determined as can be seen in Fig. 2b. The relationship between solution flow-rates (F_A, F_B), sample and reagent concentrations (C_B, C_A), sample volume (V_{B_0}), and titration end-point (x) can be readily deduced from the two sets of similar triangles:

$$C_{B_0} = \frac{C_A x}{D - x} \tag{3}$$

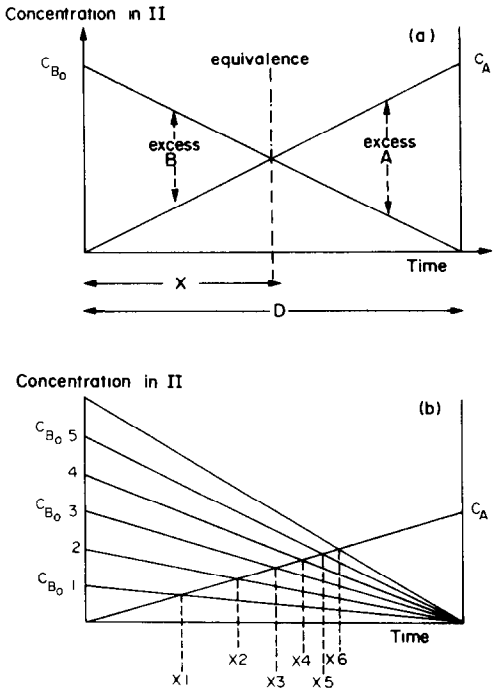

Fig. 2. Concentration *vs.* time profiles for the single-cup gradient titration. (*a*) Concentration of species A and B in vessel II as a function of time. (*b*) Titration end-point values (x) for varying initial concentrations of sample B.

$$D = V_{B_0}/(F_B - F_A) \tag{4}$$

where D is the time taken to empty vessel II.

In the present work a modified titration technique is described, where the reagent gradient is developed in the sample container with a peristaltic pump. This variant was devised for use with the AutoAnalyzer system, and the determination of chloride by differential ion-selective electrode potentiometric measurement was chosen to illustrate the technique.

EXPERIMENTAL

The differential amplifier used was an Analog Devices instrumentation amplifier (Model 603K), which has an impedance of 10^{12} ohm at both inputs, variable gain (1–2000), and includes balance and output offset controls.

The flow-cell was designed (Fig. 3) to have a minimum dead volume (< 100 μl), and to enable the two membranes to sense the ionic activity in the same portion of solution simultaneously. It also creates the minimum disturbance of solution flow.

The electrodes used for the differential titration of chloride are the sulphide and chloride ion-selective electrodes. These were made by cold-pressing Ag_2S and a mixture of Ag_2S and AgCl, respectively, in a 6-mm diameter die under a weight of 2 tons. The membranes were fixed into Delrin plastic tubes (Du Pont) which were externally threaded to allow assembly into the flow-cell. Contact between the membrane and amplifier was made by doubly-screened cables and a silver-loaded epoxy solid contact.

Stock solutions of silver nitrate and potassium chloride were made from analytical-reagent grade chemicals. The potassium chloride solution was standardized by manual titration, with a potentiometric end-point. The standard serum samples were provided by Technicon Corporation (Ardsly, New York 10068) and had a stated chloride content of 104 meq/l.

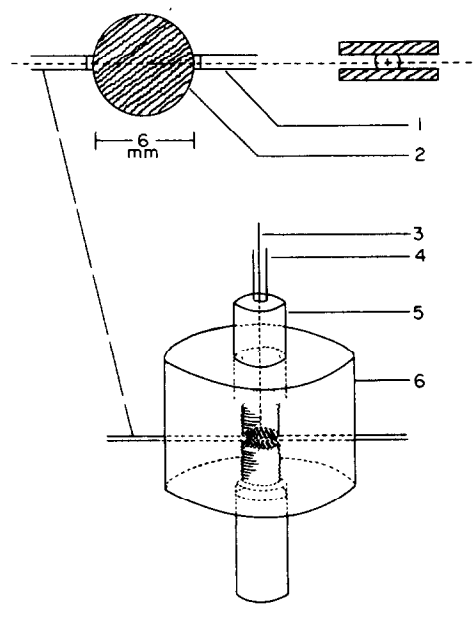

Fig. 3. Design of the flow-cell used for differential electrode potentiometry: 1, stainless-steel capillary inlet; 2, membrane; 3, silver connecting wire; 4, shielded cable; 5, "Delrin" support rod; 6, "Plexiglas" body.

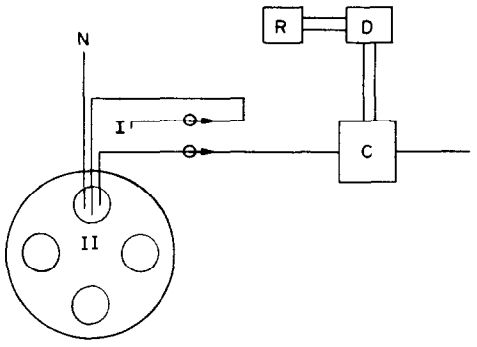

Fig. 4. Manifold design for single-cup gradient titration: I, reagent stream; II, sample vessel; N, nitrogen stream; C, measuring cell; D, differential amplifier; R, recorder.

Procedure

Samples were manipulated with a Technicon AutoAnalyzer proportioning pump and sampler. The manifold design is shown in Fig. 4. Sample solutions (5 ml) were pipetted into the cups of an AutoAnalyzer sampler turntable. Silver nitrate solution ($5 \times 10^{-3}M$) was pumped at 1 ml/min into the sample cup and the partially titrated sample was pumped into the measuring cell (C) at 2 ml/min. The solution in the sample cup was mixed by a rapid stream of nitrogen (N).

Samples, consisting of either pure aqueous potassium chloride solution or 125-μl aliquots of a standard serum diluted to 5 ml with 1.0M nitric acid, were presented for titration sequentially, interspersed by a water wash. The output from the electrodes was fed to the differential amplifier unit (D) and the potential *vs.* time profile was displayed on a potentiometric recorder (R). The system was operated at a rate of 30 samples/hr, but this could possibly be increased by using more rapid generation of the gradient.

RESULTS AND DISCUSSION

In preliminary investigations, samples of potassium chloride were titrated to a potentiometric end-point, with a calomel reference electrode and either a chloride-sensitive or silver-sensitive electrode as the indicating system. In both cases the titration curve was the expected S shape common to potentiometric titrations. The same chloride sample was then titrated, with both the silver chloride and silver sulphide electrodes in the "difference" mode to produce a peaked titration curve. In order to interpret the shape of the curves it is necessary to define the potential-determining species for both electrodes at various times during the titration process. Morf *et al.*[14] have made a detailed study of the response of solid-state membrane electrodes and have interpreted the selectivity and detection limit for the silver halide membranes in terms of the equilibrium activity of Ag^+ at the membrane surface. This silver-ion activity can result either from the finite solubility of the membrane or from the intrinsic defect level of Ag^+ within the membrane itself. These principles have been employed in an attempt to interpret the shape of the titration curves in the present system.

In the initial part of the titration both electrodes effectively respond to chloride ion, in the case of the sulphide membrane through an interference effect for which the potential can be expressed as

$$E = E^0 - \frac{RT}{F} \ln (K_{S/Cl}\, a_{Cl^-})$$

where $K_{S/Cl}$ is the selectivity ratio of the sulphide membrane towards chloride, and a_{Cl^-} is the activity of the chloride ion in solution. The potential of the chloride membrane becomes constant near to the end-point since the solubility of the membrane controls the activity of Ag^+, which is very much greater than the defect level of Ag^+. The predicted electrode potential changes are shown schematically in Fig. 5. The potentials of both electrodes will initially follow the decrease in chloride concentration until the effective pAg^+ is approximately 5.5, which represents the detection limit for chloride ion. In this region, the potentials of both electrodes remain almost constant. With increasing additions of Ag^+ titrant, the sulphide electrode will be the first to show a response, owing to its lower detection limit, defined by the defect activity of Ag^+ in the membrane. The different responses of the two membranes in this region cause the peak-shaped response. With further addition of titrant, the Ag^+ level will eventually exceed the detection limit of the silver chloride membrane, defined by the solubility product of AgCl, and both electrodes will exhibit a similar response to further increase in silver-ion concentration.

To investigate the validity of using the differential peak as an end-point, a series of chloride solutions was prepared and titrated. The timing sequence for the automated system and typical titration curves are given in Fig. 6. A statistical analysis of observed and calculated end-points from 30 titrations provided the following regression equation:

$$\text{observed end-point} = 1.13\ [\text{calculated end-points}] - 0.159$$

and the correlation coefficient was 0.997.

The deviation of the observed from the calculated values includes contributions from the dead-time of

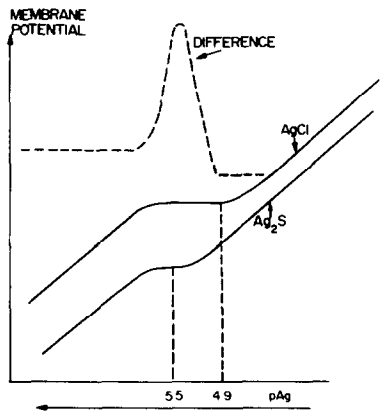

Fig. 5. Dependence of chloride and sulphide membrane potentials on silver ion activity.

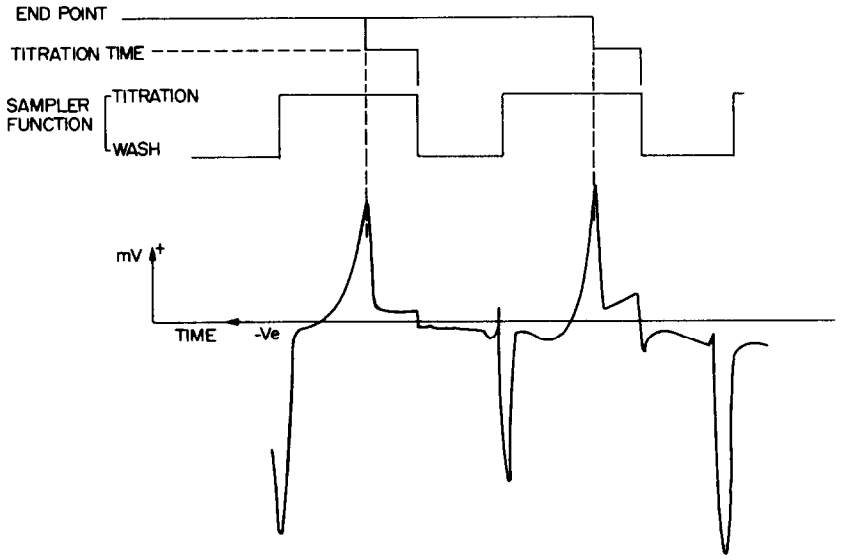

Fig. 6. Timing diagram and typical titration curves for gradient titration of aqueous chloride samples.

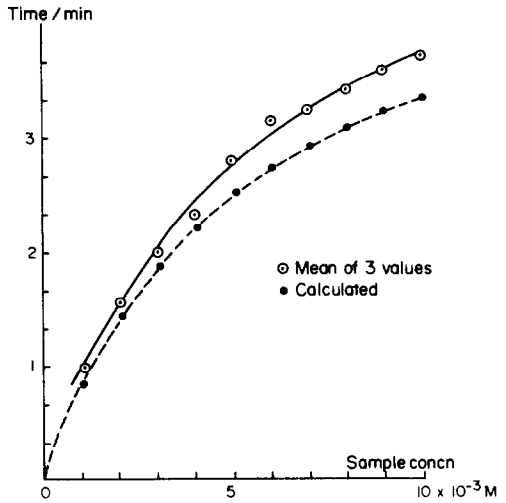

Fig. 7. Calibration graph for the gradient titration of aqueous chloride samples.

the manifold and small variations in flow-rates. This is not a serious problem as unknown concentrations are usually evaluated from an empirical calibration graph (Fig. 7).

The titration curves obtained for the determination of chloride in a standard serum (Fig. 8) are less well defined than those for pure aqueous standards. The end-points are, however, easily identifiable. Samples of diluted serum were titrated and the following results were obtained:

mean titration time (7 values) = 1.71 min
rel. standard deviation = 0.4%
chloride concentration by interpolation = $2.61 \times 10^{-3} M$
concentration of serum standard = 104.6 meq/l.
stated concentration = 104 meq/l.

The value found is within the tolerance level quoted for the sample and well within the acceptable limits

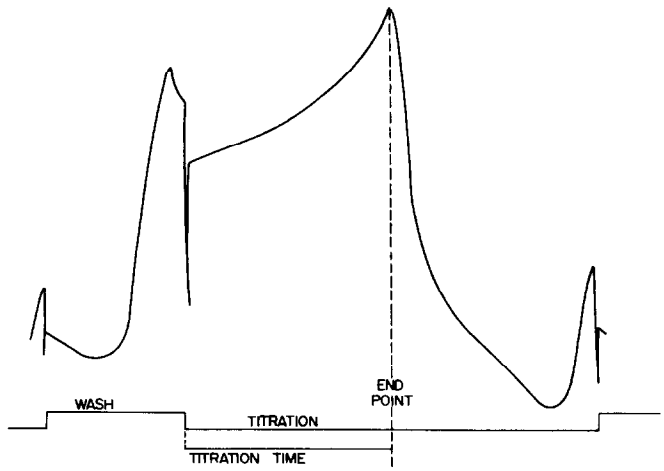

Fig. 8. Potential *vs.* time profile for the titration of a standard serum.

for most clinical chloride determinations, although interferences may be found in real serum solutions.

Conclusion

The method presents a convenient approach to the automation of a wide range of titration systems and is easily assembled from standard AutoAnalyzer modules. Although the precision of the titration is dependent to some extent on the time required for titration, acceptable results can often be obtained with very short titration times, *e.g.*, 10 sec, particularly when an automatic timing system is used. Such a system incorporating a coulometric gradient generator and a digital timing/measurement device is currently being developed in this department.

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UNIVERSAL TECHNIQUE FOR RAPID DISSOLUTION OF MATERIALS ENCOUNTERED IN THE STEEL INDUSTRY AND ITS APPLICATIONS IN THE PHOTOMETRIC DETERMINATION OF ARSENIC, PHOSPHORUS, TITANIUM AND VANADIUM IN IRON ORES*

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Summary—A rapid, simple and virtually universal technique is described for decomposing a wide range of materials such as ores, sinters, slags, ferro-alloys and other reagents and additives used in the steel industry, as well as environmental dusts and particulate matter. The procedure involves fusion with sodium peroxide, alone or with sodium carbonate (mixed flux) in a zirconium or vitreous carbon crucible. Treatment of the fused melt with water and acid results in complete dissolution. It avoids the tedious operations involved in dissolving residues left by other dissolution techniques. Losses of normally volatile elements such as arsenic, phosphorus, lead and zinc do not occur. Application of this technique is described, particularly for the photometric methods developed for determining arsenic, phosphorus, titanium and vanadium in iron ores in the ranges 0.0002–0.1% As, 0.002–0.6% P, 0.01–0.6% Ti, and 0.001–0.5% V. Both the precision and accuracy are excellent.

Some physical methods of analysis of materials encountered in the steel industry are applicable to solids, with or without initial preparation such as polishing or briquetting. However, the physical form of the sample does not always lend itself to such techniques; hence dissolution of the sample, usually with an aqueous medium, is necessary before determinations by the more traditional "wet chemical" procedures, or by such physico-chemical methods as atomic-absorption spectroscopy, d.c. or inductively coupled plasma spectroscopy, or by automatic analyser procedures designed for use with such solutions.

With the wide variety of materials with which the steel chemist is confronted, it is not surprising that a wide diversity of sample dissolution procedures has evolved, usually based on attack by acidic media chosen in the light of the material to be dissolved and the nature of the subsequent analytical steps. Sometimes this step can be rather time-consuming. In many instances some portion of the sample remains undissolved after the preliminary attack, and for completeness, it is not uncommon to filter this off, ignite it, fuse with a suitable flux, then dissolve the cooled melt and add the solution to the initial solution.

Except when prior knowledge indicates that any residue can be discarded without significant effect on the result, there seems little to be gained by using such an initial acid attack, especially when it is slow. If a fusion must be done, it might as well be done at the beginning to save time, provided the analytical procedure can tolerate the flux used.

The procedure found in our laboratories to apply almost universally to materials submitted for wet chemical analysis is to fuse the sample either with sodium peroxide or with a peroxide–carbonate mixed flux. Choice of a suitable crucible material depends on cost or on the element sought (and avoidance of possible interferences). Thus vitreous carbon crucibles avoid contaminating the subsequent solution, but are expensive and have limited life; zirconium crucibles have a wide range of usefulness but cause interference in calcium determination.[1] Similarly, choice of the acid used for dissolution of the cooled melt depends on the analytical procedure chosen, so a number of variations of this basic technique can be made.

Redox determination of total iron in iron ore, sinters *etc.*, by use of the rapid dissolution technique and reduction with stannous chloride[2] or the silver reductor[3] has been reported earlier. Similarly composite determinations of major components of sinters[4] and blast-furnace slag,[1] with use of an AutoAnalyzer, have been described. This technnique of rapid dissolution has also been successfully employed for determining aluminium complexometrically in iron ores, sinters, *etc.*,[5] and this method has been approved as an ISO standard.

To illustrate the wide applicability of the technique, rapid methods developed for determining arsenic,

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phosphorus, titanium and vanadium photometrically in iron ores, sinters *etc.*, are given here.

EXPERIMENTAL

Apparatus

Zirconium crucibles of 50 ml capacity or vitreous carbon crucibles of 20 ml capacity (available from Le Carbone Lorraine, Paris). The apparatus for generation and absorption of arsine (arsenic method) has already been described.[6]

Reagents

Only reagents that need special preparation are mentioned here. Others are given in the procedures.

Lead acetate cotton. Dissolve 30 g of lead acetate trihydrate in 300 ml of water and make just acid with acetic acid. Soak absorbent cotton in this solution. Allow excess of solution to run out, then dry at 110°.

Stannous chloride solution, 40%. Dissolve 40 g of stannous chloride dihydrate in concentrated hydrochloric acid by gentle heating, cool, dilute to 100 ml with concentrated hydrochloric acid.

Arsine absorption solution. Dissolve 0.41 g of ephedrine in 250 ml of chloroform. Weigh 0.20 g of silver diethyldithiocarbamate into a 400-ml beaker, add a few ml of the ephedrine solution to make a paste, then add the remainder and stir to dissolve. Store in a glass-stoppered amber-coloured bottle away from light. Filter, if necessary, before use.

Standard arsenic solution. Dissolve 0.6601 g of arsenic trioxide in 20 ml of 20% sodium hydroxide solution and dilute to 1 litre in a standard flask. Dilute 10 ml of this solution (As = 500 μg/ml) to 500 ml in a standard flask with water. This working solution (As 10 μg/ml) is stable for at least one month.

Ammonium molybdate solution. Add 300 ml of sulphuric acid to 500 ml of water and cool. Add 20 g of $(NH_4)_6Mo_7O_{24}.4H_2O$. Stir to dissolve and dilute to 1 litre with water.

Standard phosphorus solution (50 μg/ml). Dissolve 0.2292 g of anhydrous disodium hydrogen phosphate (Na_2HPO_4), dried at 105°, in water and dilute to 1 litre in a standard volumetric flask.

Molybdate–hydrazine sulphate solution. Add 25 ml of the ammonium molybdate solution to 55 ml of water. Add 10 ml of freshly prepared 0.15% hydrazine sulphate solution. Dilute to 100 ml with water. Prepare fresh as needed.

Acetate buffer. Dissolve 220 g of sodium acetate trihydrate in water. Add 220 ml of glacial acetic acid and dilute the mixture to 1 litre.

Fusion blank solution A (for titanium). Weigh 0.300 g of pure iron powder into a zirconium crucible. Add 4 g of sodium peroxide and mix. Fuse over a Méker burner, swirling the crucible until the melt is cherry-red and clear. Remove from the heat and swirl until the melt solidifies on the walls of the crucible. Cool for 1–2 min, then place the crucible in a 250-ml beaker. Cover with a watch-glass and cautiously add 10 ml of water to the crucible. After the effervescence ceases, empty the crucible into the beaker and wash the crucible with about 10 ml of water. Add 10 ml of concentrated hydrochloric acid to the crucible, empty it into the beaker, and rinse it into the beaker with about 10 ml of water. Boil the solution for about 2 min. Cool, transfer to a 100-ml standard flask, and dilute to the mark with water.

Fusion blank solution B (for vanadium). Transfer 1.300 g of pure iron into a zirconium crucible containing 4 g of sodium peroxide. Add 4 g more of sodium peroxide and mix thoroughly. Fuse as for fusion blank solution *A*, finally placing the cooled crucible in a 400-ml beaker. Cover with a watch-glass, cautiously add 20 ml of water to the crucible, and after effervescence ceases empty the crucible into the beaker and rinse with 10 ml of water. Add 30 ml of sulphuric acid (1 + 4) to the crucible, empty it into the beaker and rinse in with about 10 ml of water. Add 100 ml of sulphuric acid (1 + 4), then 1 or 2 drops of hydrogen peroxide until the solution is clear yellow. Boil the solution for about 2 min, cool, transfer to a 200-ml standard flask and make up to the mark with water.

Standard titanium solution. Fuse 0.1000 g of titanium metal (fine powder), with 2 g of potassium bisulphate in a platinum crucible at a low heat. When a clear melt is obtained, cool somewhat and dissolve the contents in 10 ml of sulphuric acid (1 + 1). Dilute to 1 litre in a standard flask. Dilute 20 ml of this solution to 100 ml in a standard flask to obtain the working solution (Ti 20 μg/ml).

Standard vanadium solution (50 μg/ml). Weigh 0.230 g of ammonium metavanadate (NH_4VO_3) into a 150-ml beaker. Add 60 ml of water and gently simmer to dissolve. Cool, add 1 ml of concentrated sulphuric acid and dilute to volume with water in a 100-ml standard flask. Pipette 5 ml of this solution into a 100-ml standard flask containing 50 ml of water and 1 ml of concentrated sulphuric acid, and dilute to volume with water to obtain the working solution (v 50 μg/ml).

Procedures

Arsenic. Run the samples and standards simultaneously. Prepare the standards as follows, and treat the samples in the same way. Into each of six zirconium crucibles weigh 0.500 g of ferric oxide and 3 g of sodium peroxide for the range 0.0002–0.01% As or 0.05 g of ferric oxide and 1 g of sodium peroxide for the range 0.01–0.10% As. Mix thoroughly. Fuse each as for the fusion blank solutions, place each cooled crucible in a separate 250-ml beaker, cover with a watch-glass and add 10 ml of water to the crucible. After effervescence ceases, empty the crucible into the beaker and rinse in with about 10 ml of water, 20 ml of sulphuric acid (1 + 1) and 10 ml of water. Transfer 0, 0.3, 0.5, 1.0, 2.0, and 5.0 ml of the 10-μg/ml arsenic solution into the respective beakers. Gently boil the solutions for about 2 min. Cool. At this point the arsine generation and absorption apparatus should be made ready by placing a small plug of the lead acetate cotton in position, connecting the capillary delivery tube and removing the "inner joints" with the capillary tubes attached.

Transfer the solutions to the generation flasks with a minimum of water. To each flask add 2.0 ml of freshly prepared 15% potassium iodide, mix and let stand for 5 min. Add 40% stannous chloride solution dropwise with swirling until the iron and liberated iodine have been reduced (solution becomes colourless), then add 5 drops in excess. Let stand for 15 min, during which pipette 5 ml of the arsine-absorption solution into the absorption test-tubes. Quickly introduce 3.0 g of arsenic-free 20-mesh granulated zinc into the flask through a short-necked funnel and immediately fit the delivery and absorption system. Periodically swirl the flask. Let stand until no further gases are evolved (about 15–20 min). The funnel prevents any zinc from adhering to the ground-glass joint (which would prevent a gas-tight seal).

Immediately measure the absorbance of the absorption solutions at 540 nm in 1-cm cuvettes against the absorption solution as reference. Subtract the blank to obtain net absorbance and plot this *vs.* μg of arsenic on the calibration graph. Read off the arsenic contents of the samples.

$$\%As = \frac{\mu g \text{ of As} \times 10^{-4}}{\text{sample wt (g)}}$$

Phosphorus. For the calibration curve, place 0.5 g of sodium carbonate and 2 g of sodium peroxide in each of six 150-ml beakers and treat each as follows. Add 10 ml of water, swirl to dissolve, add 10 ml of 70% perchloric acid

and mix. Add 0, 0.5, 1.0, 1.5, 2.0 or 3.0 ml of 50-μg/ml phosphorus standard, boil for a few min and cool. Transfer to a 50-ml standard flask and dilute to the mark with water. Pipette 10 ml into a 150-ml beaker and add 5 ml of water, 1 ml of 70% perchloric acid and 10 ml of freshly prepared 15% anhydrous sodium sulphite solution and bring to the boil. Add 20 ml of molybdate–hydrazine sulphate solution. Heat in a boiling water-bath for 10 min. Cool, transfer into a 50-ml standard flask and dilute to the mark with water. Within the next 2 hr measure the absorbance at 725 nm in a 2-cm cuvette against water as reference. Subtract the blank to obtain net absorbance. Plot net absorbance *vs.* μg of phosphorus.

Transfer a suitable weight of iron ore sample (containing up to 300 μg of phosphorus) into a vitreous carbon crucible containing 0.5 g of sodium carbonate, add 2 g of sodium peroxide and mix. Fuse, cool, leach and rinse with water (10 ml for each operation) into a 250-ml beaker as already described, add 10 ml of 70% perchloric acid to the crucible, empty the crucible into the beaker and rinse it with about 10 ml of water. Boil the solution for about 2 min. Cool, transfer into a 50-ml standard flask and dilute to the mark with water. Treat a 10-ml aliquot (or a smaller fraction made up to 10 ml with the blank calibration solution) as described for the calibration. Subtract the blank to obtain net absorbance. Read off μg of phosphorus from the calibration graph.

$$\%P = \frac{\mu g \text{ of phosphorus} \times 10^{-4}}{\text{sample wt (g)}}$$

Titanium. For the calibration curve, into each of a series of seven 100-ml standard flasks, pipette 10 ml of fusion blank solution *A* and add 0, 0.5, 1.0, 2.5, 5.0, 10.0 or 15.0 ml of 20-μg/ml titanium working solution.

Add 10 ml of freshly prepared 5% ascorbic acid solution, mix, wait 5 min, add 15 ml of freshly prepared 1% chromotropic acid solution and mix. Adjust (using a close-range pH paper) to pH 3.5–4.0 with acetate buffer. Dilute to the mark with water. After 15 min measure the absorbance at 470 nm in a 2-cm cuvette against water as reference. The colour is stable for at least 2 hr. Subtract the blank to obtain net absorbance. Plot net absorbance *vs.* μg of titanium.

Transfer 0.500 g of iron-ore sample into a zirconium (or nickel) crucible. Add 4.0 g of sodium peroxide and mix. Carry out the fusion, dissolution and dilution as described for preparation of fusion blank solution *A*. Pipette 10 ml into a 150-ml beaker and apply the photometric procedure described for the calibration. Read off μg of titanium from the calibration graph.

$$\%Ti = \frac{\mu g \text{ of titanium} \times 10^{-3}}{\text{sample wt (g)}}$$

Vanadium. For the calibration curve, into each of a series of 125-ml separatory funnels, pipette 25 ml of fusion blank solution *B*. Add 0, 0.25, 0.5, 1.0, 2.0, 4.0 or 5.0 ml of 50-μg/ml vanadium solution and enough water to make the total volume 30 ml. Treat each as follows. Add 0.3% potassium permanganate solution (~0.1*N*) dropwise until a faint pink persists for 2 min, and then 3 drops more. Wait for 2 min. Add 20 ml of concentrated hydrochloric acid and mix. Add 10 ml of freshly prepared 0.25% solution of *N*-benzoylphenylhydroxylamine (BPHA) in chloroform, and shake the funnel for 45 secs. Let the layers separate, draw off the organic phase into a dry 50-ml beaker, shake the aqueous phase with 10 ml of chloroform for 30 sec and draw off the organic layer into the beaker. Transfer the combined organic extracts into a dry 50-ml standard flask and make up to volume with chloroform. Measure the absorbance (within the next 2 hr) in a 1-cm cuvette at 530 nm against chloroform as reference. Subtract the blank to obtain the net absorbance. Plot net absorbance *vs.* μg of vanadium.

Transfer 0.500 g of iron-ore sample into a zirconium crucible. Add 2 g of sodium peroxide and mix. Carry out the fusion, dissolution and dilution as described for fusion blank solution *B*. Take an appropriate aliquot (25 ml for 0.001–0.1% V, 10 ml for 0.1–0.2%, 5 ml for 0.2–0.5%, plus enough fusion blank solution *B* to give a total volume of 25 ml), and apply the photometric procedure described for the calibration. Subtract the calibration blank to obtain the net absorbance. Read off μg of vanadium from the calibration graph.

$$\%V = \frac{\mu g \text{ of vanadium} \times 0.005}{\text{sample wt (g)} \times \text{sample aliquot (ml)}}$$

RESULTS AND DISCUSSION

Arsenic

The method is based on our photometric method for arsenic in steel.[6] We have established earlier that the concentration of sulphuric acid and the other parameters must be kept constant to avoid an erratic yield of arsine. Treating the peroxide fusion melt with hydrochloric acid and fuming with sulphuric acid leaves inconsistent amounts of the latter, and it is more reliable to avoid the fuming by dissolving the fusion melt directly in dilute sulphuric acid for subsequent reduction with potassium iodide and stannous chloride. This gives precise and accurate results.

In the earlier procedure pyridine was used as solvent for the silver diethyldithiocarbamate. Pyridine is not desirable, owing to its obnoxious odour, and has been successfully replaced by an ephedrine solution in chloroform. The present method is more rapid than the earlier steel method. It also avoids any distillation of arsenic trichloride such as may occur with methods based on photometry of the arsenomolybdenum blue complex. Table 1 shows the results of comparison tests with ISO iron-ore samples.

Phosphorus

In the current ASTM procedures for determining phosphorus in iron ores,[7] both the titrimetric and the phosphovanadomolybdic acid photometric methods are tedious and cumbersome. The photometric method involves the extraction of the complex with isoamyl alcohol (pungent odour). The sample dissolution technique and subsequent treatment of the residue are obviously time-consuming. The present method is direct, rapid and does not involve any separations or solvent extraction. The phosphomolybdenum blue

Table 1.

	Arsenic, %	
Sample	Expected	Found
ISO 76-12	0.0008	0.0006, 0.0005
ISO 76-16	0.002	0.0016, 0.0016
ISO 76-23	0.02	0.025, 0.025
ISO 76-24	0.055	0.063, 0.062

Table 2.

	Phosphorus, %	
Certified	Found	
BCS 302	0.71	0.73
BCS 303	0.54	0.51, 0.52
BCS 378	0.037	0.039, 0.038
BCS 172/2	0.047	0.047, 0.048
BCS 377	0.31	0 32
Minette (ISO)	0.66	0.65
Japan 800-1	0.042	0.044
Japan 830-1	0.125	0.119
Japan 850-1	0.017	0.019
NBS 27C	0.028	0.030
NBS 690	0.011	0.010, 0.010
NBS 692	0.039	0.040, 0.040

formed under the conditions of the method is stable for several hours. As is common with phosphomolybdenum blue methods, fuming with perchloric acid leaves inconsistent amounts of the acid with consequent erratic results. In our procedure the cooled melt is dissolved in perchloric acid. Absence of a fuming step results in a constant amount of perchloric acid and greatly improved precision. Since zirconium phosphate is insoluble the sample is fused in a vitreous carbon crucible. A precision test on two South African reference standard iron ores (10 replicates of each) gave a standard deviation of 0.001% phosphorus.

Table 2 shows that the results for various reference standard iron ores are quite satisfactory.

Titanium

The current ISO standard method for determining titanium in iron ores uses an acid dissolution followed by treatment and fusion of the insoluble residue. This makes the method unnecessarily time-consuming. Our universal technique for rapid dissolution obviates this time-consuming step and yields complete decomposition in a single step. We have found that pH control before formation of the titanium complex with chromotropic acid is important. The optimum pH is 4. Increasing the pH above 4 leads to a drop in the absorbance. Relatively high concentrations of elements normally encountered in iron ores, including phosphorus (*e.g.*, BCS 303: 0.5% P) and vanadium (*e.g.*, Philippine iron sand 0.3% V), do not interfere. Zirconium or nickel leached during the fusion of the sample will not interfere. Identical results were obtained with zirconium and nickel crucibles. The titanium complex is stable for 16 hr under the conditions of the procedure. Table 3 shows some results for standard samples. For standard reference samples the standard deviation was found to be 0.001% Ti at the 0.03% level and 0.008% Ti at the 0.5% level.

Table 3.

	Titanium, %	
Sample	Certified	Found
BCS 302	0.216	0.226, 0.224
BCS 303*	0.179	0.187, 0.183
BCS 378	0.070	0.060, 0.070
BCS 175/2	0.057	0.056, 0.058
BCS 377	0.114	0.117, 0.115
Philippine iron sand†	3.84	3.91, 3 92

* Contains 0.5% P
† Contains 0 3% V.

Table 4.

	Vanadium, %	
Sample	Certified	Found
Japan SRM 830-1	0.31	0.31, 0.32
Japan SRM 850-1	0.050	0.050, 0.050
South African SRM 11	0.004	0.005, 0.005
South African SRM 12	0.052	0.050, 0.050

Vanadium

Determination of vanadium in steel with BPHA has been in use in our laboratories for the last 14 years. This reagent, under the conditions of our method, is specific for vanadium. The ASTM committee on chemical analysis of metals has successfully completed round-robin testing for determining vanadium in steel, including highly alloyed steels and high-temperature alloys, by this procedure. The method is on its way to becoming an ASTM standard method. On the basis of this and the rapid dissolution technique, it was thought worthwhile to explore the application of BPHA for determining vanadium in iron ores. The cooled melt was dissolved in dilute sulphuric acid. Vanadium was oxidized with permanganate and determined with BPHA as described above. The critical point is ensuring oxidation of vanadium to the quinquevalent state before addition of hydrochloric acid and subsequent extraction and colour formation with BPHA. This is achieved in the present procedure. Precision tests on two South African reference standard iron ores (10 replicates each) gave a standard deviation of 0.001% V at the 0.005% level and 0.002% V at the 0.05% level. Table 4 shows results for some standard reference iron ores.

Other applications

In addition to the specific applications described above, the rapid dissolution procedure has a wide range of usefulness, as illustrated by Table 5, demonstrating the feasibility of its application to the analysis of a wide range of materials encountered in the steel industry.

Combined with the rapid progress in the development of automated instrumental methods for wet analysis, this procedure offers attractive alternatives

Table 5. Summary of applications of the dissolution technique

Material	Flux	Crucible	Acid	Analytical procedure	Element	Range, %
Ore–Sinter	Mixed	Zr	HCl	Redox	Fe	30–70
	Mixed	Zr	HCl	Complexometric	Al	0.25–5
	Mixed	V.C.*	$HClO_4$	Photometric	P	0.002–0.07
	Peroxide	Zr	HCl	Photometric	Ti	0.01–0.6
	Peroxide	Zr	H_2SO_4	Photometric	V	0.001–0 5
	Peroxide	Zr	H_2SO_4	Photometric	As	0 0002–0.1
Sinter	Mixed	V.C.*	HCl and H_2SO_4	AutoAnalyzer	Si	1 6
					Al	0.2–0.8
					Ca	4–12
					Mg	1–6
					Fe	50–66
B.F. Slag	Peroxide	Pt†	HCl and H_2SO_4	AutoAnalyzer	Si	20
					Al	5
					Ca	30
					Mg	9
Ferro-phosphorus	Mixed	Zr	$HClO_4$	Gravimetric	Si	0.5–4
Ferro-alloys	Mixed	Zr	HCl	Atomic absorption	Al	0.01–0.8
	Mixed	Zr	HCl	Atomic absorption	Ti	0.01–0.6
Atmospheric dusts	Peroxide	Zr	HCl	Atomic absorption	Fe	ppm
					Mn	ppm
					Pb	ppm
					Zn	ppm

* V.C. Vitreous carbon crucible.
† Sintering at 380° rather than fusing.
Mixed flux is sodium peroxide mixed with sodium carbonate.

to physical methods of analysis subject to matrix interferences. Analysis of a batch of 6 samples for any of the four elements takes less than 3 hr.

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CHELATOMETRIC DETERMINATION OF CALCIUM AND MAGNESIUM IN IRON ORES, SLAGS, ANORTHOSITE, LIMESTONE, COPPER–NICKEL–LEAD–ZINC ORES AND DIVERS MATERIALS

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Summary—Chelatometric methods for the determination of calcium and magnesium in iron ores, slags, anorthosite, copper–nickel–lead–zinc ores and various other materials are described. Potential interfering elements are masked with triethanolamine and potassium cyanide. In one aliquot calcium is titrated at pH >12, with calcein and thymolphthalein mixed indicator and in another aliquot calcium and magnesium are titrated in ammonia buffer, with *o*-cresolphthalein complexone screened with Naphthol Green B as indicator. The results compare favourably with certified values for reference materials of diverse nature.

The Canada Centre for Mineral and Energy Technology (CANMET) is engaged in a wide variety of projects, *e.g.*, electrosmelting of various ores by new processes; the characterization of various ores and materials to be used as standard reference materials as part of the Canadian Certified Reference Materials Project (CCRMP); studies of the effect of weathering on sediments and tailings; the development of new processes for the recovery of aluminium from anorthosite and other aluminium-bearing materials. Each of these programmes requires suitable accurate analytical methods for the determination of calcium and magnesium in the various kinds of materials used to meet their needs.

A previous report[1] has reviewed some of the analytical methods used for the determination of calcium and/or magnesium in various materials. Classical gravimetric procedures have long been used for the determination of macro amounts of calcium and magnesium in spite of the lengthy steps involved. Atomic-absorption spectrophotometry (AAS), on the other hand, is rapid and extremely useful for the determination of small amounts of calcium and magnesium and can frequently be employed without removing the other elements. Because of matrix effects, however, the absorption spectra may be depressed or enhanced in the presence of other elements and thus must be compensated for in the analysis by close control of the sample solution composition, *etc.*, and results must be compared with those for standards of similar composition. This requires some prior knowledge of the sample composition before the method can be applied on a routine basis. In many instances, a mercury cathode electrolysis or other separation method may have to be employed to remove heavy metals before the determination of the calcium and magnesium by AAS or classical gravimetric procedures.

In our laboratories an analytical procedure was required for the purpose of verifying results obtained by AAS, in case of dispute. One of the requirements of the method was that it should avoid the disadvantages described above and at the same time be simple, reasonably rapid, accurate and applicable to a wide variety of sample material.

Of the methods that were reviewed[1] those that involve titration with EDTA were considered to offer the greatest possibilities with regard to speed, simplicity and suitable accuracy. The review revealed that there is comparatively little information concerning the chelatometric determination of calcium and magnesium in iron ores, slags, complex copper–nickel–zinc–lead ores and tailings or anorthosite. On the other hand, there are many methods for determining calcium and magnesium in limestone, dolomite, natural waters, cement, biological matter, *etc.* These methods vary considerably in technique but with few exceptions the methods need considerable modification to be directly applicable to complex ores and other materials that are discussed in this report.

Many chelatometric methods for the determination of calcium are based on precipitation of magnesium as insoluble magnesium hydroxide at pH >12.[2–7] The sum of calcium and magnesium is then determined on a separate sample or aliquot in an ammoniacal buffer.[2–4,8] Various complexing agents are used to mask iron, aluminium, copper, zinc, *etc.*[2–15] and the choice of indicators for the titration varies widely.[2–15] The addition of poly(vinyl alcohol) has been advocated to reduce the adsorption of the indicator on the magnesium hydroxide precipitate in the calcium titration, which otherwise leads to a sluggish end-point or low results for calcium.[10,16] In the

presence of too much sodium hydroxide and large amounts of magnesium some co-precipitation of calcium occurs with consequent premature end-points. The difficulty was said to be overcome by using sucrose and sodium carbonate and controlling the addition of alkali[17] but this has not been confirmed by others.[18]

While this report was in preparation, Sinha and Dasgupta[19] described a procedure for the determination of calcium, magnesium and manganese in ceramic material and glass. Their procedure is similar in basic principles to ours and employs the same indicators and masking agents. However, these authors recommend that if more than 5 mg of iron, titanium or zirconium is present, these elements should be removed because they adsorb the indicator or affect the end-point. In contrast we have found that much larger amounts of iron and titanium can be tolerated without affecting the results.

The successful application of chelatometric titration methods to the determination of calcium and magnesium in anorthosite, clays, iron ores, limestone, dolomite, sediments and tailings containing a mixture of sand, pyrite, calcite and/or dolomite and various types of other minerals and material is described. The methods are applicable in the range of 0.1–85% CaO and/or MgO.

EXPERIMENTAL

Reagents

EDTA solution, 0.025*M*. Dissolve 9.306 g of disodium ethylenediaminetetra-acetate dihydrate in water and dilute to exactly 1 litre in a calibrated flask. Standardize the solution against a standard calcium solution.

Standard calcium solution. Dry 3 g of analytical grade calcium carbonate at 150° for 1 hr. Weigh 2.500 g of the powder, dissolve it in the minimum amount of dilute hydrochloric acid, boil, cool and transfer it to a 1-litre calibrated flask and dilute to volume. 1 ml = 1.000 mg Ca.

Ammonia solution. Dilute the concentrated reagent with an equal volume of water and store in a plastic bottle.

Potassium hydroxide solution, 30%. Store in a plastic bottle

Triethanolamine solution, 50% *v/v*.

Poly(vinyl alcohol) solution, 1% *in water*.

Screened calcein indicator. Grind together 0.2 g of calcein, 0.12 g of thymolphthalein and 20 g of dry potassium chloride.

o-Cresolphthalein complexone indicator. Dissolve 10 mg of the solid indicator in 10 ml of 95% ethanol. Prepare fresh weekly.

Naphthol Green B, 0.1% *aqueous solution.*

Procedure for anorthosite, iron ores, basic slags, dolomite, limestone, refractory slags, plastic clay, phosphate rock, burnt magnesite, sediments, soda-lime glass and zinc ores

Dissolution. Using Table 1 as a guide for the sample weight and dilution, treat the sample with 10 ml of 12*M* hydrochloric acid, 5 ml of 16*M* nitric acid, 5 ml of 72% perchloric acid and 10 ml of 48% hydrofluoric acid in a Teflon beaker and carefully evaporate the mixture to fumes of perchloric acid*. Repeat the addition of acids as necessary to decompose the sample completely and volatilize the silica and excess of hydrofluoric acid. Transfer the solution to a glass beaker and heat to fumes again to remove the last traces of fluoride. Cool the sample, transfer it to a 250-ml calibrated flask and dilute to the mark with water†.

Titration of calcium. Transfer a suitable aliquot of the sample solution to a 600-ml beaker, dilute to ~300 ml with water and add a magnetic stirring bar. Add sufficient 30% potassium hydroxide solution to neutralize the acid

* With some materials, *e.g.*, sediments, it may be possible to omit the hydrofluoric acid and simply extract the calcium and magnesium with the other acids. The silica and insoluble material can be filtered off and the filtrate used for the determination of calcium and magnesium. This procedure is valid, however, only if the calcium and magnesium are not bound with the insoluble residue.

† If magnesium is not to be determined a smaller sample weight can be taken and the dilution step omitted if desired.

Table 1. Suggested sample weights, dilution and aliquots for the determination of calcium and magnesium in various materials

Sample	Weight/Dilution/Aliquot	Approximate net titration volume of 0.025*M* EDTA*, *ml* Ca	Mg
Anorthosite	1 g/250 ml/50 ml	15–20	0.5–1
Basic slag	0.4 g/250 ml/50 ml	25–30	5–10
Bauxite	3 g—no dilution	4	—
Burned magnesite	1 g/250 ml/25 ml	2	85
Dolomite	0.4 g/250 ml/50 ml	15–20	15–20
Iron ore			
(a) Lincolnshire	0.2 g—no dilution	30–35	—
(b) Northamptonshire	2 g/250 ml/50 ml	5–10	2–6
(c) Peace River	0.5 g—no dilution	10–15	—
Limestone	0.5 g/250 ml 50 ml	35–45	0.2–0.5
Phosphate rock	0.1 g—no dilution	35–40	—
Plastic clay	2 g/250 ml/50 ml	0.5–1	1–3
Silica brick	1 g—no dilution	15–20	—
Soda lime glass	1 g/250 ml/50 ml	5–10	5–10
Zinc ore CCRMP MP-1	0.5 g—no dilution	15–20	—

* The titration volumes in this table apply only to the materials analysed in this report and are given only as a guide to the amount of Ca and Mg titrated.

(or to produce a slight precipitate of iron hydroxide). Add 2 ml of 6*M* hydrochloric acid with stirring to redissolve the precipitate. Add 20–30 ml of triethanolamine solution, 2 or 3 drops of poly(vinyl alcohol) solution and sufficient solid potassium cyanide* to complex any zinc, copper, *etc.* that may be present, and stir the solution for about 5 min. Add 30–40 ml of 30% potassium hydroxide solution and 50 ml of screened calcein indicator. At this point the colour of the solution should be bluish-green. If not, add more potassium hydroxide solution. Titrate the solution with 0.025*M* EDTA until the colour turns to a purple that lasts for 15–30 sec. Read the burette, add a slight excess of the 0.025*M* EDTA and then add 5.00 ml of standard calcium solution. Titrate the solution again with 0.025*M* EDTA to a purple end-point. Read the burette and subtract the volume of EDTA solution equivalent to the amount of standard calcium solution added, and calculate the calcium content of the sample from the net volume of EDTA solution.

Titration of calcium plus magnesium. Transfer an aliquot of sample solution equal to that taken for the calcium titration to a 400-ml beaker and add a magnetic stirring bar. Add, with stirring, 5 ml of 6*M* hydrochloric acid and 20 ml of triethanolamine solution. Add 50 ml of ammonia solution (1 + 1) and mix. Dilute to approximately 200 ml with water, add 0.5–0.75 ml of *o*-cresolphthalein complexone indicator and 1.0 ml of Naphthol Green B solution. Titrate the solution with 0.025*M* EDTA from purple-green to "pure" green. Read the burette, add 5.00 ml of standard calcium solution and titrate again with the 0.025*M* EDTA. Subtract the volume of EDTA solution equivalent to the amount of standard calcium solution added, to obtain the net volume of 0.025*M* EDTA required to titrate the calcium plus magnesium in the sample. From this volume subtract the volume of EDTA solution required for the titration of calcium alone; the difference is the volume of EDTA solution required to titrate the magnesium.

Procedure for bauxite

Fuse a 2-g sample with 10 g of anhydrous sodium carbonate in a platinum crucible. Cool the melt and dissolve it in water with the aid of an excess of dilute hydrochloric acid. Boil the solution to remove carbon dioxide and filter off any insoluble material on a Whatman No. 30 paper and wash it with hot water. Cool the filtrate to room temperature, dilute to ~ 300 ml with water and determine the calcium according to the procedure described above. The addition of cyanide may be omitted unless significant amounts of zinc, copper, *etc.*, are believed to be present.

RESULTS AND DISCUSSION

Preliminary tests

Initial tests were performed with various indicators for the titration of calcium and magnesium. The indicators used, the experimental conditions and the subjective comments on the results are given in Table 2. As a result of these tests a mixed indicator of calcein and thymolphthalein as recommended by Tucker[14] was chosen for the titration of calcium, and *o*-cresolphthalein complexone screened with Naphthol Green B as suggested by Schwarzenbach and Flaschka[2] was selected for the titration of calcium plus magnesium.

A number of tests on synthetic solutions were also performed to ascertain the recovery of known amounts of calcium and magnesium in the presence of various potential interferents that would be encountered in the analysis of ores, slags, *etc.* Complexing agents such as triethanolamine and potassium cyanide were added to overcome the interference of iron, aluminium, copper, zinc, *etc.* The large amounts of complexing agents employed in these tests did not appear to have a deleterious effect on the titrations and the indicators behaved in a satisfactory manner. The results of these tests are given in Table 3.

Analysis of various samples

The method was applied to divers samples and the results are tabulated in Tables 4 and 5. The results are in most cases in very good agreement with the certified values or with values obtained by alternative methods. The one exception is NBS 104 burned magnesite, in which the calcium value found is slightly low and the magnesium value is correspondingly high. This particular sample is extremely high in magnesium, which results in a rather large precipitate of magnesium hydroxide during the titration step for calcium and significant adsorption of calcium on the precipitate apparently occurs in spite of precautions taken to prevent it. Nevertheless, the procedure may still be satisfactory for routine purposes and the results compare favourably with the method proposed by Přibil and Veselý[20] for the analysis of magnesite.

In the analysis of basic slag (BCS No. 74/1) initial results were high because of the manganese (5% MnO) and vanadium (1% V_2O_5) in the sample. Interference from these elements was eliminated by adding an excess (~ 1 g) of potassium cyanide and 1–2 ml of hydrogen peroxide to the solution. Because of the refractory nature of bauxite it was fused with sodium carbonate. The end-point was more difficult to detect with this sample, perhaps because of the ensuing large salt content.

In agreement with Morris's observation[4] we found it best to add the triethanolamine to the sample solution all at once with vigorous stirring to avoid the formation of a slight precipitate when large amounts of aluminium were present. We have found that titration of the samples in a relatively large volume of solution aids in detecting the end-point because the turbidity is reduced and moreover the possibility of formation of calcium carbonate or insoluble magnesium ammonium phosphate is reduced.[2]

The method has subsequently been used for the determination of calcium and magnesium in iron ores, slags, sediments, anorthosite, clays, and zinc–copper–lead ores with satisfactory results. The proposed method is considerably less complex and shorter than the classical gravimetric methods, is subject to less interference and is applicable to materials of widely variable composition. It is simple, accurate and avoids separation steps that are often employed in other chelatometric methods.

* For zinc–copper ores up to 1 or 2 g of potassium cyanide may be necessary. For basic slags or samples containing manganese or vanadium it is also necessary to add sufficient hydrogen peroxide in addition to the cyanide to overcome the interference of these elements.

Table 2. Behaviour of various indicators for the titration of calcium and magnesium

Indicator	Elements present, *mg*		Colour change and comments
*For calcium**	Ca	Mg	
Murexide	10		Very poor e.p., salmon pink to blue
	10	6	Very poor e.p., salmon pink to blue
Calcon	10		Fair e.p., gradual change from pink to blue
	10	6	Fair e.p., gradual change from pink to blue
Calcein	10		Poor e.p., pure yellow to deep yellow
	10	6	Poor e.p., indeterminate
Calcein + thymolphthalein	10		Sharp e.p., blue-green to purple
	10	6	Sharp e.p., blue-green to purple
Methylthymol Blue	10		Sharp e.p., blue to pale grey-blue but quickly returns to blue after e.p. is reached
	10	6	
Glyoxal-bis(2-hydroxyanil)	10		Sharp e.p., orange-red to yellow
	10	6	Sharp e.p., salmon pink to yellow, indicator quickly decomposed to colourless
Acid Alizarin	10		Sharp e.p., red to blue
Black SN	10	2	Fair e.p., premature
	10	11	Poor e.p., premature
For calcium plus magnesium†			
Eriochrome BT	10	6	Sharp e.p., red to blue
o-Cresolphthalein complexone	10	6	Sharp e.p., purple to green with purple tinge
o-Cresolphthalein complexone + Naphthol Green B	10	6	Sharp e.p., purple to "pure" green

* Solution composed of 150 ml of water, 20 ml of 50% triethanolamine, 2 or 3 drops of 1% poly(vinyl alcohol), 10 ml of 30% potassium hydroxide solution and 0.1 g of potassium cyanide.

† Solution composed of 150 ml of water and 20 ml of ammonia buffer, pH 10.

Table 3. Titration of calcium or magnesium in the presence of various potential interferents

Element added, *mg*	KCN added, *g*	Present, *mg*	Found, *mg*
Calcium titration		*Calcium*	*Calcium*
Fe 150	none	6.00	6.00
Fe 150, Al 10, Mg 12	none	6.00	6.00
Fe 150, Al 10, Mg 12, Mn 6	none	6.00	6.00
Fe 150, Al 10, Mg 12	none	6.00	intermediate, fading e.p.
Fe 150, Al 10, Mg 12, Mg 12, Mn 6	0.1–0.2	0.50	0.51
Mg 12, Mn 6	0.1–0.2	6.00	6.00
Mg 12, Mn 6	0.1–0.2	20.0	20.0
Mg 12, Mn 6	0.1–0.2	26.0	26.2
Magnesium titration		*Magnesium*	*Magnesium†*
none	0.1	24.83	24.83
Ca 10	0.1	24.83	24.83
Ca 10, Fe 300, Al 20	0.1	24.83	24.83

* For the calcium titration the solution was composed of 300–600 ml of water, 2–3 ml of hydrochloric acid, 15 ml of 50% triethanolamine, 0.5 ml of 1% poly(vinyl alcohol), 15–25 ml of 30% potassium hydroxide solution and ~50 mg of indicator (calcein + thymolphthalein + potassium chloride mixture).

† For the magnesium titration the solution was composed of ~450 ml of water, 30 ml of 50% triethanolamine, 25 ml of 14*M* ammonia, and *o*-cresolphthalein complexone + Naphthol Green B as indicator.

Table 4. Determination of calcium and magnesium in various materials

Sample	% CaO			% MgO		
	Certified value	Range	Found	Certified value	Range	Found
Certified Reference Materials						
BCS No. 301 Lincolnshire Iron Ore	22.0	21.6–22.4	21.86, 21.87	—	—	—
BCS No. 302 Northamptonshire Iron Ore	3 30	3.15–3.42	3.16, 3.20, 3.17	1.07	0.98–1.18	1.10
BCS No. 174/1 Basic Slag	44.83	44.65–45.00	44.90, 44.88	7.13	7.08–7.20	7.15
NBS 69a Bauxite	0.29	—	0.28, 0.28	—	—	—
NBS 80 Soda Lime Glass	4.65	4.49–4.75	4.44, 4.51	3.23	3.04–3.39	3.20, 3.20
NBS 88 Dolomite	30.49	30.48–30.50	30.54, 30.47, 30.51	—	—	—
NBS 98 Plastic Clay	0.21	0.17–0.24	0.23	0.72	0.67, 0.75	0.68
NBS 104 Burned Magnesite	3.35	3.28–3.38	3.14, 3.07, 3.06, 3.21	85.67	85.59–85.88	86.00, 86.10, 86.10 85.95
NBS 120a Florida Phosphate Rock	50.3	—	50.42, 50.33	—	—	—
NBS 198 Silica Brick	2.71	2.67–2.75	2.73, 2.74	—	—	—
NBS 199 Silica Brick	2.41	2.37–2.46	2.43, 2.41	—	—	—
CCRMP MP-1 Zn–Cu–Pb Ore	4.70	—	4.70 (average of 10 results, range 4.68–4.73)	—	—	—
Miscellaneous Samples						
Peace River Iron Ore	2.55% CaO (AAS)		2.54, 2.56	—	—	—
Limestone 1	—		54.99, 54.86	—	—	0.30, 0.30
Limestone 2	52.7% CaO (Ca oxalate, grav.)		51.86, 51.67	—	—	—
Limestone 3	—		51.17, 51.33	—	—	0.60, 0.60
Anorthosite 75/30	—		10.54, 10.44, 10.50, 10.33	—	—	0.18, 0.28, 0.25 0.35
Anorthosite 75/30	—		10.36, 10.58, 10.42	—	—	0.50, 0.58, 0.53
Anorthosite 76/32	—		11.69, 11.62			0.30, 0.38
Melted Anorthosite 65046	—		14.87, 14.66	—	—	0.58, 0.46

Table 5. Analysis of sedimentary material

Sample	% Calcium (as Ca) By Ca oxalate precipitation with gravimetric finish	By Ca oxalate precipitation plus EDTA titration	By proposed direct EDTA titration method
A	0.03, 0.03	0.03	0.04, 0.04
B	—	2.49	2.43, 2.55
C	0.03, 0.05	—	0.04, 0.05
D	—	2.69	2.69, 2.70
E	—	2.46	2.44, 2.47
F	—	2.13	2.14, 2.13
G	0.07	—	0.06, 0.09
H	2.47, 2.39	2.35	2.43
I	0.12	0.12	0.12, 0.13
J	2.29, 2.29	—	2.29, 2.30

Other possibilities

EGTA has been recommended by several workers for calcium titration, because of the large difference between the stability constants for the calcium and magnesium complexes.

We examined the procedure by Burg and Conaghan[10] who used EGTA to titrate calcium. These authors, however, employed an R_2O_3 separation for the removal of Fe, Al, Ti, *etc.* and we expected difficulty to be caused by the presence of the large amounts of ammonium salts introduced by this step. Burg and Conaghan apparently encountered little difficulty with the sample material they analysed, which presumably contained relatively little R_2O_3, but no figures were reported. On the other hand, many of our samples had a very high R_2O_3 content (with consequent increase in ammonium salt content) as well as, in some cases, copper, zinc, lead, nickel, *etc.* which could not be removed by an ammonia or hexamethylenetetramine precipitation.

Flaschka and Ganchoff[6] used murexide as the indicator for the titration of calcium with EGTA and had to use a photometric titration procedure to detect the end-point. The procedure was applied only to magnesite. These authors state that the elements Co, Ni, Cu, Zn, Cd and Hg are masked by cyanide, aluminium is masked by triethanolamine and iron is converted into ferrocyanide to eliminate their interference but unfortunately they do not report the amounts of the various elements and masking agents used. This means that anyone wanting to use their procedure has to reinvestigate this aspect if they wish to apply it to other types of material, because there are few guidelines. It is debatable whether the same procedure could be applied to the complex samples that we examine here, *e.g.*, zinc–copper–lead ores, iron ores, slags, anorthosite, without considerable modification or introduction of separation steps. Moreover, not all small mining company laboratories have facilities or experienced personnel for spectrophotometric titrations or even wish to spend money for the equipment.

Bennett and Reed[21] titrated calcium with EGTA in the presence of small amounts of triethanolamine and used screened calcein as the indicator, but did not add cyanide to mask copper, nickel, *etc.* These authors reported only on the method as applied to magnesites and dolomites. The procedure for calcium is similar in many respects to ours but we employ much larger amounts of triethanolamine and potassium hydroxide (perhaps unnecessarily high for magnesites but we were investigating the procedure under the same conditions as used for iron ores, copper–zinc–lead ores, *etc.*). Bennett and Reed appeared to obtain good results for calcium by using triethanolamine in spite of Přibil and Adam's later claim[22] that it cannot be used. Bennett and Reed's procedure for the sum of calcium and magnesium is much more difficult and lengthy, owing to the incorporation of various separation steps.

Přibil and Adam[22] acknowledge their method is applicable only to pure calcium–magnesium solutions and that for the analysis of geological samples it can be applied only after separation of all interfering elements. They also state that triethanolamine cannot be used to complex iron and aluminium and removal of these elements by ammonia is ruled out because it interferes in the titration of EGTA with zinc solution (see also Burg and Conaghan[10]). Elements such as copper, nickel, manganese and others that can be titrated with EDTA must be removed or their sum with calcium determined and a correction applied. Cyanide cannot be used as a complexing agent because zinc solution is used as a back-titrant. Thus these authors' procedure is also of limited application. An examination of their results for the analysis of several magnesites shows that the method has significant and consistent positive relative errors of 4.5–11% for CaO. Our procedure gave a relative error of −60% for CaO in the analysis of a single magnesite sample. It should also be noted that the magnesium oxide content of the magnesite samples analysed by Přibil and Adam was only about half that of the NBS 104 magnesite sample analysed by us.

A final objection to the use of EGTA is its high cost, relative to that of EDTA.

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SHORT COMMUNICATIONS

PVC ION-SELECTIVE ELECTRODES BASED ON CALCIUM BIS-[DIALKYL- AND DI-(4-ALKYLPHENYL) PHOSPHATES] AND MIXED SOLVENT MEDIATORS

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Summary—PVC calcium ion-selective electrodes based on either calcium bis-di(n-decyl)phosphate or calcium bis-di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate with various solvent mediators (alone or in pairs) have been evaluated with particular respect to interference from Na^+, K^+, Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} ions. PVC calcium ion-selective electrodes based on calcium bis-di[4-(n-octyl)phenyl]phosphate with varied amounts of decan-1-ol plus di-n-octyl phenylphosphonate showed a continuous gradation in selectivity coefficients on going from a high fraction of decan-1-ol to a high fraction of the second mediator. Thus, $k\xi^{pot}_{a,Mg}$ changed from 1.6 for an electrode based exclusively on decan-1-ol to 4.9×10^{-4} for one based completely on di-n-octyl phenylphosphonate. The corresponding $k\xi^{pot}_{a,Na}$ values were 7.0×10^{-2} and 1.1×10^{-3}

The performance of poly(vinyl chloride) (PVC) calcium ion-selective electrodes is closely related to the particular solvent mediator employed.[1–7] However, the influence of mixed mediators on their behaviour has been little studied.[4–7] This note is therefore concerned with an evaluation of PVC calcium ion-selective electrodes with sensors comprising either calcium bis-di(n-decyl)phosphate or calcium bis-di[4-(1,1,3,3-tetramethylbutyl)phenyl] phosphate in conjunction with selected pairs of solvent mediators, *viz.* di-n-butyl sebacate and decan-1-ol; di-n-butyl sebacate and di-n-octyl phenylphosphonate; diethyl adipate and decan-1-ol; diethyl adipate and di-n-octyl phenylphosphonate; as well as decan-1-ol and di-n-octyl phenylphosphonate with calcium bis-di[4-(n-octyl)-phenyl]phosphate sensor.

EXPERIMENTAL

Electrodes

Ion-selective electrodes with membranes comprising the calcium sensor plus solvent mediator(s) were fabricated as described previously.[8]

Reagents

All chemicals were of reagent grade except the calcium salts and the di-n-octyl phenylphosphonate which were synthesized.[9–11]

Procedures

All emf measurements were made at 25° ± 0.1° relative to a Corning ceramic-junction calomel reference electrode (Cat. No. 476109) with a Radiometer Model PHM 64 pH-meter coupled with a Servoscribe Model RE4541 recorder. Selectivity coefficients, $k\xi^{pot}_{a,B}$, were measured by a mixed solution method[12] with a fixed level of interferent cation, B.

The permeation of calcium ions through PVC matrix membranes was measured by using ^{45}Ca as described previously.[13]

RESULTS

The composition and principal electrochemical features of 20 master PVC membranes based on either calcium bis-di(n-decyl)phosphate or calcium bis-di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate together with solvent mediators used singly or in pairs are summarized in Tables 1 and 2.

The gradation in magnesium and sodium interferences for PVC calcium ion-selective electrodes based on calcium bis-di[4-(n-octyl)phenyl]phosphate and varied amounts of decan-1-ol and di-n-octyl phenylphosphonate is shown in Table 3.

DISCUSSION

Different solvent mediators are frequently associated with considerable differences in the selectivity parameters of calcium ion-selective electrodes. Thus, Garbett and Torrance[6] found that the response of electrodes with membranes comprising calcium bis-di(n-decyl)phosphate sensor and alkan-1-ol (C_5–C_{10}) solvent mediators ranged from little selectivity for Ca, Mg, Ni and Cu to high copper selectivity when branched-chain alkan-3-ols and alkan-4-ols were used. The use of homologous alkan-1-ol solvent mediators in PVC calcium electrodes was also gener-

Table 1. Constitution and properties of PVC matrix membranes

Membrane	Solvent mediator Type	Weight, g	Sensor (0.04 g)	Slope mV per decade	Lower detection limit,* M	Linear range, M	Remarks
I	Di-n-butyl sebacate	0.36	Calcium bis-di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate	33	1.6×10^{-5}	10^{-1}–5×10^{-4}	Colourless, transparent, soft and rubbery, Gel-like inclusions.
II	Di-n-butyl sebacate Decan-1-ol	0.36 0.36		26.5	2×10^{-6}	10^{-1}–7×10^{-5}	Semi-rigid, shrunken membrane with surface exudate. Extensive crystalline inclusions. Lifetime is 6 weeks.
III	Di-n-butyl sebacate Di-n-octyl phenyl phosphonate	0.36 0.36		32	1.9×10^{-5}	10^{-1}–3×10^{-4}	Clear, transparent, soft and rubbery with no inclusions. Lifetime >2 months.
IV	Di-n-butyl sebacate Decan-1-ol	0.18 0.18		30	4×10^{-6}	10^{-1}–3×10^{-5}	Semi-rigid, shrunken membrane. Surface exudate with extensive crystalline inclusions Lifetime 1 week.
V	Di-n-butyl sebacate Di-n-octyl phenyl phosphonate	0.18 0.18		31	6×10^{-6}	10^{-1} 4×10^{-5}	Clear, transparent, soft and rubbery with no inclusions Lifetime >6 weeks.
VI	Diethyl adipate	0.36		30	1.3×10^{-6}	10^{-1}–5×10^{-5}	Opaque, soft membrane, shrunken with gel-like inclusions.
VII	Diethyl adipate Decan-1-ol	0.36 0.36		32	1.6×10^{-5}	10^{-1}–9×10^{-5}	Opaque, semi-rigid, large shrunken membrane. Surface exudate with extensive crystalline deposit. Lifetime 1 day
VIII	Diethyl adipate Di-n-octyl phenyl phosphonate	0.36 0.36		24	1.1×10^{-6}	10^{-1}–6×10^{-6}	Clear, transparent, soft and rubbery with no inclusions Lifetime >2 months.
IX	Diethyl adipate Decan-1-ol	0.18 0.18		26	1.6×10^{-6}	10^{-2} 3×10^{-5}	Opaque, semi-rigid, large shrunken membrane, surface exudate with extensive crystalline deposit. Lifetime 2 days.
X	Diethyl adipate Di-n-octyl phenyl phosphonate	0.18 0.18		31.5	1×10^{-5}	10^{-1}–3×10^{-4}	Clear, transparent, soft and rubbery with no inclusions. Lifetime >5 weeks
XI	Di-n-butyl sebacate	0.36	Calcium bis-di*n*-decylphosphate	35	3.2×10^{-5}	10^{-1}–5×10^{-4}	White transparent, soft and rubbery membrane
XII	Di-n-butyl sebacate Decan-1-ol	0.36		32	3.2×10^{-5}	10^{-1} 5×10^{-4}	White,opaque,semi-rigid,shrunken membrane, some exudate on the surface with extensive crystalline inclusions. Lifetime 1 week.
XIII	Di-n-butyl sebacate Di-n-octyl phenyl phosphonate	0.36 0.36		36	2.5×10^{-5}	10^{-1} 3×10^{-4}	Colourless, transparent, soft and rubbery membrane. Lifetime >2 months.
XIV	Di-n-butyl sebacate Decan-1-ol	0.18 0.18		32	4×10^{-5}	10^{-1} 6×10^{-4}	White opaque, semi-rigid membrane with crystalline inclusions. Lifetime 1 week
XV	Di-n-butyl sebacate Di-n-octyl phenyl phosphonate	0.18 0.18		40	2.3×10^{-5}	10^{-1} 10^{-4}	Colourless, transparent, soft and rubbery membrane, with crystalline inclusions. Lifetime >7 weeks.
XVI	Diethyl adipate	0.36		28.6	1.1×10^{-4}	10^{-1}–10^{-3}	Colourless, soft membrane.
XVII	Diethyl adipate Decan-1-ol	0.36 0.36		32	1.7×10^{-5}	10^{-1}–7×10^{-4}	White, opaque, rigid, shrunken membrane with surface exudate. Lifetime 2 weeks
XVIII	Diethyl adipate Di-n-octyl phenyl phosphonate	0.36 0.36		38	1.1×10^{-5}	10^{-1}–7×10^{-5}	Colourless, transparent, soft membrane with gel-like inclusions. Lifetime 4 weeks.
XIX	Diethyl adipate Decan-1-ol	0.18 0.18		30	3×10^{-6}	10^{-2}–5×10^{-5}	White, opaque, rigid, shrunken membrane surface exudate with crystalline inclusions. Lifetime 2 days
XX	Diethyl adipate Di-n-octyl phenyl phosphonate	0.18 0.18		39	2.8×10^{-6}	10^{-1}–3×10^{-5}	Colourless, transparent, soft membrane with gel-like inclusions. Lifetime >6 weeks

* Detection limit is that activity of calcium ions at which the emf of the electrode deviates by 9 mV (that is, $18/z_A$, where z_A is the charge on the ion of interest) from the extrapolated linear section of the calibration plot.

Table 2. Mixed-solution selectivity coefficients of various PVC calcium ion-selective electrodes ($BCl = 5 \times 10^{-3} M$; $BCl_2 = 5 \times 10^{-4} M$)

Membrane	Selectivity coefficient $k^{pot}_{Ca,Na}$	$k^{pot}_{Ca,K}$	$k^{pot}_{Ca,Mg}$	$k^{pot}_{Ca,Sr}$	$k^{pot}_{Ca,Ba}$	$k^{pot}_{Ca,Mn}$	$k^{pot}_{Ca,Cu}$	$k^{pot}_{Ca,Ni}$	$k^{pot}_{Ca,Zn}$
I	0.5	0.5	3×10^{-2}	3×10^{-2}	4×10^{-2}	5×10^{-2}	5×10^{-2}	0.3	3×10^{-2}
II	0.5	0.6	4×10^{-2}	3×10^{-2}	4×10^{-2}	0.1	0.1	9×10^{-2}	4×10^{-2}
III	0.7	0.7	0.3	9×10^{-3}	4×10^{-3}	6×10^{-3}	3×10^{-2}	3×10^{-2}	3×10^{-2}
IV	0.6	0.5	0.1	7×10^{-2}	7×10^{-2}	0.7	0.7	0.2	0.6
V	0.4	0.4	3×10^{-2}	6×10^{-2}	1×10^{-2}	8×10^{-2}	2×10^{-2}	3×10^{-2}	2×10^{-2}
VI	0.3	0.2	4×10^{-2}	3×10^{-2}	2×10^{-2}	8×10^{-2}	2×10^{-2}	0.5	*
VII†	—	—	—	—	—	—	—	—	—
VIII	0.5	0.6	3×10^{-2}	6×10^{-2}	1×10^{-2}	5×10^{-2}	2×10^{-2}	0.2	0.1
IX†	—	—	—	—	—	—	—	—	—
X	0.3	0.1	2×10^{-2}	4×10^{-2}	9×10^{-3}	3×10^{-2}	2×10^{-2}	3×10^{-2}	0.2
XI	6.7	8.7	0.3	0.2	0.3	0.3	1.8	0.4	*
XII	2.4	2.1	*	0.2	1.8	*	*	*	*
XIII	0.7	0.8	2×10^{-2}	4×10^{-2}	2×10^{-2}	7×10^{-2}	3×10^{-2}	0.3	*
XIV	4.4	4.9	2.4	0.4	0.8	8.0	17.7	12.0	*
XV	0.6	0.4	2×10^{-2}	1×10^{-2}	8×10^{-3}	0.2	9×10^{-2}	3×10^{-3}	*
XVI	2.5	17.4	0.3	1.4	0.7	*	*	3×10^{-2}	0.6
XVII	2.0	1.2	0.6	0.3	0.4	1.5	3.5	0.7	2.4
XVIII	0.4	0.4	3×10^{-2}	2×10^{-2}	1×10^{-2}	9×10^{-2}	4×10^{-2}	0.53	*
XIX	0.4	1.3	0.8	0.2	1.5	1.1	1.3	0.7	*
XX	0.4	0.2	1×10^{-2}	1×10^{-2}	8×10^{-3}	8×10^{-2}	7×10^{-2}	2×10^{-2}	*

* $k^{pot}_{Ca,B}$ could not be determined because the calibration for calcium in the presence of B ions did not coincide with the normal calcium ion calibration.

† Electrode quality too poor for selectivity evaluation (See Table 1)

ally disappointing,[4] although a membrane comprising di-n-nonyl phthalate and calcium bis-di(n-decyl)phosphate showed some promise as a copper ion-selective electrode, $k^{pot}_{Ca,Cu} = 13$.

Except for membranes VII and IX (Table 1) of the present investigation, the remaining 25 membranes provided functional ion-selective electrodes with a wide variety of electrochemical properties (Tables 1–3). Sodium and potassium interference with electrodes made from membranes containing the mixed solvent mediators or decan-1-ol is relatively severe compared with that for models based on di-n-octyl phenylphosphonate alone with di(4-octylphenyl)phosphonates,[2,3] especially in the case of membranes XI, XIV and XVI. Moreover, the superiority of the di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate compared with the di-(n-decyl)phosphate and in conjunction with any corresponding solvent system is maintained for all the selectivity coefficients in Table 2 (membranes I–X compared with membranes XI–XX).

Membranes XIV, with a decan-1-ol component, provided electrodes with some interesting bivalent cation selectivity coefficients, namely, $k^{pot}_{Ca,Cu} = 17.7$ and $k^{pot}_{Ca,Ni} = 12$, while several other decan-1-ol electrodes, *e.g.*, XVII and XIX, further demonstrated the well-known effect of reduced selectivity among bivalent cations.[4] The peculiar effect[3] of zinc is again observed and in many cases selectivity coefficients could not be measured by the mixed solution method (Table 2).

PVC matrices can impose an additional constraint on the utility of a mediator. Thus, di-n-octyl phenylphosphonate with, or indeed without, a calcium liquid ion-exchanger is perfectly compatible with PVC but

Table 3. Constitution and characteristics of electrodes with mixed-mediator membranes

Membrane*	Decan-1-ol, *g*	Di-*n*-octylphenylphosphonate, *g*	Electrode slope, *mV/decade*	Detection limit, *M*	$k^{pot}_{Ca,Mg}$†	$k^{pot}_{Ca,Na}$†
XXI‡	0.36	—	27.5	3×10^{-6}	1.6	7.0×10^{-2}
XXII‡	0.30	0.06	28.5	1.6×10^{-5}	7.9×10^{-1}	3.0×10^{-2}
XXIII‡	0.24	0.12	28.5	1.4×10^{-5}	1.0×10^{-1}	1.3×10^{-2}
XXIV‡	0.18	0.18	28.7	8.0×10^{-6}	8.6×10^{-3}	6.1×10^{-3}
XXV‡	0.12	0.24	28.5	3.8×10^{-6}	1.6×10^{-3}	3.7×10^{-3}
XXVI	0.06	0.30	28.0	2.9×10^{-6}	7.1×10^{-4}	2.1×10^{-3}
XXVII	—	0.36	29.5	2.7×10^{-6}	4.9×10^{-4}	1.1×10^{-3}

* Each membrane contained PVC (0.17 g) and calcium bis-di[4-(*n*-octyl)phenyl]phosphate (0.04 g).

† $[MgCl_2] = [NaCl] = 5 \times 10^{-2} M$, using mixed solution method.

‡ Surface exudates observed on surfaces of these membranes.

this condition is rarely observed with alkan-1-ols,[4] which fail to plasticize the cast membranes, and surface exudations are quite common. Despite this drawback, functional PVC electrodes can sometimes be fabricated (Tables 1 and 3).

Among the alcohols, decan-1-ol is a most interesting mediator and in conjunction with calcium bis-di(n-decyl)phosphate constitutes the basis of the Orion 92-32 bivalent-cation (water hardness) electrode whereas di-n-octyl phenylphosphonate maintains a fair degree of selectivity for calcium relative to other bivalent cations, in comparison with the Orion 92-20 calcium electrode. PVC matrix electrodes with varied amounts of these two classical solvent mediators showed a continuous gradation in selectivity coefficients on going from a high fraction of decan-1-ol to a high fraction of the second mediator (membranes XXI–XXVII in Table 3). Thus, $k^{pot}_{Ca,Mg}$ fell from 1.6 for an electrode based exclusively on decan-1-ol to 4.9×10^{-4} on changing completely to di-n-octyl phenylphosphonate. The same, but less dramatic, trend was evident for sodium interference (membranes XXI–XXVII).

The diffusion of Ca^{2+} and Mg^{2+} ions through the PVC sensor membrane of an ideal bivalent ion-selective electrode should be closely similar since $k^{pot}_{Ca,Mg}$ is expected to be unity. Thus, it is interesting that the migration profile of $^{45}Ca^{2+}$ between identical pairs of calcium chloride solutions ($10^{-3}M$) placed on either side of a PVC membrane containing the Orion 92-32-02 bivalent-cation exchanger matches that measured when one calcium solution is replaced by magnesium chloride ($10^{-3}M$), and $d(C''/C')/dt \sim 32 \times 10^{-7}$ sec^{-1}. The corresponding time-independent values for identical experiments undertaken with calcium bis-di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate and di-n-octylphenylphosphonate were 29×10^{-7} and 4.3×10^{-7} sec^{-1} respectively. This difference accords with a system for which $k^{pot}_{Ca,Mg} < 1$.

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ANALYTICAL UTILITY OF 2-HALOPYRIDINIUM SALTS—II*

ACIDIMETRIC DETERMINATION OF THIOL GROUPS

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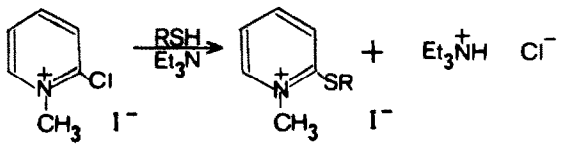
Summary—2-Chloro-1-methylpyridinium iodide reacts rapidly and quantitatively with thiol groups in the presence of excess of triethylamine to give the 2-alkyl(aryl)thio-1-methylpyridinium iodide and an equimolar amount of hydrogen chloride which is trapped by the triethylamine. The excess of triethylamine is back-titrated with hydrochloric acid, with Bromothymol Blue as indicator.

2-Chloro-1-methylpyridinium iodide reacts with many thiols in the presence of triethylamine according to the reaction:



Scheme 1

The hydrogen chloride yielded is trapped by the triethylamine, the excess of which can be back-titrated with standard hydrochloric acid, with Bromothymol Blue as indicator. This reaction provides a convenient method for semi-micro determination of –SH groups, for which there are already a large number of methods described in the literature,[1–4] but not all are universally applicable.

EXPERIMENTAL

Reagents

2-Chloro-1-methylpyridinium iodide. To 2-chloropyridine (11.35 g, 100 mmole) was added methyl iodide (14.20 g, 100 mmol) and the mixture was stirred for 2 days at room temperature. The 2-chloro-1-methylpyridinium iodide precipitate was filtered off, washed with dry ether (50 ml) and dried under reduced pressure (yield 19.9 g, 78%). It was used as a 0.1*M* solution in acetonitrile.

Triethylamine, 0.04M solution in acetonitrile.

Thiol compounds. Analytical-reagent grade, purified according to known procedures, and used as solutions of known concentration in appropriate solvents (acetonitrile, dimethylformamide, ethanol or water).

Procedure

To a mixture of 5 ml of 0.04*M* triethylamine and 2 ml of 0.1*M* 2-chloro-1-methylpyridinium iodide (both in acetonitrile) an aliquot of thiol solution was added. Then 5 ml of water and 3 or 4 drops of 0.04% Bromothymol Blue solution (in $10^{-3}M$ sodium hydroxide) were added and the solution was titrated at once with 0.01*M* hydrochloric acid to a yellow colour. Near the end-point the titration was done slowly with vigorous swirling. A blank determination was run.

RESULTS AND DISCUSSION

Several thiols, including primary, secondary and tertiary compounds, were examined. In nearly all cases, the reaction was rapid and quantitative, an exception being 2-methyl-2-propanethiol, which gave only 80% recovery. Typical results are given in Table 1.

The advantages of the proposed method over methods involving precipitation with metal ions are its fixed stoichiometry and its simplicity. Though the HMB method[5] gives more accurate results it cannot be applied for certain compounds, *e.g.*, *O,O'*-diethyl dithiophosphate, which can be determined accurately by the present method. The widely used iodometric method yields accurate results but the number of thiols to which it can be applied is limited, and it is reported[3] to give over-oxidation of some compounds and non-stoichiometric or slow reactions with secondary, tertiary and long-chain aliphatic compounds, most of which can be determined by both the present method and titration with copper(II).[3] Organic sulphides, disulphides and thiourea do not interfere.

In the case of thioglycollic acid, which contains both a carboxylic and a thiol group, the consumption of triethylamine is double because of neutralization of the carboxylic acid group as well as the hydrogen chloride formed in the reaction (see Scheme 1). When the sample is first titrated with triethylamine solution (to the Bromothymol Blue end-point) and then treated with 2-chloro-1-methylpyridinium iodide according to the recommended procedure, the volume of triethylamine used in the second reaction corresponds to the amount of thiol groups in the sample. The same technique can be used for the analysis, in

* Part I: *J. Chromatog.*, 1979, **174**, 483.

Table 1. Determination of thiol groups

Compound* (80–150 μmole)	Average recovery, %	
	Present method†	Comparison method§
Ethanethiol (8)	99.4 (0.45)	99.7
1-Propanethiol (10)	99.8 (0.30)	99.8
2-Propanethiol (11)	98.9 (0.35)	99.2
1-Butanethiol (9)	99.1 (0.40)	99.8
1-Pentanethiol (12)	98.7 (0.30)	99 5
3-Methyl-1-butanethiol (9)	99.0 (0.40)	100.0
1-Dodecanethiol (7)	99.2 (0.25)	99.8
Benzenethiol (8)	100.1 (0 30)	99.6
2-Pyridinethiol (12)	99.5 (0.42)	97.3
2-Naphthalenethiol (8)	100.1 (0.35)	99 8
O,O'-Diethyl dithiophosphate (9)	99.6 (0.40)	—
Thioglycollic acid (9)	99.8 (0.45)	100.0
2-Butanethiol (7)	98.3 (0.45)	99 0
3-Methyl-2-butanethiol (9)	96.9 (0.50)	97 7
1-Hexanethiol (10)	99.5 (0.35)	99.5
1-Nonanethiol (8)	99 3 (0.40)	99.6

* Figures in parentheses are the numbers of determinations.
† Figures in parentheses are the standard deviations.
§ HMB titration.[5]

the same sample solution, of thiol–carboxylic acid mixtures.

Further analytically useful applications of 2-halopyridinium salts are currently being explored.

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DETERMINATION OF D(+)GLUCOSE, D(+)MANNOSE, D(+)GALACTOSE OR D(−)FRUCTOSE IN A MIXTURE OF HEXOSES AND PENTOSES BY USE OF DENTAL PLAQUE COUPLED WITH A GLASS ELECTRODE

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Summary—A potentiometric sensor has been developed by coupling dental plaque with a flat-surface glass electrode. Selectivity of this electrode for hexoses and pentoses has been tested. The electrode responds linearly to β-D(+)glucose, D(+)mannose, D(+)galactose and β-D(−)fructose over a narrow concentration range between 10^{-4} and $10^{-3}M$, but has negligible response to the other hexoses and pentoses. This "plaque" electrode, using live bacterial cells, may serve as a model for the development of other selective electrodes for carbohydrates.

There are many methods for the individual determination of reducing sugars. The Sichert-Bleyer method[1,2] is widely used, and reducing hexoses and pentoses can be determined by measurement of the amount of formic acid produced after sodium periodate oxidation.[3] Aldoses may be titrated with iodine and alkali,[4] and individual polyhydric alcohols can be determined iodometrically.[4] The Lane and Eynon[5] copper reduction method has been recently modified by Khan.[6]

A mixture of sugars is normally analysed by first separating the sugars by partition chromatography[7,8] and then determining the individual sugars by oxidation.

Fermentation methods[1,9] provide a fairly reliable quantitative measure of D-glucose in a mixture of sugars, and it was shown as early as 1928 that the enzyme glucose dehydrogenase could be successfully used for manometric estimations of glucose in biological material in presence of other carbohydrates and proteins.[10]

We now describe the development and use of a selective sensor in which human dental plaque is employed at the surface of a pH-type glass electrode to prepare a sugar-sensing membrane electrode.

In this study we describe a bacterial electrode with response and selectivity to β-D(+)glucose, D(+)galactose, D(+)mannose and β-D(−)fructose, but with negligible response to the other hexoses and pentoses. The response of this plaque electrode is based on the measurement of local pH changes produced during the bacterial fermentation of some carbohydrates.

It has been shown that lactic acid is the main product during the fermentation of carbohydrates.[11–13] The acid produced in this manner is sensed by the internal pH element of the electrode and a potentiometric steady-state response proportional to the sugar concentration is obtained.

EXPERIMENTAL

Apparatus

A Corning flat-surface pH combination electrode (cat. no. 476216) was used in the construction of the bacterial electrode. Potential measurements were made with a Corning Model 12 Research pH-meter in conjunction with a Heath-Schlumberger strip-chart recorder, model SR-255-B. Measurements were made in a cell kept at 37° ± 0.2°.

Reagents

Normal 24-hr old human dental plaque in a non-fluoridated area was used as the source of the fermenting bacteria. Such plaque was collected from laboratory volunteers as described below.

The carbohydrates β-D(+)glucose, D(+)galactose, β-D-allose, L-mannose, D(+)mannose, D-idose, D-gulose, D-talose, D-altrose, β-D(−)fructose, L(−)sorbose, D(+)tagatose, L(+)arabinose, D(−)arabinose, L(−)xylose, D(+)xylose, D-ribose, L(−)ribose, D-lyxcose, L-lyxcose, D-ribulose and D-xylulose in purest form available were used without further purification. DL-Dithiothreitol was also obtained.

All other chemicals used were of analytical grade. Distilled water was used in all the experimental work.

Procedure

Day old dental plaque was collected from 25 members of the laboratory staff, whose teeth had previously been cleaned and who had fasted for 10 hr before plaque collection. The plaque was collected under sterile conditions by scraping from tooth surfaces, immediately suspended in sterilized reduced transport fluid (RTF)[14] and cooled to 4°. The suspension was homogenized by ultrasonics and stored at 4°.

A fraction of the plaque (~2 mg air-dried weight) was washed three times with Ringer's solution (0.015M sodium chloride, $3.1 \times 10^{-4}M$ potassium chloride and $2.2 \times 10^{-4}M$ calcium chloride), and before use was adjusted to pH 6.95 with dilute sodium hydroxide solution.

A dialysis membrane (molecular weight cut-off 10^4) was soaked for 1 hr in Ringer's solution and put over the flat

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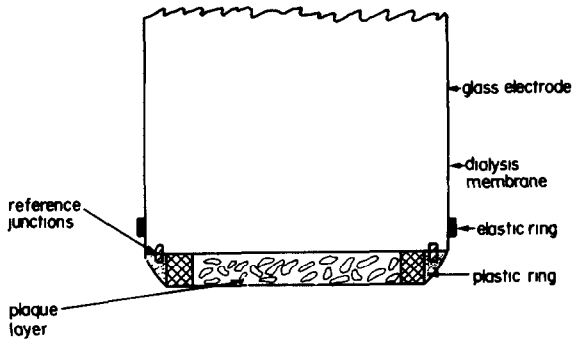

Fig. 1. Schematic diagram of the plaque electrode assembly.

surface of the glass electrode, where it was held by an elastic band. This assembly was soaked for another 2 hr in Ringer's solution to mould the membrane. The glass electrode was then inverted, the membrane removed, and a plastic ring of ~0.3 mm thickness and ~3.2 mm diameter placed between the two reference junctions and the pH-sensing portion of the glass electrode (Fig. 1). The washed plaque, suspended in Ringer's solution, was placed in the plastic ring and kept in position by replacement of the dialysis membrane. Care had to be taken not to trap air bubbles under the membrane.

Measurements were made in 1.50 ml of Ringer's solution (pH 6.95) contained in a cell sealed from the atmosphere. The space above the solution was flushed with nitrogen. Calibration curves were constructed by adding successive portions of carbohydrate solutions, previously standardized by conventional methods,[4,6] and measuring the emf of the cell.

When not in use, plaque electrodes were stored in RTF medium at 10–12°. For comparison 10 separate plaque electrodes were constructed and tested for response to glucose.

RESULTS AND DISCUSSION

Figure 2 represents the calibration curves for the plaque electrode at different ages, with β-D(+)glucose at 37°. When freshly prepared, the electrodes exhibited a response slope of 104 mV/log decade (±4 mV), in the linear range from $1.2 \times 10^{-4}M$ to $1.1 \times 10^{-3}M$ (Fig. 2, curve *a*). By the third day the slope had dropped slightly to 101 mV/log decade (curve *b*) over a linear range of 1.3×10^{-4}–$1.0 \times 10^{-3}M$. By the fourth day it was 95 mV/log decade (curve *c*) over a linear range of 1.3×10^{-4}–$7.5 \times 10^{-4}M$; electrodes were disassembled on the fifth day. We believe that the decrease in the slope and range is due to inactivation of the plaque bacteria on the electrode, on exposure to various media.[14]

The response slopes of the plaque electrode to D(+)galactose, D(+)mannose and β-D(−)fructose were also 104 ± 4 mV/log decade in the linear range 1.2×10^{-4}–$1.1 \times 10^{-3}M$, but the plaque electrode has negligible response to the other hexoses and pentoses. Plaque will also ferment some disaccharides.[15–18]

The non-Nernstian response slopes obtained throughout (104 mV/log decade) result from the fact that the yield of lactic acid increases with decreasing pH[11–13] during the fermentation process and the amounts of volatile acids and alcohols decrease.[19–23] Similar results were obtained when the plaque was mixed with carbohydrate solution, the glass electrode inserted and the pH change monitored, showing that the effect is not attributable to the electrode.

It is evident that the linear range of the response slope starts at ~30 mV (pH ≃ 6.5), possibly because

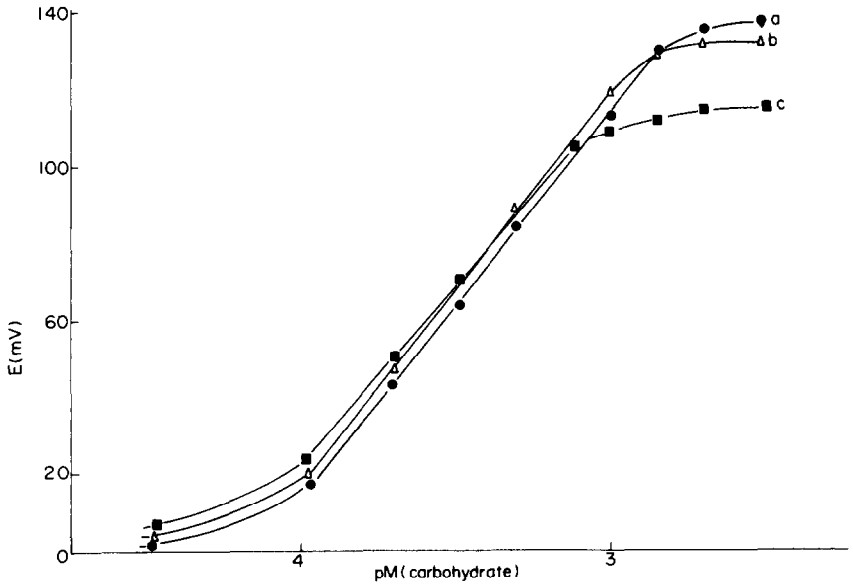

Fig. 2. Calibration curves for β-D(+)glucose in Ringer's solution on (*a*) the first day, (*b*) the third day and (c) the fourth day, at 37°.

the increase in volatile acids and alcohol products is negligible[13] at lower pH values.

Optimum results were obtained when 1.5–2.5 mg of air-dried plaque was used to prepare the electrode. Smaller amounts resulted in decreased sensitivity and larger amounts gave longer response times.

When the plaque electrode was new, the response time, *i.e.*, the time required for the emf reading to come within 2 mV of the steady-state value, increased from 20 to 40 min as the carbohydrate concentration increased from $5 \times 10^{-5}M$ to $3 \times 10^{-3}M$, and increased as the plaque electrode became older.

The response of the plaque electrode to four hexoses and pentoses is due to the presence of more than one fermenting strain found in plaque.[15] We believe that the selectivity of the electrode can be increased by using single strains.

The plaque electrode, although neither selective for a single carbohydrate (enzyme) nor effective for longer periods than 4 days, offers a very sensitive electrode for four carbohydrates in a mixture with their isomers and pentoses.

We thus believe that this biological electrode is only the beginning of biological carbohydrate electrodes and that more selective electrodes with extensive effectiveness may be constructed.

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SOLUTION OF LINEAR FIRST-ORDER EQUATIONS WITHOUT MATRIX INVERSION

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Summary—The need to solve linear first-order equations frequently occurs in several areas of analytical chemistry. Several numerical methods are available to solve these systems of equations but are cumbersome and time-consuming when the number of unknowns becomes greater than three. A simple matrix method is presented that avoids the need to calculate determinants or to invert matrices.

The occurrence of linear first-order equations in many areas of analytical chemistry is commonplace, these taking the general form

$$y_k = \Sigma x_{k,j} b_j \tag{1}$$

These equations may be solved by several numerical methods including multiple regression, constrained non-negative linear least squares[1] and linear programming.[2]

This topic has been discussed recently by Leggett[1] and Gayle.[2] The methods reviewed were applicable to an "overdetermined" set of M linear first-order equations, *i.e.*, $M > N$, if there are N unknowns.

However it frequently happens that only enough data are available to make $N = M$ and in these situations it is inefficient or inadvisable to use such techniques. Consequently other numerical methods are adopted to solve the system of equations. If $M = 2$ we have a pair of simultaneous equations, and the solution is simple. If $M = 3$ direct algebraic substitution may be employed, the equations may be solved by Cramer's Rule through the use of determinants,[3] or one of the variants of the basic Gaussian elimination methods can be employed.[4] All of these methods are amenable to hand calculation.

However, once M is greater than 3 the methods mentioned above, and other similar techniques, become excessively cumbersome to use manually. For example the hand calculation of the determinant for a 5×5 matrix or the calculation of its inverse is extremely tedious.

It is the purpose of this communication to discuss a simple matrix method, known as the **LU** method,[5] that permits the simple solution of equation (1).

THEORY

Consider the situation where the elements of y_k, ($\vec{\mathbf{Y}}$), and $x_{j,k}$, (**X**), are known and b_j, ($\vec{\mathbf{B}}$), is sought. Let $j = 4$ in this example, and $j = k$.

Any square matrix can be expressed as the product of a unit lower triangular matrix, **L**, and an upper triangular matrix, **U**.

$$\mathbf{X} = \mathbf{LU} \tag{2}$$

That is

$$\mathbf{X} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ l_{21} & 1 & 0 & 0 \\ l_{31} & l_{32} & 1 & 0 \\ l_{41} & l_{42} & l_{43} & 1 \end{pmatrix} \begin{pmatrix} u_{11} & u_{12} & u_{13} & u_{14} \\ 0 & u_{22} & u_{23} & u_{24} \\ 0 & 0 & u_{33} & u_{34} \\ 0 & 0 & 0 & u_{44} \end{pmatrix}$$

Similarly, for any vector, $\vec{\mathbf{B}}$ in this example, an equation of the form

$$\vec{\mathbf{Y}} = \mathbf{X}\vec{\mathbf{B}} \tag{3}$$

can be expressed as

$$\vec{\mathbf{Y}} = \mathbf{LU}\vec{\mathbf{B}} \tag{4}$$

or

$$\vec{\mathbf{Y}} = \mathbf{L}\vec{\mathbf{Q}} \tag{5}$$

where $\vec{\mathbf{Q}}$ is a new vector, the elements of which may be determined by the equation

$$y_i = \sum_1^J l_{ij} q_j \tag{6}$$

from which q_i may be determined if $\vec{\mathbf{Y}}$ and **L** are known. Hence $\vec{\mathbf{B}}$ can be obtained by back-substitution in the equality

$$\mathbf{U}\vec{\mathbf{B}} = \vec{\mathbf{Q}} \tag{7}$$

METHOD

Data have been taken from one of the spectra, number 10, used to obtain the results presented in an earlier publication.[1] Absorbance ($\vec{\mathbf{Y}}$) and molar absorptivity (**X**) data for four species at 570 nm were given in this publication. Other data, shown in Table 1, have been obtained from the original spectrum and computer program outputs used to produce Figs. 2 and 3 of reference 1.

The data in Table 1 form the elements of **X** and by

Table 1. Experimental data for $\vec{Y}$ and **X**

Wavelength, nm	Absorbance	Molar absorptivity			
		HBCG	BCG$^-$	HPR	PR$^-$
440	0.350_0	1.947E4	3.121E3	2.283E4	3.195E3
490	0.178_0	1.207E4	4.137E3	8 093E3	1.387E4
570	0.401_3	2.443E2	2.543E4	3.462E2	4.693E4
610	0.631_5	7.315E1	4.403E4	1.377E2	6.965E2

the rules of matrix multiplication any element of **X** is given by

$$x_{i,j} = \sum_{k=1}^{N} l_{ik}u_{kj} \tag{8}$$

In practice, the evaluation of the first row of **U** is by inspection, *i.e.*,

$$x_{1j} = l_{11}u_{1j} = 1u_{1j} \tag{9}$$

and the evaluation of the first column of **L** is obtained from

$$x_{i1} = l_{i1}u_{11} \tag{10}$$

At this point all other elements of **L** and **U** may be obtained. For example, when $i = 2$

$$x_{2j} = l_{21}u_{1j} + l_{22}u_{2j} \tag{11}$$

thereby providing the values of the second row of **U**. Repeating this process of considering integral values of i and j, in turn, leads to all elements of **L** and **U**. The results of these calculations are shown in Table 2.

The elements of $\vec{Q}$ are now evaluated in order to obtain the solution to the problem. From the rules of matrix vector multiplication and recalling equation (4) we note that

$$q_k = \sum_{j=1}^{N} u_{kj}b_j \tag{12}$$

Since **U** is an upper triangular matrix k starts at N and is decreased to unity. This enables the value of q_k to be calculated from the kth row of **U** and the previously obtained values of $q_{k+1}, q_{k+2}, \ldots, q_N$. The elements of $\vec{Q}$ in this example are shown in Table 2.

The elements of $\vec{B}$, the solution to the system of equations described by equation (1), is obtained from equation (7) by back-substitution. Thus

$$q_4 = u_{44}b_4$$
$$q_3 = u_{33}b_3 + u_{34}b_4$$
$$q_2 = u_{22}b_2 + u_{23}b_3 + u_{24}b_4$$
$$q_1 = u_{11}b_1 + u_{12}b_2 + u_{13}b_3 + u_{14}b_4$$

The calculated values of $\vec{B}$ are shown in Table 3. The values calculated by the constrained least-squares program[1] are also shown in Table 3 for comparison purposes.

DISCUSSION

The calculated values of $\vec{B}$ are in good agreement with those previously published.[1] There is a small discrepancy between the values for b_1 and b_4. However, it should be pointed out that the contributions of these components to the observed absorbances at the wavelengths considered, as detailed in Table 4, are in certain instances close to or below the reported preci-

Table 2. Elements of **L**, **U** and $\vec{Q}$

a Matrix **L**

1	0	0	0
6.199E-1	1	0	0
1.255E-2	1.153E1	1	0
3.757E-3	1.999E1	1.733E0	1

b Matrix **U**

1.947E4	3.121E3	2.283E4	3.195E3
0	2.202E3	−6.059E3	1.189E4
0	0	6.992E4	−9.020E4
0	0	0	−8.068E4

c Vector $\vec{Q}$

(3.500E-1 −3.909E-2 8.452E-1 −5.314E-2)

Table 3 Results of LU decomposition method

	b_1	b_2	b_3	b_4
LU method	4.029E-7	1.430E-5	1.294E-5	6.586E-7
CLS method*	3.214E-7	1.430E-5	1.285E-5	7.570E-7

* Data taken from Table 5, solution 10, reference 1.

Table 4. Contribution of each absorbing species to the total absorbance

Wavelength nm	h_1 (HBCG)	h_2 (BCG^-)	h_3 (HPR)	h_4 (PR^-)	A_{calc}
440	0.0078	0.0446	0.2954	0.0021	0.3499
490	0.0049	0.0592	0.1047	0.0091	0.1779
570	0.0001	0.3636	0.0046	0.0309	0.3992
610	0.0000	0.6296	0.0018	0.0005	0.6319

sion and reproducibility of the Cary 14 recording spectrophotometer.

The procedure detailed here is simple to implement and does not require a computer to perform the calculations. It is advisable to use a pocket calculator to perform the calculations in order that arithmetic precision may be maintained throughout. Since there are only a few steps in the algorithm the procedure could be adapted for use on a programmable hand-calculator although this has not been attempted by the author. Once **L** and **U** are set up they are used for any successive values of $\vec{Y}$ to give $\vec{B}$. Therefore the majority of the arithmetic need only be performed once for any one set of values of **X**.

It must be stressed that when $M > N$ the solution of a system of linear equations is *most* reliably achieved by using algorithms to be found elsewhere.[1,2] However, when $M = N$ the method presented here is an attractive alternative to the more traditional solutions to this problem.

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BIS[2-(2-PYRIDYLAZO)-5-DIETHYLAMINOPHENOLATO] COBALT(III) CHLORIDE AS A NEW EXTRACTION AND SPECTROPHOTOMETRIC REAGENT FOR TRACE ANIONS

DETERMINATION OF SULPHATED AND SULPHONATED SURFACTANTS

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Summary—The highly coloured bis[2-(2-pyridylazo)-5-diethylaminophenolato] cobalt(III) ion, $Co(PADAP)_2^+$, is proposed as a new counter-ion for use in the extraction and spectrophotometric determination of traces of colourless anions in water. Its application to the determination of sulphated and sulphonated surfactants is described. Beer's law is obeyed over a wide range and the detection limit is approximately 0.2 μg. Large amounts of diverse ions in common water samples do not interfere. The proposed method is simpler and more sensitive than the widely used Methylene Blue method.

2-(2-Pyridylazo)-5-diethylaminophenol (PADAP) forms intensely coloured chelates with many heavy metal ions.[1–6] Of the chelates formed, the cobalt chelate has unique properties: its formation is incomplete at pH values lower than 4,[4,5] but once formed, it is not decomposed even in strongly acid medium, suggesting that the cobalt is oxidized to the tervalent state during the course of chelate formation and a unipositive charge remains on the cobalt–PADAP chelate species. Thus, the extraction of the Co–PADAP chelate is possible only when anions are present which form extractable ion-pairs with the Co–PADAP cation. Hence, there is a possibility of using this cation as a counter-ion for the extraction and spectrophotometric determination of colourless anions, such as anionic surfactants.

EXPERIMENTAL

Reagents

Preparation of Co(PADAP)$_2$Cl. Dissolve zinc–PADAP, synthesized according to Florence,[6] in dilute hydrochloric acid. Add an excess of cobalt chloride solution and adjust the pH to about 7 with dilute ammonia to assist complete conversion of the PADAP into its cobalt chelate. After 10 min, adjust the pH to 1 with dilute hydrochloric acid. Extract the $Co(PADAP)_2Cl$ with chloroform to separate it from the excess of cobalt chloride. Evaporate the chloroform slowly. Dissolve the residue with 0.1*M* hydrochloric acid and dilute to the desired concentration.

The Co–PADAP obtained in this way was not pure. Since further purification is impracticable, the reagent was used without further purification. A $6 \times 10^{-4}M$ solution (0.04% solution of the chloride) was used in the present investigation. The concentration of the Co–PADAP solution was determined from the absorbance of the solution.

An analysis done on a sample repeatedly crystallized from 0.1*M* hydrochloric acid agreed with the theoretical composition: $Co(PADAP)_2Cl$, $C_{30}H_{34}N_8O_2CoCl$ requires C 56.9%, H 5.4%, N 17.7%, Co 9.3%, Cl 5.6%; found C 56.6%, H 5.7%, N 17.5%, Co 9.1%, Cl 5.4%.

$Co(PADAP)_2Cl$ forms dark violet needles, m.p. 268°, and is water-soluble. The chelate is stable for more than one month in 0.1*M* hydrochloric acid medium.

Surfactants. Sodium di-(2-ethylhexyl)sulphosuccinate (SSS) was used as a model surfactant. The purity of the surfactant was 96.5% as specified by the manufacturer.

Other reagents used were analytical grade reagents.

Procedure

Take 50 ml of sample containing less than 25 μg (as equivalent SSS) of surfactants, in a beaker. For waters containing 0.1 ppm of surfactant, use a 250-ml sample. Adjust the pH to 1 with hydrochloric acid. Add 1 ml of $6 \times 10^{-4}M$ $Co(PADAP)_2Cl$. Transfer the mixture to a separatory funnel, add 5.0 ml of benzene and shake for 1 min. Allow the layers to separate, transfer the benzene layer into a cell and measure the absorbance at 550 nm against a reagent blank. Calculate the surfactant concentration from the calibration curve constructed with SSS.

RESULTS AND DISCUSSION

Optimum conditions

The extractability of Co–PADAP from different acid media was examined with several different organic solvents. The results are summarized in Table 1 together with the dielectric constants of the solvents. It is clear that the extractability of Co–PADAP increases with increasing dielectric constant of the solvent and with decreasing charge:radius ratio of the anions present in the aqueous phase: a common feature of ion–pair extraction systems.[7,8]

Co–PADAP is not extracted from 0.1*M* hydrochloric acid medium into benzene. In the presence of large anions such as anionic surfactants, iodide and perchlorate, however, the cobalt chelate is readily extracted with benzene.

Table 1. Effect of acid and organic solvent on extraction

Solvent	NB	MIBK	$CHCl_3$	*o*-Xylene	Ethyl-benzene	Toluene	Benzene	CCl_4	C_6H_{12}
Dielectric constant[10]	34.8	13.3	4.81	2.57	2.41	2.38	2.28	2.24	2.02
0.1*M* HCl	+	+	+	−	−	−	−	−	−
0.1*M* HNO_3	+	+	+	−	−	−	−	−	−
0.05*M* H_2SO_4	+	+	+	−	−	−	−	−	−
0.1*M* $HClO_4$	+	+	+	±	±	(±)	(±)	−	−

+ ; completely extracted.
± ; almost completely extracted.
(±); partially extracted
− ; not extracted
NB; nitrobenzene
aq. phase; 10 ml of $1 \times 10^{-5}M$ $Co(PADAP)_2Cl$ soln.
org. phase; 10 ml.

Because iodide and perchlorate are not likely to be present in significant amounts in common waters, it was decided to select benzene as the solvent.

Sulphonated and sulphated surfactants are extracted successfully from about 0.1*M* hydrochloric acid medium. At this acidity, heavy metal ions likely to be present do not form complexes with PADAP.[5] The high acidity used in the proposed method suppresses ionization of soaps and makes the method selective for sulphonated and sulphated surfactants.

A shaking time of 30 sec is sufficient for the extraction. More than 99% recovery of 0.3 ppm of SSS was obtained in a single extraction.

Beer's law is obeyed up to 25 μg of SSS when 5 ml of benzene are used. Changing the sample size to 250 ml has little effect on the recovery. The molar absorptivity of the extracted species is about 5.5×10^4 l. mole^{-1}. cm^{-1} at the absorption maximum of 550 nm. The relative standard deviation is about 1.7% for 0.3 ppm of SSS.

Table 2. Effect of diverse ions on the determination of SSS

Ion	Concn. *M* (*ppm*)	Absorbance	Error, %
−	−	0.480	−
F^-	0.1 (1900)	0.488	+1.7
Cl^-	0.5 (1750)	0.496	+3.3
Br^-	0.1 (8000)	0.487	+1.5
I^-	10^{-4} (13)	0.554	+15.5
	10^{-5} (1.3)	0.475	−1.0
HCO_3^-	0.1	0.484	+0.8
NO_3^-	0.1	0.540	+12.5
	10^{-2}	0.489	+1.9
ClO_4^-	10^{-4}	0.644	+32.4
	10^{-5}	0.488	+1.7
SO_4^{2-}	0.1 (9600)	0.481	+0.2
PO_4^{3-}	0.1	0.491	+2.3
Stearate	10^{-3}	0.539	+11.9
	10^{-4}	0.486	+1.3
K^+	0.1 (3900)	0.488	+1.7
Na^+	0.1 (2300)	0.481	+0.2
Ca^{2+}	0.1 (4000)	0.492	+2.5
Mg^{2+}	0.1 (2430)	0.483	+0.6
Fe^{3+}	1.8×10^{-4} (10)	0.472	−1.7

* SSS taken; 0.4 ppm.

Stoichiometry of the extracted species

The stoichiometry of the extracted species was studied by the molar ratio method, from which it is clear that the mole ratio of Co–PADAP to surfactant in the extract is 1:1. Because sulphosuccinate species are singly negatively charged in the strong acid medium used in the proposed method, it is assumed that the species extracted is $Co(PADAP)_2^+$.surfactant$^-$. This may be further evidence that cobalt is in the tervalent state in the PADAP complex.

Effect of diverse ions

Table 2 shows the effect of various ions on the determination of SSS. The interference of anions largely depends on the size and charge of the ions. Interference is greater for large anions of small charge. Thus perchlorate and iodide interfere strongly

Table 3. Recovery tests on different surfactants—Comparisons between the MB method and the proposed method

Surfactants	Recovery, %* MB method†	Proposed method§
$C_6H_{13}SO_3Na$	not extracted	not extracted
$C_8H_{17}SO_3Na$	not extracted	not extracted
$C_{10}H_{21}SO_3Na$	44	not extracted
$C_{12}H_{25}SO_3Na$	80	15
SSS	>99	>99
LS‡	>99	>98
ABS(hard)¶	>99	>99
Commercial detergent A	>99	>99
Commercial detergent B	>99	>99

* Concentration of surfactants: $1 \times 10^{-6}M$.
† Longwell and Maniece procedure,[9] with two successive extractions.
‡ Sodium lauryl sulphate
§ Proposed method, with single extraction.
¶ Sodium dodecylbenzene sulphonate.

if present in excess of $10^{-5}M$ and nitrate in excess of $10^{-3}M$. Fortunately, however, these interfering ions are not likely to be present in excess of the maximum allowable levels in most river and ground water samples. It is to be noted that stearate does not interfere unless it is present in excess of $10^{-4}M$. Other anions present in common water samples do not interfere at all.

Surfactants determined by the proposed method

Recovery tests were made on some sulphonates and sulphates. The results are summarized in Table 3 together with the results obtained with the Methylene Blue (MB) method.[9] The range of molecular weights of the surfactants determined extends to the lower side in the MB procedure, whereas lower molecular-weight sulphonates are not determined by the proposed method. A single extraction is sufficient and a very high volume ratio of aqueous to organic phase can be used. These are distinct advantages over the widely used MB method.

The solvent, benzene, used in the proposed method is a rather poor solvent, and the range of extractable anions may be widened by choosing a better solvent.

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ANALYTICAL DATA

MICROIONIZATION CONSTANTS OF SULPHONAMIDES

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Summary—The macroionization constants and thermodynamic parameters of twelve commercial sulphonamides were determined by potentiometric titration at 15°, 25° and 35° and by spectrophotometry at 25°, in aqueous solution of ionic strength 0.1 ($NaClO_4$). The microionization constants and the tautomeric constants (concentration ratios of zwitterion to uncharged or neutral species) were obtained according to the Edsall method combined with complementary tristimulus colorimetry. By utilizing the microionization constants, the species distribution as a function of pH were calculated for the three kinds of sulphonamides.

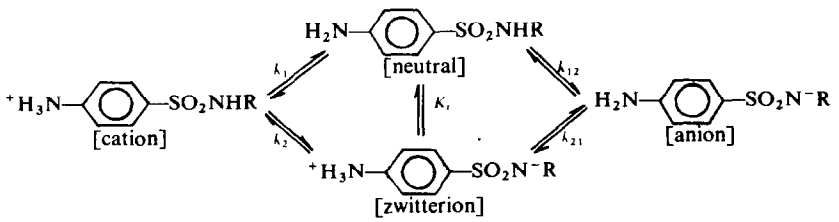
Although there is a great deal of information about the relation of the acid dissociation of sulphonamides and their chemotherapeutic activity,[1-12] the microionization constants of sulphonamides have not so far been reported. An attempt by Klotz *et al.*[12] to determine the tautomeric constant (K_t in Scheme I) of two isoelectric forms of sulphanilamide was unsuccessful. A simple comparison of the macroionization constants gives no indication of the existence of the zwitterion form in the isoelectric states of sulphonamides. However, it is well known that the second macroionization constant (pK_2) is distributed in the very wide range of 3–11, depending on the nature of the substituents on the sulphonamide groups, whereas the first macroionization constant (pK_1) falls in the narrow range of 1.0–2.5. Therefore, it is a questionable procedure to neglect the presence of the zwitterion form[3] and to predict the chemical species of a sulphonamide in the physiological pH-region purely from the macroionization constants.

We recently reported a reliable method for determining the microionization constants, which are fundamental for deducing the chemical species of biologically active substances at a particular pH-value.[13-17] Our attention has been focused further on the microionization constants of sulphonamides. Sulphonamides behaving as either proton acceptors or donors give the equilibria shown in Scheme I. In our method for elucidating microionization constants, the compound must possess a chromophore that is involved in protolytic equilibria, a condition fulfilled by the sulphonamides.

EXPERIMENTAL

Reagents

Benzenesulphonamide and sulphanilamide were obtained from Nakarai Chemical Co., Kyoto, sulphadiazine, sulphamethazine, sulphapyridine and sulphathiazole from ICN Pharmaceutical Inc., U.S.A., sulphamerazine, sulphisoxazole and sulphaguanidine from Sigma Chemical Co., St. Louis, sulphamonomethoxine and sulphadimethoxine from Daiichi Seiyaku Pharmaceutical Co., Ltd., and sulphamethizole from Eisai Pharmaceutical Co., Ltd. All other chemicals were of the highest purity available and were used without further purification.



$$K_1 = k_1 + k_2 \quad (1)$$

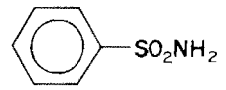
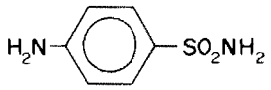
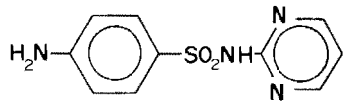
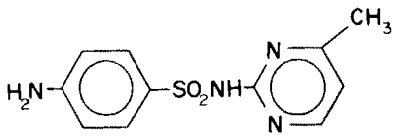
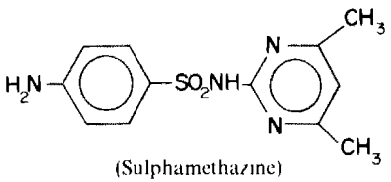
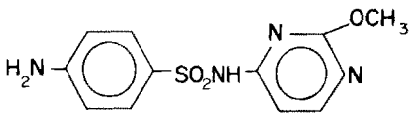
$$K_2^{-1} = k_{12}^{-1} + k_{21}^{-1} \quad (2)$$

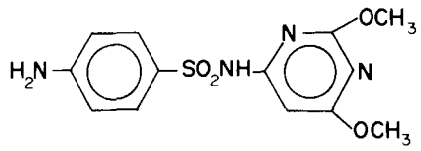
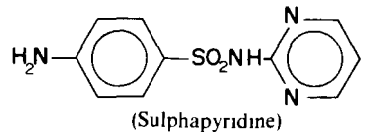
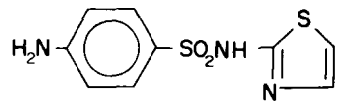
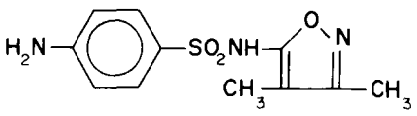
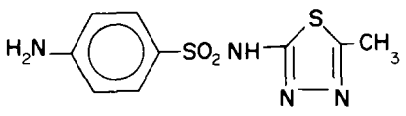
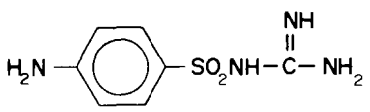
$$K_1 K_2 = k_1 k_{12} = k_2 k_{21} \quad (3)$$

$$K_t = \frac{k_1}{k_2} = \frac{k_{21}}{k_{12}} \quad (4)$$

Scheme I. Scheme of ionization equilibrium of sulphonamide derivatives.

Table 1 Macroionization constants of sulphonamides at 25 and $\mu = 0.1$ ($NaClO_4$)

Structure of compound (name)	No	Method I (Titration)		Method II (CTS method)		Method III (from microionization constants)		Literature values[1,6]	
		pK_1	pK_2	pK_1	pK_2	pK_1	pK_2	pK_1	pK_2
 (Benzenesulphonamide)	1		10.38 ± 0.06				10.54 ± 0.04		
 (Sulphanilamide)	2	2.84 ± 0.03	10.41 ± 0.05	2.54 ± 0.04	10.50 ± 0.06	2.35 ± 0.03	10.41 ± 0.04	2.36	10.43
 (Sulphadiazine)	3	2.71 ± 0.02	6.45 ± 0.02	2.48 ± 0.03	6.52 ± 0.04	2.28 ± 0.03	6.46 ± 0.07	2.00	6.48
 (Sulphamerazine)	4	2.84 ± 0.01	6.92 ± 0.01	2.53 ± 0.02	7.28 ± 0.04	2.44 ± 0.04	7.44 ± 0.05	2.26	7.06
 (Sulphamethazine)	5	2.85 ± 0.01	7.72 ± 0.01	2.62 ± 0.03	7.42 ± 0.05	2.49 ± 0.04	7.60 ± 0.05	2.36	7.38
 (Sulphamonomethoxine)	6	2.61 ± 0.03	6.48 ± 0.02	2.38 ± 0.05	6.27 ± 0.05	2.67 ± 0.05	6.19 ± 0.04	2.0	5.9

 (Sulphadimethoxine)	7	2.91 ± 0.02	6.66 ± 0.02	2.59 ± 0.03	6.78 ± 0.04	2.45 ± 0.04	7 02 ± 0 03	2.02	6.7
 (Sulphapyridine)	8	2.76 ± 0.03	8.55 ± 0.04	2.79 ± 0.04	8.61 ± 0.05	2.61 ± 0.04	8.52 ± 0.07	2.58	8 43
 (Sulphathiazole)	9	2.81 ± 0.02	7.38 ± 0.02	2.58 ± 0.03	7 42 ± 0.04	2.47 ± 0.05	7 32 ± 0.06	2 36	7.12
 (Sulphisoxazole)	10	2.82 ± 0.01	5.20 ± 0.03	2 38 ± 0 04	5.30 ± 0 04	2.19 ± 0.02	5 36 ± 0.05	1.55	5.1
 (Sulphamethizole)	11	2 88 ± 0.02	5.49 ± 0.03	2 57 ± 0.03	5.51 ± 0.04	2 31 ± 0 04	5.64 ± 0.05	2.00	5 45
 (Sulphaguanidine)	12	2.78 ± 0 01	11.70 ± 0.04	2.84 ± 0.03	11.84 ± 0.05	2.54 ± 0.03	11.91 ± 0.06	2.75	12 05

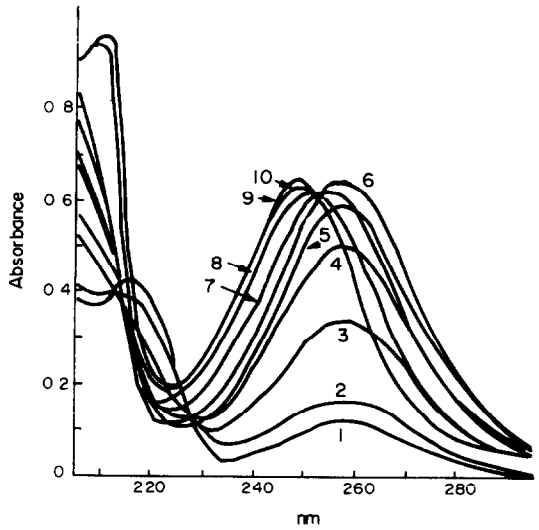


Fig. 1. Absorption spectra of sulphanilamide ($1 \times 10^{-4}M$) at various pH values. pH value: 1, 1.18; 2, 1.41; 3, 2.02; 4, 2.52; 5, 3.04; 6, 4.12; 7, 10.56; 8, 11.21; 9, 12.24; 10, 12.44.

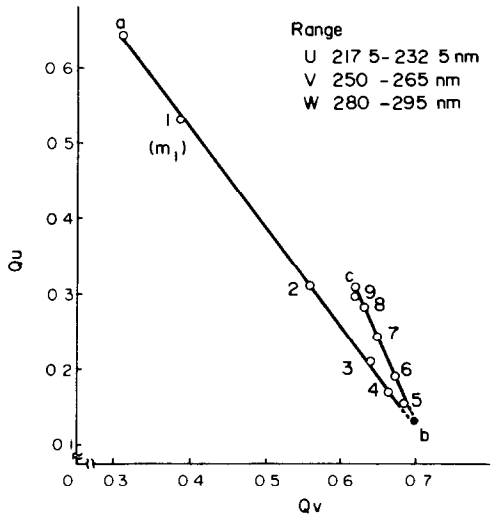


Fig. 2. $Q_u - Q_v$ plot for sulphanilamide. (a) fully protonated form; (b) singly deprotonated form; (c) doubly deprotonated form. a, pH 1.18; 1, pH 1.41; 2, pH 2.02; 3, pH 2.52; 4, pH 3.04; 5, pH 4.12; 6, pH 10.56; 7, pH 11.21; 8, pH 12.01; 9, pH 12.24; c, pH 12.44.

Titration, and measurement of absorption spectra

The pH-titration and the measurement of absorption spectra were done as described previously.[13-15] A $5 \times 10^{-4}M$ solution of the compound was acidified with hydrochloric acid and titrated at 15, 25 and 35° (±0.1°) with carbonate-free 0.1*M* potassium hydroxide at an ionic strength of 0.1 (sodium perchlorate). The spectra were measured at 25°.

Determination of the constants

The macroionization constants were calculated (*a*) by the CTS method[14-17] from the absorption spectra (Fig. 1); (*b*) from the microionization constants,[13-17] these themselves being calculated as described earlier;[15] (*c*) by the method of Schwarzenbach.[18]

The thermodynamic parameters were calculated in the same manner as in the previous paper.[15]

RESULTS AND DISCUSSION

Since the Q_r plot, obtained from the change in absorbance caused by dissociation of the ammonium and sulphonamide groups, showed two straight lines (Fig. 2), the macroionization constants could be calculated by the CTS method. They are summarized in Table 1. The values obtained by the three methods used agreed fairly well with each other and the values reported previously;[1-12] pK_1 refers to dissociation of the proton from the ammonium group, $-NH_3^+$, and pK_2 to the proton from the sulphonamide group, $-SO_2NH-$.[5] The pK_2 values are distributed over the range 5–11, as was described previously,[1] depending on the inductive effect of the NH-substituent group.

Table 2. Thermodynamic parameters for ionization of sulphonamides at 25°

Compound No.*	ΔG_1	ΔG_2	ΔH_1	ΔH_2	ΔS_1	ΔS_2
	kcal/mole		*kcal/mole*		*cal.mole*$^{-1}$*.deg*$^{-1}$	
1		14.2		9.3		−15.6
2	3.9	14.2	1.5	9.5	−7.8	−15.7
3	3.7	8.8	1.1	2.2	−8.8	−22.1
4	3.9	9.4	0.8	3.5	−10.5	−19.8
5	3.9	10.5	0.7	4.8	−10.7	−19.4
6	3.6	8.8	0.6	2.1	−10.0	−23.8
7	4.0	9.1	1.4	1 5	−8.8	−24.2
8	3.8	11.7	0.8	6.0	−10.0	−19.0
9	3.8	10.1	1.8	3.7	−6.7	−21 5
10	3.9	7.1	0.6	0.5	−11.0	−22.1
11	3.9	7.5	1.7	0.7	−7.5	−22.8
12	3.8	†	1.8	†	−8.4	†

* See Table 1.

† Thermodynamic parameters could not be calculated.

Table 3. Microionization constants and tautomeric constants of sulphonamide at 25°

Compound No.*	pK_1	pK_2	pK_{12}	pK_{21}	$K_t = (K_1/K_2)$
2	1.94 ± 0.03	6.01 ± 0.05	10.67 ± 0.03	6.61 ± 0.05	$10^{4.07}$
3	2.23 ± 0.02	4.40 ± 0.03	6.51 ± 0.03	4.34 ± 0.02	$10^{2.17}$
4	2.34 ± 0.02	5.10 ± 0.02	7.53 ± 0.03	4.77 ± 0.05	$10^{2.76}$
5	2.85 ± 0.03	5.65 ± 0.04	7.25 ± 0.04	4.44 ± 0.03	$10^{2.80}$
6	2.18 ± 0.02	4.18 ± 0.08	6.40 ± 0.02	4.41 ± 0.08	$10^{2.00}$
7	2.12 ± 0.03	4.85 ± 0.07	7.28 ± 0.03	4.38 ± 0.07	$10^{2.73}$
8	2.72 ± 0.04	5.84 ± 0.03	8.40 ± 0.04	5.28 ± 0.04	$10^{3.12}$
9	2.95 ± 0.03	5.33 ± 0.02	6.85 ± 0.03	4.46 ± 0.03	$10^{2.38}$
10	2.01 ± 0.05	4.23 ± 0.04	5.54 ± 0.05	3.42 ± 0.04	$10^{2.22}$
11	2.43 ± 0.02	4.38 ± 0.03	5.52 ± 0.03	3.57 ± 0.03	$10^{1.95}$
12	2.53 ± 0.03	6.65 ± 0.04	11.79 ± 0.03	7.67 ± 0.04	$10^{4.12}$

* See Table 1.

The thermodynamic parameters are listed in Table 2. In plots of ΔH *vs.* $T\Delta S$ for K_1 and K_2, the values for the various sulphonamides lie fairly close to straight lines. $T\Delta S$ for sulphaguanidine could not be calculated. The microionization and tautomeric constants are listed in Table 3.

An interesting aspect is revealed by examining the relative concentrations of the isoelectric neutral and zwitterion forms. These two species differ only in the siting of a proton and the consequent charge distribution. Their concentration ratio is given by the tautomeric constant (K_t) in equation (4). The K_t values ranged from $10^{1.9}$ to $10^{4.1}$, and were found to increase with degree of substitution in the homologous series from sulphadiazine to sulphamethazine. By use of these microionization constants the relative concen-

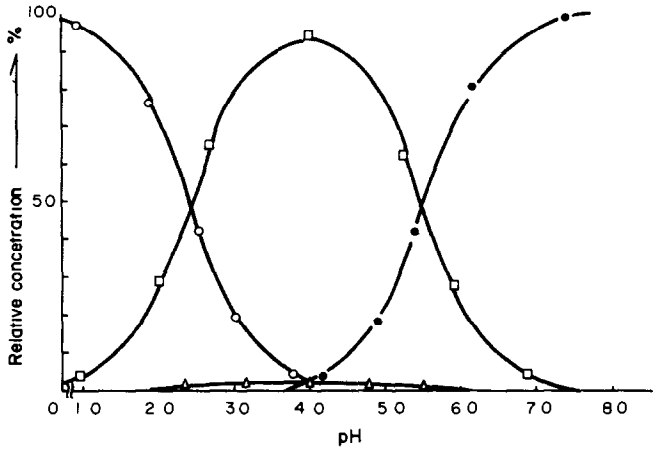
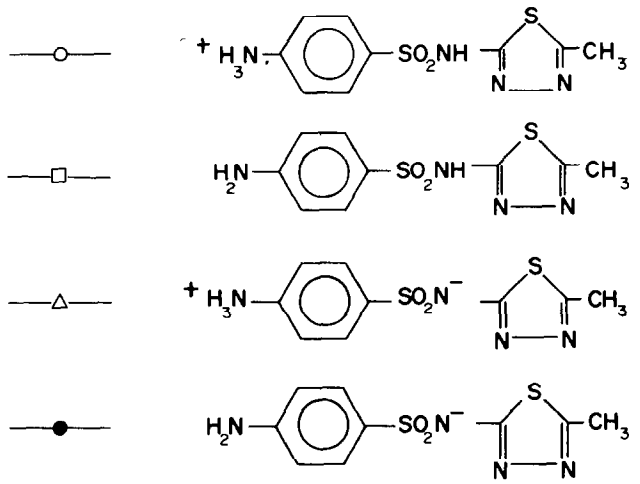


Fig. 3. Relative concentration of various ionic and neutral forms of sulphamethizole.



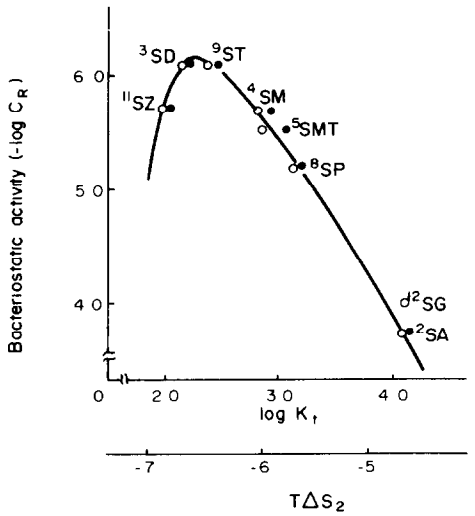


Fig. 4. Relation of *in vitro* activity to tautomeric constant or thermodynamic parameter ($T\Delta S_2$) of sulphonamides. ○ log K_t; ● $T\Delta S_2$. ^{2}SA: Sulphanilamide; ^{8}SP: Sulphapyridine; ^{3}SD: Sulphadiazine; ^{9}ST: Sulphathiazole; ^{4}SM: Sulphamerazine; ^{11}SZ: Sulphamethizole; ^{5}SMT: Sulphamethazine; ^{12}SG: Sulphaguanidine.

tration of various ionic forms of sulphonamides can be calculated as a function of pH, and Fig. 3 shows the result for sulphamethizole. Owing to the large difference between its pK_1 and pK_2 values, sulphanilamide is present completely as the neutral form from pH 4.5 to 8.5. Sulphamethizole is almost entirely in the anionic form in the physiological pH range, but sulphathiazole is in the neutral and anionic forms in approximately 1:1 ratio. The zwitterion form is relatively insignificant ($< \sim 2\%$) even for sulphamethizole, the compound with the highest contribution from this form.

The relationship between the *in vitro* activity of sulphonamides and their macroionization constants has been described in an explanation of their mechanism of action.[1,8] The *in vitro* activity of the sulphonamides examined here is related to their tautomeric constants as shown in Fig. 4. The *in vitro* activity was obtained from the report by Bell *et al.*[1] Figure 4 indicates that as log K_t increases, the bacteriostatic activity passes through a maximum and then decreases. The maximum bacteriostatic activity of these sulphonamides corresponds to a log K_t value of 2–2.8. Thus the K_t value may serve as a good guide to bacteriostatic activity of sulphonamides and be used to predict the bacteriostatic effect of any new NH-substituted sulfa drugs. A similar relation is found between the bacteriostatic activity and the thermodynamic parameters (*e.g.*, $T\Delta S$, see Fig. 4).

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PROTONATION CONSTANTS OF 1-HYDROXYETHYLIDENE-1,1-DIPHOSPHONIC ACID, DIETHYLENETRIAMINO-*N*,*N*,*N*',*N*'',*N*''-PENTA-ACETIC ACID AND TRANS-1,2-DIAMINOCYCLOHEXANE-*N*,*N*,*N*',*N*'-TETRA-ACETIC ACID

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Summary—The stepwise stability constants for the protonation of hydroxyethylidenediphosphonic acid (HEDP), diethylenetriaminopenta-acetic acid (DTPA) and diaminocyclohexanetetra-acetic acid (DCTA) have been determined potentiometrically with a hydrogen electrode at an ionic strength of 1 (KCl) and at 10–35°. The data were treated by a least-squares method for estimation of ΔH and ΔS values.

A high-precision automatic potentiometric titrator,[1–4] including a coulometer and a hydrogen electrode, developed and constructed by Merciny *et al.* in this laboratory is being utilized for the systematic investigation of mixed and double lanthanide(III) and actinide(III) complexes with multidentate ligands. Among the ligands of interest are 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), diethylenetriaminopenta-acetic acid (DTPA) and 1,2-diaminocyclohexanetetra-acetic acid (DCTA).

The dissociation properties of these acids have an important influence on their complex-formation ability, and for the evaluation of the stability constants of metal chelates, the relevant protonation constants must be known as accurately as possible. Previous determinations[5] have provided values which differ considerably, especially in the case of DCTA[6–9] and DPTA.[9–11] Usually, a glass electrode at an ionic strength of 0.1 (KNO_3) has been employed, but we preferred to use a hydrogen electrode and an ionic strength of 1 (KCl). For the less-common ligand, HEDP, (H_5L), only two sets of pK values have been reported.[12,13] It is known that 1-hydroxyethylidene-1,1-diphosphonic acid [ethane-1-hydroxy-1,1-diphosphonic acid, CH_3–C(OH)$(PO_3H_2)_2$] forms soluble complexes with most metal ions and selectively precipitates thorium, scandium and lanthanides from acid solutions.[14] It has some unique properties in biological systems[15] and it is applied as the eluting agent in the cation-exchange separation of lanthanides.[16,17] It therefore seemed desirable to make a careful study of the protonation properties of the ligands mentioned.

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EXPERIMENTAL

Reagents

HEDP. Hydroxyethylidenediphosphonic acid monohydrate was prepared at the Institute of Chemical Technology in Wroclaw. The crude reagent was recrystallized twice from water. The crystals of the monohydrate were washed twice with ethanol (HEDP is extremely soluble in water) and dried *in vacuo* overnight. The reagent was then 99% pure (by titration with standardized carbonate-free sodium hydroxide solution).

DCTA. *trans*-1,2-Diaminocyclohexane-*N*,*N*,*N*',*N*'-tetra-acetic acid monohydrate (Aldrich Inc.) was found to be 98% pure by infrared and elemental analysis. The reagent was dried *in vacuo* at 80 . No unbound water or volatile impurities were found. H_4DCTA was dissolved in boiling water by adding dropwise a 2*M* potassium hydroxide solution. Activated charcoal was added. The solution was filtered, then an amount of 2*M* hydrochloric acid slightly in excess of the amount of potassium hydroxide present was added, and crystals allowed to form in the hot solution. The crystals were washed with hot water until the filtrate did not react with a silver nitrate solution. The reagent was then found to be 100% pure by polarographic titration with a standardized zinc solution, and by titration with sodium hydroxide solution.

DTPA. Diethylenetriaminopenta-acetic acid (Aldrich) was purified similarly to DCTA.

KCl. Potassium chloride (Merck analytical grade) was dried at 350° for 16 hr.

Titration

The high-precision automatic titrator designed and developed in this laboratory[1–4] was employed. Its essential features are as follows.

(*a*) A constant-current coulometric generator of hydroxide ions.

(*b*) A digital voltmeter (10-μV sensitivity), is connected through a high-impedance electrometer to an electronic device which controls the rate of the titration according to the rate of change of the measured potential, and to a printer. A titration takes about 6 hr for each proton titrated.

(*c*) A jacketed titration cell of 150 ml capacity The temperature of the cell, Wilhelm bridge and reference electrode

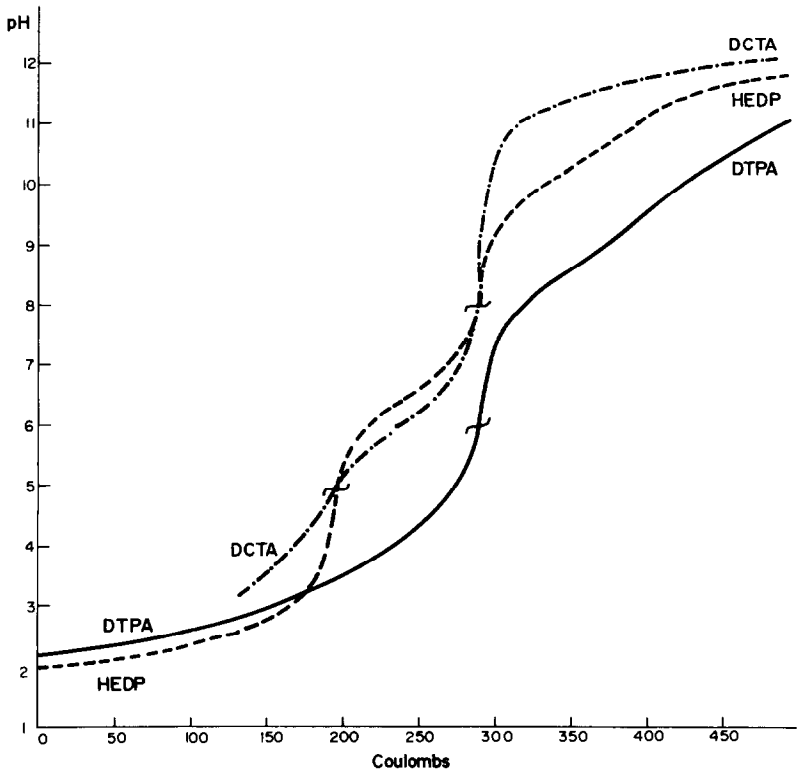

Fig. 1. Titration curves; I = 1.0 (KCl), 25°.

is controlled to within ±0.01°. Carbon dioxide- and oxygen-free water-saturated hydrogen is bubbled through the solution in the cell, and a magnetic stirrer is used.

(*d*) A salt bridge,[3] containing 1*M* potassium chloride, connects the titration cell with a second cell containing 1*M* potassium chloride and about 3 g of zinc powder.

(*e*) A hydrogen indicator electrode, based on hydrogen bubbled over platinum/platinum black.

(*f*) A silver–silver chloride reference electrode ($E°$ = 0.22230 V). The apparatus is kept in an environment of constant temperature and illumination to minimize variations of potential of the reference electrode.

According to Bates[18] the hydrogen electrode provides the most accurate method for determination of pH and can be used over the whole pH scale. Glass membranes can behave erratically in the presence of highly charged ions. Unlike the glass electrode, the hydrogen electrode does not need to be standardized and it responds perfectly linearly even in a strongly basic solution. It has been found[1] that pH measurements at every point of a titration curve can have an error of less than 0.001 pH unit.

The 100-ml samples of 0.01*M* solutions of the acids were titrated at an ionic strength of 1 (KCl) at 10, 15, 20, 25, 30 and 35°.

In order to prepare a 0.01*M* solution of the sparingly soluble DCTA, a standardized carbonate-free potassium hydroxide solution was added dropwise to a suspension of the acid in water.

The detailed experimental conditions of titration were as described previously.[1–4]

Calculations

The method of calculation recommended by Rossotti and Rossotti[19] was used. The detailed treatment of titration results was as described previously.[2,4]

Table 1. Protonation constants and estimated values of the thermodynamic quantities for protonation of hydroxyethylidene-diphosphonic acid (HEDP)

Equilibrium	$\frac{[HL]}{[H][L]}$ (1)	$\frac{[H_2L]}{[HL][H]}$ (2)	$\frac{[H_3L]}{[H_2L][H]}$ (3)	$\frac{[H_4L]}{[H_3L][H]}$ (4)	$\frac{[H_5L]}{[H_4L][H]}$ (5)
log K_p					
298.15 K I = 1 (KCl)	—	10.08 ± 0.00	6.44 ± 0.00	2.34 ± 0.01	1.21 ± 0.02
I = 0.1[12,13]	11.13[12]	10.29[12]	7.28[12]	2.47[12]	1.7[12]
		10.99[13]	6.99[13]	2.31[13]	
log $K_p = a/T + b$	—	a = 1002	a = 446.8	a = −143.1	a = −258.3
283.15 K < T < 308.15 K	—	b = 6.72	b = 4.94	b = 2.82	b = 2.08
$\Delta H = -19.168\ a$	—	−19.2	−8.6	+2.7	+5.0
kJ/mole					
$\Delta S = 19.168\ b$	—	+130	+95	+54	+40
$J.mole^{-1}.deg^{-1}$					

Table 2. Protonation constants and estimated values of the thermodynamic quantities for protonation of diethylenetriamine-*N*,*N*,*N'*,*N''*,*N''*-penta-acetic acid (DTPA)

Equilibrium	$\frac{[HL]}{[H][L]}$ (1)	$\frac{[H_2L]}{[HL][H]}$ (2)	$\frac{[H_3L]}{[H_2L][H]}$ (3)	$\frac{[H_4L]}{[H_3L][H]}$ (4)	$\frac{[H_5L]}{[H_4L][H]}$ (5)	$\frac{[H_6L]}{[H_5L][H]}$ (6)	$\frac{[H_7L]}{[H_6L][H]}$ (7)	$\frac{[H_8L]}{[H_7L][H]}$ (8)
$\log K_p$								
298.15 K $I = 1$ (KCl)	10.06 ± 0.02	8.32 ± 0.00	4.13 ± 0.00	2.50 ± 0.00	2.29 ± 0.01	(1.67 ± 0.03)	(0.88 ± 0.05)	(0.81 ± 0.05)
$I = 0.1$[5]	9.48[5]	8.26[5]	4.17[5]	2.6[5]	2.3[5]	—	—	—
$\log K_p = a/T + b$	$a = 1555.6$	$a = 1254$	$a = 623$	$a = 103.1$	$a = 452.78$	—	—	—
283.15 K < T < 308.15 K	$b = 4.84$	$b = 4.12$	$b = 2.04$	$b = 2.84$	$b = 3.81$	—	—	—
$\Delta H = 19.168\ a$	−29.8	−24.0	−12.0	−2.0	−8.7	—	—	—
kJ/mole	−33[5]	−18[5]	−7[5]	−2[5]	1[5]	—	—	—
$\Delta S = 19.168\ b$	93	79	39	54	73	—	—	—
$J \cdot mole^{-1} \cdot deg^{-1}$	88[5]	107[5]	59[5]	42[5]	38[5]	—	—	—

(Results in brackets were determined from data obtained for pH-regions where only small equilibrium amounts of the protonated species were present)

Table 3. Protonation constants and estimated values of the thermodynamic quantities for protonation of *trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetra-acetic acid (DCTA)

Equilibrium	$\frac{[HL]}{[H][L]}$ (1)	$\frac{[H_2L]}{[HL][H]}$ (2)	$\frac{[H_3L]}{[H_2L][H]}$ (3)	$\frac{[H_4L]}{[H_3L][H]}$ (4)	$\frac{[H_5K]}{[H_4L][H]}$ (5)
$\log K_p$					
298.15 K $I < 1$ (KCl)	12.13 ± 0.01	5.99 ± 0.00	3.21 ± 0.00	2.41 ± 0.01	(1.49 ± 0.05)
293.15 K $I = 1$[5]	9.30[5]	5.87[5]	3.52[5]	2.41[5]	1.72[5]
$\log K_p = a/T + b$	$a = 2269.5$	$a = 794.04$	$a = 63.11$	$-a = 131.48$	
	$b = 4.52$	$b = 3.32$	$b = 3.00$	$b = 2.85$	
$\Delta H = -19.168\ a$ kJ/mol	−43.5	−15.2	−1.2	+2.5	
	−(28–38)[20]	−(5–11)[20]			
$\Delta S = 19.168\ b$	87	64	57	54	
$J.mole^{-1}.deg^{-1}$	109–142[20]	87 98[20]			

(For results in brackets see footnotes to Table 2).

RESULTS AND DISCUSSION

Titration curves for the free acids are shown in Fig. 1. The titration curves of DTPA and DCTA are included for reference and comparison. The curve of HEDP is characterized by an acid buffer region at a pH markedly lower than that of DCTA and DTPA, and parallel to that of DTPA, followed by a strong pH rise at two meq of coulombs per mmole of ligand. This low-pH buffer region (pH 2–3) corresponds to the release of two protons from phosphonic acid groups.

The second buffer region, above pH 6 (higher than for DCTA), corresponds to the release of the next proton. It has a steeper slope than the first buffer region. The inflection observed when the third proton has been titrated is not so sharp, however, as for DCTA. The fourth and fifth equivalence points are not distinctly marked. At pH ~ 11.5, however (at about 400 coulombs), there is a small inflection and change of slope fo the HEDP curve.

Chelates of HEDP are probably of a quite different type from those of DCTA and DTPA. Some preliminary experiments suggest that in the case of tervalent lanthanides, polynuclear aggregates are precipitated as strongly hydrated micellar systems up to pH 10.

The protonation constants of the acids studied are given in Tables 1, 2 and 3, along with literature data. In the upper part of each table an abbreviated equilibrium quotient expression is included for each constant (charges have been omitted). Equilibria involving protons are written as stepwise stability constants rather than as ionization (dissociation) constants, to be consistent with the metal complex-formation constants. For the same reason, the protonation constants are expressed as concentration constants. Consequently the log K_p values are equal to the relevant pK values and ΔH and ΔS values have signs opposite to those describing dissociation constants.

The values were not corrected for complexation with ions of the medium. The protonation constants at $I = 1$ (KCl) listed include competition by ions from the supporting electrolyte and are somewhat smaller than they would be if measured in solutions of, *e.g.*, tetra-alkylammonium salts, and smaller as a rule than those determined at $I = 0.1$. The protonation constants determined at 10, 15, 20, 25, 30 and 35° are represented in the Tables by equations (where T is the temperature in K) obtained by least-squares analysis. The equations are converted into thermodynamic functions by using the van't Hoff equation. All values of thermodynamic quantities were rounded off to the nearest 0.1 kJ/mole (enthalpy) or $J.mole^{-1}.deg^{-1}$ (entropy) because of their reduced accuracy.

HEDP. The experimental protonation constants for HEDP reported in Table 1 are somewhat smaller, except for pK_5, than the only two previous determinations (at $I = 0.1$). The thermodynamic quantities have not been reported previously.

DTPA. Protonation constants for DTPA are given in Table 2. The experimental constants listed are in reasonable agreement with the most reliable set of data selected by Martell and Smith,[5] from numerous reports, except for the first protonation step. Comparison of the ΔH and ΔS values is rather difficult since all previous values refer to $I = 0.1$. There is reasonable agreement with the present values (except for the penta-protonated ligand) especially in view of the uncertainties in published data.[20]

DCTA. The experimental constants given in Table 3 differ only slightly from recommended values.[5] The ΔH and ΔS values have been reported only for the first two protonation steps.[20] The experimental enthalpy values are a little more exothermic and the entropy changes are somewhat smaller. The present data show a definite trend related to the stability of the protonated ligand. It should be possible to make a rough estimate of the complexing ability of HEDP, with respect to DTPA and DCTA, for lanthanide(III) and actinide(III) ions. It should be approximately proportional to the sum of the logarithmic constants for the first three protonation steps,[21,22] the chelates of HEDP should be the most stable ($\Sigma\log K_{p(HEDP)} = 29.39$; $\Sigma\log K_{p(DTPA)} = 22.51$; $\Sigma\log K_{p(DCTA)} = 21.33$).

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SPECTRAL BANDWIDTH IN PLANT CHLOROPHYLL DETERMINATIONS

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Summary—The apparent tendency, in recent literature concerned with the determination of chlorophylls, to ignore spectral bandwidth effects is critically discussed. Quantitative data are presented which allow the determination to be made with spectrophotometers of only moderate resolution.

The determination of plant chlorophylls by solution spectrophotometry is susceptible to numerous sources of error, primarily because of the possibility of photo-induced degradation of the pigments during sample preparation. It is particularly difficult to obtain pure solid samples of the pigments. Most workers therefore rely upon the spectral characteristics obtained by Comar and Zscheile and co-workers[1–5] or by MacKinney[6,7] to convert measured absorbances into concentrations. It was recognized very early on, that discrepancies in published values of absorptivities could arise from the presence of concomitant impurities through incomplete separation or the presence of photochemical or chemical degradation products, from variations in preparation techniques, or from instrumental factors. Of the latter, spectral bandwidth is a parameter of which the importance is often overlooked or inadequately understood.

Zscheile and Comar commented in their early work that, because the measured absorbance was an average for all wavelengths included within the spectral bandwidth of the monochromated beam, values at narrow maxima or minima would be more influenced by variation of the spectral region isolated:[1] this conclusion is valid for extremely sharp peaks. They found that the absorption values at 660 nm of a chlorophyll *a* solution in diethyl ether did not change over the bandwidth range 1–3 nm. From the chlorophyll absorption spectra included in their paper, however, careful scrutiny reveals that greater per cent changes in absorbance with slit-width could be encountered at wavelengths at which maxima or minima do not occur, but at which the rate of change of absorbance with wavelength is itself changing very rapidly. Such regions correspond to highly curved regions of the first derivative of the absorption spectrum, examples of which may readily be found in the literature.[8] When a two-wavelength method is employed for the determination of chlorophylls *a* and *b* in binary mixtures in aqueous acetone solution, such a region occurs in the chlorophyll *a* spectrum at the wavelength of the chlorophyll *b* absorption maximum (Fig. 1).

Even if spectral bandwidth is mentioned at all in the later work, a limiting value of 3 nm is quoted, based largely on the chlorophyll *a* peak absorbance as a function of bandwidth. This value appears to be accepted regardless of the solvent used, in spite of the fact that spectra may be extensively modified by changes in solvent. Comar and Zscheile did, on one occasion, study the range of spectral bandwidth over which the measured absorbance values of a typical plant extract in ether remained constant at both the chlorophyll *a* and *b* peak wavelengths.[4] This approach is of limited value, however, because a decrease in chlorophyll *b* absorbance as a result of an increased slit-width could be compensated by an increase in chlorophyll *a* absorbance. The extent of any such compensation would depend upon the ratio of chlorophyll *a* to chlorophyll *b*.

MacKinney mentioned the importance of bandwidth in comparisons of results obtained on different instruments,[6] but did not explain its particular importance in the two-wavelength method, even when discussing the poor precision of chlorophyll *b* determinations.[7] Comar demonstrated that a relatively inexpensive commercial spectrophotometer which isolated a spectral region of 4.1 nm gave results for total chlorophyll and chlorophyll *a* determinations that were similar to those obtained with a higher-resolution

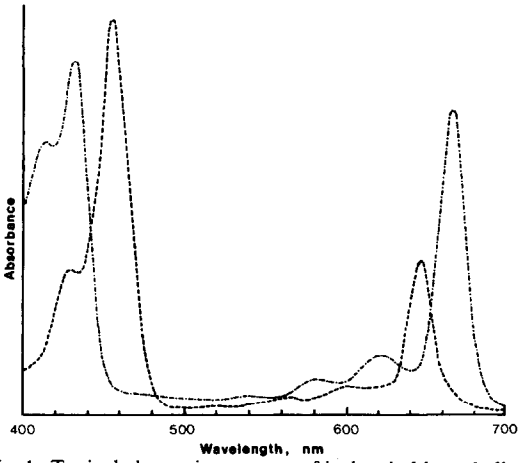

Fig. 1. Typical absorption spectra of isolated chlorophyll *a* (—·—·—) and chlorophyll *b* (----) in 80% aqueous acetone, plotted with the Cecil CE 505.

double-monochromator spectrophotometer.[9] However, since most of the radiation was concentrated in a 2.5-nm region, this could be regarded as falling within the prescribed 3-nm limit.

Arnon used the absorptivities found by MacKinney to determine chlorophylls *a* and *b* in 80% acetone extracts.[10] The spectral bandwidths of the Beckman spectrophotometer used were not specified, probably because the author was primarily concerned with the utilization of the method rather than the methodology. Vernon, also working with 80% acetone, derived equations for the two-wavelength determination of chlorophylls *a* and *b*, as well as equations for the determination of chlorophylls and pheophytins.[11] Absorptivities at appropriate wavelengths were arrived at by an experimental route which depended ultimately upon the reliability of published values for ether solutions, which are sometimes regarded as more reliable than values for aqueous acetone solutions. Using a Beckman D.U. spectrophotometer, Vernon derived a pair of equations slightly different from those based on the MacKinney[7] data, but he did not discuss any possible contribution of spectral bandwidth effects to the apparent discrepancy. Unfortunately it is impossible even to estimate the bandwidth used, particularly for a prism instrument with adjustable slits, when inadequate detail is given in the original paper.

Bruinsma critically reviewed the problems associated with the determination of chlorophylls *a* and *b* by solution spectrophotometry, but omitted to mention the need for high resolution.[12] In this work he suggested the use of a third wavelength for the determination of chlorophyll *a* plus chlorophyll *b* as a check on the reliability of the separate chlorophyll *a* and *b* determinations by the two-wavelength procedure. In another note[13] he reported the results of a detailed study of the isosbestic point of the chlorophyll *a* and *b* spectra, which was performed in an attempt to explain a systematic 5% difference between the sum of chlorophylls *a* and *b* determined separately and the direct determination of chlorophyll *a* plus chlorophyll *b* at the isosbestic wavelength. Although the apparent discrepancy between Bruinsma's results[13] and Arnon's interpretation[10] of MacKinney's results might have been explicable in terms of spectral bandwidth effects, this aspect was not considered, the difference being attributed instead to an inaccurate previous estimation of the absorption coefficient at 652 nm.

Sestak commented in qualitative terms upon the importance of spectral bandwidth when describing the construction of a simple nomogram for the two-wavelength method.[14] Differences between the results obtained on different instruments were attributed, in the author's rather loose terms, to the "accuracy" of the spectrophotometer used. Subsequent workers have either merely reiterated the need for a spectral bandwidth of less than 3 nm,[15] or totally ignored this factor.[16,17]

Although in the Official Methods of Analysis of the A.O.A.C. the need for an instrument capable of isolating a spectral bandwidth of *ca.* 3 nm near 660 nm is briefly mentioned,[18] specialized monographs on the analysis of ecological materials (*e.g.*, reference 19) or on chlorophylls (*e.g.*, reference 8) tend to ignore spectral bandwidth completely. The use of such a text recently led to the superficially surprising finding in the authors' laboratory that different results were obtained for the determination of chlorophylls *a* and *b* in the same extract when two different spectrophotometers were used.

The aims of the present study were twofold; first to quantify for the first time the effect of spectral bandwidth in the estimation of chlorophylls *a* and *b* in 80% aqueous acetone extracts of plants; secondly to see whether the determination is feasible with reasonable accuracy on a spectrophotometer with a spectral bandwidth greater than 3 nm. The latter aspect is of particular interest because of the growing trend towards the incorporation of high levels of scale expansion as a standard feature of modern spectrophotometers: this requires a high degree of signal stability, the attainment of which is often facilitated by employing fixed spectral bandwidths in the 5–8 nm range; even higher bandwidths are not unknown. It is therefore quite likely that a spectrophotometer with a bandwidth of 3 nm or less may be unavailable in laboratories in which chlorophyll determinations are required.

EXPERIMENTAL

Apparatus

Spectrophotometric measurements were made with matched, stoppered 10-mm silica cells in a Cecil CE 505 double-beam spectrophotometer or a Cary 118 spectrophotometer. The former has a nominal fixed spectral bandwidth of 5 nm. The slit-width for the Cary 118 may be varied over a wide range and the bandwidth accurately computed from information supplied by the manufacturer.

A series of cobalt sulphate standards, prepared from cobalt metal, was used to establish the accuracy of the absorbance scales on the two instruments.[20] In every case the deviation from the expected value was less than 1%.

Preparation of plant extracts and measurement of absorbance

Samples of fresh young wheat plants were extracted with 80% aqueous acetone by wet ball-milling for 45 min in subdued light. Filtered extracts were diluted to give an absorbance of less than 0.8 at 665 nm. Absorbances were measured immediately after preparation.

Separation of chlorophylls a *and* b

The pigments from a typical plant extract were extracted into diethyl ether and the extract was washed thoroughly with water and dried over anhydrous sodium sulphate in the dark. It was then subjected to liquid chromatographic separation on a cellulose column, by sequential elution first with petroleum ether (b.p. 60–80°) and then with a 1:4 v/v mixture of chloroform and petroleum ether (b.p. 60–80°). The solvent was evaporated from the chlorophyll *a* and *b* fractions under argon, and the residues were taken up in 80% aqueous acetone. All manipulations were carried out at the minimum possible light level.

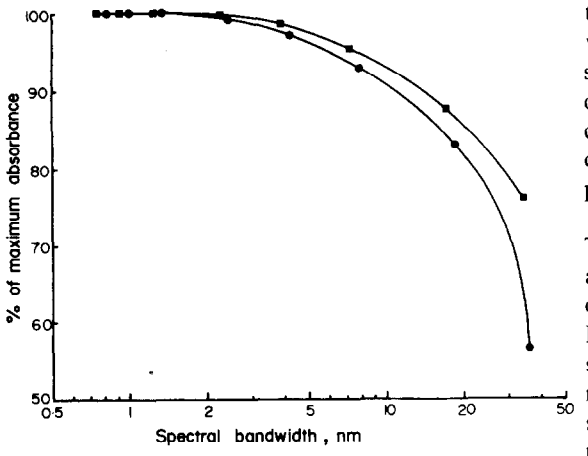

Fig. 2. Dependence of absorbance of solutions of chlorophylls *a* and *b*, expressed as per cent of maximum value, upon spectral bandwidth: ● chlorophyll *a*; ■ chlorophyll *b*.

RESULTS AND DISCUSSION

The effects of spectral bandwidth of the Cary 118 on the absorbances of chlorophylls *a* and *b* at their peak wavelengths in 80% aqueous acetone are shown in Fig. 2. Changes in the absorptivities are small at 3 nm bandwidth, but not insignificant. At the nominal spectral bandwidth of the Cecil CE 505, 5 nm, the absorptivities would be reduced to 96.1% and 97.6% of their peak values for chlorophylls *a* and *b* respectively.

The absorbances of the pure chlorophyll *a* and *b* solutions were measured on the CE 505 at both 647 nm and 665 nm. Assuming MacKinney's absorptivities at the peak wavelengths to be correct at a bandwidth of less than 2 nm, Fig. 2 was used to calculate the peak absorptivities at a bandwidth of 5 nm. These values were then combined with the measured values of the $A_{665}:A_{647}$ ratios for the pure chlorophyll solutions to calculate corrected absorptivities for chlorophyll *a* at the chlorophyll *b* peak and *vice versa*. The four corrected absorptivities were used to derive the following equations for the concentrations of chlorophyll *a* (C_a) and *b* (C_b) in mg/l.

$$C_a = 13.0A_{665} - 1.94A_{647}$$
$$C_b = 23.1A_{647} - 4.16A_{665}.$$

It is instructive to compare these equations with those derived by Arnon[10] from the MacKinney[7] data, *i.e.*,

$$C_a = 12.7A_{665} - 2.69A_{647}$$
$$C_b = 22.9A_{647} - 4.68A_{665}.$$

Because A_{665} is always larger than A_{647}, the most crucial factor in these equations is the constant by which A_{665} must be multiplied in the estimation of C_b. For reasons outlined in the introduction, the effect of spectral bandwidth is critical not only at the absorption maxima, but also in regions of the spectrum where the rate of change of absorbance with wavelength is changing rapidly. In other words a substantial error may be introduced into the estimation of chlorophyll *b* by not correcting the absorptivity of chlorophyll *a* at 647 nm, quite apart from the error caused by not correcting the absorptivity of chlorophyll *b* at this wavelength.

One other factor must be considered at this point. The Cary 118 has a double prism monochromator and the spectrophotometers used in early studies of chlorophylls also used prism monochromators. Because the dispersion is not linear, it follows that the spectral distribution within any given bandwidth is not linear. Assuming that the correct peak wavelength settings are found by using solutions of pure chlorophylls *a* and *b*, it follows that the asymmetric distribution of wavelengths within a given bandwidth (dispersion increasing with wavelength) will lead to an increased contribution to the total throughput of light at the longer wavelengths in the band. On a sharply rising region of an absorption spectrum such as the region of the chlorophyll *a* spectrum around 647 nm this will lead to an apparent increase in absorptivity. Thus the Cary 118 gave markedly higher absorbance readings than the Cecil CE 505 for all samples tested at 647 nm, because the latter instrument is a grating instrument: the difference was too great to be explained in terms of the decrease in chlorophyll *b* absorbance alone. The effect of this is to increase the ratio $A_{665}:A_{647}$ for solutions of pure chlorophyll *a* on the grating instrument compared to the prism instrument, and in turn this has a marked effect on the coefficients of the equations derived.

It would be extremely difficult to compensate theoretically for this effect for different spectrophotometers even if the spectral characteristics of their monochromators were well documented. It is highly likely that this factor is one of the major causes of the discrepancies between the equations derived by different authors for the two-wavelength method.[16]

The effect would only be pronounced on sharply changing regions of absorption spectra. It is far less important when measurements are being made at symmetrical maxima. It is therefore valid to use the data in Fig. 2, obtained with a prism instrument, to estimate values of absorptivities for a grating instrument at a bandwidth of 5 nm at the absorption maxima. To derive a pair of simultaneous equations it is still necessary to obtain pure solutions of chlorophylls *a* and *b*. This is, however, a relatively simple task compared with obtaining adequate quantities of pure solid pigments.

The results of applying the equations derived here to absorbances measured with the Cecil CE 505, and Arnon's equations[10] to absorbances measured with the Cary 118, are shown in Table 1. The agreement is quite good, considering the generally recognized poor precision of the chlorophyll *b* determination. For comparison purposes the effects of applying Arnon's equations directly to the results from the Cecil CE

Table 1. Estimation of chlorophylls *a* and *b*

Sample	Cary 118		Cecil CE 505			
			Corrected		Uncorrected	
	Ch_a*	Ch_b*	Ch_a†	Ch_b†	Ch_a§	Ch_b§
1	20.3	7.9	19.9	7.5	19.5	6.2
2	16.2	6.2	16.2	6.2	15.5	5.4
3	19.2	6.9	19.4	7.0	18.5	6.1
4	18.7	7.3	18.7	6.8	17.8	5.9

* Concentrations in mg/l., from Arnon's equations[10] based on data by MacKinney;[7] spectral bandwidth less than 2 nm.

† Concentrations in mg/l., from the absorptivity values by MacKinney,[7] correcting for slit-width effect; spectral bandwidth 5 nm.

§ Concentrations in mg/l., from Arnon's equations;[10] spectral bandwidth 5 nm.

505 are also included. The serious systematic negative error is readily discernible.

At wavelengths corresponding to rapidly changing regions of the absorption spectrum, a secondary effect of decreasing spectral bandwidth is a deterioration in the precision of absorbance measurement, primarily caused by the greater significance of random errors in resetting wavelength. Thus, for example, for the Cary 118, a high-precision instrument, the relative standard deviations of absorbance measurements at 647 nm for an extract of plant pigments were 0.14, 0.05, 0.04 and 0.02% at bandwidths of 0.18, 0.37, 1.8 and 37 nm respectively: the change in relative standard deviations at 665 nm was less pronounced. For the Cecil CE 505, the repeatability of wavelength resetting is not as good, and undoubtedly this contributes to the poor precision of the chlorophyll *b* determination. Further discussion of factors influencing precision and accuracy in spectrophotometry may be found in the literature.[20,21]

It should be noted here that the peak absorption wavelengths used throughout the present investigation for chlorophylls *a* and *b* in 80% acetone were at 665 and 647 nm respectively. These values are 2 nm higher than those reported by MacKinney,[6,7] but agree with the values found by Vernon.[11] The spectrometer calibration in the present investigation was checked by using the deuterium line at 656.10 nm. Arnon's equations were assumed to be correct at the two peak wavelengths in the present investigation. A similar finding has been reported by other workers.[16]

CONCLUSIONS

Chlorophylls *a* and *b* may be estimated by the two-wavelength method with a spectrophotometer of only moderate resolution provided the necessary equations are estabished for the instrument concerned. Absorptivities at the peak absorption wavelengths must first be corrected for the effects of spectral bandwidth. These corrected values may then be used to calculate corrected absorptivities at 647 nm for chlorophyll *a* and at 665 nm for chlorophyll *b*, from measured values of the ratio $A_{665}:A_{647}$ in each case. Equations obtained for prism instruments must not be used directly for grating instruments.

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DETERMINATION OF ng/ml LEVELS OF SULPHIDE BY A CHEMILUMINESCENT REACTION

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Summary—Sulphide ($\geq$0.1 ng/ml) is determined by measuring the chemiluminescence given by its oxidation in aqueous solution by hydrogen peroxide, catalysed by peroxidase. The detection limit is 0.05 ng/ml. Several other, rather less sensitive, systems are also described. Sulphite and thiocyanate, which depress the emission, are masked with formaldehyde.

Chemiluminescent and bioluminescent systems exhibit considerable diversity in the determination of many inorganic and organic compounds,[1-5] and newer systems are continually expanding their scope.[6,7] Few methods for the determination of sulphide by chemiluminescence measurements are available. Lukovskaya *et al.*[8,9] determined 5–20 ng of sulphide by means of its quenching of the chemiluminescent reaction of luminol with iodine, or its reduction of vanadium(V) to vanadium(IV), the vanadium(IV) then catalysing the chemiluminescent reaction of luminol with hydrogen peroxide. Klockow and Teckentrup[10] studied the determination of sulphide by means of its chemiluminescent oxidation by hypohalites in the presence of Methylene Blue. Linear calibration graphs were obtained for 1×10^{-7}–$5 \times 10^{-6} M$ sulphide.

The sensitivity of the Klockow and Teckentrup procedure is already high. However, we decided that the reaction should be studied in some detail in order to improve its sensitivity, to evaluate and eliminate interferences, and to obtain some insight into the mechanism. To improve the sensitivity of emission the use of other sensitizers, such as fluorescein and Rhodamine B, has been investigated. In addition, hydrogen peroxide (accompanied by known catalysts for peroxide reactions, such as catalase,[11,12] peroxidase[13,14] and osmium tetroxide[15,16] has been investigated in detail as an alternative oxidant.

EXPERIMENTAL

Apparatus

The instrumentation and experimental technique were as described previously.[17]

Reagents

All reagents were laboratory reagent grade, unless otherwise stated. The water used was doubly distilled.

Stock sulphide solutions (2000 μg/ml) were prepared by dissolving 1.50 g of sodium sulphide enneahydrate ($Na_2S.9H_2O$) in 100 ml of 0.1 *M* sodium carbonate or borate buffer (pH 9–12). Working solutions were prepared daily from this stock solution by appropriate dilution with the buffer solution chosen. The instability of very dilute sulphide solutions is well known. In the present investigation, flasks containing sulphide solutions were tightly stoppered, and protected from light. Under these conditions, 1–10 ng/ml sulphide solutions remained stable for 20–30 min. For 0.01–10 ng/ml solutions, no change was detected during the first 10 min after preparation; after 25 min the chemiluminescence response had decreased by 50%. For this reason, the calibration solutions used for <10 ng/ml sulphide were prepared serially from the solution of next highest concentration and analysed immediately.

Sodium hypochlorite ($1–10 \times 10^{-3} M$) and hydrogen peroxide (0.8–4.0 *M*) solutions were prepared from a commercial sodium hypochlorite solution, 12% w/v available chlorine, and 100-volume hydrogen peroxide, respectively.

Osmium tetroxide solution (0.01 *M*) was prepared by dissolving 0.253 g of 99.9% pure osmium tetroxide in 100 ml of 0.05 *M* sulphuric acid.

Sensitizer solutions ($2 \times 10^{-3} M$) were prepared by dissolving 0.0884 g of Rhodamine B or 0.0644 g of fluorescein in 100 ml of sulphide working solution.

Catalase stock solution (34×10^3 Sigma units/ml) was made by diluting 4.0 ml of catalase solution (beef liver, twice-crystallized, 25 mg/ml aqueous suspension with 0.1% thymol; activity 3×10^4–4×10^4 Sigma units/mg; Sigma Laboratories) to 100 ml with water, and stored at 4°.

Peroxidase stock solution (28.5 purpurogallin units/ml) was made by dissolving 0.0285 g of horseradish peroxidase (salt-free powder, activity 370–600 purpurogallin units/mg; Sigma Laboratories) in 10 ml of water, and stored at 2°.

Standard solutions of enzymes were prepared daily from the stock solutions by appropriate dilution with water, and were kept in an ice-bath.

Basic procedure for chemiluminescence measurements

A 0.5-ml portion of the oxidant solution was transferred to the cell and 0.5 ml of the catalyst solution added. The syringe was filled with 1.0 ml of the buffered sulphide solution of concentration 2×10^{-5}–2×10^3 μg/ml. The reaction was started by injecting the sulphide solution very rapidly (in <0.4 sec). The emission intensity was recorded at 440 nm as a function of time during 20 sec. For calibration purposes the experiment was repeated with each standard solution of sulphide. The calibration graph was a plot of maximum emission intensity *vs.* sulphide concentration.

The same procedure was used to determine "unknown" samples, with a 1.0-ml sample.

RESULTS

Injection of a sulphide solution into a sodium hypochlorite solution or hydrogen peroxide–catalyst

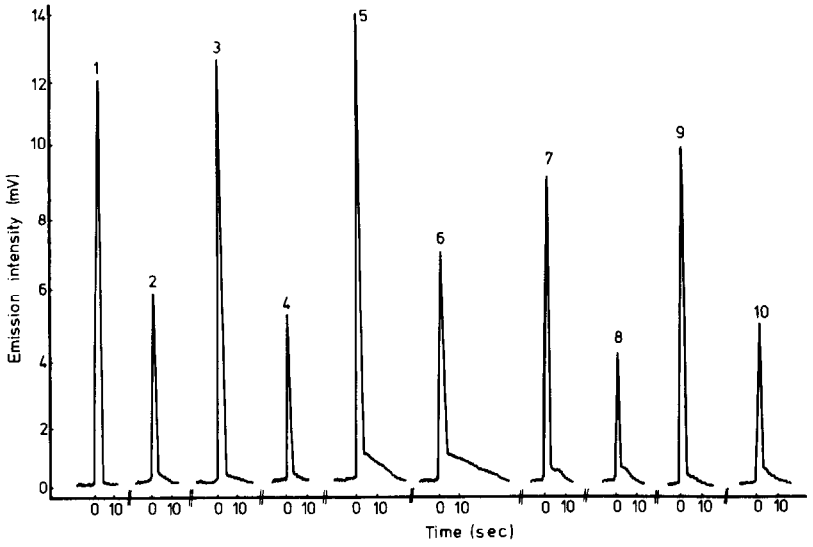

Fig. 1. Intensity *vs.* time signals from (1) 100 ppm and (2) 10 ppm sulphide, 0.1*M* NaOCl; (3) 10 ppm and (4) 1 ppm sulphide, $10^{-3}M$ fluorescein, 0.1*M* NaOCl; (5) 0.1 ppm and (6) 0.01 ppm sulphide, 1.0*M* H_2O_2, 0.25 ppm OsO_4; (7) 0.01 ppm and (8) 0.005 ppm sulphide, 0.4*M* H_2O_2, 750 Sigma units/ml catalase; (9) 0.01 ppm and (10) 0.001 ppm sulphide, 0.4*M* H_2O_2, 1.5 purpurogallin units/ml peroxidase. (1)–(4): pH 11.5; (5)–(10): pH 10.0, carbonate buffer. (1), (2), (5)–(10) at 440 nm; (3), (4) at 520 nm.

mixture produced a chemiluminescence emission which reached maximum intensity after 5 sec, and then decayed rapidly. Typical emission *vs.* time responses are shown in Fig. 1. The coefficient of variation for the determination of 200 ng of sulphide (equivalent to 100 ng/ml in the reaction mixture) based on such a response was *ca.* 5% in all cases.

The spectra of the chemiluminescence emission given on oxidation of sulphide by sodium hypochlorite or hydrogen peroxide have not been reported

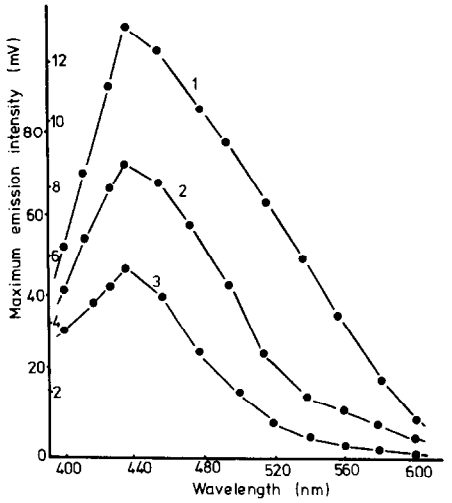

Fig. 2. Spectra of the chemiluminescence emissions given by the oxidation of sulphide under different conditions. (1) 100 ppm sulphide, 0.1*M* NaOCl, pH 11.5; (2) 100 ppm sulphide, 1.0*M* H_2O_2, 0.25 ppm OsO_4, pH 10.0; (3) 1 ppm sulphide, 0.4*M* H_2O_2, 1.5 purpurogallin units/ml peroxidase, pH 10.0. (1) 1–12 mV; (2), (3) 0–80 mV.

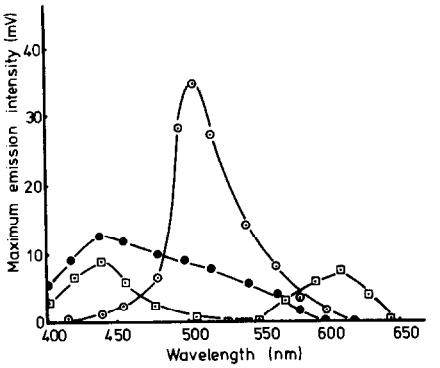

Fig. 3. Spectra of the chemiluminescence emissions given by the oxidation of 100 ppm of sulphide by 0.1*M* hypochlorite at pH 11.5 (carbonate buffer). ● without sensitizer, ⊙ with $10^{-3}M$ fluorescein, ⊡ with $10^{-3}M$ Rhodamine B.

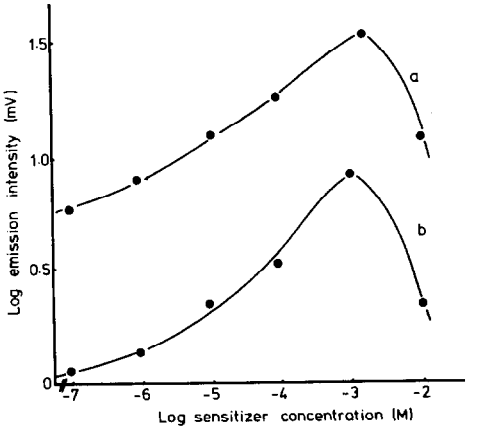

Fig. 4. Effect of sensitizer concentration on the emission intensity given by the oxidation of 100 ppm sulphide by 0.1*M* hypochlorite at pH 11.5 (carbonate buffer). (a) Fluorescein, 520 nm; (b) Rhodamine B, 610 nm.

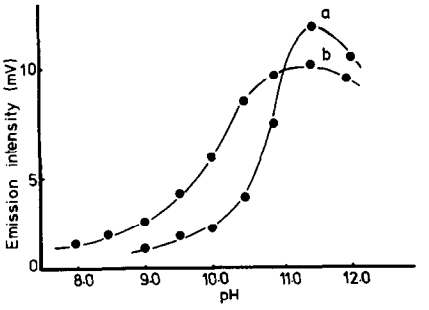

Fig. 5. Effect of pH on the chemiluminescence intensity for the uncatalysed oxidation of 100 ppm sulphide by 0.1*M* hypochlorite. (a) Carbonate buffer; (b) borate buffer.

before and are shown in Fig. 2. They were obtained by measuring the maximum emission intensity produced at various wavelengths by a constant amount of sulphide. The maximum intensity is at 440 nm in all instances, so this wavelength was used for all subsequent experiments. The spectra all have an identical single peak and differ only in their amplitude, indicating the formation of a common emitter, which has not been identified.

The effect of the sensitizers fluorescein and Rhodamine B on the emission spectrum given on oxidation of sulphide by hypochlorite is shown in Fig. 3. The spectra obtained were similar to the fluorescence emission spectrum of the sensitizer added, and confirm that the sensitizers are activated by an energy-transfer process.

The effects of sensitizer concentration on the emission intensity are shown in Fig 4. Maximum intensity was observed with a final concentration of $10^{-3}M$

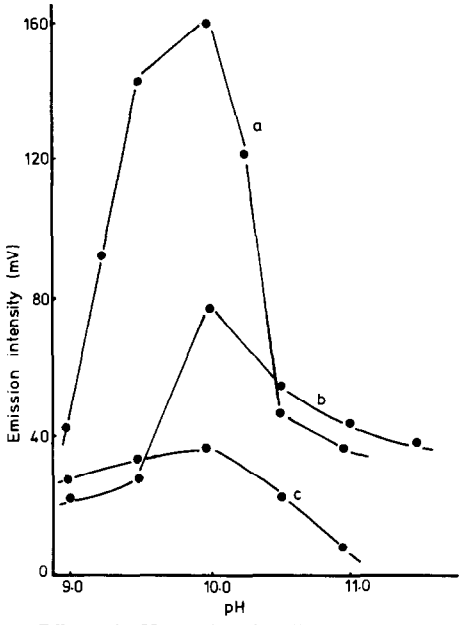

Fig. 6. Effect of pH on the chemiluminescence intensity from oxidation of 100 ppm of sulphide by hydrogen peroxide in the systems: (a) 1.5 purpurogallin units/ml peroxidase, 0.4*M* H_2O_2; (b) 0.25 ppm OsO_4, 1.0*M* H_2O_2; (c) 750 Sigma units/ml catalase, 0.4*M* H_2O_2.

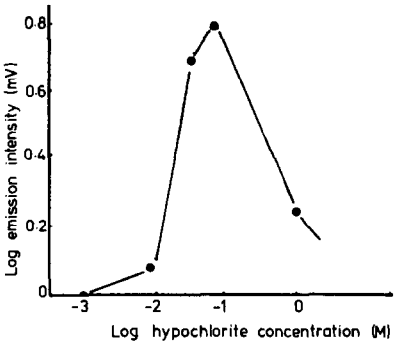

Fig. 7. Variation of chemiluminescence intensity with sodium hypochlorite concentration: 10 ppm sulphide, pH 11.5 (carbonate buffer).

fluorescein or Rhodamine B. An advantage of using fluorescein as sensitizer was an increase in sensitivity, as described later. In investigation of the optimum reagent parameters, only the quantities indicated by points on the graphs were tested, and the true optimum may not lie exactly at the value stated in the text though it will be near it.

The effect of pH (9.0–12.0) on the emission intensity was investigated for all the systems mentioned. Chemiluminescence was observed only in an alkaline medium. The uncatalysed oxidation of sulphide by sodium hypochlorite gave maximum intensity at around pH 11.5 in both buffers (Fig. 5), the emission intensity being higher for the carbonate buffer. The emission intensity during catalysed oxidation by hydrogen peroxide in a carbonate buffer was maximal at about pH 10.0 (Fig. 6).

The effect of oxidant concentration on the emission intensity was strongly dependent on the oxidant concentration, and was maximal with ~0.1*M* hypochlorite (Fig. 7), and ~0.4*M* hydrogen peroxide in the presence of either enzyme as catalyst, or ~1.0*M* hydrogen peroxide with osmium tetroxide as catalyst (Fig. 8); the concentrations refer to the reaction mixture.

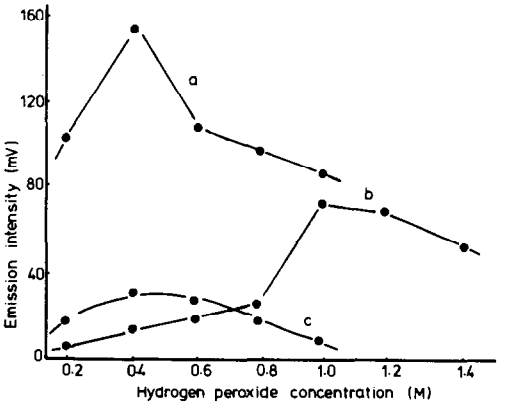

Fig. 8. Effect of hydrogen peroxide concentration on chemiluminescence intensity. (a) 1.5 Purpurogallin units/ml peroxidase; (b) 0.25 ppm OsO_4; (c) 750 Sigma units/ml catalase; 0.1 ppm sulphide, pH 10.0 (carbonate buffer).

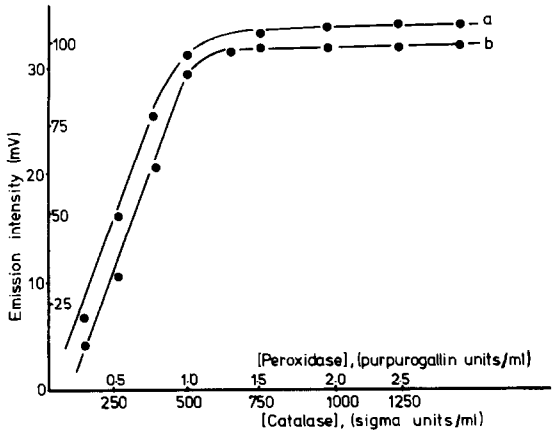

Fig. 9. Effect of enzyme concentration on the chemiluminescence emission from 100 ppm of sulphide. (a) Peroxidase, 0.8*M* H_2O_2 (0–100 mV scale); (b) catalase, 0.4*M* H_2O_2 (0–30 mV scale): pH 10.0 (carbonate buffer).

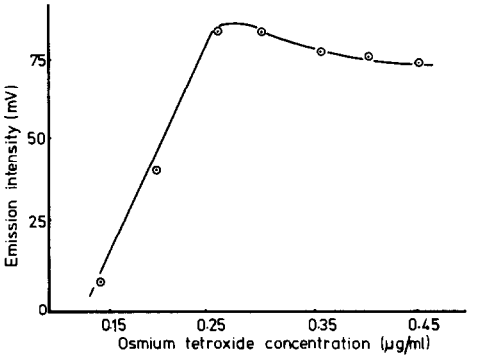

Fig. 10. Effect of osmium tetroxide concentration on the chemiluminescence intensity from 100 ppm sulphide: 1.0*M* H_2O_2, pH 10.0 (carbonate buffer).

The effect of catalyst concentration on the chemiluminescence intensity in the peroxide oxidation was also investigated (Fig. 9). The emission intensity is dependent on enzyme concentration up to ~1.5 purpurogallin units/ml final concentration of peroxidase and ~750 Sigma units/ml final concentration of catalase. The final osmium tetroxide concentration chosen was 0.25 μg/ml (Fig. 10).

Calibration graphs were obtained for each of the systems described above, and are shown in Fig. 11. They demonstrate that sulphide can readily be determined over a wide concentration range, down to $\leqslant 10^{-4}$ ppm by chemiluminescence measurements. Because the curves pass through a maximum, it is suggested that as a safety precaution in interpreting the results, a second sample should be tested, a lower concentration being used. The suggested range of application, together with detection limit, is given in Table 1. Figure 12 shows the blank and sulphide responses near the detection limit.

Interferences

The effect of some other sulphur anions (sulphate, thiocyanate and sulphite) commonly found with sulphide was studied. It was found that sulphate had no significant effect on the determination of 1 ppm of sulphide, even in 1000-fold w/w ratio, in all the systems studied. Thiocyanate (10-fold w/w ratio) suppressed the emission only in the hydrogen peroxide systems, whereas sulphite affected all the systems (Table 2).

It was found that these interference effects were eliminated by addition of formaldehyde to give a final concentration of 4.0 mg/ml (Table 2). Low concen-

Table 1. Analytical parameters for the determination of sulphide by oxidation in some different systems

System	Range of application, *ppm*	Detection limit, *ng*	*ng/ml*
NaOCl	10^{-1}–10^{2}	20	10
NaOCl–fluorescein	10^{-2}–10^{2}	2.0	1.0
H_2O_2–OsO_4	10^{-4}–10^{2}	0.2	0.1
H_2O_2–catalase	10^{-3}–10^{-1}	2.0	1.0
H_2O_2–peroxidase	10^{-4}–10	0.1	0.05

Conditions as in Fig. 11.

Table 2. Effect of interfering ions in the presence and absence of formaldehyde

System*	Change in emission intensity, % SCN^-	SCN^- + HCHO	SO_3^{2-}	SO_3^{2-} + HCHO
NaOCl†	0	0	−10	0
NaOCl–fluorescein	0	0	−12	−1
H_2O_2–OsO_4	−18	−1	−5	−2
H_2O_2–catalase	−34	0	−4	0
H_2O_2–peroxidase	−45	−2	−12	−2

* 1 ppm sulphide, 10 ppm interfering ion, 3.9 mg/ml formaldehyde.
† 10 ppm sulphide.

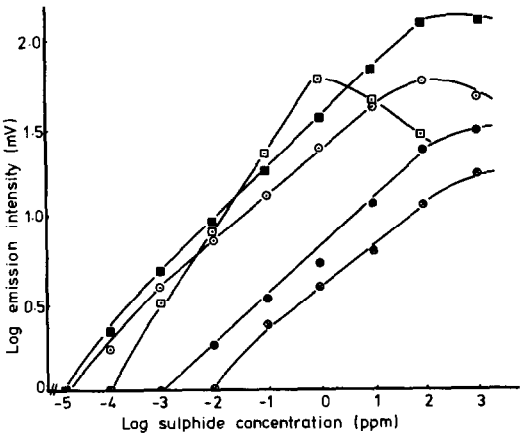

Fig. 11. Effect of sulphide concentration on maximum emission intensity. ⊗ 0.1*M* NaOCl, pH 11.5; ● 0.1*M* NaOCl, 10^{-3}*M* fluorescein, pH 11.5, 520 nm; ⊡ 750 Sigma units/ml catalase, 0.4*M* H_2O_2, pH 10.0, ⊙ 1.5 purpurogallin units/ml peroxidase, 0.4*M* H_2O_2, pH 10.0; ■ 0.25 ppm OsO_4, 1.0*M* H_2O_2, pH 10.0. All at 440 nm except where stated otherwise.

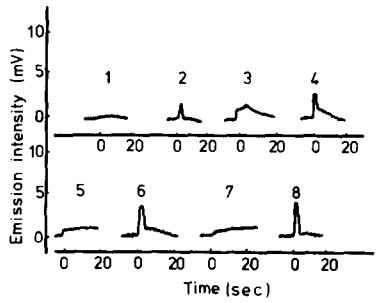

Fig. 12. Responses near the detection limit for (1), (3), (5), (7) no S^{2-} and (2) 20, (4) 0.20, (6) 0.20, (8) 2.0 ng of S^{2-} in the sodium hypochlorite and hydrogen peroxide–osmium tetroxide, –peroxidase and –catalase systems, respectively. Conditions as in Fig. 11.

trations of formaldehyde did not affect the chemiluminescence emission intensity, but ≥8.0 mg/ml increased it. Thus, the final formaldehyde concentration should not exceed 8.0 mg/ml.

DISCUSSION

The emission spectra suggest that the same excited species produces the luminescence, regardless of the oxidant or catalyst. The final oxidation product is sulphate in all instances, but the intermediate reaction steps are not well established.[18] Since $^{\cdot}OH_2$ radicals are assumed to be the source of energy for forming the excited species producing the luminescence, attempts were made to demonstrate their existence in the systems studied, by measuring the spectrum of the very weak chemiluminescence produced by reaction of the hydrogen peroxide–catalyst mixture with a buffered alkaline solution without sulphide. These attempts failed for the catalase and peroxidase systems, probably because the emission intensity was too

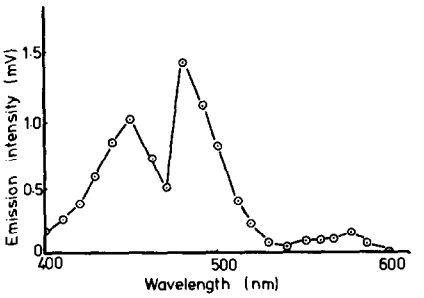

Fig. 13. Spectrum of the weak chemiluminescence blank emission given by the osmium tetroxide–H_2O_2 system: 0.25 ppm OsO_4, 1.0*M* H_2O_2, pH 10.0 (carbonate buffer).

weak. However, a spectrum for the osmium tetroxide system was obtained (Fig. 13) and the maxima at 450, 480 and 580 nm correspond to those attributed to the emission from the excited double oxygen molecule $(O_2O_2)^*$.[19] This excited molecule is produced by collision of $^{\cdot}OH_2$ radicals from the hydrogen peroxide decomposition.[20–22]

The nature of the emitting species formed by oxidation of the sulphide is not known. It is not the excited oxygen molecule described above, but it could be an excited S_2 molecule similar to that involved in flame chemiluminescence emission,[23,24] formed as an intermediate in the $^{\cdot}OH_2$ oxidation of sulphide ions. However, the chemiluminescence measurements give little further evidence about the oxidation reaction mechanism. Other studies, *e.g.*, ESR measurements, will be needed even to begin understanding the system.

It has been established that the chemiluminescence measurements provide an extremely sensitive, precise, simple and rapid (analysis time 2 min per sample) method for the determination of sulphide. The methods described should be applicable to the determination of sulphide in natural waters, such as river, rain and drinking water, with a precision of 6% at the 10^{-3}–10^{2} ppm level. Sulphate does not interfere at concentrations likely to be found in natural water systems, and ions such as sulphite and thiocyanate can readily be eliminated by masking them with formaldehyde.

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THE EXTRACTION OF IRIDIUM AND PLATINUM FROM ORGANIC SOLVENTS BY POLYURETHANE FOAM

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Summary—The feasibility of extracting iridium and platinum from organic solvents onto polyurethane foam was studied. Distribution ratios obtained were 1.1×10^4 for the extraction of iridium from ethyl acetate, 225 for the extraction of iridium from acetone and 4.8×10^3 for the extraction of platinum from ethyl acetate. Capacities of about 16% w/w were obtained for extraction of iridium from ethyl acetate, and about 2.4% for extraction from acetone.

Porous polyurethane foams have been widely used for the extraction of metals from solution. Bowen[1] pioneered work in this direction in 1970 when he used solid flexible polyurethane foam as an extractant for a number of substances from aqueous solution. Bowen noted then that most of the substances absorbed were those which could be extracted from aqueous solutions by diethyl ether and indicated that the extraction by the foams was due to absorption rather than adsorption phenomena. Several investigators have used polyurethane foams, both treated and untreated, for the absorption and recovery of inorganic and organic compounds from aqueous solutions, and Braun[2,3] has recently reviewed the uses of polyurethane foams in analytical chemistry.

To date, all applications of polyurethane foam for the extraction of various metals have been in aqueous media. The extraction of platinum metal complexes from aqueous media, however, poses several problems as these species are especially prone to hydrolysis. By use of foam extraction of these metals from suitable organic solvents, the problem of hydrolysis can be circumvented. This paper reports the extraction of iridium(IV) and platinum(IV) in the form of their hexachloro-complexes. The extraction of the palladium(IV) and rhodium(III) hexachloro-complexes was not possible because they were virtually insoluble in most organic solvents.

The extraction of iridium from aqueous medium with polyurethane foam is difficult because in the presence of the foam the iridium(IV) undergoes reduction to iridium(III), which does not appear to be extractable by polyurethane foam to any significant extent. Baghai and Bowen[4] have reported the separation of rhodium and iridium from solutions in hydrochloric acid by a silicone-rubber foam, but this required the presence of free chlorine gas in order to keep the species present in the form of the hexachlororhodium(III) and iridium(IV) complexes. Such an approach could not be employed for the extraction of iridium by polyurethane foam since polyurethane foams react rapidly with chlorine.

EXPERIMENTAL

Apparatus and reagents

A Unicam SP 500 Series 2 spectrophotometer and a Baird Atomic model 708 Iso/matic system consisting of a 2-in. NaI well-type gamma-detector, model 530 spectrometer and model 620 printer were used.

Polyether polyurethane foam sheets were obtained commercially and cut into suitable sizes.

Sodium hexachloroiridate(IV) hexahydrate and sodium hexachloroplatinate(IV) hexahydrate were supplied by Johnson-Matthey Chemicals Ltd., London.

Iridium-192 in the form of ammonium hexachloroiridate(IV) was obtained from Amersham-Searle Limited, Don Mills, Ontario.

All other chemicals used were of reagent grade.

General procedure

Foams were soaked in 1*M* hydrochloric acid for 24 hr with occasional squeezing, washed several times with distilled water, extracted with acetone in a Soxhlet apparatus for 12 hr, air-dried, placed in a vacuum desiccator for a few hr and then stored in a plastic-covered glass beaker in the dark.

Iridium(IV) and platinum(IV) stock solutions (metal concentration 500 μg/ml) were freshly prepared for each experiment by dissolving 0.1538 g of sodium hexachloroiridate(IV) and 0.1323 g of sodium hexachloroplatinate(IV) respectively, in 100 ml of the appropriate solvent.

The metal was extracted onto the foam by placing the desired amount of solution and foam into a beaker and periodically squeezing the foam by means of a glass plunger in order to bring fresh solution into contact with the foam. The plunger was operated manually, 10 strokes at a time every 15 min, or by a single automatic apparatus consisting of an eccentric motor-driven cam which pushed it up and down at an adjustable rate, or with a multiple automatic apparatus consisting of an eccentric cam turned by a heavy-duty motor which gave a 5-cm stroke at a rate of 24 per minute.

The extraction efficiency was measured by using spectrophotometry at 490 nm for iridium[5] and 263 nm for platinum[6] or a radioactive tracer technique. In the tracer technique, the ^{192}Ir was brought completely into the quadriva-

lent state by evaporation of an aliquot to dryness and treatment of the dry residue with *aqua regia*, this being repeated several times, and followed by similar treatment with concentrated hydrochloric acid to remove any oxides of nitrogen. The final dry residue was dissolved in a few ml of the solvent to be used in the extraction. Samples were mixed thoroughly with sufficient tracer to yield a count rate of at least 150 cps for 15 ml of sample contained in a test-tube of 15 mm bore. The activity of all samples was determined from the average of five successive 100-sec counts and corrected for background. The concentration of iridium in the sample measured was taken to be directly proportional to the activity.

The degree of extraction (E, %) was calculated by means of the equation

$$E = \frac{([\text{metal}]_{\text{initial}} - [\text{metal}]_{\text{final}}) \times 100\%}{[\text{metal}]_{\text{initial}}}.$$

All measurements of concentration of metal after extraction were corrected to allow for loss of solvent by evaporation during the extraction. This was determined with a "control" cell of similar dimensions and containing the same volume of solution as the "sample" cell; both "sample" and "control" were treated in exactly the same manner throughout. The amount of solvent lost was obtained by measurement of the amount of solution left in the "control" cell, or by monitoring the increase in the concentration of the metal in the "control" solution by either spectrophotometry or the radioactive tracer technique.

The distribution coefficient (D) for the extraction process was calculated from the ratio of concentration of metal in the foam to concentration of metal left in solution at equilibrium. This was equivalent to the equation

$$D = \frac{\%\text{ metal on foam}}{\text{wt of foam (g)}} \times \frac{\text{wt of solution (g)}}{\%\text{ metal left in solution}}.$$

To measure the recovery (R) from the foam, 0.1 g of foam was loaded with iridium and tracer ^{192}Ir, and the amount extracted onto the foam was measured by squeezing the foam dry between paper towels and counting it directly in a test-tube. The foam was then squeezed in the stripping solution for the desired length of time and its activity redetermined. The recovery was calculated from:

$$R = \frac{(\text{Activity}_{\text{initial}} - \text{Activity}_{\text{final}}) \times 100\%}{\text{Activity}_{\text{initial}}}.$$

RESULTS AND DISCUSSION

Preliminary studies indicated that sodium hexachloroiridate(IV) was stable in and extractable from ethyl acetate and acetone, the efficiency of extraction from ethyl acetate being especially high. It was also extractable from ethanol and propan-2-ol, but its solutions in these solvents were unstable; reduction to the unextractable tervalent species occurred, and was accelerated in the presence of polyurethane foam.

Extraction of sodium hexachloroiridate(IV) from acetone

Measurements of the extraction as a function of time indicated that equilibrium was reached in about 20 hr.

The variation of D with concentration of Ir(IV) in solution at equilibrium was investigated. D remained essentially independent of Ir(IV) concentration and had a value of 225, over the concentration range 8.56×10^{-6}–$5.06 \times 10^{-5} M$ Ir(IV), but became smaller at higher concentrations. On a log–log plot this fall-off corresponded to a straight line of slope -0.45 in the concentration range 5.06×10^{-5}–$2.4 \times 10^{-4} M$ Ir(IV).

In establishing the optimum conditions for extraction of sodium hexachloroiridate(IV) from acetone, the effect of various proportions of water on the extraction efficiency was investigated. The results are shown in Fig. 1. The efficiency of extraction decreased with increasing proportion of water.

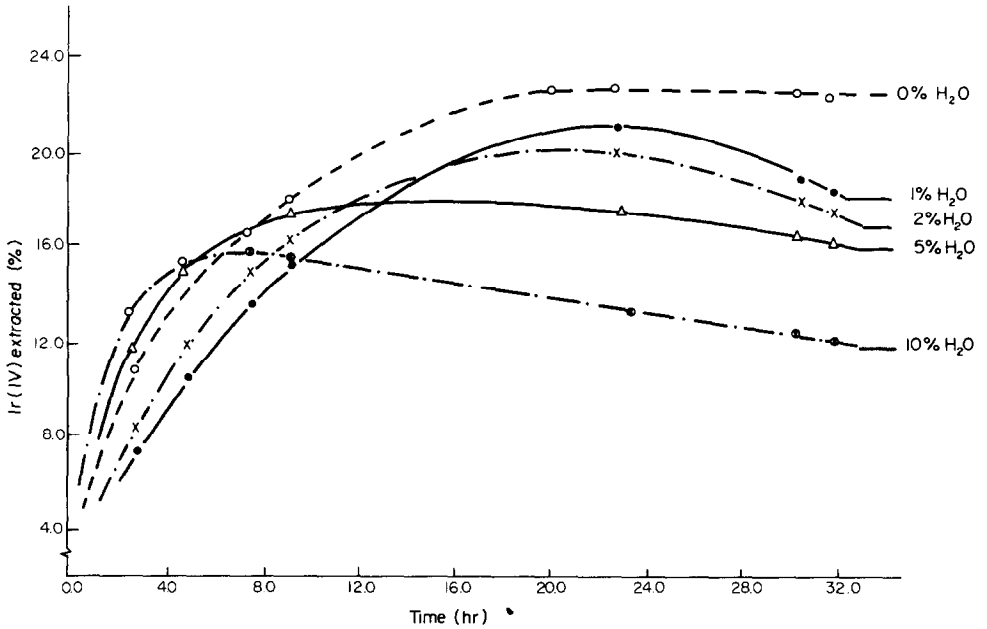

Fig. 1. Effect of water on the extraction of Na_2IrCl_6 from acetone solution. Solution 50 ml, foam 0.05 g, [Ir(IV)] 10 μg/ml, temperature 25.0 ± 0.05°, manual squeezing.

The absorption spectra of Ir(IV) solutions containing water but no foam, were monitored over a period of time. At water concentrations >2%, the intensities of the absorption peaks of $IrCl_6^{2-}$, in acetone medium, at 584, 490 and 440 nm were significantly decreased, but no additional absorbance maxima appeared in the spectrum. This suggests that there was reduction to the corresponding Ir(III) complex[5,7] in the solutions which contained water, and the rate was found to increase with increasing proportion of water. The Ir(III) complex does not appear to be extractable by the foam, which explains the decreased extraction efficiency in solutions containing water. This unfavourable effect of water makes it desirable to dry and distil the acetone to obtain better extraction efficiency.

Figure 2 shows the effect of acetic and trichloroacetic acid on the extraction efficiency. Trichloroacetic acid more than doubled the extraction efficiency when present at 0.7*M* concentration or above, but the system took about 31 hr to reach equilibrium. Acetic acid was not as effective, but equilibrium was reached more quickly (24 hr). The increase in efficiency may be due to formation of new species which are more extractable than the $IrCl_6^{2-}$ species. Spectral studies indicated that at least one more species is formed, probably through substitution of acetate or trichloroacetate for chloride as ligand. However, it was not established whether or not the new species is more extractable than the hexachloroiridate(IV) species.

Figure 3 shows the capacity of the foam for iridium under the conditions outlined. The absolute weight of iridium extracted from solutions of concentrations ranging from 50 to 600 μg/ml was measured as a percentage of the weight of foam used for the extraction. The capacity of the foam under these conditions was about 2.4%.

Extraction of sodium hexachloroiridate(IV) from ethyl acetate

Extraction equilibrium was attained after about 20 hr for solution concentrations in the range 5–20 μg/ml, and more slowly for higher concentrations. *E* slowly became smaller after about 30 hr in the case of the more dilute solutions, perhaps because of reduction to Ir(III) in the presence of the foam, but this took place so slowly that it would be important only after a long period.

A log–log plot of the distribution ratio and equilibrium Ir(IV) concentration gave a straight line of slope −0.39 for Ir(IV) concentrations ranging from 2.97×10^{-5} to $2.2 \times 10^{-4}M$. The results of this and the corresponding study for acetone suggest that the mechanism is not simple solvent extraction.

The chemical equilibria involved in the solvent extraction process can be typified by the scheme:

$$\begin{array}{ccc} HMX_0 & \overset{k_{ex}}{\rightleftharpoons} & HMX_f \\ \Big\downarrow{\scriptstyle k_0} & & \Big\downarrow{\scriptstyle k_f} \\ H_0^+ + MX_0^- & & H_f^+ + MX_f^- \end{array}$$

where HMX represents the undissociated ion-association complex which can be either H_2IrCl_6 or Na_2IrCl_6. Either of these can dissociate in two steps, *e.g.*, H_2IrCl_6 can dissociate to $HIrCl_6^-$ which can lose

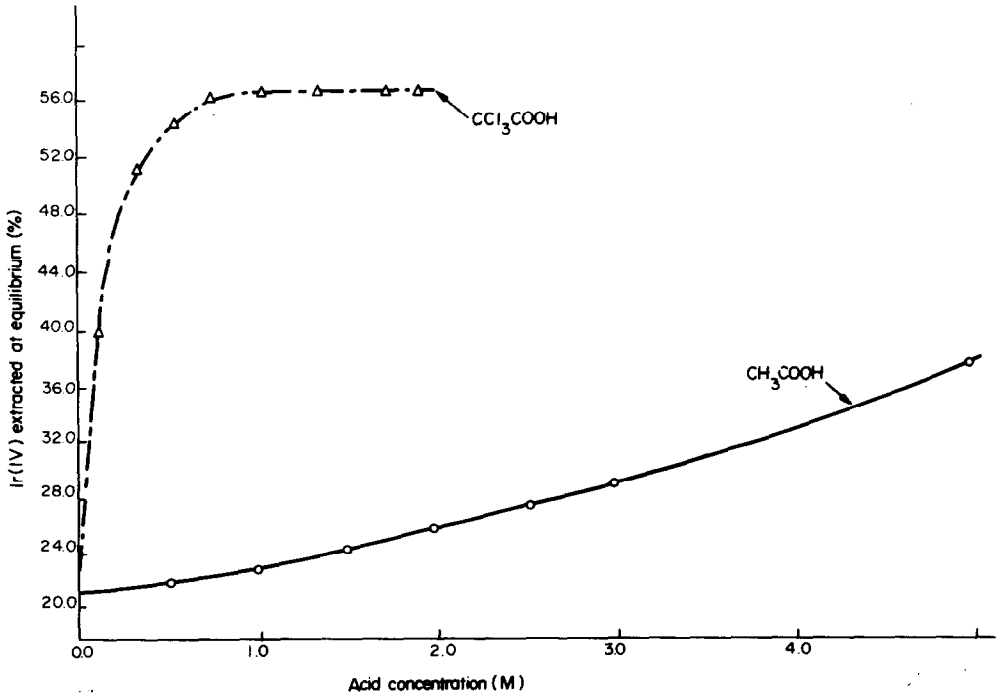

Fig. 2. Effect of acetic and trichloroacetic acid on efficiency of extraction of Ir(IV) from acetone solution. [Ir(IV)] 10 μg/ml, solution 50 ml, foam 0.05 g, temperature 25.0 ± 0.05°, manual squeezing.

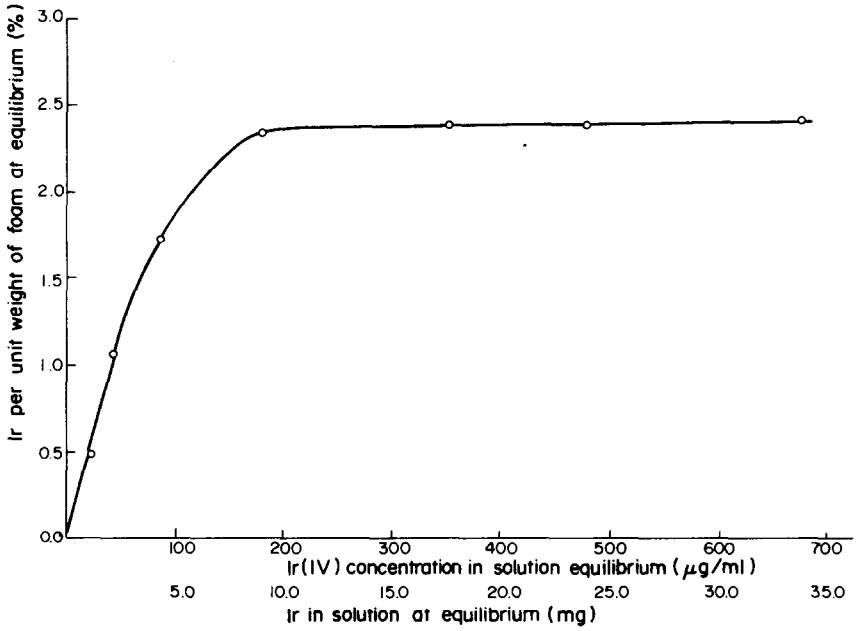

Fig. 3. Capacity of the foam for iridium extracted from acetone solution. Solution 50 ml, foam 0.04 g, temperature 25.0 ± 0.05°, manual squeezing, measurement after 24 hr.

another proton to give $IrCl_6^{2-}$. Subscripts "o" and "f" refer to the organic solution and to the foam respectively, and k_{ex} is the extraction coefficient for the undissociated complex.

In the following equations the square brackets refer to the equilibrium molar concentration of the various species, and the hydrogen species will be taken as a model.

$$k_{ex} = \frac{[HMX]_f}{[HMX]_o} \tag{1}$$

$$k_o = \frac{[H^+]_o[MX^-]_o}{[HMX]_o} \tag{2}$$

$$k_f = \frac{[H^+]_f[MX^-]_f}{[HMX]_f} \tag{3}$$

$$D = \frac{[M]_{f_{total}}}{[M]_{o_{total}}} = \frac{[HMX]_f + [MX^-]_f}{[HMX]_o + [MX^-]_o} \tag{4}$$

which on substitution of equations (1)–(3) and rearrangement gives

$$D = \frac{k_{ex} + k_{ex}k_f/[H^+]_f}{1 + k_o/[H^+]_o}. \tag{5}$$

For electroneutrality in the solution and the foam, the following equations can be written:

$$[H^+]_o = [MX^-]_o \tag{6}$$

and

$$[H^+]_f = [MX^-]_f \tag{7}$$

From equation (2)

$$[H^+]_o = (k_o[HMX]_o)^{1/2} \tag{8}$$

and from equation (3)

$$[H^+]_f = (k_f[HMX]_f)^{1/2} \tag{9}$$

Substituting (8) and (9) into (5) gives

$$D = \frac{k_{ex} + k_{ex}(k_f/[HMX]_f)^{1/2}}{1 + (k_o/[HMX]_o)^{1/2}}. \tag{10}$$

By substituting (1) into (10) and rearranging,

$$D = \frac{k_{ex}[HMX]_o^{1/2} + k_{ex}^{1/2}k_f^{1/2}}{[HMX]_o^{1/2} + k_o^{1/2}}. \tag{11}$$

If k_o and k_f both $\rightarrow 0$, then D = constant = k_{ex}.

If only $k_f \rightarrow 0$, then

$$D = \frac{k_{ex}[HMX]_o^{1/2}}{[HMX]_o^{1/2} + k_o^{1/2}} \tag{12}$$

and D will increase with increasing $[HMX]_o$ to a value which approaches k_{ex} at high values of $[HMX]_o$.

If only $k_o \rightarrow 0$, then

$$D = k_{ex} + \frac{k_f^{1/2}k_{ex}^{1/2}}{[HMX]_o^{1/2}}$$

i.e., $D\alpha[HMX]_o^{-1/2}$ and D decreases with increasing $[HMX]_o$.

If neither k_f nor $k_o \rightarrow 0$, then at very low values of $[HMX]_o$

$$D \rightarrow \frac{k_{ex}^{1/2}k_f^{1/2}}{k_o^{1/2}}$$

and at high values of $[HMX]_o$, $D \rightarrow k_{ex}$.

Only if $k_f \gg k_o$ will a fall-off of D occur with increasing $[HMX]_o$.

The dielectric constant of acetone (20.7 at 25°) can be expected to be considerably greater than that of

the foam and therefore it is very unlikely that k_f will be greater than k_0 when the complex is extracted from acetone. The dielectric constant of ethyl acetate ($\simeq 6.02$ at 25°) is much lower than that of acetone and presumably k_f might approach k_0 in this case. A fall-off of D with increasing $[HMX]_0$, however, requires that k_f should exceed k_0 by a factor greater than k_{ex}; k_{ex} for extraction from ethyl acetate can be expected to be of the order of 10^3.

This analysis shows that a solvent extraction mechanism cannot, by itself, explain the results obtained for the variation of the distribution ratio with the concentration of the complex in solution. Other processes must be involved in the uptake of iridium from acetone and ethyl acetate onto the foam. There may be specific sites on the foam which are capable of absorbing iridium; presumably an ion-exchange mechanism might play a role. It is also possible that different phenomena predominate in the extraction of the iridium complex from the two solvents.

Variation of the temperature in the range 0–25° had very little effect on the extraction efficiency.

The capacity of the foam for iridium extracted from ethyl acetate (16%) was about 6 times that for extraction from acetone (2.4%) under the same conditions. If the same species is extracted and by the same mechanism in both cases, the foam capacities would be expected to be the same. The results suggest that either interactions between the solvent and the foam influence the capacity or that different mechanisms are involved in the two extraction systems.

Iridium could be recovered from the foam by reduction to the non-extractable Ir(III) species with reagents such as sodium thiocyanate, sodium oxalate, hydroxylamine hydrochloride and hydrazine hydrochloride in acidic solutions. Hydrazine hydrochloride in 5M hydrochloric acid was the most effective stripping agent.

Most of the iridium is removed from the foam within the first hour. Hydrazine hydrochloride concentrations >1% (in 5M hydrochloric acid) gave about 93% recovery in 40 min, but the stripping rate decreased thereafter and recovery was incomplete even after 30 hr, which suggests that a small amount of iridium may not be available for recovery, possibly because of reduction to the metal in the foam either before or during stripping.

Extraction of sodium hexachloroplatinate(IV) from ethyl acetate

Preliminary work indicated that sodium hexachloroplatinate(IV) could be extracted from acetone, ethanol, propan-2-ol and ethyl acetate. The efficiency of extraction was highest with ethyl acetate, so only this system was examined quantitatively by spectrophotometry at 263 nm.

Extraction equilibrium was reached in about 24 hr. D was $\sim 4.8 \times 10^3$ and essentially independent of Pt(IV) concentration at equilibrium, in the range 1.21×10^{-5}–$2.42 \times 10^{-5} M$. The distribution ratio decreased with increasing equilibrium Pt(IV) concentration $> 2.42 \times 10^{-5} M$. On a log–log plot this fall-off corresponded to a straight line of slope −0.64.

CONCLUSION

Studies of foam extraction in non-aqueous systems provide results which can supplement the results of similar studies in aqueous systems in providing explanations of the mechanisms of the foam extraction process. More such investigations may be expected.

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DETERMINATION OF LEAD IN DRINKING WATERS BY HYDRIDE GENERATION AND ATOMIC-ABSORPTION SPECTROSCOPY, AND THREE OTHER METHODS

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Summary—Simultaneous presence of copper and nickel in potable waters interferes with the determination of lead at trace levels by the hydride–atomic-absorption spectrophotometric method. This interference is eliminated by co-precipitating lead with manganese dioxide from acidic solution. The precipitate is dissolved in 0.85% nitric acid and analysed by the automated hydride–atomic-absorption method. This method has been applied to 22 representative water samples and results compared with those obtained by using differential pulse anodic-stripping voltammetry, flame atomic-absorption and graphite-furnace atomic-absorption spectrophotometry. The precision of the three methods is reported and their accuracy checked by the analysis of reference standard water samples. The sensitivity of the three methods is of the order of 1 μg/l., compared to 100 μg/l. for flame atomic-absorption. The merits of each method are discussed.

In the last decade there has been widespread interest in the environmental and pollution problems associated with lead. Detection and measurement of traces of lead in environmental materials are of interest to analytical chemists. Atomic-absorption spectrophotometry (AAS) and anodic-stripping voltammetry have been used with varying degrees of success. AAS with flame excitation requires more than a tenfold preconcentration to achieve the necessary sensitivity, whereas the more sensitive differential pulse anodic-stripping voltammetry (DPASV) and graphite-furnace atomic-absorption (GFAAS) techniques are more suitable for natural concentrations of lead in drinking water. Manning and Slavin[1] reported the severe interference of chloride in lead determination by GFAAS. Vijan and Wood[2] successfully determined lead in drinking water by an automated hydride-generation atomic-absorption method (H–GFAAS) with a detection limit of 0.1 μg/l. However, the simultaneous presence of nickel and copper caused a severe suppression of the lead signal.

Our work was prompted by the need to determine lead in 600 samples of drinking water from homes in an area where lead plumbing was still in use, and the water contained significant concentrations of copper, nickel, iron and chloride. This paper reports a rapid procedure for co-precipitation of lead with hydrated oxides of manganese[3,4] to eliminate interference by copper and nickel, and compares the lead results obtained by DPASV and GFAAS on 22 representative samples containing 8–500 μg/lead. The relative merits of the methods are discussed.

EXPERIMENTAL

Reagents

Manganous sulphate solution, 1%.

Potassium permanganate solution, 0.25%.

Sodium borohydride solution, 4% *in* 0.1% *sodium hydroxide solution.*

Citric acid–potassium cyanide solution. Dissolve 1.0 g of citric acid and 0.5 g of potassium cyanide in 100 ml of distilled water (this reagent is poisonous and should be handled with care).

Hydrogen peroxide solution. Dilute 24 ml of 50% hydrogen peroxide to 100 ml with distilled water.

Lead stock solution (1000 *mg/l.*). Working standards were prepared by serial dilution of the stock solution with 0.7% v/v nitric acid.

Nitric acid. Suprapur grade nitric acid (5 ml) diluted to 100 ml with distilled water.

All the reagents used were analytical-reagent grade. The distilled water used was doubly distilled in glass apparatus. All glassware used was soaked with nitric acid (1 + 1) for 24 hr and rinsed several times with doubly distilled water. Nitrogen (Linde prepurified) used for deoxygenation[5,6] of the sample solution was bubbled through vanadium(II) chloride solution for oxygen removal and then through the supporting electrolyte before entering the electrochemical cell.

Instrumentation

AAS. A Techtron Model AA-5 atomic-absorption spectrophotometer equipped with a 10-mV variable-range strip-chart recorder was used in conjunction with an automated hydride generation system (Fig. 1). A Perkin–Elmer Model 603 atomic-absorption spectrophotometer equipped with Model HGA 2000 graphite furnace and Model AS-1 auto-sampler were used for flameless and flame AAS work. Instrumental settings used were those recommended in the manufacturers' manuals.

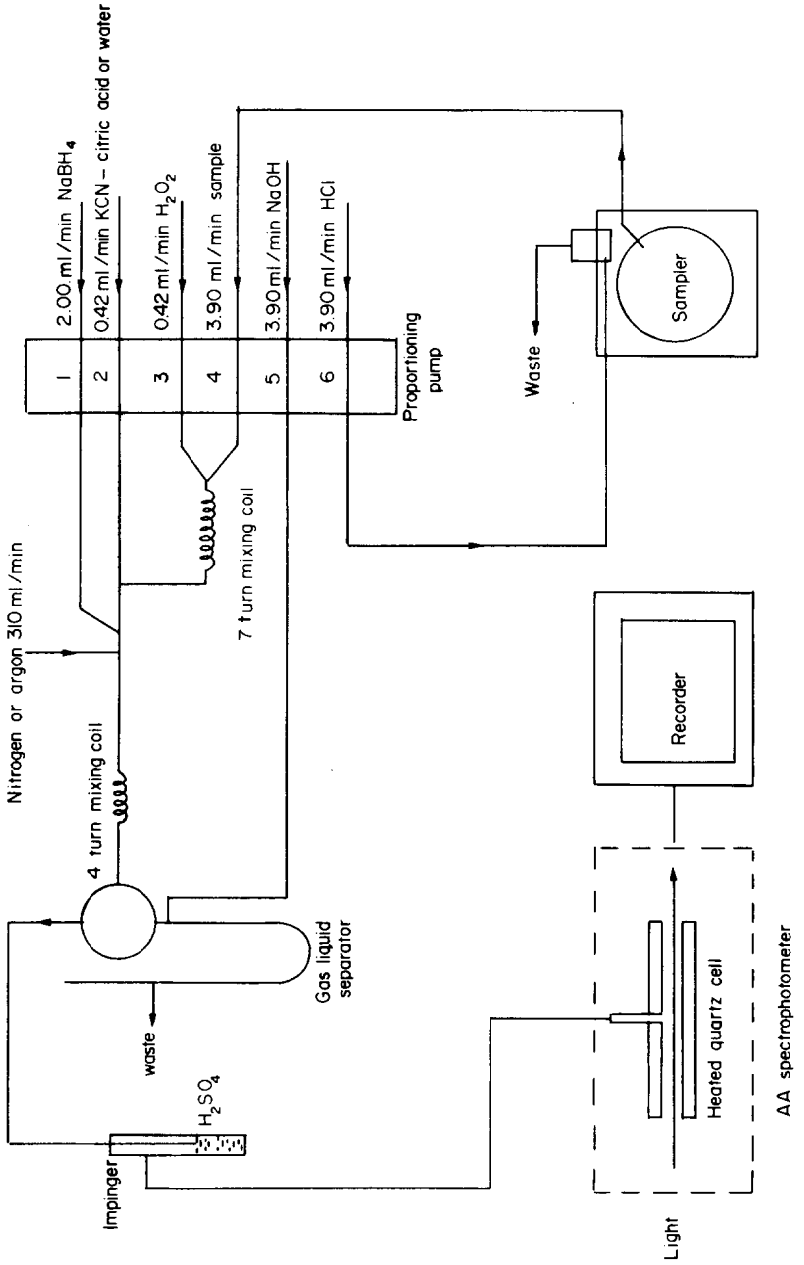

Fig. 1. System diagram.

DPASV. A Princeton Applied Research Corporation (PAR) Model 174A polarographic analyser, a PAR Model 315 automated electroanalysis controller and a Houston Omnigraphic Model 2200-3-3 X–Y recorder were used for all DPASV determinations. A Metrohm polarographic cell, equipped with a PAR Model 314 hanging mercury drop electrode (HMDE), a saturated calomel reference electrode (SCE) and a platinum wire counter-electrode, were used. The saturated calomel electrode was separated from the solution by a salt bridge with a Vycor plug.

Procedure

AAS. The drinking water samples received were preconcentrated by a factor of 10 by evaporation in 50-ml graduated centrifuge tubes in the presence of 0.5 ml of nitric acid. The samples, standards and blank (all 50 ml) were placed in racks and left overnight in an oven at 105° to reduce the volume of each to less than 5 ml. The solutions were then diluted to 5 ml with water at room temperature and analysed by AAS.

H-GFAAS. An appropriate portion of sample (1–10 ml) containing up to 500 ng of lead was transferred to a clean 15-ml graduated centrifuge tube. The sample was diluted to the 10-ml mark with distilled water. A blank and a set of standards (10, 20, 30 and 50 μg/l.) were processed along with the samples.

One ml of manganous sulphate solution and 0.5 ml of potassium permanganate were added to each centrifuge tube and mixed in immediately. The tubes were placed in an oven at 105° for 10 min and then centrifuged at 2000 rpm for 15 min. The supernatant solutions were discarded. The precipitate in each tube was dissolved with 10 ml of 0.85% v/v nitric acid and one drop of 50% hydrogen peroxide. The tubes were sealed with parafilm and mixed by a vortex mixer. The clear solutions were transferred to the sampling cups for automated lead analysis. The instrumental operating conditions and the procedure are described elsewhere.[2]

DPASV. A portion of well-mixed sample containing up to 1 μg of lead was pipetted into a 20-ml digestion tube and diluted to the 10-ml mark with 5% nitric acid. A reagent blank and a set of standards (10, 20, 30, 50, 70 and 100 μg/l.) were processed together with the samples. The tubes were heated in an aluminium heating block on a hot-plate until the solutions were reduced nearly to dryness. The residues were dissolved in 10 ml of 0.6% v/v nitric acid. The resulting solutions were transferred to the electrochemical cell, deaerated for 10 min with nitrogen, and analysed at instrumental settings for DPASV listed in Table 1.

RESULTS AND DISCUSSION

AAS

The results obtained by conventional flame atomic-absorption spectrophotometry are included in Table 3. The method requires at least a tenfold preconcentration in order for reliable signals to be obtained for the low lead levels. The accuracy of measurement at these concentrations is limited and necessitates the use of high damping and scale expansion in addition to background correction. The use of 50-ml graduated centrifuge tubes for preconcentration offers a definite advantage over the commonly used large-volume glassware, with respect to exposure to extraneous contamination. The AAS method was found to be free of interference from 200, 100, 200, 100, 45 and 8 mg/l. levels of calcium, magnesium, sodium, potassium, copper and nickel, respectively. These concentrations were twice the average amounts found in water preconcentrated by a factor of 10. Molecular absorption effects were insignificant. The sensitivity and detection limit of the AAS determination were 0.11 and 0.06 mg/l., respectively.

H-GFAAS

The lead in drinking water samples was first determined by the H–GFAAS method without any pretreatment, as outlined in the published method.[2] No lead signals were recorded for samples 1–6. Split signal peaks were recorded for sample 7, suggesting severe interference, as shown in Fig. 2A. The samples were then analysed by AAS for interfering elements such as copper and nickel. The results are summarized in Table 2. The concentration of iron in these samples ranged between 0.3 and 0.7 mg/l. It is evident from the results in Table 2 that the copper and nickel concentrations are higher than those usually found in drinking waters. The presence of either of these elements alone can be tolerated by this method. However, when present together, they cause a considerable suppression of the lead signal. The effectiveness of copper and nickel removal by co-precipitation is shown in Table 2. The average efficiency of removal

Table 1. Instrumental settings

PAR Model 174A		PAR Model 315A	
Scan-rate	2 mV/sec	Conditioning potential	0.0 V
Scan-direction	positive(+)	Deposition potential	−0.7 V
Range	1.5 V	Final potential	−0.2 V
Initial potential	0.0 V	Push-button	Purge
Modulation amplitude	25 mV	Deposition time	180 sec
Operation mode	Differential pulse	Conditioning time	0 sec
Current range	1–10 μA	Equilibration time	15 secA
Display direction	negative (−)	Purge time	10 sec
Drop time	1 sec	Override switches	Auto position
Low-pass filter	Off		
Selector switchh	External cell		
Push-button	Scan		

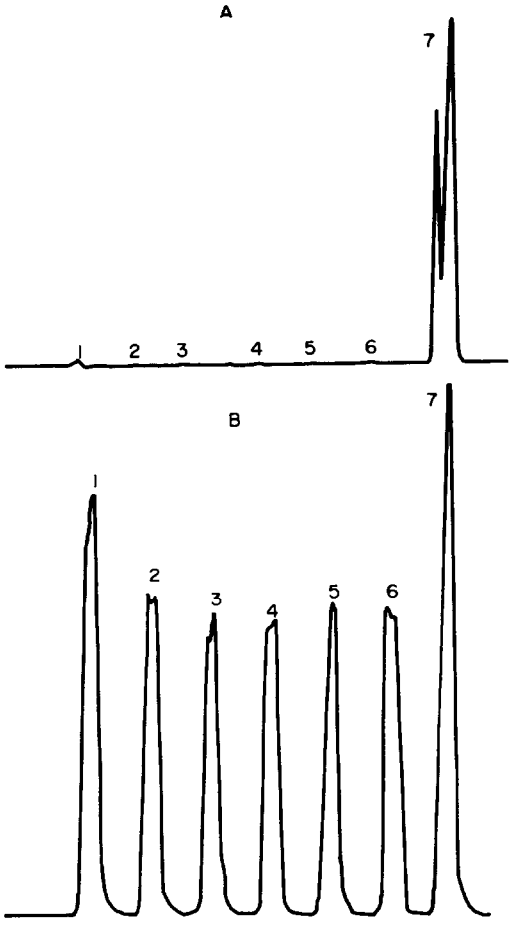

Fig. 2. Recorder tracings for lead: *A*—before, *B*—after co-precipitation.

for copper was 89% and for nickel 100%. This is in close agreement with Burke's findings.[3] The residual copper and iron, co-precipitated together with lead, are effectively complexed by the potassium cyanide–citric acid reagent. Figure 2B shows the normal tracings obtained on samples 1–7 after co-precipitation of lead from 5, 5, 2, 2, 2, 1 and 0.5 ml aliquots, respectively.

Effect of manganese. The precipitate, when dissolved in nitric acid, contributes a manganese concentration of 110 mg/ml. to the prepared sample solution. This is in close agreement with the 109 mg/l. expected according to the reaction,

$$2KMnO_4 + 3MnSO_4 + 2H_2O \rightarrow 5MnO_2 + K_2SO_4 + 2H_2SO_4. \quad (1)$$

Burke[3] stated that the co-precipitant consists of hydrated oxides of manganese but our study indicates the formation of manganese dioxide. The precipitate dissolves according to the reaction

$$MnO_2 + H_2O_2 + 2HNO_3 \rightarrow Mn(NO_3)_2 + 2H_2O + O_2. \quad (2)$$

Some nitric acid is used up in this reaction. An allowance for the amount consumed is made by dissolving the precipitate in 0.85% v/v nitric acid, thus leaving enough acid in the solution to maintain the pH close to 1.1. The quantitative generation of lead hydride is dependent on pH, as reported earlier.[2]

The addition of manganese up to 400 mg/l. to the working standard solutions did not affect the lead recovery. However, when the standard solutions were taken through the whole procedure, the recovery was only 90 ± 2% (based on more than 20 measurements taken on different days). It is therefore necessary to treat blank, standards and samples alike. The reagent blank was found to be 1.5 μg/l. or less, depending on the purity of the reagents used.

The procedure for co-precipitation has been simplified and streamlined for routine analysis so that filtration, washing and transfer steps are eliminated. The sample preparation begins in a 15-ml graduated centrifuge tube and ends in the same tube, thus minimizing the contamination error. All reagents are dispensed mechanically. A batch of 40 samples can be prepared for analysis within 3 hr. A 25% increase in sensitivity and improvement in baseline stability was achieved, as compared with earlier work,[2] by reducing the internal diameter of the quartz cell to 0.6 cm.

The H–GFAAS instrumental assembly used here is quite rugged. The use of organic solvents may poison the system. Accumulation of heavy metals in reagent lines may cause a gradual decrease in analytical sensitivity. This problem can be corrected by periodically replacing the reagent lines or cleaning them with a suitable acid.

Table 2. Copper and nickel in drinking water (*mg/l.*)

Sample	Before co-precipitation Copper	Before co-precipitation Nickel	Supernatant liquid from co-precipitation Copper	Supernatant liquid from co-precipitation Nickel
1	0.72	0.41	0.66	0.40
2	2.5	0.35	2.3	0.30
8	2.1	0.39	1.9	0.34
10	1.5	0.35	1.3	0.36
15	3.0	0.35	2.8	0.40
17	1.5	0.38	1.3	0.40
18	1.1	0.32	0.9	0.32
10	2.1	0.35	1.8	0.38
20	2.7	0.38	2.3	0.40
	Average copper removed—88.9%			
	Average nickel removed—1000.6%			

Table 3. Determination of lead in drinking water by four methods* (μg/l.)

Sample	AAS	H–GFAAS	GFAAS		DPASV	
			As received	Co-precipitated	As received	Digested
1	64	49	38	54	45	55
2	41	35	44	30	27	33
3	69	80	48	54	50	54
4	67	78	60	88	48	60
5	76	75	48	110	57	75
6	184	166	124	168	153	210
7	539	567	201	565	498	482
8	14	14	39	12	9	14
9	118	117	58	111	88	101
10	10	10	33	12	7	9
11	462	416	171	392	380	450
12	82	90	38	73	75	89
13	180	155	76	141	126	155
14	344	348	132	304	277	370
15	34	37	25	35	26	31
16	113	102	53	105	88	116
17	40	35	33	30	21	33
18	8	8	5	8	6	8
19	42	31	24	32	28	39
20	32	31	14	20	17	21
21	142	116	56	120	101	130
22	140	155	54	120	106	150

* All results are means of duplicates except for GFAAS.

Sulphuric acid in the impinger dryer should be replaced with fresh concentrated acid when the liquid level reaches the tip of the inlet tube. Certain lots of BDH (British Drug Houses) borohydride failed to generate lead signals. The reasons for this are not known. Spectrographic analysis did not reveal any basic differences between acceptable and unacceptable lots.

GFAAS

In a search for suitable alternative methods of lead determination, the GFAAS technique was applied directly to the samples as received. The results are shown in Table 3 and indicate severe matrix interferences, especially at lead concentrations above 30 μg/l. Manning and Slavin[1] have recently reported interference by chloride in the determination of lead by this technique. The average concentration of chloride found in these samples was 50 ± 2 mg/l. However, when this technique was applied to the samples prepared earlier by the co-precipitation procedure, no interference effect was noticed (Table 3). Since manganese is the predominant element present after the co-precipitation treatment, its effect on this technique was studied. A 110-mg/l. manganese/content enhanced the lead absorbance signal by 26 ± 5%. Since the manganese concentration of standards and samples taken through the procedure is the same, this enhancement provides an added advantage. A systematic study of the effect of manganese on lead determination by the GFAAS technique in the presence of other matrix elements is beyond the scope of this paper. Owing to the insufficient sample volume available for repetition of the co-precipitation and GFAAS analysis, a within-run precision study was performed on synthetic standards with 25, 75 and 150 μg/l. lead and 110-mg/l. manganese concentrations in 0.7% nitric acid. A new graphite tube was used and the relative standard deviation for ten replicates at each lead concentration was found to be 1.8%. In our experience the precision of the analysis varies significantly with the change in the characteristics of the furnace on repeated firings. The use of an autosampler has helped to improve the overall precision. However, the ruggedness of GFAAS is largely dependent on the characteristics of the graphite furnace.

DPASV

The DPASV technique is free from interference by copper, nickel, iron and chloride and requires no preconcentration (a step which is time-consuming and prone to contamination). When water samples were analysed, as received, the average recovery was 72% with respect to the AAS method (Table 1). Samples, standards and blank, digested according to the prescribed procedure, gave quantitative recovery of lead. The reason for the low recovery from undigested samples is not fully understood. However, Ediger and Coleman[7] reported that the hanging mercury drop electrode (HMDE) was sensitive only to uncomplexed metal ions and to those metals in complexes which

Table 4. Lead in standard reference material (μg/l.)

Sample	Certified value	AAS	H–GFAAS	DPASV
EPA 1	22	24	22	21
EPA 2	300	320	307	330
EPA 3	350	360	356	320

Table 5. Between-run precision of different methods

Sample	AAS			H–GFAAS			DPASV		
	n	Mean, μg/l.	RSD, %	*n*	Mean, μg/l.	RSD, %	*n*	Mean, μg/l	RSD, %
9	—	—	—	12	114	14.3	10	103	10.5
10	—	—	—	10	12	25.7	10	10	12.9
13	—	—	—	13	153	9.4	8	166	11.3
14	—	—	—	13	376	10.9	10	338	5.7
15	—		—	12	36	19.8	10	32	6.4
3	10	67	3.4	—	—	—	—	—	—
12	9	88	2.6	—	—	—	—	—	—
13	12	165	2.7	—	—	—	—	—	—
20	12	26	6.2	—	—	—	—	—	—

have rapid exchange kinetics. Drinking waters often contain traces of metals, bound as organic complexes, that are not available for reduction at the HMDE and hence only the free (aquo-complexed) metal is measured. Doubly distilled water, acidified to optimal pH with nitric acid, may also contain some low molecular-weight organic ligands. The results obtained for samples digested with nitric acid, shown in Table 3, are in good agreement with those by other methods. Digestion with sulphuric acid–potassium persulphate mixture[8] and ultraviolet irradiation[9] have also been successfully employed to decompose the complexes.

Chou and Chan[10] reported interference by iron, but no such interference was encountered in this study. This is evident from the results obtained on Environmental Protection Agency, U.S.A. (EPA) Standards 1, 2 and 3, iron contents 26, 417 and 678 μg/l. respectively (Table 4).

Samples prepared by the co-precipitation procedure did not give the expected signal when analysed by DPASV. This indicates severe interference from manganese. The sensitivity and detection limits of DPASV are comparable with those of the H–GFAAS method. The standard curve is linear up to 600 μg/l. and therefore excessive sample dilution is not necessary. The sensitivity of the DPASV technique can be further increased, if desired, by increasing the size of the HMDE, the stirring rate of the solution, the scale expansion used and the deposition time. However, an increased deposition time reduces the number of determinations per unit time and excessive stirring may cause the hanging mercury drop to fall. The instrumental settings, as shown in Table 1, were found empirically to give the optimum sensitivity.

Table 6. Regression analysis comparison of methods

Regression line	Correlation coefficient	Slope	Intercept, μg/l.
DPASV *vs.* AAS	0.993	0.960	−0.3
AAS *vs.* DPASV	0.993	1.03	+1.9
H–GFAAS *vs.* AAS	0.994	0.999	−2.6
AAS *vs.* H–GFAAS	0.994	0.998	+4.2
GFAAS *vs.* AAS	0.988	0.949	−3.3
AAS *vs.* GFAAS	0.988	1.03	+6.3

Precision and accuracy

The precision of the methods is shown in Table 5. Different samples were used for the precision study of the AAS method because of the limited sample volume. It is obvious that the AAS method, in spite of its shortcomings, has the best precision at the levels measured. With AAS as the reference method, the correlation between the three methods is quite satisfactory, as is evident from the statistics shown in Table 6. The accuracy of the methods was tested by replicate analysis of the EPA reference standards series 575 (Table 4). The results by all three methods are in good agreement with the certified values.

Although the AAS method appears to be more precise, the recorded signals for lead up to 0.25 mg/l. require high signal expansion and therefore high damping. Both the H–GFAAS and DPASV methods are ideally suited for natural concentrations of lead in potable waters. The H–GFAAS method can be applied without co-precipitation if trace amounts of either copper or nickel (but not both) are present. The simultaneous presence of these two elements in potable waters is uncommon.

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METHODOLOGICAL INFORMATION FROM THE CERTIFICATION OF CCRMP ORES AND CONCENTRATES*

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Summary—In the course of 90 certifications for 27 elements in 26 reference ores and concentrates, carried out by the Canadian Certified Reference Materials Project, much methodological information has been documented and is now made available to analysts for the selection of suitable methods for the analysis of specific materials. Information is presented for copper, gold, lead, silver, sodium, potassium, tin, tungsten, uranium and zinc. A relationship between the average coefficient of variation and element concentration makes it possible to make some generalizations about the precision to be expected for a given concentration of an element in ores and concentrates.

The Canadian Certified Reference Materials Project, CCRMP, has certified a total of 27 elements (90 certifications) for 26 reference ores, concentrates and metallurgical products (Fig. 1). Accordingly, a vast amount of information on analytical methods for various elements at different concentrations has been accumulated. Although the information pertaining to a particular reference material (RM), is presented in the certification document provided, it is unavailable to those not in possession of the RM. It was considered worthwhile, therefore, to compile this methodological information from the interlaboratory certification programmes and arrange it, by element, for use by those analysts who may be seeking suitable methods for the analysis of specific materials at various element concentrations.

Some generalizations can also be made regarding the precision to be expected for the determination of a large number of elements at different levels of concentration in ores and related materials.

CERTIFICATION SCHEME

An outline of the scheme used by CCRMP to conduct interlaboratory programmes to certify reference materials is given below so that the reader can judge the validity of the comments made on analytical methodology.

Each laboratory participating in the certification of an RM is given two randomly-selected bottles and is requested to determine the desired element(s) in quintuplicate methods of its choice. A one-way analysis of variance is used to calculate the within bottle and between bottle means and corresponding coefficients of variation for each laboratory as well as the overall mean or consensus value.

METHODOLOGICAL INFORMATION

Copper

Copper has been certified in 9 reference ores and concentrates containing from 0.114 to 24.71% Cu. Atomic-absorption, titrimetric, polarographic, spectrophotometric, electrolytic and to a lesser extent X-ray fluorescence and spectrographic methods were used in the certification programmes. No significant difference with respect to either accuracy or precision could be detected between the results of these methods. It can be concluded therefore, that the "state of the art" for copper is satisfactory and that the best criterion for selecting a particular method is probably familiarity.

Gold

Gold has been certified in a siliceous gold ore, MA-1,[1] and a copper concentrate, CCU-1.[2] In both, the gold occurs principally as the native metal. A comparison of results by fire-assay methods with those by purely wet decomposition techniques is illustrated in Table 1 and shows that there is no statistically significant difference between these methods. This suggests that the more rapid and economical acid decomposition/atomic-absorption procedure for materials similar to MA-1 and CCU-1 is preferable.

Lead

The overall means for lead in reference concentrates CZN-1[3] and CPB-1,[4] as determined by various methods, are given in Table 2. A similar pattern is clearly evident in spite of one lead concentration being almost 9 times the other. There is good agreement between the titrimetric and atomic-absorption results whereas the gravimetric results are significantly lower. No doubt this is due to inherent solubility losses during the precipitation of lead as the sulphate and/or chromate. Consequently, the gravi-

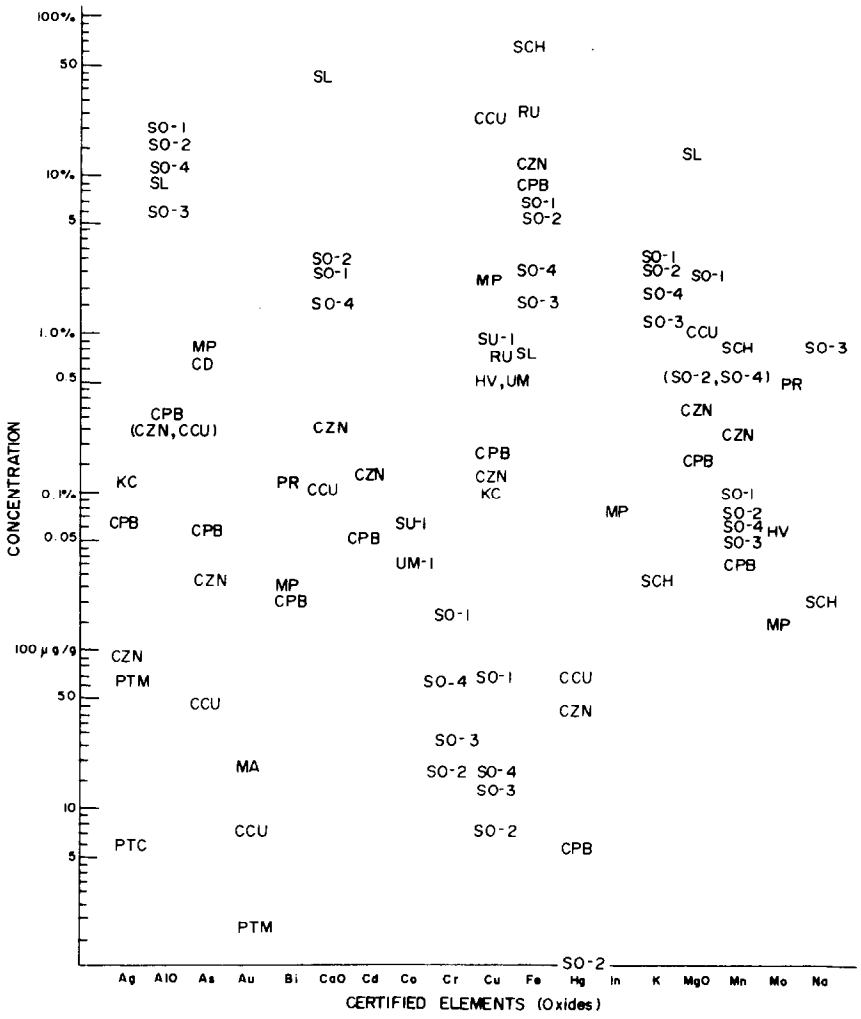

Fig. 1(a).

Fig. 1. A summary of certified elements and their concentration in CCRMP ores and concentrates.

metric methods cannot be recommended for the determination of lead.

Silver

Seven ores and related materials have been certified, with silver content ranging from 5 to 1200 µg/g. The consensus values are given in Table 3, together with the overall means obtained by wet chemical and fire-assay methods.

It is apparent that a wet-chemical (acid) decomposition with atomic-absorption finish is a popular method for the analysis of base-metal ores and concentrates containing >50 µg/g of silver. Moreover, this procedure yields results and precision comparable to those obtained by the more elaborate fire-assay method. This suggests that the wet-chemical/atomic-absorption method may be preferable to the fire-assay method, which requires more sample, reagents and time when only silver is to be determined. However, the fire-assay methods have their time-honoured place for umpire silver analysis, for the analysis of materials such as PTC-1[5] and PTM-1[6] which contain the platinum group metals together with gold and silver, and for ores to be analysed for both gold and silver.

Sodium and potassium

In 1975, an attempt to certify reference iron ore SCH-1[9] for sodium and potassium in a "free-choice" interlaboratory programme was unsuccessful because of the lack of agreement in the results for those elements. In 1977, however, a special interlaboratory programme was initiated to certify sodium and potassium in SCH-1 by the use of an atomic-absorption method that had been thoroughly assessed by a working group of Sub-Committee 2 (Chemical Analysis) of Technical Committee 102[10] of the International Organization for Standardization (ISO).

The 1977 consensus values given in Table 4 are essentially the same as the 1975 provisional values for sodium and potassium. However, the confidence intervals and coefficients of variation associated with

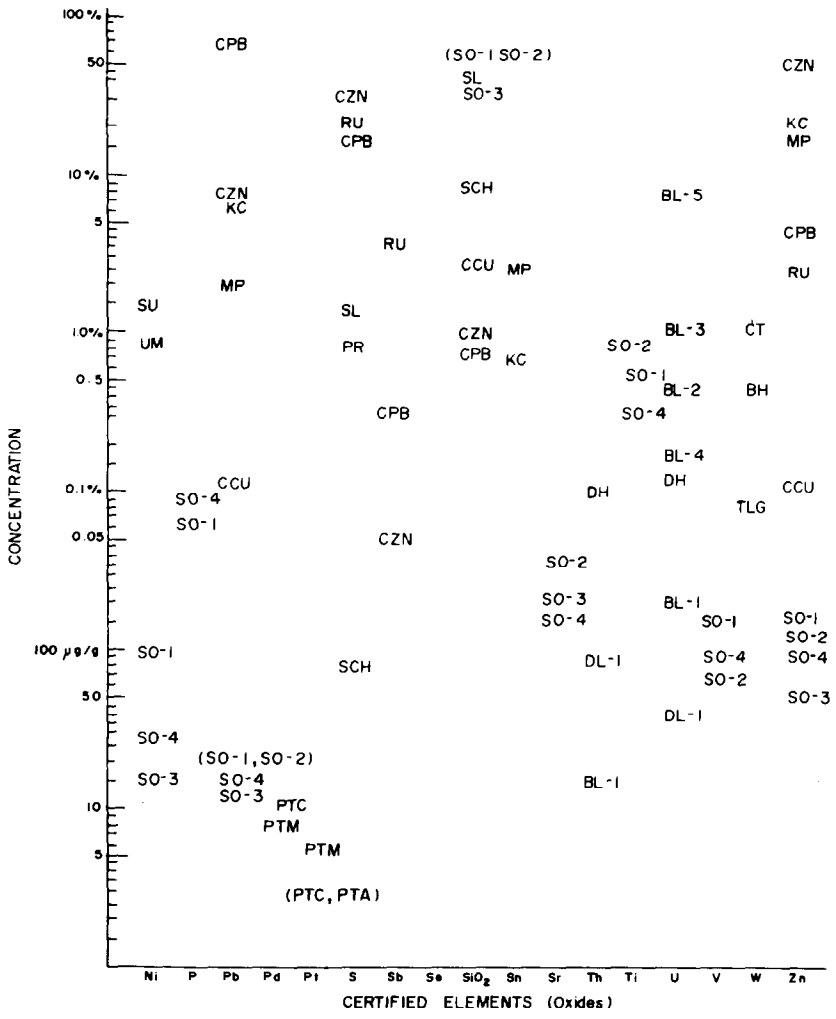

Fig. 1(b).

the 1977 means are appreciably smaller and the certification factor has decreased to below the critical value of 4.[11] The improvement in the statistical parameters of the 1977 certification programme is, of course, due to the sole use of the "standardized" ISO method. The ISO method for sodium and potassium specifies the mode of sample decomposition and use of plastic rather than glassware. Neither of these specifications was made in the 1975 certification programme. Consequently, it is likely that the greater overall range and poorer within-laboratory precision of the 1975 atomic-absorption results were due to incomplete decomposition and to contamination from the use of glassware, giving low and high results respectively.

In the 1975 certification, which involved 21 laboratories, 6 and 4 reported results by flame emission for sodium and potassium, respectively. The ranges of these results were greater than those by the atomic-absorption methods. The flame emission methods were, evidently, less satisfactory for a material like SCH-1. This is in accordance with the theoretical expectations for these two techniques.[12]

It is also of interest that the results of a "free-choice" certification programme in 1977 for sodium and potassium in a blast-furnace slag, SL-1,[13] followed essentially the same pattern as those of the 1975 programme for SCH-1. Only provisional values could be assigned for sodium and potassium.

Tin

Two base-metal ores, MP-1[7] and KC-1,[8] have been certified for tin at 2.50 and 0.68% respectively. The interlaboratory programmes for these ores, however, illustrated that those methods with an instrumental finish gave higher results than the titrimetric, *i.e.*, iodimetric method.

Also evident in Table 5 is the observation that the results of the titrimetric method are dependent on the metal used as the reducing agent. This was confirmed at CANMET, although aluminium metal was found to be as suitable as iron for the reduction.

Table 1. Comparison of fire-assay and wet-chemical results for gold in MA-1 and CCU-1

Reference material	Method	No. of results	Mean Au values, μg/g	Coefficient of variation, %
Gold ore, MA-1 (recommended Au value 17.8 ± 0.02 μg/g)	Fire-assay, gravimetry	250	17.8	2.4
	Fire-assay, atomic-absorption	104	18.1	4.3
	Wet-chemical, atomic-absorption	80	17.7	3.4
Copper concentrate, CCU-1 (recommended Au value 7.5 ± 0.3 μg/g)	Fire-assay, gravimetry	82	7.5	3.20
	Fire-assay, atomic-absorption	30	7.2	2.78
	Wet-chemical, atomic-absorption	54	7.8	2.34

A study[14] of the iodimetric method showed that whereas aluminium, iron, nickel and lead metals were able to reduce tin in synthetic solutions equally well, their ability to reduce tin in solutions from MP-1 decreased in the order aluminium ~ iron > nickel > lead. A corrective procedure, in which the iodine titrant was standardized for each metal, against known amounts of tin added as cassiterite, SnO_2, to subsamples of MP-1 before fusion with sodium peroxide, yielded comparable results for tin in MP-1 (2.43–2.44%).

No explanation can be given for the higher tin values that were obtained for MP-1 by instrumental methods during the interlaboratory certification programme. It was found at CANMET that an atomic-absorption method using the standard-additions technique yielded the same value for tin in MP-1 as the titrimetric method.[14]

Tungsten

Two scheelite ores, CT-1 and TLG-1, and a wolframite ore, BH-1, have been certified for tungsten.[15] Approximately 90% of the results were obtained by the spectrophotometric thiocyanate method. The remainder were obtained by X-ray fluorescence and were lower than the recommended overall values by 2–5% relative.

Table 6 shows the results obtained by the thiocyanate method after sample decomposition with either sodium peroxide or potassium (or sodium) pyrosulphate fusion, or by treatment with hydrochloric–hydrofluoric–phosphoric acid mixture. The fusion methods predominate because they are the most widely used by ore and rock analysts. The acid-decomposition technique was devised at CANMET in 1962.[6] The results in Table 6 indicate that there is a consistent trend in the means for tungsten, increasing

Table 2. Comparison of lead results for CPB-1 and CZN-1

Reference material	Method	No. of results	Mean Pb, %	Within-lab. means coefficient of variation, %
Lead conc. CPB-1 (recommended Pb value 64.74 ± 0.12%)	Titrimetric (EDTA)	94	64.76	0.15
	Titrimetric (molybdate)	99	64.85	0.20
	Atomic absorption	20	64.82	0.28
	Gravimetric*	50	64.55	0.16
Zn conc. CZN-1 (recommended Pb value 7.45 ± 0.05%)	Titimetric (EDTA)	68	7.44	0.41
	Titrimetric (molybdate)	40	7.42	0.47
	Titrimetric (dichromate)	10	7.45	0.27
	Atomic absorption	112	7.44	0.95
	Gravimetric*	10	7.27	2.33

* The gravimetric results were not used in arriving at recommended values for CPB-1 and CZN-1.

Table 3. Methodological comparison of silver results for CCRMP reference materials

Reference material	Method	No. of results	Mean, μg/g	Within-lab. means coefficient of variation, %
Flotation conc. PTC-1[5]	Fire-assay*	45	5.8	11.0
Nickel–copper matte PTM-1[6]	Fire-assay	44	66	4.5
Zinc–lead–copper ore MP-1[7]	Fire-assay	66	56.1	1.5
	Wet-chemical	100	60.4	
Zinc–lead ore KC-1[8]	Fire-assay	150	11.4	0.6
	Wet-chemical	115	11.4	1.1
Zinc conc. CZN-1	Fire-assay	118	92	2.3
	Wet-chemical	94	93	1.8
Lead conc. CPB-1	Fire-assay	114	625	0.8
	Wet-chemical	89	627	0.9
Copper conc. CCU-1	Fire-assay	118	139	1.3
	Wet-chemical	130	140	1.4

* Fire-assay methods involve either gravimetric or atomic-absorption finish.

Table 4. Comparison of sodium and potassium results for certification of SCH-1

Statistic	Sodium		Potassium	
	1975 Programme	1977 Programme	1975 Programme	1977 Programme
Mean	0.019*	0.019	0.027*	0.026
95% Confidence interval, %	±0.003	±0.001	±0.004	±0.002
Average within-lab. coefficient of variation, %	7.7	5.7	7.5	4.6
Certification factor	3.9	2.7	4.1	2.7

* Provisional value.

Table 5. Correlation of tin values with method for MP-1

Analytical method	Number of results	Mean tin, %
Instrumental*	109	2.50
Titrimetric—total	109	2.35
—Fe reduction	45	2.49
—Ni reduction	10	2.40
—Al reduction	10	2.23
—Pb reduction	40	2.22
—Unknown	4	2.28

* Atomic-absorption methods account for approximately 50% of instrumental results.

Table 6. Methodological comparison of statistical parameters for tungsten ores

		No. of participating laboratories	No. of results	Mean, %	Confidence interval, %	Mean within-lab. coefficient of variation, %
CT-1	Peroxide	8	84	1.035	±0.029	2.2
	Pyrosulphate	6	57	1.060	±0.024	1.8
	Acid-decomp.	3	25	1.064	±0.057	1.2
	XRF	2	20	0.989	—	—
	Overall	15	186	1.042	±0.017	2.1
BH-1	Peroxide	7	74	0.412	±0.008	2.1
	Pyrosulphate	7	65	0.427	±0.021	1.6
	Acid-decomp.	4	75	0.429	±0.002	1.6
	XRF	1	10	0.415	—	—
	Overall	15	224	0.422	±0.008	1.9
TLG-1	Peroxide	7	74	0.082	±0.009	3.7
	Pyrosulphate	4	35	0.084	±0.007	2.9
	Acid-decomp.	4	35	0.087	±0.007	4.4
	XRF	2	20	0.081	—	—
	Overall	15	164	0.083	±0.004	3.5

Table 7. Comparison of zinc results

Reference material	Certified value* and 95% confidence interval, %	Titrimetric EDTA %	Titrimetric EDTA Coefficient of variation, %	Titrimetric Ferrocyanide %	Titrimetric Ferrocyanide Coefficient of variation, %	Atomic-absorption %	Atomic-absorption Coefficient of variation, %
Zinc conc. CZN-1	44.74 ± 0.11	44.74 (185)†	0.26	44.74 (117)	0.16	44.81 (50)	0.48
Lead conc. CPB-1	4.42 ± 0.04	4.44 (49)	1.29	4.45 (36)	0.45	4.39 (145)	1.09
Copper conc. CCU-1	3.22 ± 0.04	3.25 (20)	0.78	3.24 (20)	0.58	3.22 (208)	0.96
Zinc–lead ore MP-1	15.90 ± 0.06	15.92 (107)	0.38	—	—	15.92 (35)	0.61
Zinc–lead ore KC-1	20.07 ± 0.06	20.09 (96)	0.27	—	—	19.99 (30)	0.27
Zinc–copper ore RU-1[118]	2.24 ± 0.02	2.27 (30)	0.64	2.18 (30)	0.97	2.24 (220)	0.48

* Based also on results by all methods.

† Numbers in parentheses are the number of determinations.

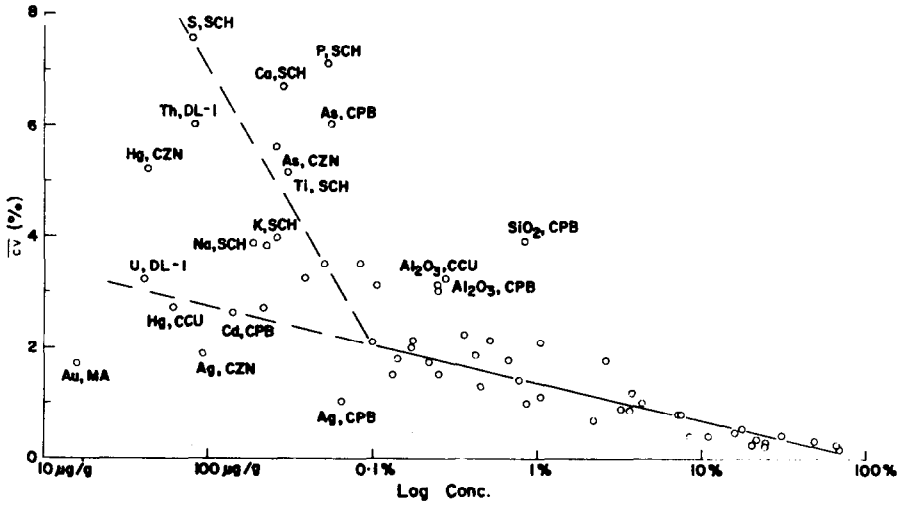

Fig. 2. The relationship between the average coefficient of variation of analysis and the concentration of the element.

in the order peroxide < pyrosulphate < acid decomposition. Statistically, there is no significant difference between the means of the three methods because of the appreciable overlap of the 95% confidence intervals except in the case of peroxide fusion *vs.* acid decomposition for BH-1. The observed trend suggests that the mode of decomposition affects, to a slight degree, the results obtained for tungsten.

In 1971, an attempt was made to certify a complex zinc–lead–copper sulphide ore, MP-1,[7] for tungsten at ~0.02%, but the range of tungsten results obtained (mostly by the thiocyanate method) was so large that a recommended value could not be assigned. This lack of agreement can be attributed to the low concentration and to the chemical complexity of the ore.

Uranium

A low-grade concentrate, BL-5,[17] has been certified for uranium at 7.09%. Of the 7 laboratories that used X-ray fluorescence, 4 found a significant difference, *i.e.*, inhomogeneity, between the two bottles they analysed. In contrast inhomogeneity was reported by only 3 of the 26 laboratories which used other methods such as titrimetry, spectrophotometry, fluorimetry, *etc.* Two of the 4 laboratories which had found between-bottle inhomogeneity re-analysed the same bottles, one by X-ray fluorescence and the other by fluorimetry, obtaining results which did not indicate a between-bottle inhomogeneity. No explanation can be given for the high degree of inhomogeneity observed by X-ray fluorescence. It can only be recommended that those wishing to apply this technique to BL-5 exercise utmost care.

Zinc

Six reference ores and concentrates have been certified for zinc; their values and confidence intervals are given in Table 7. Most of the results obtained in the interlaboratory programmes were by the titrimetric EDTA[20] and ferrocyanide[20] methods and by atomic-absorption spectrometry. Table 7 shows that the overall means for these three methods are in good agreement for the range 2–45%. It is of interest that the atomic-absorption method gives results comparable to those obtained by the more complex and time-consuming titrimetric methods. Furthermore, the older ferrocyanide method appears to be as reliable as the currently popular EDTA method.

EXPECTED PRECISION

Figure 2 shows the relationship between the average coefficient of variation, ($\overline{cv}$), and the logarithm of the concentration of all the certified elements of CCRMP ores and concentrates. The points for sodium and potassium in SCH-1 pertain only to the results obtained by the ISO method. This type of data-presentation has been used previously to assess the quality of the analytical data for rocks[21,22] and ores.[23]

Figure 2 shows that precision decreases as the concentration of the element determined decreases. The conditions prerequisite for the observed linearity for concentrations $\gtrsim$0.1%, irrespective of the element, have been discussed by Brooks *et al.*[22] Briefly, they are that the signal measured is a linear function of concentration; the constant of proportionality between signal and concentration is of the same order of magnitude for all elements; the analytical signal is much greater than the "blank" or "noise" signal; the systematic errors are relatively unimportant; and the absolute magnitude of the random errors is approximately constant, irrespective of the magnitude of the signal.

At concentrations $\gtrsim$0.01%, the linear relationship breaks down and there does not appear to be a dis-

cernible pattern between precision and element concentration. Some possible causes for this breakdown are[12] that the proportionality between measured signal and concentration differs for each element; the "blank" or "noise" signal may be significant with respect to the analytical signal; the systematic errors resulting from preconcentration or separation procedures for elements at the trace level may account for most of the variation in the data; for instrumental methods, the errors in the signal may not be related simply to the magnitude of the signal and, therefore, not be proportional to the concentration; and the variation in the data may result from "apparent" inhomogeneity of the material being analysed if too small a sample is taken for the analysis of an element at the trace level. This may also be important for elements concentrated in heavy minerals (*e.g.*, uranium in uraninite or brannerite) in finely comminuted ores and concentrates where local segregation is possible.

Figure 2 may be used as a means of estimating the precision expected for an element present at a concentration $\gtrsim 0.1\%$. For those with concentration $\lesssim 0.1\%$, there is no apparent relationship between precision and concentration. The determination of gold and silver, however, seems to be an exception, giving excellent precision even at very low levels. The relatively large sample used for fire-assay methods and the sensitivity of the method, *e.g.*, silver by atomic-absorption, may be possible explanations.

In contrast, relatively poor precision was obtained for elements at the trace level in iron ore SCH-1. One possible explanation is that many of the laboratories which participated in the certification programme had limited experience in the routine analysis of iron ore. There appears to be improved precision if only the results of the 4 laboratories which analyse iron ore on a routine basis are considered. It would, however, be presumptuous to reach such a conclusion on the basis of these results alone.

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ANALYTICAL ASPECTS OF ABSORPTION SPECTROELECTROCHEMISTRY AT A PLATINUM ELECTRODE—II

QUANTITATIVE BASIS AND STUDY OF ORGANIC COMPOUNDS*

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Summary—The theoretical basis for a new spectroelectrochemical technique, in which a narrow light-beam is passed at grazing incidence over a plane electrode surface, has been derived. Agreement between theoretical and experimental behaviour has been obtained for a number of organic molecules with well-defined redox behaviour. The advantages of this technique over other spectroelectrochemical techniques are discussed with respect to potential applications in quantitative analysis and electrochemical studies.

The transient absorption phenomena observed close to the cathode during the electrolysis of dilute aqueous solutions of metal ions have been described.[1–3] In a more recent paper,[4] a full report of the dependence of the absorbance on experimental parameters was given. There was a linear dependence on concentration, showing the potential analytical usefulness of the effect. Furthermore, experiments were described and discussed, from the results of which it was deduced that the signals observed were due to the formation of hydroxo-complexes arising from interaction of the metal ions with hydroxide ions generated as a reduction product of surface platinum oxide, formed during the anodic pretreatment of the electrode.

Although, at that stage, the various effects observed had been satisfactorily accounted for in terms of the chemistry of the system, it was felt that to pursue the potential analytical usefulness, a proper theoretical quantitative basis was required. In view of the somewhat complex nature of the process occurring at the cathode, controlled by the kinetics of reduction of the surface platinum oxide as well as by the diffusion of hydroxide ions and metal ions in solution and the kinetics of their interaction, it was decided to investigate systems in which only diffusion and electron-transfer processes would be operative. Accordingly, reactions involving a single oxidized and single reduced species, both of which remain in solution, were studied, and results obtained with organic molecules of this type as the electroactive species are reported here. The quantitative relationship between absorbance and a number of experimental parameters is derived and discussed in the light of the results obtained, and in relation to the analytical potential of the system.

* Part of the experimental work described herein was carried out at Imperial College, London and part at the University of Aberdeen, Old Aberdeen, Scotland.

THEORETICAL QUANTITATIVE BASIS

The spectroelectrochemical arrangement is shown in Fig. 1a. It is assumed that (1) the electrode reaction being monitored is $Ox + ne \rightarrow Red$, (2) that mass-transport in the solution is by semi-infinite linear diffusion and (3) that only the species Red absorbs at the wavelength of observation.

Absorbance measured normal to the electrode surfaces

Consider first the situation shown in Fig. 1b, i.e. where the light-beam passes normally through an optically transparent electrode. The variation of absorbance with the appropriate experimental parameters has already been established for chronoamperometric conditions (i.e. the absorbance is monitored following a potential step).

The usual starting point for the derivation is the Cottrell equation[5]

$$i_t = \frac{nFaD_O^{1/2}C_O^b}{\pi^{1/2}t^{1/2}} \quad (1)$$

where i_t is the instantaneous current (A) flowing at time t, n is the number of electrons transferred per mole, a is the electrode area (cm^2), D_O is the diffusion coefficient of the oxidized species (cm^2/sec), C_O^b is the bulk concentration of the oxidized species (mole/ml), t

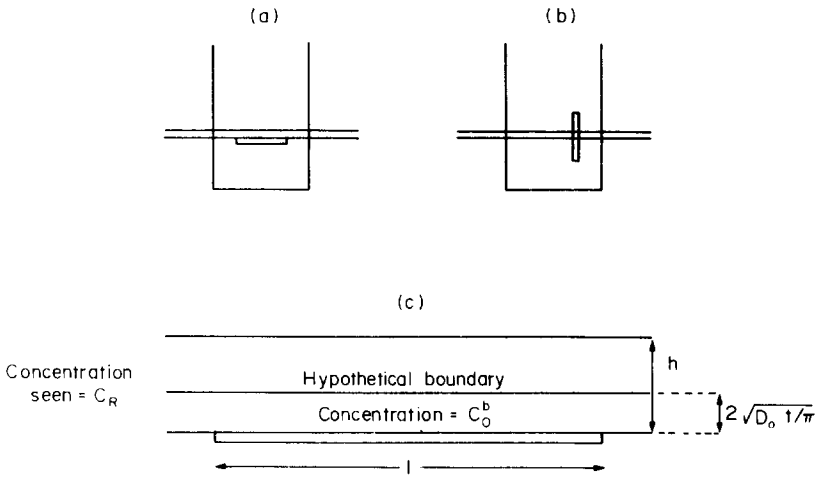

Fig. 1. Spectroelectrochemical configurations: (*a*) light-beam passing at grazing incidence over electrode; (*b*) light-beam passing normally through an optically transparent electrode; (*c*) hypothetical diffusion-layer behaviour.

is the time since the start of the electrolysis (sec), F is the Faraday constant (coulombs), which is derived from a solution of Fick's laws of diffusion under the appropriate boundary conditions[6,7] (one of which is that the concentration of the oxidized species is zero at the electrode surface during the electrolysis). Integration of equation (1) with respect to time gives the quantity of charge passed, Q, *viz.*

$$Q = \frac{2nFaD_O^{1/2}C_O^b t^{1/2}}{\pi^{1/2}} \tag{2}$$

From Faraday's laws, the amount of reduced species produced by the passage of Q coulombs is Q/Fn moles. If the reduced species occupies a volume V ml then the concentration of the reduced species is given by

$$C_R = \frac{Q}{nFV} \tag{3}$$

where the units of C_R are mole/ml.

The path-length of a beam of light passing normally through the electrode surface in the absorbing part of the solution will be V/a cm, and thus the absorbance will be given by

$$A = \epsilon_R \frac{V}{a} 1000 C_R \tag{4}$$

Substituting in equation (4) for C_R from equation (3) and for Q from equation (2),

$$A = \epsilon_R 2\left(\frac{D_O t}{\pi}\right)^{1/2} C_O^b \tag{5}$$

where C_O^b is now the bulk concentration of the oxidized species in mole/l.

Similar, though somewhat abbreviated, derivations have been given by Kuwana *et al.*[8,9] In reference 8, where this equation is first derived, the argument is confused by the use of the same symbol for electrode area and absorbance, and by not explaining the change in units of C. Equation (5) has also been derived by the method of Laplace transforms applied to the solution of Fick's equations (with the appropriate boundary conditions).[10,11] In reference 10, the unnecessary simplification is made of assuming the diffusion coefficients of the oxidized and reduced species to be equal. The equation has also been derived by integrating the appropriate function for C_R with respect to x, the distance from the electrode surface.[12]

$$A = \epsilon_R \int C_R \, dx$$

and

$$C_R = C_O^b \operatorname{erfc}(z)$$

where

$$z = \frac{x}{2D_O^{1/2} t^{1/2}}$$

Erfc(z) is the error function complement of z given by erfc(z) = 1 − erf(z) where erf(z) is the error function of z given by

$$\operatorname{erf}(z) = \frac{2}{\pi^{1/2}} \int_0^z e^{-y^2} \, dy$$

This integral is evaluated numerically and can be found in standard tables.

At first sight, it may seem strange that the absorbance–time behaviour does not depend on the diffusion coefficient of the reduced species, the chemical entity actually being monitored. However, it must be remembered that the conditions in the diffusion layer are such that both concentration *and* path-length are changing and that, qualitatively speaking, if the reduced species is moving slowly the path-length will be short but the concentration high, and conversely if

the reduced species is moving quickly then the path-length will be long but the concentration low.

It can be seen from equation (5) that, as far as absorbance is concerned, the diffusion layer behaviour is exactly equivalent to a situation in which the concentration remains constant (at a value equal to the bulk concentration of the oxidized species) and the optical path-length is given by $2(D_O t/\pi)^{1/2}$.

Absorbance measured parallel to the electrode surface

Considering now the situation in Fig. 1a, where the absorbance parallel to the electrode surface, A_p, is measured. If the height of the light-beam is h, then the concentration that the beam would detect, C_R, is given by

$$C_R h = C_O^b 2(D_O t/\pi)^{1/2}$$

This is shown in Fig. 1c.

Now for the absorbance of the reduced form, $A_{pR} = \epsilon_R l C_R$ where l is the path-length on the electrode surface. Therefore

$$A_{pR} = \epsilon_R \frac{l2}{h} (D_O t/\pi)^{1/2} C_O^b \qquad (6)$$

So far, only the reduced species has been considered as absorbing, but to account fully for the variation of absorbance with wavelength, the absorbance of the oxidized species must be taken into account. Although this aspect of the system has not been considered in any of the publications cited above it can be shown by arguments analogous to those presented for the reduced species that

$$A_{pO} = A' - \epsilon_O \frac{2l}{h}\left(\frac{D_O t}{\pi}\right)^{1/2} C_O^b$$

where A' is the initial absorbance and ϵ_O is the molar absorptivity of the oxidized species. A' is, of course, given by $A' = \epsilon_O l C_O^b$.

Thus, in the experiments described here, the complete picture during electrolysis is that the absorbance is made up of three components, namely (1) the absorbance of the oxidized species outside the ends of the electrode, (2) the absorbance of the oxidized species within the path-length on the electrode surface and (3) the absorbance of the reduced species within the path-length on the electrode surface.

If the cell path-length is L cm then the total absorbance B

$$A_{pT} = \epsilon_O(L - l)C_O^b + A' - \epsilon_O \frac{2l}{h}\left(\frac{D_O t}{\pi}\right)^{1/2} C_O^b + \epsilon_R \frac{2l}{h}\left(\frac{D_O t}{\pi}\right)^{1/2} C_O^b = \epsilon_O L C_O^b - \epsilon_O l C_O^b + \epsilon_O l C_O^b + (\epsilon_R - \epsilon_O)\frac{2l}{h}\left(\frac{D_O t}{\pi}\right)^{1/2} C_O^b$$

In the experiments described here, the absorbance before electrolysis starts, $\epsilon_O L C_O^b$, is set to zero, so

$$A_{pT} = (\epsilon_R - \epsilon_O)\frac{2l}{h}\left(\frac{D_O t}{\pi}\right)^{1/2} C_O^b \qquad (7)$$

Equation (7) is not quite the complete picture, as the absorbance will reach a maximum value when the "boundary" has diffused far enough from the electrode to fill the light-beam completely (see Fig. 1c), *i.e.*, when

$$2\left(\frac{D_O t}{\pi}\right)^{1/2} = h \qquad (8)$$

Although equation (7) has been derived for a reduction at the electrode surface, the argument applies equally to an oxidation, when the resulting equation would be

$$A_{pT} = (\epsilon_O - \epsilon_R)\frac{2l}{h}\left(\frac{D_R t}{\pi}\right)^{1/2} C_R^b$$

In deriving equation (7), no consideration has been given to longitudinal diffusion. This will only occur at the ends of the electrode, where there is a concentration gradient in a longitudinal direction. The occurrence of longitudinal diffusion will not affect the overall absorbance–time behaviour, as the number of absorbing centres in the light beam at any one time remains the same. The phenomenon has not been considered in previously reported electrochemical studies, as the regions of the solutions sampled by a light beam passing normally through an electrode contain a concentration gradient in only one direction.

EXPERIMENTAL

Apparatus

This was as described previously.[4]

Reagents

Three compounds were used: 4-amino-4′-methoxydiphenylamine (Variamine Blue), tris(5-nitro-1,10-phenanthroline)iron(II) perchlorate (nitroferroin) and 4,4′-diamino-3,3′-dimethylbiphenyl (*o*-tolidine). The Variamine Blue was recrystallized before use. Analytical reagent grade potassium sulphate or sulphuric acid was used as background electrolyte.

Procedure

The function generator was preset to provide a step function so that the electrode in the light-path became either the cathode or the anode. A 5-ml portion of the working solution, the bulk of which was continuously deoxygenated with oxygen-free nitrogen, was transferred to the electrolysis cell and deoxygenated for a further 60 sec. During electrolysis the solution was kept under nitrogen. The initial transmission at the appropriate wavelength was set to 100% by adjusting the gain. The zero of the chart-recorder was set with the light-beam interrupted by an opaque material. The chart-recorder was started and the function generator was then switched to its preset value and the absorption signal recorded as a function of time, i.e., the experiments were the spectroelectrochemical analogue of chronoamperometry. The solution was finally

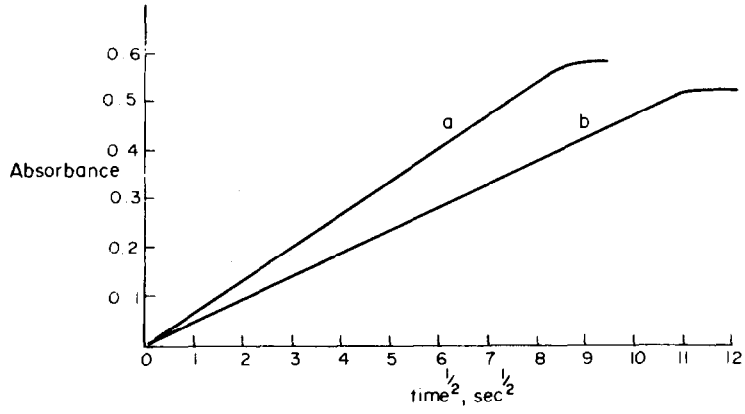

Fig. 2. Variation of absorbance with time$^{1/2}$: (*a*) nitroferroin; (*b*) *o*-tolidine.

removed from the cell by suction and the electrodes were rinsed with distilled water.

RESULTS

The variation of absorbance with a number of experimental parameters was investigated by the procedure above.

Time

The variation of absorbance with the square root of the time is shown in Fig. 2 for the reduction of nitroferroin at -0.2 V *vs.* SCE. The bulk concentration was $9.6 \times 10^{-5}M$ and the wavelength was set at 505 nm. Also shown in Fig. 2 is the same relation for the oxidation of *o*-tolidine at $+0.8$ V *vs.* SCE. In this case the bulk concentration was $10^{-5}M$ in $0.125M$ sulphuric acid, and the wavelength was set at 434 nm. The slopes of the linear portions of the plots were 0.0667 and 0.047 sec$^{-1/2}$ for the nitroferroin and *o*-tolidine respectively. The times taken to reach the maximum absorbance were 75 and 126 sec respectively.

Wavelength

The variation of absorbance with wavelength was investigated for all three compounds. A $10^{-4}M$ solution of Variamine Blue in $0.03M$ potassium sulphate was oxidized at $+2.0$ V *vs.* the counter-electrode, the oxidized form of nitroferroin ($7.8 \times 10^{-5}M$ in $0.03M$

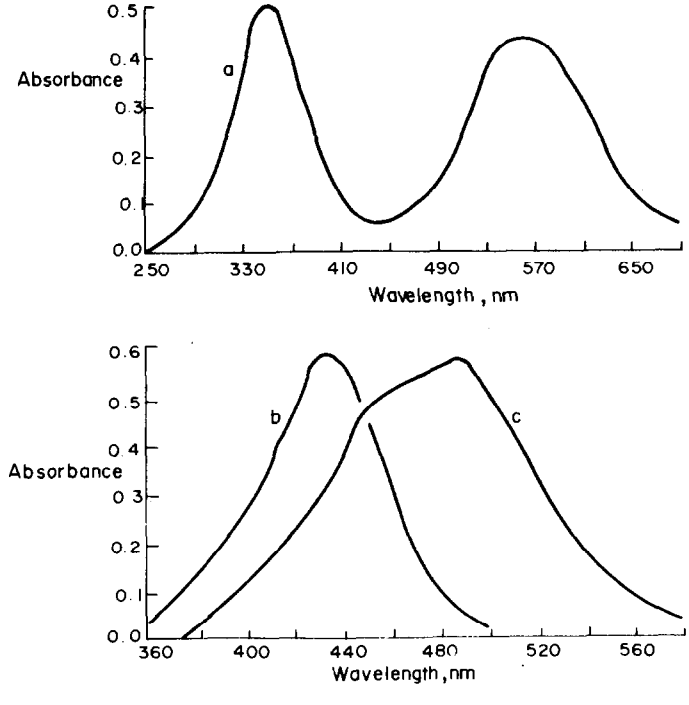

Fig. 3. Spectra obtained at the electrode surface, (*a*) Variamine Blue; (*b*) *o*-tolidine; (*c*) nitroferroin.

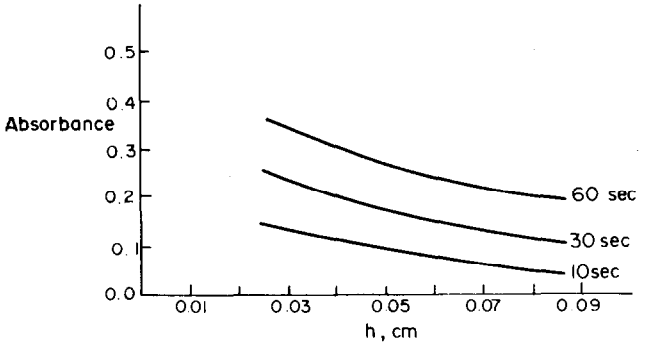

Fig. 4. Variation of absorbance of *o*-tolidine with height of light-beam for different times after the start of the electrolysis.

potassium sulphate) was reduced at −0.2 V *vs.* SCE and a $10^{-5}M$ solution of *o*-tolidine in 0.125*M* sulphuric acid was oxidized at +0.8 V *vs.* SCE. The spectra were obtained by recording the absorbance at a series of wavelength intervals (every 10 nm for *o*-tolidine, every 20 nm for Variamine Blue and every 25 nm for the nitroferroin) until a maximum was reached. After the maximum had been reached the function generator was reset, the solution removed, the wavelength changed by the appropriate amount and a fresh portion of solution introduced into the cell. The spectra are shown in Fig. 3.

Concentration

The variation of absorbance with bulk concentration was investigated for Variamine Blue (oxidation at +2.0 V *vs.* counter-electrode, measurement at 350 and at 550 nm) and *o*-tolidine (+0.8 V *vs.* SCE; 434 nm). For the Variamine Blue, straight-line calibrations were obtained over the range $0–10^{-4}M$ with slopes (equal to molar absorptivity × path-length) of 6.8×10^3 l./mole at both 350 and 550 nm. The *o*-tolidine gave a linear calibration over the range $0–1.6 \times 10^{-5}M$ with a slope of 5.5×10^4 l./mole. For convenience of comparison with atomic-absorption sensitivities these slopes can be converted into "sensitivities" defined as the analyte concentration that will give 1% absorption. For Variamine Blue and *o*-tolidine the "sensitivities" are 6.5×10^{-7} and $8 \times 10^{-8}M$ respectively.

Height of the light-beam

An estimate of the height of the light-beam was obtained by moving the working electrode into the beam (by movement of the whole cell assembly relative to the optical path) until the transmittance was reduced to zero. The electrode was withdrawn from the light-path until a beam of suitable height was obtained and the distance moved was measured with a micrometer screw-thread. The variation of absorbance with height of the light-beam was investigated for $10^{-5}M$ *o*-tolidine in 0.125*M* sulphuric acid at +0.8 V *vs.* SCE. The results are shown in Fig. 4.

DISCUSSION

Variation of absorbance with time

The theoretical expression predicts that the absorbance should vary as a linear function of the square root of the time. The experimental result (Fig. 2) show good agreement with this prediction. In the case of *o*-tolidine the height of the light-beam was set at 2.5×10^{-2} cm. Thus from a knowledge of the diffusion coefficient, 3.8×10^{-6} cm^2/ssec,[13] the time at which maximum absorbance occurs is calculated from equation (8) as 129 sec. The experimentally determined value was 125 sec. A value for the diffusion coefficient of nitroferroin is not available in the literature. The linearity of the absorbance *vs.* $t^{1/2}$ plot is considered[14] to indicate that semi-infinite linear diffusion conditions are operating and is thus a prerequisite for the validity of the experimental model. In a separate experiment the difference in molar absorptivity between the oxidized and reduced forms of *o*-tolidine was found to be 5.8×10^4 l.mole^{-1}.cm^{-1}, the length of the electrode was 0.9 cm and hence the slope of the *A vs.* $t^{1/2}$ plot was calculated to be 0.046 sec$^{-1/2}$ for a $10^{-5}M$ solution. The experimental value found was 0.047 sec$^{-1/2}$.

This experiment may thus be used to determine the diffusion coefficient of the species diffusing towards the electrode surface, provided that the parameters *l* and *h* are known. Because of the difficulty of measuring them, these may be obtained from a study of the *A vs.* $t^{1/2}$ behaviour of a compound for which the diffusion coefficient and molar absorptivities are known. Although a value of the unknown diffusion coefficient may be obtained from the time at which maximum absorbance occurs and thus does not require a knowledge of the bulk concentration, in practice this is a somewhat imprecise measurement and determination from the slope of the plot is to be preferred. From the ratio of *l*/*h* calculated for the

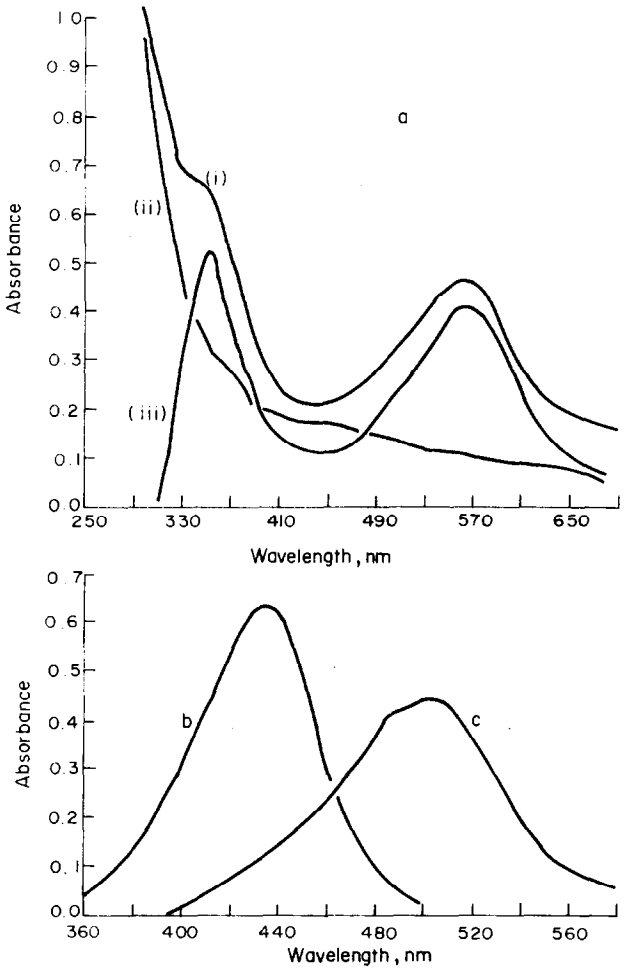

Fig. 5. Absorption spectra: (*a*) Variamine Blue; (*i*) oxidized form, (*ii*) reduced form, (*iii*) difference; (*b*) *o*-tolidine; (*c*) nitroferroin.

o-tolidine experiment the diffusion coefficient of nitroferroin is calculated to be 2.2×10^{-6} cm^2/s.

Variation of absorbance with wavelength

The term in equation (8) governing the variation of absorbance with wavelength is the term including the molar-absorptivity difference. Thus the spectra obtained at the electrode surface should be the difference between the spectra of the oxidized and the reduced forms. This was verified by obtaining the spectra of the various compounds studied, with a double-beam spectrometer. The results are shown in Fig. 5. A comparison of these spectra with those in Fig. 3 shows excellent agreement between predicted and observed spectra, particularly in the case of Variamine Blue, the oxidized and reduced forms of which both absorb over the wavelength range studied. Care must be taken in interpreting the spectra obtained during spectroelectrochemical studies, as they may not arise from just one species.

Variation of absorbance with concentration

The theory predicts that there should be a linear relation between absorbance and the bulk concentration of the species diffusing towards the electrode, provided all other factors in the equation remain constant. This, of course, is the basis for the potential analytical usefulness of the technique. The analytical growth curves described earlier were obtained by recording absorbance as a function of time until a maximum value was reached, and plotting this absorbance maximum as a function of concentration. For o-tolidine, ten calibration points were recorded over the range 0–$1.6 \times 10^{-5} M$.

A least-squares fit of the best straight line yielded a slope of 5.6×10^4 l./mole, intercept 1.2×10^{-3}, correlation coefficient 0.99948, and standard deviation of the scatter 9.2×10^{-3}. The theoretical slope was calculated to be 5.3×10^4 l./mole.

Variation of absorbance with height of light-beam

The theoretical *A vs. h* plot is shown in Fig. 6 for *o*-tolidine at 10, 30 and 60 sec after the start of the electrolysis. The experimental results in Fig. 4 show curves of a similar type though the agreement is not particularly good, most likely because of the difficulty of obtaining an accurate value for *h* with the apparatus used. However, as already mentioned, in using

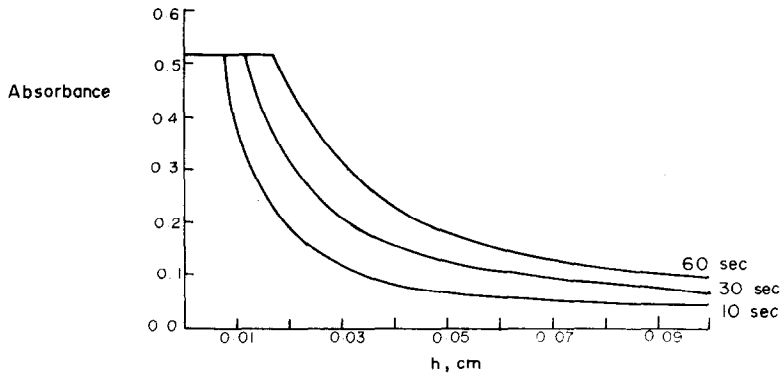

Fig. 6. Theoretical variation of absorbance of *o*-tolidine with the height of light-beam for different times after the start of the electrolysis.

the technique it is not necessary to know the individual values of l and h, only their ratio, and this may be obtained from the electrolysis of a compound of known molar absorptivity and diffusion coefficient.

APPLICATIONS

Quantitative analysis

Spectroelectrochemical techniques have potential as quantitative analytical methods as the reaction conditions within the diffusion layer are highly reproducible and so problems associated (in conventional spectrophotometry) with the kinetics of the colour-forming reaction, such as ensuring the colour is fully developed or is measured before it fades, are overcome. Obviously, the methods are only applicable to oxidation–reduction systems, but subsequent reactions of electrolysis products may also be monitored, thus extending the possible scope. This is well illustrated by the example of the analytical growth curves obtained for the reaction of metal ions with hydroxide ions generated by the electroreduction of surface platinum oxide, reported previously.[4] Existing spectroelectrochemical techniques have not found application in quantitative analysis to date; one of the reasons is probably the inherently poor sensitivity of existing methods, because of the very short path-length in the absorbing medium.

With *o*-tolidine as a model compound, it has already been shown that for the method described here, the slope of the calibration curve is 5.5×10^4 l./mole (sensitivity $8 \times 10^{-8} M$ for 1% absorption) with an absorbance maximum reached after 125 sec. This may be compared with other spectroelectrochemical methods, as follows.

Internal reflection spectroscopy. The equation for the absorbance–time behaviour is[15]

$$A = \epsilon b_{\text{eff}} C^{\text{b}}[1 - \exp(a^2 t)\text{erf}(at^{1/2})]$$

if the diffusion coefficients of the oxidized and reduced species are assumed to be equal. In this equation, $b_{\text{eff}} = \delta N_{\text{eff}}$ and $a = D^{1/2}/\delta$, where δ is the penetration depth which defines the optical path, and N_{eff} is a sensitivity factor characteristic of the type of transparent electrode.

The time taken to reach the absorbance maximum, $\epsilon\, b_{\text{eff}}\, C^{\text{b}}$, is approximately 10^{-3} sec for a δ value of 100 nm. The slope of the calibration curve as calculated from results presented by Kuwana *et al.*[16] for a tin oxide electrode, is 8.8 l./mole (sensitivity $5.0 \times 10^{-4} M$ for 1% absorption).

Normal transmission through optically transparent electrodes in a thin-layer cell. For a thin-layer thickness of 10^{-3} cm, complete electrolysis is achieved theoretically in 0.2 sec.[17] This would give rise to a calibration slope of 61 l./mole (sensitivity $7.2 \times 10^{-5} M$ for 1% absorption). This spectroelectrochemical study used a gold minigrid electrode[18] in a cell of thickness 8×10^{-3} cm. Taking the molar absorptivity of *o*-tolidine to be 6.1×10^4 $\text{l.mole}^{-1}.\text{cm}^{-1}$,[13] the calibration slope would be 4.9×10^2 l./mole (sensitivity $9.0 \times 10^{-6} M$ for 1% absorption). However, the results of the absorbance *vs.* time experiment[18] presented give a value of 1.3×10^2 l./mole (sensitivity $3.5 \times 10^{-5} M$ for 1% absorption), *i.e.*, the molar absorptivity of the *o*-tolidine used was only 1.6×10^{-4} $\text{l.mole}^{-1}.\text{cm}^{-1}$. The time taken to reach the maximum absorbance was approximately 15 sec. Nine years later, the experiment was reported as a teaching experiment.[19] The cell thickness used was 1.7×10^{-2} cm, which should theoretically give a calibration slope of 1.0×10^3 l./mole (sensitivity $4.2 \times 10^{-6} M$ for 1% absorption), but the absorbance results reported correspond to a value of 6.2×10^2 l./mole (sensitivity $7.1 \times 10^{-6} M$ for 1% absorption) *i.e.*, the *o*-tolidine used apparently had a molar absorptivity of 3.6×10^4 $\text{l.mole}^{-1}.\text{cm}^{-1}$). The absorbance–time behaviour was not reported. It should be pointed out that *o*-tolidine is carcinogenic, a fact not mentioned in the analytical literature.

Normal transmission through an optically transparent electrode. This experiment was carried out for *o*-tolidine at tin oxide optically transparent electrodes,[8,9] but the absorbance–time behaviour was not reported. The relationship between absorbance and the experimental parameters is given by equation (5), and in theory the slope of the calibration curve can be made as large as required, as there is no limit to the

path-length under "semi-infinite" conditions. However if a 1-cm path-length is taken, the theoretical slope would be 6.2×10^4 l./mole (sensitivity $7.1 \times 10^{-8} M$ for 1% absorption) but the time taken to reach the maximum absorbance, given by $t_{max} = h^2\pi/4D$, would be 2×10^5 sec.

Absorbance *vs.* time results have been reported for a gold minigrid electrode with two electroactive faces.[20] A linear A–$t^{1/2}$ relationship is reported for t between 1 and 10 sec. An absorbance of 1.0 is recorded after 10 sec, corresponding to a path-length of 0.02 cm.

In this case, and also when the light-beam is parallel to the electrode surface, it is not necessary to wait until an absorption maximum is reached, as the bulk concentration is directly proportional to the slope of the A *vs.* $t^{1/2}$ plot. Thus it is sufficient to record the absorbance–time behaviour until the slope of the A *vs.* $t^{1/2}$ plot can be established, and then to obtain a calibration by plotting this slope against bulk concentration.

Other applications

Spectroelectrochemical techniques have been applied to the determination of a number of electrochemical parameters such as diffusion coefficients, rate constants, number of electrons transferred, E^0 values, and to the elucidation of reaction mechanisms. In addition to the advantage of high sensitivity, the technique described here (giving good signal to noise characteristics without the need for repetitive accumulation of data) has a number of other advantages. The light-beam does not pass through the electrode surface and thus there is no interference from the absorption spectrum of the electrode material, the usable wavelength range being determined by the transmission characteristics of the solvent. There will be no interference from non-Faradaic absorption processes and there is no limit to the type of electrode that may be used provided the surface does not become deformed during the experiment. The appropriate equations for more complicated electrode reactions may be readily derived by modifying the equations already derived in the literature to allow for the path-length on the electrode surface and the height of the light-beam. The speed of response of the system may be changed by changing the value of h, the lower limit being set by the spectrophotometer's ability to cope with low light-levels. The disadvantages of the technique may be that layers of solution very close (100 nm or so) to the electrode surface cannot be monitored and that deviations from the theoretical behaviour may occur owing to effects at the ends of the electrode.

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THE IODOMETRIC DETERMINATION OF DITHIONITE, THIOSULPHATE AND SULPHITE IN THE PRESENCE OF ALKALI AND/OR CYANIDE

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Summary—The analysis of mixtures of dithionite, thiosulphate and sulphite in the presence of cyanide and/or in alkaline solution has been investigated. The effects of such constituents as sulphur, acetonitrile, hexafluoroarsenate, and bromide in alkaline solution were also examined. Satisfactory results were obtained by using low-temperature, oxygen-free, alkaline solutions, containing excess of alkali, at pH > 12.

The iodometric determination of dithionite, thiosulphate and sulphite was originally examined by Wollak.[1] More recently, Danehy and Zubritsky have evaluated the procedure and found it "unexceptionable".[2] However, potential analytical errors and experimental problems have been reported.[3,4]

The need for a convenient and reliable method for analysing mixtures of oxy-sulphur compounds arises from the development of high-energy lithium batteries employing oxy-sulphur oxidants such as sulphuryl chloride, thionyl chloride and sulphur dioxide. Currently under investigation at our laboratory is the Li/SO_2 cell.[5] The unknown chemistry associated with the operation of this cell may account for some of the safety hazards that have been reported.[6–8] It has been speculated that the major discharge product is lithium dithionite but formation of other oxy-sulphur compounds such as sulphite and/or thiosulphate may be possible.[9] The Li/SO_2 cell consists of a lithium anode, and a porous-carbon collector cathode immersed in a solution of sulphur dioxide in a solvent such as acetonitrile and containing a conducting salt such as LiBr (active cell) or $LiAsF_6$ (reserve cell). Decomposition of the acetonitrile or reaction with lithium also offers the distinct possibility that cyanide may be present.

The Wollak method does not allow for convenient analysis of such a heterogeneous residue. The analysis can be simplified if the residue can be effectively dissolved without undergoing reaction or major decomposition. Dissolution in aqueous sodium hydroxide has been investigated as a method for conveniently sampling such complex mixtures and providing a suitable medium for the analysis. This paper describes the experimental approach, including the effects of alkali and/or cyanide on the analysis and some room-temperature anaerobic kinetics of dithionite decomposition in aqueous alkali solutions.

EXPERIMENTAL

Reagents

Anhydrous samples of sodium dithionite were taken from unopened bottles. During use, the dithionite was stored in closed bottles in a desiccator. Only the highest purity dithionite (from J. T. Baker) was used in the alkaline solution studies. The sodium acetate–acetic acid buffer was 3.5*M* in each constituent. All chemicals used were reagent grade. All solutions were prepared and standardized by accepted methods.

*Analysis of solid mixtures of dithionite, thiosulphate and sulphite**

Titration X. In a 100-ml standard flask take a mixture of approximately 15 ml of 0.5*M* iodine, 15 ml of water and 4 g of sodium acetate trihydrate. For samples containing little or no dithionite, use 5 ml of the acetic acid–acetate buffer in place of the sodium acetate. Add 0.4 g of sample (accurately weighed through a dry funnel, slowly and with constant swirling of the solution. Rinse any residue from the funnel with a small amount of the iodine–buffer solution. The solution should be clear and have a pH between 4.5 and 5.5. Add 10% sodium sulphite solution to remove the excess of iodine and add an additional 8 ml of sulphite solution. Neutralize the solution to phenolphthalein (pH 8–10) by dropwise addition of 10*M* sodium hydroxide. Let stand for 5 min, add 4 ml of 37% formaldehyde and 10 ml of 20% acetic acid. Dilute to the mark, remove an aliquot, adjust the pH to between 3.5 and 4.0 with 20% acetic acid, and titrate (using a microburette) with 0.005*M* iodine to a violet-coloured starch end-point. Let the number of mmoles of iodine consumed be *X*.

Titration Y. Add 3 drops of 10*M* sodium hydroxide to approximately 15 ml of water and 15 ml of 37% formaldehyde contained in a 100-ml standard flask. Add 0.4 g of sample (accurately weighed) slowly, swirling the solution until dissolved. Cover and allow to stand for 20 min. Dilute to the mark with water. Remove a 10-ml aliquot. Add 150 ml of water and a few ml of the acetic acid–acetate buffer, and titrate with 0.05*M* iodine to a blue starch

* A sufficient excess of iodine must be available to accommodate samples containing cyanide.

end-point that persists for 1 min. Let the number of mmoles of iodine consumed be *Y*.

Titration Z. Add 0.4 g of sample (accurately weighed) slowly to a 100-ml standard flask containing excess of 0.15*M* iodine and either 4 g of sodium acetate trihydrate or 5 ml of buffer solution, with swirling. If the sample contains little dithionite, use the buffer. The solution should be clear and preferably have a pH between 4.5 and 5.5. Dilute to the mark. Remove an aliquot and add a few ml of the acetic acid–acetate buffer and titrate with 0.10*M* thiosulphate to a starch end-point. Let *Z* be the number of mmoles of iodine consumed.

Total sulphur. Add a 0.4-g sample to a mixture of 50 ml of 0.1*M* sodium hydroxide, 3 ml of 30% aqueous hydrogen peroxide and 1 g of sodium peroxide. Warm and let stand for 30 min. Boil to destroy excess of peroxide. Acidify with 6*M* hydrochloric acid until no more carbon dioxide is evolved. Add saturated barium chloride solution until precipitation is complete. Filter off on a sintered-glass crucible, wash with water and ethanol, dry, cool and weigh.

Analysis of samples in alkaline solution

Atmospheric oxygen can oxidize dithionite and sulphite in alkaline solution. Consequently, air must be excluded before introduction of the sample and during its manipulation. For this reason, studies in alkaline solution were carried out in a 1-litre three-necked flask under a positive pressure of argon. Residual oxygen in the 99.995% pure argon was removed by passing the argon through a saturated chromous chloride solution in 1.0*M* hydrochloric acid. Acid fumes were subsequently removed by passing the argon through sodium hydroxide solution before its entry into the flask.

A known volume of sodium hydroxide solution, prepared with freshly distilled, cooled and deoxygenated water, was introduced into the flask. Oxygen-free argon was bubbled through the magnetically stirred solution long enough to remove oxygen. After equilibration in a thermostat, the flask was removed from the thermostatic bath, its contents stirred magnetically, and a weighed sample quickly introduced under a positive argon pressure. The flask was returned to the thermostat. The solution was forced under argon into an argon-filled burette. Measured volumes, 10–25 ml, of the solution were added to rapidly swirled solutions containing excess of iodine or formaldehyde, in 100-ml standard flasks.*

THEORY

When a sample containing dithionite, thiosulphate and sulphite is added to an acidified excess of iodine, the following reactions occur.

$$S_2O_4^{2-} + 3I_2 + 4H_2O \rightarrow 2HSO_4^- + 6HI \quad (1)$$

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^- \quad (2)$$

$$HSO_3^- + I_2 + H_2O \rightarrow HSO_4^- + 2HI \quad (3)$$

* The solutions and procedures described in titrations X, Y and Z are used with the following exceptions. In titrations X and Z, acetic acid–acetate buffer must be used in place of the sodium acetate trihydrate. Excess of iodine and a sufficient volume of the buffer solution should be provided, so that when an aliquot of the alkaline sample solution has been added, the remaining iodine solution should be clear, with pH ~ 5. Dilute oxy-sulphur solutions in 2*M* sodium hydroxide required ~ 20 ml of buffer. Concentrated oxy-sulphur solutions in 0.1*M* sodium hydroxide required less buffer. In the latter case, it may be necessary to add a few grams of sodium acetate trihydrate to help buffer the acid generated by samples containing relatively large amounts of dithionite. If cloudiness due to sulphur formation occurs, repeat with a larger excess of iodine.

Determination of thiosulphate

Excess of sulphite solution is added (*a*) to remove any unreacted iodine by reaction (3) and (*b*) after adjustment of the pH to between 8 and 10, to quantitatively convert the tetrathionate, $S_4O_6^{2-}$, into half the original amount of thiosulphate, according to the reaction

$$S_4O_6^{2-} + SO_3^{2-} \rightarrow S_2O_3^{2-} + S_3O_6^{2-} \quad (4)$$

The addition of formaldehyde and acetic acid complexes any unreacted sulphite.

$$SO_3^{2-} + HCHO \xrightarrow{H^+} CHOHSO_3^- \quad (5)$$

The thiosulphate produced by reaction (4) is then titrated with standard iodine solution (titration X). Then $4X$ = mmoles of $S_2O_3^{2-}$.

Determination of dithionite

Upon addition of the sample to excess of alkaline formaldehyde, only the dithionite reacts quantitatively:

$$S_2O_4^{2-} + 2HCHO + H_2O \rightarrow HCHOHSO_2^- + HCHOHSO_3^- \quad (6)$$

The sulphite is complexed on acidification, in accordance with reaction (5). Titration with standard iodine solution (titration Y) oxidizes the thiosulphate according to reaction (2) and the sulfinate (produced mole for mole from the dithionite) is also oxidized according to

$$HCHOHSO_2^- + 2I_2 + 2H_2O \rightarrow HSO_4^- + HCHO + 4HI \quad (7)$$

Then $(Y - 2X)/2$ = mmoles of $S_2O_4^{2-}$.

Determination of sulphite

Titration Z gives the number of mmoles of iodine consumed by the sample in accordance with reactions (1), (2) and (3). Consequently, $(2Z + 2X - 3Y)/2$ = mmoles of SO_3^{2-}.

DISCUSSION

The processing and purification procedures used in the preparation of sodium dithionite lead to variability in the commercial product, as shown in Table 1. Six samples of each of the purer products were analysed by the procedure for solid mixtures. This established the composition of samples to be used later as "standards" and also validated the analytical method. Considering the heterogeneity, good reproducibility was achieved, as indicated by the standard deviation.

The removal of cyanide in the presence of oxy-sulphur compounds by chemical or distillation methods is not recommended, primarily because of the hydrolysis and decomposition reactions associated with the dithionite.[10] Various combinations of dithionite, thiosulphate and sulphite were analysed in the presence of cyanide. The results are presented in Table 2.

Table 1. Analysis of commercial dithionite samples*

Source	$Na_2S_2O_4$, %	Na_2SO_3, %	$Na_2S_2O_3$, %	Total, %
Fisher†	78.5	9.2	5.5	93.2
Mallinckrodt‡	86.5 (0.37)	8.5 (0.30)	1.3 (0.05)	96.3
J. T. Baker‡	89.9 (0.47)	6.9 (0.25)	2.5 (0.05)	99.3

† Dithionite made by the zinc base process; contains approximately 4% carbonate, 1% chloride and 1.9% of an unidentified sulphur compound (from total sulphur); lot No. 722528.

‡ Formate process, Mallickrodt samples (7672 lot No. WHAT) contained approximately 1% formate, trace chloride and 2.5% of an unidentified sulphur compound. J. T. Baker sample was lot No. 424864.

* Standard deviation from the analysis of six samples is shown in parenthesis.

The data confirm the reliability of the analytical method. Iodine reacts with the cyanide according to the reaction

$$I_2 + CN^- \overset{H^+}{\rightleftharpoons} HI + ICN \quad (8)$$

However, the equilibrium is quantitatively displaced as illustrated by the reaction with thiosulphate:

$$ICN + 2S_2O_3^{2-} + H^+ \rightarrow I^- + HCN + S_4O_6^{2-} \quad (9)$$

After the solution has been made alkaline for titration X, both the cyanide and the added sulphite can convert the tetrathionate into thiosulphate in 1:1 mole ratio. The data of Table 2 imply, however, that either the cyanide–tetrathionate reaction[11] to form thiocyanate is minimal or any thiocyanate formed reacts only negligibly with the acidified standard iodine solution. Also, the large excess of added sulphite favours the re-formation of thiosulphate by way of reaction (4). In titration Y, the excess of formaldehyde converts cyanide into cyanohydrin. This has been shown to be stable in acid,[12] and from the data of Table 2 is apparently unreactive to iodine.

The aerobic decomposition of dithionite in alkaline solution occurs rapidly.[13,14] In contrast, the anaerobic decomposition occurs slowly[15,16] and has been reported to be dependent on the alkali concentration, solution pH, and temperature.[17]

Table 3 shows some of the results obtained from iodometric analysis of various mixtures of dithionite, thiosulphate and sulphite in solutions of varying sodium hydroxide concentration and at temperatures of 0, 26 and 82°. Included are components expected to be present during the analysis of various discharged Li/SO_2 cells, including S, CH_3CN, Br^-, AsF_6^-, and CN^-.

At low temperatures, 0 and 26°, dithionite was found to undergo very slow decomposition. Consequently, the time that the sample was in solution was not critical. No discernible difference in the analytical data was noticed if the sample was kept in solution for 10 min or a few hours. Analysis is not recommended at temperatures higher than room temperature. Dithionite can have appreciable rates of decomposition at high temperatures, as is illustrated by runs 11–14 at 82°.

There must also be an excess of alkali present, preferably with the solution pH >12. Appreciable decomposition can occur if the dithionite concentration approaches or exceeds that of the alkali. However, large excesses of alkali (>10-fold) should also be avoided, as they enhance the rate of dithionite decomposition. This is noticeable even at 26°, as illustrated by runs 8 and 9.

The efficient removal of oxygen from the solution

Table 2. Determination of dithionite, thiosulphate and sulphite with and without the addition of known amounts of cyanide*

	$S_2O_4^{2-}$, *mmole*			$S_2O_3^{2-}$, *mmole*			SO_3^{2-}, *mmole*		
Run†	present	found (no CN^-)	found (CN^- present)	present	found (no CN^-)	found (CN^- present	present	found (no CN^-)	found (CN^- present)
1	—	—	—	0.633	0.614	0.622	—	—	—
2	—	—	—	—	—	—	0.910	0.900	0.921
3	—	—	—	1.270	1.252	1.272	1.205	1.190	1.210
4	2.067	2.062	2.060	0.0640	0.0639	0.0643	0.219	0.201	0.208
5	1.990	1.984	1.985	0.0329	0.0318	0.0324	0.270	0.275	0.285
6	0.761	0.762	0.766	0.629	0.636	0.620	1.270	1.260	1.247
7	1.990	1.984	1.996	0.666	0.661	0.665	1.440	1.428	1.450
8	1.990	1.973	1.973	0.666	0.674	0.673	1.440	1.459	1.432

* Run 1, 0.75 mmole KCN; run 4, 2.0 mmole KCN; all other runs, 1.5 mmole KCN.

† Runs 4 and 5; J. T. Baker and Mallinckrodt sodium dithionite samples, respectively. Runs 6, 7, 8; Mallinckrodt dithionite samples with known additions of thiosulphate and sulphite. Runs 1–7; direct analysis of solid mixtures; run 8, analysis of the mixture in 100 ml of deaerated 0.1*M* NaOH.

Table 3. Study of the effects of alkali concentration, temperature and various constituents on the analysis of mixtures of dithionite, thiosulphate, and sulphite in NaOH solution

Run*	[NaOH], *M*	Time†, *min*	Added constituents§	$S_2O_4^{2-}$, *mmole/ml* present	$S_2O_4^{2-}$, *mmole/ml* found	$S_2O_3^{2-}$, *mmole/ml* present	$S_2O_3^{2-}$, *mmole/ml* found‡	SO_3^{2-}, *mmole/ml* present	SO_3^{2-}, *mmole/ml* found‡
1	0.1	80	—	0.0499	0.0493	0.0016	0.0017	0.0053	0.0054
1A	0.1	180	Run 1 + 0.05*M* S	—	0.0492	—	0.0020	—	0.0062
2	0.1	10	—	0.0998	0.0996	0.0031	0.0034	0.0106	0.0110
3	0.1	60	$S_2O_3^{2-}$; SO_3^{2-}	0.0989	0.0986	0.0519	0.0533	0.0500	0.0499
3A	0.1	70	Run 3 + 0.45*M* CH_3CN	0.0969	0.0962	0.0507	0.0512	0.0488	0.0482
3B	0.1	75	Run 3A + 0.050*M* $LiAsF_6$	—	0.0960	—	0.0510	—	0.0480
3C	0.1	60	Run 3B + 0.018*M* S	—	0.0959	—	0.0505	—	0.479
4	0.1	15	—	0.0108	0.0106	Trace amounts		0.0011	0.0012
5	0.1	36	—	0.0494	0.0492	0.0015	0.0018	0.0052	0.0061
6	0.1	17	$S_2O_3^{2-}$; SO_3^{2-}	0.0180	0.0178	0.0116	0.0117	0.0157	0.0160
7	0.1	61	$S_2O_3^{2-}$; SO_3^{2-}	0.0484	0.0479	0.0295	0.0293	0.0352	0.0360
8	0.5	12	—	0.0504	0.0496	0.0016	0.0025	0.0053	0.0065
9	2.0	42	—	0.0511	0.0498	0.0016	0.0012	0.0053	0.0096
10	1.0	120	$S_2O_3^{2-}$; SO_3^{2-} –	0.0490	0.0487	0.0522	0.0528	0.0520	0.0522
10A	1.0	60	10 + 0.017*M* S	—	0.0486	—	0.0524	—	0.0509
10B	1.0	60	10A + 0.050*M* $LiAsF_6$ + 0.01*M* KCN + 0.29*M* CH_3CN	0.0481	0.0478	0.0518	0.0526	0.0508	0.0528
10C	1.0	60	10B + 0.02*M* LiBr	—	0.0478	—	0.0525	—	0.0521
11	0.1	50	—	0.0494	0.0445	0.0015	0.0036	0.0052	0.0094
12	0.5	30	—	0.0504	0.0469	0.0016	0.0015	0.0053	0.0092
13	1.0	10	—	0.0503	0.0478	0.0016	0.0019	0.0053	0.0063
14	2.0	14	—	0.0500	0.0443	0.0016	0.0020	0.0053	0.0197

* Initial volumes of solution and temperature of the solution: runs 1–3C, 500 ml at 0°; runs 4–9, 750 ml at 26°; runs 10–10C, 500 ml at 26°; runs 11–14, 750 ml at 82°.

† Time in minutes that the mixture was in the NaOH solution before sampling for dithionite determination. Samples for $S_2O_3^{2-}$ and SO_3^{2-} determination were generally taken within 1 min of the sample for $S_2O_4^{2-}$ determination. In runs 1, 3 and 10, the solution prepared for the first run was retained and additional materials were added immediately after the samples had been taken, so the times given in the third column are additive, e.g., in run 10C the total time elapsed between initial preparation and sampling was 300 min.

‡ Uncorrected for the amount derived from decomposition of dithionite.

§ Components typically expected in active or reserve Li/SO_2 cells and that were added to analysed Baker commercial dithionite.

was found to be the most critical factor in the low-temperature analysis. Dithionite is an effective scavenger of oxygen at 0°. Consequently, it is essential to remove oxygen before addition of the sample. This is best accomplished by deaerating before addition of the alkali. Alkaline solutions are more difficult to deaerate than water.

If elemental sulphur is present in the mixture, it can be determined by filtration in an inert atmosphere, drying and weighing. The presence of sulphur in the solution was found to have a minimal effect on the analysis of an alkaline mixture of dithionite, thiosulphate and sulphite. This is best illustrated by comparing the data for an equimolar mixture (runs 10–10C) or for a non-equimolar mixture of the oxy-sulphur compounds (runs 1 and 1A). The expected formation of thiosulphate from a sulphur–sulphite reaction was not detected. This reaction may be dependent on other factors, such as temperature, oxygen content of the solution or the nature of the colloidal sulphur dispersion. The decrease in sulphite (compare runs 10 and 10A) appears to be due to errors associated with

Table 4. Analysis of commercial J. T. Baker sodium dithionite* (0.05*M* solution in 0.1*M* NaOH)

T, *C*	$S_2O_4^{2-}$, %	$S_2O_3^{2-}$, %	SO_3^{2-}, %
0	89.3	2.53	7.1
26	88.8	2.58	7.1
82†	84.2	—	—

* See Table 1.

† Analysis at high temperatures is predictably poor, owing to accelerated dithionite decomposition. Results are strongly dependent on the solution time. The value given represents solution standing times varying over 30 min.

Table 5. Effects of NaOH concentration on the rate of decomposition of a 0.05*M* dithionite solution and the product/reactant ratio for 25% decomposition at 26°C

[NaOH], *M*	$t_{1/4}$*, *hr*	$\frac{S_2O_3^{2-}}{S_2O_4^{2-}}$
2.0	120	0.08
0.5	840	0.32
0.1	1856	0.4

* Time taken for 25% decomposition of the sodium dithionite.

calculating the sulphite by difference, from the three titrations.

One objective of this work was to determine whether components such as CH_3CN, CN^-, AsF_6^-, Br^-, characteristic of Li/SO_2 cells, would affect the determination of oxy-sulphur compounds formed during discharge of such a cell. The data in Table 3 indicate that no adverse affects occur during the analysis.

Table 4 summarizes the effects of temperature on the analysis of samples of J. T. Baker sodium dithionite in alkaline solution. Comparison with direct analysis of solid samples of J. T. Baker sodium dithionite (Table 1) indicates a slight decrease in the percentage of dithionite, $\sim 1\%$ at 26° and $<1\%$ at 0°. This is attributed to a combination of some decomposition in alkali, together with difficulty in completely removing oxygen from the sodium hydroxide solution.

Kinetic studies on the rate of dithionite decomposition at 26° revealed that increasing either the initial dithionite or hydroxide concentration increases the rate of decomposition. Table 5 illustrates the effect of alkali concentration on the rate. The product/reactant ratio appears to be dependent on the initial ratio of dithionite to alkali concentration. An equimolar solution of dithionite and sodium hydroxide behaved differently from solutions containing excess of alkali. The equimolar solution reacted slowly at first then increasingly faster. This was attributed to a corresponding drop in pH, slow at first then increasingly more rapid. After 100% reaction, the measured product/reactant ratio for thiosulphate and sulphite was 0.5 and 1.0 respectively, indicative of the reaction

$$2Na_2S_2O_4 + 2NaOH \rightarrow Na_2S_2O_3 + 2Na_2SO_3 + H_2O \quad (10)$$

However, with excess of alkali present this reaction was not observed, as the data in Table 5 indicate. With excess of alkali, the mechanism appears complicated by the transient formation of some sulphide and the appearance of a flocculent precipitate. Studies at 82° are in progress and will be reported elsewhere.[18]

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ELECTRONIC SPECTRA AND STRUCTURE OF SOME PHENOXAZONE-TYPE DYES

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Summary—Quantum-mechanical calculations of the electronic structure and spectra of a number of phenoxazone dyes are given. The distribution analysis of π-electron density in the ground and excited states makes clear the origin of the colour of these compounds and gives the possibility of direct assessment of organic reagents in terms of colour contrast and sensitivity of analytical reactions. This is illustrated for a series of dihydroxy derivatives of phenoxazone dyes.

Phenoxazone dyes (Fig. 1) are widely used in analytical chemistry, as acid–base[1–6] and redox indicators.[7–10] Many hydroxy and carboxylic derivatives of these dyes form complexes with metal ions and thus are applied in inorganic analysis for the detection and determination of metals. The phenoxazone dye reagents are used both in photometric methods and in titrimetry as metallochromic indicators. For instance, gallocyanine has been used as reagent for Sb(III), Hg(II), Pb[11,12] and Zr[13–15] and floreine for UO_2^{2+},[16] Hg(II),[17] Hg(I) and Ag.[18] Sulpho derivatives of phenoxazone dyes of the Alizarin Green type are used in aqueous medium, for instance, to determine Mo(VI)[19] (with use of surfactants), V(V)[20] and U(VI).[21] Dyes of this type are also applied in organic analysis to determine functional groups[22,23] and in pharmaceutical chemistry.[24,25]

Figure 1 shows the molecular nuclei of different phenoxazone dyes on the basis of which many analytical reagents can be synthesized by appropriate substitution with groups such as —OH, —COOH, $—NH_2$, *etc.* Up to now an empirical approach has been taken, based largely on the case of synthesis. The application of quantum-mechanical calculations, however, would allow selection of the most promising reagents (from the enormous number possible) on the basis of knowledge of the electronic structure and nature of the electronic transitions of the molecules.

With this end in view our paper gives the calculations of the electronic structure of a number of phenoxazone dyes and their derivatives in the ground and two lowest excited states. The calculations were performed in the π-approximation by the semi-empirical Pariser–Parr–Pople[26] approach to the MO LCAO SCF method. The excited singlet states were calculated, taking into account configuration interaction; 20 mono-excited configurations were considered. The following empirical data were used in the calculations, *viz.* the molecules were assumed to be planar and the bond lengths and valence angles were taken as the mean values for a great number of organic molecules.[27] The ionization potentials of the valency orbitals for the neutral molecules were those used by Hinze and Jaffe.[28] The two-centre coulomb integrals were calculated according to the formulae given by Mataga and Nashimoto.[29] In considering the ionization potentials and coulomb integrals of the oxygen atoms in the anions the dissociation mechanism[30] is taken into account: the data listed correspond to anions in which the oxygen–hydrogen bond of the hydroxyl group is broken, but the proton is still held by a strong hydrogen bond (this is analogous to a transition-state species). A Fortran-IV program composed by the authors was used for the calculations.

Table 1 compares the calculated and experimental wavelength for the maxima of the absorption bands of the longest-wavelength transitions in the molecules of the phenoxazone dye series. As seen from the table, in all cases except phenoxazone-3 the difference does not exceed 20 nm. In the case of phenoxazone-3 the interesting fact is to be emphasized that both experiment and calculation indicate a bathochromic shift of the longest-wavelength band in the transition from benzo[*a*]phenoxazone-5 to phenoxazone-3. It may be explained by the different localizations of the molecular orbitals of both molecules in the ground state. In benzo[*a*]phenoxazone-5 the atomic orbitals of carbon atoms 3 and 4 (see Fig. 1) do not make a substantial contribution to the twelfth MO (the upper occupied MO), but belong to the benzo[*a*] condensed ring. The atomic orbital of carbon atom 5 belongs to the localized carbonyl bond (atoms 5 and 15). Phenoxazone-3 has a similar localized bond (atoms 5 and 15) but atoms 3 and 4 contribute considerably to the eighth

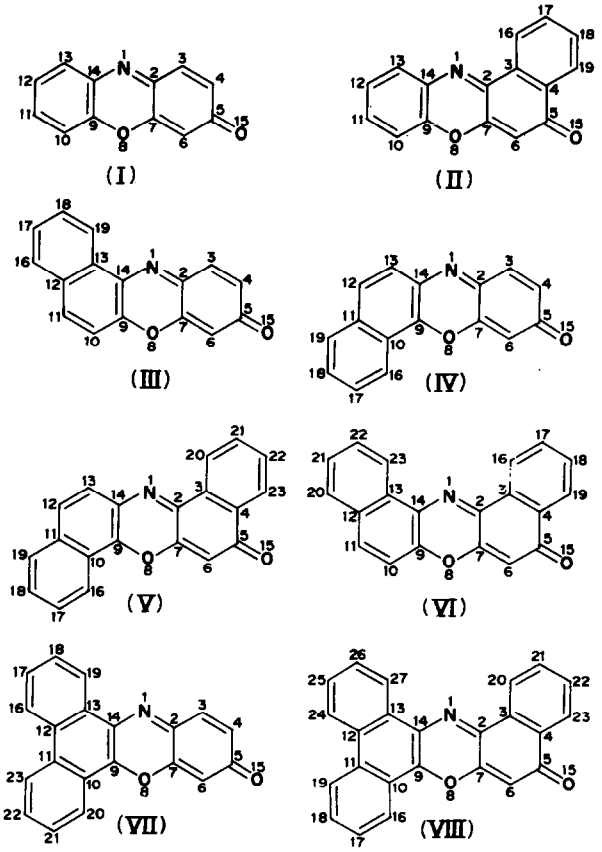

Fig. 1. Formulae of the molecular nuclei of phenoxazone dyes.

molecular orbital (the upper occupied MO) and that makes the eighth orbital more delocalized. The relatively large deviation between calculation and experiment for phenoxazone-3 ($\Delta\lambda = 28$ nm) is probably associated with distortion of the planar structure of this molecule.

As the nature of electronic spectra is determined by the value and direction of electron-density transfer in the molecules during excitation, Table 2 gives the calculated values of the electron density in the ground state and shows how they change during excitation. By analysis of the electron-density change which occurs when the molecule is excited, the positions can be determined at which the donor and acceptor groups intensify the change of electron density in the dye molecule nucleus. For instance, π-donor substituents such as —OH, —NH_2 will intensify the initial change of the electron density if they are introduced into the positions where the electron density decreases during excitation. In addition, two other factors should be taken into account. First, the direction of the electron-density transfer connected with excita-

Table 1. Wavelengths of the absorption maxima (nm) of the two longest wavelength bands. λ^E and λ^T are the experimental and theoretical values, respectively.[31,32] In brackets are given the calculated oscillator strengths (f_{ON}) for the theoretical values and the logarithms of the molar absorption coefficients (log ϵ_N)[31,32] for the experimental values

Dye	$\lambda_1^E(\log \epsilon_1)$	$\lambda_1^T(f_{01})$	$\lambda_2^E(\log \epsilon_2)$	$\lambda_2^T(f_{02})$
I	458 (4.00)	486 (0.56)	360 (4.10)	397 (0.67)
II	439 (4.12)	444 (0.92)	370 (4.06)	372 (0.22)
III	505 (4.27)	520 (1.14)	407 (3.76)*	385 (0.24)
IV	505 (3.95)	506 (0.82)	376 (3.87)	391 (0.08)
V	490 (4.18)	470 (0.96)	376 (4.01)	370 (0.02)
VI	490 (4.32)	491 (1.13)	387 (3.72)*	374 (0.09)
VII	—	525 (1.01)	—	384 (0.06)
VIII	—	495 (1.03)	—	368 (0.08)

* Values measured in 96% EtOH medium, the other experimental values were measured in 50% EtOH medium.

tion; *i.e.*, the value of the electron-density transfer is to be considered as a vector. Secondly, the substituent introduced can change the nature of the electron transfer considerably and then the data on the molecular nucleus become non-valid and the substituted molecule should be considered as a new chromophoric system.

From the nature of the two longest wavelength transitions (Table 2; the numbering of the atoms is in accordance with Fig. 1 and does not correspond to the chemical nomenclature) for the dye molecules studied, the following conclusions may be drawn.

Phenoxazone-3 (I)

From the changes in the electron density which correspond to the first* transition it is observed that only in the 3- and 4-positions† does the π-electron density increase; in other positions it decreases. According to the donor effect the positions in the molecule can be ordered as follows: 6 > 10 > 12 > 13 > 11. Table 2 shows that the effect of the acceptor groups will be much smaller than that of the donor groups. These conclusions are verified by the reagents currently used in practice: gallocyanine (1-carboxy-4-hydroxy-7-dimethylaminophenoxazone-3),[11–15] prune(methylgallocyanine),[34] floreine (1,7,9-trihydroxyphenoxazone-3).[16–18,35] As evident from our calculations, floreine must have the smallest molar absorptivity, as one of the hydroxyl groups counteracts the direction of the electron-density transfer in the molecular nucleus.

Similar conclusions may be drawn concerning the second band. The difference is that there is no position where the electron density increases, and the positions with decrease in electron density alter in another sequence: 10 > 13 > 12 ~ 6. It should be noted that the changes of electron density corresponding to the second transition are greater than those for the first transition.

Benzo[a]*phenoxazone*-5 (II)

In this molecule, in all the active locations the electron density decreases during the first excitation, in the order 6 ≫ 11 ~ 12. In the 6-position, donor substituents will exert a greater effect than in the case of phenoxazone-3. Also, a donor substituent in the 6-position makes the first band considerably stronger and the second band a little weaker. In phenoxazone-3 a donor substituent in the 6-position makes both bands stronger but the first is strengthened more than the second. The general conclusions on the spectra of this molecule agree with the fact that reagents of the Alizarin Green group, *e.g.*, 6-hydroxy-10-sulphobenzo[*a*]phenoxazone-5,[19–21] are used analytically.

Benzo[a]*phenoxazone-9* (III)

As evident from Table 2, except for the 3-position for the first band and the 11-position for the second band, in all the positions into which the substituent can be introduced the electron density decreases. For the first band it corresponds to series 6 > 19 > 11 > 17 > 16 > 10 ~ 4 > 8 and for the second band to series 10 > 19 > 16 ~ 17 > 18 > 3 ~ 4 ~ 6. The effect of the substituents on both of the bands is opposite in the 3-position and in the 11-position. The large changes of electron density observed in the benzo[*a*] condensed ring in the second excited state are of interest.

Benzo[c]*phenoxazone-9* (IV)

This molecule has several positions in which the electron density increases during excitation. For the first band an acceptor substituent will exert the greatest effect in the 4-position and a donor substituent in the 6-position. For the second band the greatest influence of an acceptor is in the 4-position and of a donor in the 13-position. It is of interest that for the first band in the 13-position the electron density increases. The effect of donor substituents on the first band must decrease in the order 12 > 16 > 18 ~ 19 > 7 and on the second band in the series 13 > 17 > 19 > 16 > 12 > 6 > 18.

Dibenzo[a,h]*phenoxazone-5* (V)

The decrease of electron density with transition to the first excited state becomes weaker in the order 6 > 12 > 16 > 18 ~ 19 > 17. The increase in electron density, and therefore the effect of acceptor groups on the first band, becomes weaker in the series 13 ~ 22 > 20 ~ 21 > 23. The effect of substituents on the second band becomes weaker in the order 13 > 6 > 17 > 19 > 21 ~ 16 for donors and 20 > 12 ~ 23 > 22 > 18 for acceptors. It is of interest that on one of the condensed rings, *a*, the electron density increases with excitation and on the other, *h*, it decreases, *i.e.*, in order to increase the intensity of the first band, acceptors must be introduced into the *a*-ring and donors into the *h*-ring.

Dibenzo[a,j]*phenoxazone-5* (VI)

As evident from Table 2 the increase in electron density and hence the effect of donor substituents must decrease in the order 6 > 23 > 11 > 20 > 21 > 22 > 10 > 16 and for the second band in the series 10 > 22 > 20 > 23 > 21 > 17 > 18 > 6. For acceptor substituents the intensity of the first band must decrease in the series 17 > 18 ~ 19 and for the second band in the order 11 > 16 > 19. These conclusions agree with the fact that in analytical practice dyes of the Alizarin Green type are widely used, *e.g.*, 6-hydroxysulpho derivatives of this molecule.[19–21]

Dibenzo[a,c]*phenoxazone-11* (VII)

In decreasing effect of donor substituents on increase of the intensity of the first band, the substi-

* Numbering of the transitions starts with the long-wavelength side.

† In all cases the term "position" refers to sites available for substitution; a negative value for Δ_{ON} represents an increase in electron density.

tutional positions may be set in the order 6 > 19 > 17 > 22 > 23 > 16 ~ 4 > 20 > 21 and for the second band 18 ~ 21 > 4 ~ 6 ~ 16 > 20 ~ 22 ~ 3. Acceptor substituents make the first band stronger only when in the 3-position.

Tribenzo[a,c,j]*phenoxazone-11* (VIII)

The greatest intensity increase of both long wavelength bands can be obtained by introducing donor substituents in the 6-position. In general the *a*- and *c*-rings intensify both bands when electron-donor substituents are introduced, and the *j*-ring does so when the electron-acceptor substituents are introduced.

Analysis of the electron-density change which occurs during excitation of the dye molecules may be made the basis for planned synthesis of reagents giving good-quality analytical reactions with metals. So that the functional introduced groups do not cause deterioration of the chromophoric properties of the nucleus of a dye molecule, π-donor substituents should be introduced in the positions at which the electron density decreases during excitation; if the

Table 2. π-Charge values on the atoms in the ground state of the molecules I–VIII and corresponding changes of charges during excitation; Δ_{0N} is the charge difference between the ground and corresponding N-excited state

Molecule number / Atom number	I			II			III		
	Ground state	*Excited state* Δ_{01}	Δ_{02}	Ground state	*Excited state* Δ_{01}	Δ_{02}	Ground state	*Excited state* Δ_{01}	Δ_{02}
1	−0.174	−0.138	−0.154	−0.204	0.092	−0.145	−0.224	−0.303	−0.341
2	0.152	−0.054	−0.069	0.165	−0.051	−0.082	0.158	0.097	0.070
3	0.024	−0.019	—	−0.008	−0.016	0.039	0.006	−0.023	0.023
4	0.063	−0.002	0.008	0.008	0.008	0.006	0.059	0.015	0.024
5	0.191	−0.077	−0.077	−0.208	−0.087	−0.089	0.047	0.006	0.012
6	0.025	0.096	0.050	0.022	0.104	−0.004	0.012	0.086	0.024
7	−0.118	−0.035	−0.030	−0.107	−0.055	−0.051	−0.134	−0.153	−0.138
8	0.243	+0.036	0.067	0.235	0.038	0.065	0.230	0.253	0.251
9	−0.138	0.009	−0.007	−0.148	0.032	−0.002	−0.150	−0.130	−0.185
10	0.027	0.047	0.121	0.027	0.004	0.157	0.017	0.019	0.109
11	0.015	0.012	0.001	0.006	0.029	−0.002	0.003	0.032	−0.017
12	0.023	0.029	0.055	0.021	0.021	0.066	0.009	0.010	0.057
13	0.014	0.022	0.080	0.006	−0.003	0.114	−0.004	−0.007	0.042
14	0.075	0.052	0.023	0.077	0.068	0.014	0.094	0.157	0.106
15	−0.420	0.022	−0.069	−0.422	0.009	−0.113	−0.146	−0.157	−0.309
16	—	—	—	0.026	0.014	−0.003	−0.001	0.022	0.068
17	—	—	—	0.019	−0.010	−0.001	0.007	0.024	0.067
18	—	—	—	0.017	0.001	0.039	0.001	0.010	0.059
19	—	—	—	0.053	0.003	−0.007	0.015	0.042	0.080

Molecule number / Atom number	IV			V			VI		
	Ground state	*Excited state* Δ_{01}	Δ_{02}	Ground state	*Excited state* Δ_{01}	Δ_{02}	Ground state	*Excited state* Δ_{01}	Δ_{02}
1	−0.223	−0.088	−0.049	−0.236	−0.079	−0.010	−0.207	−0.110	−0.138
2	0.161	−0.066	−0.057	0.173	−0.076	−0.052	0.164	−0.050	−0.098
3	0.006	−0.029	−0.003	−0.012	−0.017	0.002	−0.008	−0.019	0.014
4	0.060	−0.052	−0.028	0.017	−0.023	0.003	0.010	−0.018	0.015
5	0.048	−0.043	−0.030	0.056	−0.049	−0.033	0.204	−0.074	−0.077
6	0.013	0.080	0.012	0.016	0.082	0.041	0.014	0.092	0.007
7	−0.134	−0.024	−0.028	−0.131	−0.027	−0.031	−0.111	−0.056	−0.029
8	0.235	0.043	0.012	0.230	0.061	0.039	0.244	0.002	0.021
9	−0.154	0.040	−0.026	−0.160	0.057	−0.017	−0.146	0.020	−0.039
10	0.017	0.004	0.020	0.019	−0.002	0.010	0.018	0.008	0.105
11	−0.002	0.003	0.028	−0.003	0	0.027	0.011	0.040	−0.030
12	0.022	0.032	0.018	0.021	0.036	−0.014	0.009	0.001	0.056
13	−0.001	−0.012	0.082	−0.003	−0.019	0.053	−0.003	−0.010	0.030
14	0.091	0.055	−0.002	0.090	0.060	−0.007	0.091	0.086	0.005
15	−0.143	−0.014	−0.059	−0.150	−0.020	−0.007	−0.429	0.001	−0.060
16	−0.009	0.024	0.022	−0.010	0.028	0.002	0.024	0.003	−0.014
17	0.006	0.007	0.049	0.005	0.002	0.025	0.017	−0.016	0.022
18	0.001	0.021	0.002	−0.001	0.019	−0.003	0.017	−0.005	0.009
19	0.006	0.020	0.039	0.005	0.020	0.008	0.052	−0.005	−0.005
20				0.030	−0.014	−0.017	0.002	0.036	0.056
21				0.008	−0.012	0.003	0.009	0.019	0.037
22				0.018	−0.018	−0.008	0.005	0.012	0.061
23				0.019	−0.009	−0.013	0.014	0.043	0.053

Table 2 (*Continued*)

Molecule number / Atom number	VII			VIII		
	Ground state	*Excited state* Δ_{01}	Δ_{02}	Ground state	*Excited state* Δ_{01}	Δ_{02}
1	−0.225	−0.313	−0.309	−0.237	−0.078	−0.011
2	0.161	−0.090	0.097	−0.174	−0.077	−0.042
3	0.004	−0.029	0.005	−0.013	−0.016	0.004
4	0.063	0.010	0.038	0.019	−0.025	0.005
5	0.046	0.007	0.013	0.055	−0.043	−0.037
6	0.007	0.096	0.038	0.009	0.086	0.067
7	−0.137	−0.156	−0.158	−0.133	−0.026	−0.037
8	0.241	0.269	0.258	0.235	0.039	0.051
9	−0.154	−0.129	−0.198	−0.161	0.040	−0.004
10	0.014	0.020	0.049	0.015	0.002	0.018
11	0	0.005	0.030	−0.002	0.006	0.024
12	0.010	0.014	0.041	0.010	0.006	−0.006
13	−0.007	−0.011	0.010	−0.007	−0.006	−0.010
14	0.103	0.164	0.081	0.102	0.069	−0.032
15	−0.151	−0.152	−0.231	−0.156	−0.011	0.020
16	−0.001	0.010	0.036	−0.010	0.017	−0.004
17	0.008	0.023	−0.001	0.003	−0.002	0.016
18	0.001	0	0.057	0	0.017	0.005
19	0.016	0.038	0.023	0.004	0.006	0.001
20	−0.009	0.007	0.009	0.029	−0.013	−0.018
21	0.004	0.003	0.056	0.007	−0.012	0.001
22	0.002	0.019	0.007	0.018	−0.018	−0.004
23	0.004	0.012	0.052	0.019	−0.006	−0.017
24	—	—	—	−0.002	0.009	0.020
25	—	—	—	0.007	0.015	−0.027
26	—	—	—	−0.001	−0.002	0.023
27	—	—	—	0.015	0.023	−0.005

Table 3. Calculated positions of the longest-wavelength maxima of the bands of neutral and anionic forms of mono- and dihydroxy derivatives of dibenzo[*a,h*]- and dibenzo[*a,j*]phenoxazone-5 (V and VI, Fig. 1) and phenoxazone-3 (I, Fig. 1) (f_{0N}—the oscillator strength)

Acid–base form of reagent		H_2L (HL)				L^{2-} (L^-)			
Molecule number (Fig. 1)	Positions of hydroxy-groups	λ_1, *nm*	f_{01}	λ_2, *nm*	f_{02}	λ_1, *nm*	f_{01}	λ_2, *nm*	f_{02}
I	4	490	0.26	441	0.94	558	0.20	455	0.94
	6	555	0.37	397	0.77	731	0.23	393	0.63
	3, 4	523	0.05	453	1.08	845	0.07	457	0.82
	10, 11	489	0.67	439	0.49	630	0.27	497	0.85
	11, 12	498	0.67	414	0.59	573	0.68	449	0.60
	12, 13	494	0.56	446	0.48	646	0.10	506	0.97
II	6	484	0.67	376	0.40	592	0.40	379	0.33
V	6	504	0.73	377	0.14	607	0.45	407	0.06
	16, 17	480	0.87	396	0.26	651	0.22	449	0.99
	17, 18	474	1.01	392	0.17	502	0.91	490	0.26
	18, 19	477	0.99	384	0.18	573	0.60	424	0.65
	19, 12	478	0.87	390	0.24	554	0.43	416	0.78
	12, 13	479	0.86	425	0.30	680	0.05	487	1.13
	20, 21	480	0.89	398	0.05	579	0.45	437	0.37
	21, 22	471	0.91	401	0.27	567	0.06	471	1.03
	22, 23	480	0.99	403	0.03	576	0.75	445	0.13
VI	6	523	0.91	388	0.29	622	0.57	400	0.38
	11, 20	509	1.27	382	0.04	555	1.37	399	0.05
	20, 21	499	1.18	378	0.05	605	0.64	438	0.71
	21, 22	498	1.19	386	0.05	536	0.95	493	0.22
	22, 23	512	1.05	388	0.02	752	0.34	446	0.79
	10, 11	505	1.22	400	0.01	618	0.72	464	0.62
	16, 17	502	0.99	396	0.12	588	0.46	444	0.97
	17, 18	491	1.10	406	0.20	572	0.07	489	1.17
	18, 19	502	1.14	401	0.05	586	0.81	448	0.30

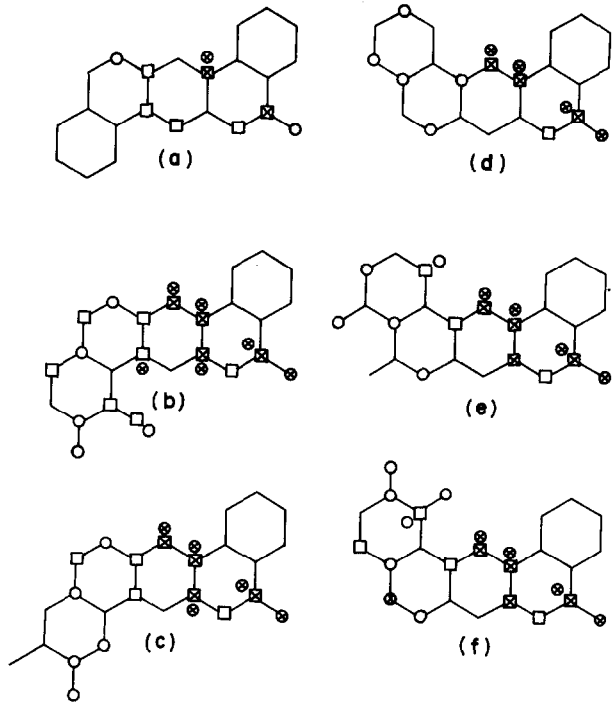

Fig. 2. Changes of the electron-density distribution in transition from the ground to the first (Δ_{01}) or second (Δ_{02}) excited state. Only the atoms with π-electron density decreasing or increasing by more than 0.05 during excitation are shown. □ $\Delta_{01} \geqslant 0.050$; ⊠ $\Delta_{01} \leqslant 0{\cdot}050$; ○ $\Delta_{02} \geqslant 0.050$; ⊗ $\Delta_{02} \leqslant 0{\cdot}050$.

electron density increases, π-acceptors should be introduced. In addition, it is necessary to take into account the polarizability of the ground and excited states. Slight polarizability will lead to a considerable change in chromophoric nature when substituents are introduced and it then becomes impossible to predict the spectral properties of the reagents from knowledge of the chromophoric nature of the molecular nuclei. To elucidate the effect of substituents on the chromophoric nature of the dyes investigated, the electronic structure and spectra of some mono- and dihydroxy derivatives of phenoxazone-3 (I), dibenzo-[*a,h*]phenoxazone-5 (V) and dibenzo[*a,j*]phenoxazone-5 (VI) were calculated. The spectral properties of these molecules are summarized in Table 3. The table indicates that the spectral properties of some of the derivatives agree with the conclusions made on the basis of study of the chromophoric nature of the molecular nuclei, for instance, the 6-hydroxy derivatives of molecules V and VI. The calculated spectral properties of these derivatives are in good agreement both with experiment[19–21] and predictions made from the properties of the molecular nuclei. The table indicates, however, that frequently the calculated spectral properties of dihydroxy derivatives cannot be explained in terms of the chromophoric nature of the corresponding molecular nuclei, because this is conditioned by the large polarization of the initial molecules, caused by the effect of the hydroxyl groups, which change sufficiently the electron-density distribution in the ground and excited states. For greater clarity Fig. 2 shows the changes of electron density which characterize the chromophoric nature of molecules V and VI and some of their dihydroxy derivatives. As evident from Fig. 2 there are comparatively small changes in distribution of the donor and acceptor centres in dibenzo[*a,j*]phenoxazone-5 and its 11,20-dihydroxy derivative (Fig. 2,d,e). This leads to the fact that in comparison with molecule VI, the longest wavelength band becomes stronger in the derivative whereas the intensity of the second band changes but little, in agreement with the conclusions drawn from the data on the spectral properties of molecule VI. In other dihydroxy derivatives (Fig. 2,b,c,f) there is a considerable change in chromophoric nature from that of the parent molecular nuclei. Therefore the calculated spectral properties of these dihydroxy derivatives cannot be understood from analysis of the chromophoric nature of the initial molecules. The molecules of these dihydroxy derivatives should be considered as independent chromophore systems. A strong influence of substituents on the chromophoric nature is evident in the case of the derivatives of phenoxazone-3. In 6-hydroxyphenoxazone-3 and its anion the intensity of the second band is stronger than that of the first band, which indicates reformation of the chromophore of phenoxazone-3. However, introduction of a carboxylic group in the 3-position, a dialkylamino group in the 11-position or an arylamino group in the 4-position leads to the longest wavelength band[33] again becoming the most intense in these derivatives.

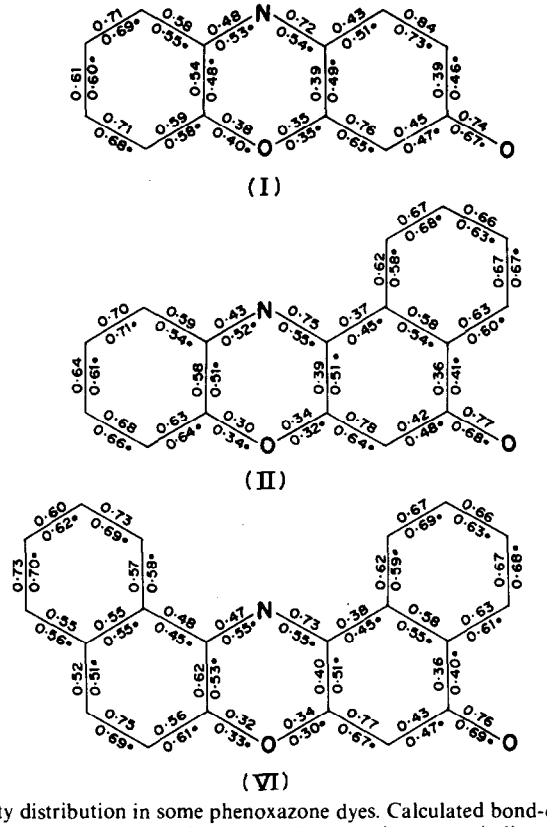

Fig. 3. π-Electron density distribution in some phenoxazone dyes. Calculated bond-orders in the ground and first excited states are shown. Values for the excited state are indicated by dots.

Some peculiarities of the properties of phenoxazone dye molecules may be elucidated from the molecular diagrams given in Fig. 3. The bathochromic shift of the longest wavelength band with transition from benzo[*a*]phenoxazone-5 to phenoxazone-3 can be explained by the deterioration of the quinonoid structure with transition to benzo[*a*] derivatives. From the diagrams shown, the slight polarizability of the π-electron cloud of these molecules and the reformation of the chromophores may be realized. For these molecules there are no well-defined boundary structures characterizing conjugation between the donor and the electron-acceptor which are peculiar to the typical dyes. This statement is more valid for the excited states.

Thus, the calculations made assist not only in realizing why derivatives of phenoxazone dyes appear to be the most effective in analytical practice, but also in projecting researches on new, more effective analytical reagents. In particular, Table 2 indicates that 18,19- and 22,23-dihydroxy derivatives of the dye V, 11,20-, 10,11- and 18,19-dihydroxy derivatives of the dye VI and 11,12-dihydroxyphenoxazone-3 should have good analytical properties (numbering of atoms and dyes as in Fig. 1).

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SHORT COMMUNICATIONS

NEW TITRANTS FOR PERCHLORATE AND FLUOROBORATE*

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Summary—Three new titrants have been evaluated for the precipitation titration of perchlorate: cetylpyridinum chloride (CPC), cetyltrimethylammonium chloride (CETAC), and benzyldimethyltetradecylammonium chloride (BDTAC). CETAC yielded the highest precision and largest potentiometric break, closely followed by CPC. BDTAC produced considerably lower precision and smaller breaks. Fluoroborate was similarly determined with CPC and CETAC. Again CETAC yielded the highest precision and largest potentiometric break. Titrations were monitored with a fluoroborate ion-selective indicator electrode and a double-junction reference electrode. The perchlorate and nitrate ISEs may also be used for monitoring the emf. Both titrants are considerably less expensive than equivalent amounts of the commonly used tetraphenylarsonium chloride titrant.

We have previously reported use of a new titrant, cetyltrimethylammonium bromide (CETAB), for the precipitation titration of perchlorate.[1] This titrant is more sensitive and its cost is less than 1/70 of that of equivalent amounts of the commonly used tetraphenylarsonium chloride (ϕ_4AsCl). The titrant strength used was 0.05*M*. Recent studies have shown that about 1 week after preparation of the titrant, crystals of CETAB will precipitate, thus causing a decrease in titre. For this reason we have investigated similar titrants which are stable indefinitely at ambient temperature.

In this paper we report the potentiometric precipitation titration of perchlorate with 0.05*M* cetylpyridinium chloride (CPC), cetyltrimethylammonium chloride (CETAC), and benzyldimethyltetradecylammonium chloride (BDTAC) and of fluoroborate with CPC and CETAC. Titrations were monitored with a fluoroborate ion-selective indicator electrode (ISE), and a double-junction reference electrode.

EXPERIMENTAL

Reagents

The titrants were approximately 0.05*M* aqueous solutions of cetyltrimethylammonium chloride (Aldrich Chemical Co.), cetylpyridinium chloride monohydrate (Aldrich Chemical Co.), and benzyldimethyltetradecylammonium chloride monohydrate (Fluka). The titrants were prepared by dissolving the required amounts in hot water and diluting to volume with cold distilled water. They were standardized against ammonium perchlorate (Fisher Certified reagent). The sodium tetrafluoroborate used was Baker and Adamson technical grade.

* Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore Laboratory under contract number W-7405-Eng-48.

Apparatus

The titration system was controlled by a Tektronix 4051 graphics system, as previously described.[2] The emf was monitored with an Orion model 93-05 fluoroborate ion-selective electrode and a double-junction reference electrode (salt bridge 0.1*M* ammonium fluoride). The Orion 93-81 perchlorate, 93-07 nitrate, and 93-20 calcium ISEs are also suitable for monitoring the emf.

Stirring was provided by a magnetic stirrer. The stirring motor was separated from the titration vessel by a water-cooled plate and an earthed aluminium plate.

Procedure

Samples were pipetted into a 50-ml beaker containing a Teflon-covered stirring bar. They were diluted with distilled water to 25 ml before titration. In all titrations the titrant was added at 0.5 ml/min. Titrations were performed at ambient temperature (23 ± 1).

Titration end-points were calculated according to Savitsky and Golay.[3] A convolute was used for a third-order second derivative based on 25 points. The zero crossing was found by linear interpolation near the change of sign.

RESULTS AND DISCUSSION

We have previously reported the use of a new titrant, cetyltrimethylammonium bromide (CETAB), at 0.05*M* concentration, for the precipitation titration of perchlorate.[1] We have since found that this concentration is near the limit of solubility at ambient temperature. After about a week crystals of CETAB precipitate, causing a decrease in titre. Therefore, in subsequent work with CETAB a concentration of 0.01*M* was used.[4]

The present work was undertaken with the purpose of finding similar titrants which can be used at concentrations of $\geqslant$0.05*M*. Suitable compounds were cetyltrimethylammonium chloride (CETAC), cetylpyridinium chloride (CPC), and benzyldimethyltetradecylammonium chloride (BDTAC). A comparison of

Table 1. Titration of 0.25 mmole of ammonium perchlorate, by use of the fluoroborate ISE: comparison of titrants

Titrant	Mean molarity	Standard deviation	Number of replicates	Mean end-point break, *mV*
CETAC	0.05281	0.00005_7	5	150
CPC	0.05016	0.00008_5	5	135
BDTAC	0.04447	0.00032_7	5	125

Table 2. Statistics for the titration of various amounts of ammonium perchlorate with 0.05*M* CPC

Ammonium perchlorate taken, *mg*	Ammonium perchlorate found, *mg*	Mean recovery, %	Number of replicates	Standard deviation, %
7.214	7.24_5	100.4_3	6	0.0_9
12.023	11.94_4	99.3_4	3	0.1_3
16.832	16.83_0	99.9_9	5	0.2_3
24.045	24.02_5	99.9_2	3	0.0_6

the precision obtained with these titrants by standardization against reagent-grade ammonium perchlorate is presented in Table 1. The highest precision was obtained with CETAC, closely followed by CPC, while BDTAC yielded considerably less precise results. Though the mean potentiometric end-point breaks are similar, they follow the same order as the precision, CETAC > CPC > BDTAC. The following compounds were also tried, but did not yield 0.05*M* solutions: cetylpyridinium bromide, cetyltrimethylammonium bromide, and cetyldimethylbenzylammonium chloride. Tetradodecylammonium bromide and tetraoctylammonium bromide were not soluble in water even at the 0.01*M* level. We have, therefore, limited ourselves in our experimental work to CPC and CETAC. These compounds form very stable 0.1*M* solutions.

Statistics for the titration of various amounts of ammonium perchlorate with 0.05*M* CPC are given in Table 2. Representative computer-generated titration curves are shown in Fig. 1. The optimum pH for this titration is from 2.2 to 11.0. Titrations are feasible between pH 1.1 and 12.2, but smaller end-point breaks are obtained at the extreme pH-values. If titrations at these extreme pH-values are required, the titrant should be standardized at the same pH.

For the perchlorate titration with CETAC the optimum pH is between 1.65 and 12.2, a somewhat wider range than for CPC. The titration is feasible down to pH 1.35, yielding smaller end-point breaks at the extreme.

The CPC and CETAC solutions were also used in the titration of technical grade sodium fluoroborate. Some representative titration curves are presented in Fig. 2. The statistics are summarized in Table 3. For the determination of fluoroborate with CETAC the optimum pH is between 2.4 and 11.0, while titrations are feasible between pH 1.6 and 12.5. As for perchlorate, at the extreme pH-values the potentiometric breaks are smaller. If it is necessary to determine fluoroborate at such pH-values, standardization of the titrant at the same pH is recommended.

For the titration of fluoroborate with CPC the optimum pH is between 3 and 9, a somewhat smaller range than for CETAC, analogous to the perchlorate determination. The titration is feasible from pH 1.7 to 11.1. The same comments as for the CPC titration apply at the extreme pH-values. The fluoroborate

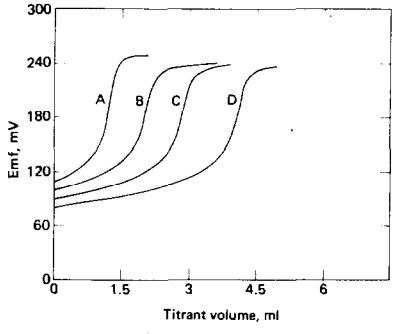

Fig. 1. Titration curves for various amounts of perchlorate with CPC (fluoroborate electrode). A, 7.2 mg; B, 12.0 mg; C, 16.8 mg; D, 24.0 mg of ammonium perchlorate.

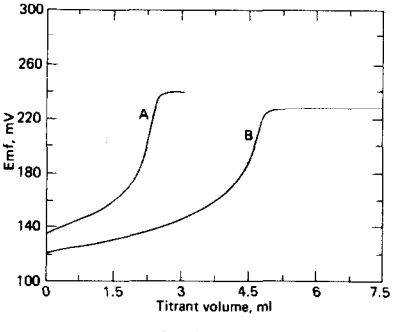

Fig. 2. Titration curves for fluoroborate with CPC (fluoroborate ISE). A, 13.7 mg; B, 27.4 mg of sodium fluoroborate.

Table 3. Statistics for the titration of fluoroborate with CPC and CETAC

Titrant	$NaBF_4$ taken, *mg*	Mean recovery %	Number of replicates	Standard deviation, %
CPC	13.723	93.9_7	6	0.1_2
CPC	27.445	93.9_1	4	0.0_5
CETAC	13.74	94.2_8	7	0.2_2
CETAC	27.48	94.3_7	6	0.1_8

salts are more soluble than the corresponding perchlorates. This is evident from the magnitude of the potentiometric breaks, a mean value of 120 mV with CETAC as titrant, and 95 mV with CPC.

On the basis of these results, CPC is the preferred titrant for perchlorate and for fluoroborate. If titrant cost is a consideration, CETAC may be preferred because its price is only 1/21 of that of equivalent amounts of CPC. A comparison of equivalent amounts of various titrants is presented in Table 4, which lists also the previously used CETAB and ϕ_4AsCl.

Some of the perchlorate and fluoroborate salts were isolated by filtration (Whatman No. 541 paper), washed with water, and air-dried. The precipitation solutions had to be heated to near boiling and then cooled to make the precipitates easy to filter off. Analytical data for the precipitates are shown in Table 5. The agreement with theory is quite good, confirming that the various titrants react on an equimolar basis with perchlorate and fluoroborate.

We have used the fluoroborate ISE in most of this work because we found previously that it yielded the highest precision.[1] However, the Orion 93-81 perchlorate ISE, the 93-07 nitrate ISE, as well as the 93-20 calcium ISE are also suitable for monitoring the emf. The 93-32 bivalent cation ISE yielded poorer titration curves, and poor precision. All the sensors mentioned function only in an aqueous medium, although extreme pH-values can be tolerated.

We must point out again that we make it a practice to initiate each series of titrations with several known perchlorate solutions. The first run of each day usually does not yield end-point breaks as large, and potentiometric breaks as sharp, as subsequent runs. However, this is also the case for the previously used perchlorate titration with ϕ_4AsCl.[5] The electrodes were stored in air between measurements, according to manufacturer's recommendations. Further work is required to find a method of conditioning the electrodes so that even the first run of a series is reliable.

Permanganate, hexafluoroarsenate, hexafluorophosphate, persulphate, tetrachloroplatinite, tetrachloroaurate, ferricyanide, tetrachloromercurate, tetrachlorothallate, hexachloro-osmate, and other similar anions were previously titrated with 0.01*M* CETAB.[4] They can possibly be determined at higher concentrations by titration with 0.05–0.1*M* CPC and CETAC.

Acknowledgement—The writer wishes to thank Lewis J. Gregory for the carbon, hydrogen, and nitrogen analyses.

Table 4. Cost comparison of 500 ml of 0.05*M* solutions of various titrants for perchlorate and fluoroborate

Titrant	Molecular weight	Supplier	Cost, $
Cetyltrimethylammonium bromide	364.5	Aldrich	0.33
Cetyltrimethylammonium chloride	320.0	Eastman	10.58
Cetylpyridinium chloride monohydrate	358.0	Aldrich	0.49
Benzyldimethyltetradecylammonium chloride monohydrate	368.1	Fluka	11.04
Tetraphenylarsonium chloride hydrochloride dihydrate	491.3	Aldrich	24.07

Table 5. Elemental analysis of various perchlorate and fluoroborate precipitates

Compound	Formula	Calculated, % C	H	N	Found, % C	H	N
(CP)ClO_4	$C_{21}H_{38}NClO_4$	62.43	9.48	3.47	62.4	9.3	3.4
(CP)BF_4	$C_{21}H_{38}NBF_4$	64.45	9.79	3.58	64.3	9.5	3.7
(CETA)ClO_4	$C_{19}H_{42}NClO_4$	59.43	11.02	3.65	59.7	10.9	3.9
(CETA)BF_4	$C_{19}H_{42}NBF_4$	61.45	11.40	3.77	61.7	11.4	3.7
(BDTA)ClO_4	$C_{23}H_{42}NClO_4$	63.94	9.80	3.24	64.0	9.7	3.2

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KINETIC DETERMINATION OF ULTRAMICRO AMOUNTS OF COPPER

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Summary—A new catalytic reaction is proposed and a kinetic method developed for the determination of ultramicro amounts of Cu(II) on the basis of its catalytic activity in oxidation of the 2-thiosemicarbazone of sodium 1,2-naphthoquinone-4-sulphonate by hydrogen peroxide in the presence of ascorbic acid. Under optimal conditions the sensitivity of the method is 0.25 ng/ml. The relative error is 4.8–18.2% for the concentration range 5–0.8 ng/ml. Most foreign ions do not change the rate of the catalysed reaction. Co^{2+} and I^- catalyse the reaction, Ni^{2+} extensively inhibits it, and in the presence of EDTA only the uncatalysed reaction takes place.

There are several indicator reactions that can be used as the basis of kinetic detection and determination of trace amounts of Cu(II) in solution.[1–6] Their sensitivity ranges from about 0.1 ng/ml to 1 μg/ml.

The oxidation of the 2-thiosemicarbazone of sodium 1,2-naphthoquinone-4-sulphonate (TNS) by hydrogen peroxide (at a concentration lower than $10^{-2}M$) at 20–25° produces a red-violet colour within 5–10 min. The colour is stable for several hours. This reaction is catalysed by trace amounts of copper, for which the sensitivity is 0.05 μg/ml. However, ascorbic acid is found to improve the sensitivity of the reaction by about two orders of magnitude. By use of a photocolorimeter for determining the reaction rate and selecting the optimal conditions, a catalytic kinetic method for the determination of ultramicro amounts of Cu(II) in solution has been developed. The sensitivity is 0.25 ng/ml, making this one of the most sensitive kinetic methods for copper determination.[1,4,6]

EXPERIMENTAL

Reagents

Hydrogen peroxide, 2*M*. The buffer was KH_2PO_4 and $Na_2HPO_4.2H_2O$.[7] TNS was synthesized by the condensation of sodium 1,2-naphthoquinone-4-sulphonate with thiosemicarbazone;[8] a $10^{-3}M$ solution of the reagent was used. A 10-μg/ml copper solution was prepared by suitable dilution of a copper sulphate solution that had been standardized electrogravimetrically. The 0.1*M* ascorbic acid was standardized by titration and adjusted with sodium hydroxide to about pH 6.5 before use; fresh solution was prepared every second day.

Analytical grade reagents, redistilled water and polyethylene vessels were used throughout.

Apparatus

A Lange photoelectric colorimeter, model J, equipped with a thermostatic system,[9] was used, with 25-mm cells and a green filter, λ_{max} 525 nm. The pH-meter was a Radiometer PHM 29b, with a combined glass-calomel electrode, GK 2311-C. All solutions were kept in a thermostatic water-bath.

Procedure

The initial concentration of each of the reactants in turn was systematically varied, the initial concentrations of the other reactants being kept constant.

The selected volumes of the reactants were put into a 50-ml standard flask, in the order TNS, buffer (5 ml), ascorbic acid, catalyst and water to make up exactly to a predetermined volume. The flask was kept in the thermostat for 10 min, then the solution was made up to the mark with hydrogen peroxide and water and vigorously shaken. The cell of the colorimeter was rinsed well and filled with the solution. The absorbance *A* was measured every 30 sec, for 5–10 min, the timing being started at the moment of hydrogen peroxide addition. Instead of the reaction rate (dc/dt), the quantity dA/dt (which is proportional to it) was measured:

$$\frac{dA}{dt} = \epsilon l \frac{dc}{dt} = \tan \alpha$$

where t is the time, ϵ is the molar absorptivity, l the cell

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path-length, c the concentration of the oxidation product, and tan α the slope of the linear part of the plot of A against t.

The initial concentrations of the reagent solutions after the dilution to 50 ml were: 4×10^{-5}–$2 \times 10^{-4}M$ TNS, 1×10^{-3}–$7 \times 10^{-3}M$ H_2O_2, 5×10^{-4}–$6 \times 10^{-3}M$ ascorbic acid, 0.25–5 ng/ml Cu(II).

The measurements were done at 25 ± 0.1°. The calibration curve was also plotted for a temperature of 20 ± 0.1°.

RESULTS AND DISCUSSION

The differential variant of the tangent method was used for processing the kinetic data, because there is a linear relation between the absorbance and time during the first 5–10 min.

To develop a method for determination of very low Cu(II) concentrations it was necessary to test the effect of ascorbic acid in the reaction system. The dependence of the reaction rate on ascorbic acid concentration is presented in Fig. 1. Ascorbic acid enhances the sensitivity of the copper-catalysed oxidation reaction by about two orders of magnitude, but it also catalyses the reaction in the absence of cupric ions (curve 2 in Fig. 1). The maximal difference between the reaction rates in the presence and absence of copper occurs at an ascorbic acid concentration of $3 \times 10^{-3}M$ (curve 3, Fig. 1). This concentration is optimal and was used in the further work.

Table 1. Accuracy and precision of Cu(II) determination

Taken, ng/ml	Found, ($\bar{x}$), ng/ml	n	S, ng/ml	W, %	G, %
5.00	5.08	6	0.23	4.5	4.8
2.00	2.01	6	0.24	12.1	12.6
0.80	0.83	6	0.14	17.4	18.2

$\bar{x}$ = mean value; n = number of determinations; S = standard deviation; W = coefficient of variation; G = relative error ($=100\ tS/\bar{x}\sqrt{n}$, where t is Student's t for 95% confidence).

There is obviously a complicated relation between tan α and pH, as a result of which the reaction between TNS, hydrogen peroxide and ascorbic acid will have a variable order with respect to hydrogen ions. The optimal pH-value, at which further work was done, was 6.6, at which there is the greatest difference between the reaction rates for catalysis by the Cu(II)/ascorbic acid combination and catalysis by ascorbic acid alone (Fig. 2).

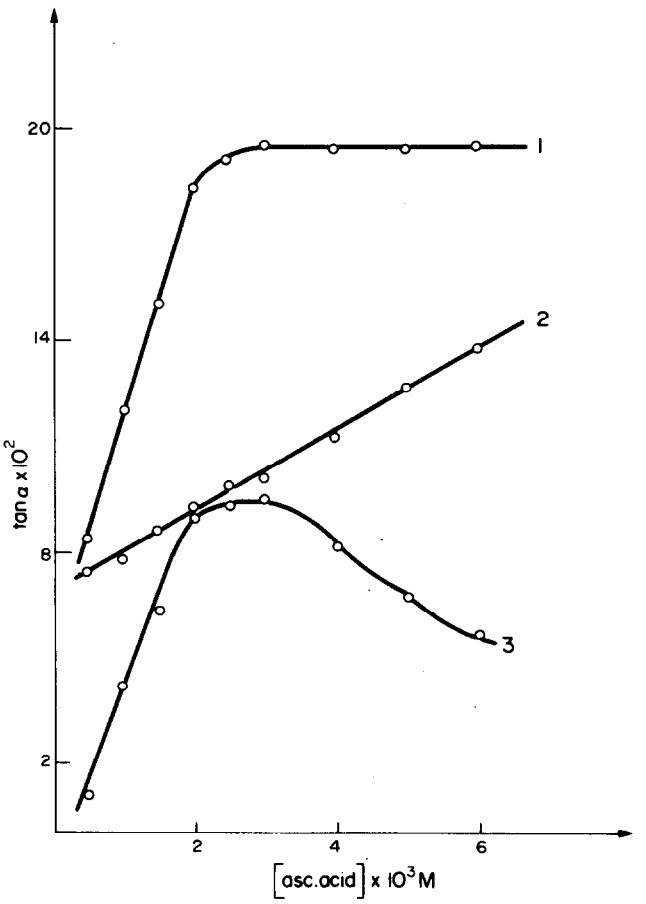

Fig. 1. Dependence of the reaction rate on ascorbic acid concentration. Initial concentrations: C_{TNS} $1 \times 10^{-4}M$, $C_{H_2O_2}$ $6 \times 10^{-3}M$, C_{Cu} 5 ng/ml, pH 6.6. Temperature 25°, 1, TNS–H_2O_2-ascorbic acid–Cu; 2, TNS–H_2O_2–ascorbic acid; 3, differential curve.

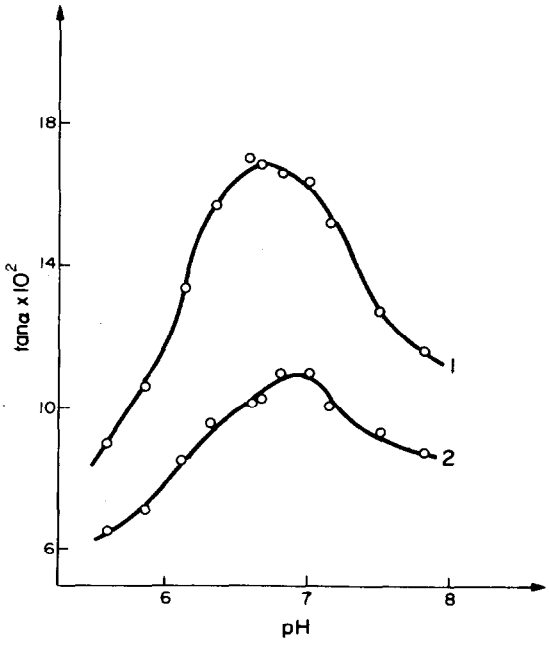

Fig. 2. Dependence of the reaction rate on pH. Initial concentrations: C_{TNS} $1 \times 10^{-4}M$, $C_{H_2O_2}$ $4 \times 10^{-4}M$, $C_{asc.acid}$ $3 \times 10^{-3}M$, C_{Cu} 5 ng/ml. Temperature 25°. 1, Catalytic reaction; 2, uncatalysed reaction.

Figure 3 shows that there is a complicated relation between hydrogen peroxide concentration and tan α. An initial concentration of $6 \times 10^{-3}M$ hydrogen peroxide was chosen as optimal.

The relation between tan α and TNS concentration is presented in Fig. 4. The maximal difference between

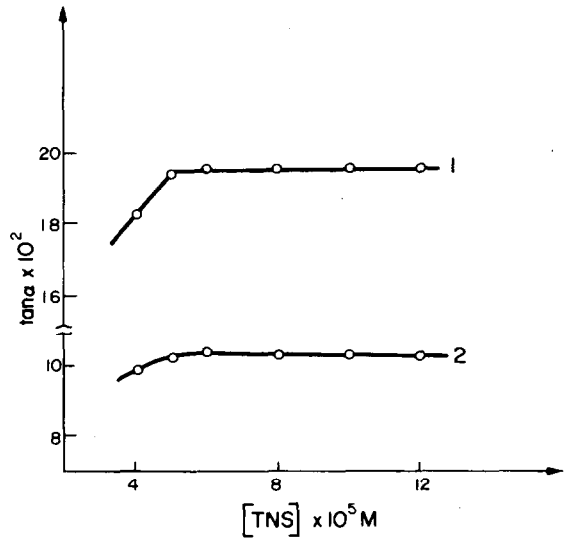

Fig. 4. Dependence of the reaction rate on TNS concentration. Initial concentration $C_{asc.acid}$ $3 \times 10^{-3}M$, other conditions as for Fig. 1 (except C_{TNS}). 1, Catalytic reaction; 2, uncatalysed reaction.

curves 1 and 2 appears at a TNS concentration $>5 \times 10^{-5}M$. For lower concentrations both reactions are first order, and at higher are of zero order with respect to TNS. A TNS concentration of $8 \times 10^{-5}M$ was selected.

Under optimal conditions, concentrations of Cu(II) between 0.25 and 5 ng/ml give linear calibration lines, as shown in Fig. 5. The line measured at 25° is steeper and more suitable for use than the line measured at 20°.

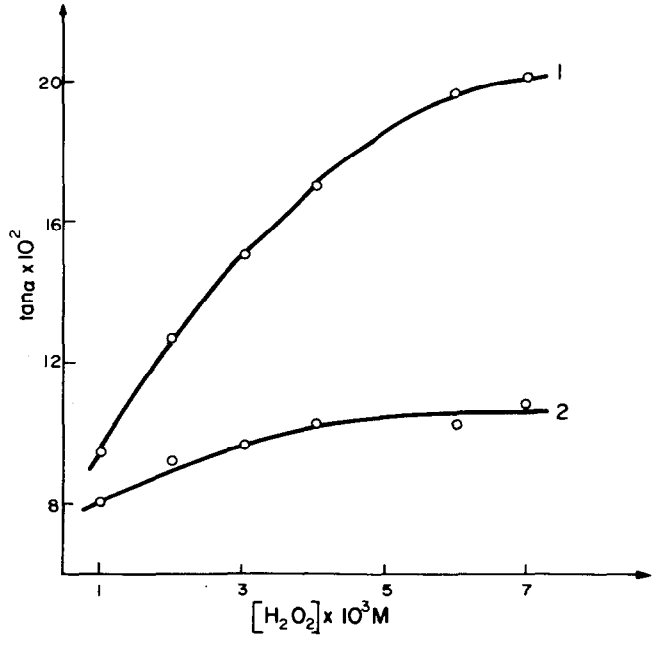

Fig. 3. Dependence of the reaction rate on H_2O_2 concentration; pH 6.6, other conditions as for Fig. 2 (except $C_{H_2O_2}$). 1, Catalytic reaction; 2, uncatalysed reaction.

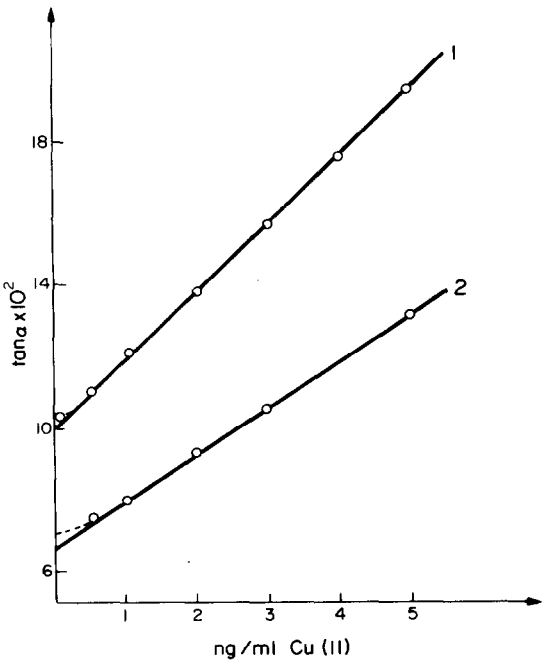

Fig. 5. Dependence of the reaction rate on Cu(II) concentration. Initial concentrations: C_{TNS} $8 \times 10^{-5}M$, $C_{H_2O_2}$ $6 \times 10^{-3}M$, $C_{asc.acid}$ $3 \times 10^{-3}M$, pH 6.6. 1. 25°; 2. 20°.

The accuracy and the reproducibility are presented in Table 1. The relative error ranges from 4.8 to 18.2% for the copper concentration interval from 5 to 0.8 ng/ml.

The selectivity was assessed by studying the effect of foreign ions on the reaction rate (Table 2). The presence (individually, in the ratio to copper that is given in brackets) of NH_4^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cl^-, SO_4^{2-}, CH_3COO^- (10^5:1), Mn^{2+}, Br^-, F^-, (10^4:1), Al^{3+}, Pb^{2+}, Fe^{3+}, $H_4C_4O_6^{2-}$, citrate, $HAsO_4^{2-}$ (10^3:1), $Mo_7O_{24}^{6-}$, WO_4^{2-}, VO_3^-, Zn^{2+}, $C_2O_4^{2-}$ (10^2:1), Cd^{2+}, I^- (1:1) has practically no influence on the reaction rate. Co^{2+} (10:1) and I^- (10^3:1) further catalyse the reaction. Ni^{2+} (10^2:1) strongly inhibits the reaction, and in presence of EDTA (10:1) only the uncatalysed reaction takes place.

Table 2. Influence of some foreign ions on the determination of ultramicro amounts of Cu(II); $C_{Cu} = 5$ ng/ml. $q = C_{Ion}:C_{Cu}$

Ion	q	100 × tan α	Ion	q	100 × tan α
—	—	19.50	Cl^-	10^5	19.35
NH_4^+	10^5	20.20	SO_4^{2-}	10^5	19.35
Mg^{2+}	10^5	19.00	CH_3COO^-	10^5	19.20
Ca^{2+}	10^5	19.35	I^-	1	19.50
Sr^{2+}	10^5	19.70	I^-	10^3	catalyses
Ba^{2+}	10^5	18.95	Br^-	10^4	19.05
Al^{3+}	10^3	18.75	F^-	10^4	19.05
Zn^{2+}	10^2	18.75	$C_2O_4^{2-}$	10^2	18.60
Mn^{2+}	10^4	18.75	$H_4C_4O_6^{2-}$	10^3	19.00
Pb^{2+}	10^3	19.05	citrate	10^3	19.70
Sn^{2+}	10	19.05	$HAsO_4^{2-}$	10^3	18.85
Ni^{2+}	10^2	15.00	$Mo_7O_{24}^{6-}$	10^2	20.50
Co^{2+}	10	catalyses	WO_4^{2-}	10^2	20.50
Fe^{3+}	10^3	20.20	VO_3^-	10^2	20.50
Cd^{2+}	1	19.20	EDTA	10	10.20

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A FIRE-ASSAY AND WET CHEMICAL METHOD FOR THE DETERMINATION OF PALLADIUM, PLATINUM, GOLD, AND SILVER IN ORES AND CONCENTRATES

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Summary—A method is presented for the determination of palladium, platinum, gold and silver in ores and concentrates by a fire-assay and wet chemical technique. After parting of the lead assay button with dilute nitric acid, and separation of the solution from the residue, the palladium and platinum in the solution are precipitated by the addition of stannous chloride, with tellurium as collector. The resulting precipitate is combined with the gold residue and dissolved in *aqua regia*, then the solution is analysed for palladium, platinum and gold by atomic-absorption spectrophotometry (AAS). Silver is determined in the original solution by AAS before the reduction step.

Recently, the author developed a short fire-assay and atomic-absorption method for the determination of gold and silver in ores and concentrates.[1] It is a variation of the lead-collection scheme and has the advantages of eliminating the steps of multiple scorifications, inquartation and cupellation usually associated with the classical procedure.

Because many types of sample must be analysed for platinum and palladium as well as gold and silver, it was thought worthwhile to extend the method to include these two platinum group metals. However, attempts to apply the method directly were unsuccessful; after the parting of the lead button with nitric acid, some of the platinum and palladium stayed with the gold in the residue and the remainder with the silver in the solution.

This paper describes a method for the recovery of the platinum and palladium remaining in the solution fraction by reduction with stannous chloride and use of tellurium as collector. The method has been applied successfully to the determination of the four precious metals in diverse certified reference materials.

EXPERIMENTAL

Apparatus

As described previously.[1]

Reagents

Stannous chloride solution. Prepare by dissolving 22.5 g of $SnCl_2.2H_2O$ in 16 ml of 12*M* hydrochloric acid. Cover, heat gently until the solution is clear, then dilute to 100 ml with distilled water.

Standard solutions of silver, gold, platinum and palladium. The silver solution is prepared by dissolving pure silver foil in dilute nitric acid (1 + 3) followed by dilution to 1 litre in approximately 10% v/v nitric acid. The gold, platinum and palladium standards are prepared by dissolving weighed quantities of the Johnson Matthey "Specpure" sponge in *aqua regia*; sodium chloride (50 mg) is added to the solution, which is evaporated to near dryness. The salts are dissolved in hydrochloric acid, and the solution evaporated again to near dryness to remove most of the nitric acid. Finally the salts are dissolved and diluted to volume with 10% v/v hydrochloric acid. The silver and gold solutions are standardized gravimetrically by the classical fire-assay procedure with lead, and the platinum and palladium solutions are standardized spectrophotometrically.

Aqua regia. Concentrated hydrochloric and nitric acids (3:1 v/v) prepared fresh as required.

Granulated lead. Free from silver and gold.

Flux. PbO 90 g, Na_2CO_3 36 g, $Na_2B_4O_7$ 18 g, SiO_2 10–20 g (according to silica content of sample), flour 3–5 g (the larger quantity for samples high in iron), Te 15–18 mg. An additional 30 g of PbO is added to this flux for samples high in copper and/or nickel.

Pretreatment of samples

Roasting of sulphides. Before fusion, sulphide samples are roasted to convert sulphides into oxides, and to volatilize arsenic and antimony to prevent the formation of matte or speiss during the fusion. All reference materials analysed, except the Magnetic Concentrate and South African ore, were roasted at 750–800° for approximately 1 hr.

The sample (−200 mesh, weighing up to 1 assay-ton, *i.e.*, 29.166 g) is roasted in a shallow fire-clay dish with intermittent stirring. If only a few grams of material are to be roasted (*e.g.*, copper–nickel matte), the sample is placed on a bed of silica to prevent possible loss of the resultant calcine to the surface of the dish; the amount of silica is then subtracted from the quantity used in the flux.

Chromite. There is at present no certified precious-metal standard containing a significant amount of chromite available to serve as a reference for testing a pretreatment. However, the following procedure is suggested, based on laboratory experience with such materials.

Chromite is not completely decomposed during the

fusion and samples containing an appreciable proportion of it require a pretreatment that will decompose it, such as sintering with sodium peroxide. The sample is mixed with 1.5 times its weight of sodium peroxide, then roasted at 700° for about 1 hr on a 10-g bed of silica in a roasting dish. The sinter cake and underlying silica are ground together in a mortar, then mixed with the flux for the fusion. The weights of sodium peroxide and silica used are subtracted from the weights of sodium carbonate and silica used in the flux described above.

Preparation of assay charge

This is done as described previously.[1]

Analysis of lead button

The button, weighing approximately 3 g, is cleansed of adhering slag, flattened and cut into pieces with a pair of snips.

Silver determination. The lead pieces are treated with 25–30 ml of nitric acid (1 + 3) in a 400-ml beaker. The beaker is covered and heated for approximately 10 min to dissolve the alloy. The cover is removed and washed with 5% v/v nitric acid, then diethylenetriamine (5–7 ml) is added cautiously with stirring until a permanent precipitate forms (Note 1). Approximately 10 ml of concentrated nitric acid are added to dissolve the precipitate, and the solution is filtered, while hot, through a Whatman No. 541 paper (9 cm) into a 200-ml standard flask. The filter paper and beaker are washed several times with 5% v/v nitric acid, and the filtrate and washings diluted to volume with water. The filter paper (containing the gold residue and part of the platinum and palladium) is retained. Silver is then determined on a 5-ml aliquot of the sample solution (containing platinum, palladium and silver) by AAS.

For milligram quantities of silver, the aliquot of the sample solution is diluted to appropriate volume with 5% nitric acid and determined by AAS.

Gold, palladium and platinum determination. The sample solution remaining in the 200-ml flask is transferred to the original 400-ml beaker, and the sample is then covered and heated to approximately 60°. Stannous chloride solution is added dropwise (6–8 ml) with constant stirring to precipitate the tellurium, palladium and platinum (Note 2).

After standing for about 30 min to allow the precipitate to coagulate and settle, the solution is filtered, by decantation, through the original filter paper. The filtrate containing lead, plus quantities of base metals if present in the original sample, is discarded after several washings of the filter paper and beaker with 5% nitric acid. The filter paper and contents are transferred to the original beaker, 15 ml of *aqua regia* are added, and the beaker is covered and heated slowly until the paper breaks down to a fine pulp, to ensure complete dissolution of the precious metals. The cover is removed, washed with 5% nitric acid, and after dilution with an equal volume of water the solution is filtered through a fast paper into a 400-ml beaker, followed by several washings of the paper with 5% nitric acid.

Approximately 50 mg of sodium chloride are added, and the sample solution is evaporated to near dryness. The salts are dissoved in hydrochloric acid and the sample evaporated again to near dryness to remove the nitric acid. This step is repeated twice.

To the cooled sample, 5 ml of cadmium–copper sulphate solution are added and the mixture is transferred to a 25-ml standard flask and diluted to volume with water. The gold, palladium and platinum contents of the sample are then determined by AAS, allowance being made in the calculation for the portion of sample solution removed from the 200-ml flask for the silver determination.

For milligram amounts of one or more of these precious metals, an aliquot is taken from the 25-ml standard flask, additional cadmium–copper sulphate solution is added to give a final 20% v/v concentration, and the sample solution diluted to the required volume with water.

Notes. 1. Treatment of the sample solution with diethylenetriamine is a modification of the procedure used by Greaves[3] to complex silver and lead compounds. Elements that precipitate as hydrous oxides under these conditions are in general those that form precipitates in dilute ammonia solution.

2. Addition of excess of stannous chloride must be avoided, otherwise difficulty may be encountered in preventing the precipitation of lead salts, subsequently resulting in low precious-metal results. Once the sample is completely darkened with finely divided tellurium, further addition of stannous chloride is stopped.

Preparation of calibration solutions

Standard silver solutions. To each of an appropriate number of 250-ml beakers containing approximately 3 g of granulated lead and various quantities of the silver stock solution corresponding to 0.1–4 ppm of silver in the final solution, 25 ml of nitric acid (1 + 3) are added, the beakers are covered, and heated gently to dissolve the lead. The covers are removed and rinsed with 5% nitric acid, diethylenetriamine (5–7 ml) is added to each beaker followed by 10 ml of concentrated nitric acid, and the standards are diluted to 200-ml volume with water. The AAS calibration curve is linear over the range 0.1–4 ppm.

Standard gold, palladium and platinum solutions. Various quantities of the gold, palladium, and platinum stock solutions are transferred by burette to separate 100-ml standard flasks containing 20 ml of the cadmium–copper sulphate solution and 50 mg of sodium chloride, and diluted to volume with water. AAS calibration curves are prepared for gold and palladium and are linear in the ranges 0.2–3 ppm and 0.4–3 ppm respectively. Because the platinum AAS response is not linear, enough standards in the range 0.8–3 ppm must be taken, or the sample must be bracketed by standards of slightly higher and lower concentration and the sample concentration calculated by assuming linear response within the bracket range.

RESULTS AND DISCUSSION

Use of tellurium as a carrier for platinum and palladium

In preliminary tests on the use of nitric acid for parting lead buttons prepared from charges salted with platinum, palladium, gold and silver, it was found that both platinum and palladium were apportioned between the residue containing the gold and the nitric acid solution containing the silver. Because tellurium has been used to co-precipitate platinum and palladium,[4] it was thought worthwhile to test its effectiveness as a carrier in the recovery of the two platinum metals after the parting step. Accordingly a number of experiments were performed in which fire-assay charges were salted with 15–18 mg of tellurium powder and various quantities of the four precious metals. After drying for approximately 1 hr the synthetic samples were fused to produce lead buttons which were analysed according to the procedure described.

The results (Table 1) confirm that recovery of the precious metals by the proposed method is complete. The weight of tellurium powder was arbitrarily chosen on the basis of laboratory tests with synthetic samples containing various quantities of tellurium and milligram amounts of platinum, palladium and

Table 1. Recovery of the precious metals from synthetic samples

Element	Added, *mg*	Found, *mg*
Silver	10.02	10.30
	0.050	0.050
Gold	4.56	4.55
	0.023	0.022
Palladium	3.00	2.90
	0.019	0.020
Platinum	4.36	4.20
	0.044	0.042

gold. In all cases, excellent recovery of the precious metals was obtained.

Application to certified reference materials

To determine its accuracy and precision, the proposed method was applied to three certified reference materials prepared by the Canadian Certified Reference Materials Project,[5–7] and to a certified ore prepared by the National Institute for Metallurgy of South Africa.[8] The analyses were performed in quintuplicate by the procedures described, and the results are shown in Table 2. They are in good agreement with the certified values. These certified values originate from many analyses performed by a number of independent laboratories, which collectively used a wide variety of assay methods.

The Flotation Concentrate and Cu–Ni Matte require special mention because these materials are both rich in copper and nickel. When the conventional fire-assay method is used on such materials, many scorifications are required to produce lead buttons that are free from these base metals. In addition, the precious metal bead that is obtained by cupellation of assay buttons must be analysed for gold and the platinum metals before silver can be obtained by difference.

CONCLUSION

The method offers several advantages over the classical fire-assay method. It is simple and rapid; a set of samples can easily be dealt with within one working day. Once the 3-g lead button is obtained, the procedure for the preparation of the silver, gold, palladium and platinum solutions for AAS is relatively fast. In addition, no major separation steps are required to isolate and separate the precious metals after fusion; inquartation and cupellations for beads containing incorrect precious metal ratios for dissolution are unnecessary.

Because the proposed method is free from these problems associated with the classical method, greater accuracy and precision of assays in the low precious-metal range is possible.

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Table 2. Application of proposed method to certified reference materials

Sample	Sample wt., *g*	Element	Mean found, *ppm**	Std. devn., *ppm**	Certified values and 95% confidence intervals	
Magnetic Concentrate PTA-1	14.58	Pt	3.20	0.11	3.05	(2.92–3.16)
Flotation Concentrate PTC-1	14.58	Ag	6.0	0.23	5.8	(5.5–6.2)
		Au	0.65	0.05	0.65	(0.55–0.72)
		Pd	12.3	0.29	12.7	(12.0–13.0)
		Pt	2.9	0.17	3.0	(2.8–3.2)
Cu–Ni Matte PTM-1	7.29	Ag	68.9	1.42	66.0	(59.0–73.0)
		Au	1.7	0.09	1.8	(1.6–1.9)
		Pd	7.8	0.30	8.1	(7.4–8.8)
		Pt	5.2	0.48	5.8	(5.5–6.2)
South African Pt–Pd Ore	29.17	Ag	0.47	0.02	0.42	(0.38–0.46)
		Au	0.37	0.03	0.31	(0.30–0.33)
		Pd	1.51	0.03	1.53	(1.50–1.56)
		Pt	3.74	0.10	3.74	(3.70–3.79)

* Based on 5 replicate determinations.

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SPECTROPHOTOMETRIC DETERMINATION OF IRON IN HIGHLY ALKALINE SOLUTION WITH 4-HYDROXY-1,10-PHENANTHROLINE

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Summary—4-Hydroxy-1,10-phenanthroline forms a stable tris-chelate with iron(II) in the range of alkalinity from pH 10 to 2*M* sodium hydroxide, with molar absorptivity 1.19×10^4 l.mole^{-1}.cm^{-1} at 545 nm. The determination of iron is performed by adding the phenanthroline, stannous chloride, and iron-free sodium hydroxide to the sample to give pH > 13; stannite is the active reductant. Beer's law is obeyed over the iron concentration range from 1×10^{-5} to 8×10^{-5}*M*. Advantages over existing methods are the use of stannous chloride instead of sodium dithionite, which avoids the problem of turbidity, and the stability of the iron(II) chelate towards oxidation by air. The conditional reduction potential at pH 11 for the iron(III)/iron(II) complex couple is 0.39 V.

The use of 4-hydroxy-1,10-phenanthroline as a spectrophotometric reagent for iron was studied by Hale and Mellon,[1] who reported the formation of a coloured complex with iron(II) in the presence of hydroxylamine, with maximum colour development at pH 7.5. Because of the strong effect of pH on the transmittance of the solutions, and a steady increase in transmittance with time, the reagent was not recommended. We have recently found, however, that a stable compound, tris(4-hydroxy-1,10-phenanthroline)iron(II), is formed in basic solutions (from pH 10 to 2*M* sodium hydroxide) in the presence of ascorbic acid, sodium dithionite or stannite, which is suitable for the spectrophotometric determination of iron.

Two other reagents for the spectrophotometric determination of iron in highly alkaline media are 4,7-dihydroxy-1,10-phenanthroline[2] and phenyl 2-pyridyl ketoxime.[3] For both the recommended reductant is sodium dithionite, a distinct disadvantage because of the tendency of dithionite solutions to form elemental sulphur. We have found that alkaline solutions of stannous chloride, in which the active reductant is the stannite ion, completely reduce the iron derivatives of 4-hydroxy-1,10-phenanthroline and 4,7-dihydroxy-1,10-phenanthroline. Phenyl 2-pyridyl ketoxime was not studied, because of its tendency to precipitate in aqueous media.

EXPERIMENTAL

Reagents

4-Hydroxy-1,10-phenanthroline. This was prepared according to Snyder and Freier[4] with the following modifications. To hydrolyse 3-carbethoxy-4-hydroxy-1,10-phenanthroline, 5 g of the ester and 50 ml of concentrated hydrochloric acid were heated with stirring until the solid had dissolved. The solution was evaporated almost to dryness, and after cooling, the moist solid was suspended in cold water, and the crystals were collected on a sintered-glass Buchner funnel and washed repeatedly with cold water. Infrared and proton nmr spectral data confirmed this compound as 3-carboxy-4-hydroxy-1,10-phenanthroline. The carboxy compound was heated at 315–345° in an electric furnace until evolution of carbon dioxide ceased, and then for an additional 10 min. After cooling, the solid was recrystallized four times from water. The resulting light yellow crystals of 4-hydroxy-1,10-phenanthroline (melting range 214–216°) were found by acid–base titration to be at least 99% pure.

A 0.01*M* solution of 4-hydroxy-1,10-phenanthroline was prepared by adding 1*M* sodium hydroxide dropwise to a suspension of 0.196 g of the solid in water, and diluting to 100 ml.

Standard iron solution, 0.01000*M*. Prepared by gently warming 0.5584 g of electrolytic iron with 41 ml of concentrated hydrochloric acid in a 1-litre standard flask until dissolution was complete, and diluting to volume. Working solutions of 5.00×10^{-4}*M* iron were prepared by diluting 25.00-ml aliquots to exactly 500 ml with distilled demineralized water.

Stannous chloride, solution 10%. Prepared by dissolving 50 g of the reagent grade dihydrate in 50 ml of hot 6*M* hydrochloric acid and diluting to 500 ml.

Sodium hydroxide. Reagent grade pellets were used to prepare a 10*M* solution which was then purified as described by Reiner and Poe.[5]

Procedure

To a series of 100-ml polypropylene standard flasks add successively, with mixing, 20 ml of purified 10*M* sodium hydroxide, 5 ml of 0.01*M* 4-hydroxy-1,10-phenanthroline, known volumes of 5.00×10^{-4}*M* iron solution or test sample, and distilled demineralized water to give a volume of 60–80 ml. Add 10 ml of 10% stannous chloride solution while swirling the flask, cap the flask, and shake vigorously until all of the precipitate redissolves. Dilute to volume with water, replace the cap loosely, and heat in an oven or water-bath for 1 hr at 80–90°. After cooling to room temperature measure the absorbance in a 1.00-cm cell at 545 nm.

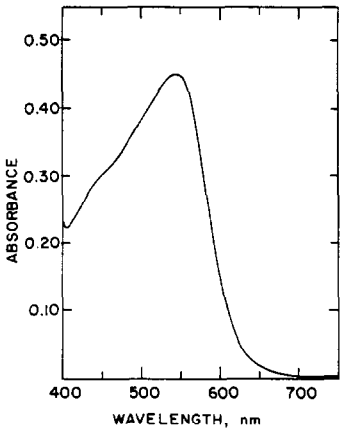

Fig. 1. Absorption spectrum of $3.75 \times 10^{-5}M$ tris(4-hydroxy-1,10-phenanthroline)iron(II) at pH 12.7 (1.00-cm cuvette).

RESULTS AND DISCUSSION

Effect of pH on absorption spectra

In the presence of sodium dithionite, complete formation of the ruby red complex occurs in the alkalinity range from pH 9 to 2*M* sodium hydroxide. If the concentration of sodium hydroxide is much greater than 2*M*, a purple precipitate forms. At pH < 9 formation of the complex is incomplete, and solutions at pH < 10 become turbid after some days. Solutions in the range from pH 10 to 2*M* sodium hydroxide are stable over a period of at least two weeks. The visible absorption spectrum of the complex is unaltered over this range (*cf.* Fig. 1). The wavelength of maximum absorbance is 545 nm, at which the molar absorptivity is 1.19×10^4 $l.mole^{-1}.cm^{-1}$. Adherence to Beer's law is observed over the range 1×10^{-5}–$3 \times 10^{-5}M$ iron, but was not investigated outside this range. A continuous-variations study showed the molar ratio of reagent to iron in the complex to be 3:1.

Choice of reducing agent

Ascorbic acid, pyrogallol, sodium sulphite, hydroxylamine, sodium thiosulphate, hydrazine sulphate and hydroquinone were rejected as unsuitable because of either insufficient reducing power or production of strong interfering colour in the basic solutions.

Sodium dithionite was recommended by Schilt *et al.*[2] for use with 4,7-dihydroxy-1,10-phenanthroline, and it works equally well with 4-hydroxy-1,10-phenanthroline. In neutral or acidic solution dithionite disproportionates to yield thiosulphate and bisulphite, and this reaction is well documented.[6] In the presence of sodium hydroxide, dithionite solutions are stable for weeks with respect to disproportionation if protected from oxygen. Most commercial preparations which we have tested, when dissolved in aqueous base, immediately produce a colloidal suspension of what is presumably elemental sulphur. Occasionally a sample is obtained which produces a clear solution, and if dithionite of such quality is available, it is an excellent replacement for stannous chloride. In that case add only 10 ml of 10*M* sodium hydroxide, and use as reductant 10 ml of 20% sodium dithionite in 10% sodium hydroxide solution.

The use of stannous chloride as reducing agent completely avoids the problems of turbidity associated with sodium dithionite. Addition of the reagent solution to excess of base results initially in the formation of insoluble stannous hydroxide. If the pH of the resulting mixture is >13, the stannous hydroxide will redissolve to yield a clear solution of hydroxostannate(II), which will rapidly and completely reduce iron(III) to iron(II) in the presence of 4-hydroxy-1,10-phenanthroline. At high concentrations (>8 g $SnCl_2.2H_2O$/100 ml) solutions of hydroxostannate(II) disproportionate to yield metallic tin. For this reason an acidic reducing solution must be used, and enough base must be present in the sample solutions to neutralize the acid and redissolve the stannous hydroxide which is formed on addition of the reducing solution.

Reagent grade stannous chloride contains significant amounts of iron, so the amount of this reagent added should be carefully measured, and all absorbance measurements made against a blank solution.

Effect of temperature and concentration of sodium hydroxide

The time required for complete formation of the iron(II) complex in 0.5–2.0*M* sodium hydroxide is 6–10 hr at room temperature. The time may be reduced to 1 hr by heating the solution at 80–90°.

When solutions of the complex were formed by heating in glass flasks, absorbance measurements were erratic and increased with concentration of sodium hydroxide. We attribute this increase not to the presence of iron in the purified sodium hydroxide, but to the introduction of iron into the solution from the glass, which is subject to attack by caustic solutions. The use of polypropylene flasks, which can be heated to 100° without damage, solves this problem.

Analytical applications and interferences

Because it is chemically similar to 4,7-dihydroxy-1,10-phenanthroline, 4-hydroxy-1,10-phenanthroline can be used as a substitute for it in the applications proposed by Schilt *et al.*[2] These are mainly the determination of iron in commercially available alkaline reagents, and we have found that complete colour formation occurs in aqueous ammonia and concentrated solutions of phosphate and carbonate salts as well as in alkalies.

The following anions (in 500-fold w/w ratio to iron at the 2-ppm level) were tested for interference in 1*M* sodium hydroxide medium: I^-, $C_2H_3O_2^-$, $B_4O_7^{2-}$, SO_4^{2-}, Cl^-, F^-, SCN^-, CN^-, and NO_3^-. Only cyanide and nitrate caused significant interference (>3% change in absorbance). Metal ions were tested only at low concentrations. At 20 ppm, Cr(III), Pb(II), Al, Ba, Ca, V(IV) and Zn did not cause significant interference, but Cu(II) caused an 8% increase in absorbance.

At 1 ppm, Hg(II) and Ni did not interfere, but Cd and Ag caused a 5% increase in absorbance.

Although tris(4-hydroxy-1,10-phenanthroline)iron(II) is stable from pH 10 to 2*M* sodium hydroxide, the use of stannous chloride as a reducing agent requires pH > 13. This is readily accomplished by adding an appropriate quantity of iron-free sodium hydroxide. Sodium dithionite may be used if conditions require pH < 13, but its use may cause turbid solutions.

At alkali concentrations > 2*M*, 4,7-dihydroxy-1,10-phenanthroline should be used instead of 4-hydroxy-1,10-phenanthroline. In the original paper[2] sodium dithionite was recommended as the reductant, but we have found that stannous chloride works equally well at pH > 13.

When oxidation by atmospheric oxygen is of concern, the use of 4-hydroxy-1,10-phenanthroline is recommended. The iron complex of the dihydroxy compound is rapidly and quantitatively oxidized by air.[7] In contrast, no visible decrease in colour was observed when air was bubbled through solutions of tris(4-hydroxy-1,10-phenanthroline)iron(II) at room temperature in the absence of a reducing agent. When heated with steam the same solutions were completely decolourized, indicating that oxidation probably occurs slowly at room temperature. This observation is consistent with a half-wave potential of 0.14 V *vs.* SCE, which we measured for a solution of the iron(II) complex at pH 11 with a rotated platinum electrode, and which we will report in greater detail in a future paper. This value, which we believe is equal to the conditional reduction potential, or 0.39 V *vs.* NHE, is significantly different from any previously reported reduction potential of an iron phenanthroline complex.[8,9] This value may make the iron complexes of 4-hydroxy-1,10-phenanthroline useful as a redox indicator system in applications where iron phenanthroline complexes have not been used in the past, or as selective reagents for spectrophotometric determination of traces of oxidants and reductants.

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9-IMINO DERIVATIVES OF DIHYDROXYANTHRAQUINONES AS ANALYTICAL REAGENTS

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Summary—The synthesis, characteristics, properties and reactions with metallic ions of the reagents isticin-9-imine, alizarin-9-imine and 3-sulphoalizarin-9-imine have been studied.

Dihydroxyanthraquinones are well known as dyes, and have been widely studied as organic reagents forming complexes with metal ions. The present work deals with the change in analytical behavior caused by substitution of an imine group for the quinonoid oxygen atom in the C-9 position. The reagents tested were 1,8-dihydroxyanthraquinone-9-imine (isticin-9-imine), and 1,2-dihydroxyanthraquinone-9-imine (alizarin-9-imine) and its 3-sulphonic acid (3-sulphoalizarin-9-imine).

EXPERIMENTAL

Reagents

The compounds were obtained by reaction of the parent dihydroxyanthraquinone at 100° in a sealed tube with a saturated solution of ammonia in methanol.[1,2] Isticin-9-imine and alizarin-9-imine were purified by recrystallization from methanol, and 3-sulphoalizarin-9-imine by recrystallization from acetone. Elemental analyses of the three substances agreed with the empirical formulae.

Solutions (0.1%) of isticin and isticin-9-imine in diethylene glycol, alizarin-9-imine and alizarin in acetone, and 3-sulphoalizarin-9-imine and Alizarin Red S in water.

Solutions (0.1%) of metal ions, prepared from analytical grade salts.

Determination of dissociation constants

3-Sulphoalizarin-9-imine solution was mixed with suitable buffers and the pH and absorbance were measured. The dissociation constants were determined by the methods of Phillips and Merritt[3] and of Hildebrand and Reilley.[4]

Qualitative tests

The reactions with 54 metal ions were tested in five media, and compared with those of the parent dihydroxyanthraquinones.

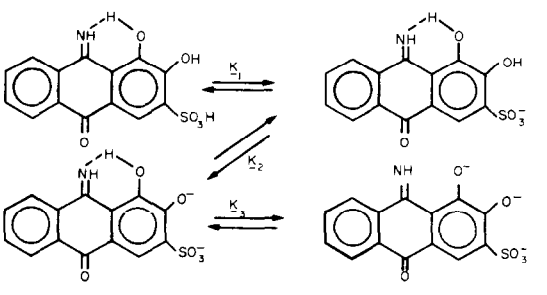
RESULTS AND DISCUSSION

The infrared spectra of isticin-9-imine and alizarin-9-imine agreed with those in the literature.[1,5] The spectrum of 3-sulpho-alizarin-9-imine differed from that of Alizarin Red S at 1620 cm^{-1} (C=N stretching) and 1550 cm^{-1} (NH deformation). As with alizarin-9-imine, the C=O and C=N stretching vibrations appeared together in one band, one of the CO bands in the spectrum of the parent compound was removed and the NH deformation band appeared.

The ultraviolet and visible region spectra showed that the chromophore systems were equivalent to those of the dihydroxyanthraquinones.[6–8] The spectra of the 9-imine derivatives in alcohol solution showed a bathochromic shift relative to those of the parent compounds, and a hypsochromic shift with decreasing pH, which makes them interesting as potential acid–base indicators.[9,10]

The three reagents are stable in methanolic solution, and unstable in alkaline and acid media. Only 3-sulphoalizarin-9-imine is soluble in water.

The acid–base behaviour of alizarin-9-imine has been described,[9] and isticin-9-imine solutions are too unstable for examination. The absorption spectra of 3-sulphoalizarin-9-imine at different pH values (Fig. 1) show three isosbestic points. The ionization equilibria may be formulated as follows:



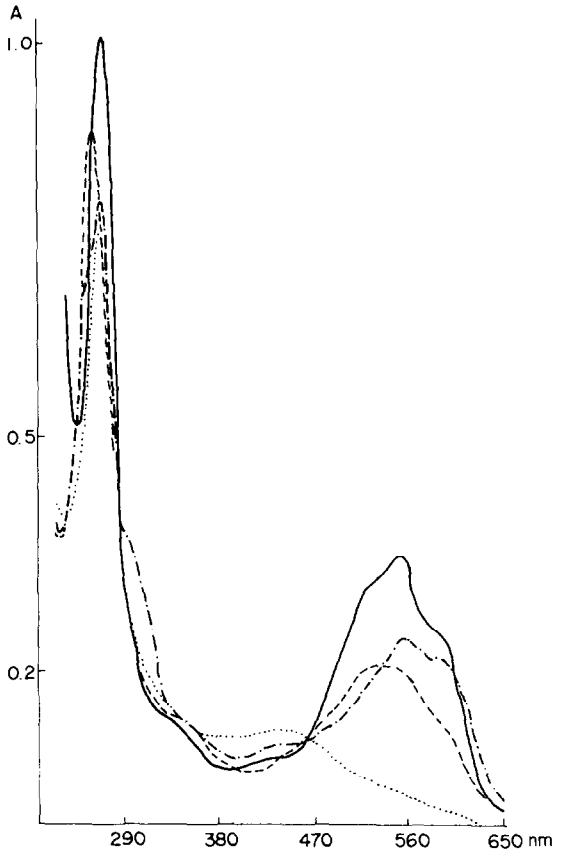


Fig. 1. Absorption spectra of 3-sulphoalizarin-9-imine in various media. —— 5*M* NaOH; —·—·— pH 8; – – – pH 3.5, ····· 6*M* HCl.

The imino group does not become protonated even in strongly acidic media. The pK values obtained were $pK_1 = 0.43$; $pK_2 = 5.74$ and $pK_3 = 9.05$, lower than those of Alizarin Red S and alizarin-9-imine (Table 1). The decrease in pK_2, relative to Alizarin Red S, is attributed to the effect of the internal hydrogen bonding of the imine and hydroxy groups. The sulphonic acid group is assumed to be responsible for the absence of an appreciable change in pK for the hydroxy group proton relative to that for the parent compound, in contrast to the marked effect for alizarin-9-imine.

Reactions with metal ions

The characteristics of the main reactions of these reagents are given in Table 2. The selectivities are not high, but improve in acid media. Isticin-9-imine is the most selective, because of the presence of two α-hydroxy groups able to establish hydrogen bonds with the imine group. The greatest reactivity is reached in nearly neutral medium, and relative to the parent compounds there is a small shift of the precipitation pH to lower values, which could be explained by the greater acidity of the 9-imine derivatives and does not appear with 3-sulphoalizarin-9-imine, because of the effect of the sulphonic group.

Isticin-9-imine and alizarin-9-imine show much greater sensitivity than the parent dihydroxyanthraquinones, which may be due to the greater ability of the imine group to form chelates; this effect is can-

Table 1. pK values of related reagents

	pK_1	pK_2	pK_3	Reference
Alizarin		8.76	10.22	11
		7.45	11.80	12
Alizarin Red S	4.73	6.72	9.35	11
Alizarin-9-imine	1.72	6.85	12.76	9
3-Sulphoalizarin-9-imine	0.43	5.74	9.05	This paper

Table 2. Detection limits (*ppm*) for principal reactions of the reagents (P = precipitate, C = colour)

	2 *M* HCl	2 *M* CH_3COOH	2 *M* CH_3COONa	2 *M* NH_3	2 *M* NaOH
Isticine-9-imine			Cu(II) red-brown (P) 4	Ca red (P) 10	
			Al red-brown (P) 2		
			Be purple (P) 0.5		
Isticine				Ca red (P) 10	
Alizarin-9-imine			Hg(II) blue (P) 4		
	Cu(II) blue (P) 5	Cu(II) blue (P) 0.8	Cu(II) purple-blue (P) 0.8	Cu(II) red (P) 7	
	Mo(VI) purple (P) 5	Fe(II) blue (P) 5	Bi(III) purple (P) 2	Cd purple (P) 5	
		Fe(III) purple (P) 2	Fe(III) blue (P) 5		
	Al pink (P) 1.2	Al red (P) 1	Al purple (P) 0.4		
	Ti(IV) red (P) 4	Ti(IV) red (P) 2	Ti(IV) red (P) 1		
		V(V) brown (P) 2	Sb(III) purple (P) 4		
			Co(II) purple (P) 2		
			Ni(II) red (P) 1.2		
			Mn(II) blue (P) 2		
3-Sulphoalizarin-9-imine		Al red (C) 4	Al red (C) 2	Ca purple (C) 4	Fe(III) red (P) 2
		V(V) orange-brown (C) 4	Cu(II) purple (P) 4		Cu(II) purple (P) 4
		Th(IV) red (C) 4	Ni(II) purple (P) 4		
		Ga(III) red (C) 1	Be red (C) 4		

celled in the case of 3-sulphoalizarin-9-imine by the presence of the sulphonic groups.

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ANALYTICAL DATA

AN EVALUATION OF THE STABILITY CONSTANTS OF THE CHLORO-COMPLEXES OF PALLADIUM(II)

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Summary—Formulae have been derived for the stepwise stability constants of the four Pd(II)–Cl complexes, for perchlorate media at 25° and ionic strengths from 0.05 to 2. The agreement with the most reliable literature data is better than 0.1 log units.

The literature data for the stability constants of the chloro-complexes of Pd(II) show rather large scatter. Victory *et al.*[1] have catalogued them but leave critical consideration to the reader. Here we attempt to deduce values for the logarithmic stepwise constants, reliable to within 0.1, for perchlorate medium at 25° and ionic strengths from 0.05 to 2.

THEORY

The empirical relations for ion activity coefficients that best satisfy both thermodynamic theory and practice for multi-electrolyte solutions are Guggenheim's modifications of the Debye–Hückel equation[2]

$$\log \gamma_R = -Az_R^2 \frac{I^{1/2}}{(1 + I^{1/2})} + \sum_{X'} B_{R,X'} m_{X'} \quad (1)$$

and

$$\log \gamma_X = -Az_X^2 \frac{I^{1/2}}{(1 + I^{1/2})} + \sum_{R'} B_{R',X} m_{R'} \quad (2)$$

where R denotes a particular cation species and R′ all cation species, X and X′ have analogous significance for anions, z is the ionic charge, m the molality and A a temperature-dependent constant (= 0.511 at 25°). For many common electrolytes the interaction coefficients B have been estimated experimentally.[3–7] Pitzer *et al.*[4,7] refined this further by assuming that B depends upon I. However, in considering chemical equilibria, where we usually deal with solutions with a complicated and changing composition, there is no point in taking these refinements into account, because these second-order corrections are small compared with the uncertainties in B and the formation constants. In this paper the interaction coefficients B will be taken as constant in the range $0.05 < I < 2$.

Equations (1) and (2) are very convenient for correlating formation constants observed in different media.

The activity and concentration constants K and Q for any chemical reaction are related through the quotient of the activity coefficients. Defining the equilibrium of interest as

$$PdCl_{n-1}^{(3-n)-} + Cl^- \rightleftharpoons PdCl_n^{(2-n)} \quad (3)$$

we have

$$K_n = \frac{a_{PdCl_n}}{a_{PdCl_{n-1}} a_{Cl^-}} = Q_n \frac{\gamma_{PdCl_n}}{\gamma_{PdCl_{n-1}} \gamma_{Cl^-}} \quad (4)$$

Substitution of equations (1) and (2) in equation (4) leads to a relation of the general form

$$\log Q_n = \log K_n + (0.511)\Delta z^2 \frac{I^{1/2}}{(1 + I^{1/2})} - b_n I \quad (5)$$

where Δz^2 is the algebraic sum of the squares of the charges on the species [so $\Delta z^2 = (2 - n)^2 - 1 - (3 - n)^2 = (2n - 6)$] and b_n is based on the separate values of B in equations (1) and (2) for the interactions of Pd^{2+}, $PdCl^+$, $PdCl_2$, $PdCl_3^-$ and $PdCl_4^{2-}$ which are at low concentration, Cl^- at varying concentrations, and Na^+ and ClO_4^- (predominant, to control the ionic strength).

Of the summation terms in (1) and (2) we can restrict ourselves to the B values for the ion-pairs Pd^{2+}–ClO_4^-, $PdCl^+$–ClO_4^-, Na^+–$PdCl_3^-$ and Na^+–$PdCl_4^{2-}$. As no data are available in the literature, however, only some general remarks can be made.

If ion-association is negligible, B will be 0.10–0.20 for uni-univalent electrolytes and 0.4–0.8 for bi-uni-

valent and uni-bivalent electrolytes.[4,5,8] As ion-association increases, these values decrease and readily become negative.[5,8]

It may be expected that extensive association will occur between Pd^{2+} and ClO_4^- because of the affinity of Pd^{2+} for Cl atoms and that for the same reason $PdCl^+$ will associate slightly with ClO_4^-. No association is expected for Na^+–$PdCl_3^-$ and a slight one for Na^+–$PdCl_4^{2-}$. In view of this the following values can be adopted for the interaction coefficients: $B_{Pd^{2+},ClO_4^-} \simeq -0.40$; $B_{PdCl^+,ClO_4^-} \simeq -0.10$; $B_{Na^+,Cl^-} = 0.15$; $B_{Na^+,PdCl_3^-} \simeq 0.15$; $B_{Na^+,PdCl_4^{2-}} \simeq 0.20$. If the difference between molality [in equations (1) and (2)] and ionic strength [in equation (5)] is neglected b_n becomes equal to the algebraic sum of the interaction coefficients B, *e.g.*, $b_1 = B_{PdCl^+,ClO_4^-} - B_{Pd^{2+},ClO_4^-} - B_{Na^+,Cl^-}$ (similar relations hold for b_2–b_4). Hence $b_1 = 0.15$, $b_2 = 0.05$, $b_3 = 0$ and $b_4 = -0.10$ as rough estimates for b_n.

DISCUSSION

Numerous investigators have determined the stability constants concerned but only a few have done so at different ionic strengths and obtained consistent sets for Q_i which are suitable for an estimation of b_n. The procedure followed by Biryukov *et al.*[9] is very suitable for low Cl^- concentrations and their results for $\log Q_1$ and $\log Q_2$ are very reliable. From their plots of $\{\log Q_n - 0.511\Delta z_n^2 I^{1/2}/(1 + I^{1/2})\}$ *vs.* I it follows that $b_1 = 0.14$ and $b_2 = 0.07$. Their set for Q_3 indicates a slightly negative value for b_3, but as their method gives less accurate values for Q_3, in principle b_3 is too uncertain for further consideration. The same remark applies to b_4. Levanda[10] determined $\log Q_4$ reliably at $I = 1$, 2, 3 and 4. Correlation of these results with earlier results obtained by Burger,[11,12] Schlenskaya[13,14] and Biryukov,[15] who determined $\log Q_4$ at $I = 0.5$ and 1 in a different way, leads to a value of -0.05 for b_4.

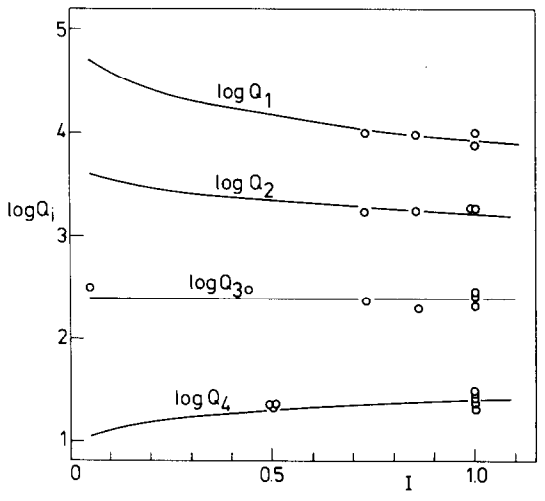

Fig. 1. The dots represent the literature values selected as the most reliable. The curves correspond to equations (6)–(9).

Comparison of these values with those deduced on a partly theoretical basis shows a satisfactory agreement. Considering the quantities $b_n I$ as correction terms for the calculation of Q_n, it will be clear that although there is some guessing in these sets, they give each other sufficient support to justify acceptance of $b_1 = 0.14$, $b_2 = 0.07$, $b_3 = 0$ and $b_4 = -0.05$.

The papers already used for the estimation of b_n also give reliable values of $\log K_n$. Q_2, Q_3 and Q_4 can also be taken from another set of values,[16] though these were determined at only $I = 1.0$. All other values can be regarded as less reliable for different reasons. Figure 1 gives a survey of the most reliable data. The following equations can be regarded as the best fit to these data:

$$\log Q_1 = 5.08 - 2.04 \frac{I^{1/2}}{(1 + I^{1/2})} - 0.14\, I \qquad (6)$$

$$\log Q_2 = 3.80 - 1.02 \frac{I^{1/2}}{(1 + I^{1/2})} - 0.07\, I \qquad (7)$$

$$\log Q_3 = 2.42 \qquad (8)$$

$$\log Q_4 = 0.88 + 1.02 \frac{I^{1/2}}{(1 + I^{1/2})} + 0.05\, I \qquad (9)$$

The corresponding curves are drawn in Fig. 1.

The equations hold for a perchlorate medium at 25° with ionic strength from 0.05 to 2. There is no practical value in extrapolating to lower values of I; $I = 0.05$ is easily reached in practice when perchlorate is present as supporting electrolyte. The values calculated with equations (7)–(9) agree very well with those selected by Martell and Smith[17] for Q_2, Q_3 and Q_4; the value[16] they take for $\log Q_1$ is less reliable. As long as we deal with a medium in which perchlorate is in much higher concentration than chloride, an uncertainty of about 0.03 in the Q values can be expected in the range $0.1 < I < 1.0$.

For other media new sets of b_n values will be needed. Large deviations may be expected for nitrate media as this ion shows a large tendency to ion-association.

The literature values considered in this paper were found in references 1, 17, 18 and 19. A computerized retrospective search of the latest literature did not add new data to this compilation.

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ANNOTATIONS

ZUM EINFLUSS DES SCHWEFELS BEI DER SAUERSTOFFBESTIMMUNG NACH DEM HEISSEXTRAKTIONSVERFAHREN

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Zusammenfassung—Bei der Sauerstoffbestimmung in hochschwefelhaltigen Materialien nach dem Heißextraktionsverfahren kann CS_2 entstehen, das die Sauerstoffbestimmung verfälscht. Die Überprüfung des Effektes mit einem Impulsofen-Gerät ergab bei Einsatz eines hochschwefelhaltigen Stahls (0,22% S) im Gegensatz zu früheren Untersuchungen mit klassischen Heißextraktionsgeräten kein Auftreten flüchtiger schwefelhaltiger Verbindungen. Bei der Analyse sulfidischen Materials tritt die Störung jedoch bereits bei Einsatz kleiner Probemengen auf.

Beim Heißextraktionsverfahren kann Schwefel die Sauerstoffbestimmung durch Bildung flüchtiger Verbindungen wie CS_2 oder COS verfälschen. Nachgewiesen wurde dieser Effekt an schwefalhaltigen Stählen,[1,2] wobei bereits Schwefelgehalte oberhalb 0,05% stören.[1] Für diese Untersuchungen wurden klassische Vakuum-Heißextraktionsgeräte eingesetzt. Ungeklärt war bisher, in welchem Maße Schwefel bei der Sauerstoffbestimmung mit modernen Heißextraktionsgeräten (Impulsofen mit Temperaturen über 2500°) stört.

Für entsprechende Untersuchungen an einem hochschwefelhaltigem Stahl (0,085% C, 0,83% Mn, 0,22% S, <0,02% Si, 0,067% P) setzten wir das Gerät TC 30 (LECO) ein und entnahmen unmittelbar nach dem Impulsofen Gasproben für die gaschromatographische Analyse. Bei der Entgasung mehrerer Proben des genannten Stahls konnten in keinem Fall flüchtige Schwefelverbindungen nachgewiesen werden. Ursache dafür ist wahrscheinlich die gegenüber den älteren Geräten wesentlich höhere Entgasungstemperatur und—in Verbindung damit—die kurze Verweilzeit der Gase im Graphittiegel. Offen bleibt, welchen Einfluß die Bindungsform des Schwefels hat.[3] Ein metallisches Standard-Referenz-Material mit noch höherem Schwefelgehalt stand nicht zur Verfügung, so daß keine Aussagen darüber gemacht werden können, bei welchen Schwefelkonzentrationen die Störung auftritt.

Analoge Versuche wurden mit folgenden Sulfiden durchgeführt: FeS and MoS_2 in reiner Form, präparativ hergestellt, und Molybdänglanz technischer Reinheit. Die Sulfide wurden unvermischt mit anderem Material in Nickelkapseln analysiert und dabei die Probenmasse zwischen 0,5 und 5 mg variiert. In allen Fällen konnte die Bildung beträchtlicher Mengen von Schwefelkohlenstoff (5 bis 10% der eingesetzten Menge an sulfidischem Schwefel) beobachtet werden. Es ist deshalb Vorsicht geboten bei der Sauerstoffbestimmung in Isolaten der metallkundlichen Analyse, wenn die Isolate Sulfide enthalten.

Insgesamt ergibt sich, daß die Impulsofentechnik nicht in dem Maße störanfällig ist wie das klassische Vakuum-Heißextraktionsverfahren, jedoch auch hier bei der Analyse jedes schwefelhaltigen Materials geprüft werden muß, ob ein entsprechender Störeinfluß vorliegt.

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Summary—The results of the determination of oxygen in materials with a high content of sulphur by the method of inert gas fusion may be erroneous because of the production of CS_2. Contrary to earlier investigations, the effect is found not to occur in the case of high sulphur steel (0.22% S) if an impulse-furnace instrument is used. However, when sulphide materials are analysed the interference appears even with very small samples.

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THE PRESENT STATUS AND FUTURE OF ROOM-TEMPERATURE PHOSPHORIMETRY*

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Room temperature phosphorimetry (RTP) has been studied by a number of research groups[1–31] and has been shown to be a highly selective, quite sensitive method of analysis for certain organic compounds in specialized environments. The advantages of RTP over conventional low-temperature phosphorimetry (LTP) are: (*i*) the simpler analytical procedure which involves solid substrates at room temperature rather than glasses in small cells at liquid-nitrogen temperatures, does not involve time-consuming degassing of solvents, and essentially avoids the difficulties of cell alignment and positioning and use of cryogenic equipment; (*ii*) the possibility of automation of RTP for routine analysis and even combination with chromatographic separations; (*iii*) the great selectivity, and in some cases near specificity, of measurement of certain species. Of course, the use of pulsed sources and gated detection for time-resolution and selective excitation and/or selective emission measurements by spectrometric means can be and is applicable to both RTP and LTP.

There are two rather minor limitations of RTP and one major limitation. The minor limitations are: (*i*) the uniformity (thickness, porosity, *etc.*) of most commercial substrates varies considerably from one lot to another or even within the same lot, resulting in precision and accuracy problems, and (*ii*) despite the simplicity of a room-temperature method compared to a low-temperature method where a coolant such as liquid nitrogen is used, the RTP procedure is at present more tedious to use and more susceptible to random and systematic errors than the LTP procedure. Item (*i*), although at present a problem, could be minimized, perhaps avoided, by technological advances, particularly within the companies producing the substrates. Item (*ii*) could also be minimized, possibly avoided, by technological advances by instrument companies developing RTP equipment; for example, the use of a continuous filter assembly with automated sample introduction[11] will solve this particular problem. Therefore, limitations (*i*) and (*ii*) can easily be dealt with by researchers who wish to use RTP, and (preferably) by industrial concerns producing the substrates and/or commercial instrumentation for RTP.

The major limitation to RTP is currently the ever-present phosphorescence background (~400–600 nm) in virtually all substrates in which the analyte also produces significant phosphorescence signal levels. In the past, we at the University of Florida, as well as others, have evaluated substrates for RTP on a rather random, haphazard basis (see Table 1) with relatively little success in overcoming the major limitation. For a wide variety of substrates, where S_A = analyte signal, S_B = background signal and N_B = background noise, S_A may vary over a wide range, but the ratios S_A/S_B and S_A/N_B rarely vary by more than a small factor, say 2 or 3, unless the substrate is completely ineffective and the analyte phosphorescence is not detectable. We have also studied a variety of pretreatments of substrates, including baking for various time periods at various oven temperatures, eluting with various polar and non-polar solvents, and irradiation with various light-sources, but have found no combination of substrates and pretreatments that will noticeably enhance the S_A/S_B and S_A/N_B ratios.

It is apparent that the future potential of RTP is immense in clinical, environmental, forensic and industrial applications because of its potential simplicity, capability of automation, and the possibility of prior chromatographic separations on the same substrate, particularly if the phosphorescence background problem can be minimized. Minimization of the phosphorescence background would lead to a greater wealth of applications because of the resulting improvement in analytical figures of merit, including detection limits, linear dynamic ranges, sensitivities and precisions, for discrete sampling units as well as for automated systems. The solution of the phosphorescence background problem can be approached in two major ways: (*i*) by researchers, who wish to use RTP, studying the mechanism of the background from commercial substrates and then developing means of minimizing it; and (*ii*) by industrial concerns, who develop filter paper, chromatographic supports, and separation materials, working with external

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‡ Present address: Proctor & Gamble Co., Cincinnati, OH 45241.

Table 1. Substrates used for room-temperature phosphorimetry

Substrate	Manufacturer	Analyte type*	Detectability of phosphorescence†	Reference
Paper and films				
S & S 604	Schleicher and	Drugs	Y	4,10
S & S 591C	Schuell	Drugs, PAHs	Y	9,10,11
S & S 903		Pesticides, drugs, PAHs	Y	4,29
S & S 904		Pesticides	Y	15,16
S & S 470W, 576C, 593, 598C, 406, 2034A, 2034AHy.		Drugs, PAHs	Y	31
E-D 613	Eaton–Dikeman	Drugs	Y	4,5
E-D 615		Drugs	Y	5
Whatman 1 (silanized, not silanized)	Whatman	*N*-Heterocyclics	Y	3,21,22
Whatman 4		Dyes	Y	25
Whatman 40		Drugs, PAHs	Y	22,31
Whatman 42		Drugs	Y	27,28
Whatman 30,41		Drugs, PAHs	Y	31
Reeve-Angel 201, 202	Reeve Angel	Drugs	Y	3,4
Curtin 7760	Curtin Scientific	Drugs	Y	4
S-P-F2402, F2401	Scientific Products	Drugs	Y	4
Will-13021, 13061	Will Scientific	Drugs	Y	4
Gelman SG, SAF	Gelman	Drugs, PAHs	Y	31
Millipore		Drugs, PAHs	Y	31
Celgard 2400, 2402, 2500		Drugs, PAHs	Y^w	31
Celgard 3501, 4510, K404A, K406A, 5511		Drugs, PAHs	Y^w	31
Teflon		Drugs, PAHs	N	10,31
Polyethylene fibre		Drugs		3
Pellets—TLC plates				
Sodium acetate (also paper impregnated with NaAc)	Reeve Angel	Drugs	Y	10,17,18
Silica gel (plastic-backed, glass-backed, aluminium-backed)	EM Laboratories	*N*-Heterocyclics Drugs	Y	19,20,21,31
Florisil		Drugs	Y^w	
Alumina		Drugs	Y^w	31
Other materials				
Glass fibres		Drugs	Y^w	1,2,3
Asbestos		Drugs, PAHs	N	31
Cellulose fibres		Drugs	Y	3
Sucrose		Drugs	Y	3
Starch		Drugs, PAHs	N	31
Charcoal		Drugs	N	3
Stainless steel		Drugs, PAHs	N	31
Saran wrap		Drugs, PAHs	N	31
Glass wool		Drugs, PAHs	N	31
Cotton		Drugs, PAHs	N	31
Glass				
Aqueous micelles		PAHs	Y	29

* Heavy-atom perturbers included I^- for ionic species and Ag^+, Tl^+, Hg^{2+}, and Pb^{2+} for polyaromatic hydrocarbons. Other components of the medium included NaOH and HCl.

† Y = yes, Y^w = yes but weak, N = no.

(or internal) researchers to minimize the background and increase the S_A/S_B and S_A/N_B ratios for a wide variety of ionic and non-ionic organic compounds. The latter approach appears to be much the more promising; we hope this plea is heard, as we strongly feel the potential analytical use of RTP is great.

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TITRIMETRIC DETERMINATION OF URANIUM IN LOW-GRADE ORES BY THE FERROUS ION–PHOSPHORIC ACID REDUCTION METHOD

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Summary—The modification and extension of the U.S.A.E.C. ferrous ion–phosphoric acid reduction method for the determination of uranium in high-grade or relatively pure material to a method for the determination of uranium with a high accuracy and precision, in ores containing 0.004–7% U is described. It is simple, rapid and requires no prior separations from elements that, in other methods, frequently interfere. For sample materials having very high concentrations of interfering elements, a prior concentration step using extraction with tri-n-octylphosphine oxide is described, but it is shown that, for most low-grade ores, this step is unnecessary.

The Canada Centre for Mineral and Energy Technology (CANMET), formerly known as the Mines Branch, has been involved in projects associated with the development of processes for the recovery of uranium from various Canadian uranium sources since the 1940s. During this time there has been a continuing interest in the investigation and application of analytical methods for the determination of uranium in the ores, mill products and high-grade uranium concentrates. In addition, the Canadian Certified Reference Materials Project (CCRMP) is a current activity of CANMET to prepare reference materials for use in analytical laboratories associated with mining, metallurgy and earth sciences. One facet of this activity is to co-ordinate an interlaboratory certification programme to certify chemically a number of radioactive ore samples for uranium and thorium content. This report describes two analytical methods developed and used in our laboratories to determine uranium in uranium ores and certified reference materials.

Previous work in our laboratory[1] has applied the ferrous ion–phosphoric acid reduction method[2] developed by the New Brunswick Laboratories (NBL) of the U.S. Atomic Energy Commission to the determination of uranium in high-grade uranium concentrates, oxides and solutions. An interference study showed that, in most cases, no prior separations from elements that frequently interfere in other methods were necessary. Most adaptations of the method have been confined to high-uranium materials such as high-purity uranium metal, oxides, alloys, uranium concentrates, *etc.* So far as the authors are aware, little work has been reported on the application of the method to uranium ores in which the uranium is a minor rather than a major constituent.

Eberle *et al.*[2] defined the optimum conditions for a 50-ml sample volume and determined 24–125 mg of uranium by potentiometric titration to a fixed potential of 640 mV. They used vanadyl sulphate as a catalyst to sharpen the end-point. They determined uranium in synthetic solutions, uranium–zirconium–niobium and uranium–stainless steel residues as well as in some calcined ash residues but did not determine smaller amounts of uranium or apply the method to uranium ores. Earlier, Cherry[3] had employed amperometric end-point detection to determine 0.1–50 mg of U_3O_8 but did not use vanadyl sulphate as a catalyst. Slanina *et al.*[4] have adapted the NBL procedure for the automatic potentiometric titration of 2–25 mg of uranium in small sample weights of high-uranium materials such as uranium dioxide, uranium alloys and "SALE" intercomparison samples.

In this report, we describe the successful extension of the ferrous ion–phosphoric acid reduction method to the determination of uranium in typical complex low-grade uranium ores including waste rock, a nickel–cobalt–arsenide concentrate, and a euxenite mineral, as well as various dissolution procedures for these ores. The potentiometric method is useful for titrating 1 mg or more of uranium and a sample weight of about 2 g is adequate for ores containing more than 0.1% uranium, but for material containing less than 0.1% uranium up to 10 g is required. The amperometric method is useful for titrating 0.05 mg or more of uranium and hence lower concentrations can be determined or smaller samples can be used. For uranium contents between 0.004 and 7% U excellent accuracy and precision are obtained by both methods.

EXPERIMENTAL

Apparatus

The equipment for amperometric end-point detection was assembled in our laboratories and consisted of a digital voltmeter (capable of measuring to the nearest 0.0001 V) to measure the current flowing through a precision 10000-ohm resistor, and a Sargent-Welch hook-type platinum wire electrode (Cat. No. S-30421) rotated by a Sargent-Welch synchronous rotator (Cat. No. S-76485). A low-resistance external saturated calomel electrode (SCE), connected by means of a saturated potassium chloride solution bridge, served as a reference electrode.

An expanded-scale or digital pH-meter equipped with calomel and platinum electrodes was used for the potentiometric titrations. The platinum electrode was a coil of sturdy wire which was cleaned daily by immersion in hot 16*M* nitric acid for several min, rinsed with water, then heated to redness in a burner flame.

A 3-min timer equipped with an alarm was used to time the oxidation of the ferrous iron in the procedure.

A 5 or 10-ml burette, reading to 0.01 ml, was used to add the titrant and a magnetic stirrer with Teflon bar was used to stir the sample solution. A Thermolyne water-cooled cooling plate (Fisher Cat. No. 14–511–110) was used, when necessary, to prevent heat from the magnetic stirrer warming the sample solution.

Reagents

Standard 0.025N potassium dichromate solution. Prepare in the usual way and prepare 0.005*N* or 0.0125*N* solutions from it by dilution with water. Standardize against NBS 950a U_3O_8 or NBS 960 uranium metal by the potentiometric procedure, taking 4 or 5 aliquots of a standard uranium solution containing from 1 to 100 mg of uranium, with calibrated silicone-coated pipettes,[1] and correcting for the temperature[5] and blank.

The other reagents required are the same as described in references 1 and 2.

Decomposition procedures

The samples may be brought into solution by (*a*) multi-acid treatment; (*b*) potassium pyrosulphate fusion; (*c*) sodium fluoroborate fusion. If a sample contains significant amounts of elements that may interfere in the reduction or titration step, the uranium can be separated from the interferents by extraction with trioctylphosphine oxide from a solution of the sodium fluoroborate melt.

Multi-acid treatment. The procedure described is for the potentiometric titration; for amperometric titration take smaller sample weights, *e.g.*, 0.1–0.5 g of ores and up to 3 g of waste material, and correspondingly smaller amounts of acid. Transfer a 1–5 g sample of the ore or a 10-g sample of waste material to a covered 400-ml Teflon beaker. Add 25 ml of 48% hydrofluoric acid and digest at low heat for 20–30 min or until the sample is decomposed*. Add 10 ml of concentrated hydrochloric acid and 10 ml of concentrated nitric acid and digest for another 30 min or until most of the black material is oxidized. Remove the cover, add 15 ml of 72% perchloric acid and evaporate the solution to fumes. Cool, rinse the walls of the beaker with a little water and fume again. Cover the beaker and boil the perchloric acid solution vigorously to remove the black coating on the walls of the beaker. Transfer the residual solution with a minimum of water to a 400-ml glass beaker. Rinse the walls of the Teflon beaker with a few ml of concentrated hydrochloric acid from a dropping bottle, cover the beaker, and warm it on the hot-plate for a few min to remove traces of sample from the walls of the beaker. Add the hydrochloric acid rinse to the perchloric acid solution and evaporate the combined solutions to near dryness. (Most samples are decomposed by this procedure but some, *e.g.*, CCRMP DL-1, BL-1 and BL-4, leave a small amount of unattacked black material. This should be filtered off and fused with sodium fluoroborate and the cooled melt combined with the original filtrate before the evaporation to near dryness.) Add 70 ml of 85% phosphoric acid to the residue, cover the beaker, and boil the solution until it is clear. (The solution will not be clear if hydrofluoric acid has not been added to remove the silica.) Cool to room temperature and add 50 ml of 20% v/v sulphuric acid. Determine the uranium by the potentiometric procedure. If the amperometric procedure is to be used add 28–30 ml of phosphoric acid and 20 ml of 9*M* sulphuric acid instead of the quantities stated and use a 150-ml beaker.

Potassium pyrosulphate fusion. Fuse a 2-g sample of ore with 8–10 g of potassium pyrosulphate in a platinum crucible at red heat until decomposition of the ore is complete. Place the cooled crucible and melt in a 400-ml beaker, add 50 ml of 20% v/v sulphuric acid, and digest at just below the boiling point on the hot-plate until the melt is dissolved. Remove the crucible and rinse it with water. Adjust the sample solution to 50 ml, by evaporation if necessary, and determine the uranium by the potentiometric procedure.

Sodium fluoroborate fusion. Mix 1–2 g of the ore sample with 4–5 times its weight of sodium fluoroborate and fuse the mixture in a platinum crucible over a Méker burner until dissolution is complete (~5 min). Place the cooled crucible in a 400-ml beaker, add 50 ml of 20% v/v sulphuric acid, and heat on the hot-plate until the melt has been dissolved. Remove the crucible and rinse it with water. Determine the uranium by the potentiometric procedure.

Sodium fluoroborate fusion and TOPO extraction. Fuse the ore with sodium fluoroborate, dissolve the cooled melt in 20 ml of 5*M* nitric acid, transfer the solution to a 125-ml separatory funnel and dilute to 50 ml with water. Add 0.3 g of sodium fluoride and 0.1 g of ascorbic acid and mix to dissolve. Add 10 ml of 0.1*M* trioctylphosphine oxide in cyclohexane and shake for 1–2 min. Allow the phases to separate, then discard the aqueous phase. Drain the organic phase into a 400-ml beaker, rinsing with several ml of chloroform or carbon tetrachloride.

Evaporate the combined extract and rinsings to dryness on the hot-plate. Add 10 ml of concentrated sulphuric acid, 10 ml of concentrated nitric acid, and 2 ml of 72% perchloric acid and carefully evaporate to fumes of sulphuric acid to destroy the organic material. Repeat the treatment with small additions of nitric acid to ensure the destruction of the organic material. Dilute the solution to 50 ml with water and determine the uranium by the potentiometric procedure.

Reduction and titration of uranium

Potentiometric procedure. This is applicable to the determination of from 1 to 300 mg of uranium but normally between 1 and 70 mg of uranium is determined in ore samples.

To the sample solution add 5 ml of 1.5*M* sulphamic acid and 70 ml of 85% phosphoric acid (unless this is already present). Warm the solution to about 48° and stir the solution continuously, but moderately, with a Teflon-coated magnetic stirring bar. Pipette 5 ml of 1*M* ferrous sulphate into the solution without touching the walls of the beaker. When the temperature of the solution reaches 41°, gener-

* It has been found that some samples need not be treated with hydrofluoric acid to remove silica, because the uranium is completely leached by the other acids. This may not be true of all minerals and the behaviour of any particular ore should be examined by experiment before a procedure for routine purposes is chosen.

ally within 5 min*, wash down the beaker sides with 15 ml of nitric acid–sulphamic acid–molybdate solution. Exactly 3 min (use a timer equipped with an alarm) after the disappearance of the brown-black colour, add 100 ml of water and 5 ml of 0.1*M* vanadyl sulphate or 100 mg of solid vanadyl sulphate. Immediately insert the platinum and calomel electrodes in the solution, stir briskly without spattering, and titrate with 0.005*N* or 0.0125*N* potassium dichromate to a fixed potential near 630 mV. Complete the titration within 3 min of adding the water. Correct the volume of titrant used for the ambient temperature[5] and, if necessary, for the blank†.

Amperometric procedure. This is applicable to the determination of 0.05–50 mg of uranium but is usually confined to the determination of less than 10 mg. It can be used only if the sample is decomposed by the multi-acid method or if a TOPO extraction has been made, because the large amount of salts introduced by a fusion method renders the method impractical.

Adjust the solution in a 150-ml beaker to a volume of 20 ml containing 5–10 ml of concentrated sulphuric acid. Add 2 ml of 1.5*M* sulphamic acid solution and 28–30 ml of 85% phosphoric acid (unless already present). Stir the solution continuously, but moderately, with a Teflon-coated magnetic stirring bar. Pipette 2 ml of 1*M* ferrous sulphate into the solution without touching the walls of the beaker. After 30 sec wash down the beaker sides with 6 ml of the nitric acid–sulphamic acid–molybdate solution from a pipette. Exactly 3 min (use a timer equipped with an alarm) after the disappearance of the brown-black colour, add 40 ml of water and 40–50 mg of vanadyl sulphate crystals. Immediately insert the platinum and calomel electrodes into the solution, rotate the platinum electrode and apply to it a voltage of +1.0 V *vs.* the SCE. Titrate with 0.005*N* or 0.0125*N* potassium dichromate solution and record the current readings *vs.* volume of titrant added. Complete the titration within 3–5 min of adding the water. Determine the end-point graphically. Standardize the dichromate solution by titrating known amounts of uranium, *e.g.*, 0.5–10 mg, in the same way. Establish the blank graphically and correct for it if necessary.

PRELIMINARY EXPERIMENTS

Potentiometric method

Preliminary tests established that the potentiometric titration curves have well-defined inflexion points at 630 mV with a slope of 1200 mV/ml and 3000 mV/ml for 0.005*N* and 0.0125*N* potassium dichromate titrant respectively. In a titration to a fixed potential of 630 mV an error of ±30 mV at the end-point would be equivalent to approximately ±0.015 mg of uranium with either titrant. In practice, use of a fine burette tip permitted the addition of titrant in 0.01-ml increments so that the end-point could be approached to within ±10 mV, *i.e.*, an error equivalent to ±0.006 mg of uranium when 0.005*N* potassium dichromate was the titrant. With this titrant a minimum of about 0.1 mg of uranium could be titrated, with a precision of about 10–15%. For higher amounts of uranium the relative error is much less (Table 1). There appears to be no bias, and the precision for higher concentrations is about the same as in the original method.[2]

Table 1. Potentiometric determination of small amounts of uranium

$[K_2Cr_2O_7]$, *N*	U taken, *mg*	U found, *mg*	Difference, *mg*
0.005	0.103	0.096	−0.007
	0.509	0.498	−0.011
	1.025	1.026	+0.001
	5.112	5.118	+0.006
	12.76$_2$	12.75$_6$	−0.006
0.0125	15.27$_0$	15.26$_5$	−0.005
	30.00$_0$	30.00$_9$	+0.009
	50.38$_6$	50.38$_3$	−0.003

The blank correction was established without titration, by carrying a uranium-free solution through all the steps of the procedure and measuring the potential of the final solution prepared for titration. The volume of titrant needed to bring the potential to 630 mV was then estimated from the previously determined slope of the titration curve. For example, if the potential of the blank solution was 590 mV a correction of (630–590)/1200 ml was subtracted from the volume of 0.005*N* dichromate solution required to titrate the uranium. On the other hand, if the potential reading was 690 mV, a correction of (690–630)/1200 ml was added. Significant blank corrections, *e.g.*, from −0.03 to +0.07 ml of 0.005*N* potassium dichromate were necessary only for amounts of uranium less than 15 mg; blank corrections for amounts of uranium greater than 15 mg that were titrated with 0.0125*N* potassium dichromate were virtually zero. The blank corrections are generally constant for each batch of reagents but must be determined each time new reagents are prepared.

The vanadyl sulphate catalyst was found to be the dominant factor in the blank. The blank correction could be nil, negative or positive, depending on the method and care used in the preparation of the vanadyl sulphate solution, owing to the possible presence or absence of traces of reducing or oxidizing agents. The catalyst solution can be prepared by reducing ammonium metavanadate with hydrazine sulphate as described by Eberle *et al.*[2] and Lund and Yamamura[6] or from vanadyl sulphate crystals.

If the vanadyl solution is prepared from ammonium metavanadate, traces of reducing or oxidizing agents may not be completely destroyed unless great care is taken in the fuming stage. The use of vanadyl sulphate is more convenient, but we have found that it sometimes contains traces of reducing or oxidizing substances which can vary from bottle to bottle. These impurities can be eliminated by treating the vanadyl sulphate with sulphuric acid and a small amount of hydrazine sulphate followed by fuming very carefully to destroy the impurities and excess of

* Frequently, cooling to 41° takes longer, especially at ambient temperatures up to 32°. In addition, the magnetic stirring motor unit warms up and can heat the sample solution. If this occurs, use a water-cooled cooling plate between the magnetic stirring unit and the beaker.

† Correction for ambient temperature need not be made if the volume of titrant is less than 10 ml or if high accuracy is not required but a blank correction is necessary for a low titrant volume.

Table 2. Effect of decomposition procedure on recovery of uranium

Procedure	U taken, mg	U found, mg	Difference, mg
Potassium pyrosulphate fusion			
Blank (8 g of $K_2S_2O_7$)	Nil	Nil	—
Blank + 2 g of NBS 99	Nil	Nil	—
Blank + 2 g of NBS 99	5.112	5.172	+0.060
Blank + 2 g of NBS 99	12.762	12.660	−0.102
Sodium fluoroborate fusion			
Blank (8 g of $NaBF_4$)	Nil	Nil	—
Blank + 2 g of NBS 99	Nil	0.018	+0.018
Blank + 2 g of NBS 99	5.112	5.136	+0.024
Blank + 2 g of NBS 99	12.762	12.750	−0.012
Multi-acid			
Blank (30 ml of HCl, 20 ml of HNO_3, 10 ml of HF, 25 ml of 1:1 H_2SO_4)	Nil	0.015	+0.015
Blank + 2 g of NBS 99	Nil	0.015	+0.015
Blank + 2 g of NBS 99	5.112	5.130	+0.018
Blank + 2 g of NBS 99	12.762	12.762	0.000

hydrazine sulphate. Either vanadyl sulphate crystals or the prepared solution can be used satisfactorily in the titration step, but each new batch of either should be checked before use to ensure the absence of reductants or oxidants. This is done by means of a blank determination on all reagents, by the potentiometric procedure just described. The potential of the solution should be about 630 mV and should not change when either 100 mg or 5 ml of the vanadyl catalyst is added. A change of ±20 mV can normally be neglected or can be accounted for in the blank correction, depending on the accuracy desired.

Attempts made to titrate less than 0.1 mg of uranium potentiometrically with 0.005*N* potassium dichromate were unsuccessful because of the relatively slow response of the electrodes, as a result of which the titration could not be completed within the prescribed time limit of 3 min. Moreover, the blank correction was much greater and less reproducible. Further attempts to titrate less than 0.5 mg of uranium potentiometrically were therefore abandoned.

Effect of the decomposition procedure. Each dissolution procedure was investigated for its possible effect on the recovery of uranium, NBS 99 Feldspar being used to simulate a uranium ore matrix. After dissolution, the feldspar samples were "spiked" with different amounts of uranium. Other samples served as blanks. The results in Table 2 show that none of the decomposition procedures has any effect on the recovery of uranium added as a spike.

Interferences. Scarborough and Bodnar[7] found that all the platinum metals (except rhodium) caused slightly high results in the Eberle method. For example, in the titration of about 100 mg of uranium, the presence of 1–15 mg of platinum caused the results to be correspondingly 0.14–1.5% high. In our experiments in which fusions were done in platinum crucibles, there was no evidence that platinum interfered, presumably because not enough was dissolved from crucibles.

No study was made of other potentially interfering elements because previous studies[1,2] had shown that, in most cases, no prior separations were required.

Amperometric end-point detection method

The potentiometric procedure was suitable for the titration of as little as 0.1 mg of uranium in a pure solution but was unsatisfactory for the determination of this amount in ores, *i.e.*, 0.001% in a 10-g sample, because of the sluggish response of the electrodes and the uncertainty of the blank corrections at low levels of uranium.

Cherry[3] used amperometric end-point detection but we observed that his procedure in the end-point was sluggish. The titration was time-consuming and the recoveries were low, presumably owing to air-oxidation in the time required to complete the titration. Cherry did not use vanadyl sulphate as a catalyst in the titration and we believe this omission is the reason for the sluggish end-point.

We therefore re-examined the system to find the smallest amount of uranium that could be satisfactorily titrated. The amounts of reagents were scaled down from those used in the potentiometric procedure.

Effect of sulphuric acid concentration. Controlling the temperature to 41°, as required for destruction of the excess of ferrous iron in the potentiometric method, is not as critical because of the smaller amounts present, so the reduction–oxidation step can be done at room temperature. However, the concentration of sulphuric acid has an effect on the amount of uranium recovered (Table 3).

The results in Table 3 show that the recovery of uranium is complete only when the concentration of sulphuric acid exceeds 2*N*, *i.e.*, 3 ml of concentrated acid per 50 ml of solution before the titration. With the sulphuric acid concentration used in the potentiometric procedure, 1.4*N*, the results are significantly lower and less precise. In all subsequent tests a concentration of at least 3.5*N* was used for the solution for titration.

Reagent blanks carried through the entire procedure corresponded to about 0.01 mg of uranium. The actual uranium content of the blank was virtually zero, however, and the apparent blank was due to the behaviour of the system, which sets a lower limit to the amount of uranium that can be determined, *i.e.*, about 0.03 mg.

Effect of delay in titration time. Known amounts of uranium were carried through the reduction and oxi-

Table 3. Effect of concentration of sulphuric acid on the recovery of uranium by amperometric titration of 50 ml of solution

$[H_2SO_4]$, *N*	No. of detns.	U taken, mg	U found, mg
1.4	4	0.103	0.094 ± 0.009
1.4	6	0.515	0.425 ± 0.106
2.1	3	0.515	0.513 ± 0.006
3.5	5	0.103	0.103 ± 0.003
3.5	10	0.515	0.514 ± 0.005
7.0	1	0.103	0.104

Table 4. Effect of delay in titration time on recovery of uranium by amperometric titration

Standing time, min	Uranium taken, mg	Uranium found, mg
0–1	0.515	0.517, 0.513
15	0.515	0.482, 0.491
30	0.515	0.453, 0.457

dation steps and then let stand for various lengths of time before titration. Table 4 shows that low recoveries of uranium occur if the solutions are left for too long before titration, the error being a linear function of delay time. Accurate results will be obtained only if the titration is completed within 5 min of adding the water and vanadyl catalyst.

The low recoveries may be due to aerial oxidation, but oxidation by nitric or nitrous oxide is also a possibility if the solution is not stirred sufficiently rapidly to disperse the gas bubbles quickly during the oxidation of the excess of ferrous iron.

Several tests were made to establish the range over which uranium could be successfully titrated amperometrically (Table 5).

The results in Table 5 show that good accuracy and precision are obtained in the titration of 0.1–10 mg of uranium. Similar results were obtained if the volume of solution for titration was 25 or 100 ml. In practice it is easier to use the larger volumes of 50 or 100 ml.

RESULTS AND DISCUSSION

Analysis of uranium ore

Potentiometric method. A number of international standard and other uranium ores were analysed after decomposition by all the procedures as described, and the results compared with the certified values or those

Table 5. Amperometric determination of small amounts of uranium (50 ml of solution)

No. of detns.	U taken, mg	U found, mg	Std. devn, mg
1	0.005	N.D.*	
1	0.010	N.D.*	
2	0.020	N.D.*	
8	0.051	0.051 ± 0.013	±0.008
5	0.103	0.103 ± 0.003	
10	0.515	0.514 ± 0.005	±0.003
1	5.111	5.111	
1	10.19	10.18	

* Not determinable, titrations were of same magnitude as the blank.

Table 6. The potentiometric determination of uranium in ores

Sample	Weight, g	Decomposition procedure	U_3O_8 found, %				
			Fe^{+2}–H_3PO_4 reduction	Certified	Colorimetr.†	Fluorimetr.†	X-ray†
EMM-228	2	$K_2S_2O_7$	0.474, 0.474	0.471	0.472	0.473	—
Torbernite*	2	Multi-acid	0.470, 0.470, 0.469	—	—	—	—
(Australia)	2	$NaBF_4$	0.471, 0.471	—	—	—	—
EMM-229*	2	$K_2S_2O_7$	0.308, 0.307	0.313	0.314	0.304	—
Torbernite	2	Multi-acid	0.308, 0.306	—	—	—	—
(Spain)	2	$NaBF_4$	0.308	—	—	—	—
	2	$NaBF_4$ + TOPO	0.308	—	—	—	—
EMM-230	2	$K_2S_2O_7$	0.363, 0.365, 0.371	0.375	0.366	0.366	—
Uraninite*	2	Multi-acid	0.362, 0.369, 0.358, 0.370	—	—	—	—
(Australia)	5	Multi-acid + Hg cathode	0.370, 0.365	—	—	—	—
	2	$NaBF_4$ + TOPO	0.361	—	—	—	—
EMM-231	2	$K_2S_2O_7$	0.415, 0.418, 0.418	0.418	0.417	0.413	—
Carnotite*	2	Multi-acid	0.417, 0.413	—	—	—	—
(USA)	2	NaBF	0.415	—	—	—	—
	2	$NaBF_4$ + TOPO	0.421	—	—	—	—
EMM-5598§	5	Multi-acid	0.126, 0.124	0.126	—	—	—
Uranium Ore	5	Multi-acid + Hg cathode	0.124	—	—	—	—
Eldorado Port	10	Multi-acid	0.027, 0.022, 0.023, 0.021	0.028	0.023	—	—
Radium Mine Waste	10	Multi-acid + Hg cathode	0.020	—	—	—	—
Rock Standard							
EMQ-3876 Australian AEC Standard Ore S-317‡	2	$K_2S_2O_7$	0.134, 0.137	0.137	0.132	0.13	0.13
EMQ-3877 Australian AEC Standard Ore S-318‡	2	$K_2S_2O_7$	0.768, 0.752	0.748	0.752	0.75	0.75
EMQ-3878 Australian AEC	1.5	$NaBF_4$ + TOPO	0.272¶	0.31	0.295	0.29	0.28
Ore S-319‡	1.5	$NaBF_4$ + TOPO	0.274	—	—	—	—
EMP-3052 Ni-Co-As Concentrate	0.5	$K_2S_2O_7$	1.34	—	1.30	—	—
EMD-4153 Euxenite	0.3	Multi-acid	5.75	—	5.80	—	5.75

* International Atomic Energy Agency (Vienna) Standard.
† Average of several determinations.[8]
‡ Australian Atomic Energy Commission, Lucas Heights, N.S.W.
§ Canadian Uranium Producers' Standard CUP 115 (Elliot Lake, Ont.).
¶ Sample said to be segregated.[10]

Table 7. Potentiometric determination of uranium in CCRMP uranium ores (multi-acid decomposition)

Sample	Weight, g	No. of detns.		U found, % Certified	Titrimetric
DH-1	1	5		0.177	0.177 ± 0.001
DL-1	10	5	(a)	0.0041	0.00400 ± 0.00006
DL-2	10	10	(b)	0.011–0.012	0.0113 ± 0.0002
	10	10	(c)	(tentative value)	0.0114 ± 0.0001 (f)
BL-1	5	5	(d)	0.022	0.0205 ± 0.0003
	5	5	(a)		0.0210 ± 0.0004
BL-2	1	5		0.453	0.453 ± 0.001
BL-3	0.5	5		1.02	1.021 ± 0.005
BL-4	1	5	(d)	0.173	0.170 ± 0.001
	1	5	(a)		0.172 ± 0.001
BL-5	2.5 g/250 ml + 10 ml	5		7.09 (e)	$7.15_3 \pm 0.03_1$

(a) Fluoroborate fusion of residue from multi-acid treatment.
(b) Hydrofluoric acid omitted from multi-acid dissolution procedure.
(c) Hydrofluoric acid included in multi-acid dissolution procedure.
(d) No fluoroborate fusion of residue from multi-acid treatment.
(e) Work in our laboratories and others indicates the "true value" to be closer to 7.13–7.14% U than to the consensus value.[13]
(f) Standard deviation = ±0.00004%.

obtained by a colorimetric,[8] fluorimetric or X-ray procedure,[9] as shown in Table 6. Some uranium ores to be used as certified reference materials in the CCRMP programme were also analysed (Table 7).

The TOPO extraction is fairly specific and serves to separate uranium from many common interfering elements.[11,12] Therefore, to decide whether substantial amounts of potentially interfering elements would cause error in the proposed method a few samples of ore were decomposed by the sodium fluoroborate–TOPO procedure and the uranium was determined by the potentiometric method. Another batch of samples was subjected to a mercury cathode electrolysis to remove possibly interfering species. The results in Table 6 show that it is unnecessary to remove these other elements, but also show that no uranium is lost if the removal *is* applied.

All the methods are equally reliable, but the fusion with sodium fluoroborate has been found to be more convenient in practice, so is used whenever possible. This flux is also more suitable if the uranium is to be extracted with TOPO. The multi-acid treatment is useful if large samples are taken and/or the samples contain much silica, *e.g.*, are waste or tailings.

Amperometric method

Several uranium-bearing ores were analysed by the amperometric method and the results compared with the potentiometric, certified, colorimetric,[8] fluorimetric and X-ray[9] values (Table 8).

Because of the smaller weights taken the ores were dissolved by the multi-acid method. The amounts of acid used, however, were only about a fifth of the amounts given in the procedure. Some of the ore samples were titrated after adjustment to 50 ml, and others were diluted to 100 ml after appropriate amounts of sulphuric acid and other reagents had been added. The results show that the amperometric method also gives satisfactory accuracy and precision.

Table 8. Amperometric determination of uranium in ores

Sample	Weight, g	Volume of solution titrated, ml	% U_3O_8 Certified	Found
EMQ 3875	2.5	50	0.0100	0.0081, 0.0084,
Australian AEC				0.0106
Standard ore S-316*	2.5	100		0.0091, 0.0100
EMQ 3876	0.5	50	0.137	0.131, 0.132,
Australian AEC				0.139, 0.136
Standard ore S-317*				
EMQ 3877	0.1	50	0.748	0.754, 0.754,
Australian AEC				0.745
Standard ore S-318*	0.1	100		0.755, 0.749
EMQ 3878	0.2	50	0.31	0.296, 0.277,
Australian AEC				0.278, 0.303
Standard ore S-319*	0.2	100		0.303, 0.301
EMM 228	0.1	50	0.471	0.469, 0.466
Torbernite (Australia)†				
EMM 230	0.1	50	0.375	0.370, 0.367
Uraninite (Australia)†				
EMM 231	0.1	50	0.418	0.420, 0.409
Carnotite (U.S.A.)†				
EMM 229	0.1	50	0.313	0.314, 0.302
Torbernite (Spain)†				

* Australian Atomic Energy Commission, Lucas Heights, N.S.W.
† International Atomic Energy Agency (Vienna) Standard.

It can be used to titrate smaller amounts of uranium than the potentiometric method, and hence only $\frac{1}{20}$–$\frac{1}{3}$ of the sample weight is required, with no sacrifice in accuracy or precision. This enables a multi-acid treatment to be used and simplifies the decomposition procedure. Moreover, the reagent consumption is lower and the blank is virtually zero. The amounts of uranium titrated in the samples listed in Table 8 range from 0.2 to 1 mg whereas for the samples in Tables 6 and 7 the amounts were 3–20 and 1–10 mg respectively. Small sample size may introduce some error, however, if the sample is not homogeneous. A disadvantage of the method is the need to plot graphs to determine the end-point.

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A METHOD FOR REDUCING THE FLOW-SENSITIVITY OF A POLAROGRAPHIC DISSOLVED-OXYGEN SENSOR

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Summary—A method for reducing the flow-sensitivity of polarographic dissolved-oxygen sensors has been modelled mathematically and tested experimentally. The improved probe uses a two-layer membrane: the first layer is made of Teflon and the second of silicone rubber, which is more permeable than Teflon to oxygen. This arrangement reduces the flow-sensitivity to about 4% of that for a single-membrane 1-mil Teflon; second membrane 2.5-mil silicone rubber. (*b*) First membrane 0.5-mil Teflon; second membrane 5-mil silicone rubber.

Membrane-covered dissolved-oxygen (DO) probes have found acceptance in a wide range of research and industrial applications.[1] Most, if not all, practical sensors may be termed polarographic devices (although the method is clearly voltammetry) following the concepts of Clark's design.[2] The sensor is composed of a pair of electrodes immersed in an electrolyte solution and separated from the test-solution by a gas-permeable membrane. Oxygen diffuses through the membrane and is reduced cathodically at the surface of a noble metal. The electrolytic current thus generated is proportional to the partial pressure of oxygen at the surface of the membrane and to the diffusivity of oxygen through the membrane. The consumption of oxygen by the probe necessitates a certain rate of flow at the surface of the membrane to replenish the oxygen removed; otherwise the sensor will register a pO_2 lower than the true value for the test-solution. The effect of the flow-rate on the response is referred to as the flow-sensitivity of the probe, and it should be kept as low as possible.

The requirement of solution flow past DO sensors is a major disadvantage of conventional probes: it calls for a pump or other flow-producing mechanism (such as a stirrer) if an accurate reading is to be obtained. Here we describe a method for reducing the flow-sensitivity of polarographic DO sensors. Its use should eliminate the need for pumping or stirring in most applications.

THEORETICAL CONSIDERATIONS

Various methods for reducing flow-sensitivity of DO sensors have previously been reported.[3–5] Unfortunately, these attempts have generally resulted in sluggish probes with long response-times, ranging from minutes to hours. In most applications, such long response-times are unacceptable, and in addition, calibration and standardization of very slow sensors is impractical.

Butler *et al.*[5] demonstrated that the flow-sensitivity of DO electrodes can be considerably reduced if the cathode is made hair-like. This approach is an application of the well-known fact that sensors with an extremely small cathode are relatively insensitive to changes in flow. Unfortunately, the output current of the DO sensor is proportional to the cathode size, and this sets a practical lower limit to the size of the cathode. Butler *et al.* improved the situation somewhat by using a spiral of 40-μm thick platinum as the cathode, which resulted in a sensor with the flow-sensitivity of a 40-μm thick cathode but with a much higher output current, owing to the relatively larger area. We have found that sensors of this type still require stirring in most applications.

The flow-sensitivity of DO sensors may be improved by reducing the oxygen consumption and distributing it over a larger volume of the test-solution. Once the consumption per unit area is reduced to a low enough level, eddy diffusion within the solution may suffice to replenish the oxygen consumed by the sensor. With large-area cathodes, oxygen diffusion paths are approximately perpendicular to the surface of the cathode and hence the oxygen is consumed from the solution directly overlying the cathode area (Fig. 1). It is therefore evident that in this case oxygen consumption per unit area can be reduced only by reducing the permeability of the membrane separating the cathode from the test-solution. Unfortunately, this will slow down the response of the electrode and reduce its sensitivity.[3] However, at the edge of the cathode, oxygen is consumed from a larger area

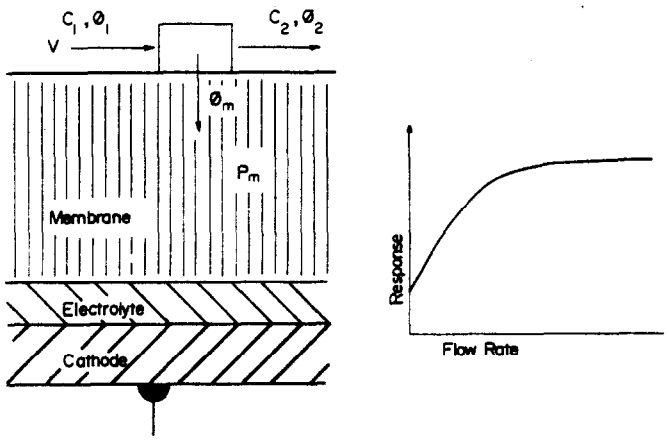

Fig. 1. Schematic representation of the origin of flow-sensitivity in a membrane-covered polarographic sensor with large surface area. (*a*) Cross-section of cathode and membrane. V is the solution flow-rate, ϕ the flux of the electroactive species and P_m is the permeability of the membrane. (*b*) Resulting flow-sensitivity of a large-area cathode sensor.

owing to the spreading out of the diffusion paths. This "edge effect" becomes much more important if the total surface area of the cathode is reduced (Fig. 2).

A further reduction of oxygen consumption per unit area (or volume) of solution can be obtained by further spreading out the diffusion stream-lines. This could be accomplished by introducing a second gas-permeable membrane between the primary membrane and the solution. The second membrane should have a much higher permeability to oxygen than the primary membrane has. An analogue of this configuration would be a point heat-source separated from a solution by a layer of a thermal insulator, above which there is a layer of a thermal conductor. It is obvious, intuitively, that the layer with high thermal conductivity will help in distributing the heat over a larger area than would be the case in its absence. This approach, of using two gas-permeable membranes, is considered here.

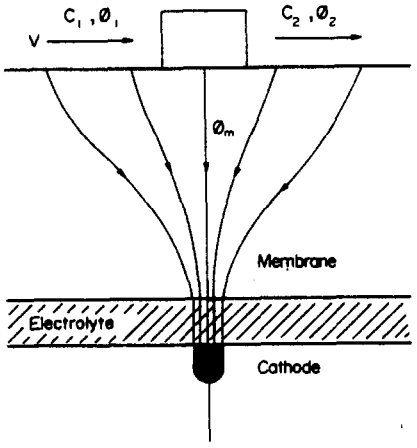

Fig. 2. Schematic representation of diffusion paths in a polarographic sensor with a small-area cathode. Since consumption of electroactive species per surface area is smaller than for a large-area electrode, the sensor is less sensitive to flow.

Among the various gas permeable-membranes applicable for DO sensors, Teflon (Trade-Mark of Du-Pont) is usually preferred on account of its relatively high diffusivity to oxygen, and its mechanical strength, mechanical stability and inertness.[6] The extra layer required for reducing flow-sensitivity should thus have a much higher permeability to oxygen than that of Teflon. Only one material, silicone rubber, was found to meet this criterion. Its permeability to oxygen is about 60 times that of Teflon.[7]

MODEL CALCULATIONS

Migration of gases through membranes is usually treated in terms of the permeability coefficient (P) rather than the diffusion coefficient (D). This simplifies calculations because the gas transport can be treated as a function of the partial pressure, which is a continuous function for boundary layers. Permeability is related to diffusivity by[7]

$$P = SD \tag{1}$$

where S is the solubility coefficient of the gas in the membrane.

The diffusion flux of oxygen, J, at any point in a cross-section through a membrane can thus be expressed as:

$$J = -P\left(\frac{dv}{dx} + \frac{dv}{dy}\right) \tag{2}$$

where v is the partial pressure of oxygen and the permeability P is assumed to have no directional dependence but may vary along the plane.

Under the steady-state conditions, the net flux at any given point must be zero. This can be simulated numerically by calculating the gradients of pO_2 from each point, along the co-ordinates of the cros-section, deriving the net flux and setting it to zero. From this

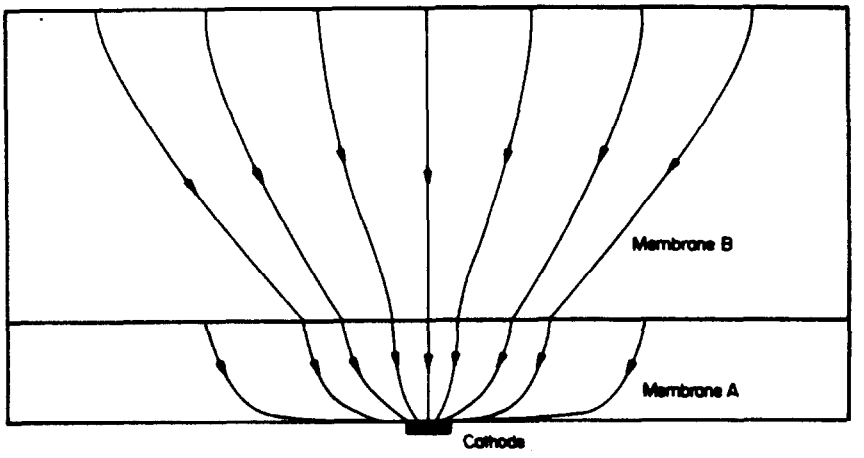

Fig. 3. Diffusion path of a double-membrane polarographic sensor, computed for the model given. The permeability of the silicone rubber membrane (B) was assumed to be 60 times that of the Teflon membrane (A).

equality $v_{i,j}$ at the centre point can be derived:

$$v_{i,j} = \frac{P_{i-1}v_{i-1,j} + P_i(v_{i,j-1} + v_{i,j+1} + v_{i+1,j})}{P_{i-1} + 3P_i} \quad (3)$$

where i is the line number of the grid, j the column number, and the grid is assumed to be perpendicular to the cathode surface, *i.e.*, it represents a cross-section through the membrane. The cathode is assumed to be a long and narrow bar so that diffusion parallel to its length (the z axis) is negligible. The boundary conditions are $v = 0$ at the cathode surface and $v = 1$ in the test-solution. It is also assumed that

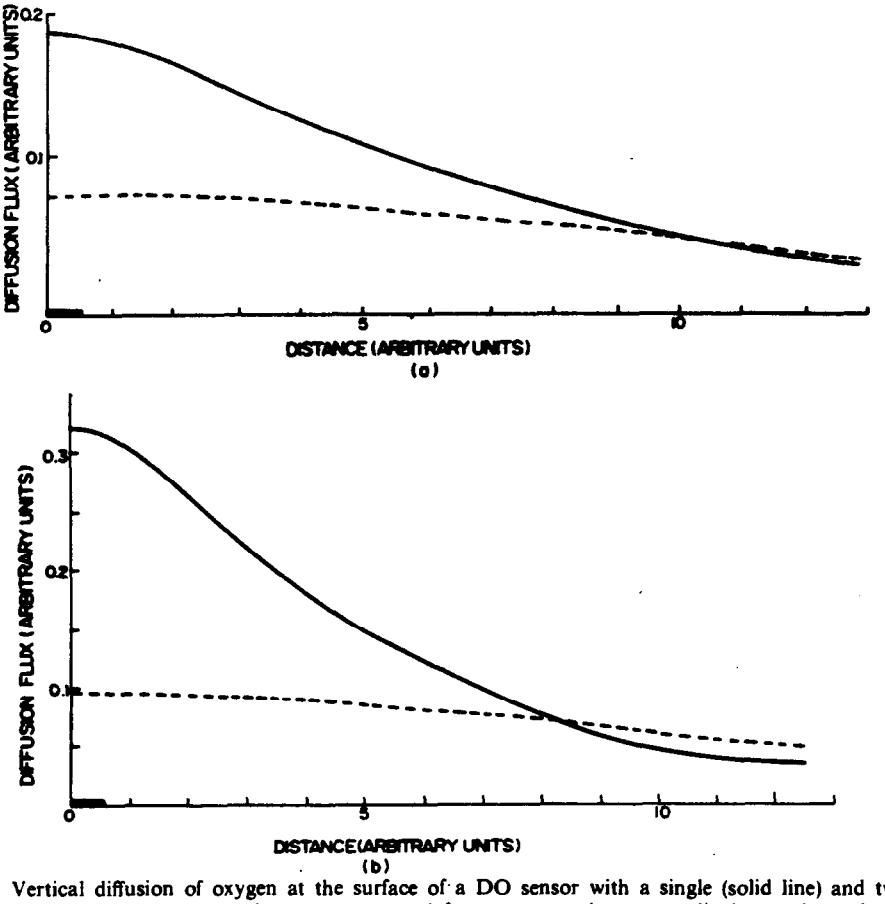

Fig. 4. Vertical diffusion of oxygen at the surface of a DO sensor with a single (solid line) and two (broken line) gas-permeable membrane(s), computed for a cross-section perpendicular to the cathode. Only one half of the plane is shown. The heavy bar represents one half of the cathode width. (*a*) First membrane 1-mil Teflon; Second membrane 2.5-mil silicone rubber. (*b*) First membrane 0.5-mil Teflon; second membrane 5-mil silicone rubber.

a thin electrolyte layer separates the cathode and the first membrane.[8] The calculations are done for the two membranes in contact.

Model calculations of this type confirm the assumption that a layer with high diffusivity will reduce the oxygen consumption per unit surface area of test-solution. This is illustrated in Figs. 3 and 4 which summarize three computer runs. It is clear that the introduction of a second membrane, of high permeability, has the effect of spreading out the oxygen consumption (Fig. 3). Furthermore, the localized high-consumption peak of the single membrane sensors is significantly damped out (Fig. 4) in the two-membrane sensors.

EXPERIMENTAL

The performance of the double-membrane electrode was tested with the sensor shown in Fig. 5. It comprised a Plexiglas body in which a cavity was formed. The anode was a 1-mm thick silver wire, about 5 cm long, wound around the central stem in which the gold cathode was cast. The gold cathode surface was shaped in the form of a thin bar 3 mm long and about 15 μm wide.

Sensitivity to flow was tested in demineralized water in a 500-ml beaker at room temperature (25 $\pm$ 2°). The solution was stirred with a magnetic bar and was kept saturated with respect to the atmosphere by bubbling air through it. All measurements were made with a 0.8 V polarization potential and 2M potassium chloride as internal electrolyte solution.

The signal processer was a home-made electronic circuit built around a MOSFET operational amplifier (RCA CA 3130).

The sensitivity of the sensor to stirring, for a given membrane pair, was found by comparing the response at full stirring speed and the response with no stirring or air-bubbling. A rest period of about 15 min was allowed before the response for the unstirred system was read. It should be noted, though, that even if forced stirring is not used, some solution movement is still expected, because of convective diffusion. However, since a similar effect is expected in practical applications we consider the present results to represent a realistic measure of the flow-sensitivity of the sensor.

RESULTS AND DISCUSSION

The flow-sensitivity of the DO sensor was tested for various membrane pairs (Table 1). The response was found to be mainly dependent on the thickness of the Teflon membrane. This would be expected, considering that the permeability of silicone rubber to oxygen is about 60 times the permeability of Teflon. Hence, the diffusion is primarily controlled by the Teflon membrane. The flow-sensitivity is expressed as the relative difference in response for the stirred and unstirred systems.

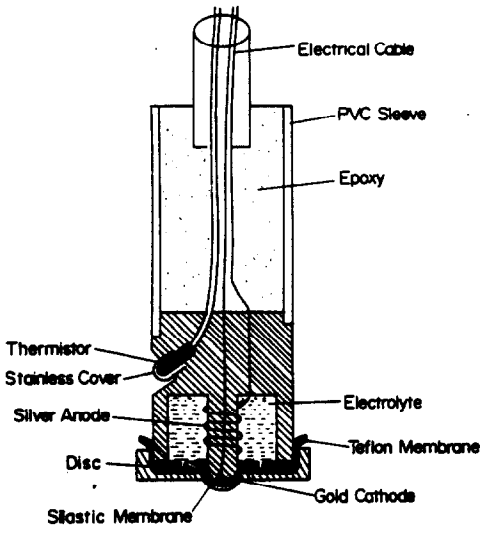

Fig. 5. Cross-section of the DO sensor used in the present study.

The flow-sensitivity of the sensor is greatly reduced when a two-membrane configuration is used (Table 1), as predicted by the model calculation. The highest flow-sensitivity (*ca.* 58% change in response) was registered with the thinnest Teflon membrane (0.5 mil), in complete agreement with our assumption that the flow-sensitivity is a function of the rate of oxygen consumption per unit area. The 0.5-mil Teflon membrane had the lowest resistance to diffusion and hence the rate of oxygen consumption should be the largest. This is substantiated by the magnitude of the response in an air-saturated solution (440 nA), the largest value for this set of experiments.

Table 1. Performance of the proposed sensor with different membranes

Membrane thicknesses, *mil*		Response in air	Response in stirred air-saturated solution	Decrease in response,*	Response time,† *sec*	
Teflon	Silicone rubber	*nA*	*nA*	%	t_{63}	t_{90}
0.5	—	490	440	58	0.9	1.2
1	—	331	320	25	3.0	6.5
2	—	281	268	23	4.5	14
1	10	238	240	9	18.0	32
2	10	270	269	4	14.5	24
1	40	285	301	4	115	220

* Difference in response to well-stirred and unstirred solutions, expressed as fraction of response in stirred solution.

† Time to reach 63% (t_{63}) or 90% (t_{90}) of final value.

The flow-sensitivity of a sensor with a single Teflon membrane decreases progressively as thicker membranes are used (Table 1). With a 1-mil membrane the reduction in response is about 25%, much too high for most practical applications. However, this flow-sensitivity is already considerably smaller than that expected for a cathode of large area, because of the enhanced "edge effect" of the hair-like electrode (Fig. 2). The addition of the extra membrane, highly permeable to oxygen, was found to be effective in further reducing the sensitivity to flow (Table 1), the difference in response between stirred and unstirred solutions decreasing with increasing thickness of the silicone rubber membrane. Minimum flow-sensitivity was obtained with a combination of a 1-mil Teflon film and a 40-mil silicone rubber membrane. However, the response time to reach 90% of maximum signal ($t_{90\%}$) for this membrane pair was about 220 sec, which is probably too high for most applications. Fortunately, a similar flow-sensitivity (*ca.* 4%) was found for the 2-mil Teflon film and 10-mil silicone rubber membrane combination. The response time for this pair ($t_{90\%}$ = 24 sec) is considerably shorter and probably acceptable even for manual individual measurements.

A second bonus of the double-membrane system is the similarity in response to air (the gas phase) and air-saturated solution. Ideally, these responses should be identical because the electrode is sensitive to pO_2, which is identical in the two phases. However, experience has shown that the response of conventional DO sensors to solutions is much lower than that to the gas phase. This could be explained by the existence of a hydrodynamic boundary layer[9] adjacent to the membrane when it is in contact with a stirred solution. This stationary film provides an extra resistance to diffusion and hence the diffusion flux should be smaller than the diffusion flux obtained in the gas phase, where there is no additional resistance to oxygen migration towards the cathode. An equality of response to air and air-saturated solution has an important practical advantage. Sensors exhibiting this behaviour can be calibrated with air instead of an air-saturated solution (or any other standard solution), because the pO_2 in air is constant. This calibration procedure is very convenient and time-saving in field measurements with portable instruments.

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EFFECT OF HALOGENATED SOLVENTS ON IRON ATOMIZATION IN GRAPHITE-FURNACE ATOMIC-ABSORPTION SPECTROSCOPY

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Summary—When an iron solution in an organic solvent is examined by atomic-absorption spectroscopy, the signal decreases to below that for a comparable aqueous solution. This effect is most pronounced for halogen-containing solvents because of volatility of $FeCl_2$, and a change in the atomization curve with a shift towards higher temperatures for atomization. When the cuvette is pretreated with halogenated solvent a double peak is observed. The first peak corresponds to that found for aqueous solvents, the second may be correlated with the peak for iron in a halogenated solvent. The peak-splitting effect of such a solvent increases with halogen:hydrogen ratio in the solvent. The halogen penetrates the structure of the graphite cuvette and probably forms strong carbon–halogen compounds which modify the atomization conditions for iron. When water is introduced hydrogen halide is formed, which removes the excess of halogen from the atomization region.

Determination of metals by flameless atomic-absorption spectroscopy is influenced by the presence of many accompanying substances[1] as well as by the atomizer parameters.[2,3] Many authors have pointed out the effect of halide ions on the determination of lead,[1,4,5] copper,[6,7] nickel[8] and iron.[9] Such effects may be due to the volatility of halides[8] or to occlusion of metal atoms in the matrix crystals.[10] The unfavourable influence of chlorine was also noted when it was introduced as gaseous carbon tetrachloride into the stream of inert sheath gas.[11] Taking into account that halogenated solvents are widely used in analytical chemistry and may be used in preliminary concentrations or separations it seemed necessary to investigate in more detail their effect on the processes in the graphite atomizer.

EXPERIMENTAL

Apparatus

A Perkin–Elmer model 300 A atomic-absorption spectrophotometer with an HGA-72 graphite cuvette and a Hitachi–Perkin–Elmer model 159 recorder were used throughout this work. The Perkin–Elmer hollow-cathode lamp was run at 30 mA.

Eppendorf–Merburg micropipettes were used.

Reagents and solutions

Standard aqueous iron(III) chloride solution, 1 mg/ml, diluted as necessary to give the working solutions.

Standard non-aqueous iron(III) solution, 0.1 mg/ml, was prepared from a Merck reagent. The solution contained 98.5 mg of tris(1-phenyl-1,3-butanedione) iron in 100 ml of methyl isobutyl ketone (MIBK). The working solutions, 1 μg/ml, were prepared by dilution with n-hexane or carbon tetrachloride.

Measurements

For iron a three-step temperature programme was used: drying for 20 sec at 100°, pyrolysis for 20 sec at 1000° and atomization for 15 sec at 2600°. All measurements were made at 248.3 nm (0.2-nm band-pass). In the atomization study no separate pyrolysis stage was used and the atomization curves were recorded for temperature increase from room temperature to 2660° at an average rate of 65°/sec. Argon was used as sheath-gas, at a flow-rate of 1.5 l./min.

The cuvettes were modified by injection of 10 or 20 μl of an organic solvent, drying for 20 sec at 100°, and heating for 15 sec at 1500°. After cooling of the cuvette the sample was injected and the absorbance measurements were made, either the peak-height or the peak-area being used. Under similar conditions the dependence of the absorbance on time or temperature was measured. The temperature was generally measured according to the manufacturer's instruction and occasionally checked with a photoelectric pyrometer.

RESULTS AND DISCUSSION

Influence of atomizer conditions on determination of iron

Atomization of iron from the graphite cuvette is a process which strongly depends on the composition of the injected solution. In the case of aqueous solutions of iron(III) chloride the maximal absorbance was obtained at 1800°. When the sample contained the metal as tris(1-phenyl-1,3-butenedione)iron dissolved in n-hexane the atomization temperature did not change, but the peak-height decreased by approx. 25% (Fig. 1). A much greater suppression was observed when the organic iron standard solution was diluted with carbon tetrachloride instead of n-hexane. Under these conditions the peak-height was less than a tenth of that for corresponding aqueous solutions of iron, and the atomization was also significantly slower in reaching the maximal value at 2300°. This indicates an entirely different mechanism in the presence of carbon tetrachloride.

The effect of carbon tetrachloride on atomization of iron was also investigated through introduction of the

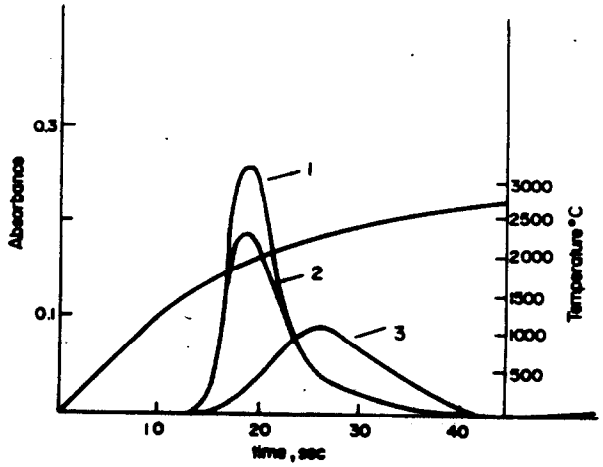

Fig. 1. Atomization curves for iron in various solvents: 1–10 ng Fe in aqueous solution; 2–10 ng Fe in n-hexane solution; 3–40 ng Fe in carbon tetrachloride solution.

solvent into the cuvette before injection of the sample, according to the procedure described above. When either the aqueous or the organic solution of the analyte was introduced into such a modified cuvette, splitting of the absorption peak was observed (Fig. 2). This indicates that iron is atomized by two different routes. The first is that for the non-modified cuvette, *i.e.*, the atomization temperature is approx. 1800°. The second peak occurs at 2200°, which is about 100° lower than that for atomization of iron injected in solution in carbon tetrachloride. Experiments were performed with the impregnated cuvette heated to different temperatures before introduction of the iron solution. Usually, for chlorine-containing solvents, prior heating to 2100° is sufficient to remove the solvent effect. The gradual disappearance of splitting of the iron peak as the temperature to which the cuvette is preheated is increased from 1000 to 2010° is shown for the case of carbon tetrachloride (Fig. 3). For bromine- or iodine-containing solvents this temperature should be about 100° higher. Thus cleaning of such solvents from cuvettes may be done at a temperature slightly higher than those indicated, but in our experiments was done at 2660°.

To ensure that such phenomena are connected with the contact of graphite with organic solvent, a tantalum boat was placed in the cuvette and the analytical samples were injected into it. Atomization in these conditions obviously proceeds through a different mechanism (Fig. 4) and the tantalum boat somewhat inhibits the formation of free atoms, the formation being maximal at 2200°. In contrary to the case when carbon tetrachloride is introduced before the sample into the graphite surface, its addition to the tantalum atomizer has no observable effect. During the drying and heating periods the solvent undoubtedly evaporates completely from the smooth metal surface, whereas it penetrates the graphite surface, modifying its structure or reacting with it chemically.

Effect of various halogenated solvents

The observed effect of treating the cuvette with carbon tetrachloride suggested that similar effects may also be observed in the case of other solvents contain-

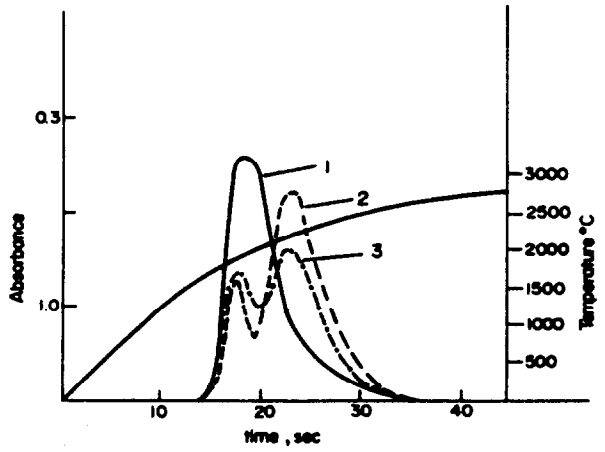

Fig. 2. Atomization curve for 10 ng of iron. 1—Non-modified cuvette, aqueous analyte solution; 2—modified cuvette with CCl_4, aqueous analyte solution; 3—modified cuvette with CCl_4, n-hexane analyte solution.

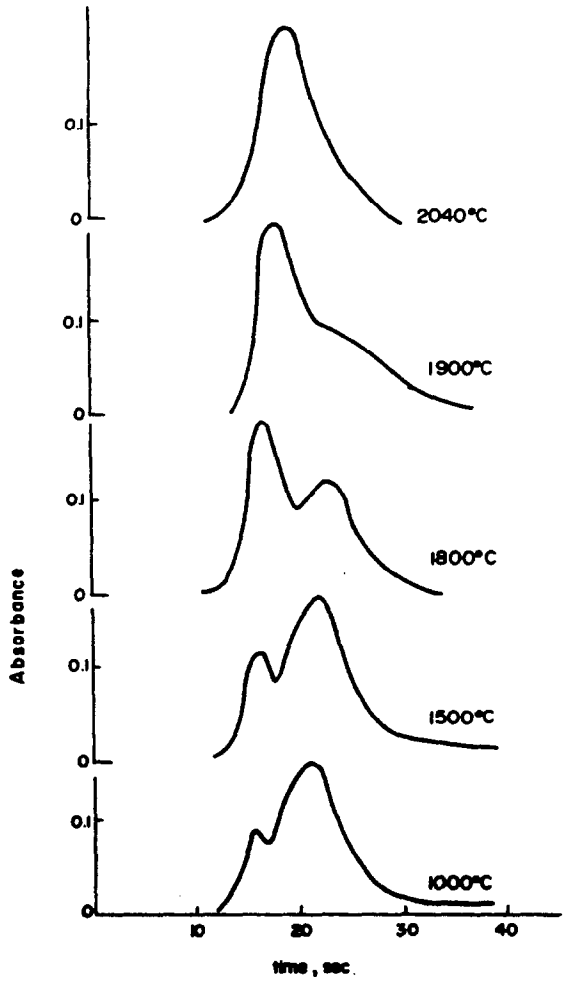

Fig. 3. Effect of preheating tempearature of the cuvette impregnated with 20 μg of carbon tetrachloride, on the subsequent atomization of 10 ng of iron.

ing halogen atoms. Several of them, listed in Table 1, were investigated and compared with hydrocarbon solvents such as hexane, benzene and toluene. All halogenated solvents or solutions of halogens in organic solvents exhibit a similar effect and a double peak is always observed on the atomization curve, whereas other solvents do not exert such behaviour (Fig. 5). This indicates that the presence of halogens is

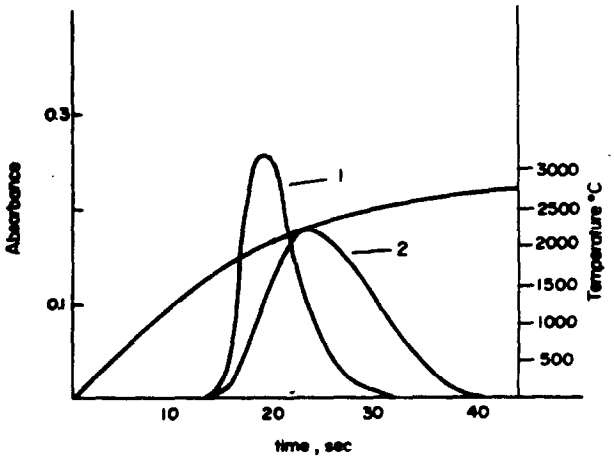

Fig. 4. Atomization curves for 10 ng of iron in aqueous solution: 1—graphite cuvette; 2—tantalum boat.

Table 1. Substances used for modifying the graphite cuvette

Carbon tetrachloride	Bromoform	1-Iodopropane	1-Hexane
Chloroform	1-Bromohexane	Iodine in n-hexane	Benzene
1-Chlorohexane	Bromine in n-hexane		Toluene
Chlorobenzene			
1,2-Dichloroethane			

responsible for the occurrence of the second peak. The relative heights of both peaks depend on the amount of the halogenated compound as well as on its nature. Increase in halogen content suppresses the first peak, which always appears at an atomization temperature of 1800°, and increases the second peak at higher temperatures. This effect is more pronounced for organic solvents which contain a greater ratio of halogen to hydrogen in the molecule and is the largest when bromide or iodine solutions in n-hexane are used (Table 2; Fig. 5). A significant difference was noted between the effects of carbon tetrachloride and 1-chlorohexane but no systematic changes in the peak-area were observed (Table 2). For chlorine and bromine compounds the position of the second peak is similar and corresponds to an atomization temperature of approx. 2200°. In the case of iodine compounds the second peak appears somewhat earlier, *i.e.*, at 2000°.

The first peak for iron atomization from the modified graphite cuvette seems to be connected with that part of the sample which does not penetrate into the graphite, as it also occurs with untreated graphite atomizers. The second peak should be the result of formation in the modified graphite of more thermally stable compounds, which need higher temperatures for their decomposition.

When measurements are made under the usual conditions for analytical determination, *i.e.*, when the maximal atomization temperature is achieved in 2–3 sec this solvent effect is less significant. The iron peak is suppressed by only about 25%, and a small

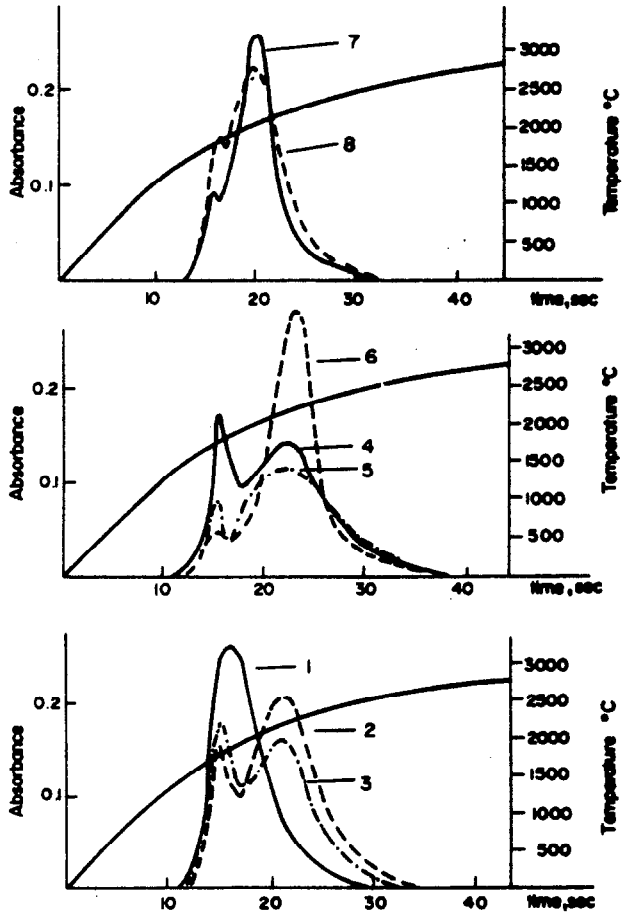

Fig. 5. Atomization curves for 10 ng of iron from cuvettes modified with various halogenated solvents: non-modified cuvette (1), cuvette modified with chlorobenzene (2), 1-chlorohexane (3), 1-bromohexane (4), bromoform (5), bromine in n-hexane (6), 1-iodopropane (7), iodine in n-hexane (8).

Table 2. Effect of halogenated solvents (20 μl) used for cuvette impregnation, on the peak-heights and peak-areas for 10 μl of iron solution

Solvent	Amount of iron, *ng*	Peak I, height, *mm*	Peak II, height, *mm*	Peak height ratio (II/I)	Peak area, *arbitrary units*
CCl_4	5	15	27	1.8	60
	10	36	49	1.4	120
	15	59	72	1.2	160
	20	99	83	0.83	225
$C_2H_4Cl_2$	5	12	23	1.9	48
	10	43	44	1.0	107
	15	57	62	1.1	164
	20	93	76	0.82	221
C_6H_5Cl	5	19	28	1.5	72.5
	10	41	54	1.3	135.5
	15	69	62	0.90	166
	20	93	76	0.82	201
$C_6H_{13}Cl$	5	23	23	1.0	—
	10	48	38	0.79	—
	15	90	55	0.62	—

distortion of the peak-shape is visible when the chart-speed is 160 mm/min. The integrated absorbance remains constant under these conditions.

Graphite treated with certain chemicals, *e.g.*, chlorine and bromine, can form two types of compounds.[14,15] The less thermally stable compounds may be easily decomposed and removed, whereas 10–20% of the halogen is more firmly bound as so-called residual compounds. It is supposed that under the influence of halogen (X), species of the formula $C_n^+X^- \cdot 3X_2$ are formed. Partial decomposition with liberation of the halogen occurs at slightly above 1000°, whereas the residual compounds need heating to at least 1900° for decomposition.[16] These graphite compounds are more reactive than the untreated graphite, and in the presence of iron(III) chloride form a new species, $C_n^+FeCl_4^- \cdot 3FeCl_3$.[17,18] Its formation needs an excess of halogen.

Organic solvents injected into a graphite cuvette, in contrast to aqueous solvents penetrate the atomizer walls, giving favourable conditions for subsequent formation of stable graphite compounds. If the excess of halogen is freed from the graphite cuvette it may react with the iron-containing sample, producing relatively volatile iron(III) chloride.

Effect of water on the iron determination

Effects similar to those due to the injection of organic solvent into the graphite cuvette were not observed when aqueous solutions of hydrochloric acid or iodine chloride was used. This seems to be connected with two factors. First, the aqueous solution does not penetrate into the graphite, and remains as a drop on the surface, and secondly the amount of active halogen is diminished because of formation of volatile hydrogen chloride, as a conse-

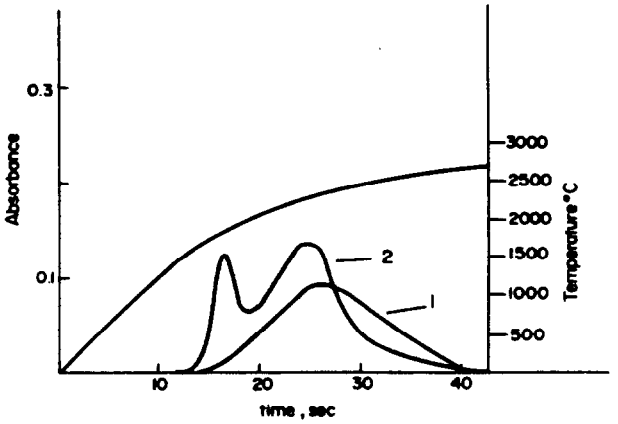

Fig. 6. Atomization curve for 40 ng of iron in carbon tetrachloride solution: 1—in the absence of water; 2—with injection of 10 μl of water.

quence of hydrogen formation in the reaction between water and graphite.[12,13]

The influence of water is also observed when 10 μl of water are added to the cuvette after evaporation of an organic iron solution in carbon tetrachloride. The peak-height increases and some atomization of iron occurs at lower temperature than in the absence of water. This effect is more pronounced if in the pyrolysis step at 1200° the gas flow is stopped. In the absence of water the iron atomization occurs at temperatures as low at 1200°, but iron(II) chloride, which exhibits non-specific absorption, is also produced. During 15 sec the whole of the iron is removed and none is observed when the temperature is increased to 2500°. In the presence of 10 μl of water the only absorption peak recorded is at 2500°, and is significantly higher than that with continuous gas flow.

Conclusions

The experiments performed on the effect of halogen-containing solvents show that they strongly influence the atomization processes of iron in the graphite cuvette. Such behaviour must always be taken into account when halogenated compounds are involved in a routine analytical procedure. It seems probable that effects of this kind can be explained by formation of residual halogen compounds in graphite, and that these, being more active, interact with the iron compounds present as analyte. It is also shown that the presence of water can significantly influence these processes by formation of hydrogen halide and consequent removal of excess of halogen from the cuvette material.

The effect of halogenated solvents on atomization of other metals indicates a somewhat different mechanism and will be the subject of subsequent studies.

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AN INVESTIGATION OF METHODS FOR THE ANALYSIS OF ZINC CONCENTRATES FOR THEIR TOTAL FLUORIDE CONTENTS

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Summary—Accurate determination of trace fluoride down to 6.6 μg/g in zinc concentrate samples is found to be most expediently performed potentiometrically with a fluoride ion-selective electrode and a single-point standard addition after decomposition of the sample by potassium hydroxide fusion. The lower limit of measurement may be reduced to 1.8 μg/g if distillation from tungsten oxide flux is used in place of the fusion, but the analysis takes longer. The need for reliable fluoride data for zinc concentrate standard reference materials is exemplified and results for CANMET zinc concentrate MP-1 are given. The failure of some of the methods used to analyse standard siliceous materials is discussed.

Accurate determination of total fluoride content in geochemical samples has been one of the more difficult of inorganic analyses. During the early part of the last three decades trace fluoride was most commonly determined by spectrophotometric or titrimetric methods. Because of the relatively high sensitivity of the methods to interferences, for most samples the fluoride is separated from the sample matrix in a step applied either during or after opening-out of the sample. Distillation, ion-exchange, precipitation and solvent extract methods have been used for the separation, and the first two seem to have been the most successful.

Steam distillation of hexafluorosilicic acid from sulphuric or perchloric acid (Willard and Winter distillation) has been suggested as a "standard" separation method, generally applicable to most geochemical samples. Although the distillate is relatively free from the matrix components except for halides, nitrate, phosphate and sulphate,[1,2] this method of separation requires caution and constant supervision by the analyst owing to the high temperatures used (120–180°). Also, large volumes (up to 500 ml) of distillate need to be accumulated in order to remove all the fluoride when aluminium, boron, calcium, silicon or zirconium is present.

Although distillation from a solid flux (pyrolytic distillation or pyrohydrolysis) uses higher temperatures (700–1200°), less supervision is required and the distillate volume is as small as convenient for the analyst. The heat can be supplied by flame,[5] electrothermally,[1–4] or by induction heating.[6] The distillate is also relatively free from matrix elements except for, depending on the temperature, halogens,[7] boron[8] and possibly bismuth, cadmium, thallium, tellurium and other volatile metals.[9] Comparisons between the Willard and Winter and pyrolytic distillation methods have been made.[5,10] A variety of pyrolytic distillation fluxes have been reviewed.[4,11,12]

Owing to the volatility of hydrogen fluoride, silicon tetrafluoride and other fluorides, methods of opening out geochemical samples other than by distillation involve a fusion with a basic flux. Carbonates, hydroxides, peroxides, borates and various mixtures of these have been used.[1–4,13,14] For spectrophotometric analysis, a separation step is necessary after opening-out by fusion, whereas direct analysis is possible if an ion-selective electrode is used.

The introduction of the fluoride ion-selective electrode has allowed accurate and precise measurements of trace fluoride in aqeous solution, provided careful calibration is done. Interference caused by complexation with various ions or by excessive ionic strength can be dealt with by using a standard addition method. This determination method has been used successfully for many types of geochemical samples after distillation[5,6,14,16] or after fusion but without separation of the fluoride before measurement.[5,13–16]

In industry, the determination of fluoride can be required in certain cases besides those solely concerned with environmental or health hazards. An example is the production of high-purity zinc. Fluoride, at certain concentrations, is used to condition the silver-lead anodes in order to produce purer electrolytic zinc metal more efficiently.[17] Obviously, during the development, modification or monitoring of such a process, accurate determination of fluoride in any samples related to the process would be necessary. On the other hand, fluoride can also cause problems in the production of high-purity zinc and again its accurate determination is essential.[14]

Five zinc concentrate samples were submitted to this laboratory for determination of their total fluoride contents. The most expedient method was sought by investigating several sample preparation methods in conjunction with two fluoride detection methods.

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The preparation methods were wet digestion with sodium hydroxide and hydrogen peroxide, distillation from tungsten oxide, tungsten oxide–sodium tungstate or tungsten oxide-uranium pentoxide as fluxes, and fusion with sodium carbonate or potassium hydroxide. The detection methods were spectrophotometry by the Alizarin Fluorine Blue method and potentiometry with a fluoride electrode.

EXPERIMENTAL

Reagents

All chemicals used were of reagent grade. According to colorimetric analysis the sodium hydroxide collector solution contained less than 0.08 μg of fluoride per ml (less than 0.05 net absorbance) which corresponds to less than 2 μg/g in the solid reagent used.

The sodium carbonate for fusions was prepared by heating anhydrous sodium bicarbonate at 105° for 24 hr. The 0.1*M* sodium bicarbonate wash solution was also prepared from this reagent.

The preparation and characteristics of the fluoride electrode buffer (5*M* acetate–0.5*M* citrate) have been described elsewhere.[18]

A few drops of 0.5% phenolphthalein solution (in 50% methanol) were put in all sample solutions to give a rough guide to the pH.

Wet digestion with sodium hydroxide and hydrogen peroxide

The sample (0.5 g) was placed in a 250-ml polyethylene beaker. Fifty ml of 0.1*M* sodium hydroxide were added and, cautiously, 50 ml of 30% hydrogen peroxide were slowly added. The whole was heated at 100° for 10 min. The volume was made up to 100 ml and the solution was transferred to a polyethylene storage bottle.

Distillation from solid fluxes

The apparatus used for distillation was similar to that described elsewhere[19] except that the quartz tube was 1 in. in bore instead of 1.25 in., two sodium hydroxide collector solutions were used instead of one, and the burette and stirrer were not used.

The sample (1 g) was mixed in a mortar and pestle with 3 g of anhydrous tungsten oxide, or 7.5 g of anhydrous tungsten oxide–sodium tungstate mixture (1:4) or 3.1 g of anhydrous tungsten oxide–uranium pentoxide mixture (30:1) and transferred into a platinum boat (9.5 × 1 × 1 cm). The furnace was set at about 825°, and the boat was placed in its centre for 45 min. Moist oxygen gas was passed over the sample at a flow-rate of about 1.5 l./min and through two consecutive sodium hydroxide (0.2 or 1*M*, depending on the sample) collector solutions (60 and 40 ml). Glass frits were used to disperse the gases in the collector solutions. The volume was made up to 200 ml and the solution was transferred to a polyethylene storage bottle.

Fusions

The siliceous geochemical samples (0.5 g) were fused for 30 min with 3.5 g of sodium carbonate–zinc oxide flux (6:1) in a platinum crucible, in a muffle furnace at 900°. After cooling, the fusion cake was broken and warmed with distilled water for 2 hr. The mixture was filtered with a Whatman No. 42 filter paper. The volume was made up to 100 ml and the solution was transferred to a polyethylene storage bottle. The zinc concentrate samples were similarly fused with 3.5 g of sodium carbonate, with no zinc oxide.

The zinc concentrate samples (0.5 g) were fused for 30 min with 6 g of potassium hydroxide preheated (to remove moisture) in a nickel crucible in a muffle furnace at 425°. After cooling, the fusion cake was broken and warmed with distilled water for 10 min. The mixture was filtered with a Whatman No. 42 filter paper. The volume was made up to 100 ml and the solution was transferred to a polyethylene storage bottle.

Spectrophotometry

The method used was that recommended by Burdick and Jackson for their reagent "Amadac-F".[20,21] Five ml of the reagent were required for 20 ml of sample solution. The method was direct but there was an appreciable blank value due to the reagent colour. A typical uncorrected graph was linear up to a fluoride concentration of about 0.7 μg/ml with a slope of 0.625 ml/μg and an intercept of 0.36 on the ordinate. The reagent-fluoride complex developed fully in 1 hr and was stable for an additional 24 hr. The absorbance was measured in a 1-cm glass cell at 620 nm with a Beckman DU spectrophotometer.

Potentiometry

The sensing and reference electrodes used were the Orion model 94-09A fluoride and Orion single-junction model 90-01 reference electrodes respectively. The pH-meter used was the Orion microprocessor-ionalyzer/901 which digitally displayed the single-point standard addition results (or other modes when selected) immediately after each analysis. The electrode blank and slope values were included in the microprocessor calculation automatically. Before an analysis the electrode blank value was determined by allowing the electrode to equilibrate in the buffer solution, and the electrode slope value was determined by measuring the potential difference between a 1.0 and a 10.0 μg/ml standard fluoride solution. Both steps are described in the Orion methods manual and were done twice daily. All measurements were made at room temperature without temperature compensation.

To check the calibration, standard solutions in the appropriate range were analysed; for example, when measurements at about the 0.4 μg/ml fluoride level were being made, 0.1–1.0 μg/ml standard solutions, 0.1 μg/ml, were analysed. The results varied from the nominal values by no more than 0.002 μg/ml.

It was assumed that the electrode slope value remained the same for the sample media. This assumption was tested by preparing a calibration curve by adding known amounts of fluoride to aliquots of a solution made from zinc concentrate number 3 fused with potassium hydroxide, and found to be correct. The electrodes were connected to the pH-meter so that the sample solution was grounded. A grounded magnetic stirrer was used with a Teflon-coated stirrer bar for all measurements. Insulation was placed between the sample beaker and magnetic stirrer.

Ten ml of the buffer (5*M* acetate–0.5*M* citrate) were required for 40 ml of sample solution. Hydrochloric acid was used to neutralize most of the hydroxide or carbonate flux before addition of the buffer. Also, when the carbonate fusions were neutralized the sample was vigorously agitated before analysis to remove most of the carbon dioxide which formed.

Plastic vessels (other than Teflon) were used during all potentiometric measurements.

RESULTS AND DISCUSSION

Analysis of standard reference materials

All fluoride determinations discussed were performed, unless stated otherwise, with an ion-selective electrode and single-point standard addition.

We considered the closest commercial standard materials, at least in terms of total element concentration if not mineralogy (Table 1), to be the Canadian CANMET zinc ore concentrates CZN-1, KC-1, MP-1 and RU-1, and the American NBS zinc ore

Table 1. Analysis of zinc concentrate samples (%) by flame atomic absorption spectrophotometry and, for S, by X-ray fluorescence analysis

	Sample				
	1	2	3	4	5
Zn	45	55	60	17	3.4
Pb	6.7	1.2	2.8	12	17
Fe	11	3.8	7.2	26	25
Ca	0.35	1.1	0.78	7.8	0.47
S	31	29	3.0	7.7	12

concentrates 113a and 329. Analysis of one or more of these was necessary to show which preparation method recovered all the fluoride. Unfortunately, only MP-1 had been analysed for total fluoride content (provisional value 4.04%), and this was higher than those in the zinc concentrate samples by a factor between 200 and 2000, depending on the sample.

When we used MP-1 as a test standard we initially doubted the efficiency of the sample preparation steps, because the results were all low, but later, because of the agreement between results obtained by using sodium carbonate and potassium hydroxide fusion, we concluded that the provisional value was erroneously high (Table 2). We are now in the process of collecting the other zinc concentrate standard materials mentioned. The results for total fluoride will be reported elsewhere. It is hoped that some trace fluoride concentrations will be found.

Because distillation and fusion preparation methods have been reported to be successful for the analysis of rocks and minerals for fluoride, two USGS standard rocks, W-1 (recommended fluoride value[22] 250 μg/g) and G-1 (recommended value[22] of 690 μg/g) were analysed. For rock W-1 acceptable recoveries were obtained only with the potassium hydroxide fusion (Table 2).

Failure of the sodium carbonate–zinc oxide fusion in this case was particularly surprising since it was considered a "standard" method. Attempts to improve the efficiency by increasing the heating time to 60 min, doubling the amount of flux to 6 g and changing the ratio of flux components to 4:1 from 6:1 yielded only slightly higher recoveries for W-1 in three trials: 208, 210 and 224 μg/g.

Inspection of previously reported fluoride results for W-1 and G-1 (Table 3) revealed that many values were lower than the potassium hydroxide fusion results reported here. Considering those data and this work, we concluded that the sodium carbonate–zinc oxide fusion was not applicable to these samples and therefore should not have been considered as being generally applicable, *i.e.*, as a "standard" method, for all siliceous geochemical samples. The use of this fusion could be the main reason for such large scatter in the previously reported results. Furthermore, though we did not make a comparison between the

Table 2. Average fluoride concentration in USGS W-1 (250 μg/g), USGS G-1 (690 μg/g) and CANMET zinc concentrate MP-1 standards (s = standard deviation, n = number of replicate determinations)

	Distillation		Fusion	
	WO_3	Na_2WO_4–WO_3	Na_2CO_3–ZnO	KOH
W-1, μg/g	75	93	175–225	253
G-1, μg/g	221	400	524	745
MP-1, %	n.a.*	n.a.*	3.75%†	3.88%‡

* n.a. = not analysed.
† no ZnO, s = 0.05, n = 4.
‡ s = 0.05, n = 4.

Table 3. Literature values for total fluoride (μg/g) in W-1 and G-1 (col. = colorimetry, ISE = ion-selective electrode, dist. = distillation)

W-1	G-1	Method	Reference
205, 210	690, 710	Na_2CO_3–ZnO fusion, col.	7
210, 210	715		
230, 230, 250	630, 650, 630	V_2O_5 dist. (730–750°) col. and ISE	5
220, 220, 230	650, 650, 640		
230	630	Willard and Winter dist.	5
240	724	Na_2CO_3–ZnO fusion, ISE	13
220, 220, 230	610, 620, 620	Willard and Winter dist., col.	23
230, 230, 240	630, 630		
250	700	Values suggested from wide scatter of data from several analysts..	24

two temperature ranges 700–850° and 850–1200°, we feel that pyrolytic distillations in the lower of these temperature ranges are also not applicable to all siliceous geochemical samples.

Recent work on sample preparation by fusion reports the use of sodium peroxide as a flux component,[14,15,25] which offers a more complete opening-out, and distillation from various solid fluxes has been thought or actually found to be efficient only when higher temperatures (1000–1200°) are used.[3,12,26–30]

Though W-1 and G-1 are not guaranteed as homogeneous with respect to trace elements, we feel that the failure to obtain consistent results for fluoride in them is indicative of failure of the method of opening-out rather than of inhomogeneity in the sample.

Analysis of the zinc concentrate samples

Spectrophotometry. Although the Amadac-F reagent was used mainly because of its insensitivity to interfering species, such as sulphate,[20,21] that commonly affect other reagents, only limited success was achieved when the zinc concentrate samples were analysed, depending on which preparation step was used.

The distillates from the tungsten oxide flux were analysed without interference, but those from the tungsten oxide–sodium tungstate flux exhibited strong interference effects. Often, in the case of the latter flux, a red spectral shift occurred (visible to the eye) instead of the blue shift that should have occurred. In worse cases, the Amadac-F reagent coagulated. Because tungsten oxide is considered to be a less efficient flux, we supposed that the red shift or coagulation of the Amadac-F reagent was due to the presence of more of the sample matrix elements in the distillates. This conjecture was born out by a dc-arc spectrographic analysis of the distillate residues.

Solvent extraction of the Amadac–fluoride complex[31] was tried in an attempt to prevent the red spectral shift, but the extracted complex also exhibited a red shift.

There were, during this work, some problems with the Amadac-F reagent owing to the upward drift of standard and sample solution absorbances during the period when the absorbance should have been stable. This was considered to be due to the particular reagent batch, since previous batches did not act in this way. As a result, the lower limit of measurement was defined as a net absorbance value of 0.05 (fluoride 0.08 μg/ml). This corresponded to 20 μg of fluoride per g in the zinc concentrate samples if 0.5 g of sample were distilled to give 100 ml of test solution.

Potentiometry. The theory, operation and applications of this method of fluoride detection have been well documented.[32–34] Comparisons between this method and some colorimetric methods have been made for geochemical samples opened out by distillation or fusion.[5,35]

The blank level of the electrode varied daily, between potentials equivalent to about 0.020 and 0.055 μg/ml fluoride levels. The fluoride level was reproducible during each day to within about 0.002 μg/ml, which corresponded to 0.50 μg/g in the zinc concentrate samples if 0.5 g of sample was dissolved to give 100 ml of test solution. A conservative lower limit of fluoride measurement with the electrode would have been 0.010 μg/ml.

Sodium hydroxide–hydrogen peroxide wet digestion. This method did not open out any of the zinc concentrate samples completely. For example, only 40% of the fluoride in zinc concentrate no. 5 was recovered. It had been hoped that this method would allow very quick separation of fluoride from the sample by simple leaching of the samples with the reagent mixture.

Distillation from solid fluxes. All three fluxes, tungsten oxide, tungsten oxide–sodium tungstate and tungsten oxide–uranium oxide, were applicable to the zinc concentrate samples, the results agreeing with those obtained when fusion was used. Table 4 shows some of the results obtained and we feel that the potassium hydroxide results were the most reliable.

Though the only reagent blank for these methods came from the sodium hydroxide used for collection of distillate, they suffered from the disadvantage of requiring more elaborate equipment and longer analysis time because only one distillation at a time was performed. The lower limit of measurement was dependent on the reproducibility of the total blank value. Ignoring the blank contribution of the sodium hydroxide collector solution, if 0.5 g of sample was distilled to give a test volume of 100 ml and the lower

Table 4. Analytical results (μg/g) for fluoride by potentiometric detection (s = standard deviation, n = number of replicate determinations)

Zinc concentrate	Na_2CO_3 fusion	WO_3 distillation	KOH fusion
1	65 $s = 5, n = 4$	55 $s = 9, n = 10$	58 $s = 10, n = 5$
2	20 $s = 1, n = 4$	33 $s = 6, n = 4$	28 $s = 9, n = 5$
3	<20 $n = 4$	<1.8 $n = 4$	<6.6 $n = 4$
4	18 $s = 2, n = 4$	20 $s = 0.6, n = 4$	22 $s = 5, n = 4$
5	166 $s = 5, n = 4$	140 $n = 1$	165 $s = 10, n = 4$

Table 5. Comparison between single fluoride results obtained by using calibration with simple standards and single-point standard addition

	Preparation	Simple calibration	Standard addition
MP-1, %	Na_2CO_3 fusion	3.76	3.94
MP-1, %	KOH fusion	3.31	3.89
G-1, μg/g	KOH fusion	271	745

detection limit was defined as twice the variability of the electrode blank value (0.002 μg/ml), then the lower limit for this method was 1.0 μg/g for the zinc concentrate samples. However, if the blank from the collector solutions is included and the fluoride in the solid sodium hydroxide is assumed not to exceed 0.2 μg/g, the lower limits when 1*M* and 0.2*M* sodium hydroxide solutions were used were 5.0 and 1.8 μg/g respectively.

These values could possibly be reduced further by using lower concentrations of sodium hydroxide. This would necessitate using an oxidant such as sodium bismuthate in the flux in order to oxidize sulphur to sulphate, to avoid evolution of hydrogen sulphide and its conversion into sulphur dioxide and/or trioxide in the gas phase and passage into the collector solutions. Alternatively, fluoride could perhaps be removed from the collector solutions by ion-exchange chromatography.

It should be noted that for homogeneous samples, *e.g.*, MP-1, a relative standard deviation of about 1.3% is possible (Table 2). According to the relative standard deviations shown in Table 4, at least some of the zinc concentrate samples were inhomogeneous.

Fusions

Although both fusion methods were also applicable to the zinc concentrate samples (Table 4), the potassium hydroxide fusion offered the advantages of shorter analysis time, lower measurement limit and a fusion cake that was more easily broken up for leaching.

Potassium hydroxide was a more efficient flux overall since it dissolved more of the sample matrix, and, as a result, single-point standard addition was used to avoid matrix interference (Table 5). It is possible that use of the sodium carbonate fusion would allow calibration with simple aqueous standards because less matrix interference occurs (Table 5), but this is not recommended, since single-point standard addition is more efficient.

Several flux components were analysed to determine their blank contributions (Table 6). Most contained approximately 1.7 μg of fluoride per g, except for the peroxide salts which contained higher amounts, but the precision was better for the potassium hydroxide. If 0.5 g of sample was fused with 6 g of potassium hydroxide and dissolved to yield 100 ml of solution and the lower detection limit is taken as twice the variability of the overall blank value, then the lower limit for this preparation method was about 6.6 μg/g for the zinc concentrate samples. We feel that this value is a conservative estimate and that concentrations around 2 μg/g could be measured.

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Table 6. Average fluoride content in several reagent fluxes, determined potentiometrically (n = number of replicate determinations, s = standard deviation)

	Reagent	F, μg/g	n	s
KOH	Anachemia AC-7650	1.8	8	0.22
NaOH	Anachemia AC-8371	6.1	1	—
Na_2CO_3	Baker 3604	1.5	1	—
$Na_2CO_3.10H_2O$	Fisher S-264	0.7*	1	—
$NaHCO_3$	Anachemia AC-8248	2.1†	7	0.7‡
Na_2O_2	Merk 7423	4.2	1	—
Na_2O_2	Anachemia AC-8452	2.5	1	—

* 1.9 based on Na_2CO_3.
† 1.7 based on Na_2CO_3.
‡ 0.6 based on Na_2CO_3.

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THE USE OF APPROXIMATION FORMULAE IN CALCULATIONS OF ACID–BASE EQUILIBRIA—IV

MIXTURES OF ACID AND BASE AND TITRATION OF ACID WITH BASE

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Summary—The pH of mixtures of mono- or diprotic acids and a strong base is calculated by use of approximation formulae and the theoretically exact equations. The regions for useful application of the approximation formulae (error <0.02 pH) have been identified. The results obtained are used to calculate the curves for titration of mono- or diprotic acids with a strong base.

In the previous papers in this series, the solutions of the theoretically exact equations and the approximation formulae for the pH of solutions of acids,[1] salts[2] and their mixtures[3] were compared, and the regions in which the approximation formulae give the pH correctly to within ± 0.02 were identified. This paper deals with mixtures of mono- or diprotic acids and a strong base. The calculations were done by the same method as that used in Part II.[2] The results are used to allow the calculation of the curves for titration of mono- or diprotic acids with a strong base. In this paper the pH is calculated stepwise with the approximation formulae relevant at different stages of the titration. The quadratic formulae are used as the approximation formulae, since they can be solved directly and the ranges of application are relatively broad.[3]

THEORY

Mixtures of a weak monoprotic acid and a strong base

For a mixture with an initial concentration of acid C_A and of base C_B, material balance gives

$$C_A = [HA] + [A^-] \tag{1}$$

and charge balance gives

$$[H^+] + [Na^+] = [H^+] + C_B = [OH^-] + [A^-]. \tag{2}$$

These equations give

$$[H^+] = K_A \frac{C_A - C_B - [H^+] + [OH^-]}{C_B + [H^+] - [OH^-]} \tag{3}$$

which yields the exact equation

$$[H^+]^3 + (K_A + C_B)[H^+]^2 - \{K_A(C_A - C_B) + K_w\}[H^+] - K_A K_w = 0. \tag{4}$$

When C_B is less than C_A, the solution will be acid, and the term $[OH^-]$ in equation (3) can be neglected. This provides the approximate solution

$$[H^+] = \frac{-(K_A + C_B) + \sqrt{(K_A + C_B)^2 + 4K_A(C_A - C_B)}}{2} \tag{5}$$

When C_B is in excess of C_A, the solution will be basic, and the term $[H^+]$ on the right of equation (3) can be neglected. This provides the approximate solution

$$[H^+] = \frac{-\{K_A(C_B - C_A) - K_w\} + \sqrt{\{K_A(C_B - C_A) - K_w\}^2 + 4K_A K_w C_B}}{2C_B}. \tag{6}$$

Mixtures of a weak diprotic acid and a strong base

For such a mixture in which the acid has successive dissociation constants K_1 and K_2, material balance gives

$$C_A = [H_2A] + [HA^-] + [A^{2-}] \tag{7}$$

and charge balance gives

$$[H^+] + [Na^+] = [H^+] + C_B = [OH^-] + [HA^-] + 2[A^{2-}]. \tag{8}$$

These equations give the exact equation

$$[H^+]^4 + (K_1 + C_B)[H^+]^3 + \{K_1(K_2 - C_A + C_B) - K_w\}[H^+]^2 - K_1\{K_2(2C_A - C_B) + K_w\}[H^+] - K_1K_2K_w = 0. \tag{9}$$

When C_B is less than C_A, the solution will be acid, and the terms $[A^{2-}]$ and $[OH^-]$ in equations (7) and (8) can be neglected. This provides the approximate

expression

$$[H^+] = K_1 \frac{C_A - C_B - [H^+]}{C_B + [H^+]} \quad (10)$$

and the solution

$$[H^+] = \frac{-(K_1 + C_B) + \sqrt{(K_1 + C_B)^2 + 4K_1(C_A - C_B)}}{2}. \quad (11)$$

Addition of the hydrogen-ion contribution from the second dissociation step modifies equation (11) to[3]

$$[H^+] = \frac{-(K_1 + C_B) + \sqrt{(K_1 + C_B)^2 + 4K_1(C_A - C_B)}}{2} + nK_2 \quad (12)$$

where n is a positive integer.

When C_B is in excess of C_A, there are two cases. When the solution is acid for the most part, the terms $[H_2A]$ in equation (7) and $[OH^-]$ in equation (8) can be neglected. This provides the approximate expression

$$[H^+] = K_2 \frac{2C_A - C_B - [H^+]}{C_B - C_A + [H^+]} \quad (13)$$

and the solution

$$[H^+] = \frac{-\{(C_B - C_A) + K_2\} + \sqrt{\{(C_B - C_A) + K_2\}^2 + 4K_2(2C_A - C_B)}}{2}. \quad (14)$$

In the case where the solution is basic, the terms $[H_2A]$ in equation (7) and $[H^+]$ in equation (8) can be neglected. This provides the approximate expression

$$[H^+] = K_2 \frac{2C_A - C_B + [OH^-]}{C_B - C_A - [OH^-]} \quad (15)$$

and the solution

$$[H^+] = \frac{K_2(2C_A - C_B) + K_w + \sqrt{\{K_2(2C_A - C_B) + K_w\}^2 + 4K_2K_w(C_B - C_A)}}{2(C_B - C_A)}. \quad (16)$$

Addition of the hydroxyl-ion contribution from the first dissociation step modifies equation (16) to[3]

$$[OH^-] = \frac{-\{K_2(2C_A - C_B) + K_w\} + \sqrt{\{K_2(2C_A - C_B) + K_w\}^2 + 4K_2K_w(C_B - C_A)}}{2K_2} + n\frac{K_w}{K_1}. \quad (17)$$

Titration of a weak monoprotic acid with a strong base

If V_A ml of a weak monoprotic acid with original concentration C_A are titrated with a strong base of concentration C_B, material balance with the analytical concentration of the acid a, after the addition of v_B ml of the base, gives

$$a = [HA] + [A^-] = \frac{C_A V_A}{V_A + v_B} \quad (18)$$

and material balance with the analytical concentration of the base b gives

$$b = [Na^+] = \frac{C_B v_B}{V_A + v_B}. \quad (19)$$

Introduction of a dilution correction factor $\rho = V_A/(V_A + v_B)$ and the stoichiometric fraction[4] $t = b/a$ simplifies equations (18) and (19) to

$$a = C_A\rho \quad (20)$$

$$b = at = C_A\rho t. \quad (21)$$

Charge balance gives

$$[H^+] + [Na^+] = [H^+] + C_A\rho t = [OH^-] + [A^-]. \quad (22)$$

These equations give

$$[H^+] = K_A \frac{C_A\rho(1 - t) - [H^+] + [OH^-]}{C_A\rho t + [H^+] - [OH^-]}. \quad (23)$$

Since the solution will be acidic before the equivalence point, the term $[OH^-]$ in equation (23) can be neglected. This provides the approximate solution

$$[H^+] = \frac{-(K_A + C_A\rho t) + \sqrt{(K_A + C_A\rho t)^2 + 4K_AC_A\rho(1 - t)}}{2}. \quad (24)$$

At the equivalence point, $C_AV_A = C_BV_B$, $t = 1$ and if the base used is sodium hydroxide, the equivalence mixture is a solution of NaA.[2] The hydrogen-ion concentration is

$$[H^+] = \frac{K_w + \sqrt{K_w^2 + 4K_AK_wC_A\rho}}{2C_A\rho}. \quad (25)$$

Since the solution is basic after the equivalence point, the term $[H^+]$ on the right of equation (23) can be neglected. This provides the approximate solution

$$[H^+] = \frac{-\{K_AC_A\rho(t - 1) - K_w\} + \sqrt{\{K_AC_A\rho(t - 1) - K_w\}^2 + 4K_AK_wC_A\rho t}}{2C_A\rho t}. \quad (26)$$

Titration of a weak diprotic acid with a strong base

Material balance of the acid gives

$$a = C_A\rho = [H_2A] + [HA^-] + [A^{2-}] \quad (27)$$

and charge balance gives

$$[H^+] + C_A\rho t = [OH^-] + [HA^-] + 2[A^{2-}]. \quad (28)$$

Since the solution will be acidic before the first equivalence point, the terms $[A^{2-}]$ and $[OH^-]$ in equations (27) and (28) can be neglected. This provides the approximate expression

$$[H^+] = K_1 \frac{C_A\rho(1-t) - [H^+]}{C_A\rho t + [H^+]} \quad (29)$$

and the solution

$$[H^+] = \frac{-(K_1 + C_A\rho t) + \sqrt{(K_1 + C_A\rho t)^2 + 4K_1C_A\rho(1-t)}}{2}. \quad (30)$$

Addition of the hydrogen-ion contribution from the second dissociation step modifies equation (30) to[3]

$$[H^+] = \frac{-(K_1 + C_A\rho t) + \sqrt{(K_1 + C_A\rho t)^2 + 4K_1C_A\rho(1-t)}}{2} + nK_2. \quad (31)$$

At first equivalence point, $t = 1$ and if the base used is sodium hydroxide, the equivalence mixture is a solution of NaHA.[2] The hydrogen-ion concentration is

$$[H^+] = \sqrt{\frac{K_1(K_2C_A\rho + K_w)}{K_1 + C_A\rho}}. \quad (32)$$

Between the first and second equivalence points, there are two cases. When the solution is acidic, the terms $[H_2A]$ in equation (27) and $[OH^-]$ in equation (28) can be neglected. This provides the approximate expression

$$[H^+] = K_2 \frac{C_A\rho(2-t) - [H^+]}{C_A\rho(t-1) + [H^+]} \quad (33)$$

and the solution

$$[H^+] = \frac{-\{C_A\rho(t-1) + K_2\} + \sqrt{\{C_A\rho(t-1) + K_2\}^2 + 4K_2C_A\rho(2-t)}}{2}. \quad (34)$$

In the case where the solution is basic, the terms $[H_2A]$ in equation (27) and $[H^+]$ in equation (28) can be neglected. This provides the approximate expression

$$[H^+] = K_2 \frac{C_A\rho(2-t) + [OH^-]}{C_A\rho(t-1) - [OH^-]} \quad (35)$$

and the solution

$$[H^+] = \frac{K_2C_A\rho(2-t) + K_w + \sqrt{\{K_2C_A\rho(2-t) + K_w\}^2 + 4K_2K_wC_A\rho(t-1)}}{2C_A\rho(t-1)}. \quad (36)$$

Addition of the hydroxyl-ion contribution from the first dissociation step modifies equation (36) to[3]

$$[OH^-] = \frac{-\{K_2C_A\rho(2-t) + K_w\} + \sqrt{\{K_2C_A\rho(2-t) + K_w\}^2 + 4K_2K_wC_A\rho(t-1)}}{2K_2} + n\frac{K_w}{K_1}. \quad (37)$$

At the second equivalence point, $C_AV_A = 2C_BV_B$, $t = 2$ and the equivalence mixture is a solution of Na_2A.[2] The hydrogen-ion concentration is

$$[H^+] = \frac{K_w + \sqrt{K_w^2 + 4K_2K_wC_A\rho}}{2C_A\rho}. \quad (38)$$

Equation (38) can be obtained by substituting $t = 2$ in equation (36).

Addition of the hydroxyl-ion contribution from the first dissociation step modifies equation (38) to[2]

$$[OH^-] = \frac{-K_w + \sqrt{K_w^2 + 4K_2K_wC_A\rho}}{2K_2} + n\frac{K_w}{K_1} \quad (39)$$

where the last term is derived as described in Part II.[2]

After the second equivalence point, the solution is basic and equation (36) is still used.

RESULTS AND DISCUSSION

Mixtures of a weak monoprotic acid and a strong base

Figure 1 shows the range of applicability of equation (5) [to give results within 0.02 pH unit of the value given by equation (4)]. The ranges become narrower as C_A and C_B decrease. As can be seen from equation (3) in Part III and equation (3) in Part IV, if the terms C_A and C_S in Part III are replaced with $(C_A - C_B)$ and C_B respectively in Part IV, then equation (5) in Part III will be identical with equation (5) in Part IV. Therefore the range is the same as that shown on the right-hand side of Fig. 2 in Part III.[3]

Figure 2 shows the conditions for which equation (6) give results differing by $\leqslant 0.02$ pH unit from those obtained from equation (4). The ranges become broader as C_A and C_B decrease.

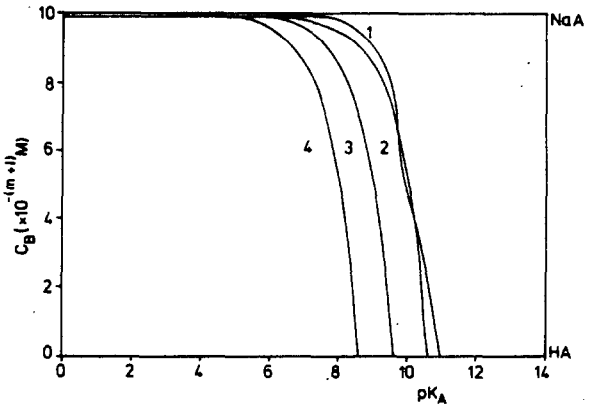

Fig. 1. The range of application of equation (5). Numbers indicate $-\log C_A$ and m corresponds to the number.

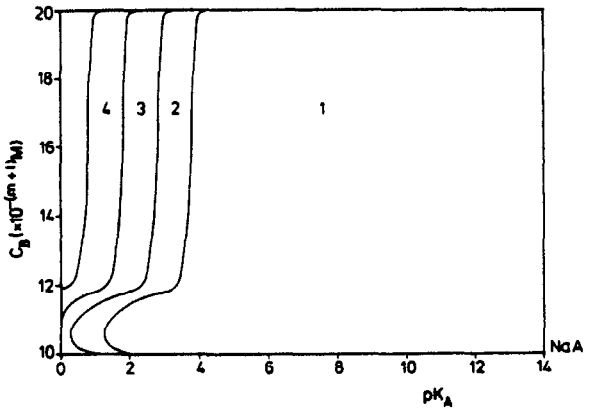

Fig. 2. The range of application of equation (6). Numbers indicate $-\log C_A$ and m corresponds to the number.

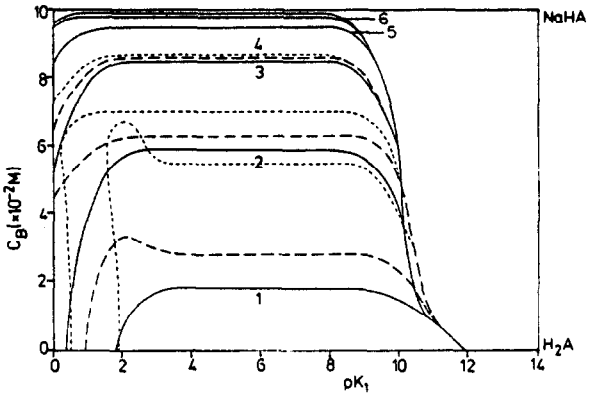

Fig. 3. The range of application of equation (11) (——), and equation (12) with $n = 1$ (– – –) and $n = 2$ (-----) when $C_A = 10^{-1}M$ and $C_B < 10^{-1}M$. Numbers indicate $-\log (K_2/K_1)$.

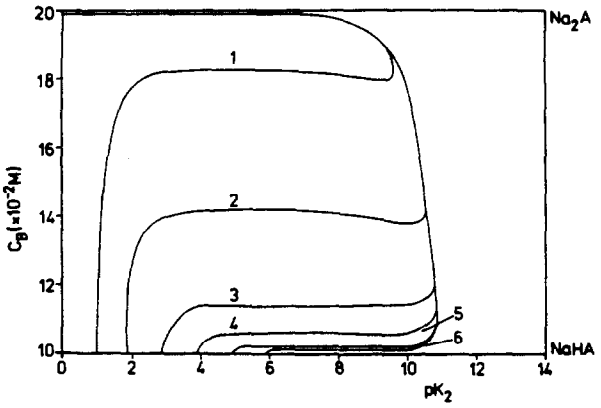

Fig. 4. The range of application of equation (14) when $C_A = 10^{-1}M$ and $C_B > 10^{-1}M$. Numbers indicate $-\log(K_2/K_1)$.

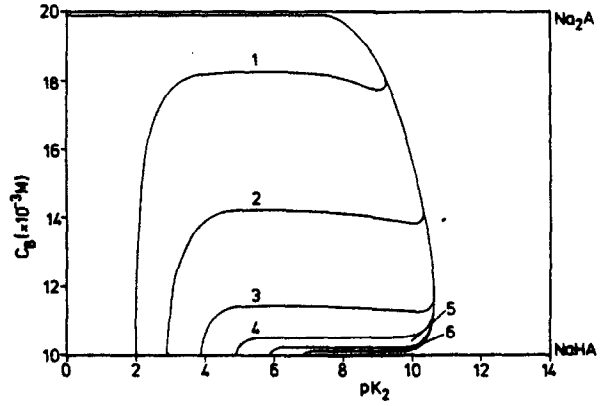

Fig. 5. The range of application of equation (14) when $C_A = 10^{-2}M$ and $C_B > 10^{-2}M$.

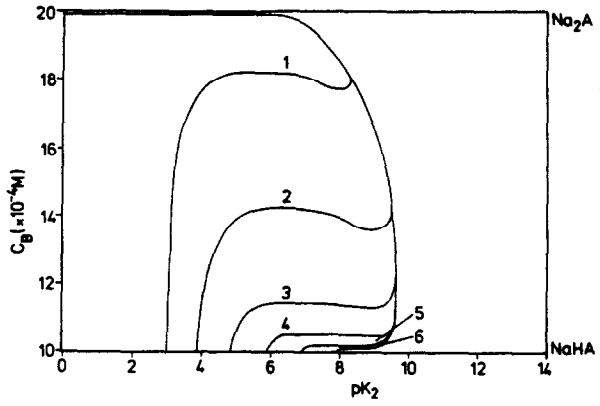

Fig. 6. The range of application of equation (14) when $C_A = 10^{-3}M$ and $C_B > 10^{-3}M$.

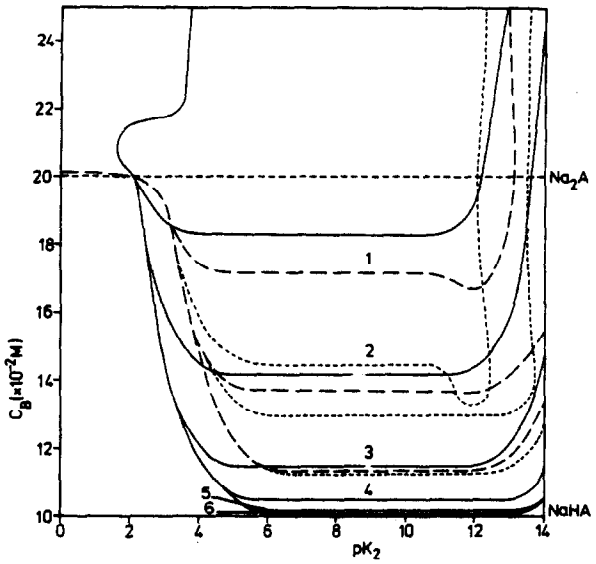

Fig. 7. The range of application of equation (16) (——), and equation (17) for $n = 1$ (---), and $n = 2$ (-----) when $C_A = 10^{-1}M$ and $C_B > 10^{-1}M$.

Mixtures of a weak diprotic acid and a strong base

Figure 3 shows the range of applicability of equations (11) and (12) when the concentration of the mixture is $0.1M$ in acid and up to $0.1M$ in base. The range spreads out as K_2/K_1 decreases and is modified by nK_2, with $n = 1$ and $n = 2$ in equation (12), for a given ratio of K_2/K_1 up to 10^{-3}. As can be seen from equation (19) in Part III and equation (10) in Part IV, if the terms C_A and C_H in Part III are replaced with $(C_A - C_B)$ and C_B respectively in Part IV, then equations (20) and (21) in Part III will be identical with equations (11) and (12) in Part IV. Therefore the range is the same as shown in Fig. 6 in Part III.[3] When the concentration is $10^{-2}M$ and $10^{-3}M$, the range of application of equations (11) and (12) will become narrower, as can be seen from Figs. 7 and 8 in Part III.[3]

Figure 4 shows the range of application of equation (14) when the concentration of the mixture is $0.1M$ in acid and more than $0.1M$ in base. The range spreads out as K^2/K_1 decreases but equation (14) cannot be used when $C_B = 2C_A$. When the concentration is less than $10^{-2}M$, the range becomes narrower, as shown in Figs. 5 and 6.

Figure 7 shows the range for use of equations (16) and (17) when the concentration of the mixture is

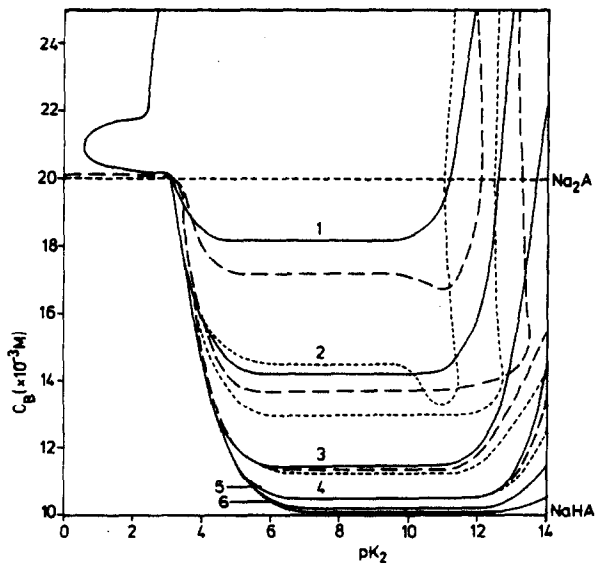

Fig. 8. The range of application of equation (16) (——), and equation (17) for $n = 1$ (---), and $n = 2$ (-----) when $C_A = 10^{-2}M$ and $C_B > 10^{-2}M$.

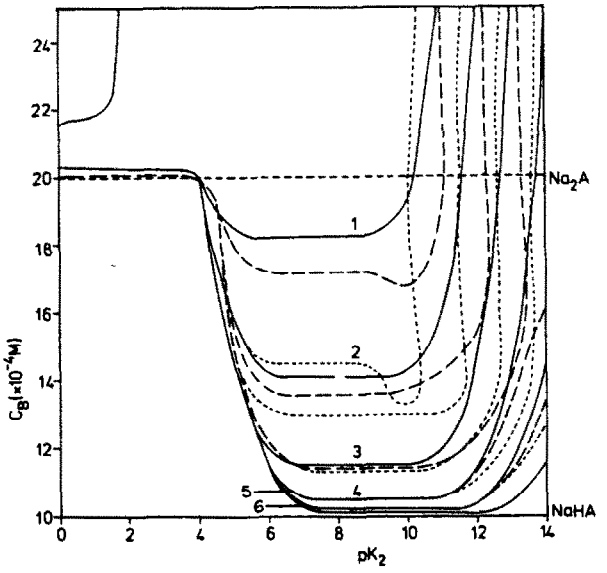

Fig. 9. The range of application of equation (16) (——), and equation (17) for $n = 1$ (---), and $n = 2$ (-----) when $C_A = 10^{-3}M$ and $C_B > 10^{-3}M$.

0.1M in acid and more than 0.1M in base. The range spreads out as K_2/K_1 decreases and is modified by nK_w/K_1, with $n = 1$ and $n = 2$ in equation (17), for a given ratio of K_2/K_1 up to 10^{-3}. When the concentration is less than $10^{-2}M$, the range becomes narrower as shown in Figs. 8 and 9. As can be seen from equation (36) in Part III and equation (15) in Part IV, if the terms C_H and C_S in Part III are replaced with $(2C_A - C_B)$ and $(C_B - C_A)$ respectively in Part IV, then equations (37) and (38) in Part III will be identical with equations (16) and (17) in Part IV. Figures 7–9 are applicable to the range of the mixture of NaHA and Na_2A in Part III.[3]

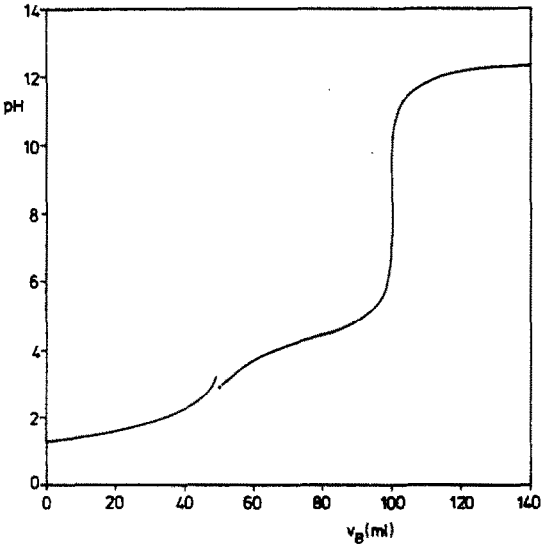

Fig. 10. A titration curve for 0.1M oxalic acid with 0.1M strong base by equations (30), (32) and (34).

Titration of a weak monoprotic acid with a strong base

The titration curve may be drawn from equations (24), (25) and (26) to within 0.02 pH unit except just before the equivalence point as can be seen from Figs. 1 and 2, if activity corrections are omitted.

Titration of a weak diprotic acid and a strong base

The titration curve may be drawn from equations (30) and (32) up to the first equivalence point. Between the first and second equivalence points, if the solution is still acid, the curve may be drawn from equation (34) and if the solution is basic, the curve may be drawn from equation (36). When K_2/K_1 is larger, the curve may be drawn only in the vicinity of start of titration and of the second equivalence point as can be seen from Figs. 3–9. The range spreads out as K_2/K_1 decreases. When this ratio is less than 10^{-4}, the curve may be drawn over the whole area except before and after the first equivalence point, but the range becomes narrower as the concentrations of acid and base decrease. The range is modified by nK_2 in equation (31) and by nK_w/K_1 in equation (37), with $n = 1$ and $n = 2$, for a given ratio up to 10^{-3}.

Figure 10 shows a titration curve calculated for 0.1M oxalic acid[5] ($pK_1 = 1.25$ and $pK_2 = 4.28$) by use of equations (30), (32) and (34). The acid (50 ml) is titrated with 0.1M strong base.

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WATER ANALYSIS BY SPARK-SOURCE MASS-SPECTROMETRY AFTER PRECONCENTRATION ON ACTIVATED CARBON

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Summary—For trace analyses of environmental waters, spark-source mass-spectrometry has been combined with a preconcentration procedure involving chelation of the dissolved trace elements with oxine and subsequent adsorption of the oxinates and naturally occurring organic and colloidal metal species onto activated carbon. The activated carbon is filtered off and ashed at low temperature. The residue is dissolved, an internal standard and pure graphite are added and, after drying, the electrodes are prepared. The photographically recorded mass spectrum is evaluated by a suitable computer routine. The error of the procedure is around 30%. While this preconcentration and analysis procedure is capable of measuring about 40 elements quantitatively, in practice 10–25 trace elements are determined simultaneously above the 0.1-μg/l. detection limit, as is illustrated by analyses of drinking water, surface and ground water samples. Although a sophisticated technique, SSMS can be considered for regular panoramic survey analyses.

The knowledge of the trace element content of various environmental waters has become increasingly of interest during recent years. Routinely, atomic-absorption spectrometry and spectrophotometry are applied most often to measure selected elements in monitoring of surface and drinking water *etc.* However, the ability to perform truly panoramic analyses at low concentration levels is of prime importance when checking for unexpected pollutants, identifying causes of a pollution accident or characterizing waters in the context of geochemical studies. Spark-source mass-spectrometry (SSMS) with photographic detection is inherently a multi-element analysis technique with comparable sensitivity for all elements and it could be a powerful tool for such broad-spectrum analysis of waters.

The literature on water analysis by SSMS is relatively scanty; very few sample preparation procedures have been proposed. Ahearn[1] and Kai and Watanabe[2] applied direct drying of one or a few drops on an electrode with enlarged, smooth surface; the surface impurities originating from the evaporated liquid were then sparked with a pointed metallic counter-electrode. With this electrode-tip evaporation, however, the analysed material is derived from only a small volume and the detection limit is accordingly unfavourable. Also, the inhomogeneity of the electrodes makes accurate quantitative analysis questionable. Chupakhin *et al.*[3] and Owens[4] used direct sparking of frozen aqueous solutions. Again, this approach makes use of a sample electrode configuration that is likely to be inhomogeneous and insufficiently robust to resist either high spark voltages or rapid spark pulse-rates. Most authors[5–10] hitherto have reduced large sample volumes by freeze-drying or evaporation, and then mixed either the concentrated liquid or the solid residue with graphite powder for electrode preparation; sometimes the organic material in the residue was ashed. This technique is indeed very simple, it sometimes allows analysis at low concentration levels and, apart from some volatility and container-wall adsorption losses, all elements present in the water sample are available in the final electrode for determination. In many situations this technique can be applied straightforwardly and advantageously. However, it is only useful for samples of low salinity; for sea-water and brines evaporation results in preconcentration factors of only 30 or less, hence in unfavourable detection limits. Also, the SSMS spectra can be considerably complicated by prominent molecular ions, such as $NaCl^+$, SO_2^+, CaF^+, NO^+, from the major components in the sample, and, for samples with higher salinity, this feature sets stringent requirements for the resolution of the mass spectrometer and for the sophistication and power of the photoplate evaluation routine.

For truly versatile and widely applicable analysis, SSMS should be combined with a physico-chemical preconcentration that leaves the abundant alkali and alkaline earth metal ions in solution and collects all trace elements of interest. The use of ion-exchange resins can hardly be considered for this purpose, since these are difficult to destroy before the electrode preparation and thus cause problems with respect to electrode strength and conductivity and spectral interferences.

In this work, SSMS is combined with a newly developed enrichment procedure[11] in which the transition metals in solutions are chelated by an organic reagent and subsequently adsorbed onto activated carbon. A broad elemental range is obtained with 8-hydroxyquinoline (oxine) as chelating agent.

The metal ions are collected after chelation but naturally occurring metal–organic and colloidal components are also concentrated.[12] Very high enrichment factors and recoveries and a favourable precision are obtained. The collection substrate is also suitable for direct analysis by X-ray fluorescence[13] or neutron-activation analysis. For analysis by SSMS however, the substrate must be ashed to remove organic material and to improve the electrode strength after the mixing with graphite.

EXPERIMENTAL

Reagents

All reagents were of analytical or ultrapure grade. The water used for synthetic solutions was demineralized and then doubly distilled in quartz apparatus. The 8-hydroxyquinoline was obtained from Union Chimique Belge. The activated carbon, ("Baker Analyzed" reagent) was treated with hydrofluoric acid and hydrochloric acid, washed with water and dried at 110° to reduce the metal contamination by a further factor of five.[14] Ultrapure graphite (Johnson–Matthey) was used for making the electrodes. Experiments were done in glassware because polyethylene appeared to adsorb some oxine. The standard solutions used for determining the relative sensitivity coefficients for 41 elements in a graphite matrix were made from Johnson–Matthey "Specpure" products.

Apparatus

The mass spectrometer used was a radiofrequency spark-source double-focusing instrument with Mattauch-Herzog geometry (JEOL JMS 01 BM 2). It has a spherical electric field for focusing the ion-beam in the *z*-direction. Ilford Q2 38 × 5 cm ion-sensitive plates were used as detectors. The transmittance of the exposed plates was measured with a single-beam microdensitometer (JEOL JMD-2C) controlled by a JEOL JEC-6 minicomputer. Photoplate measurement data were punched on paper tape and processed off-line by a 64-K computer (PDP 11/45). Recently the microdensitometer and the larger computer were linked by magnetic tape, instead of the minicomputer and paper tape.[15]

Preconcentration procedure

The characteristics of the preconcentration by oxine chelation and activated carbon adsorption were thoroughly studied earlier.[11] From this investigation, the following optimal recipe resulted. The water sample, *e.g.*, 1 litre in a glass conical flask, is adjusted to a pH value near 8, and a saturated solution of oxine in acetone is added. If a significant amount of metal oxinate precipitate is formed, it is filtered off. Activated carbon is added, and after one hour of shaking, is filtered off.

If this preconcentration is combined with an analysis technique in which the detection limit is proportional to the final sample mass, as in X-ray fluorescence, the quantities of oxine and activated carbon can be kept minimal. Since, however, activated carbon cannot be directly compressed into electrodes for SSMS analysis and has to be ashed afterwards anyway, a safe excess of activated carbon can be added to the water to be analysed, *e.g.*, 700 mg/l. In this way complete metal collection is ensured even for waters containing a significant humic material concentration.[12] The oxine is added to give a concentration of about 100 mg/l.; this is sufficient to chelate all transition metals in natural waters. An excess of oxine does not hamper the transition metal collection,[11] but it increases the collection of alkaline earth ions, resulting in deterioration of the mass spectrum and detection limit. The quantity of activated carbon or of oxine is not very critical in the SSMS analysis.

Sample preparation

For analysis by SSMS the adsorber is ashed at low temperature together with the previously collected precipitate, if any. The apparatus used was a Tracerlab ICN-LTA-302 with two ashing chambers and maximum forward power of 300 W. The ash in the glass ashing boats (25 mm long, 12 mm broad) is dissolved with a minimum amount of suprapure concentrated nitric acid (200–400 μl) and 200 μl of 100-ppm indium standard are added as an internal reference. The solution is poured into a 25-ml glass flask and the boat is thoroughly washed with demineralized doubly distilled water and finally with 2–3 ml of acetone, which also serves as a wetting agent for the graphite powder. To this solution 300 mg of suprapure graphite are added and the slurry is continuously mixed while the solution is evaporated in a rotary evaporator (Büchi Rotavapor M-HB 140) under reduced pressure and at a temperature below 50°. The moist powder is then dried in an oven at 100° for 12 hr, shaken for 5 min in a Wig-L-Bug and compressed into cylindrical electrodes (12 mm long, 2 mm diameter) under a pressure of 10 ton/cm^2.

Mass spectrometric procedure

Indium was chosen as the internal standard because its concentration in common environmental samples is very low, hence systematic errors are at a minimum. It has only two stable isotopes (^{115}In, 95.72% and ^{113}In, 4.28%) and singly and multiply-charged ions cause very few spectral interferences. Its low boiling point (2000°) and low first ionization energy (5.77 eV) make indium a sensitive element for SSMS analysis.

The cylindrical electrodes were positioned top-to-top, with one electrode fixed and the other vibrating, to maintain a mean gap of about 300 μm. The instrumental parameters are listed in Table 1. The spectra of water samples can be very complex, especially when a graphite matrix is used, and the maximum resolution of electrical detection is insufficient for resolving the SSMS spectra.

Moreover electrical detection is not very suitable for panoramic analysis of unknown samples. Even the peak-switching mode is useless in this case. Therefore photographic detection is advisable. Indeed, photographic detection has a truly multi-element character and at a first visual inspection of the photoplate qualitative information is available for an experienced operator. With some manual measurement and calculation a rough quantitative analysis can be obtained. For more accurate quantitative results, however, a microdensitometer and computer interpretation are necessary. The automatic analysis of the photographically recorded mass spectra has been described in the literature.[16] On the average a precision of ±16% is obtained and by use of relative sensitivity factors the mean error is 30%.

Typically, a series of 15 graded exposures is made on each plate, ranging from 0.1 to 150 nC. All samples are presparked for at least 5 min.

RESULTS AND DISCUSSION

Preconcentration procedure

In earlier work,[11–13,17] it has been shown that the preconcentration method involving chelation by oxine and adsorption on activated carbon, while being applicable to natural waters, allows reproducibilities of 5–10% and enrichment factors of around

Table 1. Instrumental conditions for SSMS analysis of graphite electrodes

Spark:	
radiofrequency	1 MHz
spark voltage	40 kV
pulse length	20 μsec
repetition frequency	1 kHz
Width of the slits:	
main slit	10–20 μm
α-slit	0.6 mm
β-slit	0.8 mm
Analysis:	
accelerating voltage	28 kV
electrostatic sector voltage	2.9 kV
magnetic field	14000 gauss
Vacuum:	
source	$<1 \times 10^{-7}$ mmHg
analyser	$<1 \times 10^{-8}$ mmHg
Plate development:	
temperature	18°
developing time	2.5 min
fixation time	5 min

10^4. For the most common ionic species of the following elements, a recovery above 80% has been demonstrated: V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Cd, rare earths, Hf, Re, Hg and Pb. In addition, the high stability constants of oxine complexes suggest high recoveries for Al, Ga, Zr, Nb, Pd, In, Sn, Ta, W, Tl, Bi, Th and U. It might be expected that all these elements could be determined simultaneously in the SSMS spectrum, except when important spectral interferences exist, as *e.g.*, for Cr, Co, Al.

Low-temperature ashing

The sample, contained in a horizontal glass boat, is positioned in the ashing chamber, at 0.1 mmHg pressure. Pure oxygen gas streaming at 250 ml/min flow-rate is activated in a radiofrequency (13.6 MHz) electromagnetic field. The energetic oxygen molecules, radicals, ions and atoms oxidize the sample. The temperature is a function of the ashing rate, the material and mainly the power of the field, but is always below 100°.

The ashing of pure activated carbon proceeds very easily: even with a forward power of only 50 W, 100 mg can be ashed within 1 hr (Fig. 1) and the temperature is in this case between 40 and 50°. With larger amounts the ashing rate decreases with time because a layer of metal oxides and salts is formed over the sample and hampers the contact of the activated oxygen with the underlying sample. The ashing rate also decreases with increasing metal concentration. Samples with high metal concentrations [*e.g.*, ground water containing much $Fe(OH)_3$] can take up to several days for ashing. This, of course, is a serious drawback to the procedure.

Possible losses of Co, Zn, Cd, Ag and Hg during ashing were tested for with radioactive tracers (^{60}Co, ^{65}Zn, ^{109}Cd, ^{110m}Ag and ^{203}Hg). Carrier and tracer were added in the same chemical form, *i.e.*, chloride

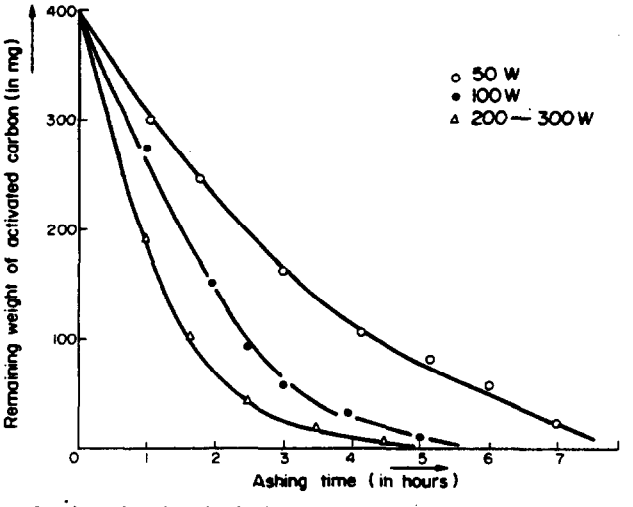

Fig. 1. Weight loss of activated carbon in the low-temperature ashing unit, as a function of ashing time.

or nitrate. In the same experiment, the recovery of the ash was tested, as described earlier. For all five elements a recovery of over 99% was found after ashing and over 98% after preparation of the electrodes from the graphite powder. Others[18,19] have also obtained high recoveries, except for As and Se, but there is evidence for influence of ionic speciation[18] and even for matrix influence.

Interlaboratory intercomparison test

As a first test of the applicability of SSMS to inorganic water analysis, a synthetic water sample from the International Atomic Energy Agency for interlaboratory intercomparison,[20] was analysed in the routine way without special precautions. In ionic composition the sample simulated surface water, but no organic material was present. To 500 ml of solution at pH 8, 30 mg of oxine were added and after 30 min the metal oxinate precipitate was filtered off. To the clear solution, 85 mg of activated carbon were added and after 90 min of shaking the carbon was collected on the same filter as the oxinate precipitate. The filter was analysed by X-ray fluorescence spectrometry. Subsequently the filter was ashed at low temperature for 20 hr. The ash was dissolved in 200 μl of nitric acid, and a few ml of water and acetone, 200 μl of indium standard and 300 mg of graphite were added. The slurry was dried and three electrodes were pressed. The electrodes were sparked under the conditions given in Table 1, and exposures ranging from 0.3 to 115 nC were recorded on the ion-sensitive plate. The mass spectrum was scanned with the microdensitometer and processed by computer.

The results of the XRF and SSMS analyses are listed in Table 2 together with the averages of the results reported by the participating laboratories. There might be a systematic positive deviation of the SSMS data from the XRF data, presumably due to an inaccuracy in adding the internal standard solution. Our high Zn values are probably due to some contamination through the distilled water used for diluting the IAEA sample. The reason for the high value for AS by SSMS is unclear. For the other elements the mean relative deviation between the SSMS results and the round-robin averages, calculated with respect to the former, is 23%, and the overall bias (the algebraic sum of the relative deviations) is +8%. In general this agreement seems acceptable for a procedure that allows many elements to be determined in one run.

Analysis of tap water

Tap water from the Antwerp drinking water supply was filtered through a Nuclepore 0.4-μm membrane filter. To seven 1-litre samples 80 mg of oxine and, after 30 min, 150 mg of activated carbon were added. After 90 min, the seven samples were filtered and the residues analysed by XRF. Four samples were ashed at low temperature and analysed by SSMS. The results are given in Table 3.

The average precision for the XRF is 5%. At this low concentration level the precision for SSMS is rather unfavourable, with an average relative standard deviation of 36%. The drinking water is inorganically very pure; only 9 elements appear above the 0.1 μg/l. detection limit and no unexpected elements show up. Except for iron, the agreement between both measurement techniques is satisfactory for these low concentration levels.

Analysis of ground and surface water

Some ground and surface water samples from Flanders, with varying pollution levels, were also analysed by SSMS after preconcentration on activated carbon. Owing to the high concentration of colloidal $Fe(OH)_3$ and humic substances it was hardly possible to filter immediately. Therefore the samples were

Table 2. Results on IAEA Interlaboratory Intercomparison Sample W-3

	Concentration, μg/l.		
Element	Present results by SSMS	XRF results on same preconcentrated sample[12]	Average results reported to IAEA by participating laboratories[20]
V	13	8.9	8.4
Mn	9.0	10.3	10.8
Fe	73	50	50
Co	8.7	5.9	11.9
Ni	15	11.2	10.7
Cu	15	12.2	12.5
Zn	21	19.1	13.6
As	31	12.5	15.6
Zr	6.2		7.5
Mo	7.2		
Ag	0.7		
Cd	5.2		5.2
La	4.2		
Pb	33	27.8	30.3

Table 3. Analysis of drinking water

Element	Concentration and standard deviation, μg/l. Present results by SSMS	XRF results on same preconcentrated sample[12]
Mn	1.6 ± 0.6	0.9 ± 0.05
Fe	9.2 ± 1.7	4.5 ± 0.3
Co	1.3 ± 0.7	
Ni	3.8 ± 1.2	2.4 ± 0.1
Cu	2.5 ± 0.7	2.2 ± 0.1
Zn	3.9 ± 1.2	3.5 ± 0.2
As	0.15 ± 0.04	
Mo	1.0 ± 0.5	
Cd	1.7 ± 0.9	

acidified with suprapure nitric acid to a final concentration of 0.1%. In this treatment the $Fe(OH)_3$ redissolves and some metal complexes with fluvic or humic substances are broken.[21] After 3 days the supernatant liquid was decanted and filtered. To 600 ml of the solution 7 μg of Eu were added as an internal reference to check the preconcentration recovery. In the case of the Scheldt river estuarine sample, the levels of oxine and activated carbon added were increased to 250 and 330 mg/l. respectively to compensate for the partial consumption of oxine by the abundant alkaline earth ions. The preconcentration procedure, the electrode preparation and SSMS analysis were the same as for the two earlier examples. The mean recovery of the Eu standard, measured by SSMS, was 91%. The analysis results are listed in Table 4. The SSMS lines of Mn and Fe were so intense that they were overexposed at all stages for some samples. For comparison purposes some elements were also determined by flameless atomic-absorption spectroscopy. These results are listed in Table 4b. A dozen elements are commonly determined. In the river Scheldt sample,

Table 4a. Analysis of ground and river waters by SSMS

Element	Concentration, μg/l. Ground water: Sampling site A	B	C	River water: Aa	Kleine Nete	Grote Nete	Mark	Scheldt
Sc	0.7	0.3	0.8					
V	5			1.7	7.6			7
Mn	29	54	48	230	>270	>380	>760	>920
Fe	3.3*	4*	11*	>4*	>4.7*	>5.8*	>2.5*	>8.3*
Co	0.4	0.2	0.8	3.7	13	22	5.7	3.3
Ni		17						18
Cu	50	4.7	3.7	135	29	12	8	43
Zn	30	17	40	170	110	1300	160	11
As	0.3	0.1	1.6	0.13	0.17		0.15	0.6
Y				0.2	0.9	0.7	0.6	0.3
Zr							0.8	5
Mo								2
Pd								1
Ag	4.5	2	1.5	0.5	0.5	1	0.8	2
Cd		1.6		4		8	6	5
Sn								1
Sb	8.5	0.4		4.2				1
La	22	0.8			0.4	1	0.5	2
Pb	15	1	2.7	8	5	13	2.5	14
Ce						1	0.5	1.5
Nd								4
Dy								3.5
Pr						0.2		0.5
U						1		2.5
Mg†	8.5*	7.5*	2.5*	6.5*	6.5*	6*	10.5*	430*
Ca†	70*	116*	18*	68*	44.*	45*	78*	640*

* Concentrations in mg/l.
† Determined by EDTA titritation.

Table 4b. Analysis of ground and river water by AAS

	Concentration, μg/l.							
	Ground water			River water				
Element	A	B	C	Aa	Kleine Nete	Grote Nete	Mark	Scheldt
Fe	5.4*	4*	17*	4.5*	5.6*	7.2*	2.5*	
Cu	52	2	3	103	46	9	8	
Zn	44	24	24	360	260	1400	160	
Cd	0.5	0.3		7		6	10	5
Pb	12	3	2.1	2.1	7	14	0.7	

* Concentrations in mg/l.

which is highly contaminated, 23 elements are measured simultaneously.

Detection limit, accuracy and precision

The detection limits for SSMS analysis of graphite electrodes[22] amount typically to 0.5–0.1 μg. Since a 1-litre water sample is reduced in volume and finally incorporated in a 300-mg graphite electrode, representing an enrichment factor near 3300, the detection limit relative to the original water sample is in the 0.1 μg/l. range.

The accuracy is mainly limited by the uncertainties in the relative sensitivity factors for the elements in graphite electrodes, which are around 30%.[22] Such a value is confirmed by the present results: the average discrepancies between SSMS data and X-ray fluorescence or atomic-absorption results, or the results from an intercomparison run, are of this magnitude.

The mean precision for repeated preparation and analysis of graphite electrodes is 16%.[22] The variations on repeated analysis of water samples appear to be larger and around 35%. This is due to added variabilities from the sample preparation procedure and to the low concentration levels involved.

CONCLUSION

SSMS can be combined with a chemical preconcentration step involving chelation with oxine and adsorption on activated carbon, for multi-element analyses of environmental waters. While this preconcentration procedure is capable of collecting about 40 trace elements nearly quantitatively, in practice 10–25 trace elements are measured simultaneously above the 0.1-μg/l. detection limit. Because the abundant alkali and alkaline earth metal ions are not collected, the procedure is applicable to samples with high or variable salinity, and the SSMS spectra are less complex, so that a mass spectrometer resolution of 4000 and mass calibration accuracy of 0.02 amu are quite sufficient (a simple physical preconcentration by evaporation or freeze-drying collects the abundant ions as well but necessitates a mass calibration accuracy of 0.005 amu[10,15]). The proposed technique is time-consuming and complex, yields precisions and errors around 30% and is definitely not indicated for routine analyses; it can be considered, however, for regular survey analyses.

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SHORT COMMUNICATIONS

METAL CHELATES OF PHOSPHONATE-CONTAINING LIGANDS*—III

ANALYTICAL APPLICATIONS OF *N,N,N',N'*-ETHYLENEDIAMINETETRA-(METHYLENEPHOSPHONIC) ACID

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Summary—*N,N,N',N'*-Ethylenediaminetetra(methylenephosphonic) acid is used as a titrant for the direct determination of Cu, Co and Ni, with murexide as indicator. Indirect titrimetric procedures are suggested for the determination of silver, mercury, zinc and cyanide and both direct and indirect methods are applied for the analysis of binary mixtures of silver (or mercury) and copper (cobalt or nickel). The stoichiometry of the reaction, interferences of some metal ions and the pH effects on the complexation reactions are discussed. The values of the equilibrium constants of the protonated CuH_nL (n = 1, 2, 3 and 4) as well as the unprotonated CuL chelates have been measured.

The use of ethylenediaminetetra-acetic acid in chelatometry has been extensively studied.[1–4] Complexones having more donor groups than EDTA have also been reported.[3] Schwarzenbach and co-workers were the first to report the high affinity of the anions of aminomethylphosphonic acid-*N,N*-diacetic acid (AMPADA) and aminoethylphosphonic acid-*N,N*-diacetic acid (AEPADA) toward calcium and magnesium ions.[5] The fact that the stability constant of the calcium chelate of AEPADA is lower than that of the AMPADA chelate suggests that the phosphonic acid group features prominently in the complex formation rather than acting only as a solubilizing group.

Because of the structural similarity of ethylenediaminetetra(methylenephosphonic) acid (ENTMP) to EDTA, it was felt that it might yield results of analytical significance and reflect the different natures of the phosphonic and carboxylic groups.

In an earlier report,[6] the values of the acid dissociation constants and the stability constants of the alkaline earth metals with ENTMP were given. The present paper describes the use of this reagent as a titrant for the direct titration of copper(II), cobalt(II) and nickel and the indirect determination of silver, zinc, cyanide and mercury(II). Mixtures of silver or mercury with copper, cobalt or nickel are also analysed.

* Part II: E. N. Rizkalla and M. T. M. Zaki, *Talanta*, 1979, **26**, 979.

EXPERIMENTAL

Reagents

The recrystallized tetrasodium salt, Na_4H_4ENTMP, was used. The purity was nearly 100% as measured by potentiometric titration with standard sodium hydroxide. A $10^{-3}M$ solution was prepared by dissolving the appropriate amount in 1 litre of doubly distilled water.

Solid potassium tetracyanonickelate was prepared.[7] Ammonium chloride–ammonia buffer (pH ~ 9.8) and powdered murexide–sodium chloride mixture (1:100) were prepared according to standard methods.

All reagents were analytical grade.

Potentiometric measurements

The various equilibrium constants were calculated from the data obtained from the potentiometric titration of ENTMP ($10^{-3}M$) in the absence and the presence of copper(II) at 1:1 and 1:2 metal:ligand molar ratios. The hydrogen-ion concentration was measured with an Orion Research Digital Ionalyzer, Model 801 A, fitted with a combined glass and calomel electrode, type 91-04. The ionic strength was kept constant at 0.1 with potassium nitrate.

Titrimetric procedures

Determination of copper, nickel or cobalt. To a solution containing microamounts of metal ion (as low as 0.3 mg in 5 ml of water), ammonia–ammonium chloride buffer is added dropwise and the pH is adjusted approximately to 8–9. The solution is then titrated with ENTMP ($10^{-3}M$) to the murexide end-point.

Determination of silver, mercury or zinc. The sample solution is added to a solution of potassium tetracyanonickelate and the mixture made alkaline with ammonia buffer. The solution is then titrated with ENTMP, with murexide as indicator.

In the zinc determination, the displacement reaction

$$Zn^{2+} + Ni(CN)_4^{2-} \rightleftharpoons Zn(CN)_4^{2-} + Ni^{2+}$$

is a bit slow but can be accelerated by gentle heating.

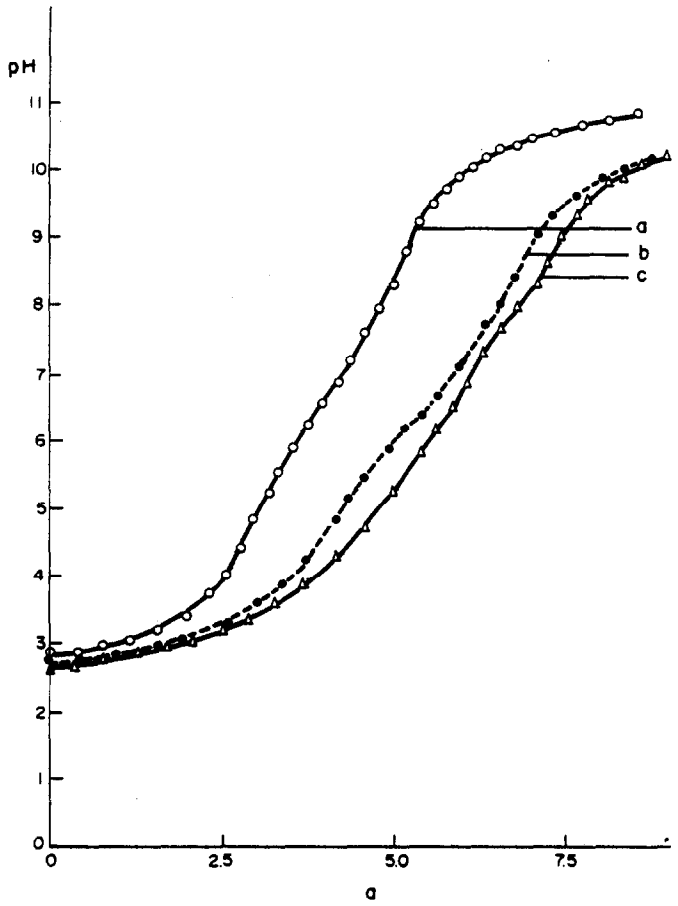

Fig. 1. Potentiometric titration curves of (a) ENTMP; (b) Cu–ENTMP (1:1); (c) Cu–ENTMP (1:2) at 25°, I = 0.1 (KNO_3); (a = moles of base added per mole of ENTMP).

Determination of cyanide. Cyanide is determined by adding a known excess of nickel solution, followed by back-titration of the excess of nickel as described above.

Determination of silver and copper (nickel or cobalt) in a mixture. In an aliquot of the sample solution, the copper (nickel or cobalt) content is determined by direct titration with ENTMP (silver does not interfere). To a second aliquot potassium tetracyanonickelate is added and the total of the two cations is determined titrimetrically.

Determination of mercury and nickel (or cobalt) in a mixture. Nickel (or cobalt) is determined in the presence of mercury(II) by masking the latter with potassium iodide. The total metal content is determined by adding potassium tetracyanonickelate to an aliquot of the sample, followed by direct titration with ENTMP to the murexide endpoint.

RESULTS AND DISCUSSION

Effect of pH on complex formation

The efficiency of complex formation of ENTMP with metal ions is greatly influenced by pH. Figure 1 (a, b and c) illustrates the potentiometric titration curves of ENTMP alone and in the presence of copper(II) at molar ratios 1:1 and 1:2 respectively. It is clear that an increase in the metal ion concentration leads to a shift of the buffer regions to lower pH values. Appreciable complex formation takes place only after the addition of four equivalents of base (a = 4; pH ≃ 3). Beyond this a value, the copper–ENTMP curves are characterized by two distinct inflections in the buffering regions a = 4–6 and 6–8. which indicates the presence of fairly stable CuH_nL (n = 1, 2, 3 and 4) complexes in addition to the normal CuL chelate.

The equilibria in solution may be represented by the equations

$$Cu^{2+} + H_4L^{4-} \xrightleftharpoons{K_4} CuH_4L^{2-} \quad (a = 4)$$

$$CuH_4L^{2-} \xrightleftharpoons{K_3K_2} CuH_2L^{4-} + 2H^+ \quad (a = 4\text{–}6)$$

$$CuH_2L^{4-} \xrightleftharpoons{K_1K_0} CuL^{6-} + 2H^+ \quad (a = 6\text{–}8)$$

The values of the corresponding equilibrium constants are presented in Table 1. The mathematical treatment of the data is reported elsewhere.[8] A value of 13.79 is used for pK_w in the present calculations.

Microdeterminations with ENTMP

As indicated from the potentiometric curves and the titrimetric conditions for the determination of copper, the most probable reaction that takes place in

Table 1. Equilibrium constants of Cu–ENTMP chelates [25°, I = 0.1(KNO$_3$)]

Reaction	log K_{MH_nL}
$Cu^{2+} + H_4L^{4-} \rightleftharpoons CuH_4L^{2-}$	7.34
$Cu^{2+} + H_3L^{5-} \rightleftharpoons CuH_3L^{3-}$	10.42
$Cu^{2+} + H_2L^{6-} \rightleftharpoons CuH_2L^{4-}$	13.87
$Cu^{2+} + HL^{7-} \rightleftharpoons CuHL^{5-}$	16.77
$Cu^{2+} + L^{8-} \rightleftharpoons CuL^{6-}$	18.67

a medium of pH 8–9 may be represented as

$$H_4L^{4-} + Cu^{2+} \rightleftharpoons CuL^{-6} + 4H^+$$

In order to discuss the analytical validity of this titration, the conditional stability constant, log K'_{CuL}, is calculated. Taking into account the protonation of the metal complex and the fraction of copper that is present as the ammine or hydroxo complexes, it can be shown that

$$\log K'_{CuL} = \log K_{CuL} + \log \alpha_{CuL(H)} - \log \alpha_{Cu} - \log \alpha_{L(H)} = 16.02$$

where $\alpha_{CuL(H)}$ is the side-reaction coefficient for protonated metal chelate, α_{Cu} that for the hydroxo and ammine copper complexes, and $\alpha_{L(H)}$ that for the protonated ligand respectively. Although the formation constant of the Cu–EDTA complex[9] is almost identical to that reported here for the Cu–ENTMP chelate, the conditional stability constant of the latter is ~ 50 times that of the EDTA complex under the same experimental conditions.

At the equivalence point, the conditional copper-ion concentration, $[Cu']_{eq}$, is calculated by using the relationship

$$p[Cu']_{eq} \sim \tfrac{1}{2}(\log K'_{CuL} - \log C_{Cu})$$

Similarly, from the conditional constant of the Cu–murexide complex, the concentration of free copper ions at the transition point, $[Cu^{2+}]_t$, is calculated and the titration error, Δ, is determined to be -0.02% from the relationship

$$\Delta = \left[\frac{1}{[Cu']_t K'_{CuL}} - \frac{[Cu']_t}{C_{Cu}}\right] 100\%$$

Table 2. Complexometric titration of copper, nickel, cobalt mercury, silver, zinc and cyanide with ENTMP

Ion	Taken, *mg*	Found, *mg*	Recovery, %
Cu^{2+}	0.0737	0.075	101.2
	0.1472	0.148	100.7
	0.3685	0.368	99.9
Ni^{2+}	0.0788	0.079	100.4
	0.1578	0.158	100.1
	0.7880	0.788	100.0
Co^{2+}	0.0767	0.078	101.6
	0.2301	0.232	100.8
	0.3835	0.384	100.1
Ag^+	0.5778	0.574	99.3
	1.1556	1.151	99.6
Hg^{2+}	0.3900	0.386	99.0
	0.9750	0.974	99.9
Zn^{2+}	0.1235	0.122	98.9
	0.3705	0.369	99.5
CN^-	0.7694	0.780	101.3
	0.9843	0.988	100.4

Table 2 summarizes the results obtained for the direct determination of copper, nickel and cobalt ions. Interference is expected from ions having conditional stability constants above the threshold of analytical utility (log $K'_{ML} > 7$). Magnesium and silver do not interfere even when present in concentrations equimolar with those of copper, cobalt and nickel.

Although calcium forms relatively weak complexes with ENTMP (log $K'_{CaL} = 4.025$),[10] it forms a fairly stable complex with murexide indicator (log $K'_{CaIn} \sim 4.8$), which makes the transition interval at the end-point rather wide and leads to erroneous results.

Silver, mercury and zinc are determined indirectly by using potassium tetracyanonickelate. Stoichiometric results are obtained within experimental error. The same method is applied to determine silver and mercury in their mixtures with copper, cobalt or nickel. In the case of mercury mixtures, potassium iodide is used as a masking agent for mercury, in order to determine nickel or cobalt contents. It is worth noting that addition of the iodide must precede the addition of murexide otherwise a very stable

Table 3. Complexometric titration of binary mixtures of copper (cobalt or nickel) and silver (or mercury) with ENTMP

Mixture M_1–M_2	Taken, *mg*		Found, *mg*		Recovery, %	
	M_1	M_2	M_1	M_2	M_1	M_2
Cu^{2+}–Ag^+	0.0635	0.5778	0.062	0.567	98.0	98.1
	0.1270	0.5778	0.126	0.574	99.0	99.2
Ni^{2+}–Ag^+	0.1508	0.5778	0.152	0.582	100.7	100.7
	0.3016	0.5778	0.300	0.578	99.6	100.0
Co^{2+}–Ag^+	0.0590	0.5778	0.584	0.569	99.0	98.5
	0.1180	0.5778	0.117	0.576	99.0	99.6
Co^{2+}–Hg^{2+}	0.0590	0.3600	0.060	0.360	101.9	100.0
	0.0590	0.7200	0.060	0.712	101.9	98.9
Ni^{2+}–Hg^{2+}	0.0464	0.3600	0.047	0.360	101.2	100.0
	0.1392	0.7200	0.138	0.720	99.2	100.0

mercury–murexide complex is formed, which masks the end-point.

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DENSITY OF ANHYDROUS ACETONE AND ESTIMATION OF WATER CONTENT OF ACETONE FROM DENSITY MEASUREMENTS

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Summary—An equation is proposed which allows estimation of the water content of acetone samples from their densities.

In the course of a conductometric and calorimetric study of electrolytes in acetone it appeared necessary to estimate the water content of the purified solvent used. Karl Fischer titrations present some difficulties as far as ketones are concerned.[1] The recent development of electromagnetic densimeters led us to develop the routine estimation of water in acetone by density measurements.

Except for water, the possible contaminants of purified acetone, *viz.* propanal, butan-2-one, ethanol and methanol, are thought not to interfere because of their densities. Methanol is frequently cited as a major contaminant (commercial analytical grade acetone is generally certified as containing not more than 500 ppm). In fact it was found by chromatographic analysis in some of our purified samples, but at a concentration less than 25 ppm. Adding methanol to a batch of acetone free from this compound (<4 ppm) yields a density change of only 0.000023 g/cm^3 for 1000 ppm.

Density variations can thus be mainly attributed to changes of the water content. It is, unfortunately, not possible to obtain any samples of acetone absolutely free from water, so a standard addition procedure is needed to obtain the density of anhydrous acetone.

EXPERIMENTAL

The solvent used was purified as follows. Acetone (grade normapur, Prolabo Co.) was stored for 1 or 2 days over anhydrous calcium sulphate, then distilled and the middle fraction retained; this was then shaken for 24 hr with 4-Å molecular sieve. A final slow distillation was then performed in an anhydrous atmosphere, with a 1-m column packed with glass helices. Acetone thus purified has a very low conductivity: 1–2 × 10^{-8} Ω^{-1}.cm^{-1}.

Weighed amounts of water were added to known amounts of the purified acetone. The solutions obtained (100–2000 ppm water) and the acetone were then submitted to chromatographic analysis on a Porapak Q column. The water/acetone peak-area ratio varies linearly according to the concentration of water (Fig. 1). By extrapolation to zero ratio, the water content of the purified acetone is obtained.

The densities of the solutions were then measured with a SODEV flux densimeter model OID, following the method described by Picker *et al.*[2] Standardization was done with water (ρ = 0.997047 g/cm^3). All measurements were made at 25.00 ± 0.01°.

RESULTS

A parametric equation:

$$d = d_0 + Bc + Dc^2 \quad (1)$$

was fitted to the experimental data. This allowed, by least-squares analysis, determination of the density d_0 of pure acetone. The value chosen was obtained from a treatment using all the points resulting from experiments with two different stock solutions. The coefficients B and D are related to the partial molar volume of water in acetone at infinite dilution ($\bar{V}^0$) and b_v, the concentration coefficient, respectively.

From the expression for the apparent molar volume:

$$\phi_v = \bar{V}^0 + b_v c \quad (2)$$

equation (3) may be obtained:

$$d = d_0 + \left(\frac{M_w - d_0 \bar{V}^0}{1000}\right)c - \left(\frac{b_v d_0}{1000}\right)c^2. \quad (3)$$

It was found that at infinite dilution $\bar{V}^0$ (water) = 14.3 cm^3/mole and $b_v \sim 0$ (see Fig. 2).

The partial molar volume of water in acetone then is considerably smaller than the molar volume of water (18 cm^3/mole). The same, though to a lesser extent, is observed for methanol: $\bar{V}^0$ 39.6 cm^3/mole, as compared to 40.7 cm^3/mole. This decrease can be related to the loss of structure in these solvents when they are diluted with acetone. Finally, the measurements were extended to concentrated solutions and for water concentrations up to 1M. The following formula is recommended when the water concentration (c) is expressed as molarity:

$$d = 0.78430_9 + 6.854 \times 10^{-3}c - 1 \times 10^{-4}c^2 + 1.5 \times 10^{-4}c^3 \quad (4)$$

If the concentration (p) is expressed in ppm, the equation is

$$d = 0.78430_9 + 2.983 \times 10^{-7}p - 7.6 \times 10^{-14}p^2 + 1.3 \times 10^{-17}p^3 \quad (5)$$

Since the error in the density determination is at worst 5 × 10^{-6} g/cm^3, absolute accuracy in the esti-

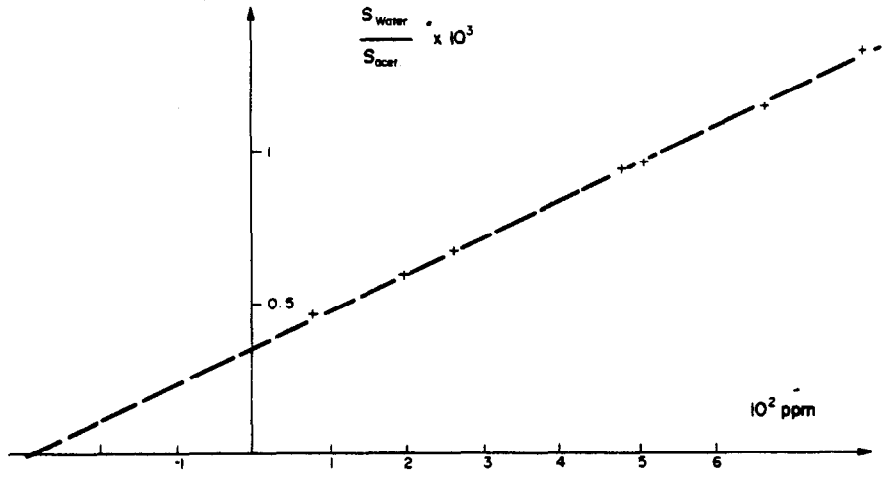

Fig. 1. Determination of the water content of purified acetone by plotting the ratio of the areas of the chromatographic peaks for water and acetone against the concentration of water added to the pure acetone.

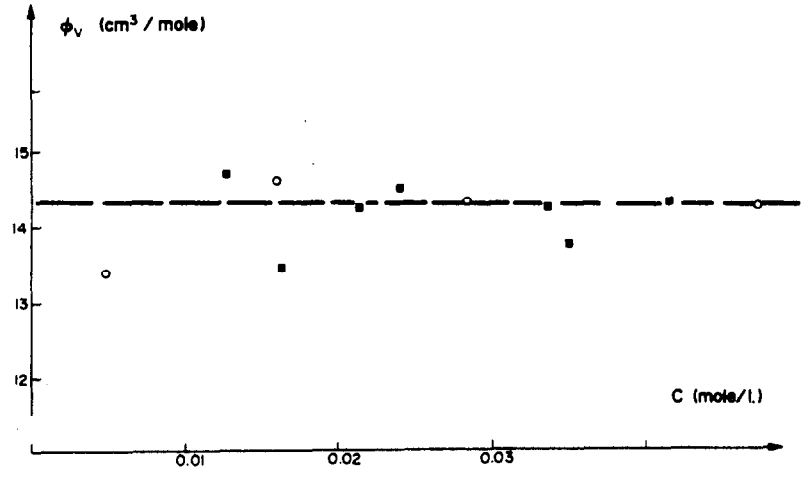

Fig. 2. The apparent molar volume of water calculated for various concentrations of water in acetone (two independent series of experiments denoted by ○ and ■).

mation of the water content is expected to be better than 20 ppm or 0.001*M*.

For the routine estimation of water in acetone the use of standardization curves obtained from equation (5) might be recommended. However, for a water content below $5 \times 10^{-2} M$ (1000 ppm) the following equations give a satisfactory result.

$$c = 146(d - d_0) \tag{6}$$

$$p = 3.35 \times 10^6 (d - d_0) \tag{7}$$

With these equations the average relative deviation would vary from 10% at the 100-ppm level to 1% at the 1000-ppm level.

The value generally accepted for the density of acetone is 0.7845 g/cm³. Hughes's measurements, which give 0.7840 for an acetone containing 1800 ppm of water[3] and 0.78345 for pure acetone (a value extrapolated from densities of rather concentrated water solutions) are certainly erroneous. As far as we know, the most elaborate purification treatment has been carried out by Evans.[5] It gives an acetone with a density of 0.78433 ± 0.00002 g/cm³, in good agreement with our result of 0.78431 g/cm³.

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SPECTROPHOTOMETRIC CHARACTERISTICS OF DISSOCIATION OF Zr AND Hf COMPLEXES WITH METHYLTHYMOL BLUE

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Summary—Two modifications of the spectrophotometric method for determination of the stability constants of ML and ML_2 complexes are derived and used for determination of the stability constants of Zr and Hf complexes with Methylthymol Blue. The dependence of the degree of dissociation of the complexes on the Zr and Hf concentrations is discussed.

The conditional stability constant of the 1:1 zirconium complex of Methylthymol Blue (MTB) in 1*M* perchloric acid was found by Cheng[1,2] to be about 1×10^5, but nothing is known about the stability constant of the Hf(MTB), $Zr(MTB)_2$ and $Hf(MTB)_2$ complexes (the last two exist in weakly acidic solutions).

In the present work the stability constants of Zr(MTB), Hf(MTB), $Zr(MTB)_2$ and $Hf(MTB)_2$ were determined by a modification of the spectrophotometric method.[3–5]

THEORY

Spectrophotometric method for determination of stability constants of ML-complexes

If only a 1:1 ML-complex is present, its conditional stability constant can be calculated from its degree of dissociation determined by using the absorbances of two solutions: A_1 for a solution which contains the same total concentrations of metal and ligand ($c_M^\circ = c_L^\circ = c$), and A_2 for a solution which contains the same concentration of metal as the first solution, but twice the concentration of ligand ($C_M^\circ = 0.5c_L^\circ = c$).

The absorbance of the first solution is:

$$A_1 = (1 - \alpha')\epsilon cd \tag{1}$$

and of the second:

$$A_2 = (1 - \alpha'')\epsilon cd \tag{2}$$

where ϵ is the molar absorptivity of the complex, d is the path-length and α' and α'' are the degrees of dissociation in the first and second solutions respectively.

The ratio of the absorbances is

$$R = \frac{1 - \alpha''}{1 - \alpha'} = \frac{A_2}{A_1} \tag{3}$$

The dependence between the conditional stability constant (K'_{ML}) and degrees of dissociation α' and α'' is given by the equations:

$$K'_{ML} = \frac{1 - \alpha'}{c(\alpha')^2} \tag{4}$$

$$= \frac{1 - \alpha''}{c\alpha''(1 + \alpha'')} \tag{5}$$

Combination of equations (3)–(5) yields:

$$R = \frac{\alpha''(1 + \alpha'')}{(\alpha')^2} \tag{6}$$

Equations (3) and (6) allow calculation of α' and α'' from the equations:

$$\alpha' = \frac{R - 1 + \alpha''}{R} \tag{7}$$

$$\alpha'' = \frac{R - 2 + [(2 - R)^2 + 4(R - 1)^3]^{0.5}}{2(R - 1)} \tag{8}$$

The values of α' and α'' can also be obtained graphically (Fig. 1). They are then used to calculate the conditional stability constants of the ML-complex from equations (4) and (5).

Spectrophotometric method for the determination of stepwise stability constants of ML_2-complexes

This method uses the absorbances of two solutions of the ML_2-complex measured at the isosbestic point of the ML and ML_2 spectra.

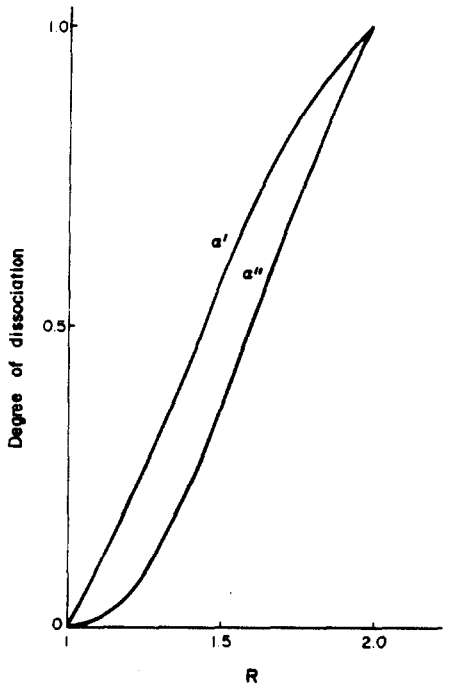

Fig. 1. Absorbance ratio of ML-complex solutions *vs.* degree of dissociation: α' for $c_L^\circ : c_M^\circ = 1$; α'' for $c_L^\circ : c_M^\circ = 2$.

The first and the second stepwise stability constants, determined at constant pH, as a function of the total concentration of metal (c) and the first and second degrees of dissociation (α_1 and α_2) of ML_2, are obtained from the equations

$$K_1 = \frac{[ML]}{[M][L]} = \frac{1 - \alpha_2}{c\alpha_1\alpha_2(1 + \alpha_2)} \quad (9)$$

$$K_2 = \frac{[ML_2]}{[ML][L]} = \frac{1 - \alpha_1}{c\alpha_1^2(1 - \alpha_2^2)} \quad (10)$$

The absorbance A_i of the solution containing both complexes, measured at the isosbestic point ($\epsilon_{ML} = \epsilon_{ML_2} = \epsilon_{ML_n}$), is:

$$A_i = [\epsilon_{ML_n} + \epsilon_L\alpha_1 - (\epsilon_{ML_n} - \epsilon_L)\alpha_1\alpha_2]c_i d_i \quad (11)$$

where ϵ_L is the molar absorptivity of the ligand, and ϵ_{ML_n} is the molar absorptivity of the complexes at the isosbestic point, c_i is the concentration and d_i the path-length used.

The insertion of the absorbances of two solutions with different ML_2-complex concentrations (c' and c'') into equations (9)–(11) gives a system of 6 equations (3 for each concentration) with 6 unknowns: K_1, K_2, α_1', α_2', α_1'' and α_2''.

The way in which both stability constants are determined is given below.

First the dependence between the degrees of dissociation of the ML_2-complex is determined by means of the equation

$$\alpha_2 = \frac{E_i + E\alpha_1}{\alpha_1} \quad (11a)$$

where

$$E = \frac{\epsilon_L}{\epsilon_{ML_n} - \epsilon_L} \quad (11b)$$

$$E_i = \frac{\epsilon_{ML_n} - (A_i/c_i d_i)}{\epsilon_{ML_n} - \epsilon_L} \quad (11c)$$

From the condition that both α_1 and α_2 must be < 1, and from equation (11a) for a weakly dissociated complex, the result

$$\frac{E_i}{1 - E} < \alpha_1 \ll 1$$

is obtained. Hence for determination of both stability constants of $Hf(MTB)_2$ and $Zr(MTB)_2$ it is sufficient to calculate values of α_2 for α_1 values between $E_i/(1 - E)$ and $2E_i/(1 - E)$.

Next the K_1 and K_2 values can be calculated from equations (9) and (10) and the values of α_1', α_1'', α_2' and α_2''. The K-values calculated for two concentrations of complex give two curves when K_2 is plotted against K_1. The co-ordinates of the intersection of the curves are the desired values of K_1 and K_2.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade unless otherwise stated.

Standard zirconium solution. A stock solution (~0.01 *M*) of $ZrOCl_2$ was prepared in 1 *M* hydrochloric acid and standardized by gravimetric zirconium determination as ZrO_2.

Standard hafnium solution. Hafnium dioxide (Johnson, Matthey, spectral purity grade) was fused with a mixture of sodium carbonate and borax at 1100 ± 20°. The cooled melt was washed several times with water and dissolved in 6 *M* hydrochloric acid. Hafnium hydroxide was precipitated with ammonia, filtered off, washed with water and dissolved in 1 *M* hydrochloric acid to give ~0.01 *M* hafnium concentration. This stock solution was standardized by gravimetric hafnium determination as HfO_2.

The standard zirconium and hafnium solutions were diluted to prepare working solutions.

Solutions of Methylthymol Blue (Merck). Used for not longer than two days.

Solutions of Zr(MTB) and Hf(MTB). To a zirconium or hafnium solution in 1 or 0.3 *M* hydrochloric acid a solution of MTB in the same concentration of acid was added and diluted with the appropriate acid (1 or 0.3 *M*) to known volume. The concentration ratio M:MTB was 1:1 or 1:2. After mixing, the solution was kept in a water-bath at 95 ± 1° for 15 min, then kept at room temperature for at least 45 min.

Solutions of $Zr(MTB)_2$ and $Hf(MTB)_2$. Prepared in a similar way to those of Zr(MTB) and Hf(MTB), but after addition of the MTB solution a buffer solution was added to keep the pH between 2.05 and 2.25.

RESULTS AND DISCUSSION

The results obtained by the "ML" method for Zr(MTB) and Hf(MTB), at pH = 0.26 (1 *M* acid) and pH = 0.68 (0.3 *M* acid) are shown in Table 1. The conditional stability constants of Zr(MTB) and Hf(MTB) were calculated by using equations (4)–(8)

Table 1. Some properties of zirconium and hafnium complexes of Methylthymol Blue

[HCl], M	Zr(MTB) 1.0	Zr(MTB) 0.3	Hf(MTB) 1.0	Hf(MTB) 0.3
λ_{max}, nm	590	590	580	580
Molar absorptivity, ϵ, $l.mole^{-1}.cm^{-1}$	2.52×10^4	2.47×10^4	2.80×10^4	2.73×10^4
Standard deviation of ϵ, $l.mole^{-1}.cm^{-1}$	5×10^2	4×10^2	9×10^2	7×10^2
Conditional stability constant, K'	3.18×10^5	6.65×10^5	2.27×10^4	2.64×10^5
Standard deviation of K'	0.38×10^5	0.70×10^5	0.53×10^4	0.29×10^5

Table 2. Stepwise and overall conditional stability constants of 1:2 zirconium and hafnium complexes with Methylthymol Blue

	$Zr(MTB)_2$	$Hf(MTB)_2$
$K_1 \pm s$	$2.24 \pm 0.26 \times 10^5$	$1.90 \pm 0.20 \times 10^5$
$K_2 \pm s$	$0.93 \pm 0.21 \times 10^7$	$1.74 \pm 0.28 \times 10^7$
$\beta_2 \pm s$	$2.08 \pm 0.53 \times 10^{12}$	$3.31 \pm 0.64 \times 10^{12}$

All mean values of K_i and their standard deviations (s) were calculated for $n = 6$

with the mean values from 8 independent determinations.

The dependence of the degree of dissociation of the ML-complexes on concentration is shown in Fig. 2. It is seen that in $1M$ hydrochloric acid the quantitative spectrophotometric determination of hafnium with MTB is impossible owing to the high degree of dissociation of Hf(MTB) even if a 10-fold ratio of MTB to Hf is used. However, it is possible at higher pH values (*e.g.*, in the $0.3M$ acid) since $K'_{Hf(MTB)}$ is then about 12 times as great as at the lower pH and approximates to the value of $K'_{Zr(MTB)}$ at pH = 0.26.

The stepwise and overall conditional stability constants, obtained by the "ML_2" method [equations (9)–(11c)] for $Zr(MTB)_2$ and $Hf(MTB)_2$ are shown in Table 2.

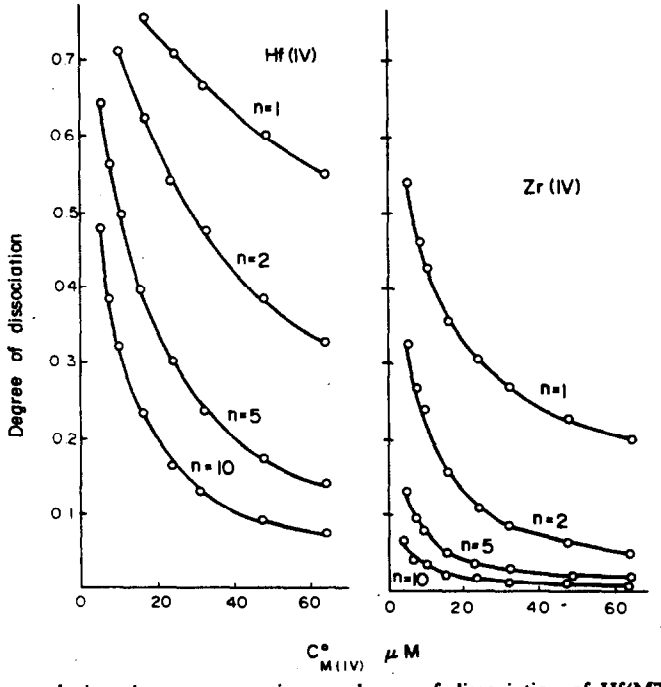

Fig. 2. Hafnium and zirconium concentration *vs.* degree of dissociation of Hf(MTB) and Zr(MTB) complexes for different values of $n = c^{\circ}_{MTB}:c^{\circ}_{M}$.

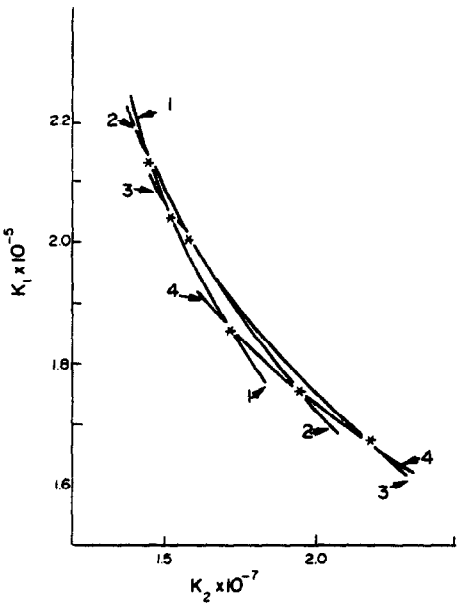

Fig. 3. K_1 *vs.* K_2 values calculated by using equations (9) and (10) for $Hf(MTB)_2$ complex concentrations: 1–3.2μM, 2–8.0μM, 3–16μM and 4–32μM.

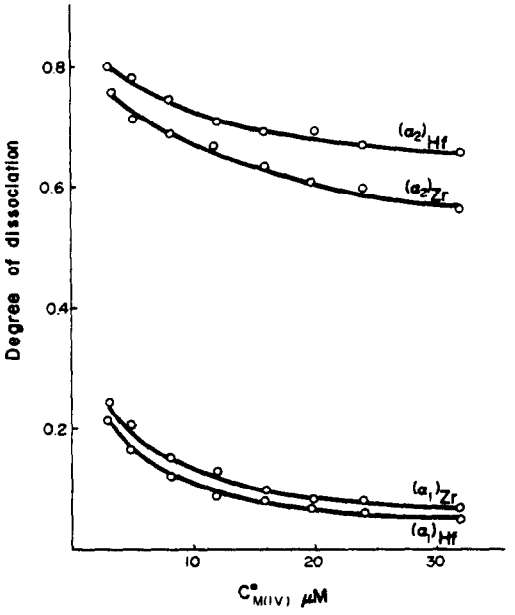

Fig. 4. Hafnium and zirconium concentrations *vs.* α_1 and α_2 for $Hf(MTB)_2$ and $Zr(MTB)_2$ complexes.

Mean K_i values and their standard deviations, given in Table 2, were calculated from 6 intersection points of 4 curves, as shown for $Hf(MTB)_2$ in Fig. 3. The absorbances used in calculations of E_i [equation (11c)] for Zr–MTB complexes were taken at 575 nm ($\epsilon_{Zr(MTB)_2} = \epsilon_{Zr(MTB)} = 2.33 \times 10^4$ $Lmole^{-1}.cm^{-1}$ and $\epsilon_{MTB} = 980$) and for Hf–MTB complexes at 580 nm ($\epsilon_{Hf(MTB)_2} = \epsilon_{Hf(MTB)} = 2.82 \times 10^4$ and $\epsilon_{MTB} = 950$).

The dependence of the first (α_1) and second (α_2) degrees of dissociation of $Zr(MTB)_2$ and $Hf(MTB)_2$ complexes on total concentration of Zr or Hf respectively is given in Fig. 4. It is seen from Fig. 4 that the dominant Zr or Hf species is the 1:2 complex.

The molar ratio of $M(MTB)_2$:M(MTB):M is $(1-\alpha_1)\alpha_1(1-\alpha_2):\alpha_1\alpha_2$, *e.g.*, when $c^{\circ}_{Hf} = 20\mu M$ and $c^{\circ}_{MTB} = 40\mu M$, $c_{Hf(MTB)_2}:c_{Hf(MTB)}:c_{Hf} = 0.930:0.022:0.048$, and for the zirconium system $c_{Zr(MTB)_2}:c_{ZrMTB}:c_{Zr} = 0.915:0.033:0.053$. In such cases the small quantities of Zr and Hf unbound by MTB can be bound quantitatively when the concentration of MTB used is twice the stoichiometric value; in that case $c_{M(MTB)_2}:c_M > 5000$.

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STUDIES ON OPTIMIZATION OF CONDITIONS FOR SEPARATING RHODIUM AND PLATINUM BY CATION-EXCHANGE

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Summary—It has been established that, owing to the amphoteric properties of rhodium(III) hydroxide, by making a rhodium chloride solution alkaline (pH ~ 13) with sodium hydroxide and then acidifying to pH 2 with nitric acid it is possible to convert at least 99% of the rhodium into cationic forms. This fact is utilized for separation of rhodium(III) and platinum(IV) from chloride solutions on a sulphonic acid cation-exchanger in hydrogen form. Loss of rhodium in the separation process is <1%. Platinum elution is complete. This method is suitable for separation of mixtures of rhodium and platinum (present in molar ratio between 1:200 and 20:1).

Most of the work on ion-exchange separation of Pt, Rh, Ir and Pd has been based on anion-exchange. Cation-exchange has been used by Berg and Senn,[1] McNevin and McKay,[2] Stevenson *et al.*[3] and Pshenitsyn *et al.*[4] The most interesting was the work of McNevin and McKay, who separated platinum and rhodium by utilizing the ability of rhodium to form cationic aquo- and hydroxyaquo-complexes. Rhodium in chloride medium was transformed into cationic complexes, sorbed on the resin and eluted with 6*M* hydrochloric acid at 60°. The authors stated that 1–10% of the rhodium was not recovered but recovery of platinum was complete.

The aim of the present work was to study the quantitative transformation of rhodium(III) into cationic complexes, and their subsequent separation from platinum(IV) with a cation-exchanger.

EXPERIMENTAL

Reagents

Rhodium(III) solutions (~7 g/l.) were prepared by dissolving $RhCl_3$ in hydrochloric acid, and platinum(IV) solution (~2 g/l.) was made from chloroplatinic acid. They were diluted as required.

Cation-exchange resin

Varion KS (H^+ form, capacity 4.6 meq/g) was used. In the column studies 3 g of resin were used in an 8-mm bore tube; the flow-rate was 0.5 ml/min.

Apparatus

A Perkin-Elmer 300 A atomic-absorption spectrophotometer with an HGA-12 graphite furnace.

Determination of rhodium and platinum

Atomic-absorption was used, under optimum conditions already found,[5,6] with the heating regime given in Table 1. Rhodium concentrations <2.5 ppm were measured at 340.3 nm, higher concentrations at 350.4 and 351 nm; band-pass was 2 Å; lamp current 30 mA.

Platinum concentrations <3 ppm were measured at 266 nm, band-bass 7 Å, and higher concentrations at 273.4 nm, band-pass 2 Å; lamp current 30 mA.

RESULTS AND CONCLUSIONS

Transformation of rhodium into cationic form

On the basis of the literature, the reaction scheme

$$Rh(H_2O)_nCl_{(6-n)} \xrightarrow{OH^-} Rh(OH)_3 \xrightarrow{OH^-} Rh(OH)_6^{3-} \xrightarrow{H_3O^+} Rh(H_2O)_6^{3+}$$

Table 1. Heating regime for determination of rhodium and platinum

	Drying, Rh and Pt	Ashing, Rh	Ashing, Pt	Atomization, Rh and Pt
Temperature, °C	103	1270	1367	2650
Time, *sec*				
5- or 10-μl sample	15	15	15	15
20-μl sample	25	20	20	15

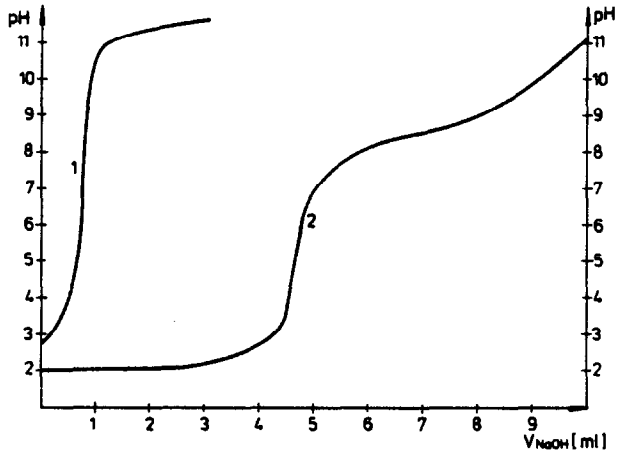

Fig. 1. Titration curves of rhodium(III) chloride solutions with 0.2*M* NaOH. 1, C_{Rh} = 70 ppm; 2, C_{Rh} = 70 ppm.

was investigated. Rhodium chloride solutions (C_{Rh} 70.4 ppm, pH 2.8; C_{Rh} 704 ppm, pH 1.98) were titrated with 0.2*M* sodium hydroxide to pH ~11.9, left for 24 hr, by which time the pH had fallen to about 10.3, and then titrated with 0.4*M* hydrochloric acid to pH 3. This pH was chosen because, according to Jørgensen,[7] at pH 3.5 the solution is clear and contains complexes of the type $Rh(H_2O)_6^{3+}$. The titration curves are presented in Figs. 1 and 2. The number of OH^- groups bound per rhodium ion was calculated from the total hydroxide added and the amount theoretically required to reach the pH observed before and after the final titration with acid (Table 2).

Rhodium binds far more OH^- groups than expected theoretically, an effect which is most likely due to adsorption of hydroxide ions on hydroxyl- or oxo-bridged polymers (*cf.* Alimarin[8]). Comparison of the spectra of the re-acidified solutions with those given by Jørgensen[7] indicates that no chloride ions are co-ordinated to the rhodium(III), but complexes such as $Rh((H_2O)_6^{3+}$, $Rh(OH)(H_2O)_5^{2+}$ and $Rh(OH)_2(H_2O)_4^+$ may be present.

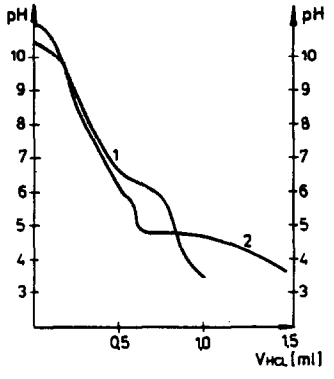

Fig. 2. Titration curves of alkaline rhodium solutions with 0.4*M* HCl. 1, C_{Rh} = 70 ppm; 2, C_{Rh} = 70 ppm.

The rate at which equilibrium is reached decreases with increasing rhodium concentration, and with high initial rhodium concentration, displacement of chloride may not be complete. Heating cannot be used, because it favours formation of the chloro-complexes.[8]

Batch cation-exchange studies

Ion-exchanger (1 g per 100 ml of solution) was shaken for 8 hr with 4.3 × 10^{-4}*M* rhodium(III) and rhodium left in the aqueous phase was determined within the next 24 hr. The effect of hydroxide concentration and of aging before and after acidification was investigated. If the pH became > 13 at any stage the sorption of rhodium on the resin was reduced, presumably because inert anionic hydroxo-complexes were formed. Hydroxide should be added only until dissolution of $Rh(OH)_3$ is complete (*i.e.*, pH ~ 13). The optimum aging time for the alkaline solution is about 4 hr, but any aging period up to 24 hr will do (≥99% sorption on the resin). Even 72 hr of aging causes only just over 1% loss of rhodium to the aqueous phase. Once acidified, however, the solution should be subjected to ion-exchange as soon as possible, to prevent formation of neutral or anionic chloro-complexes.

Table 2. Separation of rhodium(III) and platinum(IV) from chloride solutions on cation-exchanger Varion KS; the results given in the table are mean values of three measurements

Taken		Recovered	
Rh, *μg*	Pt, *mg*	Rh, *μg*	Pt, *mg*
73.3	28.6	73.1	28.5
73.3	5.7	72.6	5.6
73.3	5.7	72.6	0.6

Column ion-exchange studies

The influence of the nature of the acid used for the acidification was investigated, by exchange experiments on a resin column. It was found that the use of nitric or perchloric acid resulted in a solution from which 99.7 ± 0.1% of the rhodium was retained by the cation-exchanger, irrespective of aging of the solution for up to 72 hr, for low rhodium concentrations, and 98.1 ± 0.2% for high concentrations. With hydrochloric acid, however, the retention was lower, and decreaased with age of the solution (99.5% for 30 min aging, 97.3% for 24 hr, 75.8% for 72 hr, with 44 ppm Rh; 94.8% for 30 min, 95.6% for 24 hr, 88.2% for 72 hr, with 282 ppm Rh).

The pH of the rhodium solution was practically without influence on the degree of retention when nitric acid was used (98.3% retention at pH 3.5, 99.0 ± 0.1% at pH 1–2).

It was thought that the slightly lower retention obtained with the higher rhodium concentration might be caused by inert polymeric species being more readily formed during the addition of hydroxide. To test this, the same amount of rhodium but at two different concentrations ($C_1 = 50C_2$) was treated with alkali and acid, and the more concentrated solution was then diluted to the same concentration as the other (~150 ppm) before the ion-exchange. The initially more concentrated solution gave slightly lower retention (99.0% against 99.6%). It was also found that provided the solution was sufficiently diluted before the treatment with alkali and acid, 99.7 ± 0.1% retention was obtained for rhodium concentrations up to 300 ppm.

Thus the optimum conditions for conversion of rhodium(III) chloro-complexes into cationic complexes are to add enough 1*M* sodium hydroxide to the rhodium solution (≤300 ppm Rh) to give pH 13, age the solution for 4 hr, and acidify to pH 2 with 4*M* nitric acid.

The solution is then passed through the exchanger column at 0.5 ml/min. The best eluent is 4*M* hydrochloric acid, passed at a rate of 1 ml/min, 200 ml giving practically complete elution of 14.4 mg of rhodium.

Separation of rhodium and platinum

Solutions containing rhodium and platinum (as their chloro-complexes) in 1:200, 1:40 and 1:5 molar ratio were treated as described to convert the rhodium into cationic form, then 20-ml portions were fed onto exchange columns at 0.5 ml/min. The platinum was still present as the anionic chloro-complex, so was washed out with 80 ml of water (at 0.5 ml/min), and the rhodium was then eluted with 100 ml of 1*M* hydrochloric acid (at 1 ml/min). The results are shown in Table 2.

A precipitate eventually appeared in the platinum fraction separated, presumably because of the low concentration of chloride ions.

The analysis of an Rh–Pt alloy (Rh:Pt = 10:1) was then attempted. The sample was decomposed by chlorination. The absorption spectrum of the resultant solution was different from that of a solution of $RhCl_3$ in hydrochloric acid, however, and the sample solution was therefore boiled with *aqua regia* and evaporated to dryness, this procedure being repeated. The salts were then taken up with distilled water (the spectrum was then similar to that of a pure rhodium chloride solution) and the conversion and exchange procedure was applied.

The recovery was 100% for platinum and 99.2% for rhodium. The loss of less than 1% of the rhodium was a better result than any quoted in the literature, the rhodium loss usually being between 1 and 10%. The method is therefore well suited for separation of these two elements.

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DETERMINATION DES CONSTANTES D'ACIDITE DE DIACIDES MOYENNEMENT FORTS

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Résumé—La mesure précise de la concentration en proton provenant de la dissociation d'un diacide moyennement fort se fait par l'intermédiaire d'un indicateur coloré en utilisant un montage spectrophotométrique différentiel. Les constantes du diacide sont déterminées par ajustement à une droite de la fonction appropriée. La méthode est testée sur les deux premières ionisations de la *O*-phosphosérine en solution aqueuse (25°; KNO_3 0,1 *M*); on obtient $pK_1 = 0{,}72$ ($3\sigma = 0{,}08$) et $pK_2 = 2{,}14_6$ ($3\sigma = 0{,}01$).

Les acides moyennement forts ($pK \lesssim 2$) sont presque totalement dissociés aux concentrations analytiques usuelles, ce qui rend délicate la détermination de leurs constantes d'acidité. Dans un travail antérieur,[1] nous nous sommes intéressés à l'obtention de ce type de constantes, les méthodes introduites ayant été appliquées à la première ionisation d'acides phosphoniques et aminoalkylphosphoniques. Nous décrivons ici l'élargissement de la méthode aux diacides moyennement forts; elle sera testée sur la *O*-phosphosérine protonée, $\overset{+}{N}H_3CH(CO_2H)CH_2OP(O)(OH)_2$, qui est un tétraacide dont les deux premières dissociations sont nettement distinctes des suivantes et peut d'abord être considéré comme un diacide.

PRINCIPE

L'acide étudié est symbolisé, en tenant compte de sa charge, par H_4A^+, avec:

$$K_1 = \frac{[H_3A]\,[H^+]}{[H_4A^+]} \text{ et } K_2 = \frac{[H_2A^-]\,[H^+]}{[H_3A]}$$

$$(K_4, K_3 \ll 10^{-2} \lesssim K_1, K_2).$$

Considérons une première solution qui contient l'acide moyennement fort, de concentration totale C_A ($C_A = [H_4A^+] + [H_3A] + [H_2A^-]$), un indicateur coloré HI de concentration C_I ($C_I = [HI] + [I^-]$) et éventuellement de l'acide fort HX à la concentration C_1 pour protoner le groupement $-NH_2$ et faire rétrograder la dissociation. La relation entre les différentes concentrations est alors:

$$[H_4A^+] + [H^+] = [H_2A^-] + [X^-] + [I^-]$$
$$= [H_2A^-] + C_1 + [I^-] \quad (1)$$

Une seconde solution ne contient que de l'acide fort HX de concentration totale C_2 et de l'indicateur coloré à la même concentration C_I que la précédente, donc:

$$[H^+] = [X^-] + [I^-] = C_2 + [I^-] \quad (2)$$

La seconde solution est diluée jusqu'à ce que les absorbances de l'indicateur coloré soient égales (pour simplifier, l'absorbance de l'acide moyennement fort est supposée nulle). Les solutions ont alors mêmes $[I^-]$ et [HI] puisque C_I est constante; comme $[H^+] = [HI]\,K_I/[I^-]$, où K_I est la constante de dissociation de l'indicateur, les concentrations en $[H^+]$ sont identiques dans les deux solutions. D'aprés (2):

$$[H^+] = C_2 + [I^-] = C_2 + \left(\frac{a - a_{HI}}{a_I - a_{HI}}\right) C_I$$

a étant l'absorbance de la solution, a_I et a_{HI} les absorbances limites.

Les relations (1) et (2) entraînent alors:

$$[H_4A^+] = [H_2A^-] + C_1 - C_2$$

En y introduisant les valeurs connues ainsi que K_1 et K_2, on aboutit à:

$$\frac{C_A + C_1 - C_2}{(C_2 - C_1)\,[H^+]} = \frac{1}{K_2} + \frac{1}{K_1K_2}\left(\frac{C_A - C_1 + C_2}{C_2 - C_1}\right)[H^+] \quad (3)$$

qui est équivalente à la fonction bien connue[2]

$$\frac{\bar{n} - 2}{(3 - \bar{n})\,[H^+]} = \frac{1}{K_2} + \frac{1}{K_1K_2}\left(\frac{4 - \bar{n}}{3 - \bar{n}}\right)[H^+]$$

où $\bar{n}$ est le nombre moyen de H liés [$\bar{n} = 3 + (C_1 - C_2)/C_A$].

Il suffit donc d'effectuer plusieurs relevés pour différentes valeurs de C_A, C_2, C_1. Une régression linéaire

Tableau 1. Détermination des constantes d'acidité K_1 et K_2 de la *O*-phosphosérine; 25°C; milieu KNO_3 0,1 *M*; dinitro-2,6 phénol: $\epsilon_I = 7.8.10^3$ l.mole^{-1}.cm^{-1}; $\epsilon_{HI} = 91$.mole^{-1}.cm^{-1}; $\lambda = 432$ nm

C_1 m*M*	C_A m*M*	C_I m*M*	$C_2(\Delta C_2)$§ m*M*	$[I^-]$ m*M*	$[H^+]$ m*M*	$\bar{n}$	$y(\Delta y)$§.10^{-2}	$x(\Delta x)$§.10^2	$[H_4A^+]$ m*M*
1,00	$3,42_0$		$2,47_7$ (0,00$_6$)	0,121	$2,59_8$	2,276	$1,46_5$ (0,04$_5$)	$0,61_9$ (0,00$_3$)	0,01
1,00	$5,08_0$		$3,38_0$ (0,00$_9$)	0,093	$3,47_3$	2,335	$1,44_8$ (0,03$_8$)	$0,86_9$ (0,00$_4$)	0,03
1,00	$6,18_4$		$3,89_9$ (0,01$_2$)	0,079	$3,97_8$	2,370	$1,47_3$ (0,03$_7$)	$1,02_9$ (0,00$_5$)	0,05
1,00	$6,85_2$		$4,19_5$ (0,01$_3$)	0,074	$4,26_9$	2,388	$1,48_4$ (0,03$_6$)	$1,12_4$ (0,00$_5$)	0,06
2,00	$5,42_2$	$5,41_8$	7,82 (0,02)	0,170	$7,99_0$	2,557	$1,57_4$ (0,05$_1$)	$2,60_3$ (0,02$_3$)	0,12
2,00	$5,75_2$	$5,75_4$	8,2 (0,02)	0,164	$8,37_1$	2,574	$1,60_7$ (0,05$_3$)	$2,80_0$ (0,02$_6$)	0,13
2,00	$5,99_9$	$6,00_2$	8,48 (0,02$_5$)	0,158	$8,63_9$	2,587	$1,64_4$ (0,05$_5$)	$2,95_4$ (0,02$_9$)	0,15
2,00	$7,38_1$	$7,37_6$	10,11 (0,03)	0,135	$10,24_5$	2,630	$1,65_9$ (0,05$_7$)	$3,79_0$ (0,04$_3$)	0,23
2,00	$8,48_0$	$8,48_3$	11,35 (0,04)	0,122	$11,47_3$	2,662	$1,70_6$ (0,06$_2$)	$4,54_0$ (0,05$_9$)	0,30
2,00	$8,65_8$	$8,65_2$	11,47 (0,04)	0,121	$11,59_3$	2,674	$1,78_6$ (0,06$_8$)	$4,71_9$ (0,06$_6$)	0,31

§ Erreur maximale attendue

sur la fonction précédente permet d'obtenir $1/K_2$ et $1/K_1K_2$.

Notons que pour atténuer les variations de coefficients d'activité par variation de force ionique, chacune des solutions précédentes contient un sel de fond. La différence de force ionique entre les deux solutions, égale à $[H_4A^+]$, est d'ailleurs insignifiante (voir le tableau 1).

PARTIE EXPERIMENTALE

Mode opératoire

Nous ne rappellerons que les points principaux du procédé expérimental, détaillés par ailleurs.[1] Un montage spectrophotométrique différentiel est utilisé (spectrophotomètre Jouan, Spectral DF 170). La première cellule contient la *O*-phosphosérine (C_A), le dinitro-2,6 phénol (C_I), l'acide chlorhydrique (C_1), en présence de nitrate de potassium 0,1 *M*. Dans la seconde cellule, qui reçoit un volume v_0 de solution, l'acide à étudier est remplacé par l'acide chlorhydrique dont la concentration totale initiale devient C_2°. On y ajoute progressivement une solution isotonique en indicateur (C_I) et en nitrate de potassium: si v est le volume de diluant qui annule la différence des absorbances, on a $C_2 = C_2^\circ\, v_0/(v_0 + v)$. Pour atténuer les incertitudes, les concentrations C_A, C_1, C_2° sont amenées aux valeurs désirées par pesées de solutions stocks.[1]

RÉSULTATS ET DISCUSSION

Les résultats obtenus sont rassemblés dans le tableau 1. Les domaines d'incertitudes limites sur $y = (C_A + C_1 - C_2)/(C_2 - C_1)\ [H^+]$ et $x = (C_A - C_1 + C_2)\ [H^+]/(C_2 - C_1)$ ont été évalués par le calcul d'erreur classique en prenant: $\Delta C_A/C_A = 5.10^{-3}$; $\Delta C_1/C_1 = 10^{-3}$; $\Delta[I^-]/[I^-] = 1,5.10^{-2}$; l'erreur $\Delta C_2/C_2$ comprend l'incertitude sur la concentration initiale en acide fort (10^{-3}) à laquelle s'ajoute l'erreur photométrique: celle-ci est évaluée expérimentalement à partir de la courbe d'extrapolation des différences d'absorbance en fonction du volume de diluant. La figure 1 représente le tracé de $y = f(x)$. L'ajustement à une droite des valeurs y, x est mené par la méthode des moindres carrés en minimisant $\Sigma W(y - y_c)^2$ où

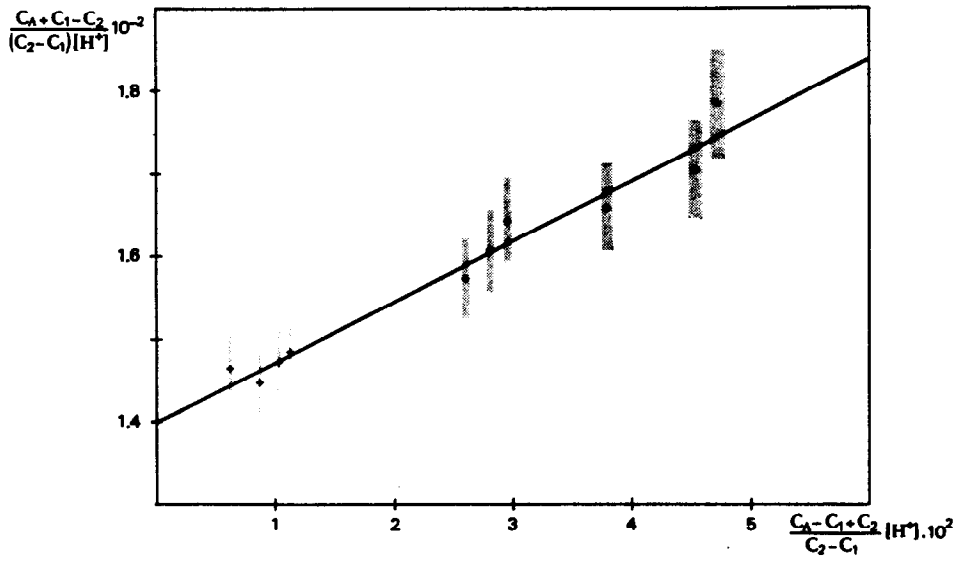

Fig. 1. Détermination de $1/K_2$ (ordonnée à l'origine) et de $1/K_1K_2$ (pente).

W est un poids inversement proportionnel au carré de l'erreur sur y. Son équation est $y = 734\,x + 140$ avec un coefficient de détermination de 0,984; d'où: $pK_1 = 0{,}72$ (0,08); $pK_2 = 2{,}14_6$ (0,01).

Les domaines de confiance indiqués entre parenthèses correspondent à 3 fois l'écart-type (calculé à partir des écarts-types sur la pente et l'ordonnée à l'origine à l'aide de la formule classique de propagation des erreurs).

Dans le tableau 1, on peut relever que $\bar{n}$ est loin d'atteindre la valeur 4 car les concentrations en H_3A et surtout en H_4A^+ restent faibles. Il est donc indispensable d'optimiser la précision des expériences car ce sont les derniers chiffres significatifs sur les données qui permettent d'aboutir à un résultat. Les expressions initiales doivent évidemment être modifiées suivant la charge des espèces en présence, les calculs pouvant être étendus à un plus grand nombre d'acidités. Cette méthode est également applicable aux acides qui absorbent à la longueur d'onde de travail: il suffit simplement de tenir compte de leur absorbance propre. Elle est même préférable dans ce cas aux méthodes spectrophotométriques classiques de détermination des constantes d'acidités : en effet, les coefficients d'extinction deviennent alors difficilement accessibles pour un diacide moyennement fort.

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Summary—The proton concentration resulting from the dissociation of a moderately strong dibasic acid can be precisely determined by means of an acid–base indicator by differential spectrophotometry. The dissociation constants are then calculated by linear regression of the appropriate function. The method was tested on the first two dissociation constants of protonated *O*-phosphoserine in aqueous solution (25°, KNO_3 0.1 *M*): the values found are $pK_1 = 0.72$ ($3\sigma = 0.08$) and $pK_2 = 2.14_6$ ($3\sigma = 0.01$).

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SPECTROPHOTOMETRIC DETERMINATION OF TRACES OF LEAD WITH BROMOPYROGALLOL RED AND CETYLTRIMETHYLAMMONIUM OR CETYLPYRIDINIUM BROMIDE

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Summary—A method is described based on the sensitizing effect of cetyltrimethylammonium or cetylpyridinium bromide on the lead–Bromopyrogallol Red colour reaction. The reaction is instantaneous and the colour remains stable for over 120 hr in the presence of Triton X-100. Both colour systems obey Beer's law up to 5.5 ppm of lead. Methods are described for dealing with interferences.

Dithizone[1] is often recommended for the spectrophotometric determination of lead, but has well-known drawbacks. Metallochromic reagents, which allow the direct determination of lead in aqueous media, have been proposed, but none except 4-(2-pyridylazo)resorcinol[2] is sensitive enough to be useful for low concentrations of lead.

The sensitizing effect of surfactants on colour reactions is now well known. In the present work it was found that addition of cetyltrimethylammonium bromide (CTAB) or cetylpyridinium bromide (CPB) to the lead–Bromopyrogallol Red (BPR) complex causes a large shift in the wavelength of maximum absorption, providing the basis for a sensitive spectrophotometric method.

EXPERIMENTAL

Reagents

Lead solution. Dissolve 0.1000 g of lead nitrate in water and dilute to 250 ml. Dilute appropriate volumes of this 250-ppm stock solution with water to provide a 20.0 ppm solution.

Bromopyrogallol Red solution, 0.01%. Dissolve 0.1 g in hot water or 1% sodium acetate solution and dilute to 1 litre.

Triton X-100 solution, 0.5%.

CTAB or CPB solutions, 0.05%.

Acetate buffer, pH 5.0, 0.1*M*.

Procedure

Transfer a suitable volume (up to 15 ml) of sample solution (containing not more than 140 μg of lead) to a 25-ml standard flask. Add, with mixing, 2 ml of buffer, 5 ml of BPR solution, 1 ml of Triton X-100 solution and 1 ml of CTAB or 0.5 ml of CPB solution. Dilute to the mark with water and measure the absorbance in 10-mm cells at 630 nm against a reagent blank. Prepare a calibration graph for 10–140 μg of lead by the same procedure.

RESULTS AND DISCUSSION

The colour reaction of lead with BPR at pH 5 was already known.[3] We found in the present investigation that in the presence of 1 ml of 0.05% CTAB or CPB solution, the lead–BPR reaction proceeds instantaneously but gives a blue precipitate. Though large amounts of CTAB solubilize the complex, excess of CPB is without effect. However, the neutral surfactant Triton X-100, which by itself does not sensitize the lead–BPR colour reaction, effectively solubilizes both the CTAB and CPB colour systems. The best results are obtained when 1 ml of 0.5% Triton X-100 solution is added before the addition of CTAB or CPB to lead–BPR system at pH 5 to give a final volume of 25 ml. Under these conditions the colour development is instantaneous and the colour stable for over 120 hr.

Absorption spectra

The spectra of BPR, Pb–BPR, Pb–BPR–CTAB and Pb–BPR–CPB in the presence of Triton X-100 are shown in Fig. 1. No new absorption band is produced by the CTAB or CPB and their presence merely strongly enhances the 630-nm band for the Pb–BPR complex. The absorbance at 630 nm, under the conditions of the procedure is constant over the pH range 4–7. Hence pH 5 (acetate buffer) was chosen.

Effect of reagent concentrations

With other variables held constant, for 75 μg of lead, the absorbance increased with increasing amount of 0.01% BPR solution up to 4.5 ml and then remained constant with amounts up to 10 ml. Analogous studies with the other reagents showed that at least 3 ml of 0.01% CTAB solution or 1.5 ml of 0.01%

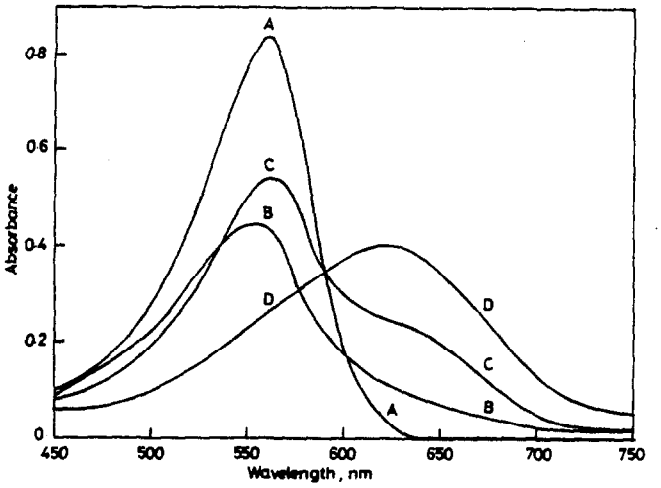

Fig. 1. Absorption spectra (pH 5; total volume 25 ml; 10-mm cells): A, 1.0 ml of $4.8 \times 10^{-4}M$ BPR and 1 ml of 0.5% Triton X-100; B, as in A, with 200 μg of lead; C, as in A, with 50 μg of lead and 1 ml of 0.05% CTAB; D, as in A, with 100 μg of lead and 1 ml of 0.025% CPB.

CPB solution should be added, and at least 1 ml of 0.25% Triton X-100 solution. At lower concentration of Triton X-100, both colour systems are unstable and a precipitate forms.

The order of addition of reagents is not critical provided the Triton X-100 is added before the CTAB or CPB.

Beer's law and precision

Both systems obey Beer's law over the range 10–140 μg of lead in a final volume of 25 ml. The apparent molar absorptivities at 630 nm are 2.6×10^4 and 2.0×10^4 $l.mole^{-1}.cm^{-1}$ for the CTAB and CPB systems respectively. Ten determinations on standard solutions that contained 75 μg of lead showed a mean recovery of 100.4% by the CTAB procedure and 101.0% by the CPB procedure, with relative standard deviations of 0.6% and 0.8% respectively.

Interferences

At the 5-mg level, Li^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, NH_4^+, AsO_2^-, NO_3^-, SO_3^{2-}, $S_2O_3^{2-}$, SO_4^{2-}, Cr^{3+}, SeO_3^{2-}, F^-, Cl^-, I^-, ClO_4^-, SCN^-, tartrate, thiourea, ascorbic acid, hydroxylamine hydrochloride, hydrazine sulphate and sulphamic acid do not interfere in the determination of 50 μg of lead, but WO_4^{2-}, MoO_4^{2-}, VO_3^-, Sn^{2+}, Ti^{4+}, Bi^{3+} and Zr^{4+} precipitate on addition of CTAB or CPB or as hydroxides, AsO_4^{3-} and IO_3^- cause a decrease in the absorbance by oxidizing the reagent, and Cu^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Pd^{2+}, La^{3+}, UO_2^{2+}, Ce^{4+}, Pt^{4+}, Sb^{5+}, Mn^{2+} and Fe^{3+} enhance the absorbance. Sb^{3+} interferes by being precipitated. The interference of Cd^{2+}, Co^{2+}, Ni^{2+} and AsO_4^{3-} can be overcome by addition of tartrate and that of Bi^{3+}, Pd^{2+}, Pt^{4+} and Cu^{2+} (in presence of sulphite) by adding thiourea. Addition of fluoride eliminates the interference due to Be^{2+}, La^{3+} and Ce^{4+} (after reduction with ascorbic acid). Sb^{5+} and Sb^{3+} interference is eliminated by adding mannitol and that of Mn^{2+} by adding triethanolamine. Other interferences can only be overcome by selectively holding Pb^{2+} on a cation-exchanger (Dowex 50 $\times$ 8 Na^+-form) from neutral solutions containing fluoride (to exclude Ti^{4+}, UO_2^{2+}, Sn^{2+}, Sn^{4+}, Ce^{3+} and Fe^{3+}) and thiocyanate (to exclude Zn^{2+}) and then eluting with $1N$ nitric acid. Such an approach, even in the absence of complexing ligands, also permitted the selective determination of lead in the presence of MoO_4^{2-}, WO_4^{2-} and VO_3^-.

Stoichiometry of the complex

Dhupar *et al.*[3] reported formation of the 1:1 Pb–BPR complex. With a constant volume of Triton X-100 present, the composition of the complexes was determined by the mole-ratio and continuous variation methods. Both methods show a 1:1:1 Pb:BPR:CPB complex; the continuous variation method shows a 1:1:1 Pb:BPR:CTAB complex but the mole-ratio method indicates the existence of both 1:1:1 and 1:1:2 complexes. The equilibrium shift method also gives evidence for existence of the 1:1:2 complex, indicating that CTAB not only forms an ion-pair system with the sulphonate group but also displaces the proton of the phenolic group of the BPR molecule, which otherwise is held by hydrogen bonding. Triton X-100, being a neutral surfactant having a much higher aggregation number in its micelles[4] ($>10^3$) than cationic surfactants (10–100) evidently stabilizes the CTAB and CPB systems, by inclusion of the complex species in the interior of the micelles.

Determination of lead in brass

The method was tested for the analysis of brass samples after separation of the lead on calcium carbonate as described elsewhere.[5] Systematic studies revealed that lead can be selectively separated from iron, zinc and copper, if the precipitation is done in

Table 1. Analysis of brass samples

Sample	Lead found, % Atomic absorption	Proposed method*
1	2.80	2.85 (0.2 ml)
		2.77 (0.4 ml)
		2.82 (1 ml)
2	Nil	Nil
2 with 0.125% added lead		0.12$_5$ (5 ml)
		0.12 (10.0 ml)
2 with 0.62% added lead		0·60 (2.5 ml)
		0.61 (5.0 ml)

* Volume taken for analysis is shown in parentheses.

the presence of triethanolamine. Such an approach is simpler in that it does not require subsequent extractive separation of lead from interfering elements that accompany it during the co-precipitation step. However, a slight decrease in the recovery of lead in presence of excess of calcium necessitates the preparation of a calibration graph with the collection procedure applied to lead standards.

Table 1 gives the results for two solutions prepared by dissolving 1 g of sample in 10 ml of nitric acid, filtering off the metastannic acid, diluting to 250 ml in a standard flask, treating 0.2–10 ml of the solution with 2 ml of calcium solution (prepared by dissolving 2 g of calcium carbonate in the minimum amount of hydrochloric acid and diluting to 50 ml with water) followed by 2 ml of 10% triethanolamine solution, sufficient 2*M* ammonia to raise the pH to ~9, and 2 ml of 10% sodium carbonate solution (with stirring), the suspension obtained then being centrifuged and the supernatant liquid discarded, the precipitate washed twice with water and finally dissolved in dilute nitric acid. The results agree with those obtained by atomic absorption spectrometry.

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SPECTROPHOTOMETRIC DETERMINATION OF URANIUM WITH ANTHRANILIC ACID AND RHODAMINE 6G

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Summary—The reaction of the uranium–anthranilic acid complex to form an ion-association complex with Rhodamine 6G provides a means for its estimation. The anionic primary complex is suggested to be a mixed-ligand complex of uranium with anthranilic acid and its oxidation products. The method is sensitive ($\epsilon = 6.25 \times 10^4$ l.mole^{-1}.cm^{-1} at 575 nm) and fairly selective, and obeys Beer's law for 0.04–4.00 ppm of uranium. It has been applied to analysis of monazite sand.

A number of ion-association methods involving the use of basic dyes for the determination of uranium have been reported.[1–5] Although these methods are more sensitive than those based on many of the binary systems, they all require extraction of the complex into an organic phase. Recently, Rhodamine 6G has been used in a highly selective spectrophotometric method (in aqueous medium) for the estimation of mercury[6] as its tetraiodo complex. A similar approach has resulted in the development of the procedure (presented here) for the estimation of uranium with anthranilic acid and Rhodamine 6G.

EXPERIMENTAL

Reagents

Uranium solution (500 ppm). Dissolve 0.1055 g of $UO_2(NO_3)_2.6H_2O$ in 100 ml of distilled water containing 1 ml of conc. nitric acid. Dilute to give a 20-ppm solution.

Anthranilic acid reagent. Dissolve 2.0 g of recrystallized anthranilic acid in the minimum of sodium hydroxide solution and dilute to about 50 ml with water. Add 2.0 ml of hydrogen peroxide (100-vol.) and heat on a water-bath for 30 min. After cooling, add hydrochloric acid to neutralize the alkali and dissolve the separated anthranilic acid as the hydrochloride. Dissolve 8.0 g of pure anthranilic acid in the minimum of 6*M* hydrochloric acid, mix this with the oxidized anthranilic acid solution and dilute to 500 ml with distilled water. This procedure produces the optimum concentration of oxidized products in the anthranilic acid solution.

Rhodamine 6G solution (0.1%).

Acetic acid-sodium acetate buffer (1 M, pH 4.5).

Procedure

Transfer 5 ml of sample solution, containing not more than 100 μg of uranium, into a dry or well-drained 25-ml standard flask. Add, with mixing, 1.0 ml of 0.1*M* EDTA, 5.0 ml of anthranilic acid reagent, 1.0 ml of acetate buffer, 1.0 ml of Rhodamine 6G solution and 1.0 ml of 0.5% gelatin solution. Dilute to the mark after 15 min and read the absorbance at 575 nm in a 10-mm cell against a reagent blank carried through the procedure. Establish the concentration of uranium by reference to a calibration graph prepared for 10–100 μg of uranium by the same procedure.

RESULTS AND DISCUSSION

Preliminary studies were carried out with a commercial sample of anthranilic acid by the procedure described, but with the pH adjusted to various values. The optimum pH for the reaction was found to be 3.8–4.7. When the reagent concentrations were varied at pH 4.5, the absorbance increased with increase in anthranilic acid concentration. This behaviour was attributed to the presence of impurities in the anthranilic acid and hence a recrystallized sample was tested. Though EDTA had had no effect on the uranium reaction with the commercial anthranilic acid (absorbance 0.210), the absorbance obtained with the recrystallized anthranilic acid in the absence of EDTA was 0.130 and in the presence of EDTA was 0.080. This suggested that the impurity in the anthranilic acid was also involved in the reaction. This impurity was thought to be a product of oxidation of anthranilic acid. As the concentration of the oxidized product in the commercial sample was very low, it was increased by oxidation with peroxide. A black product, soluble only in alkali, acetone and methanol to give an intense red solution, was obtained. The compound could not be characterized, however. An extremely low concentration of this product was found to increase the absorbance obtained with recrystallized anthranilic acid, even in the presence of EDTA. No reaction was obtained with the same concentration of oxidized product alone. Also, low concentrations of azo compounds such as diazotized anthranilic acid self-coupled or coupled with salicylic acid produced, along with pure anthranilic acid, ion-association complexes which were more stable to EDTA. Therefore it is suggested that the oxidized product is an azo compound, by analogy with the presence of azobenzene in aerially oxidized aniline,[7] and that it forms a more stable mixed-ligand complex with anthranilic acid and uranium. In order to produce anthranilic acid containing the optimum concen-

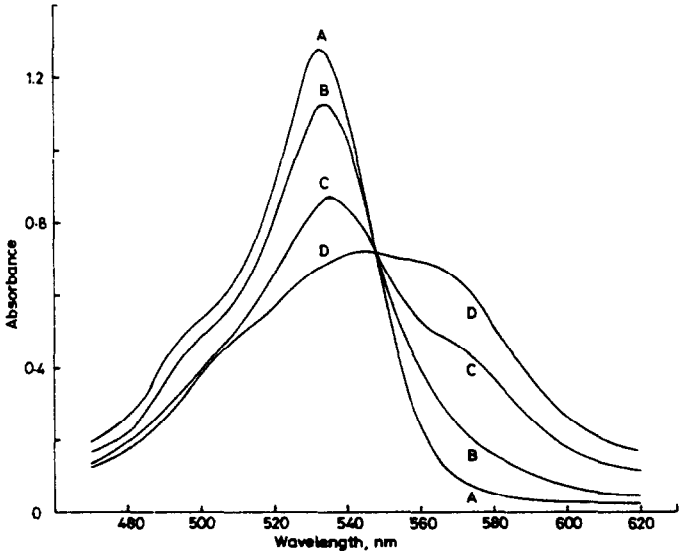

Fig. 1. Absorption spectra of uranium–anthranilic acid–Rhodamine 6G system: (pH 4.5; total volume 25 ml; 10-mm cells): A, 1.0 ml of $4.3 \times 10^{-4}M$ Rhodamine 6G and 5.0 ml of 2% anthranilic acid solution; B,C,D, as in A, with 20, 50 and 100 μg of uranium respectively.

tration of oxidized product the procedure described above was developed.

The absorption spectra shown in Fig. 1 clearly show the bathochromic shift from the absorption maximum of the dye (530 nm) to that of the complex (575 nm).

Beer's law is obeyed over the range 0.04–4.0 ppm of uranium and the molar absorptivity is 6.25×10^4 $1.mole^{-1}.cm^{-1}$.

The ratio of uranium to Rhodamine 6G is shown to be 1:1 by the mole-ratio plot shown in Fig. 2. In spite of the increased stability of the mixed-ligand primary complex, the overall stability is not high, as evident from Fig. 2 and the fact that the absorbance for a given amount of uranium and a fixed volume of reagent decreases with increase in sample volume. With sample volumes of 4, 5, 6 and 10 ml, the absorbances due to 20 μg of uranium were 0.230, 0.210, 0.190 and 0.140 respectively. This effect is attributed to the primary mixed-ligand complex also having comparatively low stability, and thus being dissociated to an extent determined by the concentrations of the reactants. Thus for reproducible results, all volumes must be kept constant.

Interference studies

The recommended procedure was applied to solutions containing 20 μg of uranium and 1 mg and 0.5 mg amounts of various ions and the results are summarized in Table 1.

Up to 0.5 mg of Sb(V), Cr(VI) and Ce(IV) could be masked by reduction with 1 ml of 1% hydroxylamine hydrochloride solution. Up to 1 mg of Fe(III) or Fe(II) was masked with 1 ml of 0.5% potassium cyanide solution. Addition of 1 ml of 5% thiourea masked 1 mg each of Pt(IV) and Pd. No method for removing the interference of Zr, Hf, and Mo could be found.

Analysis of monazite sand

A 0.5-g sample of finely ground monazite sand was attacked by the method of Hughes and Carswell[8] and the solution was made up to 100 ml. Then 1 ml of this solution was treated with 5 drops of saturated aluminium nitrate solution followed by addition of dilute ammonia solution till the precipitation of aluminium was complete. The precipitate was centrifuged, washed with dilute ammonia solution and dissolved in 8 ml of saturated aluminium nitrate solution[9] (~2.7*M*). The solution was transferred quanti-

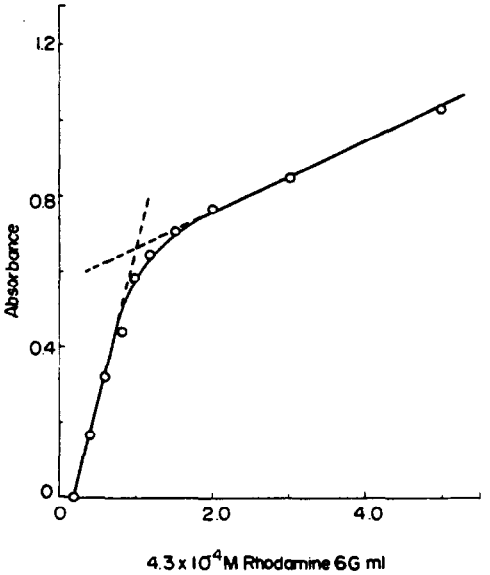

Fig. 2. Mole-ratio plot (pH 4.5; total volume 25 ml; 10-mm cells; 575 nm): 1.0 ml of $4.3 \times 10^{-4}M$ uranium (100 ppm) and 5.0 ml of 2% anthranilic acid solution.

Table 1. Interference studies

Interferent	Remarks
Cu(III, Ca, Mg, Ba, Sr, Zn, Pb, Ni, Mn(II), Co(II), Al, Hg(II), V(V), Tb, Dy, Pr, Sm, Ho, PO_4^{3-} AsO_4^{3-}, AsO_3^{3-}, $B_4O_7^{2-}$ (1 mg each)	No interference
Th, La, Nd, Gd, Y, Cd, Be, Se(IV) and W(VI) (0.5 mg each)	No interference
Fe(II) and (III), Pt(IV), Pd, Cr(VI), Ce(IV), Zr, Hf, Mo(VI), Sb(V), F^-, ClO_4^-, SCN^- and oxalate (0.5 mg each)	Interfere

Table 2. Analysis of monazite sand

Sample	Reported U_3O_8, %*	Uranium added per gram of sample (% as U_3O_8)	U_3O_8 found, % Proposed method	Arsenazo III method
1	0.35	—	0.32, 0.34, 0.34	0.36, 0.35, 0.33
2	0.35	—	0.35, 0.34, 0.33	0.35, 0.35, 0.36
3	0.35	1 mg (0.115%)	0.46	0.46
4	0.35	2 mg (0.23%)	0.57	0.59

* By Bhabha Atomic Research Centre, Bombay.

tatively into a 60-ml separatory funnel, with 1 ml of saturated aluminium nitrate solution for rinsing purposes, and shaken with 5 ml of methyl isobutyl ketone for 3 min. The aqueous phase was discarded.

The uranium was stripped from the organic phase with two portions of 0.1*M* hydrochloric acid. The acid phase was treated with 10% ammonium carbonate solution till the precipitation of aluminium was complete. Uranium was held in solution as the $[UO_2(CO_3)_3]^{4-}$ complex.[10] The precipitate was digested, centrifuged, washed with 10% ammonium carbonate solution and discarded. The solution and washings were combined, evaporated to dryness, and heated to sublime the ammonium salts. The residue was dissolved in 5 ml of 0.1*M* hydrochloric acid and the determination of uranium was completed as described above.

Table 2 shows that similar results were obtained when the same two sample solutions were repeatedly analysed by the proposed method and by the arsenazo III procedure[11] after separation of the uranium by the method described above.

Conclusions

The present paper establishes the feasibility of developing an aqueous procedure for the estimation of uranium with basic dyes. Owing to the instability of the primary complex, there is need to use a high concentration of reagents and rigorous control of conditions. If a suitable water-soluble ligand which forms a more stable complex with uranium could be found, this approach is very promising, as it combines the ease of colour development in the aqueous phase with the sensitivity attainable with the use of basic dyes.

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POTASSIUM HEXACYANORUTHENATE(II) AS AN ANALYTICAL REAGENT FOR IRON

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Summary—Fe(III) undergoes a reaction with colourless $Ru(CN)_6^{4-}$ to produce an intensely violet-blue complex that absorbs at 550 nm and obeys Beer's law over the iron concentration range 0.04–2 μg/ml in acidic medium. Some common cations and anions are tolerable at low concentrations. The procedure is applicable for determination of total iron in potable water. Destruction of organic matter is required for contaminated surface waters or soil samples.

There are many selective and sensitive analytical reagents for iron,[1,2] and new ones are introduced every year.[3–7] We have observed that potassium hexacyanoruthenate(II) reacts selectively with Fe(III) to produce a coloured complex, and is a useful analytical reagent for iron in aqueous samples. The product is violet-blue, with maximum absorption at 550 nm, and its analytical sensitivity extends down to the μg/L level. Most common cations and anions normally present in natural waters give negligible interference when present at the usual concentrations in these samples.

EXPERIMENTAL

Reagents

Potassium hexacyanoruthenate(II) (PHCR) 0.0038*M*. Dissolve 1.777 g in 1 litre of doubly distilled demineralized water.

Iron(III) solution, 10 *ppm*. Dilute a commercial atomic-absorption standard appropriately, or prepare from ferric alum or other suitable salt.

Iron(II) solution, 10 *ppm*. Prepare by dissolving ferrous ammonium sulphate hexahydrate in 0.1*M* sulphuric acid.

Buffer solution. A 9:1 mixture of 0.02*M* acetic acid and 0.02*M* sodium acetate.

Procedure

To prepare the calibration curve, take 2 ml of PHCR solution in each of a series of 25-ml standard flasks and add 10 ml of buffer solution and enough 10-ppm iron(III) solution to give final iron concentrations of 0.04–2 μg/ml. Dilute to volume with demineralized distilled water and after 10 min measure the absorbance at 550 nm against a blank prepared similarly. Use the same procedure for samples. For determination of iron(II), heat in a water-bath at 75° for 1 hr to oxidize the iron, then cool, before measuring the absorbance.

RESULTS AND DISCUSSION

The reaction is simple, rapid and relatively free from interference by some common cations and anions at low levels. The absorbance is stable at pH up to ~3.5, but decreases at higher pH. The violet-blue complex has maximum absorption at 550 nm (Fig. 1) and is stable for about 4 hr after formation. The reagent concentration is not critical over a wide range and the concentration 0.0038*M* was chosen for convenience.

Beer's law is obeyed at pH < 3 over the range 0.04–2 ppm. At higher concentrations a turbidity develops. The coefficient of variation at 0.2 ppm is 6% but below 4% at 2 ppm. The sensitivity is adequate for analysis of environmental samples (*e.g.*, potable and surface waters and soils, *etc.*), which are naturally associated with iron contamination. Since no special sample preparation is required, the method is particularly useful for routine analysis.

The interference caused by other ions has been examined (Table 1); of the ions tested only Zn^{2+}, Cd^{2+} and Cu^{2+} at 10-ppm concentration cause positive interference. Zn^{2+} and Cd^{2+} form a white turbidity with PHCR, and Cu^{2+} forms a yellow species that absorbs at around 400 nm under similar experimental conditions. However, these interferences are negligible at 2-ppm concentration of these ions.

A continuous variations plot shows that the blue species results from reaction of Fe^{3+} with PHCR in 1:1 ratio. The product results neither from hexacyanoferrate(II) or (III), nor from any reaction of iron(III) with these ions, since such species absorb in the 690–770 nm range, far removed from the 550 nm observed for the blue species. Figure 1 shows that PHCR itself has practically no absorption in the visible range and only one coloured species is generated in the Fe^{3+}–PHCR reaction. The mechanism of this reaction has not been investigated, but possibilities are an exchange reaction or production of an ion-association species.

The reaction has been tested for determination of total iron in an aqueous sample. Iron(II) and iron(III)

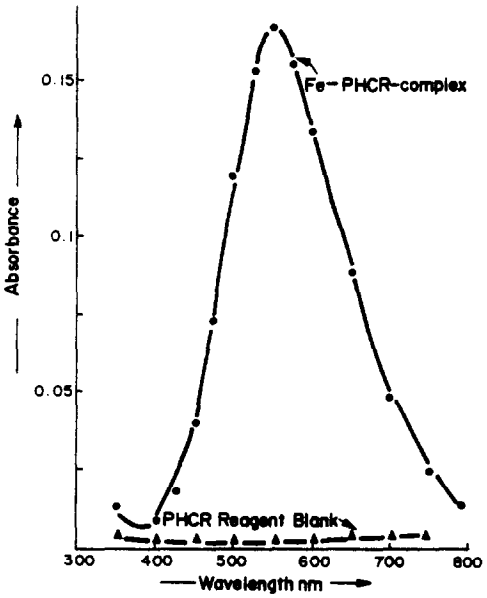

Fig. 1. Absorption spectrum of Fe^{3+}–$Ru(CN)_6^{4-}$ complex with 1 ppm Fe^{3+} and $3.8 \times 10^{-3}M$ $Ru(CN)_6^{4-}$ reagent in acidic medium (pH ~2.5).

were mixed in various proportions and the system was heated at 75° for 1 hr to complete the oxidation of the iron(II). The results in Table 2 show that the method works satisfactorily. The procedure was also applied to determination of iron in potable city water, both directly and by the method of standard additions; in both cases triplicate analysis gave a mean value of 0.04 ppm. Some difficulty was encountered when surface waters from open lakes were analysed by this method. The absorbance was generally decreased by 10–20% even for a standard sample, and a yellowish tinge was always noticed, even in filtered samples of these waters. The procedure was consequently modified to remove this organic matter. A 50-ml sample was evaporated to dryness with a 1:1 mixture of nitric and perchloric acids and the residue taken up with pure water, this procedure being repeated until a colourless solution was obtained. The normal procedure was then applied. The same procedure was applied to soil samples, 1 g of dry soil being repeatedly digested with 10 ml of the acid mixture and evaporated to dryness, till a clear solution was obtained. Each extract was analysed by the spectrophotometric procedure and by atomic-absorption. Table 3 shows an acceptable agreement between the results.

Table 1. Effect of various ions on the Fe^{3+}–PHCR reaction; Fe^{3+} 0.8 ppm, PHCR $3.8 \times 10^{-3}M$, pH ~2.5, absorbance 0.141 in absence of added ions

Concn. / Ion	Absorbance 10 ppm	20 ppm	40 ppm	80 ppm	160 ppm	320 ppm	500 ppm	Interference
Zn^{2+}	0.233	0.355	—	—	—	—	—	positive, strong
Cu^{2+}	0.178	0.210	—	—	—	—	—	positive, strong
Cd^{2+}	0.154	0.176	0.219	—	—	—	—	positive, strong
Mn^{2+}	0.148	0.149	0.262	—	—	—	—	positive, weak
Pb^{2+}	0.141	0.135	0.060	—	—	—	—	negative, strong
Cr^{3+}	0.146	0.148	0.148	0.160	—	—	—	positive, weak
Al^{3+}	0.143	0.142	0.143	0.136	0.129	0.095	—	positive, weak
Mg^{2+}	0.146	0.144	0.142	0.142	0.140	0.152	0.154	negligible
Ca^{2+}	0.143	0.140	0.139	0.142	0.140	0.140	0.135	negligible
Hg^{2+}	0.141	0.145	0.147	0.142	0.140	0.135	0.133	negligible
Na^{+}	0.138	0.139	0.141	0.141	0.143	0.138	0.134	negligible
K^{+}	0.142	0.140	0.142	0.146	0.143	0.138	0.133	negligible
SO_4^{2-}	—	0.138	0.139	0.140	0.141	0.136	0.126	negative, weak
Cl^{-}	0.138	0.142	0.141	0.141	0.138	0.133	0.128	negative, weak
NO_3^{-}	—	0.139	0.142	0.138	0.141	0.130	0.127	negative, weak
HCO_3^{-}	0.141	0.139	0.141	0.139	0.141	0.132	0.130	negative, weak

Table 2. Total iron determination (Fe^{3+} + Fe^{2+}) at 550 nm and 75°C, pH ~2.5, volume 25 ml

Fe^{3+}, ppm	Net absorbance*	Range	Fe^{3+} + Fe^{2+}, ppm	Net absorbance*	Range	Error, %
0.60	0.118	0.114–0.121	0.4 + 0.2	0.122	0.119–0.124	+3.4
			0.2 + 0.4	0.116	0.115–0.121	−1.7
1.0	0.197	0.193–0.203	0.6 + 0.4	0.199	0.196–0.208	+1.0
			0.4 + 0.6	0.195	0.193–0.200	−1.0
2.0	0.376	0.358–0.385	1.2 + 0.8	0.379	0.357–0.389	+0.8
			0.8 + 1.2	0.364	0.340–0.380	−3.2

* Average of four determinations.

Table 3. Determination of Fe^{3+} in some environmental samples

Sample	Atomic absorption, *ppm*	Present method, *ppm*
Petitcodiac Lake water (Moncton)	2.37	2.38
Jones Lake water (Moncton)	7.9	8.09
Road soil Edinburg street	52.3	51.8
Road soil Elmwood street	36.2	34.3
Potable city water Moncton	Not detected*	0.04†

* AAS signal hardly distinguishable from background in direct analysis (air/acetylene flame).

† By direct analysis and method of standard additions.

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SILVER-REDUCTOR METHOD FOR DETERMINATION OF IRON IN IRON ORES AND IRON-ORE SINTERS

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(*Received* 13 *July* 1979. *Revised* 19 *October* 1979. *Accepted* 11 *December* 1979)

Summary—A new technique for the quick dissolution of iron ore, magnetite and sinter products has been developed. The sample is dissolved with thioglycollic acid and hydrochloric acid, the excess of thioglycollic acid is oxidized, and the iron is reduced in the silver reductor.

Several reductants[1–4] have been proposed for reduction of iron(III) before its titration with standard oxidants, and the silver reductor[5] has been used in analysis of iron ores.[6] The Bhargava method[6] requires use of expensive zirconium or glassy carbon crucibles. Nowadays the stannous chloride reduction method is regarded as disadvantageous because of the question of disposal of the mercury residues. The method is still widely used, however, perhaps because when it is used with hydrochloric acid it promotes the dissolution of iron ore and sinter products and produces iron(II).[7] It cannot be used in conjunction with the silver reductor, because of the difficulty in removing the excess of stannous chloride. We therefore conceived the idea of trying another reductant in the dissolution step, namely thioglycollic acid, which was already used in our laboratory for other purposes. Hydrazine sulphate and hydroxylamine hydrochloride were also tried but thioglycollic acid was found the most suitable. The dissolution takes about 15 min.

EXPERIMENTAL

Procedure

Weigh 0.5 g of prepared dried sample into a 400-ml beaker and add 20 ml of concentrated hydrochloric acid and 0.5 ml of thioglycollic acid, and cover the beaker with a watch-glass. Digest the sample on a hot-plate until dissolution is complete. Add 30 ml of distilled water and 1 ml of concentrated nitric acid and immediately cover again. The solution becomes deep red, a vigorous reaction takes place and the excess of thioglycollic acid is decomposed.

Boil off nitrous fumes and add a pinch of sodium nitrite. If no more red colour is formed, it may be assumed that all the thioglycollic acid has been destroyed. Add 1 g of urea and boil for 2 min. Cool somewhat and filter through a thin paper-pulp pad into a 250-ml standard flask, washing with distilled water, hot hydrochloric acid (1 + 1) and finally distilled water. Cool the solution and make up to the mark with distilled water.

Pass 100 ml of the solution (measured by pipette) through the reductor[5,6] at 25–30 ml/min into a 500-ml conical flask, washing the reductor with 1*M* hydrochloric acid (six 20-ml washes suffice). Add 5 ml of phosphoric acid and 5 or 6 drops of diphenylamine indicator and titrate with 0.1*N* potassium dichromate. Run a blank with the volume of hydrochloric acid used and correct the result accordingly.

$$\text{Iron} = \frac{(A - B) \times C \times 0.01396 \times 100}{m} \%$$

where A = volume (ml) of dichromate solution consumed by the 100 ml of sample solution; B = volume (ml) of dichromate used for blank; C = normality of dichromate solution; m = sample weight (g).

RESULTS AND DISCUSSION

Typical results for iron ores and iron-ore sinters are shown in Table 1.

The elements likely to be reduced along with iron are Mo, Cu, V, W and Pt. Of these only vanadium is present in iron ore and sinter products. If V or Pt is present, a sharp end-point will be observed when all the ferrous iron has been titrated, but there will be a slow return of the colour of the reduced form of the indicator as slow oxidation of the reduced form of these elements occurs [vanadium(IV) and platinum(II)]. Titanium does not interfere.

Table 1.

Sample	Total iron, %	
	Certified	Found
BCS 175/2, Nimba iron	66.1	65.9
BCS 377, iron-ore sinter	52.5	52.4
BCS 303, iron-ore sinter	36.0	35.8
Japanese standard iron 800-I, hematite (Rompin iron-ore)	62.92	62.8
Japanese standard iron 810-I, magnetite (Texada)	64.86	65.0

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AMPEROMETRIC DETERMINATION OF OPIUM AND STRYCHNOS ALKALOIDS WITH POTASSIUM IODOTRI-IODOTHALLATE(I)

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Summary—The reagent potassium iodotri-iodothallate(I) is used for amperometric titration of alkaloids. at −0.7 V *vs.* SCE.

Determination of opium and strychnos alkaloids is of importance to toxicologists and pharmaceutical and forensic chemists. Silicotungstic[1,2] and phosphotungstic[3] acids, picric, picrolonic, styphnic and flavianic acids,[4] and nitranilic acid[5] have been used for the amperometric determination of these alkaloids. Picric acid needs careful pH control and nitranilic acid has limited use because it forms relatively soluble addition products with organic bases. Picrolonic and styphnic acids have been reported to yield unreliable results.[6] Reinecke's salt,[6] sodium tetraphenylborate[7] and sodium alizarin sulphonate[4] have also been used but have the disadvantage of either instability of the reagent solution or the necessity for an indirect determination.

The most commonly used precipitants for alkaloids are the iodo-complexes of metals such as Bi, Hg, Sb and Cd and they have been used for amperometric determinations.[8,9] Their chief advantage is the ease of preparation and stability of the reagent. Horák and Zýka[10] proposed potassium iodotri-iodothallate(I) as a very sensitive precipitant for alkaloids and used it for their indirect determination by photometric estimation of the thallium with Crystal Violet. The reagent is easy to prepare and does not require strict pH control. We have therefore investigated its use for the amperometric determination of opium and strychnos alkaloids. A drawback, of course, is that the method is applicable only to the individual alkaloids, and a preliminary separation may be needed; there are several methods available.[11–17]

EXPERIMENTAL

Reagents

Codeine, morphine, narcotine, papaverine, thebaine, strychnine and brucine solutions (10^{-2}–$10^{-4}M$) were prepared in distilled water containing 1–2 ml of 0.01*N* hydrochloric or sulphuric acid.

Potassium iodotri-iodothallate(I) solution. Mix 1 volume of 0.05*M* Tl_2SO_4 solution and 3 volumes of 0.1*N* iodine solution in 0.15*M* potassium iodide. The reagent was found to be stable over a period of weeks. In the present work, the concentration of the precipitant is expressed with respect to the concentration of thallium(I). The reagent was assigned the formula $KTl^{III}I_4$ by Horák and Zýka.[10] It is stated in the literature[18] that addition of iodine to thallous iodide in the presence of potassium iodide will lead to the formation of a soluble iodide complex containing the triiodide ion.

Procedure

Direct titrations. A suitable portion of the alkaloid solution (10 ml of 0.25×10^{-3}–$0.25 \times 10^{-2}M$) is transferred to a polarographic cell, 1.0 ml of 1*M* potassium chloride and 0.1 ml of 0.001% Triton X-100 solution are added and the solution is deaerated by passage of a stream of pure hydrogen. A potential of −0.70 V *vs* SCE is applied to the dropping mercury electrode. Measured volumes of the reagent solution (0.25×10^{-2}–$0.25 \times 10^{-1}M$) are added from the burette and mixed in by passage of hydrogen after each addition. The current obtained after each addition is plotted against the volume of the titrant added. The endpoint is determined by linear extrapolation of the two arms of the curve.

In reverse titrations, the reagent solution ($10^{-3}M$) is taken in the cell and titrated with the alkaloid solution ($10^{-2}M$) under the conditions stated above.

RESULTS AND DISCUSSION

Polarographic reduction of the thallium(I) complex

In supporting electrolytes such potassium nitrate, potassium chloride and ammonium chloride solution, the reagent gives a reversible reduction wave on polarography with a dropping mercury electode. The half-wave potential in 1*M* solutions of these electrolytes is −0.47 V *vs.* SCE, which corresponds to the reduction of Tl(I). The plateau of the wave is from −0.60 to −0.85 V. If a maximum appears it can be suppressed by adding 0.001% Triton X-100 solution. For amperometric titration of alkaloids, −0.70 V was selected as the operating potential. The alkaloids do not given any current at applied potentials up to −1.0 V.

Effect of acidity

To study the effect of acidity on precipitation of the alkaloids, radiometric titrations were performed with ^{204}Tl-labelled reagent. Precipitation was found to be practically quantitative over the acidity range from 0.01 to 0.4*N* in all cases studied.

Composition of the precipitate

Direct and reverse amperometric titrations were performed with different concentrations of the reagent

Table 1. Sensitivity of amperometric titrations of alkaloids with potassium iodotri-iodothallate(I)

Alkaloid	Reverse titration	Direct titration
Codeine	$1.0 \times 10^{-3}M$	$0.5 \times 10^{-3}M$
Morphine	$1.0 \times 10^{-3}M$	$0.5 \times 10^{-3}M$
Thebaine	$1.0 \times 10^{-3}M$	$0.5 \times 10^{-3}M$
Narcotine	$1.0 \times 10^{-4}M$	$1.0 \times 10^{-4}M$
Papaverine	$1.0 \times 10^{-3}M$	$1.0 \times 10^{-3}M$
Strychnine	$0.75 \times 10^{-3}M$	$0.75 \times 10^{-3}M$
Brucine	$2.5 \times 10^{-4}M$	$1.0 \times 10^{-4}M$

and the alkaloid. The alkaloids always form precipitates in 1:1 ratio with the reagent, suggesting formation of a precipitate of the type $[RH^+][TlI_4^-]$, where RH^+ is the protonated alkaloid. This agrees with the observations made by Horák and Zýka.[10] The composition of the precipitates was also confirmed by radiometric titrations with ^{204}Tl-labelled reagent.

Sensitivity

The minimum alkaloid concentration that can be determined was evaluated by both direct and reverse titrations and the results are presented in Table 1. The direct titrations (alkaloid in the cell) give relatively better sensitivity.

Interferences

Quinine, cinchonine, atropine sulphate, ephedrine hydrochloride, berberine and acetylsalicylic acid interfere at all concentrations.

Calcium carbonate, starch, sucrose, glucose, sodium benzoate, menthol, terpin hydrate, camphor, sodium barbiturate, saccharin and cyclamate do not interfere even in 100-fold ratio to the alkaloid.

Precision and accuracy

Results for codeine, narcotine and strychnine as representative alkaloids are given in Table 2. The accuracy of the method is fairly good, the error being within $\pm 1\%$.

Determination of codeine and narcotine in some pharmaceutical preparations

Codopyrin (Glaxo, India), codeine suphate tablets (India-Pharma, India) and coscopin syrup (Biological Evans, India) were treated for extraction of codeine and narcotine by the methods laid down in the Indian Pharmacopoea,[19] and the codeine and narcotine titrated. The results given in Table 3 indicate that the method can be applied to the analysis of pharmaceutical preparations.

Table 2. Results of determination of codeine, narcotine and brucine by direct and reverse amperometric titrations

Alkaloid	Taken mg	Reverse titration Found,* mg	Error, %	Direct titration Found,* mg	Error, %
Codeine	29.90	30.25	+1.0	30.15	+0.8
Narcotine	4.00	4.02	+0.5	4.00	0.0
Brucine	3.96	3.93	−0.8	3.94	−0.5

* Average of five determinations.

Table 3. Results of determination of codeine and narcotine in some pharmaceutical preparations by amperometric titration

Preparation	Nominal content	Found*
Codeine sulphate tablets	Codeine sulphate 15 mg/tablet	15.22 mg/tablet
Codopyrin tablets	Codeine phosphate 8 mg/tablet	7.76 mg/tablet
Coscopin syrup	Narcotine 7 mg/5 ml	6.85 mg/5 ml

* Average of 5 determinations.

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LIQUID–LIQUID EXTRACTION OF THORIUM FROM MALONATE SOLUTION WITH LIQUID ANION-EXCHANGERS

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Summary—Thorium is quantitatively extracted with 4% Amberlite LA-1 or LA-2 in xylene, from 0.01*M* malonic acid medium at pH 3.0 and stripped from the organic phase with 1*M* hydrochloric acid, then determined spectrophotometrically at 545 nm as its complex with thoron. It is separated from other elements by selective extraction and stripping.

Most extractions of thorium with liquid anion-exchangers have been from nitric or sulphuric acid. Trioctylamine in benzene has been used for the extraction from 5–6*M* nitric acid[1] (and also 2.5*M* aluminium nitrate[2]). Extraction from nitrate media with Amberlite LA-1 or LA-2 is possible in the presence of methanol[3] or ethanol.[4] Various organic solvents have been used as diluents.[5,6] Thorium cannot be extracted from chloride media,[7] but is extracted from 1.5*M* sulphuric acid with Primene JM-T or Amberlite LA-2[8] or tributylamine.[9] Mixtures of sulphuric and hydrobromic acid,[10] or phosphoric and nitric acid have also been used with dodecylamine[11] or trioctylamine[12] as the extractant. Trilaurylamine[13] and Aliquat 336 in xylene have also been used[14] as extractants, with mineral acid media. No studies of organic acid media seem to have been made. We have therefore extended our studies[15,16] of malonic acid media used with various liquid anion-exchangers, to include thorium, and find it can be separated from a large number of elements with which it is generally associated in fission products and minerals.

EXPERIMENTAL

Reagents

A stock solution of thorium was prepared by dissolving 1.312 g of thorium nitrate hexahydrate in 500 ml of demineralized water containing 1% of nitric acid, and standardized gravimetrically.[17] A 51-μg/ml thorium solution was prepared by appropriate dilution.

Amberlite LA-1, Amberlite LA-2, Primene JM-T, Alamine 335 S, Aliquat 336 S and TIOA (tri-iso-octylamine) were used without further purification, and converted into the malonate form as described earlier.[15]

General procedure

To a solution containing 102 μg of thorium 5 ml of 0.02*M* malonic acid were added, the pH was adjusted to 3.0 with 0.02*M* sodium hydroxide or malonic acid, and the volume was made up to 10 ml. The solution was transferred to a separatory funnel and shaken with 10 ml of 4% Amberlite LA-1 in xylene for 5 min on a wrist-action flask-shaker. The aqueous phase was discarded and the organic phase shaken with 10 ml of 1*M* hydrochloric acid to strip thorium. The aqueous layer containing the thorium was then mixed with 2.5 ml of 0.1% thoron solution and made up to 25 ml with demineralized water. After about 30 min for full colour development the absorbance was measured at 545 nm against a reagent blank. The concentration of thorium was calculated from a calibration curve.[17]

RESULTS AND DISCUSSION

Extraction as a function of pH

The pH for the extraction was varied between 1.0 and 7.0. Figure 1 shows the results for the 4% solutions of the various liquid anion-exchangers in xylene. The optimum pH for quantitative extraction was 2.0–5.0 for Amberlite LA-1 or LA-2, 2.5–6.0 with Primene JM-T and 2.5–5.5 with Aliquat 336 S. The extraction was only 95% complete with Alamine 336 S and poor with TIOA. The problem of emulsification with Primene JM-T or Aliquat 336 S could be circumvented by separation of the phases with a high-speed centrifuge (4000 rpm), but not by the use of long-chain alcohols such as decanol or octanol.

Effect of various diluents

The phase-volume ration was kept at unity, to avoid emulsion formation. Xylene, hexane and benzene were found to be the most efficient diluents (Table 1). Other diluents either caused turbidity or formed emulsions. Cyclohexane and kerosene proved to be poor diluents. Extraction equilibrium was generally reached within 5 min, and stripping was complete in 2 min. For practical work 5 min is recommended for both extraction and stripping.

Extraction was quantitative only with Amberlite LA-1 and LA-2, Primene JM-T and Aliquat 336 S in

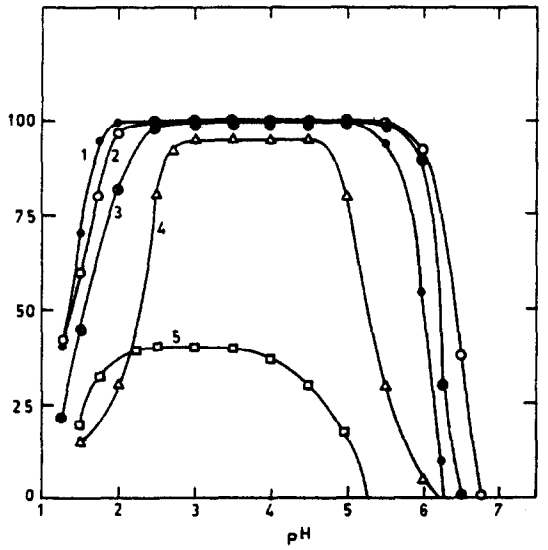

Fig. 1. Extraction of Th from malonic acid solution by various 4% amine solutions in xylene. 1, Amberlite LA-1; 2, Primene JM-T; 3, Aliquat 336 S; 4, Alamine 336 S; 5, Tri-iso-octylamine.

benzene, xylene or hexane, but the last two caused either turbidity or emulsion-formation whatever diluent was used, so Amberlite LA-1 was chosen for further study.

Malonic acid and Amberlite LA-1 concentration

Extraction starts at a malonic acid concentration of $10^{-4}M$ and is quantitative at concentrations $> 10^{-3}M$ (Table 2). Hence for practical purposes, $0.01M$ malonic acid is used. Table 3 shows that $0.01M$ Amberlite LA-1 (*i.e.*, a 4% solution) in xylene is adequate for quantitative extraction of thorium. The mechanism of extraction is the same as that for zirconium.[16]

Choice of stripping agent

Stripping was found to be complete with any $1M$ mineral acid solution (Table 4). With alkali metal hydroxides and carbonates, it was essential to use a more dilute solution (0.01–$0.1M$). Stripping was not complete with sodium or lithium chloride. Stripping could be achieved with higher concentrations of sulphuric or hydrochloric acid but with nitric acid concentrations $> 2M$ the stripping was incomplete because of formation of anionic nitrate-complexes which were re-extracted into the organic phase. Hydrochloric acid ($1M$) is perhaps the best stripping agent.

Table 1. Effect of various diluents (Th = 102 μg; pH = 2.5; 4% Amberlite LA-1)

Diluent	Extraction, %
Benzene	99.8
Toluene	96.0
Xylene	99.8
Hexane	99.7
Cyclohexane	88.5
Chloroform	87.0
Carbon tetrachloride	85.2
Nitrobenzene	91.2
Kerosene	92.0

Table 2. Effect of malonic acid concentration (Th = 102 μg; 4% Amberlite LA-1 in xylene)

[Malonic acid], mM	Extraction, %
0.30	50.0
0.50	67.0
0.60	79.4
0.70	86.1
0.80	94.0
0.90	97.4
1.00	99.8
1.20	99.8

Separation of thorium from other elements

The effect of other ions was examined as in the previous work,[15,16] the same tolerance limits being set. The results are given in Table 5. Alkali and alkaline earth metal ions, thallium(I), iron(II), silver, arsenic(III), yttrium, tin(IV) and all lanthanides except lanthanum, praseodymium, neodymium and cerium(III) do not form anionic complexes at pH 3.0 and hence are not extracted along with thorium.

Zinc, cadmium, nickel, copper, cobalt, aluminium, lanthanum, praseodymium and neodymium formed weak complexes with malonic acid at pH 3.0. Such complexes can be destroyed by scrubbing the organic phase with water, leaving thorium in the organic phase, for later stripping with $1M$ hydrochloric acid.

Gallium, bismuth, uranium, iron, antimony and mercury form relatively strong complexes with malonic acid and are extracted along with thorium. Because thorium does not form chloro-complexes, it is readily stripped with $5M$ hydrochloric acid, the other metals (which form strong chloro-complexes) being re-extracted by the exchanger. After stripping of the thorium the other elements can be stripped with $1M$ sodium hydroxide.

Table 3. Effect of Amberlite LA-1 concentration (Th = 102 μg; pH = 3.0; xylene as diluent)

[Amberlite LA-1], mM	Extraction, %
5	14.0
6	16.0
8	33.1
9	44.5
10	75.0
60	90.0
80	99.8
100	99.8

Table 4. Effect of different stripping agents (Th = 102 μg; pH = 3.0; 4% Amberlite LA-1 in xylene)

Stripping agent	Extraction, %						
	0.05*M*	0.1*M*	0.5*M*	1.0*M*	2.0*M*	4.0*M*	8.0*M*
Hydrochloric acid	—	35.8	95.0	99.8	99.8	99.8	99.8
Hydrobromic acid	—	—	92.0	99.8	99.8	—	—
Sulphuric acid	0.0	2.5	8.0	99.2	99.8	99.6	99.6
Nitric acid	< 10.0	25.0	40.0	99.6	99.5	77.0	< 5.0
Sodium hydroxide	99.5	99.6	99.2	—	—	—	—
Ammonium hydroxide	99.8	99.7	—	—	—	—	—
Sodium carbonate	99.0	99.2	99.8	—	—	—	—
Lithium chloride	40.0	55.5	60.5	60.5	—	—	—
Sodium chloride	45.0	59.6	60.1	62.5	62.5	—	—

Table 5. Effect of diverse ions (Th = 102 μg; pH = 3.0; Amberlite 4% in xylene)

Foreign ion	Added as	Tolerance limit, *mg*
Ag^{+}	$AgNO_3$	4.0
Tl^{+}	Tl_2SO_4	1.5
Tl^{3+}	$TlCl_3$	1.0
In^{3+}	$In_2(SO_4)_3 \cdot 5H_2O$	0.5
Ga^{3+}	$GaCl_3$	1.0
Cu^{2+}	$CuSO_4 \cdot 5H_2O$	2.2
Cd^{2+}	$Cd(NO_3)_2 \cdot 4H_2O$	2.5
Hg^{2+}	$HgCl_2$	0.5
As^{3+}	$AsCl_3$	1.2
Sb^{3+}	$SbCl_3 \cdot 3H_2O$	1.0
Bi^{3+}	$Bi(NO_3)_3 \cdot 5H_2O$	1.0
Pt^{4+}	$H_2PtCl_6 \cdot XH_2O$	1.5
Fe^{2+}	$FeSO_4 \cdot 7H_2O$	2.5
Fe^{3+}	$Fe_2(SO_4)_3 \cdot 7H_2O$	1.0
Cr^{3+}	$Cr(NO_3)_3 \cdot 9H_2O$	0.9
Al^{3+}	$Al(NO_3)_3 \cdot 9H_2O$	2.5
Ti^{4+}	$Ti(SO_4)_2$	0.6
Sn^{4+}	$SnCl_4$	1.0
Zr^{4+}	$Zr(NO_3)_4 \cdot 5H_2O$	0.8
U^{6+}	$UO_2(NO_3)_2 \cdot 6H_2O$	0.5
Y^{3+}	$Y(NO_3)_3$	1.1
La^{3+}	$La(NO_3)_3$	1.0
Ce^{3+}	$Ce(NO_3)_3$	0.5
Nd^{3+}	$Nd(NO_3)_3$	1.5
Pr^{3+}	$Pr(NO_3)_3$	0.5
Sm^{3+}	$Sm(NO_3)_3$	1.3
Gd^{3+}	$Gd(NO_3)_3$	1.5
Dy^{3+}	$Dy(NO_3)_3$	1.0
Be^{2+}	$Be(NO_3)_2 \cdot 3H_2O$	2.5
Mn^{2+}	$MnSO_4 \cdot 7H_2O$	2.0
Co^{2+}	$Co(NO_3)_2 \cdot 6H_2O$	2.5
Ni^{2+}	$Ni(NO_3)_2 \cdot 6H_2O$	2.5
Mg^{2+}	$MgSO_4 \cdot 7H_2O$	3.0
Ca^{2+}	$Ca(NO_3)_2$	3.0
Sr^{2+}	$Sr(NO_3)_2 \cdot 2H_2O$	2.5
Ba^{2+}	$Ba(NO_3)_2 \cdot 4H_2O$	2.0
Li^{+}	$Li_2SO_4 \cdot 4H_2O$	6.0
Na^{+}	NaCl	6.0
K^{+}	KCl	5.0
Rb^{+}	RbCl	4.5
Cs^{+}	CsCl	2.5
SeO_3^{2-}	Na_2SeO_3	0.4
TeO_3^{2-}	Na_2TeO_3	0.6
WO_4^{2-}	Na_2WO_4	0.6
$B_4O_7^{2-}$	$Na_2B_4O_7$	0.9
$Mo_7O_{24}^{6-}$	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.5
SiO_3^{-}	$NaSiO_3$	1.0

Thorium, scandium and cerium can be separated by extraction of all three, followed by stripping of cerium with 0.05*M* sulphuric acid and scandium with 0.25*M* sulphuric acid. Thorium, which is retained as an anionic sulphato-complex in the organic phase, is then stripped with 1*M* hydrochloric acid.

Titanium, zirconium, vanadium and indium can be separated in nitrate media. With 8*M* nitric acid, thorium forms an anionic nitrato-complex which is retained in the organic phase. Thus after extraction of thorium along with these metals from malonate media, all these elements were stripped with 8*M* nitric acid, leaving thorium behind in the organic phase as its nitrato-complex; the thorium was subsequently stripped with 1*M* hydrochloric acid.

Thorium is separated from oxy-anions such as selenite, tellurite, tungstate, borate and silicate by stripping it with 1*M* hydrochloric acid. The oxy-anions can then be stripped with 1*M* sodium hydroxide.

The separation from titanium, zirconium, silver, antimony, uranium and bismuth is important as they are usually associated with thorium in fission products. Cerium, yttrium, lanthanum, aluminium and silicate are associated with thorium in monazite. From ten runs with 102 μg of thorium, the recovery was 99.8% ± 0.2. The proposed method is rapid, simple and selective and is applicable at microgram levels.

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ANALYTICAL DATA

POLAROGRAPHY OF MIXED-LIGAND COMPLEXES OF COPPER WITH SOME AMINO-ACIDS AND OXALATE

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Summary—Mixed-ligand complexes formed by copper(II) with an amino-acid (aspartic acid, glutamic acid or lysine) and oxalic acid have been investigated polarographically. These 1:1:1 complexes all undergo a two-electron reduction at the dropping mercury electrode. The stability constants computed from the shift in half-wave potential with changing ligand are discussed from the point of view of steric, electrostatic and statistical considerations.

Mixed-ligand complex formation has been studied by various techniques, but polarographic studies have attracted little attention. In this investigation we studied the mixed chelates of oxalic acid and aspartic acid, glutamic acid or lysine with Cu(II) ion as a continuation of earlier studies[1–3] on mixed chelates. Such studies may be of interest in providing models for enzyme–substrate complexes with metals and other mixed-ligand species in biological species.

Since the co-ordination number of copper(II) is unlikely to exceed four, only one mixed species of the type Cu(XY) can be formed with bidentate ligands.

The DeFord and Hume[4] expression for $F_0(X)$ may be extended to give a new function $F_{00}(X, Y)$ given by

$$F_{00}(XY) = \text{antilog}\left[\frac{0.4343\,nF}{RT}\Delta E_{\frac{1}{2}} + \log\frac{I_m}{I_c}\right]$$

where the symbols have their usual significance.

By Leden's approach[5]

$$F_{00}(X, Y) = \{\beta_{00} + \beta_{01}[Y] + \beta_{02}[Y]^2\}[X]^0 + \{\beta_{10} + \beta_{11}[Y]\}[X] + \{\beta_{20}\}[X]^2$$

or

$$F_{00}(X, Y) = A + B[X] + C[X]^2$$

where

$$A = \beta_{00} + \beta_{01}[Y] + \beta_{02}[Y]^2;$$

$$B = \beta_{10} + \beta_{11}[Y]; C = \beta_{20}.$$

In practice, if the concentration of the weaker ligand is maintained constant while that of the stronger is varied, the original graphical method may be extended to the F_{00} data. The intercept on the F_{00} axis in the plot of F_{00} *vs.* [X] gives A, so

$$F_{10} = \frac{F_{00} - A}{[X]} = B + C[X].$$

By a similar plot of F_{10} *vs.* [X] and taking the intercept on the F_{10} axis as B,

$$F_{20} = \frac{F_{10} - B}{[X]} = C.$$

From knowledge of B, the mixed stability constant β_{11} for the Cu(X, Y) complex can be evaluated.

EXPERIMENTAL

All chemicals used were reagent grade. Potassium oxalate, L-aspartic acid, L-glutamic acid, and L-lysine monohydrochloride were used as complexing agents. Potassium nitrate was used as supporting electrolyte to maintain the ionic strength constant at 1.0*M*. All solutions were prepared in doubly distilled water. Gelatin solution (0.004%) was used as maximum suppressor. The temperature was held at 30 ± 1°.

The procedure was the same as that described earlier.[3]

RESULTS AND DISCUSSION

Binary systems

The formation constants of the complexes of copper(II) with oxalate, aspartate, glutamate and lysinate were measured separately before the study of the mixed-ligand systems, by the method of DeFord and Hume.[4] The conditions used corresponded as closely as possible to those for the mixed systems, *i.e.*, 1*M* potassium nitrate and 30°. The results, which are in good agreement with values in the literature,[6–8] are summarized in Table 1.

Mixed (ternary) systems

The copper–oxalate–aspartate, copper–oxalate–glutamate, and copper–oxalate–lysinate systems were studied by keeping the concentration of the weaker ligand (oxalate) constant, while varying the concen-

tration of the second ligand. The total copper concentration was kept at $5 \times 10^{-4} M$.

The concentrations of aspartate, glutamate and lysinate were calculated from the pH of the solution and the pK values of the acids determined by the method of Albert and Serjeant[9] (9.61, 9.44 and 8.95 respectively) which agree well with those reported by others.[8,10] All the systems were studied at pH > 5.6 because it is then experimentally much easier to hold the oxalate concentration constant and to vary the aspartate, glutamate and lysinate concentrations.

In all cases a single well-defined wave was obtained, for which the plot of $-E_{d.e.}$ *vs.* log $i/(i_d - i)$ gave a slope of 30 ± 2 mV, indicating that the reduction is reversible and involves the transfer of two electrons. The direct proportionality of the diffusion current to the square root of the effective height of the mercury column indicates that the reduction is entirely diffusion-controlled.

A shift of the half-wave potential to more negative values with increase in aspartate, glutamate or lysinate concentration was observed. This shift is greater in the presence of oxalate than in its absence, and signifies mixed-ligand complex formation. The results were calculated by the method of Schaap and McMasters.[11]

The results of this study are conveniently summarized as shown below, where the logarithm of the equilibrium constant is indicated for each reaction.

A number of interesting observations can be made. The tendency to add a given ligand X to CuX and to CuY (where X is oxalate and Y is aspartate, glutamate or lysinate) can be compared. The log K values are (3.6, 4.4), (3.6, 4.6) and (3.6, 4.7) respectively. Because the statistical factor is only 2 in favour of the mixed complex the largest part of the difference in log K must be attributed to entropy effects, which would favour formation of a charged complex. The corresponding tendency to add Y to Cu(X) and to Cu(Y) can also be compared. The log K values (7.3, 6.8), (7.1, 6.9) and (6.4, 6.3) for the oxalate–aspartate, oxalate–glutamate, and oxalate–lysinate systems respectively show that formation of the mixed complex is favoured by an amount corresponding to that predicted by the statistical factor.

For comparing the stabilities of simple and mixed complexes, it is convenient to measure the disproportionation constant for the equilibrium

$$2Cu(XY) \rightleftharpoons Cu(X)_2 + Cu(Y)_2$$

Statistically,[12] the concentration [Cu(XY)] should be $2[Cu(X)_2]$ or $2[Cu(Y)_2]$, so the value of the disproportion constant should be $0.25 = 10^{-0.60}$.

However, the observed values are found to be $10^{-1.3}$, $10^{-1.2}$ and $10^{-1.2}$ for the Cu(ox, asp), Cu(ox, glu) and Cu(ox, lys) systems respectively. Such a difference is normally attributed to factors such as steric and electrostatic effects. The steric effect should be appreciable since aspartate, glutamate and lysinate ions are larger than oxalate ions. The electrostatic effect, however, should be negligible compared to the steric effect.

Table 1. Formation constants for the single-ligand complexes

Complex species	Formation constant
Cu(oxalate)	$\log \beta_1 = 5.7$
$Cu(oxalate)_2^{2-}$	$\log \beta_2 = 9.3$
$Cu(aspartate)^+$	$\log \beta_1 = 8.6$
$Cu(aspartate)_2$	$\log \beta_2 = 15.5$
$Cu(glutamate)^+$	$\log \beta_1 = 8.2$
$Cu(glutamate)_2$	$\log \beta_2 = 15.1$
$Cu(lysinate)^+$	$\log \beta_1 = 7.4$
$Cu(lysinate)_2$	$\log \beta_2 = 13.7$

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ANNOTATIONS

ELECTROANALYTICAL STUDIES OF COMPLEXES OF TETRACYCLINES WITH Cd(II), Pb(II) AND Cu(II) IN AQUEOUS MEDIUM

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Summary—The dissociation constants of protonated tetracycline, chlorotetracycline and desmethylchlorotetracycline and the overall stability constants of the complexes of Cd, Pb and Cu(II) with these tetracyclines have been determined.

Tetracyclines are important antibacterial compounds. Their antibacterial activity is approximately the same[1,2] but its mechanism has not been definitely established, though it is believed to be associated with the formation of mixed complexes of antibiotic, ribosomes and a metal ion such as Mn(II).[3–5] The metal complexes are less toxic than the antibiotics themselves.[6–8] These findings have stimulated work on the behaviour of tetracyclines with metal ions.

EXPERIMENTAL

Reagents

The tetracyclines were obtained as the hydrochlorides, and used without purification, as fresh solutions in redistilled water. All other chemicals were of analytical grade. Sodium perchlorate was used to maintain the ionic strength constant at 0.1 *M*. Gelatin solution (5×10^{-4}%) was used as the maximum suppressor. Pure nitrogen was used for deaeration.

Apparatus

A manual polarograph was used. All experiments were done at 30 ± 0.5°.

Procedures

The dissociation constants of the tetracyclines were determined potentiometrically by the method of Irving and Rossotti.[9] The overall stability constants of the complexes were calculated by the graphical method of DeFord and Hume[10] as improved by Irving[11] and by the mathematical method of Mihailov.[12]

RESULTS

Effect of pH

The effect of pH on the polarographic behaviour was examined over the pH ranges 1.4–4.9 for the Cu(II) and 3.0–6.95 for the Cd and Pb antibiotic systems. The electrode processes were found to be diffusion-controlled, and the slopes of the usual log plots corresponded to two-electron reversible reductions.

The half-wave potentials were independent of pH up to about 5.2 for the cadmium systems and 4.9 for the lead systems, becoming more negative with increasing pH. For the copper systems $E_{1/2}$ became more negative with increasing pH throughout the range studied.

Effect of antibiotic concentration

This effect was studied at pH 5.8, 5.6 and 3.3 for the Cd, Pb and Cu systems respectively. The electrode processes were diffusion-controlled and reversible.

DISCUSSION

To calculate the stability constants the successive dissociation constants of the tetracyclines must be known. The hydrochlorides, $LH_3^+Cl^-$, have three sites of almost similar basicity and the protons may be dissociated from these sites in proportions which vary with the degree of neutralization. These sites are

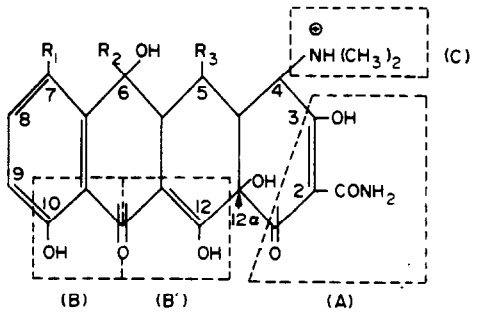

Fig. 1.

shown in Fig. 1 as the amide system (A), the phenolic β-diketone moieties (B) and (B'), and the dimethylammonium cation (C). The first dissociation constant (pK_1^H) is generally attributed to site (A).[14,15] Stephens *et al.*[14] have assigned the second and the third dissociation constants, pK_2^H and pK_3^H, to site (C) and the combined (B) and (B') system, respectively, while Leeson *et al.*[15] reverse these assignments in the case of protonated tetracycline, on the basis of comparison with those of tetracycline methiodide and desmethylammoniumtetracycline, considering a complete dissociation scheme for all. Leeson *et al.*[15] also felt that the similarities in structure and dissociation constants make their conclusions generally applicable to other members of the tetracycline group of compounds. On the basis of our polarographic results, we think Leeson *et al.*[15] were correct.

Co-ordination sites of tetracyclines for complexation

According to Conover,[16] on the basis of absorption spectra, the normal site for metal binding by tetracyclines is the enolized β-diketone group at C_{11} and C_{12}, *i.e.*, group (B') in Fig. 1. From potentiometric studies, however, Doluisio and Martin[17] concluded that the chelation occurs through the co-ordination with the nitrogen atom of the dimethylamino group at C_4 and the oxygen atom of the hydroxyl group either at C_3, or at C_{12a}. On the basis of reflectance spectra, Baker and Brown[18] decided that the dimethylamino group does not take part in the co-ordination, and that the chelation is through oxygen atoms, probably in system (A). In addition, they pointed out the need for a more detailed study because they believed that groups (B) and (B') cannot be excluded from consideration.

Thus, there is lack of agreement as to the assignment of the site of co-ordination. We suggest the following considerations:

1. Albert[19] pointed out that only the functional group having a dissociation constant of approximately 7 undergoes ionization when chelation takes place.

2. Doluisio and Martin[17] concluded that the second dissociating hydrogen atom of TC–HCl was the one displaced by the Cu(II) ion.

3. The dimethylamino group of tetracycline and anhydrotetracycline is not involved in the co-ordination in the Ni(II) and Co(II) complexes.[18]

4. On the basis of their NMR studies of tetracycline, 4-epi-tetracycline and tetracycline methiodide, Rigler *et al.*[20] have shown that group (B') is always protonated.

5. The antibacterial activity of tetracyclines is approximately the same[1,2] and the strong, or fairly strong complexes formed by these compounds with bivalent transition metal ions are of similar stability for a given metal ion.[17,19,21,22]

6. The half-wave potentials, when plotted against $-\log[LH^-]$ obtained from pH studies at constant antibiotic concentration and from concentration studies at constant pH, fall on the same curve.

From these, the following conclusions may be drawn: (*a*) the views of Conover[16] and Doluisio and Martin[17] are not tenable (points 3 and 4); (*b*) for each metal ion, the co-ordination sites are the same for all the tetracyclines under study (point 5); (*c*) it is only pK_2^H which is responsible for the variation in the half-wave potential for each system (point 6); (*d*) the co-ordination sites of the molecules of TC, CTC and DMCTC are the oxygen atoms of system (B) (points 5 and 6); (*e*) pK_2^H is due to system (B) (points 2–4 and 6). If metal complexation is indeed involved in the antibacterial activity[3–5] and the phenolic group in tetracycline[23] and chlorotetracycline[24] is responsible for part of their antibacterial activity, then that is further support for our conclusions.

Stability constants

Protonated tetracyclines, LH_3^+, behave as tribasic acids, with the three pK values around 3, 7 and 9.

Table 1. Successive dissociation constants of protonated tetracyclines

Antibiotic*	Temperature, °C	Ionic strength	pK_1^H	pK_2^H	pK_3^H	Reference
TC	30	0.10	3.52	7.27	8.80	Present work
	25	0.10	3.42	7.52	9.07	22
	20	0.01	3.35	7.82	9.57	21
	30	0.01	3.69	7.63	9.24	17
	25	0.00	3.30	7.68	9.69	14
	25	—	3.30	7.75	9.61	15
CTC	30	0.10	3.40	7.08	8.44	Present work
	25	0.10	3.26	7.20	8.77	22
	20	0.01	3.30	7.44	9.27	19
	30	0.01	3.66	7.40	9.06	17
	25	0.00	3.30	7.44	9.27	14
	25	—	3.27	7.36	9.22	15
DMCTC	30	0.10	3.57	6.90	8.70	Present work
	25	0.10	3.46	6.96	8.96	22
	30	0.01	3.85	7.31	9.23	17

* TC = tetracycline; CTC = chlorotetracycline; DMCTC = desmethyltetracycline.

Table 2. Values of overall stability constants of complexes of tetracyclines

Metal ion	Temperature, °C	Ionic strength	TC log β_1	TC log β_2	CTC log β_1	CTC log β_2	DMCTC log β_1	DMCTC log β_2	Reference
Cd(II)	30	0.10	3.20	5.72	3.08	5.49	2.99	5.33	Present work*
	30	0.10	3.21	5.72	3.08	5.50	2.99	5.32	Present work†
	25	0.10	3.32§	5.18‡	—	5.12‡	3.07§	4.84‡	22
Pb(II)	30	0.10	3.81	6.59	3.62	6.31	3.51	5.87	Present work*
	30	0.10	3.82	6.55	3.63	6.29	3.50	5.87	Present work†
Cu(II)	30	0.10	7.61	12.82	7.54	12.69	7.47	12.45	Present work*
	30	0.10	7.61	12.81	7.54	12.69	7.49	12.42	Present work†
	30	0.01	7.50	12.80	7.50	12.40	6.90	12.30	17
	20	0.01	7.80	12.80	7.60	12.60	—	—	19, 21
	25	0.10	7.58§	12.23‡	7.46§	10.71‡	7.66§	12.40‡	22

* DeFord and Hume method.
† Mihailov method.
§ Referred to as log K_{HML}.
‡ Referred to as log K_{ML}.

These are the "macroscopic" constants, referring to the overall dissociation processes. Rigler *et al.*[20] have given a complete "microscopic" dissociation scheme and deduced the values of the corresponding dissociation constants from NMR data. The microscopic and macroscopic constants are mathematically related in various ways,[25] but Silva and Dias[26] have demonstrated that either set can be used for the calculation of the stability constants of metal complexes, provided that in the complexes formed the metal is co-ordinated to a fixed chelation site. There is general agreement that a ligand having more than two co-ordination sites always uses the same ones for complex formation with Cd, Pb, Cu(II), Ni and Zn. Since the complexing species of protonated tetracyclines is LH^- and group (B) (Fig. 1) is involved in the co-ordination, the concentration of LH^- in each system can be calculated from the pH of the solution and the pK_2^H value of the protonated tetracycline.

The plots of $-E_{1/2}$ *vs.* $-\log[LH^-]$ were smooth curves, indicating successive complexation in each system and so the overall stability constants were evaluated by the graphical method of DeFord and Hume[10] as improved by Irving.[11] Plots of $F_2[LH^-]$ were parallel to the $[LH^-]$-axis for all the systems, indicating the formation of two complexes with metal-to-ligand ratios of 1:1 and 1:2. The results are given in Table 2 and were checked by the method of Mihailov.[12] They are in good agreement with those in the literature.

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THE BINDING OF TRACE AMOUNTS OF LEAD(II), COPPER(II), CADMIUM(II), ZINC(II) AND CALCIUM(II) TO SOIL ORGANIC MATTER

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Summary—The binding by peat of Ca(II), Cd(II), Zn(II), Cu(II) and Pb(II) present at trace-level concentrations in 0.0010, 0.010 and 0.10*M* sodium chloride, has been studied as a function of the degree of neutralization of the soil organic acid. The theoretically-based method used to express the complexation equilibria requires values for the concentrations of the several mobile counter-ions in the peat phase [$\overline{M(II)}$, $\overline{H^+}$ and $\overline{Na^+}$] and permits estimation of the nature of the complexed species formed in the peat as well as of reasonable values for the formation constants of the species formed. The values of the formation constants thus obtained are independent of the ionic strength of the equilibrating solution, as they should be. This result was unattainable with the earlier methods of computation used for studying these equilibria. The species formed are $Ca(II)A^+.HA$ and $M(II)A^+$, where M(II) represents Cd(II), Zn(II), Cu(II) and Pb(II).

The availability of metal ions in the soil for plant nutrition, soil pollution, *etc.* is effectively controlled by the ability of the soil organic matter to bind them rather strongly. It is, therefore, not surprising that these interactions, which involve in many cases the formation of a complex between the soil organic matter and the metal ions, have been studied for many years by various approaches. For excellent reviews on this subject the reader is referred to articles by Scheffer and Ulrich,[1] Scheffer and Schachtschabel,[2] Schnitzer and Kahn,[3] Flaig *et al.*[4] or Zunino *et al.*[5]

In this connection the determination of the stability constants of the complexes formed is of special interest, because these quantities characterize the strength of the interaction and make possible a quantitative comparison of the capability of soil organic matter of different origin to bind the various metal ions. The determination of the stability constants, is, however, complicated by the fact that soil organic matter is present as a negatively-charged, weakly acidic polymeric material, the carboxylic and phenolic groups of which are involved in the interaction mechanism with the metal ions. It has been pointed out by Marinsky[6] that study of metal-ion binding in such polyelectrolyte systems is complicated by the large and variable electric field at the surface of the polymer, which makes binding under different conditions of electric charge very difficult to interpret. This difficulty has, however, been successfully overcome, as shown by Marinsky and co-workers[7–9] for metal-ion binding by weakly acidic synthetic polymers. In these studies a suitable correction term was incorporated in the equations employed for the evaluation of stability constants. This correction term, for deviations from ideality, was determined experimentally by using the measurement of pH during neutralization with base to estimate the potential differences between the surface of the partially dissociated polyacid and the region of the solution where this potential vanishes. In the earlier examination of the gel analogues of the linear polyacid[8,9] the Donnan potential was included in the correction term so obtained.

In the present paper we have extended this approach to the examination of binding of metal ions by natural weakly acidic polymers, in this case the humic substances of the soil. The Donnan potential term has been eliminated in the eventual description of the resin phase of these systems.

Since the composition of the organic matter extracted from the soil can be affected by the chemical extractants usually employed[5] in their preparation for study, we have used only sphagnum peat washed with hydrochloric acid and water, and with no further pretreatment. The concentration of the metal ions in solution was kept at trace levels in order to ensure that, at equilibrium, the concentration of unbound polymer ligand was always measurable no matter whether one or two ligands were bound per metal ion. The deviation from ideality deduced from the apparent dissociation constant of the organic polyacid was then easily obtainable through the pH measurement. Carrier-free radioactive Pb^{2+}, Cd^{2+}, Zn^{2+}, and Ca^{2+} were employed to study the distribution equilibrium

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of these metal ions between sodium chloride solution and peat. Because only short-lived radioactive nuclides of copper can be prepared, the copper was initially at a concentration level sufficient to permit accurate atomic-absorption determination of the free copper at equilibrium. All experiments were performed at three different ionic strengths by using 0.001, 0.01 and 0.1 M sodium chloride media.

THEORETICAL

At equilibrium, during each step of the potentiometric titration of a weakly acidic polymer gel, $(HA)_\nu$, in the presence of a simple strong electrolyte, MX, the chemical potentials, μ, of the diffusible components, HX, MX and H_2O, are equal in both phases:

$$\mu_{HX} = \bar{\mu}_{HX}$$
$$\mu_{MX} = \bar{\mu}_{MX}$$
$$\mu_{H_2O} = \bar{\mu}_{H_2O} \quad (1)$$

where the bars indicate the gel phase and HX is a strong acid. On the assumption that the chemical potential in isothermal systems can be divided into two additive terms, one of which depends only on composition and the other only on pressure, the chemical potential of each component i (i = HX, MX, H_2O) in a solution of molarity M and under a pressure P is given by

$$\mu_i(P, M) = \mu_i(P^0, M) + (P - P^0)(V_i) \quad (2)$$

where P^0 is the standard pressure, preferably taken as 1 atm, and V_i is the partial molar volume of component i. The activity, a_i, of the ith component is defined by

$$\mu_i(P, M) = \mu_i^0(P) + RT \ln a_i, \quad (3)$$

where μ_i^0 is the chemical potential of the ith component in the standard state. From equations (2) and (3):

$$\mu_i(P, M) = \mu_i^0(P^0) + RT \ln a_i + (P - P^0)V_i. \quad (4)$$

We can assume that V_i is independent of composition and pressure, without introduction of serious error.

The osmotic pressure, π, of the water in the gel phase is equal to $(\bar{P} - P)$ and is related to the water activity, a_w, in the two phases by

$$\pi = -RT/V_w(\ln \bar{a}_w/a_w). \quad (5)$$

The chemical potentials of HX and MX in each phase are given by

$$\mu_{HX} = \mu_{HX}^0 + RT \ln a_{HX} + PV_{HX}$$
$$\bar{\mu}_{HX} = \bar{\mu}_{HX}^0 + RT \ln \bar{a}_{HX} + \bar{P}V_{HX}$$
$$\mu_{MX} = \mu_{MX}^0 + RT \ln a_{MX} + PV_{MX}$$
$$\bar{\mu}_{MX} = \bar{\mu}_{MX}^0 + RT \ln \bar{a}_{MX} + \bar{P}V_{MX}. \quad (6)$$

For the system described above, the equilibrium expression for the reaction

$$\overline{HX} + MX \rightleftharpoons HX + \overline{MX} \quad (7)$$

is

$$RT \ln \frac{(a_{HX})(\bar{a}_{MX})}{(\bar{a}_{HX})(a_{MX})} + \pi(V_{MX} - V_{HX}) = \bar{\mu}_{HX}^0 + \mu_{MX}^0 - \mu_{HX}^0 - \bar{\mu}_{MX}^0. \quad (8)$$

If the same standard state in the gel and solution phases is chosen for the reacting components the sum on the right-hand side of equation (8) is zero and

$$RT \ln \frac{(a_{HX})(\bar{a}_{M_X})}{(\bar{a}_{HX})(a_{MX})} = \pi(V_{HX} - V_{MX}). \quad (8a)$$

The partial molar volume of the common ion, X, cancels in equation (8a) and $(V_H - V_M)$ can be substituted for $(V_{HX} - V_{MX})$.

In most gel phases the term $\pi(V_H - V_M)$ is sufficiently small to be neglected and

$$\ln \frac{(a_{HX})}{(a_{MX})} \sim \ln \frac{(\bar{a}_{HX})}{(\bar{a}_{MX})}. \quad (9)$$

By substitution of the product of the molar concentration and activity coefficient $(M_i y_i)$ for the activity of each ionic constituent of the system (H^+, M^+, X^-) in equation (9):

$$\ln \frac{(M_{H^+})(y_H)(M_{X^-})(y_{X^-})}{(M_{M^+})(y_{M^+})(M_{X^-})(y_{X^-})} \sim \ln \frac{(\bar{M}_{H^+})(\bar{y}_H)(\bar{M}_{X^-})(\bar{y}_{X^-})}{(\bar{M}_{M^+})(\bar{y}_M)(\bar{M}_{X^-})(\bar{y}_{X^-})} \quad (9a)$$

all the $(M_{X^-} y_{X^-})$ terms in both phases cancel and

$$\ln \frac{(M_{H^+} y_{H^+})}{(M_{M^+} y_{M^+})} \sim \frac{(\bar{M}_{H^+} \bar{y}_{H^+})}{(\bar{M}_{M^+} \bar{y}_{M^+})} \quad (9b)$$

so that

$$\ln \frac{a_{H^+}}{a_{M^+}} \sim \ln \frac{\bar{a}_{H^+}}{\bar{a}_{M^+}} \quad (9c)$$

or

$$pM - pH = p\bar{M} - p\bar{H} \quad (9d)$$

where pM *etc.* refer to the activity of the species indicated.

The values of pM and pH are experimentally measurable or calculable in these systems; the concentration of M in the gel phase ($p\bar{C}_M$) is also obtainable since to preserve electroneutrality in the gel during neutralization with standard base (MOH) it must be at least equal to $p\bar{C}_{A^-}$ (because $\bar{C}_{M^+} + \bar{C}_{H^+} = \bar{C}_{A^-} + \bar{C}_{OH^-}$). Additional M will be present in the gel phase because of imbibement of MX ($\bar{C}_{MX}$). The concentration of A^- is calculable from the stoichiometry of the neutralization reaction and the measured volume (V_g) of the gel, while $\bar{C}_{MX}$ can either be determined experimentally or estimated from

equation (10)*:

$$(\bar{C}_{MX} + \bar{A}^-/V_g)(\bar{C}_{MX}) \sim (C_{MX})^2 \qquad (10)$$

where $\bar{A}^-$ is the quantity of A^- in the gel phase. Since $p\bar{M} = p\bar{C}_M - \log \bar{y}_M$, equation (9d) can be rewritten as

$$pM - pH - p(\bar{C}_{MX} + \bar{A}^-/V_g) = -\log \bar{y}_M - p\bar{H}. \qquad (9e)$$

By definition,

$$p\bar{H} = pK^{int}_{(HA)_v} + \log \bar{A}^-/HA + \log \bar{y}_{A^-} \qquad (11)$$

where $K^{int}_{(HA)_v}$ is the intrinsic dissociation constant of the polyacid functional unit that is repeated v times in the gel, $A^-/\overline{HA}$ is the mole ratio of the dissociated and undissociated functional units of the polyacid and $\bar{y}_A$ is the activity coefficient assigned to describe the deviation of this ratio from ideality. By substituting this definition of $p\bar{H}$ in equation (9e) and rearranging, we obtain the following useful expression:

$$pH - pM - \log\left(\bar{C}_{MX} + \frac{\bar{A}^-}{V_g}\right) - \log \frac{\bar{A}^-}{\overline{HA}} = pK^{int}_{(HA)_v} + \log \bar{y}_{A^-} + \log \bar{y}_M \qquad (9f)$$

There is much experimental evidence available in the literature to suggest that $\bar{y}_M = \bar{y}_H$ in these systems. In the research by Travers and Marinsky[7] study of the complexation of bivalent metal ions by poly(methacrylic acid) (PMA) showed that deviation from ideality of bivalent ions exposed to the same potential as the hydrogen ion is described exactly by the deviation term deduced from the potentiometric properties of the PMA. Further support comes from the research of Noguchi *et al.*,[12] who demonstrated that the activity coefficients of Na^+ and K^+ ions in carboxymethyldextran are equal. Additional strong evidence for the equivalence of $\bar{y}_H$ and $\bar{y}_M$ is available in the ion-exchange literature.[13] At relatively low degrees of cross-linking (2% w/w divinylbenzene) $\bar{y}_H$ is about equal to $\bar{y}_{Na}$ as shown by the ion-exchange distribution of Na^+ and H^+ between a polystyrene sulphonate resin and a simple dilute electrolyte solution containing Na^+ and H^+ (Na^+, H^+, X^-). The selectivity coefficient measured over the complete composition range of the resin in mixed form deviates very little from unity, demonstrating this equivalence of $\bar{y}_{Na}$ and $\bar{y}_H$ as an experimental fact ($K^{Na}_H = 1.02 \pm 0.02$ at $\bar{x}_{Na} = 0$, $K^{Na}_H = 1.07 \pm 0.02$ at $\bar{x}_{Na} = 0.5$ and $K^{Na}_H = 1.12 \pm 0.03$ at $\bar{x}_{Na} = 1$, where x_{Na} is the mole fraction of Na^+ in the cations sorbed by the resin).[14]

Since $\bar{y}_M \sim \bar{y}_H$ the right-hand side of equation (9f) can be rewritten as $pK^{int}_{(HA)_v} + \log \bar{y}_{A^-} + \log \bar{y}_{H^+}$, and the sum of these terms is seen from equation (11) to be equal to $p\bar{C}_H - \log(\bar{A}^-/\overline{HA})$ which in turn is equal to $pK^{app}_{(HA)_v}$, where $K^{app}_{(HA)_v}$ is the apparent dissociation constant of $(HA)_v$. Equation (9f) can thus be rewritten in the form:

$$pH - pM - \log(\bar{C}_{MX} + \bar{A}^-/V_g) - \log \frac{\bar{A}^-}{\overline{HA}} = pK^{app}_{(HA)_v} \qquad (12)$$

This equation has been used to examine the potentiometric properties of carboxymethyldextran (Sephadex CM-50) and a poly(methacrylic acid) resin (IRC-50) cross-linked with approximately 5% divinylbenzene.[15] By plotting the left-hand side of equation (12) *vs.* α, the degree of dissociation, and by extrapolating to $\alpha = 0$, where deviations from ideality that are caused by electrostatic interactions at the surface of the polymer become zero, $pK^{int}_{(HA)_v}$ was resolved for both the Sephadex and IRC-50. The $pK^{int}_{(HA)_v}$ values of 3.25 and 4.83 so obtained for these polyacids were the same as the values obtained for the linear uncross-linked polyelectrolyte form of carboxymethyldextran, and for both poly(methacrylic acid)[6,17] (the linear analogue of IRC-50) and isobutyric acid[18] (its repeating monomeric unit). This result is considered an unambiguous demonstration of the successful culmination of earlier studies in this direction by Nagasawa,[19] Katchalsky,[20,21] and Michaeli.[22]

A reliable approach to evaluation of the intrinsic pK values of weakly acidic polyacids in gel (resin) form as well as to estimation of $pK^{app}_{(HA)_v}$ as a function of their dissociation has thus been well documented and we have employed equation (12) to facilitate computation of formation constants for the complexes formed by Pb^{2+}, Ca^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+} with the soil organic acids in peat during their neutralization with sodium hydroxide.

For examination of the complexation of these bivalent ions by functional units of the peat it is useful to consider their competition with hydrogen ions for these sites:

$$\frac{\bar{M}_b(\bar{a}_H)}{(\bar{a}_{M(II)})(\overline{HA})} = D. \qquad (13)$$

Recalling that

$$p\bar{H} = pH - pNa + p\overline{Na} \qquad (9d)$$

and that similarly

$$p\bar{M}(II) = pM(II) - 2pNa + 2p\overline{Na} \qquad (9g)$$

we can substitute these expressions for $\bar{a}_H$ and $\bar{a}_{M(II)}$ in equation (13) to obtain

$$\frac{\bar{M}_b(a_{Na})(a_H)}{(a_{M(II)})(\bar{a}_{Na})(\overline{HA})} = D. \qquad (13a)$$

If only the species $[M(II)A]^+$ is formed,

$$D = \beta^{app}_{(M(II)A)_v}/\beta^{app}_{(HA)_v}. \qquad (14)$$

As we point out later, the observed deviation of $\beta_{(HA)_v}$ from its intrinsic value may either be electrostatic in origin or may originate in the heterogeneity of the natural material. If the former is the dominant factor the variation of D will parallel the variation of $\beta_{(HA)_v}$ with α because of the double charge on the metal ion (see reference 7); a plot of log D *vs.* $pK^{app}_{(HA)_v}$ should

* From equations (1), $\bar{a}_{MX} = a_{MX}$ and $(\bar{y}_\pm)^2_{MX}(\bar{C}_{M^+})(\bar{C}_{X^-}) = (\bar{y}_\pm)^2_{(M^-)}(C_{X^-})$. Now $C_{M^+} = C_{X^-} = C_{MX}$; $\bar{C}_M = (\bar{A}/V_g + \bar{C}_{MX})$; $\bar{C}_{X^-} = \bar{C}_{MX}$. Any small difference between $(\bar{y}_\pm)_{MX}$ and $(y_\pm)_{MX}$ is neglected, to obtain equation (10).

yield a straight line. The value of log D at $p\bar{K}^{int}_{(HA)_v}$ leads to evaluation of $\beta^{int}_{(M(II)A)_v^+}$.

If log D increases more rapidly than $p\bar{K}^{app}_{(HA)_v}$ the concurrent presence of a second species, *e.g.*, $M(II)A_2$, could be indicated. To examine this possibility, D can be multiplied by $(\bar{a}_H)/(\overline{HA})$ to give a new D' value which can be equated with the ratio $\beta_{(M(II)A)_v^+}/(\beta_{(HA)_v})^2$, the non-ideality terms cancelling. If D' is plotted *vs.* $(\bar{a}_H)/(HA)$ the presence of both species will be indicated by a line of positive slope with a finite intercept on the ordinate. The slope is $D(\beta^{app}_{(M(II)A)_v^+}/\beta^{app}_{(HA)_v})$ and the intercept at $(\bar{a}_H)/(HA) = 0$ is $\beta_{M(II)A_2}/(\beta_{(HA)_v})^2$.

If, on the other hand, the source of deviation in $\beta_{(HA)_v}$ is predominantly statistical in nature, its value will be essentially independent of the charge of the metal ion; if there is a parallel disturbance in the value of $\beta_{(M(II)A)_v^+}$ because of the heterogeneity of the sample, the value of D should tend to be constant.

In our study of the binding of bivalent metal ions to peat, as a function of α and ionic strength, we have examined the properties of D. With this approach it has been possible to obtain a fairly quantitative estimate of the complexation of these ions in peat.

EXPERIMENTAL

Materials

Sphagnum, peat, as characterized earlier,[10] was used throughout. It was converted into the acid form by repeated treatment with 1*N* hydrochloric acid, followed by exhaustive washing with demineralized water.

Apparatus

All batch experiments were performed at room temperature (25 ± 2°) in quartz or polyethylene bottles in order to avoid sorption of the metal ions on the container walls. In order to keep the reaction mixture CO_2-free, a nitrogen atmosphere was always maintained above it.

Reagents

All chemicals used were Merck p.a. grade. Carrier-free radioisotopes (^{45}Ca, ^{212}Pb, ^{115}Cd and ^{65}Zn) were purchased from Amersham-Buchler GmbH.

Analytical determinations

The ^{45}Ca content of the solutions was determined by evaporation of the liquid phase on a 2-cm planchette and subsequent beta-counting of the residue, with a methane gas-flow proportional counter. The ^{212}Pb, ^{115}Cd and ^{65}Zn were determined directly in solution with a well-type NaI scintillation detector. Since no suitable radioactive tracers for copper were available, this metal ion was determined by flame atomic-absorption spectroscopy (Beckman type 1233 instrument) after preconcentration by extraction with ammonium 1-pyrrolidinecarbodithioate solution and methyl isobutyl ketone followed by stripping with concentrated nitric acid, according to a procedure given by Schmidt and Dietl.[11] The pH-measurements were made with a glass electrode (N-64, Schott u. Gen.) and a digital pH-meter (Knick, type 644).

The relative error of the analytical determinations was ±1% for the radioanalytical determinations and ±5% for the AAS.

Procedure

Exactly 0.15 g of peat (corresponding to 0.013 g of material oven-dried at 105°) was equilibrated with 20 ml of a stock solution which contained the carrier-free radioactive tracer of the metal ion to be investigated and the neutral salt, sodium chloride, at concentrations of 0.001, 0.01 or 0.1*M*. The degree of neutralization of the peat was varied by adding 0, 0.003, 0.005, 0.008, 0.01 or 0.015 meq of sodium hydroxide (standard solution). To exclude carbon dioxide from the system the samples were covered with an atmosphere of nitrogen and stoppered lightly. For the potentiometric examination of the peat (metal-free) and in the experiments with copper(II), 1.5 g of peat (0.13 g of dry material) and 200 ml of sodium chloride solution (0.001, 0.01 or 0.1*M*) were employed. In the Cu(II) study 0.02 meq of Cu^{2+} was added in order to give a sufficient amount in the supernatant solution for its subsequent determination by AAS. In a number of experiments with ^{45}Ca the volume of salt solution and the quantity of peat were varied. The sample was equilibrated by being shaken continuously for 6 hr. The peat was allowed to settle overnight and the pH and radioactivity of the supernatant solution were measured. At least duplicate runs were made for each experiment.

RESULTS AND DISCUSSION

Potentiometric properties of peat

The potentiometric data obtained during the neutralization study are listed in Table 1 together with a summary of the results of their analysis by use of equation (12).

To compute $\bar{C}_{Na}$ for use in equation (12) the volume of the gel phase, V_g, was estimated to be 1.37 ml (corresponding to the quantity of water contained by the fully hydrated sample). With the concentration of $\overline{Na}^+$ corresponding to $\bar{A}^-/V_g$ deduced from the stoichiometry of each batch experiment at equilibrium, the additional sodium ion concentration imbibed by

Table 1. Potentiometric titration of peat

Base added *meq*	pH	α	$p\bar{K}^{app}_{(HA)_v}$*
	0.0010*M* NaCl		
0.00	4.15	0.077	4.041
0.03	4.95	0.170	4.249
0.05	5.45	0.267	4.304
0.08	5.96	0.422	4.313
0.10	6.36	0.526	4.436
0.12	6.69	0.632	4.499
0.15	7.36	0.790	4.732
0.18	8.22	0.947	4.831
	0.010*M* NaCl		
0.00	3.85	0.164	4.089
0.05	4.53	0.298	4.221
0.08	5.06	0.431	4.349
0.10	5.42	0.531	4.502
0.15	6.43	0.790	4.765
0.18	7.38	0.947	4.961
	0.10*M* NaCl		
0.00	3.63	0.310	3.884
0.03	3.84	0.350	4.015
0.06	4.13	0.414	4.157
0.09	4.46	0.520	4.272
0.10	4.61	0.559	4.342
0.12	4.95	0.647	4.499
0.15	5.71	0.742	4.901

* $p\bar{K}^{app}_{(HA)_v} = pH - pNa - \log \bar{C}_{Na} - \log \bar{A}^-/\overline{HA}$.

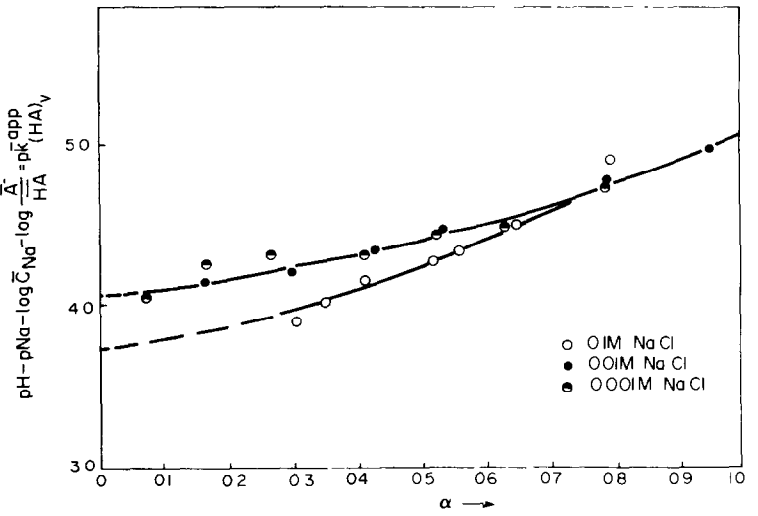

Fig. 1. The value of $pK^{app}_{(HA)_2}$ as a function of α.

the gel phase was computed by presuming that the Donnan distribution of sodium chloride in both phases

$$(\bar{a}_{Na})(\bar{a}_{Cl}) = (a_{Na})(a_{Cl})$$

was adequately described, as shown below, by using concentration units in place of activity units in this expression. With this approximation giving

$$(\bar{A}^-/V_g + \bar{C}_s)(\bar{C}_s) = (C_s)^2$$

$\bar{C}_s$ was calculable for every experimental situation.

The $pK^{app}_{(HA)_v}$ values so obtained for the peat are plotted against α in Fig. 1 for the three salt systems studied. It is observed that, for the 0.001M and 0.01M sodium chloride systems, one curve can be drawn through the set of points. For the 0.1M system, however, the $pK^{app}_{(HA)_v}$ values fall below this curve; as α increases there is convergence of the two curves.

This result can be rationalized as follows. In the absence of electrolyte invasion the intercept value of $pK^{app}_{(HA)_v}$ at $\alpha = 0$, where electrostatic interaction is absent, should correspond to the $pK^{int}_{(HA)_v}$ value of the soil organic acid. With the more dilute salt systems where imbibition of sodium chloride is minimized by the relatively higher concentration of the sodium ions associated with $\bar{A}^-$, and the lower outside concentration of sodium ions even at the lowest α value, the effect of presence of salt on the value of $pK^{app}_{(HA)_v}$ should be negligible. As a consequence the parallelism of $pK^{app}_{(HA)_v}$ in 0.001 and 0.01M sodium chloride is not surprising. Indeed the intercept value of the extrapolated curve should correspond closely to $pK^{int}_{(HA)_v}$. In the presence of 0.1M sodium chloride, however, imbibition of salt at the lowest α values is appreciable. There is significant screening of the potential field due to the charged poly-ion as well as an increase in the deviation from ideality of the hydrogen ions owing to long-range Debye interactions, and the observed value of $pK^{app}_{(HA)_v}$ is lowered appreciably. At the higher α values the concentration levels of sodium ions in the gel phase of all three systems become similar, which explains the convergence of the two curves.

The discussion above presumes the deviation in $pK^{int}_{(HA)_v}$ to be due exclusively to the variable charge at the surface of the polyion. There is, however, an additional factor which can contribute to the observed variation of $pK^{app}_{(HA)_v}$. These soil organic acids are poorly defined materials which could be composed of a mixture of acids of different strengths. Such heterogeneity probably contributes to the shape of the curves presented in Fig. 1 and may even be the dominant factor.

No direct measurement of the volume of the peat samples as a function of α or ionic strength has been made. It has been assumed that to a first approximation the peat samples are sufficiently rigid to yield a constant solid phase volume. Any variation in the volume of the peat phase will lead to error in the analysis of the data and could account for some of the differences observed at the highest ionic strength.

Complexation of bivalent metal ions by peat

The data are presented in Table 2. Except for the Cu(II) study and about half the Zn and Cd experiments the α value was directly calculable from the quantity of standard base added and the equilibrium concentration of hydrogen ion.

With the Cu(II) systems, the quantity of metal ion bound to the dissociated groups had to be taken into account. For this purpose CuA^+ was presumed to be the dominant complex species formed. The α value so obtained was employed for the computation [with equation (12)] of the $pK^{app}_{(HA)_v}$ value given in column 4 of the table. A modified α value, α_{eff}, is listed in column 3 for the Cu(II) system. To obtain an α value characterized by a polymer charge-density comparable to that of the other systems, twice the quantity of bound Cu was subtracted from the amount of $\bar{A}^-$ initially formed, calculated as the sum of the amount of base added and of acid entering the aqueous phase.

Table 2. Binding of M(II) by peat [$pK^{app}_{(HA)_v} = pH - pNa - \log \bar{C}_{Na} - \log(\bar{A}^-/\overline{HA})$]

[NaCl] M	pH	α	$\rho K^{app}_{(HA)_v}$	Wet peat g	V_s	D^*
A: ^{45}Ca(II); NaCl						
0.10	3.673	0.281	3.996	0.15	20.0	0.808
	3.863	0.340	4.050	0.15	20.0	0.689
	3.820	0.363	3.955	1.50	100.0	0.668
	3.988	0.400	4.046	0.15	20.0	0.644
	4.316	0.485	4.198	0.15	20.0	0.518
	4.480	0.548	4.243	1.50	100.0	0.508
	4.562	0.563	4.286	0.15	20.0	0.436
	5.470	0.792	4.662	1.50	100.0	0.576
	5.435	0.794	4.608	1.50	200.0	0.549
	5.373	0.795	4.555	0.15	20.0	0.569
0.010	3.640	0.132	4.097	1.50	100.0	0.935
	4.500	0.282	4.290	1.50	100.0	0.721
	5.290	0.529	4.366	1.50	100.0	0.577
	5.442	0.531	4.535	0.15	20.0	0.410
	6.270	0.79	4.656	1.50	100.0	0.418
	6.480	0.79	4.863	0.15	20.0	0.303
0.0010	3.910	0.067	4.080	1.50	100.0	1.258
	5.480	0.265	4.358	1.50	100.0	1.228
B: Cu(II); NaCl; 0.15 g of wet peat						D
0.10	3.584	0.314	3.783		200.0	0.359
	3.760	0.335	3.881		200.0	0.514
	3.774	0.336	3.905		200.0	0.432
	3.927	0.367	3.979		200.0	0.428
	4.413	0.498	4.149		200.0	0.551
	4.520	0.502	4.302		100.0	0.233
	5.198	0.702	4.412		200.0	0.623
0.010	3.781	0.120	4.078		200.0	0.404
	3.775	0.123	4.065		200.0	0.352
	3.580	0.106	4.046		100.0	0.534
	4.097	0.144	4.196		200.0	0.388
	4.386	0.218	4.207		200.0	0.405
	4.861	0.341	4.275		200.0	0.309
0.0010	3.988	0.018	4.165		200.0	0.176
	4.551	0.087	4.037		200.0	0.117
C: ^{212}Pb(II); NaCl; 0.15 g wet peat; V_s = 20.0 ml						
0.10	3.675	0.280	4.002			1.284
	3.606	0.328	3.820			2.134
	3.870	0.337	4.063			1.843
	3.803	0.366	3.931			1.891
	3.942	0.415	3.968			2.353
	4.229	0.500	4.080			2.376
	4.303	0.487	4.181			1.678
	4.656	0.556	4.396			1.209
	5.406	0.795	4.589			0.786
0.010	3.834	0.170	4.085			2.165
	3.93	0.137	4.365			3.009
	4.295	0.217	4.332			1.563
	4.14	0.242	4.075			1.997
	4.353	0.315	4.030			15.90
0.0010	4.137	0.080	4.154			0.630

[NaCl] M	pH	α	$\rho K^{app}_{(HA)_v}$	D
D: ^{113}Cd(II); NaCl; 0.15 g of wet peat; V_s = 20.0 ml				
0.10	3.548	0.251	3.948	0.0153
	3.758	0.265	4.121	0.0348
	3.888	0.310	4.142	0.0160
	3.850	0.345	4.025	0.0454
	3.983	0.401	4.037	0.0268
	4.072	0.409	4.109	0.0295
	4.312	0.467	4.231	0.0228
	4.233	0.499	4.087	0.0283
	4.569	0.562	4.305	0.0295
	5.000	0.679	4.478	0.0186
	5.508	0.794	4.694	0.0215
0.010	3.718	0.106	4.350	0.0611
	3.962	0.168	4.226	0.0595
	3.875	0.155	4.206	0.0819
	4.175	0.225	4.183	0.0600
	4.270	0.220	4.157	0.0620
	4.672	0.329	4.304	0.0549
	4.539	0.297	4.275	0.0713
	5.004	0.421	4.364	0.0511
	5.035	0.432	4.365	0.0601
	5.448	0.527	4.501	0.0373
	5.777	0.675	4.484	0.0340
	6.283	0.790	4.664	0.0145
0.0010	3.788	0.066	3.975	0.0587
	4.182	0.108	3.922	0.0517
	4.149	0.077	4.191	0.1048
	4.506	0.185	3.739	0.0475
	4.883	0.172	4.187	0.0358
	5.105	0.318	3.793	0.0338
	5.348	0.268	4.214	0.0127
E: ^{65}Zn(II); NaCl; 0.15 g of wet peat; V_s = 20.0 ml				
0.10	3.548	0.251	3.948	0.0345
	3.640	0.303	3.910	0.0303
	3.758	0.265	4.121	0.0448
	3.888	0.310	4.142	0.0211
	3.850	0.345	4.025	0.0396
	3.983	0.410	4.037	0.0642
	4.072	0.409	4.109	0.0440
	4.312	0.467	4.231	0.0466
	4.233	0.499	4.087	0.0573
	4.596	0.562	4.305	0.0602
	5.000	0.679	4.478	0.0468
	5.508	0.794	4.694	0.0515
0.010	3.718	0.106	4.350	0.0827
	3.962	0.168	4.226	0.0764
	3.875	0.155	4.206	0.0959
	4.175	0.225	4.183	0.0749
	4.270	0.220	4.157	0.0711
	4.672	0.329	4.304	0.0643
	4.539	0.297	4.275	0.0831
	5.004	0.421	4.364	0.0648
	5.035	0.432	4.365	0.0710
	5.448	0.527	4.501	0.0399
	5.777	0.675	4.484	0.0388
	6.283	0.790	4.664	0.0149
0.0010	3.788	0.066	3.975	0.0602
	4.182	0.108	3.922	0.0512
	4.149	0.077	4.191	0.0957
	4.506	0.185	3.739	0.0475
	4.883	0.172	4.187	0.0330
	5.105	0.318	3.793	0.0380
	5.348	0.268	4.214	0.0128

In several of the Cd(II) and Zn(II) samples the carrier-free radioactive nuclides added were sufficiently acidic to require a correction to be made for the extra acid present before equilibration with the peat.

The last column lists the values of D computed from the initial and equilibrium concentrations of bivalent metal ion. The volume of solution and the weight of peat are included in Table 2 when these quantities varied.

With the activity of H^+ and Na^+ in solution known, only the activity coefficients of Na^+ in the gel phase, $\bar{y}_{Na}$, and of the M(II) ion in the solution phase, $y_{M(II)}$, at equilibrium, were needed for the evaluation of D with equation (13). The method of Kielland[23] was used for this purpose. For this purpose as shown

$$\log \bar{y}_{Na} = \frac{-0.358(1)\sqrt{\bar{C}}}{1 + 0.2325a\sqrt{\bar{C}}} \qquad (15a)$$

where $\bar{C} = \sum_i \bar{C}_i Z^2 = 2\bar{M}_s + \bar{A}/V_g$, $a = 4$ Å and $\bar{M}_s$ is computed from the Donnan distribution [equation (10)]. For computation of $y_{M(II)}$:

$$\log y_{M(II)} = \frac{-0.358(4)\sqrt{C_s}}{1 + 0.2325(a)\sqrt{C_s}} \qquad (15b)$$

where $C_s = 0.2$, 0.02, and $0.002M$ at the three different concentrations of sodium chloride used. A value of 5.0 Å is taken for a for Pb(II) and Cd(II) by Kielland; for Cu(II), Ca(II) and Zn(II) it is 6 Å. In equations (15a) and (15b) the figure in brackets in the numerator is the square of the charge on the cation.

The value of D thus obtained with equation (13) neglects one experimental aspect. The amount of metal ($\bar{M}_b$) bound to the peat is calculated from the amount of free metal ion removed from the solution phase at equilibrium, and is actually equal to the sum of bound and free metal, $\overline{M(II)A^+}$ plus $\overline{M(II)}$ in the resin phase. Thus

$$D^{exp} = \frac{(\bar{M}_b)(a_H)(a_{Na})}{(a_M)(\bar{a}_{Na})(\overline{HA})}$$

$$= \frac{[\overline{M(II)} + \overline{M(II)A^+}](a_H)(a_{Na})}{(a_M)\bar{a}_{Na})(\overline{HA})}$$

$$= D^{real} + \frac{[(\overline{M(II)}](a_H)(a_{Na})}{(a_M)(\bar{a}_{Na})(\overline{HA})}. \qquad (13b)$$

Since

$$\overline{M(II)} = \frac{(a_M)(\bar{a}_{Na})^2}{(a_{Na})^2\bar{y}_{M(II)}}$$

$$D^{exp} = D^{real} + \frac{(\bar{a}_{Na})(a_H)}{(a_{Na})(\bar{y}_{M(II)})(\overline{HA})}$$

$$D^{exp} = D^{real} + \frac{1}{\beta^{app}_{HA}(\bar{A}^-)\bar{y}_{M(II)}} \qquad (13c)$$

The magnitude of the extra term in equation (13d) was never greater than $0.05D^{exp}$ and no correction for this perturbation of D^{exp} has been made.

With equation (13) employed as described it was found that for all systems save the Ca(II) system the value of D was relatively constant. In the case of Ca(II), D became significantly smaller as α increased and $1 - \alpha$ decreased. Dividing D by ($\overline{HA}$) removed this trend in D to a considerable extent. We have reported the value of $D/(\overline{HA})$ instead of D in Table 2 for the ^{45}Ca–peat–NaCl system. It is designated by D^*.

It may be observed from inspection of Table 2 that the α range examined in the 0.01 and $0.001M$ sodium chloride systems is increasingly smaller, especially in the case of the more strongly bound metal ions. The reason for this is experimental. At the lower ionic strength the peat samples are exposed to a higher pH at a particular degree of neutralization. At the higher pH-values encountered the peat is slightly soluble. As a consequence a small percentage of the bound metal ion is also dissolved. With the more strongly complexed ions the quantity of metal ion dissolved along with the peat is sufficiently great to mask the presence of free, unbound metal ion. The result is a significant lowering of the computed D value even at the lowest α values in the $0.001M$ sodium chloride system.

The tendency for D to remain nearly constant or become smaller with increasing charge of the gel matrix would appear to indicate that electrostatic interaction between counter-ion and poly-ion is not the major factor contributing to the observed change in $pK_{(HA)_\nu}$ with α. As was pointed out earlier in the theoretical section of this paper, if the source of the observed deviation is electrostatic, it should produce a parallel change in the value of D with the formation of $\overline{M(II)A^+}$ as the dominant species. Had $\overline{MA_2}$ been formed, the expected rise in D would be even bigger.

On the basis of this result, then, we must conclude that the deviation observed in $pK_{(HA)_\nu}$ with change in α is principally a consequence of sample heterogeneity. The tendency for the value of D to be fairly insensitive to change in α is apparently due to cancellation of heterogeneity effects on the formation of $\overline{HA}$ and $\overline{M(II)A^+}$, recalling that

$$D = \frac{\beta^{app}_{M(H)A^+)_\nu}}{\beta^{app}_{(HA)_\nu}}.$$

Reconsidering the potentiometric data (Fig. 1) we must conclude that the overall shape of the curves is due primarily to the presence in the peat sample of a mixture of organic acids with different acid strengths. The interpretation of the differences observed between the curves at lower and higher ionic strength at the lower degrees of neutralization can still be attributed to (1) the more effective screening of the electric field at the surface of the charged polymer by imbibed salt in $0.10M$ sodium chloride and (2) incorrect assessment of the gel phase volume, the second factor being much the more important.

In the presentation of the binding data in Table 2, it was pointed out that the values of D that were obtained for the ^{45}Ca systems became progressively smaller as the value of α increased. This result was contrary to the apparent constancy of D for the other metal ion systems. On the basis of this result, it was assumed that $^{45}Ca(II)$ is bound to two neighbouring ligand groups of the gel matrix with the release of only one proton; co-ordination to the oxygen atom of the phenol group in the second functional group without displacement of the hydrogen atom has been postulated. Redefinition of D on this basis as follows

$$D^* = D/(\overline{HA}) = \beta^{app}_{(\overline{Ca(II)A\cdot HA})^+_{1/2}}/\beta^{app}_{(\overline{HA})_1}(\overline{HA})$$

resulted in a better correlation of the data. The fluctuation in the value of D^* was then similar to that in D for the other metal ions, with which the complexed species $\overline{(M(II)A)}^+$ is presumed to be formed.

The two different equilibria that describe the complexation of Ca(II) and the other M(II) ions studied are thus

$$Ca(II) + \bar{A}^- + HA \rightleftharpoons \overline{Ca(II)A\cdot HA}$$

and

$$M(II) + \bar{A}^- \rightleftharpoons \overline{M(II)A}^+$$

By multiplying the computed D^* or D value by $\beta^{app}_{(\overline{HA})_1}$ we can obtain estimates of $\beta^{app}_{(\overline{Ca(II)A\cdot HA})^+_{1/2}}$ and $\beta^{app}_{(\overline{M(II)A})^+_1}$, respectively, as a function of α and ionic strength. The respective β values at $\alpha = 0$ and $\mu = 0.01$ or 0.001 for Ca, Cd, Zn, Cu and Pb are $\sim 10^4$, 4×10^2, 6×10^2, 4×10^3, and 2×10^4.

Use of the theoretically-based equation (13), by requiring expression of the concentration of the several mobile counter-ions in the peat phase [$\overline{M(II)}$, $\overline{H}^+$ and $\overline{Na}^+$] has permitted estimate of the nature of the complexed species formed in the peat as well as a reasonable value for the formation constant of each of the species formed. The values of D thus obtained are independent of the ionic strength of the equilibrating solution, as they should be. This result was unattainable with the earlier methods of analysis and computation employed to study these kinds of equilibria. There is no question that the interpretation of ion-binding by materials such as peat, humic acids, colloidal silica and even hydrous iron oxide must proceed by the path that is outlined in this study.

Conclusion

In the examination of two-phase equilibria of the kind studied in this research, it is essential to know accurately the concentration of the mobile counter-ions in both phases. We have attempted to accomplish this by (1) assuming that the simplified expression for the Donnan equilibrium of electrolytes applies and (2) measuring the solvent content of the peat samples examined.

A more careful analysis of the solid phase than has been conducted in this research is desirable, however. It is achievable by a more detailed examination of solvent uptake by peat as a function of α and the solution phase ionic strength. To eliminate the difficult problem of quantitative estimation of electrolyte invasion a fully dissociated polyelectrolyte such as sodium polystyrenesulphonate can be substituted for the simple salt employed in these studies. Such an experimental approach to avoid the problem of electrolyte imbibition has been reported.[15]

With the more precise information about the gel phase available in these ways a more accurate assessment of the nature of the complexation reactions between bivalent cations and peat than has been obtained in this study will most certainly result. Only then will extension of these studies to consideration of the reactions between the soil organic acids and increasing quantities of these metal ions be meaningful.

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A RAPID HYDRIDE-EVOLUTION ELECTROTHERMAL ATOMIC-ABSORPTION METHOD FOR THE DETERMINATION OF TIN IN GEOLOGICAL MATERIALS

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Summary—A rapid, semi-automated electrothermal atomic-absorption spectrophotometric method involving lithium metaborate fusion and hydride evolution has been developed for the determination of tin in geological materials. The sample is fused with lithium metaborate and brought into solution with hydrochloric acid: the tin in the solution is then converted into its hydride with sodium borohydride in an autosampler. The hydride is decomposed and atomized in an electrically heated quartz furnace, and the atomic absorption of tin at 286.3 nm is measured. The detection limit and the linear working range for tin are 1 ng/ml and 0–140 ng/ml respectively. The method has wide tolerance for variation in reagent concentrations and possible interferences, and when tested on some certified rocks and soils having a wide range of tin values, was found to be satisfactorily accurate and precise. The procedure is now used routinely in our laboratories. At least 10–12 samples can be analysed per hour.

Traces of tin are commonly found in many silicate rocks, soils and sediments, partly as a constituent of the silicate lattice (lattice-bound tin), and partly as cassiterite, SnO_2. The determination of tin at below the μg/g level has assumed importance because of its geochemical[1] and environmental[2] implications. Methods that are at present in use for determining low levels of tin in geological samples include spectrophotometry,[3] flame atomic-absorption spectrometry,[1] polarography,[4] X-ray fluorescence[5] and neutron activation.[6] All of these methods, with the possible exception of neutron activation, are subject to considerable error because they are not sufficiently sensitive or selective; also, they require a multistage process to complete the analysis. The inaccuracy associated with these methods is clearly reflected in the wide range of values obtained in the interlaboratory certification programme for two certified Canadian reference ores.[7] The technique of neutron activation, although sensitive and specific, is not accessible to many laboratories. Furthermore, all the existing methods are slow.

In our laboratories a rapid, simple, sensitive and selective method was required because of the large throughput of samples and also because most of the geochemical samples we encounter contain tin in amounts less than 5 μg/g.

Recently, Vijan and Chan[8] described a hydride-generation–electrothermal atomic-absorption method for determining ng–μg amounts of tin in airborne dust. As this approach seemed to satisfy most of our requirements, we decided to explore the feasibility of adapting it for determining tin in some samples of rocks and soils. The results are reported in this paper.

EXPERIMENTAL

Apparatus

A Varian Techtron Model AA-5 atomic-absorption spectrophotometer was used with a Cathodeon tin hollow-cathode lamp. An electrically heated open-ended quartz tube, 15 cm long and 1.2 cm internal diameter with a 4-mm diameter inlet tube fused in the middle was used for atomizing tin in the gaseous stream. The argon flow-rate was regulated by means of a calibrated flowmeter. The temperature of the tube furnace was regulated by means of a 0–110-V Variac transformer. The furnace was mounted on the burner-head and aligned in the usual manner to let maximum light from the hollow-cathode lamp reach the detector. A Technicon autosampler, proportionating pump and manifold were used in conjunction with a 10-mV recorder for achieving automatic operation as described by Vijan and Wood.[9] The manifold is represented schematically in Fig. 1.

Reagents

A 1000-mg/l. tin stock solution was prepared by dissolving 1.000 g of tin metal (BDH, ACS certified) in 100 ml of concentrated hydrochloric acid at 40° and making up to 1 litre with distilled and demineralized water. Lower concentrations were obtained by dilution of the stock solution and adjusted to be 0.1*M* in hydrochloric acid content.

A 1% solution of sodium borohydride (Baker Analyzed) in 0.2% sodium hydroxide solution was used for hydride generation. All other acids and salts used were of analytical reagent or ACS grade.

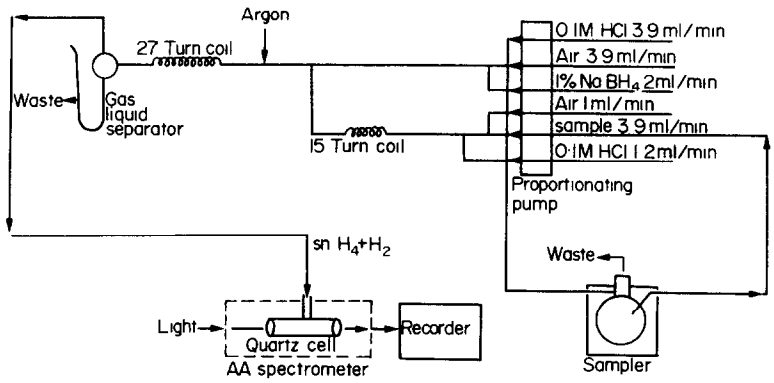

Fig. 1. Schematic diagram of autosampler and atomic-absorption spectrophotometric system for tin.

Procedure

Sample decomposition. A 0.25-g sample is mixed with 0.8 g of lithium metaborate in a vitreous carbon crucible, and the crucible is placed for 15 min in a furnace preheated to 950 ± 30°. The molten mass is rapidly poured into 10–15 ml of 1*M* hydrochloric acid contained in a 50-ml polyethylene beaker and immediately stirred magnetically until dissolution is complete (3–5 min). The solution is made up to 100 ml (or less, if desired) with distilled, demineralized water just before measurement. Blanks and standards are subjected to the same procedure.

Measurement. The atomic-absorption spectrometer and the quartz furnace are allowed to warm up until a stable baseline is obtained (usually 30 min) under the optimized operating conditions listed in Table 1. The vent is closed to the lowest position in order to prevent air turbulence in front of the cell openings and thereby minimize any baseline drift. The argon is turned on at the predetermined flow-rate. The manifold tubes are inserted into the solutions of hydrochloric acid and sodium borohydride, as shown in Fig. 1. The proportionating pump is started, and when the system has attained equilibrium (15–20 min), as indicated by minimal baseline noise on the strip-chart recorder, the spectrometer is adjusted to zero absorbance. The autosampler containing the test solutions is switched on, and the tin hydride vapours are then swept sequentially in the argon stream through a U-shaped spray trap into the resistance-heated quartz tube where they are atomized. The atomic-absorption peaks due to blanks, standards and samples are recorded, and the concentration of tin in the test solutions is obtained by reference to linear working curves prepared from the calibration standards.

RESULTS AND DISCUSSION

Sample decomposition

The lack of a generally applicable method of decomposition renders the determination of tin in geological materials very difficult. For example, ammonium iodide is supposed to decompose cassiterite quantitatively, but is known to be totally ineffective for lattice-bound tin and even for cassiterite entrapped in silicates.[10] The reverse appears to be true with wet digestion using hydrofluoric acid–sulphuric acid or nitric acid–perchloric acid mixtures.[3] Also, a number of other decomposition procedures such as fusion with $K_2S_2O_7$, Na_2O_2, Na_2CO_3, Na_2O_2–Na_2CO_3 or Na_2CO_3–S have been found to be useful only at levels of tin greater than 20 μg/g.[3,11] Several other problems encountered with the use of these decomposition methods have been discussed by Bond *et al.*[4]

Fusion with lithium metaborate has been used successfully for the quantitative decomposition of a number of ores and minerals which are otherwise difficult to decompose,[12] but this method does not seem to have been used for tin ores, especially low-grade ores, before. We found it to be equally effective for the low-grade ores used in this study and for cassiterite concentrates. In all cases, the recovery of tin was found to be close to 100%.

Optimization of instrumental parameters

The manifold tube sizes were selected by trial and error until the optimum sensitivity was attained. A 1-min sampling and 2-min washing cycle governed by a two-lobed cam was found to give absorption peaks with the least distortion and which also returned smoothly to a fairly steady baseline. Introduction of air into the system through the manifold tubes (Fig. 1) made the peaks sharper and also gave faster return to the baseline. Without the segmenting, the peaks were somewhat irregular and took longer to return to the baseline. The performance was better when air was supplied through both tubes rather than through only one. The optimum argon flow lay in the range 0.25–0.4 l./min, with the absorption signal decreasing at higher or lower flow-rates, for example, to about 35% of the maximum at 0.1 l./min. Moreover, lower

Table 1. Optimized operating conditions for tin

Line	286.3 nm
Band-width	0.15 nm
HCL current	4.0 mA
Damping	Maximum (D)
Argon flow	220 ± 20 ml/min
Atomization temp.	850 ± 30°C
Recorder span	5 mV full scale
Chart speed	0.5 cm/min
Sample time	1.0 min
Wash time	2.0 min

Table 2. Interferences for 20 ng of tin per ml in 0.1*M* HCl

Interferent	Concentration, *ng/ml*	Recovery of tin, %	
		Direct	Co-pptn.†
As(III)‡	100–300	98 ± 2	97 ± 2
	400	92 ± 3	97 ± 3
	500	85 ± 3	98 ± 1
	1000	67 ± 2	96 ± 2
	3000	58 ± 1	96 ± 2
Co^{2+}	100–800	97 ± 2	97 ± 2
	1000	95 ± 2	100 ± 3
	3000	89 ± 3	96 ± 2
	5000	64 ± 1	98 ± 1
	10000	41 ± 2	97 ± 1
Cu^{2+}	100–1000	102 ± 1	98 ± 2
	3000	73 ± 2	99 ± 2
	5000	51 ± 1	94 ± 2
Ni^{2+}	100–800	101 ± 2	100 ± 1
	1000	92 ± 2	97 ± 2
	3000	77 ± 1	97 ± 1
	5000	58 ± 2	96 ± 2

* Average and range of triplicate measurements.
† Co-precipitated with manganese hydroxide according to the procedure of Burke.[13]
‡ Sb(III) behaved similarly.

flow-rates produced distorted peaks with an unstable baseline, while higher flow-rates decreased the sensitivity because of the combined effects of dilution and shortened residence time. In this work, the flow-rate was fixed at 0.35 l./min. In agreement with Vijan and Chan[8] we found the optimum atomization temperature for tin to be around 850 ± 30°. Raising the temperature to around 1000° lowered the sensitivity by 35%. A decrease in temperature to around 720 ± 20° increased the signal by about 18% but broadened the peaks and resulted in baseline drift.

Optimization of solution conditions

We were interested in a rapid routine method capable of handling large numbers of samples, and therefore one with a wide tolerance in operating conditions and concentrations of reagents. In the final procedure, sample volumes could vary from 3 to 5 ml, the concentration of the hydrochloric acid solution pumped through the manifold could be in the range 0.05–0.20*M*, and the borohydride concentration could vary between 0.4 and 5.0%. In four months of routine work, the slope and intercept of the calibration curve remained constant from day to day (coefficient of variation at the 95% confidence interval was ±2%).

The use of solution concentrations outside the ranges mentioned resulted in a loss of sensitivity. For example, with 0.01*M* and 0.05*M* hydrochloric acid the yield of tin hydride was only 50 and 33%, respectively, while at higher acidities, the yield decreased progressively, reaching values of 70, 50, 12 and 4% with 0.5, 1.0, 3.0 and 5.0*M* hydrochloric acid, respectively. Similarly, the use of 0.3 and 0.1% sodium borohydride solutions resulted in the formation of only 85 and 42% of the expected amount of tin hydride.

Interferences

At the 20-ng/ml level of tin, no interference occurred from 5000 mg/l. each of Na^+, K^+, NO_3^-, PO_4^{3-}, SO_4^{2-} and Cl^-, 250 mg/l. each of Ca^{2+}, Mg^{2+} and Al^{3+}; 50 mg/l. each of Fe^{3+}, Mn^{2+}, Pb^{2+} and Zn^{2+}, 10 mg/l. each of Cd^{2+}, Cr(VI), Hg^{2+}, Mo(VI), Se(IV) and V(V). Interferences did occur from As(III), Co^{2+}, Cu^{2+}, Ni^{2+} and Sb(III) as shown in Table 2. It is clear from Table 2, however, that at least a 50-fold ratio of Co, Cu or Ni and a 20-fold ratio of As or Sb to tin can be tolerated. Thus, the method is limited to samples containing less than 1 mg/l. each of Co, Cu and Ni and 0.4 mg/l. each of As and Sb in the final test solution. Above these levels the interferences may be prevented or at least minimized by separating the tin by co-precipitation with manganese hydroxide as described by Burke.[13]

Analytical parameters

The sensitivity (concentration corresponding to 1% absorption), detection limit (concentration corresponding to 3 × standard deviation of the blank), and linear working range, for tin in 0.1*M* hydrochloric acid are 1.0, 0.5 and 0–140 ng/ml, respectively. Thus, levels as low as 0.1 and as high as 2.8 μg/g of tin can be determined in a 0.25-g sample made up to a final volume of 50 ml. The lower limit of detection can be improved by decreasing the final sample volume or increasing the sample size. The coefficient of variation (95% confidence) was 7, 3 and 2% at levels 4, 10 and 20 times the detection limit. Considering the levels involved, the range of precision may be deemed acceptable.

Table 3. Determination of tin in some ores and soils

Sample*	Concentration of tin, μg/g	
	Certified/provisional value	This work†
SO-1	3	2.9 ± 0.2
SO-2	3	2.8 ± 0.2
SO-3	1	1.1 ± 0.1
SO-4	3	2.8 ± 0.2
CRX-1	53–57	52.6 ± 0.9
SY-2	5	4.7 ± 0.2
SY-3	6	5.6 ± 0.3
MRG-1	3	3.1 ± 0.1
G-2	1.5	1.4 ± 0.1
GSP-1	6.3	6.0 ± 0.3
AGV-1	4.2	4.5 ± 0.3
GH	10	10.3 ± 0.4
BR	8	7.8 ± 0.2
BCR-1	2.6	2.3 ± 0.2

* SO-1 to SO-4 are soil standards obtained from the Canada Centre for Mineral and Energy Technology; CRX-1 is a U.S. Geological survey soil standard; SY-2, SY-3 and MRG-1 are Canadian Certified Reference rocks; BR and GH are rocks obtained from the Centre de Recherches Pétrographiques et Géochemiques, France, and all the others are U.S. Geological Survey Reference rocks. In all these samples the levels of As, Co, Cu and Ni were much below the interference levels and therefore all the values of tin were obtained by the direct method.

† The measure of precision is the standard deviation derived by analysing in triplicate three different weights of the same standard.

Application to geological samples

The values obtained for tin in some reference standards of rocks and soils are compared with the certified values in Table 3. The precision, expressed as coefficient of variation at the 95% confidence interval, was obtained by analysing in triplicate three different sample sizes of each reference standard (*i.e.*, a total of 9 determinations for each standard). Although the testing is by no means exhaustive, the data do show that the method is capable of yielding reasonably accurate and precise results. This rapid procedure is now being routinely used in our laboratories for determining tin in samples of rocks and soils. At least 10–12 samples can be analysed per hour.

The procedure can easily be extended to the determination of tin in sediments, and with minor modifications to a variety of other matrices.

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USE OF TRANSITION ELEMENTS TO ENHANCE SENSITIVITY FOR SELENIUM DETERMINATION BY GRAPHITE-FURNACE ATOMIC-ABSORPTION SPECTROPHOTOMETRY COMBINED WITH SOLVENT EXTRACTION WITH THE APDC–MIBK SYSTEM

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Summary—A microanalytical method for the measurement of selenium in waters and biological materials by a flameless atomic-absorption technique has been developed. The ammonium pyrrolidinedithiocarbamate–methyl isobutyl ketone extraction system is used for separation from interfering materials such as large amounts of alkali and alkaline earth metal salts and mineral acids. The atomic-absorption sensitivity for selenium is found to be enhanced to a large extent by co-extraction of some transition metal ions. Copper(II) has been used successfully as such an additive to diminish the volatility of selenium in the graphite furnace during the ashing step of the atomization cycle. When the aqueous phase/organic solvent volume ratio is 5 and the volume injected into the graphite furnace is 20 μl, the sensitivity for selenium is 0.3 ng/ml for 1% absorption. The relative standard deviation is *ca.* 2%. Interference by other metal ions is prevented by masking with EDTA. The method has been applied satisfactorily for the determination of minute amounts of selenium in waters and various biological materials.

In a previous paper the differential determination of selenium(IV) and selenium(VI) by graphite-furnace atomic-absorption spectrophotometry combined with the diethyldithiocarbamate (DDTC)–carbon tetrachloride extraction system was described.[1] With ammonium pyrrolidinedithiocarbamate (APDC), selenium(IV) is also well extracted into methyl isobutyl ketone (MIBK) over a wide pH range. In the electrothermal excitation atomic-absorption method, however, the resulting sensitivity is lower than that of the DDTC–CCl_4 extraction system.

In further investigations, it was found that the addition of some transition metal ions (Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, *etc.*) to the aqueous phase leads to a remarkable enhancement of the atomic-absorption sensitivity for selenium. This paper mainly describes the effect of copper(II) on the sensitivity and its application to the determination of selenium in biological materials by graphite-furnace atomic-absorption spectrophotometry following an APDC–MIBK extraction procedure.

EXPERIMENTAL

Reagents

All the solutions were prepared as described previously[1] from analytical-reagent grade chemicals and demineralized water, and stored in polyethylene bottles. It was established that the distilled water and the demineralized water used were both free from detectable selenium impurity.

Apparatus

The apparatus was that described before,[2] except that in the present work the light-source was a Westinghouse selenium hollow-cathode lamp.

Recommended general procedure

Transfer an aliquot of a sample solution containing not more than 2 μg of selenium(IV) to a separatory funnel. Add 2 ml of 100-ppm copper(II) solution ($CuSO_4$), 5 ml of 5% EDTA solution, 2 ml of 1% APDC solution and 5 ml of acetate buffer solution (pH 4.5). Dilute the mixture to 25 ml with water and shake the funnel for 2–5 min with 10.0 ml of MIBK. Leave to stand for 20–30 min and then separate the organic phase.

Inject 20 μl of the organic phase by micropipette into the graphite furnace. Pass argon through the furnace at a flow-rate of 3 l./min, and then atomize the sample by the following heating sequence: drying for 30 sec at 20 A (*ca.* 200°), ashing for 120 sec at 70 A (*ca.* 1050°), and atomizing for 8 sec at 250 A (*ca.* 2600°). Record the absorption signal at 196.1 nm.

Apply the whole procedure to the aqueous standard solutions.

RESULTS AND DISCUSSION

Optimization of the atomization system

The preheating conditions were examined in the same way as described in the previous paper,[1] and followed the same pattern, the useful limits being 20–30 A for 30 sec (200–300°) for drying, 65–85 A for 120 sec (1000–1300°) for ashing, and 220–260 A for 10 sec (2300–2700°) for the atomization.

The shielding-gas flow-rate should be the same as in the previous paper:[1] 3 l./min.

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Table 1. Enhancement of sensitivity for 2 μg of Se/25 ml, by some transition elements

Element	Absorbance	Enhancement factor
none	0.110	—
Cu(II)	0.605	5.5
Zn(II)	0.600	5.5
Co(II)	0.330	3.0
Ni(II)	0.250	2.3
Fe(III)	0.240	2.2

[Ion]/[Se(IV)] = 100:1.

Extraction

Extraction behaviour of Se(IV) and Se(VI). In the previous paper,[1] the extraction behaviour of selenium(IV) and (VI) with APDC, DDTC and dithizone in MIBK, nitrobenzene and carbon tetrachloride was investigated. Although selenium(IV) is 95% extracted with APDC into MIBK (as determined by the radioactive-tracer technique with ^{75m}Se), its atomic-absorption sensitivity is very low. In this study, it has been found that if a transition metal ion such as copper(II), zinc(II), cobalt(II), nickel(II) or iron(III) is present in the aqueous solutions, the height of the absorption peak given by the extract is markedly increased. The enhancements in the sensitivity obtained by the addition of these cations, each added in 100-fold amount relative to selenium, are shown in Table 1. The sequence of the enhancement of factors reflects the order of the stability of the metal selenides as well as of the metal sulphides. Hence co-extracted metal–APDC complexes are assumed to be converted into the more stable selenides at higher temperatures in the ashing stage of the atomization cycle. This is why addition of the transition elements suppresses the appreciable volatilization loss of selenium(IV).

Copper(II) is very much to be preferred as the additive, because copper(II)–APDC is considered to be more stable than copper(II)–EDTA and therefore

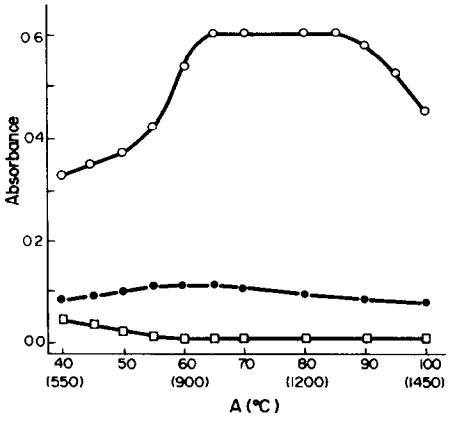

Fig. 1. Effect of current (temperature) of the ashing stage on atomic-absorption of 2 μg of selenium(IV). ○, Se(IV) with 200 μg of Cu(II); ●, Se(IV) without Cu(II); □, reagent blank; aqueous phase, 25 ml; organic phase, 10 ml.

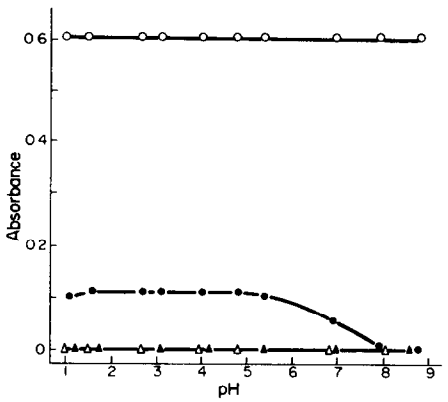

Fig. 2. Effect of pH on the extraction of 2 μg of selenium(IV) and selenium(VI). ○, Se(IV) with 200 μg of Cu(II); ●, Se(IV) without Cu(II); △, Se(VI) with 200 μg of Cu(II); ▲, Se(VI) without Cu(II); aqueous phase, 25 ml; organic phase, 10 ml.

EDTA is available as a masking agent for many interfering cations. The effect of copper(II) on the optimum current (temperature) required for the ashing stage is shown in Fig. 1.

Figure 2 shows the influence of pH on the absorption signal when selenium is extracted with and without copper(II) present. It was confirmed by use of ^{75m}Se radiochemical tracer that the absorption signals were equated with the degree of extraction of selenium over the pH range tested, for all selenium levels, when copper(II) was present. The atomic-absorption of selenium(IV) in the extracts from solutions containing copper(II) was maximal and constant over the pH range 1–9, whereas in the absence of copper(II) constant (but lower) atomic-absorption was obtained only in the pH range 1.5–5. It is interesting to note that in the presence of copper(II) the selenium(IV) is extracted very well over the pH range 5–9 where selenium(IV)–APDC is not extracted from pure solution. Further, selenium(VI) is not extracted at all over the entire pH range 1–9. Accordingly, to determine the amount of selenium(VI) it is necessary to reduce selenium(VI) to selenium(IV) before the extraction, as described in the previous paper.[1]

Effect of copper(II) concentration. Different amounts of copper(II) were added to an aqueous solution of selenium(IV) and the extraction was done as described above. The result is shown in Fig. 3. To obtain maximum sensitivity, a minimum 50-fold w/v ratio (60-fold molar ratio) of copper(II) to selenium(IV) is enough. However, large amounts of copper(II) consume APDC and appreciably lower the degree of extraction of selenium. Therefore, a copper(II) concentration of *ca.* 8 μg/ml is recommended when the selenium(IV) concentration is 80 ng/ml or less.

Effect of shaking time and stability of the extracts. Extraction is quantitative in 15 sec, and shaking for up to 20 min produces no further change in absorp-

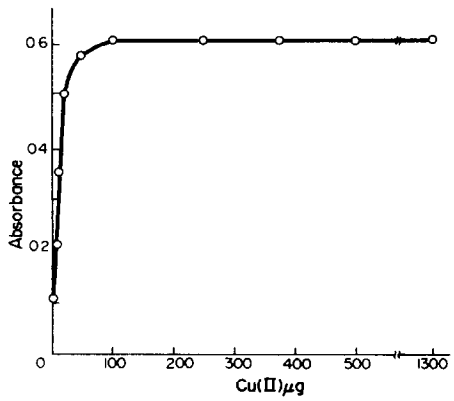

Fig. 3. Effect of copper(II) concentration on the atomic-absorption determination of 2 μg of selenium(IV). Aqueous phase, 25 ml; organic phase, 10 ml.

tion. The extract gives an absorption signal that is almost constant for measurement at any time up to at least 5 hr after the extraction.

Degree of extraction. This was investigated by the radioactive-tracer technique with ^{75m}Se and a 1:1 volume-ratio of aqueous to organic phase. The degree of extraction of selenium(IV) is 95% over the pH range 1.5–5.0 when Cu(II) is absent and the pH range 1.0–9.0 when copper(II) is present (in up to not more than 100:1 ratio to selenium). When copper(II) is present, selenium(IV)-APDC is extracted completely over the whole pH range 1.0–9.0, but when Cu(II) is absent the degree of extraction decreases at pH >5.5 and becomes zero at pH 8–9. The difference is presumably due to the co-extraction of selenium(IV)–APDC with copper(II)–APDC. Selenium(VI) is not appreciably extracted over the whole pH range.

Table 2. Permissible amount of foreign ions in determination of 2 μg of Se/25 ml (within negative error of 10%)

Ion	Added as	Limit [ion]/[Se]	
		No EDTA	1% EDTA
Fe^{3+}	Chloride	3	1000
Co^{2+}	Chloride	15	1000
Ni^{2+}	Chloride	550	1000
Mn^{2+}	Chloride	35	1000
Zn^{2+}	Chloride	60	1000
Cr^{3+}	Nitrate	45	1000
Pb^{2+}	Nitrate	5	7
Ag^{+}	Sulphate	900	1000
Hg^{2+}	Chloride	115	115
Sn^{2+}	Sulphate	6	50
Cd^{2+}	Sulphate	130	160
As^{3+}	Arsenious acid	35	35
Sb^{3+}	Chloride	27	27
Bi^{3+}	Chloride	200	250
Te^{4+}	Tellurous acid	20	20
CN^{-}	Potassium salt	20	20
S^{2-}	Sodium salt	75	75

The following are tolerable in 1000-fold ratio to Se: Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Al^{3+}, Cu^{2+}, As(V), NH_4^{+}, F^{-}, Cl^{-}, Br^{-}, I^{-}, NO_3^{-}, CH_3COO^{-}, SO_4^{2-}, PO_4^{3-}.

Table 3. Analytical results for water samples

Sample	Se added, *ng/ml*	Se found, *ng/ml*
River water	—	2
	5	7
	15	17
Waste water A	—	280
Waste water B	—	25
	5	31
Sea-water	—	ND
	5	5
	15	15
	25	25

ND: not detected.

Effect of phase-volume ratio on the sensitivity. To examine the effect of the volume ratio on the sensitivity of the atomic-absorption procedure, 10 ml of MIBK were taken for the extraction from various volumes of the aqueous phase, each containing 2 μg of selenium(IV). With increasing aqueous–organic phase-volume ratio (V_w/V_o 1–10), the measured absorbance values remained almost unchanged. If an appropriate V_w/V_o ratio is chosen according to the selenium content of the samples, it is possible to determine selenium over a wide concentration range from ng/ml to μg/ml levels.

Calibration curve and precision

Under the established optimum conditions, the calibration curve was made with a selenium(IV) standard solution containing a constant amount of Cu(II). Similar results were obtained with a selenium(VI) solution, when the selenium(VI) was reduced to selenium(IV) by hydrogen chloride as described previously.[1] The linearity is good over the range 2–40 ng/ml with an aqueous/organic solvent ratio of 5. The sensitivity for 1% absorption was found to be 0.3 ng/ml. The relative standard deviation was estimated to be *ca.* 2% for 40 ng/ml of selenium(IV) (10 determinations, three injections for each).

Interference study

In the previous paper on selenium determination by the DDTC–CCl_4 extraction system,[1] diverse ions, such as iron(II, III), cobalt(II), nickel(II), copper(II), were found to interfere considerably. In this study, such interferences were minimized by the addition of EDTA, as shown in Table 2.

Matrix interferences were further investigated with wet-ashed biological samples. The absorption peaks were directly proportional to the amount of the wet-ashed meat and fish samples analysed. When different amounts of selenium were added to the solutions made after ashing a series of meat and fish samples, recovery was found to be complete. The absence of significant spectral or chemical interference from the samples confirmed that the extraction method allows the determination of selenium in wet-ashed samples of biological materials to be performed simply through

Table 4. Analytical results for biological materials

Sample*	Se added, *ng/g*	Se found, *ng/g*
Rice A	—	33
Rice B	—	13
Meat (Chicken) A	—	73
Meat (Chicken) B	—	51
	100	155
Meat (Hog) A	—	31
Meat (Hog) B	—	41
	100	137
Egg yolk A	—	72
Egg yolk B	—	60
	100	166
Fish (Mackerel)	—	93
Fish (Flatfish)	—	137
	100	237

*Fresh material.

comparison with similarly extracted aqueous selenium standards.

Applications

Determination of selenium in water. Small amounts of selenium in various types of water can be conveniently determined by this method.

Take an aliquot of sample solution containing not more than 2 μg of selenium in a beaker. Add hydrochloric acid to adjust the acidity of the solution to *ca.* 4*M*. Heat in a boiling water-bath or a steam-bath for 20 min [selenium(VI) is reduced to selenium(IV)]. Cool, and add 5 ml of the EDTA solution and 2 drops of Methyl Orange solution. Neutralize with ammonia solution. Add 2 ml of copper(II) solution and 2 ml of APDC solution. Adjust the pH to *ca.* 4.5 with 5 ml of buffer. Transfer the mixture to a separatory funnel and adjust the volume to *ca.* 50 ml with washings from the beaker. Extract and measure the atomic-absorption as in the general procedure described above. Prepare a calibration curve by extracting selenium(IV) from standard solutions. Some results are presented in Table 3 together with those of a recovery test on the samples. According to the method proposed here, ng/ml levels of selenium in waste water or seawater can be determined satisfactorily.

Determination of selenium in biological materials. Biological materials are wet-ashed in Kjeldahl flasks and small amounts of selenium in samples can be determined by the method proposed here.

Put 5–10 g of wet biological sample, followed by 10 ml of conc. nitric acid and 5 ml of conc. sulphuric acid, in the flask. Place each flask on a copper heating-plate. Warm slightly and discontinue heating if foaming becomes excessive. When the reaction has quietened, heat the flask cautiously and rotate it occasionally to prevent caking of sample. Maintain oxidizing conditions in the flask at all times during the digestion by cautiously adding small amounts of nitric acid whenever the mixture turns brown or darkens. Continue the digestion until organic matter is destroyed and sulphur trioxide fumes are copiously evolved. The final solution should be colourless, or at most a light straw colour. Cool, and dilute with water to 50 ml in a standard flask. Take an aliquot of the sample solution (containing not more than 2 μg of selenium) and continue according to the procedure described above for water analysis.

Some results for biological samples are presented in Table 4 together with those of a recovery test on the samples.

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GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF ORGANOSULPHUR COMPOUNDS AND OTHER LABILE MOLECULES

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Summary—A survey is made of investigations into the adsorption of polar and labile substances on surfaces within gas chromatographic equipment and of the methods which have been developed to minimize it. A description is given of a gas chromatograph—mass spectrometer in which such methods have been applied for the analysis of trace quantities of sulphur compounds.

Gas chromatography (GC) combines unexcelled separation capability with facile compound detection. The technique in one form or another has become the premier method used by analytical chemists for the detection and determination of traces of organic and organometallic compounds. The recent analytical literature shows that despite the rise of HPLC, gas chromatography still accounts for approximately 50% of work published in trace organic analytical chemistry. The choice of column materials for a particular analysis becomes extremely important, not only for the complete separation of all the components in the sample, but also to reduce losses due to adsorption. This has been a source of concern for many years in the chromatographic analysis of highly reactive and polar molecules, such as organosulphur compounds, which are highly sensitive to adsorption and catalysis. A considerable number of papers have stressed the need to use inert materials in the gas chromatographic determination of these substances at trace levels, since any interaction with either the column walls or the support material results in serious errors in quantification. It has been suggested that these losses are due to processes more complicated than simple adsorption effects. For instance, catalysis by the metal surface can lead to structural changes in the component. In any event, these effects need to be minimized so that the separation depends entirely upon the partition process.

THE PROBLEMS OF ADSORPTION

Hodges and Matson[1] reported that an inert support was required to reduce tailing in the chromatographic analysis of sulphur-containing gases. Adsorption problems were also encountered by other workers in the same field.[2–4] Losses of sulphur compounds seem to occur when the vapours come into contact with metal surfaces. Harrison and Coyne[5] believed that the non-reproducibility encountered in the detection of thiols and hydrogen sulphide in beer or water headspace samples at the 0.01–0.1 ppm level, when stainless-steel, nylon or glass columns were used, was due to an interaction with the metal capillary tube connecting the column exit to the flame photometric detector (FPD). Freedman[6] noted the poor recovery of low molecular-weight alkyl thiols and sulphides at low concentrations from stainless-steel capillary columns. He believed that it could be improved by using an all-glass system. A loss of hydrogen sulphide was reported in the quantitative determination of sulphur compounds in the gas phase of cigarette smoke,[7] and this was attributed to the use of stainless-steel tubing in the lines. By replacing them with Teflon tubing an improvement was obtained. Vitenberg *et al.*[8] also advocated the replacement of metal surfaces by glass or Teflon in the determination of sulphur compounds in industrial effluents. Adsorption problems are not confined solely to the chromatographic system, but can occur with the vessels used to prepare standard samples. Thompson[9] used permeation tubes to eliminate such effects. A copper evaporator used in the gas-chromatographic determination of heterocyclic compounds[10] was found to cause transformations to occur within some of the molecules before they entered the column. This was related to the presence of a tertiary butyl group at a sulphide sulphur bond with the thiophene ring, or the sulphide sulphur bond with this ring through a methylene group. Again, in the determination of organosulphur species extracted from marine sediment,[11] at least one sulphur compound was believed to have undergone a photoreaction.

These phenomena have been observed with other types of molecules. For instance, the calibration curves for chelates of cerium-group lanthanides[12] give an intercept of 0.18 μg at zero response. This is due to a loss of sample to the column material. Cadmium, lead and cobalt chelates of monothioacetylacetone[13] showed considerable decomposition during separ-

ation on a chromatographic column and none was eluted unchanged. The nickel chelate gave a barely discernible peak when less than 0.3 μg was injected. A similar adsorption pattern was observed for the other chelates, especially those of zinc and cobalt, whilst lead thioacetylacetonate underwent thermal decomposition. Utsunomiya[14] investigated the chromatographic behaviour of rare-earth chelates of isobutyrylpivalylmethane and reported that the peak height for the terbium chelate decreased on repeated injection of sample. The terbium chelate was not eluted when pure solvent (benzene) was injected into the column, but a peak corresponding to terbium appeared when isobutyrylpivalylmethane (IBPM) was used. This peak also decreased in height on repeated injection of IBPM and further injection of terbium samples produced a reasonable peak. It was thought that sample decomposition was the reason for these peculiar results, and that the terbium might be oxidized to Tb(IV). This effect was not observed in the case of the other rare-earth chelates. During a week's storage of standard solutions of chromium(III) hexafluoroacetylacetonate [$Cr(hfa)_3$] in 2-dram vials with polyethylene-lined caps, the concentration of $Cr(hfa)_3$ decreased.[15] The loss of $Cr(hfa)_3$ when contained in borosilicate glass flasks for the same period was negligible. Stainless-steel columns containing silicone grease and Apiezon M supported on firebrick have been shown to adsorb completely tin(IV) chloride, titanium(IV) chloride and iron(III) chloride. This is thought to be due to a reaction between the chlorides, the greases and the walls of the column.[16]

Isoprene, acetaldehyde and acrolein have been identified as constituents in the gas phase of cigarette smoke.[17] All of the isoprene and acrolein, but only 80% of the acetaldehyde was recovered after adsorption and desorption on Tenax, followed by gas chromatography. Srinivasan *et al.*[18] reported an assay for pentadecylcatechols in poison ivy extracts by gas chromatography. When pure pentadecylcatechol was injected into the columns (glass, with 1% SE-30 or 1% NGS on Chromosorb W) a broad peak was observed. A second injection produced a sharp peak superimposed on the first peak. Subsequent injections showed that the sharp peak increased in height at the expense of the broad peak, which finally disappeared. If the compound was injected after a period of 1 hr, the broad peak was once more observed, but disappeared again with further injections. It is believed that pentadecylcatechol reacted with the support or liquid phase. This problem was overcome by preparation of a less reactive derivative. When the poison ivy extract was injected into the column, no peak corresponding to pentadecylcatechol was seen until the column was first saturated by injection of that compound.

These problems are also encountered when a gas chromatograph is linked with a mass spectrometer (GC–MS). Bruner *et al.* used a Watson–Biemann separator and compared the results with those obtained in a direct coupling method, with cholesterol as the test material.[19] The signal on the total ion-current monitor was lost when the separator was installed. A reversal of the ion intensities showed that adsorption and thermal decomposition had occurred. It was reasoned therefore that direct coupling was needed when polar, high-boiling and thermally unstable compounds were being handled. A palladium separator used by Lovelock *et al.*[20] produced similar results. The structure of certain compounds could be changed by catalysis at the metal surface. Honour *et al.*[21] found that the steroids present in the urine of patients with hypertension were degraded in the GC–MS system used for analysis. Modifications of the all-glass interface were necessary to overcome the problem.

EVALUATION OF COLUMN MATERIALS

Large numbers of reports have been published in recent years concerning the adsorptive properties of different column materials and methods for their deactivation. It appears that the adsorption phenomenon can be related to either the solid support or the column material used in a particular analysis. Grob[22] studied the effects of different metals (gold, gold/platinum, platinum and platinum/iridium) used for the tubing connecting a capillary column with a flame-ionization detector (FID). A glass capillary column was used with OV-1, SE-52, OS-124, Ucon HB 5100, Emulphor ON 870 (E), PEG 20,000 or Silar 10C as stationary phase. No permanent inactivation of the surface was obtained, in fact he observed intense adsorption and only transient inactivation could be produced, as in the case of the PEG column. To achieve low activity on the surface, the inactivating agent needed to be applied constantly. The most effective agent was found to be the carrier leaving the column containing Ucon HB 5100. When the platinum tubing was replaced by inactivated glass, practically permenent inactivity was obtained, which seemed to be independent of the column coating. For these reasons Grob recommended the use of glass instead of metal in GC–MS systems. 2-Mercaptobenzothiazole (2-MBT) and benzotriazole (BTA) were assessed as reagents for the deactivation of a stainless-steel transfer line between a SCOT capillary column and a flame photometric detector.[23] The line was first conditioned with hydrogen sulphide, 2-MBT or BTA and any adsorption effects were detected by passing butanethiol through the column. The detector gave no response when an untreated line was used. Hydrogen sulphide was found to be partially effective as a deactivating agent, but the effect decreased with time. 2-MBT gave a better performance but was found to bleed off eventually and was not suitable for temperature programming. The detector response was highest when the line was coated with BTA but again the effect decreased, although over a longer period of time. Welsch *et al.*[24] tested a variety of silaning agents for the deactivation of glass capillary columns,

viz. dimethyldichlorosilane (DMCS), trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS). Three different column coatings were used, squalane, OV-1 and Ucon 550 LB. Silaning was accomplished by pushing a plug of the reagent through the column, sealing the ends and applying heat. It was found that deactivation by heating with HMDS at 300° for about 20 hr produced the best results. Silanation of glass capillary columns has received further attention from Novotný and Bartle.[25] They saturated glass capillary columns with the vapour of DMCS, TMCS, HMDS, allyltrichlorosilane (ATS) or phenyltrichlorosilane (PTS) at 150° for 48 hr. The columns were then coated with 10% SF-96 and dinonyl phthalate. They found that silanation with HMDS and TMCS was only effective for non-polar stationary phases and a negative effect was observed for polar phases. Results with the dinonyl phthalate stationary phase were best if ATS-treated columns were exposed to oxygen at high temperatures.

Adsorption on aluminium tubing[26] manifests itself by causing very long tailing peaks, double peaks due to dehydration of alcohols, or loss of sensitivity due to irreversible adsorption. Tailing was found to be more common with old tubing, probably because of oxide layer formation on the inner wall. In order to reduce this tailing effect, columns of aluminium and glass were packed with 80/100 mesh Porapak Q, or 10% SE-30 on 80/100 mesh Celite 560 AW, treated with DMCS and tested by measuring the peak shapes obtained for n-butanol, that obtained with n-butanol on Porapak Q in glass columns being used as reference ("zero" adsorption). Initially the inner walls were coated with polar tailing-reducers, such as Gas-Quat L, Antarox CO 880, sodium laurate and FFAP. These gave a small improvement if the original tailing was not serious, but were ineffective if serious adsorption was encountered. Removal of the oxide layer with methanolic hydrogen chloride made tailing much worse. Good peak shape and detection limits were obtained by treating a packed column with trifluoroacetylacetone (TFAA). This treatment appeared permanent (up to a period of 6 months). Similar results were given by hexafluoroacetylacetone (HFAA).

The materials used for columns and connections in the gas chromatography of sensitive, high molecular-weight compounds were compared by Arnold and Fales.[27] They compared copper, aluminium and stainless steel with silaned glass as standard. Test solutions of codeine, adrenosterone, phenazocine, cholestane, cholesterol-3-methyl ether, cholesterol and narcotine, were separated on 1% SE-39 on siliconised Gas Chrom P, 100/120 mesh. Glass was found to be only slightly superior to aluminium or stainless steel. Basic substances (codeine, phenazocine) were almost completely adsorbed and the other compounds showed a diminished response when a copper system was used. The peaks reappeared to some extent upon subsequent injections. It was noted that the Teflon connections used on the glass column caused peaks to tail and some compounds were lost at elevated temperatures. Teflon was later compared with stainless steel for the determination of sulphur-containing pollutants such as hydrogen sulphide.[28] Teflon was found to be adsorbent, in contradiction of the common assumption. Different types of Teflon, *e.g.*, FEP and TFE, were also investigated. In studies carried out on the chromatographic analysis of Kraft Mill sulphides,[29] three types of glass container were examined for adsorptive properties and compared with "Scotchpak" bags. It was found that the latter were unsuitable as containers and therefore glass was used (which showed little adsorption). Greased glass stopcocks caused losses of methanethiol when compared with Teflon-clad stopcocks, whilst neoprene and silicone-rubber stoppers showed no loss over 48 hr. Farwell *et al.*[30] noted the problems encountered when trying to determine sulphur-containing gases with an all-glass cryogenic enrichment and capillary gas-chromatographic system. The sample was collected in a glass U-tube (containing glass beads) cooled in liquid oxygen. The sample was then transferred (by heating) to a glass capillary trap before entering the gas-chromatographic column. Four types of glass were evaluated for use as the U-tube *viz.*, soda-lime, borosilicate, conventional quartz and clear fused quartz. It was found that untreated glass, Pyrex and conventional quartz showed a minimum of adsorption. Pyrex was chosen since the U-tube made with it compared well with a Teflon tube packed with Teflon (FEP)* (40/60 mesh), but this unfortunately produced non-quantitative recoveries and possessed memory effects. It was reported that a surface-deactivated WCOT column was required to minimize peak tailing and to achieve good separation of polar compounds. For this reason all glass parts were deactivated, with a variety of chemicals. Glass capillary columns were not recommended for the determination of sulphur dioxide or dimethyl sulphoxide, because of bad tailing and peak broadening. Some conditioning of the column was required for hydrogen sulphide, carbon oxysulphide (COS), carbon disulphide, methanethiol and dimethyl disulphide. The most efficient deactivating agent proved to be a combination of polysiloxane and methyl silicone (SE-30 or SP-2100).

Uden and Jenkins[31] investigated the adsorption and displacement effects of aluminium(III), chromium(III) and iron(III) β-diketonates, using different types of column materials, liquid phases and supports. Peak broadening and tailing were more evident for iron than for chromium, especially before the column had been conditioned by successive injections. Aluminium showed less tailing than iron, but more than chromium. Peak shapes were improved by repeatedly silaning the column. Firebrick and Phase Sep P showed more tailing and adsorption than Chromosorb W or Phase Sep N. Adsorption of all the chelates was much reduced by using a Teflon support.

* FEP = fluorinated ethylene propylene co-polymer.

Severe adsorption occurred with Carbowax 20M and DEGA as liquid phases. Apiezon L and M were found to be better than SE-30. The nature of the column material, copper, glass or stainless steel, had little effect. One chelate which had been adsorbed could later be displaced by another, *e.g.*, chelates of aluminium and iron, and this effect was not changed by silanation of the column or the use of a Teflon support. However, the stationary phase had considerable effect, the displacement being more apparent with polar liquids. A strange phenomenon was observed in the chromatography of iron compounds in that a portion of the chelate was gradually eluted before the rest of the sample. Substantial column interactions have been observed when glass or Teflon columns and deactivated supports are used for the chromatography of β-diketonates, except for those of chromium(III), aluminium(III), beryllium(II) and a few others.[32] These interactions manifest themselves in the form of asymmetrical or spurious peaks and sample losses. Quantitative analysis below the μg level becomes difficult. Nickel heptafluorobutanoylpivalylmethanate showed considerable tailing on a Teflon column containing 30% silicone gum on 60/85 mesh Universal B. Chelate samples less than 0.2 μg were not eluted. Substitution of stainless steel or copper for Teflon or glass resulted in almost complete loss of the chelate. Other more polar stationary phases appeared to adsorb and/or decompose these complexes. A stainless-steel column containing 3% QF-1 on Varaport 30 (80/100 mesh) showed no response for $Ni(ATFP)_2$ when the sample level was less than 5 ng [H(ATFP) = 4-amino-1,1,1-trifluoropent-3-en-2-one]. The detection limit rose to 20 ng when the column was inserted into a different gas chromatograph containing a steel injection port. Samples of less than 0.5 μg of $Cu(ATFP)_2$ were not detected, owing to interaction with the column. Koppe and Adams[33] evaluated a large number of support materials and stationary phases on stainless-steel columns for the determination of gaseous sulphur compounds below the ppm level. With empty columns losses occurred. Recoveries were quoted for hydrogen sulphide, sulphur dioxide and methanethiol from glass, stainless-steel and Teflon columns. No sulphur compounds were recovered when aluminium tubing was used, and stainless steel was chosen as the column material. Hydrogen sulphide and sulphur dioxide were determined in an inert gas at the 10–1000 ppm level, with a variety of column materials.[34] Reproducible results could be obtained only by reducing contact between the effluent and the metal surfaces and by a general use of Teflon. Adsorption problems were encountered and it was found necessary to condition columns by repeated injections of the sample to obtain a uniform response. Figures 1 and 2 illustrate this point, for two different colums. Some adsorption was still present, but it was much less pronounced when the stainless-steel column was replaced by Teflon (with which the response was 8 times as great).

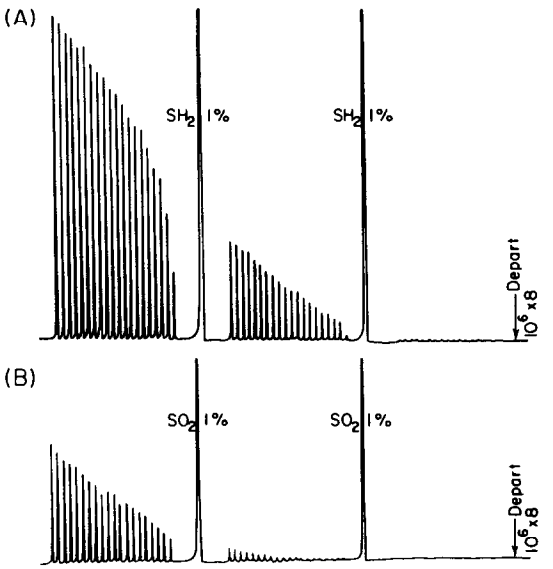

Fig. 1. (A) Successive injections of H_2S (1000 ppm) at 120°.[34] Column: stainless steel 1 m × 1/8 in. with 20% Carbowax 400 on Diatoport S. (B) Successive injections of SO_2 (1000 ppm) at 120°.[34] (Reproduced by kind permission of the author.)

When the Diatoport S support was replaced by Teflon 6, a great improvement was observed in the case of sulphur dioxide, but little change was noticed for hydrogen sulphide. The adsorption of the latter practically disappeared when dinonyl phthalate was used instead of Carbowax 400 as the stationary phase, but the reverse took place with sulphur dioxide, there being a reduction in peak height after the first injection.

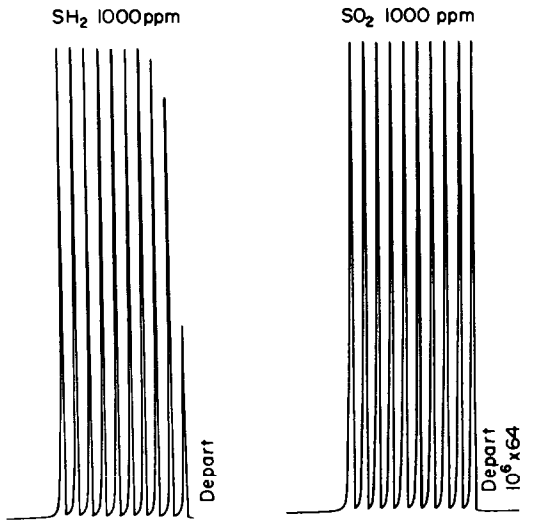

Fig. 2. Successive injections of H_2S and SO_2 (1000 ppm) at 120°.[34] Column: Teflon 1 m × 1/8 in. with 20% Carbowax 400 on Teflon 6. (Reproduced by kind permission of the author.)

Sokolov *et al.*[35] also found it necessary to condition the column with the compound under study, *e.g.*, copper trifluoroacetylacetonate [$Cu(tfa)_2$]. Glass or Teflon columns were used with a variety of liquid phases and supports. The first sample of $Cu(tfa)_2$ injected on Chromaton NAW and Polychrom-1 coated with SE-54 was completely adsorbed. Subsequent injections resulted in gradual distribution of the adsorbed compound along the length of the column. Significant adsorption was caused by the glass-wool plugs (25–30% of the total amount adsorbed) compared with 10–12% on PTFE wool. Adsorption was also found to increase with increase in the amount of liquid phase used. Little difference was found between silaned and non-silaned Chromaton but a large increase in adsorption was found when Polychrom-1 was used. An increase in temperature caused a decrease in adsorption, however.

Non-ideal column behaviour has been reported for metal β-diketonates. The peak shape is usually asymmetric and the column HETP is lower than that for organic compounds of comparable volatility. A mixture of liquid phases and supports was suggested for the determination of mixed-ligand complexes of lanthanides.[36] Dexsil required the least loading by successive injections of the terbium complex. With QF-1 and SE-30, but not Dexsil 300, there was displacement of europium by terbium. Glass columns were then evaluated after silanation to various degrees. All the silaned columns showed unsatisfactory chromatographic behaviour. Spurious peaks and shoulders occurred when QF-1 and SE-30 were used, possibly owing to chelate decomposition and exchange of the ligand.

A chromatographic study of several volatile metal halides on different stationary phases (n-octadecane, squalane, Apiezon T, silicone oil and paraffin) showed that tin(IV) chloride and titanium(IV) chloride were completely adsorbed on Apiezon L. Branched alkanes, such as Apiezon grease and silicone oil, led to reactions on the column, therefore normal alkanes were prescribed. Kusy[38] reported the separation of polar and non-polar compounds on columns containing various stationary phases. He noted that adsorption took place, especially with polar compounds. Substances able to form hydrogen bonds were believed to undergo adsorption, with the strength of the bond dependent on the structure of the molecule. He found evidence of irreversible adsorption on the support (Chromosorb P), but this decreased with increase in column loading.

The contribution of the support materials to adsorption phenomena has received a great deal of attention. Ettre[39] investigated firebrick, Chromosorb, Chromosorb W, Celite and Teflon for the separation of polar and non-polar substances. In accordance with popular opinion he reported that Teflon should only be used for highly polar samples. Another study compared Chromosorb W, Firebrick P, Carborundum, Fluoropak, glass beads, nichrome beads and stainless-steel beads,[40] for a mixture of nine ketones. Adsorption decreased, especially for polar compounds, when the supports were silaned, except for Fluoropak, which showed no adsorption properties anyway. Ottenstein[41] has discussed diatomite and non-diatomite supports and methods for their deactivation. Sze *et al.*[42] realized the need to eliminate adsorption on the solid support in their separation of lower aliphatic amines. Potassium hydroxide, tetrahydroxyethylethylenediamine (THEED) and tetraethylenepentamine (TEP) were used as deactivating agents for Chromosorb W 60/80 mesh. Potassium hydroxide (2%), when used with 15% Carbowax 400 or 1540, eliminated tailing. THEED showed no deactivation, whereas TEP improved tailing. A combination of these two reagents was eventually decided upon for the best separation. The trifluoroacetylacetonates of chromium(III) and ruthenium(III) have been separated on a glass column containing squalane or Apiezon L on 80/100 mesh Chromosorb W and DMCS.[43] The chelates reacted with the uncoated unsilaned support and no peaks were observed unless the material was silaned. Figure 3 shows the chromatograms for $Cr(tfa)_3$ at various stages of silanation. No further improvement was obtained after 500 μl of dimethyldichlorosilane (DMCS) had been added to the support. At low stationary-phase loadings, some adsorption was seen even with silanation. It was

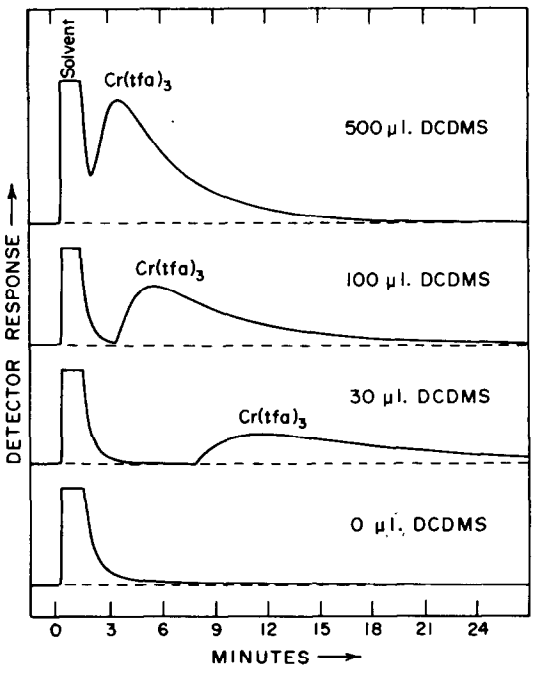

Fig. 3. Chromatograms for $Cr(tfa)_3$ at various stages of silanation.[43] Column: 3.4 g of Chromosorb W (no liquid loading), at 120°; Sample: 30 μg of $Cr(tfa)_3$ in 3 μl of solution. Carrier-gas velocity 4.0 cm/sec. (Reproduced from *Journal of Gas Chromatography* by permission of Preston Publications Inc.)

stated that highly loaded columns should be used to minimize adsorption with these support materials. Acid-washed Chromosorb W, alone or silaned with DMCS, and containing 6% OV-101 or 6% DC-200 was evaluated for column performance with five organophosphorus compounds.[44] The conditioned columns were treated with Carbowax 20M vapour and ng amounts of material were injected into the column. Only ronnel exhibited no adsorption on silaned Chromosorb with OV-101. Improvements were achieved after Carbowax treatment, as shown by the azinphosmethyl oxygen derivative which could not be detected on either column (AW or DMCS) before deactivation. It was noted that very gradual bleeding of the Carbowax occurred with time. Chromosorb 102 gives tailing of the peaks observed with amines, reported by Hertl and Neumann,[45] who ascribed the problem to unreacted vinyl groups behaving as active sites on the support material. They treated the support with hydrofluoric acid, followed by a coating of 2% Carbowax 20M and found that less interaction was observed with the Carbowax-treated support, *i.e.*, the peaks were more symmetrical. It was conjectured that the Carbowax blocked the active sites by covering the support surface, while hydrofluoric acid was supposed to react with the vinyl groups. Suprynowicz *et al.*[46] reported that silaning agents react with active sites to produce trimethylsilyl groups. They illustrated this point by using pure and silaned Diatomite D covered with 1%, 2% and 5% dinonyl phthalate. Silanation decreased the adsorptive nature of the support. In many of the papers mentioned in this article, Carbowax 20M has been used as a deactivating agent. Its performance in this role was evaluated with Celite 545 as support and 1%, 3% and 10% Apiezon L and OV-210 as stationary phases.[47] Bare Celite and coated Celite columns were run side by side, a valve being used to switch flows to an electron-capture detector (ECD). The difference became less pronounced as the load or polarity of the liquid phase increased. With bare Celite, no peaks were observed for the first injections. On subsequent injections the peaks obtained were still smaller than those obtained with the modified Celite.

METHODS FOR DEACTIVATION OF CHROMATOGRAPHIC MATERIALS

Schieke and Pretorius[48] recently described several different methods of deactivating whisker-walled open-tubular glass columns. The whiskers increase the surface area but are highly active, causing excessive tailing, and therefore need to be removed. Silanation was attempted with a solution of DMCS in toluene, which was passed through the column with a stream of dry nitrogen. A second column was filled with HMDS and TMCS vapours and sealed at the ends before being heated at 200° for 48 hr. The vapour was also heated at 200° for 24 hr. Adsorption of surface-active agents, such as benzyltriphenylphosphonium chloride, was tried. Several materials were chosen as reagents for surface carbonization. Dichloromethane vapour was sealed in a column and heated at 550° for 30–45 min; acetylene was passed through the column, the ends were sealed and the acetylene pyrolysed at 550° for the same length of time; n-hexane was injected until 10% of the column was filled and the heating process was repeated. The application of non-extractable polymer layers was investigated by saturating a column for 3–6 hr with a 2% solution of Carbowax 20M in dichloromethane. After flushing with dry nitrogen, the ends were sealed and the column was heated at 280° for 24 hr. A mixture of polar and non-polar compounds was injected into each prepared column and the amount of tailing was calculated. The most effective deactivation involved the passage of HMDS and TMCS vapours through the column at 200° for 24 hr.

Column conditioning

There is some controversy regarding the "carrier effect" observed in GC–MS systems. This phenomenon was recently discussed by Blazer and Chait.[49] They reported that it was advisable to inject a large quantity of the carrier substance (a compound which the adsorbing system is unable to distinguish from the compound of interest,[50] usually the same compound, but containing a stable heavy isotope) either simultaneously with the sample, or before the analysis. This covers any active sites in the column and connections between the GC and MS. This effect is only useful for selected ion monitoring analysis of small quantities. The need to coat gas chromatographic columns before analysis has received special attention in many cases where highly reactive or polar compounds are used. For instance, Gumbmann and Burr[51] reported that the initial response to sulphur compounds extracted from potatoes was low and erratic on three different columns, but improved with repeated use of the columns. Two reports[52, 53] concerned with the separation of a mixture of gases on two columns connected to a thermal conductivity detector (TCD), both stated that the systems needed to be conditioned in the case of sulphur dioxide.

The determination of elemental sulphur with an ECD and an FPD[54] required repeated sulphur injections on glass columns containing three different liquid phases before reproducible results could be obtained. Black *et al.*[55] found it necessary to condition a Teflon column packed with Supelpak S by using high concentrations (five 60-ng/ml injections) of hydrogen sulphide and sulphur dioxide. Devonald *et al.*[56] minimized the adsorption of sulphur-containing species on flasks by pretreating the flasks with sulphur vapour. They found that sulphur dioxide and dimethyl disulphide were adsorbed onto the syringes used. This effect has been observed for other types of molecules, such as imidan and imidoxon, which are phosphorus-containing pesticides.[57] The glass column used for this particular determination contained 10%

DC-200 on Gas Chrom Q 50/100 mesh and needed to be conditioned by repeated injections. The same authors[58] encountered similar problems in the analysis of 138 pesticides and their metabolites with glass columns containing a variety of stationary phases on Gas Chrom Q 80/100 mesh.

An all-glass system was utilized by Juvet and Durbin[59] to reduce reactions with metal chelates to a minimum. They found that the columns used needed to be conditioned, especially for iron(III) hexafluoroacetylacetonate. Chelates of chromium and aluminium (acetylacetonates, trifluoroacetylacetonates and hexafluoroacetylacetonates) were separated on a stainless-steel column containing 20% Dow Corning Silicone Fluid 710R on Gas Chrom Z.[60] At least 6 μl-injections containing about 1 mg/ml were necessary to condition the column. Gallium, aluminium, indium and beryllium trifluoroacetylacetonates have also been investigated.[61] It was found necessary to condition a silaned glass column containing silaned glass microbeads as support, by successive injections of the gallium and indium complexes. Brunnée *et al.*[62] tested different types of material as connection lines between a GC and an MS. They found that the surface was deactivated by the sample itself, which in this case was cholesterol.

Silanation

Glass columns have been advocated for the chromatographic analysis of drugs, pesticides and other compounds that undergo thermal decomposition in metal columns. However, glass itself must be pretreated. A solution of DMCS (5%) in toluene was suggested by Bach[63] as a useful silaning agent which should react with and block any active sites. A solution of HMDS was used by Dewar and Maier[64] to deactivate glass beads used as a solid support for squalane. Novotńy and Tesařik[65] used a combination of HMDS and TMCS to silane glass capillary columns after they had been internally etched with a gaseous mixture of hydrogen chloride and hydrogen fluoride. Several stationary phases were then applied and the separation efficiency of silaned and unsilaned columns was determined. The silaned surface showed a favourable effect only for non-polar stationary phases, whereas a strongly negative effect was observed for polar phases. It is now common practice to treat columns and column packings with silaning agents. An example is the determination of trace mercaptans and sulphides in natural gas[66] with stainless-steel columns treated with Siliclad and packed with 5% polyphenyl ether (PPE) on acid-washed Chromosorb G, 80/100 mesh, treated with DMCS. Stainless steel was preferred to Teflon for the columns and a glass sample loop gave a greater response than either Teflon or stainless steel. A Siliclad solution was used by the same author in a later determination of sulphur gases in hydrocarbon streams.[67] There was still some adsorption on one column containing PPE and phosphoric acid on a silaned support. Goode[68] recognized the need to use silaned columns for the determination of sulphur compounds in North Sea natural gas, in preference to conditioning unsilaned columns by repetitive sample injection. A solution of Silyl-8 was placed in the appropriate column and heated at 250° for 12–16 hr. Adsorption losses on treated and untreated aluminium cylinders used for collecting samples were also studied. In any given cylinder, adsorption losses were found to increase with increasing molecular weight of the compound under investigation and to be proportional to the initial concentration, this loss occurring entirely within the first few hours. It is of interest that this author used an aluminium tube to connect the column to the GC detector. Heating tape maintained the temperature of this tube at 300–400°, which was believed to reduce adsorption of sulphur compounds. Nickel columns and connections have been used in a heart-cutting technique in high-resolution gas chromatography applied to the analysis of sulphur compounds in cigarette smoke.[69] Although nickel is now regarded as being as inert as glass, these authors realized the need to silane all metal parts coming into contact with the sample.

A film of SE-30 containing fine particles of silaned silicic acid was deposited on silaned glass capillary columns for the determination of human urinary steroids.[70] The silaning agent, DMCS in toluene, was also used in the precolumn tubing and splitter. This precaution was taken since metal columns destroy many biological samples. No change in the column properties was apparent after 6 months. Glass separators used in a combined GC–MS system can be the cause of sample losses if precautions are not taken. MacLeod and Nagy[71] found it necessary to treat their fritted glass molecular separator *in situ* by injection of bistrimethylsilylacetamide (BSA) through a septum into the transfer lines. The compound was drawn by vacuum into the separator for reaction with surface OH groups. The sensitivity for selected terpenoids increased after treatment with BSA.

Carbowax 20M

Carbowax 20M has proved particularly useful in gas chromatography, not only as a stationary phase, but more recently as a deactivating agent. Schomburg *et al.*[72] found that compounds were adsorbed if glass capillary columns were not treated with Carbowax. This was not true, however, if polar stationary phases were used. Free silanol groups on the glass surface have been suggested to be the cause of active sites.[73] The remedy proposed was to coat the column with Carbowax and heat it under nitrogen. The coating was removed and then reapplied. The coated capillary column showed excellent separation power and long-term stability when used for 2-undecanone and low-boiling aliphatic alcohols. Before treatment the column showed tailing of the alcohol peaks, but after treatment resolution was improved and tailing totally absent. Blomberg[74] also deactivated Pyrex capillary

columns, using a thin layer of non-extractable Carbowax 20M coated with SF-96, for the separation of sulphur compounds.

Miscellaneous liquids

Several other liquid phases have been assessed as deactivating agents. A glass tube packed with 5% PEG 20M on Chromosorb W, AW was inserted into the injection port of a gas chromatograph and connected to a glass capillary column with PTFE tubing.[75] PEG 20M was allowed to bleed through the capillary column overnight. The deactivated column was coated with SE-30 and its performance compared with that of one that had not been deactivated. Dieldrin and endrin (a substance very sensitive to adsorption) were used as the test materials. It was found that endrin was not eluted from the non-deactivated column containing a thin film of SE-30, but decomposed to produce two products. Decomposition was thought to be due to the column wall activity, since this effect was not observed for the deactivated column containing a thin film, or the non-deactivated column with a thick film. Withycombe *et al.*[76] noted the need to replace metal surfaces with glass tubing. However, the sample splitter alone was deactivated by injecting SE-30 and heating to 400°. Heckman *et al.*[77] used 6*M* hydrochloric acid as the deactivating agent for glass capillary columns. The ends were sealed and the column was heated overnight at 100–150°. The presence of benzene on molecular sieve 13X was found to reduce the adsorption capacity of the sieve for thiophene[78] in the determination of low concentrations of sulphur compounds. Averill[79] showed that if the stainless-steel tubing used on a GC–MS system was treated with 2,4-pentanedione, greatly improved peak shapes and lowered detection limits for steroids were obtained.

Gases

The mechanical properties of glass make it preferable to Teflon for chromatographic supports, according to Diez *et al.*[80] The surface activity of glass is due to the presence of silanol (Si–OH) and siloxane (Si–O–Si) groups, which behave as electron donors and acceptors respectively. Glass columns were therefore deactivated by high temperature treatment with a mixture of nitrogen and hydrazoic acid (1:3). Compounds such as ethanol, benzene, methyl ethyl ketone, nitromethane and pyridine were eluted from the treated columns. It was observed that adsorption decreased for those products which did not contain nitrogen atoms, probably because an Si–N–B bond had been formed. Bruner *et al.*[81] conditioned their glass columns by heating in a nitrogen atmosphere and found that no adsorption occurred with sulphur compounds and therefore concluded that there was no need to use PTFE. Bruner *et al.*[82] also found that a glass system gave similar results to PTFE, provided that it was conditioned by passage of dry helium or hydrogen at 130° for 6 hr.

The use of columns at higher temperatures, *e.g.*, 55°, was advocated by Adams *et al.*[83] to minimize surface adsorption of certain compounds, *viz.*, hydrogen sulphide, sulphur-dioxide and methanethiol.

Teflon columns and connectors

Stevens *et al.*[84–86] have found that Teflon columns and supports give superior separation and detection of reactive sulphur compounds. They report the loss of a peak corresponding to sulphur dioxide when Teflon (FEP) lines are replaced by stainless steel. Soft glass and borosilicate glass also show retention of sulphur dioxide at levels below 10 ppm. Teflon (FEP) was found not to give this effect and was therefore adopted as the column material. Further sample–metal interaction was eliminated by fitting the column exit directly into the base of the flame-photometric GC detector. Several packing materials were evaluated and all were found to be unsatisfactory, even after silanation. Powdered Teflon was the only one sufficiently inert to be of use. The same was true for the variety of liquid phases evaluated. A mixture of polyphenyl ether (5-ring) and phosphoric acid was finally chosen. For further experiments on the determination of low concentrations of sulphur compounds, a Teflon (FEP) column containing polyphenyl ether and phosphoric acid on (40/60 mesh) Teflon was used.

Many workers in this field have since adopted the use of Teflon for chromatographic materials. Bruner *et al.*[87] used a Teflon column for the GC determination of sulphur compounds in air. All gas lines, sampling loops and exponential dilution flasks were constructed of Teflon. In this way all adsorption effects were minimized. Baumgardner *et al.*[88] reported the use of a Teflon column, connectors and 3-way valve in the measurement of sulphur compounds with an FPD. A similar column was prepared by Blanchette and Cooper[89] for the determination of hydrogen sulphide and methanethiol in mouth air at ng/ml levels. Teflon was also used as the material for sampling probes and connections. No memory effects were noted with this system. Teflon is now being used whenever adsorption losses need to be minimized. This is shown by its use in the construction of permeation tubes for calibration purposes,[90] and for parts in which the surfaces are contacted by the sample, such as sampling valves, columns and even the wool used to plug the ends of columns.[91]

There seems to be general agreement that there is a need to reduce or minimize effects arising from the interaction of reactive or polar molecules with the column material, stationary phase, support, sample lines and valves. The exact method used seems to vary from one author to another. In fact, some conflicting results are given, which would suggest that a great deal of care must be taken in making such evaluations.

AN APPROACH TO THE STUDY OF ORGANOSULPHUR SPECIES BY AN INERT GC–MS TECHNIQUE

The amount of sulphur present in the atmosphere results largely from natural causes, such as volcanic activity, sea spray and decomposition of organic materials rather than from industrial or man-made emissions from paper mills, fossil combustion and petroleum refining.[92] The latter sources, however, are increasing in importance and are becoming of major concern to environmentalists. Consequently there is a requirement to monitor trace quantities of low molecular-weight air pollutants such as hydrogen sulphide and sulphur dioxide. Attempts to identify the origin of petroleum spills by using the sulphur "fingerprint" is a very recent application.[93–95] Other materials which have been investigated for sulphur content include pesticide residues,[96–98] flavour volatiles,[51,76] beers and wines,[5,99–101] combustion products of tobacco,[4,7,69,102,103] sulphonamides,[104,105] town and natural gas,[66,106–109] marine sediments,[11] coal[110] and petroleum samples.[111–116] This is not intended as a complete list, but merely serves to indicate the various industries relying on sulphur chemistry.

The development of the flame photometric detector by Brody and Chaney[117] made possible the determination of sulphur compounds at trace levels by observation of the blue emission from the S_2 species produced in a hydrogen-rich flame, through a filter having maximum transmittance in the 394 nm region. The selectivity and sensitivity of this technique allowed sulphur compounds to be determined at nanogram and picogram levels with relative ease, compared with previous methods.

A new detector introduced by HNU Systems Inc.[118] is based on the photoionization of the species being eluted and is claimed to be ten times more sensitive to low molecular-weight sulphur compounds than the FPD. It has a wide linear range and since it does not use a flame, a supply of hydrogen and air is not required. A recent development by Photovac Inc.[119] would appear to be capable of detecting compounds at a level at least one order of magnitude lower than that detectable with the HNU system.

Gas chromatography–mass spectrometry couples the most powerful separation technique available to the analytical chemist with what can be referred to as the ultimate detector. Determination of sulphur compounds by this combined technique should attain detection limits at least comparable with GC-FPD, as well as providing structural identification. Two major problems with this system, however, are removal of the carrier gas, and the pressure differences between the GC and the ion-source. For these reasons, enrichment devices or molecular separators are preferred when using packed columns. Three basic types of separator are available,[120] the one of choice here being the semi-permeable membrane separator, developed by Llewellyn and Littlejohn.[121] This incorporates a silicone-rubber membrane selectively permeable to organic compounds and largely impermeable to the carrier gas.

Earlier, mention was made of some of the problems encountered in the analysis of highly reactive and polar compounds and methods were suggested for alleviating the situation. Although Teflon is not completely inert, it does show the least adsorption of sulphur species and for this reason we chose it for use in an inert GC–MS system instead of glass, which would require deactivation, and constant conditioning by repeated sample injection. By replacing with Teflon all surfaces that are contacted by the sample, any adsorption is kept to a minimum. In a recent article, Fujiwara and Ogata[122] showed that hydrogen sulphide reacts with silicon(111) surfaces. The material was exposed to hydrogen sulphide at room temperature and and the amount adsorbed was measured by Auger electron spectroscopy and low-energy electron-loss spectroscopy. It was found that hydrogen sulphide molecules were adsorbed non-dissociatively on silicon surfaces at room temperature. After annealing at 550° dissociation produced desorption of hydrogen and formation of silicon–sulphur covalent bonds. Above 650° the sulphur atoms were desorbed to leave a clean silicon surface. This evidence would seem to indicate that labile sulphur molecules react not only with metal surfaces, but also with those made of glass, and extreme caution should be used in the choice of column materials for any such determination at low concentration levels.

Here we describe an inert system in which the sample contacts only Teflon surfaces from the point of injection to its entry into the ion-source. By use of another detector (FPD) in parallel with the MS, checks can be made on both losses in resolution and decreases in the signal, caused by adsorption.

EXPERIMENTAL

Chemicals

The compounds studied were of reagent grade and were used without further purification.

Gas chromatography

Chromatograms were obtained with a dual-column dual-electrometer Varian Aerograph GC (Model 2740) coupled to an FPD (Tracor Inc., Austin, Texas) mounted at the side of the GC, complete with a 750-V power supply. The response was monitored with a dual-pen Linear Instruments (Model 385) recorder. In this way both FPD (sulphur) and FID (solvent) signals were obtained. The column consisted of a 40 ft × 1/8 in. (outer diameter) Teflon (FEP) tube packed with 40/60 mesh Chromosorb T coated with 12% polyphenyl ether and 0.5% orthophosphoric acid (Chromatographic Specialties, Brockville, Ontario). The column exit was fitted directly into the base of the detector to minimize dead volume and reduce effluent–metal interactions. All gases used (nitrogen, hydrogen, air) were dried with "Gas-dry" filter traps (Chromatographic Specialties). In addition, an oxygen trap ("Oxisorb") was placed on the nitrogen cylinder and a hydrocarbon trap was present on the hydrogen cylinder.

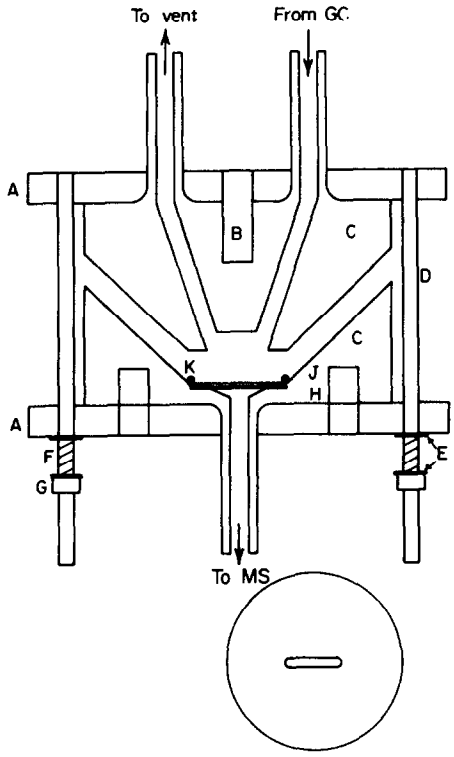

Fig. 4. Teflon membrane molecular separator.

Mass spectrometry

The instrument used was an AEI Model 902, high-resolution (variable between 1000 and 30000) double-focusing mass spectrometer using an electron impact source and coupled to an AEI DS-50 data system. Source temperature 150°; electron energy 70 eV; accelerating voltage 8 kV. Diffusion pumps capable of pumping air through the lines at 2400 l./sec were installed in preparation for chemical ionization.

Separator. The membrane separator (Fig. 4) was constructed completely from Teflon and had an outside diameter of 5 cm. The inside surfaces were tapered at 45° to prevent tearing or creasing of the membrane when the unit was assembled. The inside path was 1 cm long, 0.24 cm wide and 0.25 cm deep. The silicone-rubber membrane (J) (0.1 mil thick, General Electric, Schenectady, New York) was placed over a Teflon-coated stainless-steel support 1.5 cm in diameter (Millipore Ltd., Mississauga, Ontario) covered by a Teflon filter (H) (Millipore Ltd.) and secured with a rubber O-ring (K). Aluminium plates (A) and brass bolts (D) were used to hold the separator halves (C) together after sealing with a high vacuum sealant (Space Environment Labs., Boulder, Colorado). Connections were made to 1/8-in. outside diameter Teflon (FEP) tubing (Chromatographic Specialties) at the GC end and 1/4-in. outside diameter Teflon tubing (Alltech Associates, Arlington Heights, Illinois) at the MS end, with special Teflon unions (Fluoroware, Chaska, MN).

Re-entrant tube. The tube is shown in Fig. 5. A length of 1/4-in. outside diameter Teflon tubing (A) (Alltech Associates) was covered with a double layer of aluminium foil (J) [holding a chromel–alumel thermocouple (K) in place] and glass tape (L) soaked in leak-sealant. Heater wire (M) (nichrome, 28 gauge) was then wrapped round this and covered by another layer of soaked glass tape. The framework consisted of standard 12-mm (C) and 1/4-in. (H) Swagelok fittings (Avon Valve and Fitting Ltd, Scarborough, Ontario) welded to a stainless-steel metal flange (F). Four feedthroughs (G) (Quality Hermetics, Toronto, Ontario) with enamel wire (N) attached were soldered into the flange. Ceramic tubing (P) was used as an insulator on all wires. Pins 2 and 4 were connected to the heater wires and pins 1 and 3 were connected to the thermocouple. A short length of 12-mm outside diameter Pyrex glass tubing (B) which had been internally etched with hydrofluoric acid to produce a wall thickness of 0.05 cm was placed over the end so that the Teflon tubing protruded about 1/4 in. Teflon front and back ferrules (Avon Valve and Fitting Ltd) were used as a seal between the tubing and the stainless-steel framework. Once complete, the re-entrant tube was bolted to the source housing by means of the metal flange, with the Teflon tip touching the ion-source.

Procedure

The system is shown schematically in Fig. 6. Liquid or

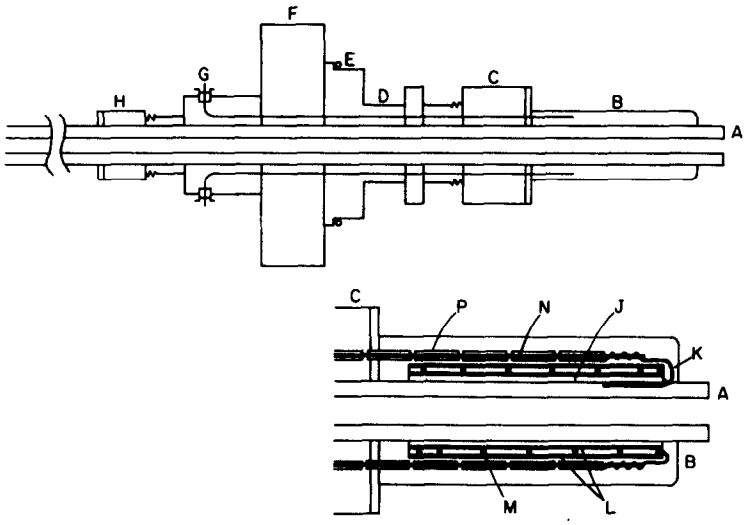

Fig. 5. Teflon re-entrant tube.

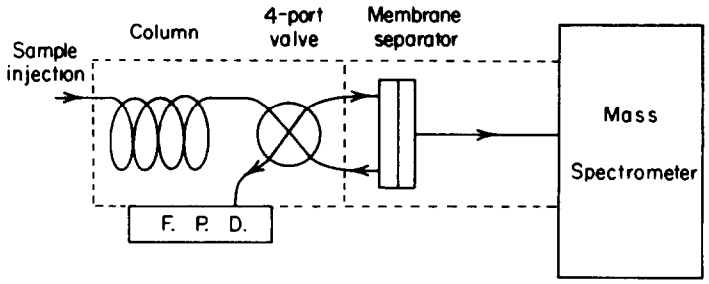

Fig. 6. Schematic diagram of GC–MS arrangement showing the alignment of the 4-port valve.

headspace samples are injected into the GC by syringe. On emerging from the column, the effluent passes into a 4-port Teflon valve (Hamilton 2x valve with 1/8-in. NPT CTFE fittings) situated in the GC detector oven and heated at 120°, whence it can pass either directly into the detector base of the FPD or to the membrane separator. Since no stream splitter is used, samples need to be injected twice to obtain both an FPD recording and a mass spectrum. From the separator, the sample passes through a silicone-rubber membrane and into the ion-source. The carrier gas (nitrogen) and any effluent not entering the MS pass back through the 4-port valve and are detected by the FPD. The separator and lines to the MS are heated in a specially built aluminium oven. All lines and connections that make contact with the sample from the GC inlet to the ion-source are made of Teflon. The connections between the GC and MS are made as short as possible to minimize dead volume. A triple temperature-control unit is built to provide and control heat to the separator, line and re-entrant tube. The temperatures are adjusted manually to those specified, and measured with chromel–alumel thermocouples. Heat is transferred by wrapping all the parts in aluminium foil. Heating tapes (Briscoe Mfg. Co., Columbus, Ohio) are wrapped round the separator and re-entrant line and connected to the triple control unit. The third connection is made to the nichrome heating wire through two of the four feedthroughs.

RESULTS AND DISCUSSION

The detector response and all chromatographic conditions were optimized with a solution containing methanethiol, ethanethiol, n-butanethiol, dimethyl sulphide, diethyl sulphide and dimethyl disulphide in absolute ethanol (100 μg of S per ml). Sample sizes were usually 1 or 10 μl injected into the column. The following conditions were then used for GC and GC-MS experiments: column temperature 120°; injector and FPD temperature 140°; nitrogen carrier gas flow-rate 30 ml/min; hydrogen flow-rate 60 ml/min; air flow-rate 100 ml/min.

All solutions were prepared in Nalgene polypropylene flasks (Canlab, Toronto, Ontario) which had been thoroughly cleaned. The sulphur response was recorded by one recorder pen linked through the electrometer to the photomultiplier tube and the solvent response by a second pen (by measuring the response at a collector-ring housed in the FPD body). The collector ring is sensitive to carbon ions produced during ionization of the effluent in the flame; this system is less sensitive (by a factor of $\sim 10^3$) than a normal FID used in gas chromatography but it is adequate for the measurement of the ethanol concentration. The FPD response was measured as peak area (peak height × width at half-height) and the efficiency of the column was calculated at different carrier-gas flow-rates. There seemed to be an increase in the number of theoretical plates with a decrease in flow-rate, as predicted by the van Deemter equation. The optimum nitrogen flow-rate would correspond, however, to a long analysis time and so a compromise was made between analysis time and efficiency by using a flow-rate of 30 ml/min ($\sim$4000 theoretical plates). Under these conditions a good separation of the six sulphur compounds was achieved, as shown in

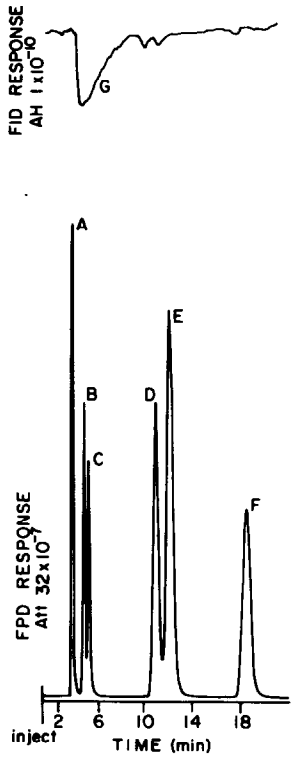

Fig. 7. Chromatogram of organosulphur compounds in ethanol (S 100 μg/ml). Sample size 1 μl. A, Methanethiol; B, ethanethiol; C, dimethyl sulphide; D, diethyl sulphide; E, butanethiol; F, dimethyl disulphide; G, ethanol.

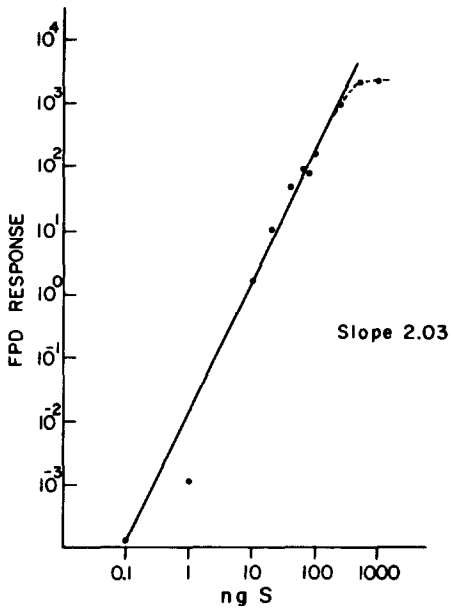

Fig. 8. Log–log plot of FPD response *vs.* concentration of n-butanethiol (as ng of S).

Fig. 7. Sharp, narrow peaks were obtained in a reasonable time with a minimum of tailing.

The response of the detector was examined by injecting sample sizes in the range 1000–0.1 ng of S. A log–log plot of response *vs.* concentration (Fig. 8) was linear in the range 250–0.1 ng for all six species examined and the slope varied from 1.69 for methanethiol to 2.03 for n-butanethiol. These values were obtained by a least-squares method. This is in agreement with previous reports that the FPD response varies as the square of concentration[90,123,124] and that a log–log plot has a slope of approximately 2.

The lowest limit of detection, defined as a signal equal to twice the standard deviation of the noise, was found to correspond to about 10 pg of S. Peaks corresponding to 100 pg of S were easily seen for all six compounds (Fig. 9). These values seem to be better by a factor of 10–1000 than results previously reported. Table 1 shows a list of GC–FPD or GC–MS determinations for inorganic and organic sulphur species in a variety of matrices, together with the columns used and detection limits. In most cases a Tracor FPD was utilized, except in the case of reference 135, where a Varian dual flame detector was used. In less than a quarter of the reports listed is the detection limit in the picogram range. Most authors in these cases used some method to deactivate the column so that losses would not be experienced when handling small quantities of material. When this step was omitted, the detection limit rose considerably. This paper confirms that by careful choice of the chromatographic materials and elimination of dead volumes, labile species can be determined at extremely low concentration levels.

Some preliminary work was carried out with the separator connected to a vacuum system to simulate an MS. All six sulphur species were injected into the GC and the fraction passing through the membrane was determined. These experiments served to emphasize two aspects associated with this system. First, the detector flame was not extinguished on rotation of the 4-way valve to divert the effluent flow either to the separator or to the FPD. Secondly, no loss in resolution was produced by the 1/8-in. Teflon tubing used in the connections between the valve and FPD. In fact, the peaks remained sharp, with no perceptible broadening.

The separator and re-entrant tube were attached to the MS-902. A pressure of about 1×10^{-6} mmHg was maintained in the source. This occasionally rose to about 4×10^{-6} mmHg when the separator and re-entrant were heated to above 100°. The yield for methanethiol, butanethiol, diethyl sulphide and dimethyl disulphide was determined at different separator temperatures and different carrier-gas flow-rates. Only four sulphur species were used for these experiments, because the solvent peak (ethanol) was not permitted to overlap with any peaks due to

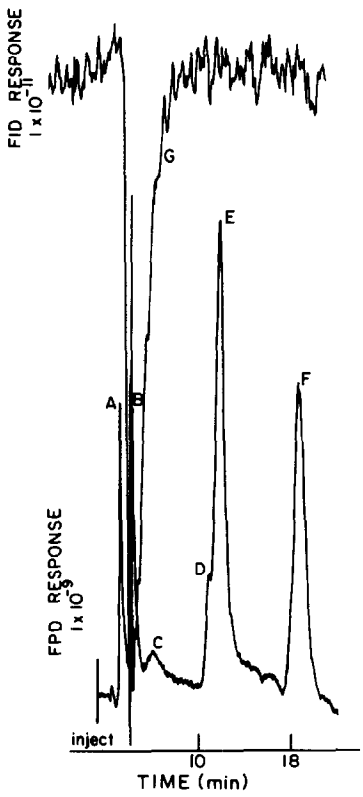

Fig. 9. Chromatogram of organosulphur compounds in ethanol (S 0.1 μg/ml). Sample size 1 μl. Peaks as shown in Fig. 7.

Table 1. Detection limits for sulphur determination by GC–FPD and GC–MS

Column	Compounds*	Limit of detection	Reference
GC–FPD			
(1) 34′ × 0.085″ I.D. Teflon (FEP), 12% PPE, 0.5% H_3PO_4, on Chromosorb T 40/60	SO_2, H_2S, CS_2, COS, SF_6, CH_3SH, $(CH_3)_2S_2$, C_2H_5SH,	1–4 ng	126
(2) 4.6′ × 0.085″ I.D. Teflon (FEP), Carbopack B-HT-100	$(CH_3)_2S$, $(C_2H_5)_2S$,		
(3) 6′ × 0.085″ I.D. Teflon (FEP), Chromosil 310	$(C_2H_5)_2S_2$, $CH_3SC_2H_5$,		
(4) 1′ × 0.085″ I.D. Teflon (FEP), Deactigel 120–140	1-Pr, 1-Bu, 2-Bu		
(1) 6 m × 2 mm I.D. Teflon (FEP), 10% Triton X-305 ± H_3PO_4	COS, H_2S, SO_2, CH_3SH, C_2H_5SH, $(CH_3)_2S$, CS_2,	5–20 ng	127
(2) 12 m × 2 mm I.D. Teflon (FEP), 0.5% Triton X-305 on Chromosorb G (AW, DMCS) 70/80	$(C_2H_5)_2S_2$, $(CH_3)_2S_2$, $CH_3SC_2H_5$, $(CH_3)_2S_3$		
80 m × 0.28 mm I.D. glass capillary coated with FFAP	H_2S, SO_2, CS_2, CH_3SH, C_2H_5SH, $(CH_3)_2S$, $(C_2H_5)_2S$, $(CH_3)_2S_2$, thiophen, TMS, 2-Pr, 1-Pr, 2-Bu, 1-Bu	Trace amounts	128
20 m × 0.25 mm I.D. glass capillary coated with SF-96	CS_2, CH_3SH, $(CH_3)_2S$, $(C_2H_5)_2S$, $(CH_3)_2S_2$, 1-Bu, 2-Bu, thiophen	75–300 pg	74
18′ × 1/8″ O.D. Teflon, Porapak QS 80/100 acetone-washed	H_2S, COS, SO_2, CH_3SH, $(CH_3)_2S$, $(CH_3)_2S_2$	10 μg	129
1.83 m × 6 mm O.D. glass, 9% OV-101, 1% HIEFF 8 AP on Gas Chrom Q 80/100	SO_2, aldicarb, malathion	1.2 ng	130
3 m × 3 mm glass containing	SO_2, COS, H_2S, CS_2,	4 ng $(C_2H_5)_2S$	106
(1) 25% TCEP on Shimalite AW, DMCS 60/80	CH_3SH, $(CH_3)_2S$, C_2H_5SH,	as internal	
(2) 25% TCP on Shimalite AW, DMCS 60/80	$(CH_3)_2S_2$, $(C_2H_5)_2S$, thio-	standard	
(3) 10% PPE on Shimalite TPA 60/80	phen, THT, 2-Pr,		
(4) Porapak Q 50/80	1,1-dimethylethanethiol,		
(5) Silica gel 60/80	1-hexanethiol allyl sulphide		
300′ × 0.02″ I.D. stainless-steel capillary, Polyethylene glycol 400	$(C_2H_5)_2S$,	2.5 ng	5
	$(CH_3)_2S_2$	5 ng	
(1) 6′ × 1/8″ stainless steel, 5% Silicone Oil QF-1 on Porapak QS 80/100	C_2H_5SH, $(CH_3)_2S$, 1-Bu, 2-Bu, 1-Pr, 2-Pr, THT	μg range	66
(2) 10′ × 1/8″ stainless steel, 5% PPE on Chromosorb G, AW, DMCS 80/100			
30 m × 0.5 mm I.D. glass capillary (SCOT), SE-30	Organosulphur compounds	1 ng	11
6′ × 0.02″ O.D. glass with	H_2S, COS, CS_2, thiophen	5–50 μl smoke	4
(1) Porapak Q 80/100			
(2) Chromosorb 104 80/100			
5.5 m × 3 mm I.D. glass, 25% 1,2,3-tris-2-cyanoethoxypropane on Chromosorb W, AW 60/80	37 sulphur compounds	1 ng $(CH_3)_2S_2$	102
(1) 6′ × 1/8″ O.D. Teflon (FEP), Tracor Special Silica	COS, H_2S, CS_2, SO_2,	μg range	7
(2) 18′ × 1/4″ O.D. glass, 20% FFAP on Chromosorb W, AW, DMCS 60/80	$(CH_3)_2S$, $(CH_3)_2S_2$, $(C_2H_5)_2S$, thiophen and higher boiling compounds		
34′ × 0.085″ I.D. Teflon (FEP), 12% PPE, 0.5% H_3PO_4 on Teflon 40/60	SO_2, H_2S, CH_3SH, C_2H_5SH, $(CH_3)_2S$	20–100 ng	84–86
36′ × 1/8″ O.D. Teflon (FEP), 9% PPE, H_3PO_4 on Teflon T6 40/60	H_2S, SO_2, CH_3SH	< 100 ng	28
1.25 m × 3 mm I.D. Teflon, graphitized carbon black 40/60 treated with 0.5% H_3PO_4, 0.3% Dexsil	SO_2, H_2S, CH_3SH, $(CH_3)_2S$	ng range	131
1.6 m × 0.4mm I.D. glass, 0.7% H_3PO_4, 0.7% XE-60 on Carbopack B 40/60	H_2S, SO_2, CH_3SH	ppM range†	82
80 cm × 0.4 cm I.D. Teflon, 0.7% H_3PO_4, 0.7% XE-60 on graphitized carbon black 40/60	H_2S, SO_2,	62.5 ng	87
	CH_3SH	187.5 ng	
30′ × 1/8″ O.D. Teflon (FEP), 5% PPE, 0.05% H_3PO_4 on Teflon 30/60	H_2S,	70 ng	89
	CH_3SH	150 ng	
30′ × 1/8″ O.D. Teflon, Supelpak S	SO_2, H_2S	ppM range†	55
122 cm × 0.175 cm stainless steel, 5% Carbowax 20M, 10% DC200 on Gas Chrom Q 60/80	Insecticides, UC-21149	5–60 pg	96
240 cm glass, 5% OV-101 on Gas Chrom Q 80/100	Pesticides	0.5–2.5 ng	97
2′ × 1/8″ O.D. stainless steel, 4% Igepal CO-880 on Anachrom ABS 80/90	Malathion, parathion	1 ng range	117
(1) 10′ × 1/4″ O.D. glass, 10% Carbowax 20M on Chromosorb W	Thiophen	200 pg	132
(2) 6′ × 1/4″ O.D. glass, 3% SE-30 on Chromoport XXX 80/90			
240 cm × 6 mm O.D. glass, 5% DC-200 on Gas Chrom Q 80/100	Pesticides	ng range	133

Table 1—*continued*

Column	Compounds*	Limit of detection	Reference
3′ × 4 mm I.D. glass, 10% OV-1 on Chromosorb W-HP 80/100	Methyl parathion	1 ng	134
200 cm × 6 mm O.D. glass, 5% OV-101 on Chrom W‡	1-Hexanethiol, methyl parathion	50 pg S/sec	135
1 m × 1/4″ O.D. Teflon (FEP), Tenax-GC 35/60	SO_2, H_2S, COS, CH_3SH, $(CH_3)_2S$	<1 ppm (v/v)	91
(1) 1.8m × 3 mm O.D. stainless steel, silica gel	H_2S, COS, CS_2, SO_2	μg range	67
(2) 1.8 m × 3 mm O.D. stainless steel, 5% QF1 on Porapak QS 80/100			
(3) 7.3 m × 3 mm O.D. stainless steel, 10% PPE 6R, 0.4% H_3PO_4 on Chromosorb G, AW, DMCS 80/100			
(1) 120 cm × 3.17 mm O.D. glass, 5% QF-1 on Chromosorb W, AW, DMCS 60/80	Soluble elemental S	2.2 ng	54
(2) 103 cm × 3.17 mm O.D. glass, 5% QF-1, 4% SE-30 on Chromosorb W, AW, DMCS 60/80		3 ng	
(3) 122 cm × 3.17 mm O.D. glass, 5% OV-17 on Chromosorb W, AW, DMCS 60/80		4 ng	
5′ × 1/8″ O.D. glass, 5% DC-200, 7.5% QF-1 on Chromosorb W, HP 80/100	Methyl parathion	80 pg/sec	136
24′ × 1/8″ O.D. Teflon, 5% PPE, 0.05% H_3PO_4 on Teflon	H_2S, SO_2, CH_3SH, $(CH_3)_2S$, $(CH_3)_2S_2$	0.3 ng	56
50 m × 0.5 mm glass, Carbowax 20M	1-Bu	1×10^{-10} g	23
150 cm × 6 mm O.D. aluminium, 20% tricresyl phosphate on Celite 545 60/85 mesh	$(CH_3)_2S$, $CH_3SC_2H_5$, $(C_5H_5)_2S$, $(C_2H_5)_2S_2$, thiophene, THT, methyl n-butyl sulphide, other organosulphur species	<20 pg S	68
GC–MS			
3 m glass, 5% DEGSE on Chromosorb W	$(CH_3)_2S$	<10 ppM†	137
1000′ × 0.02″ I.D. glass capillary, Squalane	Thiophenes	<1 ng	138
1.83 m × 4 mm I.D. column, 3% OV 225 on Chromosorb 750 80/100	6-Mercaptopurine	20 ng	139

* 1-Pr = 1-propanethiol; 2-Pr = 2-propanethiol; 1-Bu = 1-butanethiol; 2-Bu = 2-butanethiol; TMS = tetramethylene sulphide; THT = tetrahydrothiophene.
† ppM = parts per milliard (10^9).
‡ Dual-FPD.

sulphur-containing compounds. It was important to prevent ethanol from entering the separator, where it would flood the membrane. Therefore the valve was switched before elution of the ethanol peak, so that the solvent was diverted to the FPD. Any sulphur species eluted at the same time would also be diverted to the detector and be vented.

Solutions of the four sulphur-containing species in absolute ethanol (S 100 μg/ml) were used with a sample size of 1 μl, which corresponded to 100 ng of sulphur injected into the column. The ion-current at *m/e* 47 was monitored for each of the compounds studied. This is the most intense peak in the spectrum of methanethiol, the second most intense in that of diethyl sulphide and the fifth most intense in that of both butanethiol and dimethyl disulphide.[125] All MS parameters were optimized to give good peak shape with a flat top and maximum sensitivity. Figure 10 gives examples of the traces obtained.

The temperature of the separator, line and reentrant tube was varied and the response of the FPD noted. In the first run all the sample was diverted to the detector. In the second run the effluent was diverted to the membrane separator. Any sulphur species not dissolving in the membrane was monitored by the FPD. From the difference in the areas of these two peaks, the amount of material entering the MS could be calculated (yield), assuming no loss due to condensation or leakage. The amount of material passing through the silicone membrane depends on the diffusion rate, the solubility of the gas and the membrane thickness. The yields (Y) were calculated according to the equation prescribed by McFadden:[111]

$$Y = \frac{Q_{MS}}{Q_{GC}} \times 100\%$$

where Q_{GC} is the quantity of sample leaving the chromatograph and Q_{MS} is the quantity of sample entering the mass spectrometer.

Table 2 shows the results obtained for a number of sulphur species examined at various separator temperatures. Values as high as 47% were obtained at the lower temperatures, but this figure decreased with a decrease in the molecular weight of the material under investigation. It is believed that by optimiza-

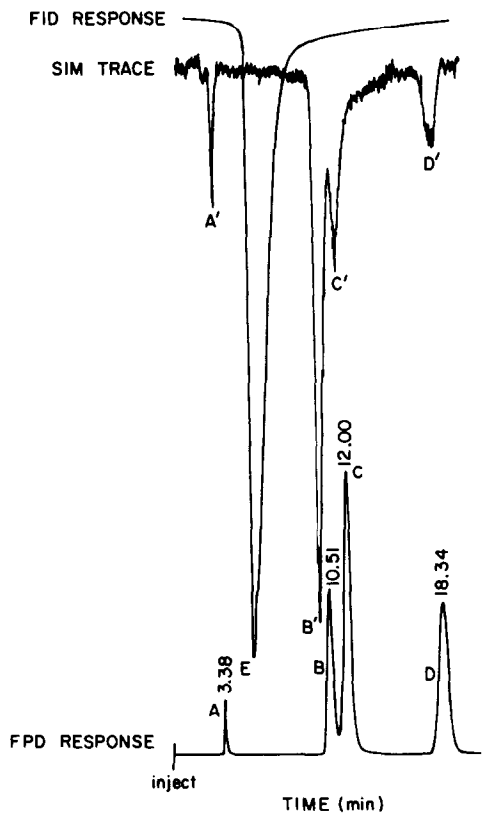

Fig. 10. FPD/FID response and SIM trace for 4 organosulphur compounds (equivalent of 100 ng of sulphur injected). A,A', Methanethiol; B,B', diethyl sulphide; C,C', butanethiol; D,D', dimethyl disulphide; E, ethanol.

tion of the separator temperature and flow-rate of the carrier gas, yields greater than 50% could be achieved. It can be seen that with increasing temperature the yield decreases. For diethyl sulphide, a reduction of the nitrogen flow-rate to 15 ml/min gave an increased yield, presumably because more time was available for the sample to dissolve in the membrane before being carried away by the carrier gas. Values for the separation factor N were also calculated by using the equation:

$$N = \frac{Y}{100} \times \frac{V_{GC}}{V_{MS}}$$

where Y is the yield, V_{GC} is the carrier-gas volume measured at the chromatograph and V_{MS} is the carrier-gas volume measured at the mass spectrometer. Because the value for V_{MS} was extremely low and could not be accurately measured, the resulting enrichment figures were approximate but certainly very high.

Table 2. Yields of different compounds for membrane separator at various temperatures (flow-rate 30 ml/min)

Compound	Temperature of separator, °C			
	50	60	80	100
Methanethiol, %	35.7	37.0	29.6	3.4
n-Butanethiol, %	38.1	36.9	24.0	
Diethyl sulphide, %	39.4	39.3	25.9	
Dimethyl disulphide, %	47.1	45.1	30.7	

An unusual phenomenon was observed on repeated exposure of the separator to the GC effluent. The value of the yield for a particular compound decreased with time, *i.e.*, the amount dissolving through the membrane was reduced when the separator was in constant use. The membrane therefore requires a recovery period between samples although the reason for this has not yet been established. This phenomenon has been observed at different temperatures. The yield for diethyl sulphide at 80° changed from 25.9% to 18.2% in 150 min. At 60° the change was from 39.3% to 23.1% in only 90 min. This effect is being examined further.

Peaks arising from 40 ng of S in material passing through the separator were of a comparable size to those obtained from the same amount inserted from a glass reservoir fitted with a silicon carbide leak. The large signals obtained with 40 ng of S suggest that much lower quantities could be detected by using single-ion monitoring, possibly extending into the picogram range if suitable care and adequate precautions were taken.

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CONSTRUCTION OF A LIQUID-MEMBRANE TYPE PERIODATE ION-SELECTIVE ELECTRODE AND ITS APPLICATION TO THE POTENTIOMETRIC TITRATION OF α-DIOLS AND α-AMINO-ALCOHOLS

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Summary—The construction of a liquid-membrane type periodate ion-selective electrode and its application to the potentiometric titration of α-diols and α-amino-alcohols are described. The ion-pair of periodate anion with Capriquat (tri-n-octylmethylammonium chloride), is easily extracted into nitrobenzene, and this extract is employed as a liquid ion-exchange membrane. The calibration curve shows Nernstian response towards periodate ion over the concentration range from $10^{-1}M$ to $10^{-7}M$ with a slope of 60 mV/pIO_4^-. Selectivity coefficients with respect to various ions were evaluated. The electrode potential was independent of pH in the range 2.5–7.5. Some α-diols and monoethanolamine were successfully titrated potentiometrically with the aid of the present electrode.

Periodate is a good oxidizing agent for α-diols, α-amino-alcohols and similar compounds. Efstathiou and Hadjiioannou reported some potentiometric[1–3] and catalymetric[2,4] determinations of vicinal glycols, α-amino-alcohols, carbohydrates and metal ions by means of a perchlorate ion-selective electrode which was used as a periodate sensor.

Baczuk and Dubois[5] briefly described a periodate ion-selective electrode in which tris(1,10-phenanthroline)iron(II) was used as the counter-ion.

This paper describes the construction, and application in some potentiometric titrations, of an ion-selective electrode sensitive to periodate, based on a nitrobenzene solution of the Capriquat(tri-n-octylmethylammonium)–periodate ion-pair as an ion-exchange membrane.

EXPERIMENTAL

Reagents

All chemicals used were of analytical reagent grade.

Sodium periodate solution (0.25*M*). Dissolve 53.47 g in 1 litre of demineralized water and standardize by iodometric titration.

Capriquat solution (1m*M*). Dissolve 0.0404 g in 100 ml of nitrobenzene. (The reagent was obtained from Dojindo Laboratories.)

Sodium thiosulphate solution (0.1*M*). Dissolve about 25 g of the reagent in 1 litre of water and standardize against potassium iodate solution.

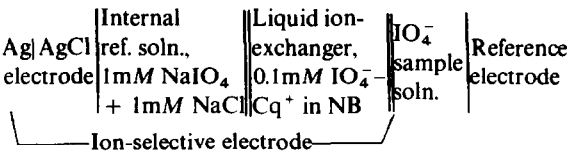
RESULTS AND DISCUSSION

Electrode construction

The electrode barrel used in this study is the same as that described previously.[6,7] The liquid ion-exchange membrane was prepared as follows: to a 1-ml portion of 1m*M* sodium periodate in a 100-ml separatory funnel were added 8 ml of water, 1 ml of 1m*M* Capriquat (Cq) in nitrobenzene (NB) and 9 ml of nitrobenzene. The mixture was shaken for 10 min and then left to stand for 30 min. After separation of the layers, the organic phase was dried with anhydrous sodium sulphate.

Potential measurement

The cell composition including the ion-selective electrode is shown as follows.



The potential difference of the cell was measured with an Orion microprocessor "Ionalyzer", model 901, connected to a Hitachi recorder, type 056. An Orion electrode, type 90-01, was employed as the outer reference electrode. The reading was taken 30 sec after immersion of the electrodes because the response of the ion-selective electrode is almost instantaneous.

Selection of counter-cation

Several quaternary onium cations were tested as the counter-cation in the membrane. Figure 1 shows the calibration curves. Among them, water-insoluble Capriquat was found to show the best behaviour with respect to sensitivity and reproducibility of potential at lower concentrations of periodate.

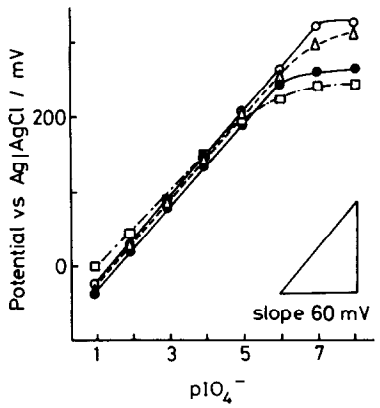


Fig. 1. Effect of counter-cation: —○— Cq^+, —●— Methylene Blue$^+$, --△-- tetraphenylarsonium$^+$, —·-□-·— zephiramine$^+$ (benzyldimethyltetradecylammonium$^+$). Ion-pair concentration: 0.1m*M*. Internal reference solution: 1m*M* $NaIO_4$ in 1m*M* NaCl. Internal reference electrode: Ag/AgCl electrode. Temperature: 18.0°C.

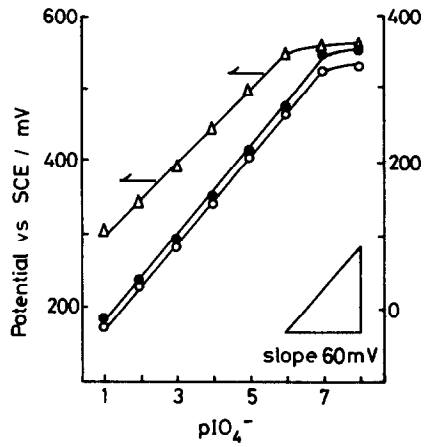


Fig. 3. Selection of internal reference electrode: —△— platinized Pt, —○— Ag/AgCl, —●— Ag. Ion-pair concentration: 0.1m*M*. Temperature 18.0°C.

Selection of ion-pair concentration in the membrane

The effect of the IO_4^-–Cq^+ ion-pair concentration in the membrane phase is shown in Fig. 2. When the ion-pair concentration was below 5μM, the electrode hardly responded to low concentrations of periodate. Because of the high electrical resistance of the membrane, the potentials were unstable when the ion-pair concentration was below 0.05m*M*. Further, the electrode with 0.5m*M* ion-pair concentration showed a sudden potential jump at lower periodate concentrations. Consequently, the ion-pair concentration of 0.1m*M* was considered to be the best.

Selection of internal reference electrode

Three kinds of internal reference electrode, *i.e.*, platinized platinum, silver, and silver–silver chloride, were constructed and tested. As shown in Fig. 3, the silver and silver–silver chloride electrodes both gave good results: the silver–silver chloride electrode, with an electrolyte which was 1m*M* in sodium periodate and in sodium chloride, was chosen as the internal reference electrode. The ion-selective electrode exhibited linear Nernstian response to periodate from $10^{-1}M$ to $10^{-7}M$ with a slope of 60 mV/pIO_4^- at 18°C.

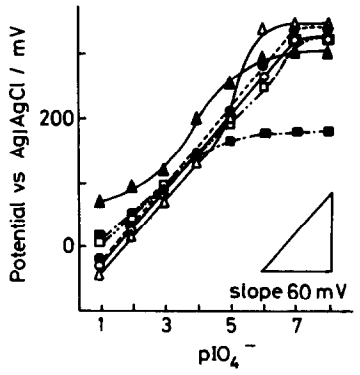


Fig. 2. Effect of IO_4^-–Cq^+ ion-pair concentration in ion-exchange membrane: —△— 0.5, ---●--- 0.25, —○— 0.1, —▲— 0.05, —·-□-·— 0.01, --·■·-- 0.005m*M*. Temperature 18.0°C.

Influence of pH

The change in electrode potential with pH of the test solution is shown in Fig. 4. The pH of the 0.1m*M* periodate test solution was adjusted by addition of 0.1m*M* periodate solution in 0.1*M* hydrochloric acid or sodium hydroxide. The total ionic strength was kept at 0.1*M* by addition of sodium chloride. The potential of the cell was independent of pH over the range 2.5–7.5. The positive shift of the potential at both ends of the graph may be attributed to a decrease in the concentration of IO_4^-, since the two break-points occur at pH values almost equal to the reported values for the dissociation constants[8] of H_5IO_6, $pK_1 = 2.20$, $pK_2 = 8.01$.

Selectivity

The effect of diverse ions on the periodate ion-selective electrode is given by the selectivity coeffi-

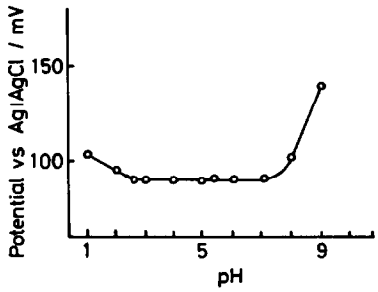


Fig. 4. Electrode response as a function of pH: 1m*M* $NaIO_4$ solution. Ionic strength: 0.1*M*. Temperature 17.0°.

Table 1. Selectivity coefficients $K_{IO_4^-,j}$ for the periodate ion-selective electrode

Anion	$-\log K_{IO_4^-,j}$
ClO_4^-	0.37
SCN^-	0.74
ClO_3^-	2.09
IO_3^-	2.23
Cl^-	3.57
CH_3COO^-	3.82
HCO_3^-	3.37
BrO_3^-	2.13
SO_4^{2-}	3.94
NO_3^-	2.55
OH^-	1.84
$H_2PO_4^-$	2.52
BO_2^-	1.28

cient, $K_{IO_4^-,j}$, in the familiar Nikolskii–Eisenman equation:[9]

$$E = E_0 - \frac{RT}{F} \ln [a_{IO_4^-} + \Sigma K_{IO_4^-,j} a_j^{1/z_j}] \quad (1)$$

where $a_{IO_4^-}$ and a_j are the activities of the periodate and ion j respectively and z_j is the absolute value of the charge on ion j. The selectivity coefficient was obtained by the separate solution method proposed by Srinivasan and Rechnitz.[10] It can be calculated from the difference in the potentials measured for equimolar solutions of iodate and the interfering ion by means of the equation:

$$-\log K_{IO_4^-,j} = (E_2 - E_1)F/2.303RT \quad (2)$$

where E_1 is the potential measured for the solution containing only the periodate ion and E_2 is that for the solution of the foreign ion. Results are summarized in Table 1.

The present electrode shows good selectivity with respect to most common anions. It should be emphasized that iodate, which is produced by the reduction of periodate, does not interfere. Selectivity coefficients for iodide and bromide were not evaluated, because these two ions are oxidized by periodate. The electrode, however, shows only a poor selectivity for periodate with respect to perchlorate and thiocyanate.

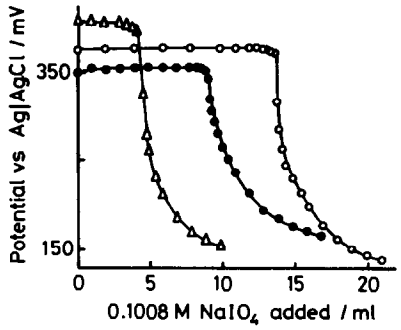
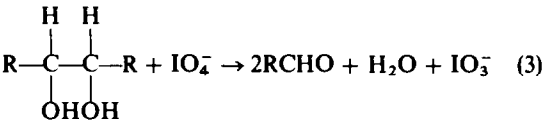


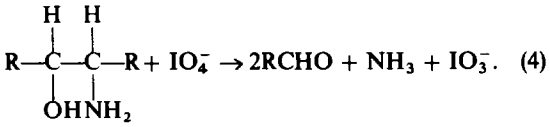
Fig. 5. Potentiometric titration of monoethanolamine: $9.54 \times 10^{-2}M$ monoethanolamine taken: —△— 5, —●— 10, —○— 15 ml with 5 ml of $0.1M$ $NaHCO_3$ and water to 25 ml.

Potentiometric titrations

Generally, α-glycols and α-amino-alcohols may be oxidized by periodate as follows:[11,12]



$$R{-}\underset{OH}{\overset{H}{C}}{-}\underset{OH}{\overset{H}{C}}{-}R + IO_4^- \rightarrow 2RCHO + H_2O + IO_3^- \quad (3)$$



$$R{-}\underset{OH}{\overset{H}{C}}{-}\underset{NH_2}{\overset{H}{C}}{-}R + IO_4^- \rightarrow 2RCHO + NH_3 + IO_3^-. \quad (4)$$

Potentiometric titration curves for the determination of monoethanolamine by titration with periodate followed with the help of the ion-selective electrode are shown in Fig. 5.

The titration procedure was as follows. To a known amount of sample in a 50-ml beaker were added 5 ml of $0.1M$ sodium bicarbonate and water to a final volume of 25 ml, and the mixture was titrated potentiometrically with $0.1008M$ sodium periodate. However, because of the slowness of the reaction, the electrode only reached a stable potential 5 min after the addition of titrant. Before the end-point, the electrode potential showed a sharp change just after the addi-

Table 2. Comparison of the potentiometric and iodometric titration methods

Sample	Factor of the sample solution: Potentiometric titration*	Iodometric titration†	F_0	t_0
Ethylene glycol	0.9641 ± 0.0002	0.9645 ± 0.0001	7.89	0.53
Propylene glycol	1.0025 ± 0.0001	1.0020 ± 0.0001	3.03	0.87
2,3-Butanediol	0.9823 ± 0.0001	0.9820 ± 0.0001	2.00	0.52
Monoethanolamine	0.9533 ± 0.0001	0.9535 ± 0.0001	2.22	0.44

* A 10-ml portion of *ca.* $0.1M$ sample solution was titrated with $0.1008M$ $NaIO_4$.

† Concentration of sample solution: $0.01M$. Four titrations.

$F(3,3; 0.025) = 15.4 > F_0$; $t(6; 0.05) = 2.447 > t_0$.

tion of titrant and then gradually fell as reaction took place. After the end-point, however, the electrode gave a stable potential within 30 sec of the addition of periodate. Results by the present titration were compared with those obtained by the iodometric titration method, which was carried out as follows. A 5-ml portion of 0.025*M* sodium periodate was added to a 5-ml portion of *ca.* 0.01*M* glycol or amino-alcohol solution buffered appropriately and left to stand for an hour. The solution was acidified by addition of sulphuric acid (1 + 1) then 1 g of potassium iodide was added. The iodine liberated was titrated with standard sodium thiosulphate solution with starch as indicator. A blank titration was also carried out. Results for four compounds titrated by the two methods are given in Table 2. The *F*- and *t*-tests revealed that there were no significant differences between the means and the variances obtained by the two methods.

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DETERMINATION OF TIN IN ORES, IRON, STEEL AND NON-FERROUS ALLOYS BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY AFTER SEPARATION BY EXTRACTION AS THE IODIDE

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Summary—A simple and moderately rapid method for determining 0.001% or more of tin in ores, concentrates and tailings, iron, steel and copper-, zinc-, aluminium-, titanium- and zirconium-base alloys is described. After sample decomposition, tin is separated from the matrix elements, except arsenic, by toluene extraction of its iodide from a 3*M* sulphuric acid–1.5*M* potassium iodide medium containing tartaric and ascorbic acids. It is finally back-extracted into a nitric–sulphuric acid solution containing hydrochloric acid to prevent the formation of an insoluble tin–arsenic compound and the resultant solution is evaporated to dryness. Tin is subsequently determined by atomic-absorption spectrophotometry in a nitrous oxide–acetylene flame, at 235.4 nm in a 10% hydrochloric–0.5% tartaric acid medium containing 250 μg of potassium per ml. Co-extracted arsenic does not interfere. Results obtained by this method are compared with those obtained spectrophotometrically with gallein after the separation of tin by iodide extraction.

A reasonably simple and moderately rapid atomic-absorption (AAS) method for the determination of moderate and small amounts of tin in diverse ores and mill products was required for use in the Canadian Certified Reference Materials Project (CCRMP) and in routine work in the CANMET chemical laboratory. The need for a reliable instrumental method for determining low levels of tin was apparent from the wide range of values obtained for a zinc concentrate, CZN-1 (20–209 μg/g),[1] and a lead concentrate, CPB-1 (0.016–0.041%),[2] by AAS and other methods (mostly emission spectrography), during a recent interlaboratory certification programme. A wide range of values at higher levels (~0.7 and 2.5%) was also obtained by AAS and mostly by titrimetric methods during earlier interlaboratory certification programmes for a zinc–tin–copper–lead ore, MP-1,[3] and a zinc–lead–tin–silver ore, KC-1.[4] As part of the project involving the certification of KC-1, a spectrophotometric method was developed for the determination of small amounts of tin in ores and other materials.[5] This method involved the preliminary separation of tin by toluene extraction of tin iodide from a 2*M* sulphuric acid–1.5*M* potassium iodide medium, stripping into dilute sodium hydroxide solution, and determination with gallein. Because of its high sensitivity (molar absorptivity = 4.11×10^3 l. mole^{-1}. mm^{-1}) and the relatively high absorbance exhibited by gallein under the conditions required for the formation of the tin complex, this method is not really suitable for the determination of >1% of tin. However, it was considered that the relatively specific iodide-extraction preconcentration step described in this method, in conjunction with an atomic-absorption finish, should provide a simple and reliable method for determining tin in ores.

In recent years, various AAS methods for tin have been based on its separation and preconcentration by extraction of tin iodide with high molecular-weight amines from hydrochloric[6] and sulphuric acid media,[7] or with trioctylphosphine oxide–methyl isobutyl ketone solutions from hydrochloric acid media.[8–13] In these methods, tin is determined by direct aspiration of the organic phase into the flame. Another recent method involves the extraction of tin iodide into benzene from a hydrochloric–perchloric acid medium and the subsequent determination of tin after its back-extraction into dilute hydrochloric acid containing magnesium chloride.[14] However, these extraction procedures are not as selective as that described in the gallein method[5] because many other elements are co-extracted as molecular and ion-association compounds from hydrochloric acid media and/or in the presence of amines and phosphine oxides.[15] Therefore, the applicability of the toluene–iodide extraction step used previously[5] was investigated in the present work. Because of the greater ease of preparation of aqueous calibration solutions, particularly for routine work, the back-extraction of tin into an acidic medium, as described previously,[14] was also examined.

This paper describes the successful determination of tin in ores and mill products, iron, steel and copper-, zinc-, aluminium-, titanium- and zirconium-base alloys, after its separation by extraction of tin iodide into toluene from a 3*M* sulphuric acid–1.5*M* potas-

sium iodide medium containing tartaric and ascorbic acids, and its subsequent back-extraction into a mixture of nitric, hydrochloric and sulphuric acids. Results obtained by this method are compared with those obtained previously by the gallein method.[5]

EXPERIMENTAL

Apparatus

A Varian Techtron Model AA6 spectrophotometer equipped with a 6-cm laminar-flow nitrous oxide–acetylene burner and a high-intensity tin hollow-cathode lamp was used for the determination of tin. The following instrumental parameters were employed (Note 1).

Wavelength: 235.4 nm
Lamp current: 6 mA
Spectral band pass: 0.20 nm
Height of light-path above burner: 6 mm
Acetylene flowmeter reading: 7.0 (~5 l./min)
Nitrous oxide flowmeter reading: 6.5 (~9 l./min)
Flame: non-luminous, ~1. 5–2 cm "red feather"
Aspiration rate: 2 ml/min

Reagents

Standard tin solution, 1000 μg/ml. Dissolve 0.5000 g of pure tin metal by heating gently, in a covered 400-ml beaker, with 50 ml of concentrated sulphuric acid and 5 ml of 30% hydrogen peroxide, then remove the cover and evaporate the solution to fumes of sulphur trioxide. Cool in a water-bath and carefully add ~25 ml of water. Transfer the solution to a 500-ml standard flask containing 150 ml of 50% v/v sulphuric acid, dilute almost to the mark with water and mix gently by swirling the flask. Cool to room temperature and dilute to volume with water.

Potassium (2500 μg/ml)–tartaric acid (5%) solution. Dissolve 4.77 g of potassium chloride and 50 g of tartaric acid in ~500 ml of water and dilute the solution to 1 litre with water. Filter if necessary.

Potassium iodide solution, 5.3M. Prepare sufficient just before use.

Sulphuric acid, 3M. Dilute 65 ml of 50% v/v sulphuric acid to 200 ml with water. Store in a plastic wash-bottle.

Sulphuric acid (9M)–potassium iodide (0.05M) wash solution. Add 0.5 ml of 5.3*M* potassium iodide to 50 ml of 50% v/v sulphuric acid and mix. Prepare immediately before use (Note 2).

Sulphuric acid (10% v/v)–nitric acid (50% v/v) solution.

Tartaric acid, 20% solution.

Sulphuric acid, 5 and 50% v/v.

Calibration solutions

Add 10 ml of 10% sulphuric acid–50% nitric acid solution, 2 ml of concentrated hydrochloric acid and 5 drops of concentrated perchloric acid to each of eleven 100-ml beakers; then, by burette, add to the first 10 beakers, 0.2, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 ml, respectively, of the standard 1000-μg/ml tin solution. The contents of the last beaker constitute the zero calibration solution. Cover the beakers, evaporate the solutions to fumes of perchloric acid or sulphur trioxide, then remove the covers and evaporate the solutions to dryness. Cool, wash down the sides of the beakers with water and evaporate the solutions to dryness again to ensure the complete removal of sulphuric acid. Add 10 ml each of concentrated hydrochloric acid and 2500-μg/ml potassium–5% tartaric acid solution to each beaker and mix to dissolve the salts. Cool, transfer the solutions to 100-ml standard flasks, dilute to volume with water and mix (Note 3).

Ores and mill products

Run a blank with each set of samples. Transfer 0.1–1 g of powdered sample, containing up to ~5 mg of tin and not more than ~10 mg of tungsten, to a 30-ml zirconium crucible. Add 2 g each of sodium carbonate and sodium peroxide and mix thoroughly. Cautiously fuse the mixture over an open flame and keep it molten for ~30 sec to ensure complete decomposition. Allow the melt to cool, then transfer the crucible to a covered 400-ml Teflon beaker containing ~30 ml of water and 55 ml of 50% sulphuric acid. When the melt has dissolved, remove the crucible after washing it thoroughly with water, then cover the beaker (Note 4) and evaporate the solution to ~75 ml. Remove the cover, add 5 ml of concentrated hydrofluoric acid and evaporate the solution to copious fumes of sulphur trioxide to remove silica, hydrogen peroxide and hydrofluoric acid. Cool, wash down the sides of the beaker with water and evaporate the solution to fumes of sulphur trioxide to ensure the complete removal of hydrofluoric acid (Note 5). Cool to room temperature, cover the beaker and add 5 ml of 20% tartaric acid solution and 50 ml of water. Heat the solution to dissolve the soluble salts, then cool it to room temperature and, if necessary, filter it (Whatman No. 541 paper) into a 400-ml beaker. Wash the beaker three times with small portions of water, then wash the paper once with 5% sulphuric acid (a plastic wash-bottle is convenient), followed by water. Discard the paper. Evaporate the filtrate to ~85 ml and cool it to room temperature.

Add 1 g of ascorbic acid to the blank and sample solutions and mix thoroughly. Transfer the solutions to 250-ml separatory funnels, marked at 100 ml, and dilute to the mark with water. Add 40 ml of freshly prepared 5.3*M* potassium iodide solution and 30 ml of toluene, stopper and shake for 2 min. Allow several min for the layers to separate, then drain the lower aqueous layer into a second 250-ml separatory funnel. Wash the stem of the first funnel with 3*M* sulphuric acid and collect the washings in the second funnel. Add ~1 ml of 5.3*M* potassium iodide solution to the first funnel containing the extract, and without mixing, drain the resulting aqueous layer into the second funnel and wash the stem of the first funnel again with 3*M* sulphuric acid (Note 6). Add 20 ml of toluene to the second funnel and re-extract the solution by shaking for 2 min. Allow the layers to separate, then drain off and discard the aqueous phase. Run the second extract into the first funnel, and wash the second funnel with toluene from a plastic wash-bottle. Drain off and discard any residual aqueous phase in the first funnel, then add 10 ml of freshly prepared 9*M* sulphuric acid–0.05*M* potassium iodide wash solution (Note 2) and, without delay, shake the funnel gently for ~30 sec. Allow the layers to separate, then drain off and discard the aqueous layer. Repeat the washing step with 10 ml of a second freshly prepared wash solution. Drain off the aqueous layer and wash the stem of the funnel with water to remove the residual wash solution.

Add 10 ml of 10% sulphuric acid–50% nitric acid solution and 2 ml of concentrated hydrochloric acid to the combined extracts, stopper and shake for 1 min. Allow the layers to separate, then drain the aqueous layer into a 100-ml beaker and wash the stem of the funnel with water. Wash the toluene phase by shaking for ~30 sec with each of two 5-ml portions of water and add the washings to the beaker (Note 7). Add 5 drops of concentrated perchloric acid to the resulting solution, cover the beaker, evaporate the solution to fumes of perchloric acid or sulphur trioxide, then remove the cover and evaporate the solution to dryness. Cool, wash down the sides of the beaker with water and evaporate the solution to dryness to ensure the complete removal of sulphuric acid. Add 1 ml each of concentrated hydrochloric acid and 2500-μg/ml potassium–5% tartaric acid solution to the beaker containing the blank. Depending on the expected tin content, add sufficient concentrated hydrochloric acid and potassium–tartaric acid solution to the beaker containing the sample for 1 ml of each to be present for each 10 ml of final solution. Heat the

resulting solutions for ~5 min in a hot water-bath (Note 8) and cool to room temperature. Transfer the blank solution to a 10-ml standard flask, and the sample solution to a flask of appropriate size (10–100 ml). Dilute each solution to volume with water and mix.

Measure the absorbance at 235.4 nm when the solution is sprayed into a strongly reducing nitrous oxide–acetylene flame (Note 9). Determine the tin content (in mg) of the solution by relating the absorbance to the values obtained concurrently for calibration solutions of slightly higher and lower tin concentrations. Correct the result obtained for the sample by subtracting that obtained for the blank.

Iron and steel

Transfer 0.1–1 g of sample (Note 10), containing up to ~5 mg of tin and not more than ~10 mg of tungsten, to a 400-ml Teflon beaker. Cover the beaker and add 25 ml of water and 10 ml of concentrated nitric acid. Heat gently until the sample is decomposed, then add 55 ml of 50% sulphuric acid and heat until the evolution of oxides of nitrogen ceases. Remove the cover, add 1 ml of concentrated hydrofluoric acid and evaporate the solution to copious fumes of sulphur trioxide. Cool, wash down the sides of the beaker with water and evaporate the solution to fumes of sulphur trioxide to ensure complete removal of hydrofluoric acid (Note 5). Cool to room temperature, cover the beaker and add 5 ml of 20% tartaric acid solution and 50 ml of water. Heat the solution to dissolve the salts, then proceed with the filtration of the solution, if necessary, the extraction (Note 11) and the subsequent determination of tin as described above.

Zirconium-base alloys

Depending on the expected tin content, transfer up to 1 g of sample to a 400-ml Teflon beaker. Cover the beaker and add 55 ml of 50% sulphuric acid and 1 ml of concentrated hydrofluoric acid. Heat gently until the sample is decomposed, then remove the cover and evaporate the solution to copious fumes of sulphur trioxide, and continue as from the corresponding stage in the analysis of iron and steel (Note 12).

Titanium-base alloys

Transfer up to 0.25 g of sample (Note 13) to a 400-ml beaker, cover and add 55 ml of 50% sulphuric acid. Heat until the sample is decomposed, then remove the cover, evaporate the solution to fumes of sulphur trioxide and proceed as described above for zirconium-base alloys (Note 12).

Aluminium- and zinc-base alloys

Decompose up to 1 g of sample and determine tin by the method described for iron and steel (Note 12).

Copper-base alloys

Decompose up to 0.2 g of sample, containing not more than ~100 mg of copper, and determine tin by the method described for iron and steel (Note 14).

Notes

1. A strongly reducing nitrous oxide–acetylene flame is required to obtain the highest sensitivity for tin. The observation height in the flame is also extremely important.[16] Consequently, after all other instrumental parameters have been set, the acetylene flow-rate should be adjusted to give the maximum "red feather" without producing a luminous flame. Under these conditions, very little carbon is deposited in the burner slot. Subsequently, the height of the light-path above the burner should be adjusted to give maximum absorbance when a tin solution is aspirated into the flame.

2. This solution must be used *immediately* after preparation because potassium iodide is rapidly oxidized by air to iodine in strongly acidic solutions.

3. The calibration solutions are stable for at least 1 week.

4. The solution should be kept almost completely covered during the initial evaporation, to avoid loss by spray.

5. Tin will not be quantitatively extracted as the iodide if hydrofluoric acid is not completely removed and if too much sulphuric acid is removed by evaporation.

6. By this procedure, the aqueous phase that remains in the bore of the stop-cock is transferred to the second funnel.

7. The toluene can be used for subsequent extractions if the toluene phases are combined in a large separatory funnel and washed twice by shaking with ~3% sodium hydroxide solution, followed by three washes with ~10% sulphuric acid.

8. If arsenic is present in the sample, heating is necessary to ensure the complete dissolution of the tin–arsenic compound that is formed on evaporation of the sample solution to dryness in the presence of nitric and sulphuric acids. This compound is only sparingly soluble in cold hydrochloric acid.

9. Scale expansion (~2–5-fold is recommended for the determination of tin. Because of the moderately high "noise" level of the analytical signal, a 10-sec integration time is also recommended to improve the precision of the determination.

10. This decomposition procedure is not suitable for samples of high chromium content (*i.e.*, stainless steel). However, up to 0.5 g of such steels can readily be dissolved by treatment with 55 ml of 50% sulphuric acid and 20 ml of *aqua regia*. After the removal of oxides of nitrogen by boiling, proceed as described. The use of more than 0.5 g of sample is not recommended because the chromium(III) sulphate salts that are formed may not dissolve completely when the solution is diluted with water and heated.

11. Because of the high iron content, add 2 g of ascorbic acid before the extraction of tin.

12. The addition of ascorbic acid before the extraction step is not necessary if the sample contains very little iron.

13. The use of more than ~0.25 g of sample is not recommended because a precipitate forms during the extraction step.

14. If silicon is absent, use a Pyrex beaker and omit the addition of hydrofluoric acid.

RESULTS

Calibration solutions

Calibration solutions containing ~5–10% by volume of hydrochloric acid to prevent the hydrolysis of tin are usually used for the determination of tin by AAS because this acid has no effect on the absorption by tin in air–hydrogen or hotter flames.[14,16–18] Initial tests showed that the absorbance of these solutions decreases on standing. However, solutions that are stable for at least 1 week can readily be prepared by adding 0.5% of tartaric acid. This also results in a slight increase in absorbance.

In subsequent work, slightly high results were obtained for tin—after its extraction as the iodide and treatment of the extract and the resulting solution as described in the proposed method—when calibration solutions containing 10% hydrochloric acid and 0.5% tartaric acid, and prepared by dilution of appropriate volumes of freshly prepared standard tin (metal) solution in 50% hydrochloric acid, were used for comparison purposes. It was found that this error can be

avoided by using a stock solution prepared by dissolving tin metal in concentrated sulphuric acid containing hydrogen peroxide, and evaporating the calibration solutions to dryness with nitric, hydrochloric and perchloric acids as described for the sample solutions. This ensures that the tin in the calibration solutions will be present in the same form as in the sample solutions. The removal of the excess of sulphuric acid by evaporation to dryness is necessary because even small amounts enhance the absorption by tin in the nitrous oxide–acetylene flame.

Separation of tin by extraction as the iodide

Although it was found previously[5] that up to 2 mg of tin can be quantitatively extracted into toluene in two successive extractions from a 2*M* sulphuric acid–1.5*M* potassium iodide medium containing tartaric acid to prevent possible hydrolysis of tin, low results were obtained when this procedure was applied to the separation of $\geqslant$2.5 mg. Subsequent work showed that this was because the acid concentration was too low and that up to at least 5 mg of tin can be quantitatively extracted, in two extractions, from a 3*M* sulphuric acid medium.

Back-extraction of tin iodide

Earlier,[5] tin was back-extracted from the toluene phase with dilute sodium hydroxide solution. However, this was not considered to be suitable for the subsequent determination of tin by AAS because of the high sodium content of the resultant solution. Tests in which tin was back-extracted into 6*M* hydrochloric acid, rather than into 0.6*M* hydrochloric acid as described by Terarshima,[14] yielded results that were initially high. However, the absorbance of the resulting 10% hydrochloric acid–0.5% tartaric acid solutions decreased on standing. Further work showed that the high results were caused by the retention of some of the aqueous phase containing potassium iodide in the toluene extract. The potassium in the resultant solution enhances the AAS signal for tin by suppressing ionization of tin in the flame[17,18]. Tests showed that, in a nitrous oxide–acetylene flame, this enhancement is constant for potassium levels from ~250 to $\geqslant$3000 μg/ml. It was later found that this interference from potassium can be eliminated by washing the extract with a strongly acidic dilute potassium iodide solution (9*M* sulphuric acid—0.05*M* potassium iodide)[19] to reduce the amount of entrained potassium iodide, and by adding ~250 μg/ml of potassium to both the sample and calibration solutions. The decrease in absorbance of the solutions on standing may be due to kinetic effects in formation of tin complexes coupled with concomitant effects on the atomization efficiency.

Subsequent work showed that tin can be readily back-extracted by shaking the toluene phase with a mixture of nitric and sulphuric acids, and determined by AAS after evaporation of the resultant solution to dryness and dissolution of the salts as described in the proposed method. This acid mixture is advantageous because the iodide that is retained in the extract is oxidized to iodine which remains in the toluene phase. However, in the presence of arsenic, which is partly co-extracted as the iodide, this procedure yielded low results for tin because of the partial formation of a tin–arsenic compound [probably containing tin(IV) and arsenic(V)] that is insoluble in nitric–sulphuric acid media. Interference from this compound was also observed in previous work involving the determination of arsenic in tin-bearing ores and copper-base alloys.[20] Because the compound is soluble in hydrochloric acid, interference from arsenic can be readily eliminated by adding this acid to the nitric–sulphuric acid solution used for the back-extraction of tin. No loss of tin occurs during the subsequent evaporation of the solution to dryness because it is not volatilized as the chloride in the presence of sulphuric acid.[21] However, if the resultant salts contain arsenic, heat is required for the complete dissolution of the tin–arsenic compound in dilute hydrochloric acid.

Effect of diverse ions

A high concentration (1.5*M*) of potassium iodide and a 2*M* sulphuric acid medium were chosen for the extraction of tin in the gallein method[5] to minimize the co-extraction of antimony and germanium, respectively, which interfere in the determination of tin with gallein. Under these conditions, and from the 3*M* sulphuric acid medium used in this work, only arsenic is significantly co-extracted as the iodide.[19] Thallium forms an insoluble yellow iodide that remains, to a large extent, in the toluene phase. Up to 20 mg of arsenic and 50 mg of antimony will not interfere in the determination of tin by the proposed method when the final solution is diluted to $\geqslant$10 ml. The effects of germanium and thallium were not investigated because more than trace amounts of these elements are not usually present in ores. Lead precipitates as the sulphate and tungsten forms an insoluble compound in the sulphuric acid medium used for the extraction of tin. However, large amounts of lead and up to ~10 mg of tungsten will not interfere if the precipitates are removed by filtration before the extraction step. More than ~100 mg of copper may interfere with the extraction of tin, by precipitating as cuprous iodide. Iron(III) is reduced to iron(II) with ascorbic acid before the extraction step. This prevents its reaction with iodide, which results in the presence of a large amount of iodine in both phases, making the interface difficult to see.

Applications

To test the reliability of the proposed method, it was applied to the analysis of two CCRMP ores that have been certified for tin,[3,4,22] to the CCRMP zinc and lead concentrates, CZN-1 and CPB-1, for which values are given for information purposes,[1,2] and to four diverse CCRMP ores to which a known amount

Table 1. Determination of tin in CCRMP reference ores and concentrates

Sample	Nominal composition, %	Certified value and 95% confidence limits, % Sn	Sn found, %	
			Spectrophotometric gallein method	Atomic-absorption spectrophotometry
MP-1 Zinc–tin–copper–lead ore	19.4 Si, 3.6 Al, 5.7 Fe, 3.4 Ca, 11.8 S, 15.9 Zn, 2.1 Cu, 1.9 Pb, 0.8 As	2.43 (2.32–2.54)	—	2.36†
KC-1 Zinc–lead–tin–silver ore	11.1 Si, 0.8 Al, 16.1 Fe, 28.1 S, 20.1 Zn, 6.9 Pb	0.67 (0.66–0.68)	—	0.651†
CPB-1 Lead concentrate	64.7 Pb, 4.4 Zn, 8.4 Fe, 17.8 S, 0.06 As, 0.4 Sb	0.022 (0.016–0.029)‡	0.019§	0.020, 0.021
CZN-1 Zinc concentrate	44.7 Zn, 7.5 Pb, 10.9 Fe, 30.2 S, 0.03 As, 0.05 Sb	0.0074 (0.0052-0.0096)‡	0.0078§	0.0076, 0.0079
UM-1 Nickel–copper–cobalt ore	17.6 Si, 13.4 Fe, 1.7 Ca, 21.7 Mg, 0.9 Ni, 0.4 Cu, 3.5 S	0.020‖	—	0.020
SU-1 Nickel–copper–cobalt ore	16.2 Si, 5.0 Al, 22.9 Fe, 2.9 Ca, 2.5 Mg, 0.5 Ti, 1.5 Ni, 0.9 Cu, 12.1 S	0.020‖	—	0.018
PR-1 Molybdenum ore	39.2 Si, 2.4 Al, 1.2 Fe, 1.4 Ca, 0.6 Mo, 0.8 S	0.020‖	—	0.020
CD-1 Antimony ore	3.6 Sb, 0.7 As, 32.9 Si, 5.5 Al, 1.4 Ca, 2.8 Fe, 3.1 S	0.020‖	—	0.020

† Mean of 3 values.
‡ CCRMP value given for information only (not certified).
§ Mean of 10 values obtained at CANMET during the interlaboratory certification programme.
‖ 200 μg of tin added to a 1-g sample.

Table 2. Determination of tin in NBS and BCS iron, steel and non-ferrous alloys

Sample	Nominal composition, %	Certified value and range, % Sn	Sn found, %	
			Spectrophotometric gallein method	Atomic-absorption spectrophotometry
NBS-19g Acid open-hearth steel	0.6 Mn, 0.2 Si, 0.4 Cr, 0.03 Nb	0.008 (0.008–0.009)	0.0095	0.0081
NBS-32E Nickel–chromium steel	0.8 Mn, 0.3 Si, 1.2 Ni, 0.7 Cr	0.011*	0.013	0.011, 0.011
NBS-33C Nickel steel	0.7 Mn, 0.3 Si, 3.3 Ni	0.003 (0.003–0.004)	—	0.0025
NBS-36A Chromium–molybdenum steel	0.4 Mn, 0.4 Si, 2.4 Cr, 0.9 Mo	0.011 (0.010–0.012)	0.012	0.010
NBS-55E Open-hearth iron	0.01 As	0.007 (0.006–0.008)	—	0.0054, 0.0054
NBS-101E 18 Chromium–9 nickel steel	1.8 Mn, 0.4 Si, 9.5 Ni, 18.0 Cr, 0.4 Mo	0.020 (0.019–0.023)	—	0.020
NBS-125 High silicon steel	5.0 Si	0.007 (0.005–0.008)	0.0092	0.0066
NBS-152 Basic open-hearth steel	0.8 Mn, 0.2 Si	0.036 (0.035–0.039	—	0.037
NBS-160A 19 Chromium–14 nickel–3 molybdenum steel	1.6 Mn, 0.6 Si, 14.1 Ni, 18.7 Cr, 2.8 Mo	0.013 (0.010–0.017)	—	0.012
BCS-218/2 Carbon steel	0.6 Mn, 0.2 Si, 0.04 As	0.035 (0.032–0.037)	0.034	0.033, 0.033
BCS-273 Mild steel		0.065 (0.060–0.068)	0.065	0.065
NBS-37E Sheet brass	69.6 Cu, 27.9 Zn, 1.0 Pb	1.00 (0.98–1.02)	1.02	0.995
NBS-62D Manganese bronze	59.1 Cu, 37.1 Zn, 1.2 Al, 0.7 Mn	0.38 (0.37–0.40)	0.403	0.397
NBS-157A Copper–nickel–zinc alloy	58.6 Cu, 29.1 Zn, 11.8 Ni	0.021 (0.016–0.026)	0.024	0.022
NBS-158 Silicon bronze	90.9 Cu, 2.7 Si, 2.1 Zn, 1.3 Mn	0.97 (0.96–0.99)	0.985	0.990
NBS-164 Manganese–aluminium bronze	63.8 Cu, 21.9 Zn, 6.2 Al, 4.7 Mn	0.63 (0.60–0.64)	0.664	0.632
NBS-94B Zinc-base alloy	4.1 Al	0.006 (0.005–0.006)	0.0056	0.0055
NBS-87 Silicon–aluminium alloy	6.2 Si, 0.6 Ni, 0.2 Ti	0.063 (0.05–0.077)	0.063	0.063
BCS-268 Silicon–aluminium alloy	4.9 Si, 1.3 Cu	0.03	0.029	0.031
NBS-176 Titanium-base alloy	5.2 Al	2.47*	—	2.49
NBS-360 Zircaloy-2		1.43*	1.44	1.45

* NBS provisional result.

of tin was added. It was also applied to certified reference iron and steel samples and to copper-, zinc-, aluminium-, titanium- and zirconium-base alloys. The results of these analyses are given in Tables 1 and 2.

DISCUSSION

Table 1 shows that the results obtained for the CCRMP reference ores MP-1 and KC-1 are lower than the certified values.[22] However, that obtained for MP-1 is within the 95% confidence limits for the recommended value, and that obtained for KC-1 is very close to the lower confidence limit. The results obtained for the lead and zinc concentrates, CPB-1[2] and CZN-1,[1] are in good agreement with the CCRMP values given for information purposes, and with the mean values obtained by the gallein method during the interlaboratory certification programmes. Those obtained for the CCRMP ores, to which a known amount of tin was added, agree with the amount added. Analysis of these ores by the proposed method showed that they each contain <5 μg of tin per g.

Table 2 shows that the results obtained for the NBS and BCS iron, steel and non-ferrous alloys are in good agreement with the certified values and, where applicable, with the results obtained by the gallein method.

Tin can be quantitatively extracted as the iodide into toluene from $>3M$ sulphuric acid and $<1.5M$ potassium iodide media.[19] However, a high acid concentration is not recommended because iodide is rapidly oxidized by air to iodine in strongly acidic solutions. Considerable heat is also evolved when a fairly concentrated sulphuric acid solution is diluted with water and potassium iodide solution before the extraction step. Extraction from $<1M$ potassium iodide media is also not recommended. This would probably necessitate an increase in the sulphuric acid concentration because the concentration of potassium iodide required for the complete extraction of tin is a function of the acid concentration; it decreases as the acidity increases. More antimony is also co-extracted from dilute iodide media.[19]

Fusion with a mixture of sodium peroxide and sodium carbonate, which decomposes silicates, is recommended for the decomposition of ores and mill products. Several investigators[23,24] have described rapid, routine AAS methods for tin which are based on its separation from the matrix elements by heating with ammonium iodide and dissolution of the sublimed tin iodide in dilute acid. However, depending on the type of tin-bearing minerals present, low results can be obtained by this method because tin bound in a silicate lattice, or in cassiterite entrapped in silicates, is not converted into tin(IV) iodide by this procedure.[12,25]

Greater sensitivity can be obtained for tin by using an air–hydrogen flame.[16] However, a nitrous oxide–acetylene flame was chosen because effects from other elements, anions and acids, which interfere strongly in the air–hydrogen flame,[16,17] are minimized in this flame.[23,26] The 235.4-nm line was used because, with the nitrous oxide–acetylene flame, it provides greater sensitivity for tin than the 224.6- or 286.3-nm lines.[27]

The proposed method is suitable for samples containing as little as $\sim 0.001\%$ of tin if a 1-g sample can be taken. However, the accuracy that can be obtained at this level depends on the magnitude of the reagent blank. In this work, the blank varied from ~ 2 to 8 μg of tin after sample decomposition by fusion, and from ~ 2 to 6 μg after decomposition with acids. The advantages of this method compared with the gallein method[5] are that it is quicker and requires fewer and less exacting manipulations, and that a relatively large amount of antimony will not interfere.

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THERMAL DECOMPOSITION OF CHEMOTHERAPEUTIC PREPARATIONS AND EXPECTORANTS

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Summary—The thermal decomposition of 31 drugs containing sulphonamides, antituberculous agents, dyes, an oxidant, carboxylic acid derivatives and formaldehyde, expectorants and codeine phosphate has been studied by differential thermal analysis (DTA) and thermogravimetry (TG). The DTA, TG and differential TG curves have been used for identification of the drugs and their qualitative and quantitative analysis. The results for 18 of the drugs were in good agreement with those calculated from the formulation.

The determination of active components in pharmaceutical dosage forms by conventional analytical procedures is sometimes difficult owing to the necessity for separation of the matrix materials. This inconvenience can sometimes be overcome by using thermoanalytical techniques.

Differential thermal analysis (DTA), thermogravimetry (TG) and differential thermogravimetry (DTG) have been used for the quantitative analysis of mixtures containing *N*-butylscopolamine and novalgine[1,2] and for assaying water in model powders and granulates consisting of lactose, gelatin and/or caffeine, amidopyrine and phenacetin.[3] γ-Hexachlorocyclohexane in talc has been determined by isothermal TG and by measuring DTA peak areas.[4]

The complicated composition of pharmaceutical dosage forms together with lack of information about it often makes it impossible to use DTA, differential scanning calorimetry (DSC) and TG in the quantitative analysis of powders, capsules and tablets containing analgesics,[5] antacids[6] and vitamins.[7] However, our previous studies, supported by analysis of the thermal decomposition of both the active components and the excipients, in conjunction with knowledge of the composition of drugs, have shown the usefulness of the DTA, TG and DTG curves for qualitative and quantitative investigation of both hard and soft formulations.[8] These studies also enabled us to specify those thermal processes which permit assay for an active component.

In this article, the results of these studies are presented, with reference to preparations containing sulphonamides, antituberculous drugs, dyes, an oxidant, derivatives of carboxylic acids and formaldehyde, expectorants and Codeinum phosphoricum tablets.

EXPERIMENTAL

Reagent and materials

The drugs tested were all manufactured by the Pharmaceutical Works "Polfa" at various factories in Poland.

Both the active components and the excipients of all these drugs conformed to the purity requirements established for pharmaceutical substances.

Apparatus and technique

A MOM OD-130 derivatograph was used. All runs were made under identical conditions which were as follows: 100-mg samples were placed in Pt crucibles (9.5 mm diameter) and heated to 600–1000° under atmospheric pressure at a rate of 5 deg/min, with α-Al_2O_3 as a reference.

All materials, except for powders and ointments, were finely ground. Three tablets were used for a single determination.

To detect transient decomposition products, the heating was interrupted at a predetermined temperature and the contents of the crucible were analysed by thin-layer chromatography, infrared spectroscopy and elemental analysis. The interruption occurred at temperatures corresponding either to horizontals or distinct inflections in the TG and DTG curves, which indicated completion of a given decomposition step. Owing to the complexity of the drugs, the results obtained in this way gave only preliminary information as to the composition of the residues, and were not taken into account in further considerations.

RESULTS AND DISCUSSION

The chemotherapeutic preparations, expectorants and the Codeinum phosphoricum tablets all had complex composition. About 45% of the drugs contained 3–5 components, about 39% had 6–9 components and the rest were two-component mixtures. In multicomponent preparations the content of individual components varied widely, *e.g.*, they frequently occurred in ratios from 1:250 to 1:500, and in some cases the ratio ranged from 1:5000 to 1:10000. In the preparations studied the major component was usually an active one. In 71% of the drugs only one active component was detected, in about 16% there were two active components, and in about 13% three or more.

From these data it might be concluded that the complex composition of the drugs (different elemental composition, chemical structure, molecular weight and physical and chemical properties of the components) should markedly affect the shape of the

DTA, TG and DTG curves for the thermal decomposition of these drugs. Their decomposition is entirely different in character from that of the pure components. In only a few cases, namely decomposition of the sulphonamide drugs containing a relatively high percentage of the active components, does the shape of the curves closely resemble that for the pure sulphonamides.

Qualitative analysis

The identification of the components of sulphonamide drugs, except for the Pabiamid dusting powder and the Trisulfan tablets, was easy owing to the fact that the sulphonamide content exceeded 50%. However, the similar mechanism of their thermal decomposition, due presumably to the similarity of chemical structure of sulphonamides, rendered difficult the identification of the active components of the drugs on the basis of the TG curves. The DTA curves proved more useful, especially the temperature ranges, peak areas and shapes of the endothermic peaks associated with polymorphic transformations and melting of individual sulphonamides.

The steps of the thermal decomposition of sulphanilamide are well developed on the decomposition curves of the Pabiamid dusting powder because its other component is thermally stable over the temperature range studied. Because the trimethoprim content is low, thermal effects due to its decomposition are absent on the decomposition curves of the Biseptol 120-mg and 480-mg tablets. On the other hand, the close similarity in the thermal decomposition of sulphathiazole, sulphamethazine and sulphacetamide means that there are no differences between the decomposition steps of the individual drugs, thus precluding the possibility of identification of any component of the Trisulfan tablets. In all the other drugs it was possible to identify the main sulphonamide component (Fig. 1).

Except for the Etionamid suppositories, the content of the active components in all remaining antituberculous drugs exceeds 50%, thus facilitating qualitative analysis. This is particularly evident in the thermal decomposition of the PAS-Kalium granulate and the PAS-Natrium and Napashin tablets. Individual steps of the thermal decomposition of the potassium and sodium *p*-aminosalicylate are well developed on the DTA, TG and DTG curves of the respective drugs. Thermal effects accompanying decomposition of isoniazid are absent on the decomposition curves of the Napashin and Isoniazidum tablets. In the latter case the effect is concealed by that of the decomposition of starch. A similar effect was observed with the Pyrazinamidum tablets. On the other hand, owing to the narrow range of evaporation of ethionamide, which only slightly overlapped the peaks of the remaining components of the Etionamid tablets and suppositories, this compound could be detected in both drug forms. It is to be emphasized that the endothermic peaks associated with the melting of isoniazid and pyrazinamide in the Isoniazidum and Pyrazinamidum tablets, and the melting and vaporization of ethionamide, are well developed on the DTA curves of the drugs (Fig. 2).

Owing to the low content of the dyes and the oxidant tested, in chemotherapeutic preparations, the shape of the DTA, TG and DTG curves is determined by decomposition of the excipients. Thermal effects

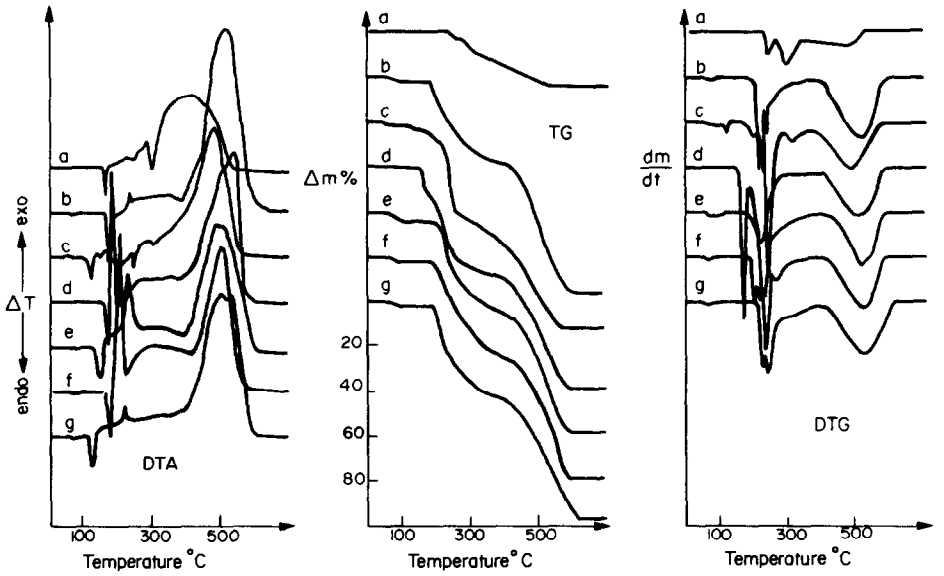

Fig. 1. DTA, TG and DTG curves of the thermal decomposition of sulphonamide drugs. *a*—Pabiamid (DP), *b*—Sulfamethazinum (T), *c*—Urenil (T), *d*—Amidoxal (T), *e*—Biseptol 480 mg (T)*, *f*—Madroxin (T), *g*—Trisulfan (T).

* DTA, TG and DTG curves of the thermal decomposition of Biseptol 120 mg (T) resemble closely those of Biseptol 480 mg (T). For symbols in brackets see Table 1.

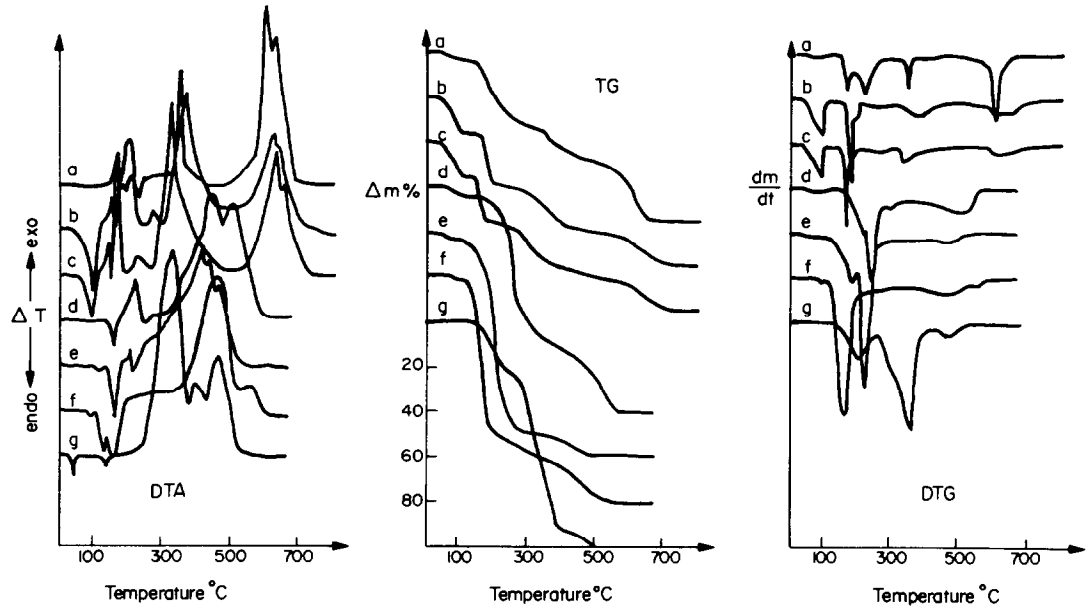

Fig. 2. DTA, TG and DTG curves of the thermal decomposition of antituberculous drugs. *a*—PAS-Kalium (G), *b*—PAS-Natrium (T), *c*—Napashin (T), *d*—Isoniazidum (T), *e*—Pyrazinamidum (T), *f*—Etionamid (T), *g*—Etionamid (S). For symbols in brackets see Table 1.

due to the thermal decomposition of the active components do not appear on the thermal analysis curves of these drugs, except for the Nitrofurantoin and Pantocidum tablets. The decomposition of nitrofurantoin, occurring over a very narrow temperature range, permits detection of the compound in tablets. Well-defined endothermic peaks due to the melting of the excipients appear on the DTA curves (Fig. 3).

Well-developed steps in the thermal decomposition of active components facilitate their identification in drugs. This was confirmed by analysing the DTA, TG and DTG curves of chemotherapeutic drugs containing derivatives of carboxylic acids and formaldehyde. The active components of all these drugs could be readily identified, the steps concerned being the dehydration of boric acid in Ung. Acidi borici ointment and the dehydration of sodium tetraborate and decarboxylation of sodium hydrogen carbonate in the Gargarin powder, vaporization of phenyl salicylate from the Salolum and Salotannal tablets, and of urotropine from the Urotropinum tablets. The endothermic peaks due to melting and the aforementioned processes were well defined on the DTA curves. The thermal decomposition curves for sodium benzoate closely resembled those of the decomposition of the Natrium benzoicum tablets, thus permitting their identification (Fig. 4).

In the group of expectorants, the results of investi-

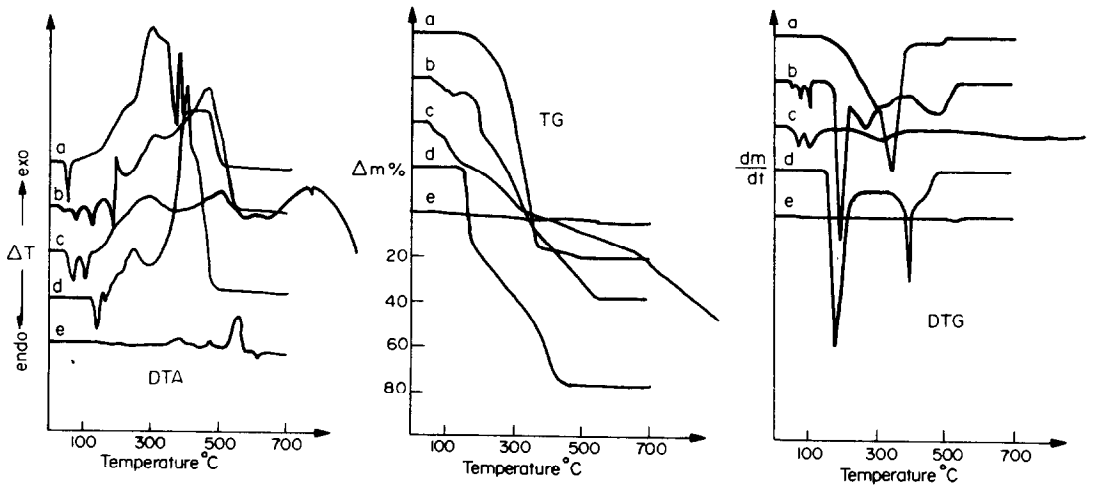

Fig. 3. DTA, TG and DTG curves of the thermal decomposition of drugs containing dyes and a drug containing an oxidant. *a*—Nitrofurazon (DP), *b*—Nitrofurantoin (T), *c*—Rivanolum (T), *d*—Akron (T$_s$), *e*—Pantocidum (T). For symbols in brackets see Table 2.

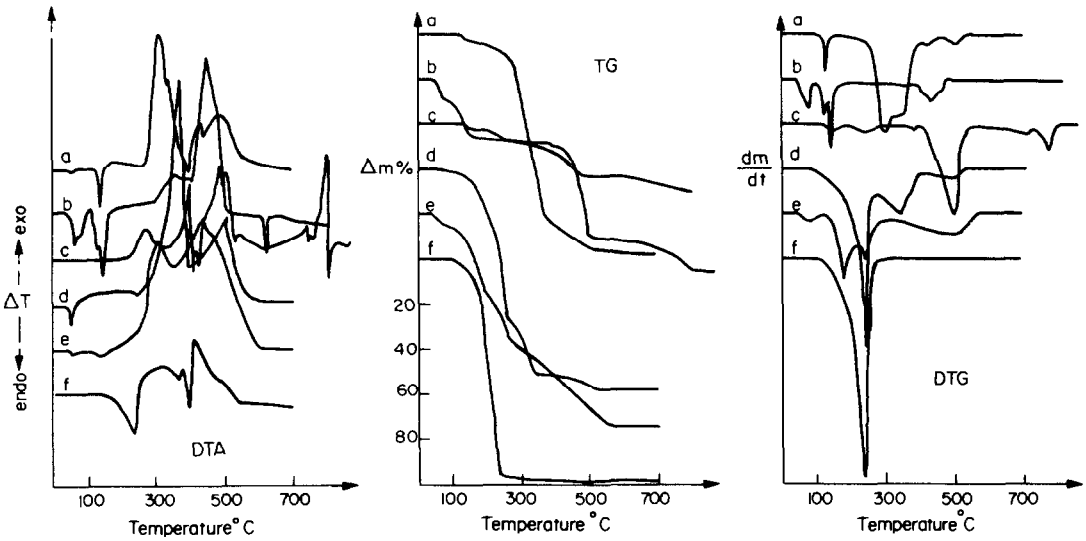

Fig. 4. DTA, TG and DTG curves of the thermal decomposition of drugs containing carboxylic acid derivatives or formaldehyde: *a*—Ung. Acidi borici (O), *b*—Gargarin (P), *c*—Natrium benzoicum (T), *d*—Salolum (T), *e*—Salotannal (T), *f*—Urotropinum (T). For symbols in brackets see Table 2.

gation of the Codeinum phosphoricum tablets (antitussives) are included. The DTA, TG and DTG curves allow the identification of codeine phosphate, except in the Thiocodin and Azarina tablets, where its content is too low for detection in this way. Individual steps of the thermal decomposition of potassium guaiacolosulphonate are well defined and permit its identification in the Thiocodin and Kalium guajacolosulfonicum tablets. On the other hand, the decomposition of the effervescent tablets of Sal Ems factitium is accompanied by a reaction between the components, tartaric acid and sodium hydrogen carbonate, and this is confirmed by an endothermic DTA peak associated with the loss of the volatile products carbon dioxide and water, which overlaps the exothermic effect of the reaction. Moreover, the individual decomposition steps of the sodium tartrate formed in this reaction are also seen on the curves. In the Azarina tablets, none of the pharmacognostic components present could be identified. The final product of the thermal decomposition of these drugs was mineral salts that are stable up to 1000° (Fig. 5).

Quantitative analysis

The very varied composition of chemotherapeutic tablets, expectorants and the Codeinum phosphoricum tablets makes their quantitative analysis far more difficult than the qualitative analysis. In about 60% of the drugs, the percentage of the active component exceeded 50% and in another 20% of them exceeded 25%. Hence, it was possible to assay the active components in the majority of the drugs on the basis of the loss in weight indicated on the TG curves. The DTG curves allowed distinct differentiation of suc-

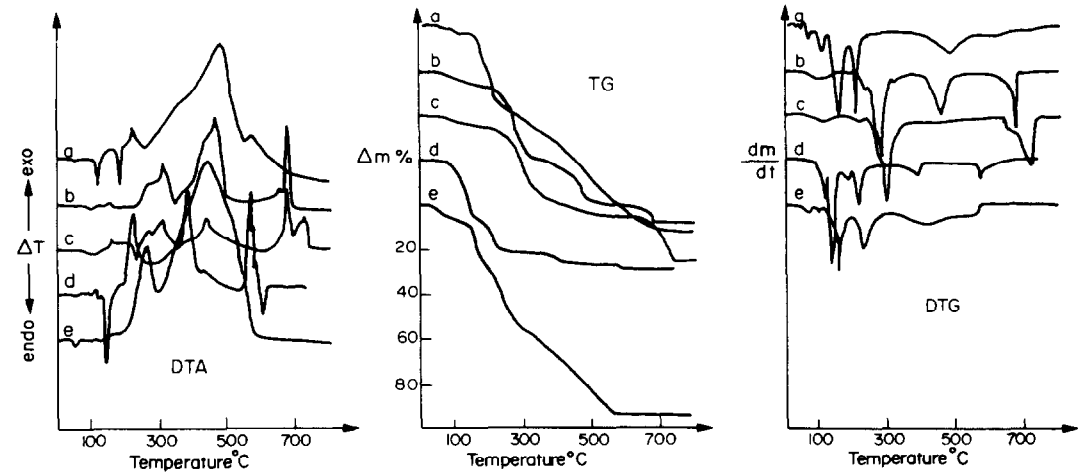

Fig. 5. DTA, TG and DTG curves of the thermal decomposition of expectorants. *a*—Codeinum phosphoricum (T), *b*—Thiocodin (T), *c*—Kalium guajacolosulfonicum (T), *d*—Sal Ems factitium (T_e), *e*—Azarina (T). For symbols in brackets see Table 2.

Table 1. Results of analysis of the thermal decomposition of the sulphonamide and antituberculous drugs

Trade name	Drug form	Active component	Fig	Temperature intervals, C	Active component, % Found	Active component, % Calc.	Residue in crucible, % Found	Residue in crucible, % Calc
Pabiamid	DP	Sulphanilamide	1a	220–530	23 0	25 0	77 0	75 0
Sulfamethazinum	T	Sulphamethazine	1b	190–280	78 1	76 9	3 5	2 4
Urenil	T	Sulphonylurea	1c	160–215	87 8	87 7	7.0	2 4
Amidoxal	T	Sulphafurazole	1d	160–280	87 5	87 7	1 5	0 9
Biseptol 120 mg	T	Sulphamethoxazole				58 8	3 5	2 9
		Trimethoprim				11 8		
Biseptol 480 mg	T	Sulphamethoxazole	1e			58 8	1 0	2 9
		Trimethoprim				11.8		
Madroxin	T	Sulphadimethoxine	1f	180–270	89 7	83 3	2 0	2 7
Trisulfan	T	Sulphathiazole	1g			33 3	3 0	3 9
		Sulphamethazine				25 0		
		Sulphacetamide				25 0		
PAS-Kalium	G	Potassium *p*-aminosalicylate	2a	130–180	69 6	70 0	25 0	25 3
PAS-Natrium	T	Sodium *p*-aminosalicylate	2b	40–110	91 2	92 6	25 0	26.9
Napashin	T	Sodium *p*-aminosalicylate	2c	40–100	88 2	90 9	26 0	24 7
		isoniazid				2 4		
Isoniazidum	T	Isoniazid	2d			50.0	0 0	0.0
Pyrazinamidum	T	Pyrazinamide	2e			76 9	1 5	2 5
Etionamid	T	Ethionamide	2f	120–200	68.0	62 5	0 5	0 8
Etionamid	S	Ethionamide	2g	130–250	23 4	20 0	0 0	0 0

Key: DP—dusting powder, G—granulate, T—tablet, S—suppository.

cessive steps of the thermal decomposition. The amount of a particular compound was calculated by simple proportion from the losses in weight of the pharmaceutical and the analogous losses in weight of the pure compound. The amount of the residue left in the crucible was read directly from the TG curve. This procedure has been reported in detail elsewhere.[8]

The values obtained in this way are shown as "found" in Tables 1 and 2, and those denoted as "calc." were calculated from information supplied by the manufacturers.

The temperature intervals listed in Tables 1 and 2 refer to the losses in weight recorded in the TG and DTG curves shown in Figs. 1–5, on which the analyses were based.

The dehydration of sodium *p*-aminosalicylate was used for its determination in the PAS-Natrium and Napashin tablets. The boric acid content of Ung. Acidi borici ointment was determined on the basis of its condensation step. The decarboxylation of sodium hydrogen carbonate was used for assaying it in the Gargarin powder. The total content of tartaric acid and sodium hydrogen carbonate in the Sal Ems factitium tablets was determined from the loss in weight due to volatile reaction products and to decarboxylation of the sodium hydrogen carbonate remaining in excess. Owing to the proximity of the temperature ranges of the two processes, discrimination of the corresponding weight losses is problematic.

Use was also made of steps associated with the

Table 2. Results of analysis of the thermal decomposition of drugs containing dyes, an oxidant, carboxylic acid derivatives, formaldehyde, expectorants, and of Codeinum phosphoricum tablets

Trade name	Drug form	Active component	Fig	Temperature intervals, C	Active component, % Found	Active component, % Calc	Residue in crucible, % Found	Residue in crucible, % Calc
Nitrofurazon	DP	Nitrofurazone	3a			0 2	0 0	0 0
Nitrofurantoin	T	Nitrofurantoin	3b	150–215	30 8	28 6	2 0	2 7
Rivanolum	T	Ethacridine lactate	3c			50.0	15 0*	12 7
Akron	T_s	Ethacridine lactate	3d			0 2	3 0	1 2
Pantocidum	T	Pantocide	3e	100–540	4.5	4 6	95 5	95 4
Ung. Acidi borici	O	Boric acid	4a	110–140	9 0	10 0	4 5	6 4
Gargarin	P	Sodium hydrogen carbonate	4b	40–80	34.0	35 0	57 0†	61 1
		Sodium tetraborate				35 0		
		Other mineral salts						
Natrium benzoicum	T	Sodium benzoate	4c	130–540	100 0	100 0	36 5	36 8
Salolum	T	Phenyl salicylate	4d	80–260	74 4	76 9	2 0	2 3
Salotannal	T	Phenyl salicylate	4e			36 0	6 0	3 7
		Tannin proteinate				36 0		
Urotropinum	T	Urotropine	4f	100–250	98 0	98 2	1 0	1 8
Codeinum Phosphoricum	T	Codeine phosphate	5a			25 0	8 5	3 8
Thiocodin	T	Potassium guaiacolosulphonate	5b			75 0	33 0	27 6
		Codeine phosphate				3 8		
Kalium Guajacolosulfonicum	T	Potassium guaiacolosulphonate	5c			86 2	34 0	26.5
Sal Ems factitium	T_s	Effervescent mixture	5d	40–170	73 4	79.9	51 0	50 4
		Other mineral salts						
Azarina	T	Codeine phosphate	5e			1 3	6.0	0 0
		Pharmacognostic materials						

Key: DP—dusting powder, P—powder, T—tablet, T_s—suction tablet, T_e—effervescent tablet, O—ointment.
* Residue at 900°.
† Residue at 500–600°.

formation of a transient product. The knowledge of the composition and chemical structure of a decomposition product of potassium *p*-aminosalicylate enabled us to determine its content in the PAS-Kalium granulate. In the Sulfamethazinum, Urenil, Amidoxal, Madroxin, Nitrofurantoin and Natrium benzoicum tablets the composition and chemical structure of transient products could not be determined, however. The content of the active components was determined by comparison of weight losses taking place between two successive inflections on the TG and DTG curves of a given drug and of an authentic sample.

The step due to vaporization of ethionamide, phenyl salicylate and urotropine was used for the determination of their contents in the Etionamid, Salolum and Urotropinum tablets, respectively, as well as in the Etionamid suppositories. The vaporization occurs at relatively low temperatures and overlaps only slightly the temperature range of decomposition of the excipients. When the excipients do not decompose over the temperature range studied, the content of active component can be determined from the loss in weight associated with its total decomposition. In this way sulphanilamide in the Pabiamid dusting powder and pantocide in the Pantocidum tablets were determined.

CONCLUSIONS

This study has confirmed the possibility of employing the thermal decomposition curves of chemotherapeutic drugs, expectorants and Codeinum phosphoricum tablets for qualitative and quantitative analysis. Identification of individual components is most conveniently accomplished on the basis of simultaneously recorded DTA, TG and DTG curves of their thermal decomposition, by using temperature ranges, peak areas and peak shapes of the corresponding DTA peaks, and weight losses from the TG curves. In quantitative assays only the TG and DTG curves are used because they permit discrimination of individual steps of the thermal decomposition of the active components and precise determination of the weight losses.

On the basis of the TG and DTG curves the content of the active components has been determined in 18 out of 31 drugs without the necessity to separate the excipients. Unfortunately, the determination by this method of very small amounts of active components (usually strongly acting) is practically impossible because the thermal effects of their decomposition cannot be detected. The results obtained in this study are in good agreement with those calculated from the manufacturer's data.

Attention should also be paid to the possibility of using the DTA, TG and DTG curves for identification of particular drug forms.

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MICROPROCESSOR-CONTROLLED SYSTEM FOR AUTOMATIC ACQUISITION OF POTENTIOMETRIC DATA AND THEIR NON-LINEAR LEAST-SQUARES FIT IN EQUILIBRIUM STUDIES*

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Summary—A microprocessor-controlled potentiometric titration apparatus for equilibrium studies is described. The microprocessor controls the stepwise addition of reagent, monitors the pH until it becomes constant and stores the constant value. The data are recorded on magnetic tape by a cassette recorder with an RS232 input–output interface. A non-linear least-squares program based on Marquardt's modification of the Newton–Gauss method is discussed and its performance in the calculation of equilibrium constants is exemplified. An HP 9821 desk-top computer accepts the data from the magnetic tape recorder. In addition to a fully automatic fitting procedure, the program allows manual adjustment of the parameters. Three examples are discussed with regard to performance and reproducibility.

Potentiometric titrations are widely used to study the stoichiometry and stability of metal complexes.[1] Generally, solutions containing ligand and metal ions in different ratios and at different total concentrations are titrated with a suitable base. The titration can be followed with an electrode responsive to pH, or the metal ion or the ligand. In order to obtain reliable results the collection of a large number of precise data is required.

The use of microprocessors for automatic control of titration operations and for digital data-acquisition has now become readily available. Several systems[2–4] have been described and several automatic titrators are now commercially available.[5] However, these are not suitable for equilibrium studies, since they are programmed for end-point titrations. Recently, two papers describing versatile microprocessor-controlled titrators have appeared. Leggett[3] uses an Intel 8080A microprocessor to control the titration steps, to read the pH-values, once they are constant, and to store the data, which can be printed out or recorded on magnetic tape. Minor changes in the software are possible since the information is on programmable read-only memory (PROM). A second very flexible system was developed by Wu and Malmstadt[4] who used an ADD8080 microprocessor.[6] This system is capable of handling spectrophotometric, potentiometric and amperometric titrations. The programs are written in BASIC.

Once the data from a titration have been collected, programs are necessary for the calculation of the equilibrium constants. Several non-linear methods for the refinement of titration curves have been published and their advantages and disadvantages discussed.[7] All of them must be run on big computers with large memories.

The present paper describes a fully automatic titrator based on a Zilog Z80 microprocessor chip, which drives the burette, reads the pH-values and stores them on magnetic tape. A program based on Marquardt's extension of the Newton–Gauss method is used to calculate the "best" equilibrium constants, on a Hewlett-Packard HP9821 desk-top calculator. Examples are given to demonstrate the performance and reproducibility attainable.

EXPERIMENTAL

Apparatus

Titration system. The titration system (Fig. 1) consists of a digital pH-meter (Metrohm E600) with a BCD-output, a 1-ml motor-driven burette (Metrohm E415) capable of delivering the reagent in 0.01-ml portions, a controlled-temperature titration vessel, a magnetic stirrer, a thermostat (Haake FS), a Dolphin microprocesssor system with parallel and serial interfaces (Stoppani Ltd.) and a magnetic tape recorder (Zinniker ZE601) with FSK modem 1200 band data transfer and an RS232 compatible input–output.

Operation. The program is started and the following instructions read in: (1) allowed pH tolerance (generally 0.001 or 0.002 pH units), (2) user identification, (3) and (4) experiment numbers, (5) volume of each addition in multiples of 0.01 ml and (6) number of additions. After this the titration proceeds automatically.

A simple interface card (Fig. 2) was developed, consisting of an address decoder which activates the interface once it is called by the program. Two 74LS373 data buffers store each two of the four digits of the BCD output of the pH-meter and transfer them to the data bus of the microprocessor when the output control is set low by the 74LS138 address decoder. On the interface card there is also a relay, which switches the burette motor on when the processor asks for a further addition of reagent. This is

* Programs and constructional details available on request.

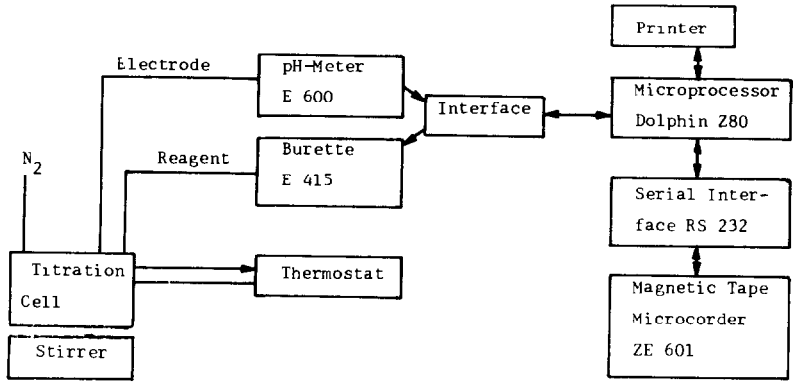

Fig. 1. Titration system with microprocessor.

done by a fast change of the $6 output of the microprocessor, which activates the timing circuit NE555 (used as a missing pulse detector), closing the relay.

The software is written in CALM Z80 and translated into machine language by a cross assembler, available from the University computing centre.[8] The program (Fig. 3) is stored on an EPROM 2708 and is 300 bytes long. It needs three subroutines which determine the waiting time between two pH-readings, read the pH-value and display it. In addition, monitor subroutines are used for starting, writing on and stopping the magnetic tape. Once the control data have been read in and printed out for checking purposes the first pH-value is accepted from the 74LS373 data buffers. The microprocessor then waits for 15 sec, reads the pH-value again and compares it with the previous value. If the two do not differ by more than the pH tolerance ΔpH, the pH value and a control code (zero) are stored in the stack. However, if they differ by more than ΔpH the waiting subroutine is called again. At the end of each waiting cycle the time is doubled until a total waiting time of 4 min is reached. After each waiting interval the pH is read and compared with the previous value. If after 4 min the pH is still not constant, the pH-value is stored with the control code set to 1, in order to identify the less reliable values. During the waiting subroutine the elapsed time and the three lowest digits of the pH are displayed. After the pH and its code have been stored, the number of points is decreased. As long as this number is not zero the microprocessor activates the burette to add the next portion of reagent and the cycle starts again. At the end all stack registers are transferred and stored on magnetic tape. The data obtained can then be read from the recorder by any computer with an RS232 interface or, after reloading of the data into the memory of the microprocessor, by an HP9821 desk-top calculator through the parallel interface of the microprocessor and the HP11203A BCD-input interface. A specific program was written for this type of data transfer, whereby the slower HP9821 calculator is the master and the microprocessor is the slave. The calculator reads the data and creates a data file containing all the information necessary for the curve-fitting procedure.

Chemicals

Benzoic acid was recrystallized from water; 1,4,8-triazacyclodecane (TACD) was prepared and checked for purity as described elsewhere[9] and 1,4,7-triazacyclononane trihydrochloride[10] (TACN) was converted into the free base and used as an aqueous stock solution of its bisulphate. $Cu(CH_3CN)_4BF_4$[11] was synthesized by the procedure given for $Cu(CH_3CN)_4ClO_4$.[12] All other chemicals were of analytical grade and used without further purification.

Titration

The digital pH-meter (Metrohm E600) was calibrated and the calibration checked by titrating a mixture of dilute nitric and acetic acids. Then the following were titrated, at least twice: (1), 50 ml of 5×10^{-3} *M* benzoic acid in 0.1*M* potassium chloride; (2), 50 ml of 3×10^{-3}*M* TACD with 45% and 90% of the equivalent amount of Cu^{2+} and 100 ml of 1.5×10^{-3}*M* TACD with 45% and 90% of the equivalent amount of Cu^{2+} in 0.5*M* potassium nitrate; (3), 25 ml of a mixture that was 4×10^{-3}*M* in TACN and 2×10^{-3}*M* in $Cu(CH_3CN)_4BF_4$ in 16% acetonitrile at $I = 0.2$ (Na_2SO_4).

Calculation procedure

The stability constants are calculated by the computer program MARFIT which consists of the following parts.

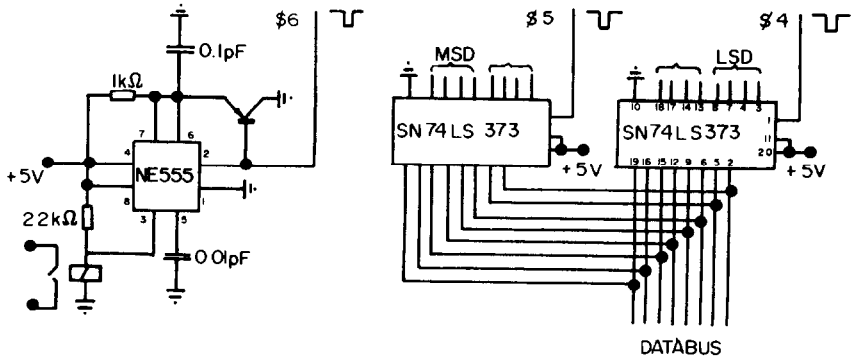

Fig. 2. Interface card for pH-reading and burette-motor activation relay.

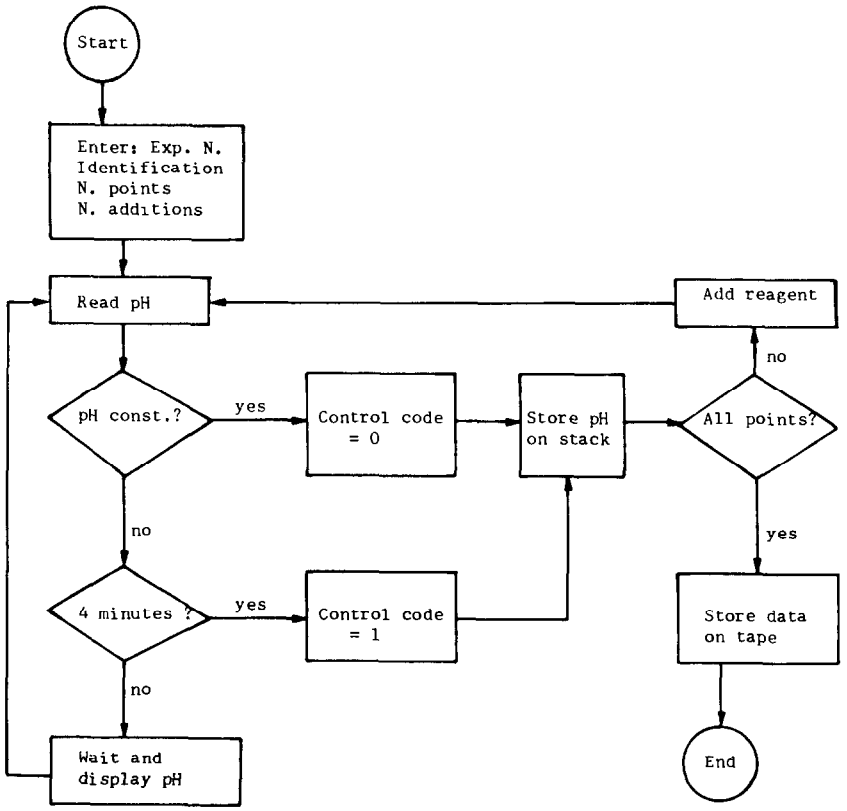

Fig. 3. Data-acquisition program flow-chart for pH (or mV) titrations.

(*a*) Data input and subroutine needed to describe the equilibrium system. The program can accept either one or several titration curves.

(*b*) Adjustment of each parameter and plotting of the calculated curve to visualize the changes induced.

(*c*) Calculation of the "best" parameters by minimizing the error-square sum (1),

$$SQ = \sum_i (ml_{obs,i} - ml_{calc,i})^2 \qquad (1)$$

by the Newton–Gauss algorithm[13] supplemented by the Marquardt technique.[14]

(*d*) Results are printed out and stored on magnetic tape. Another program then allows plotting of any combination of titration curves.

The input consists of the data files generated after the transfer from the microprocessor to the HP calculator and contains as information the identification, the pH-values pH_i and the corresponding values of the volume of reagent $ml_{obs,i}$ (i up to 45 per file), a series of constants k_e (volume, concentration, known equilibrium constants, ...) and the parameters p_j (unknown equilibrium constants).

For each mathematical model describing a chemical system a specifically tailored subroutine is needed by the program making optimum use of the limited memory of the calculator. Its main advantages are increased speed and reliability of convergence,[15] although general subroutines might be easier to handle on big computer systems.[16]

If the option of manual adjustment is set, the program plots the experimental points, draws the theoretical curve and calculates SQ [equation (1)] for the given set of parameters. It then asks for a new estimate of a parameter and repeats the drawing and calculation. The visual interaction allows a rough fit and rapidly gives reasonable estimates for the non-linear least-squares procedure. When no further manual adjustment is needed the calculator starts the automatic search for the minimum of SQ. The shifts of the parameters and SQ are calculated alternately until the relative change in SQ is less than 0.001. If, however, the system becomes unstable the Marquardt technique is introduced. The Marquardt parameter is first chosen to be equal to the largest diagonal element of the correlation matrix and is added to all diagonal elements. After each step of convergence it is reduced by a factor of 3. In the case of divergence it is increased by a factor of 3. Only SQ values are printed out after each iterative cycle, but optionally all parameters and their shifts can be obtained if inspection of the convergence process is desired.

The subroutine calculates the concentrations of the species and the equivalents of reagent added. Depending on the nature of the species considered, the solutions for (2) and (3)

$$C_L = \sum_k [H_k L] + \sum_m \sum_n \sum_p p[M_m H_n L_p] \qquad (2)$$

$$C_M = [M] + \sum_m \sum_n \sum_p m[M_m H_n L_p] \qquad (3)$$

can be obtained either by solving a quadratic equation (if only complexes of the type MLH_n are present) or by using the Newton–Raphson method.[15] Once the minimum of SQ is found, the "best" parameters with their standard deviations and the values of pH, ml_{obs} and ml_{calc} are printed and stored on tape for later use (Fig. 4).

RESULTS AND DISCUSSION

Since the titration system was designed to obtain data for equilibrium studies, adding fixed amounts of reagent in multiples of 0.01 ml is adequate. End-point

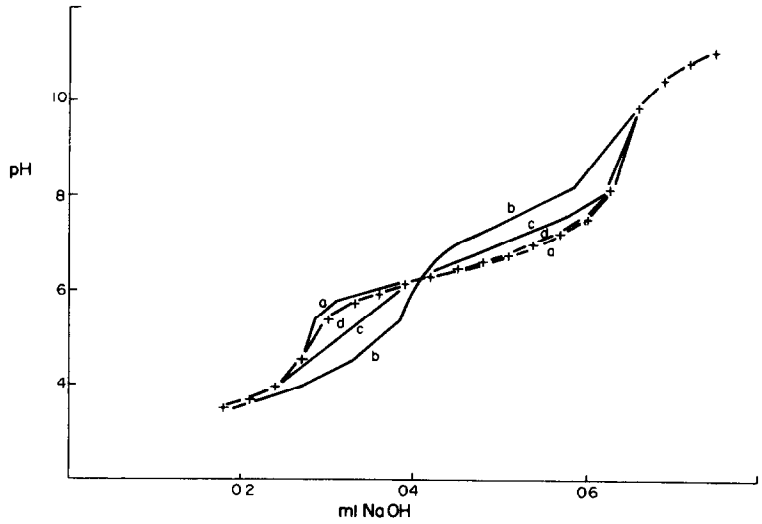

Fig. 4. Interactive estimation and refinement of parameters for the Cu^+/TACN system. Curve *a*, $CuTACN^+$ with log $K = 8.23 \pm 0.04$; *b*, $CuTACN^+$ with log $K = 8.23$ and $CuTACNH^{2+}$ with log $K = 2$; *c*, with log K values 8.23 and 3 respectively; *d*, best fit with log K values of 8.28 ± 0.02 and 3.79 ± 0.05 respectively. Experimental points shown as crosses.

values, if required, could still be obtained by fitting the titration points to a theoretical curve. A pH-meter capable of measuring to 0.001 pH is necessary to follow the pH-drift in a relatively short time in order to decide whether a pH-value is constant and acceptable, before proceeding. The pH-values are stored when the difference between two subsequent readings is equal to or less than 0.001 pH. The time intervals of 15, 30, 60 and 120 sec, after which the pH is read in and compared with the previous value, proved appropriate for most titrations. The pH is stable after 15 sec except near the equivalence point. The maximum time of 4 min, after which the pH is accepted even if not constant, was programmed as a safety measure in order to avoid an undesired stop of the data-acquisition in the event of a pH-drift over a longer time.

The titration procedure consists of the following steps.

(*a*) The pH-meter is repeatedly calibrated with two buffers of pH 4 and 7 at constant temperature, until the response slope and calibration voltage are constant.

(*b*) A mixture of nitric and acetic acids is titrated to check the calibration of the pH-meter. From this control titration α_H and pK_w can also be determined and used for the following experiments.

(*c*) The titrations for the equilibrium study are then run under identical conditions. Duplicates which do not agree within 0.01 pH in their buffer regions are rejected.

(*d*) The minimum experimentation used to study metal-ion complexation equilibria consists of two sets of four curves at two total concentrations each with two different metal-to-ligand ratios. This is necessary to be able to distinguish between monomeric and polymeric compounds, as well as to determine the exact stoichiometry.

The experimental data stored on magnetic tape by the microprocessor are transferred to the desk-top calculator. Additional information such as the volume, α_H, pK_w, known equilibrium constants and estimates of the unknown are read in and a new file compatible with the non-linear least-squares program MARFIT is created and stored on the magnetic tape recorder. Before a complete calculation is performed, each single titration curve is analysed with the computer, the manual adjustment option being used. This allows checking of the data and identifying which of the unknown parameters may be changed to obtain a better fit. With this type of interactive parameter refinement it is possible to fit the experimental points to a degree which then permits the final numerical treatment. It is also possible to see whether the model chosen is able to fit the data. As pointed out by Gans,[17] both a good fit and a model appropriate to the system under investigation are required. With our program and the manual adjustment it is easy to test several models and to see which additional species are necessary for a better fit.

Once a model and meaningful estimates of the equilibrium constants have been found, the non-linear least-squares calculation can be started. If only one titration curve is involved, it is possible to stop the manual adjustment and start the automatic refinement at once. If, however, several curves are to be calculated together a modified program must be used.

The program MARFIT contains the Newton–Gauss–Marquardt algorithm. It has the speed of the Newton–Gauss approximation, and when it diverges the Marquardt parameter is introduced and increased

Table 1. Equilibrium constants obtained from the microcomputer-controlled titration system with the non-linear least-squares program MARFIT

System	Constants*		No. of titrations	α_H	pK_H	Temperature °C	I
Benzoic acid	$pK_H = 4.09_0 \pm 0.01$	(4.08_2)†	4	0.837	13.82	25	0.1(KCl)
Cu^{2+}/TACD§	$pK_1 = 16.22 \pm 0.01$ (16.14)[9]	$pK_2 = 10.34 \pm 0.02$ (10.26)[9]	4 + 4‡	0.877	13.85	25	0.5(KNO_3)
		$pK_3 = 14.51 \pm 0.02$ (14.52)[9]					

Mixed constants with proton activity and molar concentrations.
† $pK_H = 4.00_5$ given by Leggett[3] was converted into a mixed constant by using $\alpha_H = 0.837$.
§ $K_1 = [Cu^{2+}][TACD]/[CuTACD^{2+}]$, $K_2 = [CuTACD^{2+}][TACD]/[Cu(TACD)_2^{2+}]$, $K_3 = [CuTACD^{2+}]^2[OH^-]^2/[(CuTACDOH)_2^{2+}]$.
‡ Two sets of four curves each were calculated.

until convergence is attained. In the case of the complexation of Cu^{2+} with TACD, for which the Newton–Raphson iteration procedure is used in the subroutine to calculate the concentrations of the species, the time required for 4 curves with a total of 120 experimental points is about 40 min.

Three examples are now discussed. Benzoic acid has been titrated and the mean pK_H value of four titrations (Table 1) shown to agree with the literature value.[3] The calculation is simple, since the species present are obvious and a guess of the pK_H is trivial. The standard deviation in ml of reagent ($\sigma_{ml} = 0.0012$–0.0014 ml for 33 points) is about 0.1% of the total volume used for the titration and comparable to the value of 0.001 ml given by the manufacturer.

In the second system three species have been identified.[9, 18] They are $CuTACD^{2+}$, $Cu(TACD)_2^{2+}$ and $(CuTACDOH)_2^{2+}$. In a 1:1 metal-to-ligand mixture, only $Cu(TACD)^{2+}$ and $(CuTACDOH)_2^{2+}$ are formed, whereas in 1:2 metal-to-ligand mixtures $Cu(TACD)^{2+}$ and $Cu(TACD)_2^{2+}$ are present. The dimeric nature of the hydrolysed species can only be ascertained when two different total concentrations are used. For this calculation we have taken two sets of four curves each with 33 data points. The mean values are given in Table 1 together with the constants obtained previously. The results of the two sets were practically identical and the standard errors of the pK values were $\leqslant 0.02$. For the four titration curves σ_{ml} was 0.0024 and 0.0023 ml, about twice that for a single curve. Figure 4 shows an example of the modification of a mathematical model and the estimation of unknown parameters by visual interaction. In a first trial a titration curve of Cu^+ and TACN ($pK_H^1 < 2$, $pK_H^2 = 7.30$, $pK_H^3 = 10.92$) in aqueous acetonitrile[16] was fitted, assuming CuL^+ to be the only complex species in solution. For log K ($Cu^+ + TACN \rightleftharpoons CuTACN^+$) $= 8.23 \pm 0.04$ as the best value (curve *a*, Fig. 4) the standard deviation σ_{ml} was 0.0095, roughly three times the normal value. The slope of the calculated curve is too small and the presence of a partially protonated species $Cu(TACNH)^{2+}$ was considered. From the estimated value, log K ($Cu(TACNH)^{2+} + H^+ \rightleftharpoons Cu^+ + TACNH_2^{2+}$) = 2, curve *b* was obtained. A better estimate, 3, gave curve *c*. From there the automatic procedure took over to give the final curve *d* in four iterations with $\sigma_{ml} = 0.0022$ and log K values of 8.28 ± 0.02 and 3.79 ± 0.05 for the equilibrium constants of $CuTACN^+$ and $Cu(TACNH)^{2+}$ respectively.

The system has been tested for more than a year with very different metal–ligand titrations and has shown high flexibility, good reproducibility and accuracy.

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ANALYTICAL APPLICATIONS OF CONDENSED PHOSPHORIC ACID—III*

IODOMETRIC DETERMINATION OF SULPHUR AFTER REDUCTION OF SULPHATE WITH SODIUM HYPOPHOSPHITE AND EITHER TIN METAL OR POTASSIUM IODIDE IN CONDENSED PHOSPHORIC ACID

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Summary—Novel methods for the reduction of sulphate to hydrogen sulphide with hypophosphite–tin metal or hypophosphite–iodide in condensed phosphoric acid (CPA) are proposed. The reduction of sulphate with hypophosphite alone does not proceed quantitatively. Sulphate, however, is quantitatively decomposed with hypophosphite when tin metal or potassium iodide is used together with it. The determination of sulphur by the hypophosphite–tin metal–CPA and tin(II)–CPA methods is interfered with by copper on account of the stabilization of copper(I) sulphide, but this interference can be eliminated by adding iodide, *e.g.* potassium and lead salts. Alum and barytes are quantitatively decomposed within 15 min at 140 and 280°, respectively. The hydrogen sulphide evolved is absorbed in zinc acetate solution at pH 4.5 and then determined by iodometry.

Hypophosphite[1–6] and tin(II)[7–12] have been widely used for the reduction of sulphate to hydrogen sulphide. The reducing power of hypophosphite seems to be stronger than that of tin(II), and it exerts a marked reducing power at far lower temperatures than does tin(II). Hypophosphite methods are usually applied in organic solvents, owing to the fact that the presence of water is detrimental to the reduction. In the hypophosphite methods, therefore, samples must be subjected to oxidative decomposition before the reduction to hydrogen sulphide.[5,6] In this respect, the method proposed by Kiba *et al.* [tin(II)–CPA method][7] is very attractive, considering that the condensed phosphoric acid (CPA) used as solvent is a very powerful agent for dissolution of solid materials, and that the decomposition of samples and the evolution of hydrogen sulphide are carried out in the same reaction vessel.

CPA may also be used as a solvent for the reduction of sulphate with hypophosphite, because CPA is a kind of non-aqueous solvent. No information on this kind of usage of CPA and hypophosphite, however, has so far been reported. We have therefore investigated some novel methods for determination of sulphur in which these properties of CPA and hypophosphite are utilized, and report our findings here.

EXPERIMENTAL

Apparatus

The reaction was very much faster owing to the vigorous convection, when the reaction vessel was heated from the bottom, than when it was heated uniformly all round.[13] Therefore, the apparatus shown in Fig. 1 was used in the present investigation. To obtain accurate data, the temperature was measured by means of an alumel–chromel thermocouple and regulated as described previously.[13] Use of an ordinary thermometer,[7,12] however, may be more convenient for practical purposes.

Reagents

Condensed phosphoric acid (CPA).[7] Prepared as described previously,[13] by heating orthophosphoric acid to 280°.

Tin(II)–CPA. According to the method proposed by Kiba *et al.*,[7] 150 g of CPA and 30 g of tin(II) chloride dihydrate are heated to 280° in a 300-ml quartz flask on an electric heater.

Standard thiosulphate solution, 0.05M. Prepared and standardized as described previously.[14]

Standard iodine solution, 0.05N. About 3.2 g of iodine and 8 g of potassium iodide are dissolved in about 20 ml of water and made up to 1 litre with water.

Zinc acetate solution, 2%. Adjusted to pH 4.5 with acetic acid.

Starch indicator solution, 0.2%.

Nitrogen. Commercial product purified by passage through an alkaline pyrogallol solution.

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

Samples

The barytes and alunite used were supplied by the Department of Mining and Mineral Engineering, Faculty of Engineering, Tohoku University. They were finely ground in a mortar and then used in the experiments. The sulphur contents obtained by gravimetry[15] as barium sulphate were: alum 13.4%, alunite 12.9%, barytes 13.8%.

General procedure

Transfer a suitable weight of powdered sample, containing 2–10 mg of sulphur, to the reaction vessel D (Fig. 1)

* Part II: T. Mizoguchi and H. Ishii, *Talanta*, 1979, **26**, 33.

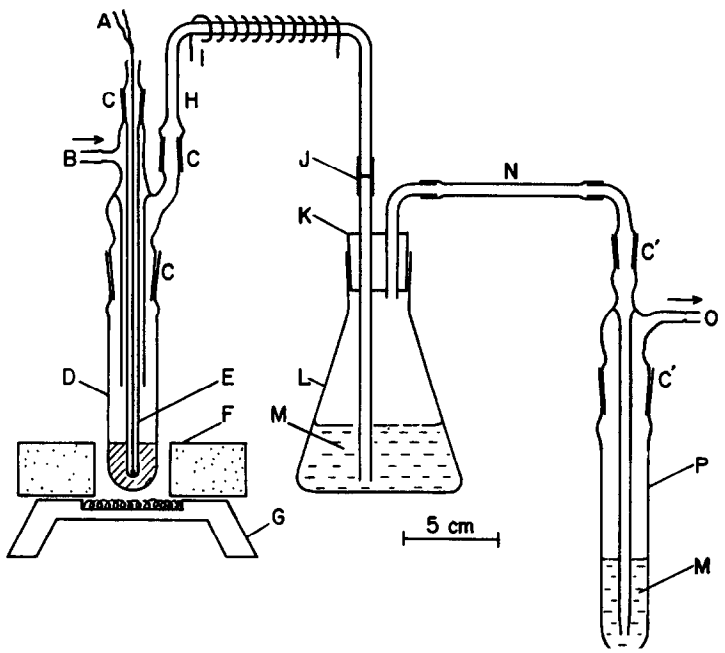

Fig. 1. Apparatus for the determination of sulphur. A, alumel–chromel thermocouple; B, gas inlet; C.C', glass joints; D, reaction vessel (quartz tube); E, protective tube (lower part: quartz tube); F, brick; G, electric heater; H, gas-delivery tube; I, nichrome heater (used for the removal of condensate) (*ca.* 15 Ω, applied voltage *ca.* 30 V); J, silicon rubber tubing; K, rubber stopper; L, first absorbing vessel (200-ml Erlenmeyer flask); M, 2% zinc acetate solution; N, vinyl tubing; O, gas outlet; P, second absorbing vessel.

and, depending on the method chosen for reduction (see Table 1), add the recommended amount of CPA (if necessary) and the reducing agent(s). Put 120 and 20 ml of 2% zinc acetate solution into the absorption vessels L and P, respectively. Connect all the parts of the apparatus as shown in Fig. 1, glass joints C being lubricated with CPA. Pass nitrogen through the apparatus for a few minutes to expel air. Heat the reaction vessel to 300° in 20–30 min by using an electric heater, with nitrogen flowing at a rate of about 2 bubbles/sec. After keeping the reaction vessel at 300° for the desired time, disconnect the absorption vessels from the reduction unit and then transfer the contents of the second (P) into the first (L) with a small amount of water. Place the Erlenmeyer flask on a boiling water-bath for 10 min, with nitrogen flowing, to expel the phosphine formed in the reduction step and then cool to about 15° or lower in an ice-bath. Add exactly 20 ml of 0.05*N* iodine and titrate with thiosulphate solution, using starch as indicator. Carry out a blank determination on the reagents.

The sample taken for the determination contains $f(V_B - V_A)$ mg of sulphur where f is the sulphur equivalent (mg/ml) of the 0.05*M* thiosulphate, V_B is the volume of thiosulphate solution required by the blank, and V_A is the volume of thiosulphate solution required for the titration of the excess of iodine.

Note 1. When hypophosphite is used as a reducing agent, the heating must be stopped at 100–150°, just before the vigorous foaming due to the decomposition of hypophosphite.

Note 2. If elemental sulphur is liberated in the tin(II)–CPA and hypophosphite–tin metal–CPA methods, the samples must be reductively decomposed at lower temperature (*e.g.*, 280°) with prolonged heating time.

Note 3. To accelerate the reaction between zinc sulphide and iodine, it is recommended to apply ultrasonic waves to the contents of the Erlenmeyer flask for about 1 min each time before and after the addition of standard iodine solution.

RESULTS

Effect of pH on the absorption of hydrogen sulphide

Hydrogen sulphide was not absorbed completely at pH below 4. However, the iodometric reaction cannot be used at pH above 5 because iodine then reacts with acetate to some extent. Therefore, the pH value of the absorption solution was adjusted to 4.5 in the recommended procedure. The reaction between iodine and

Table 1. Recommended conditions for the reduction of sulphate

Reduction method	Reducing agents added, *g*		CPA added, *g*	Decomposition
Tin(II)–CPA	Tin(II)–CPA	15	0	30 min at 300°
Hypophosphite–tin metal–CPA	$NaH_2PO_2.H_2O$	1.0	15	15 min at 300°
	Sn metal	0.2		
Hypophosphite–iodide–CPA	$NaH_2PO_2.H_2O$	0.5	15	20 min at 300°
	KI	0.2		

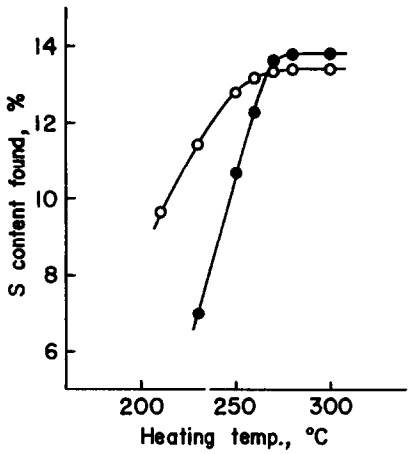

Fig. 2. Effect of heating temperature on the determination of sulphur by the tin(II)–CPA method. Heating time 30 min. ○ Alum, ● barytes.

acetate can be suppressed by cooling the absorption solution to 15° or lower before titration.

Reduction of sulphate with tin(II)–CPA

The reduction of sulphate in alum and barytes was quantitative at 280° or higher when the samples were heated for 30 min (Fig. 2). The minimum heating time at 300° required for the quantitative decomposition of barytes, which is the least soluble sample, was 20 min. (Fig. 3).

Reduction of sulphate with sodium hypophosphite

Effect of the amount of sodium hypophosphite added. The reduction of sulphate in barytes was not quantitative even when the amount of sodium dihydrogen hypophosphite monohydrate added was as much as 1 g (Fig. 4). A constant sulphur content, in good agreement with that obtained by gravimetry, was obtained when tin metal or potassium iodide was used together with the hypophosphite. The minimum amount of

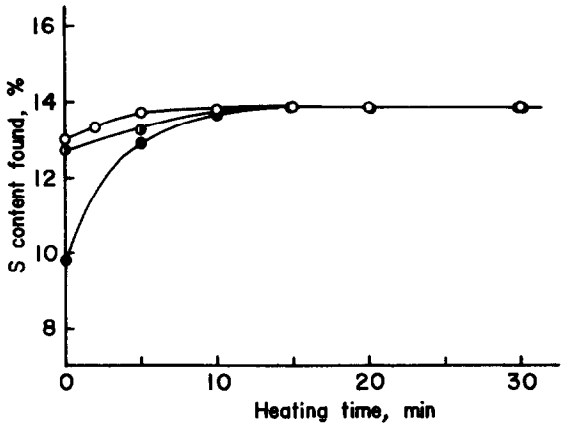

Fig. 3. Effect of heating time on the determination of sulphur. Sample barytes, heating temperature 300°, reducing agent: ○ hypophosphite–metallic tin, ◑ hypophosphite–iodide, ● tin(II).

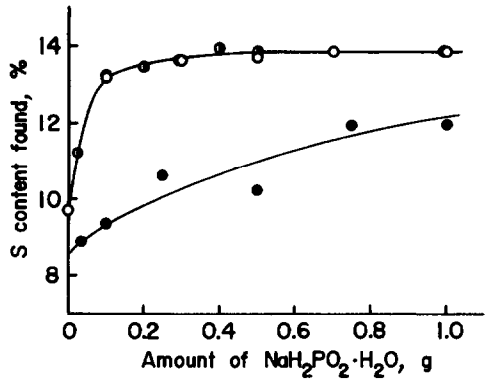

Fig. 4. Effect of the amount of hypophosphite on the determination of sulphur by the hypophosphite–CPA methods. Sample barytes, decomposition 20 min at 300°. ○ Sn 0.2 g, ◑ KI 0.2 g, ● hypophosphite alone.

sodium dihydrogen hypophosphite monohydrate required for the quantitative reduction of sulphate in barytes was 0.5 and 0.4 g for the hypophosphite–tin metal and hypophosphite–iodide reducing systems, respectively.

Effect of the amount of auxiliary reducing agent. Sulphate in barytes was reduced quantitatively with 0.1–0.5 g of tin metal and 0.1–0.3 g of potassium iodide (Fig. 5). When more reductant than this was added a small amount of elemental sulphur or iodine was liberated.

Effect of heating temperature and time. Alum was quantitatively decomposed with hypophosphite–tin metal and hypophosphite–iodide mixtures at temperatures above 140 and 220°, respectively (Fig. 6). Barytes required a temperature above 280° in both methods, on account of its low solubility in CPA, and the minimum heating times at 300° are shown in Fig. 3.

Effect of iodide on the reduction of sulphate in copper and silver sulphates

When the hypophosphite–iodide–CPA method was used, the sulphur recoveries were almost quantitative

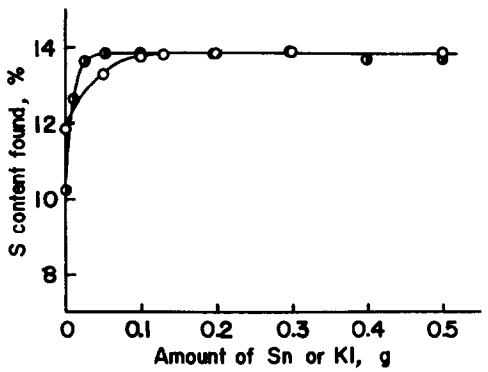

Fig. 5. Effect of the amount of auxiliary reducing agents on the determination of sulphur by the hypophosphite–CPA methods. Sample barytes. ○ Sn, $NaH_2PO_2 \cdot H_2O$ 1 g, decomposition 15 min at 300°, ◑ KI, $NaH_2PO_2 \cdot H_2O$ 0.5 g, decomposition 20 min at 300°.

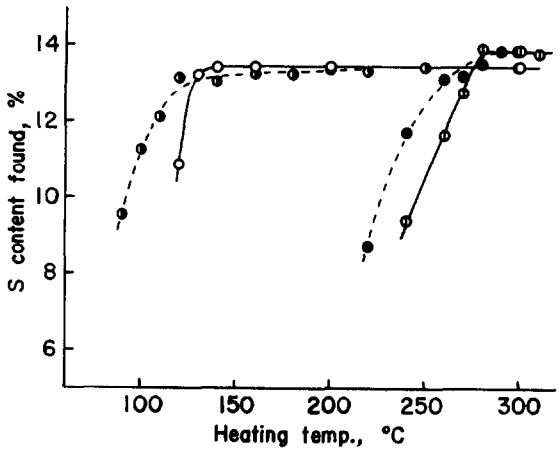

Fig. 6. Effect of heating temperature on the determination of sulphur by the hypophosphite–CPA methods. Heating time 30 min. —○— alum (metallic tin), —◑ barytes (metallic tin), --◑-- alum (iodide), --●-- barytes (iodide). The compounds shown in parentheses are auxiliary reducing agents.

for both copper and silver sulphates. The sulphur content found for copper sulphate was somewhat lower, however, when the tin(II)–CPA and hypophosphite–tin metal CPA methods were used (Table 2). The recovery of sulphur from silver sulphate was not quantitative only with the tin(II)–CPA method.

Effect of diverse ions

Table 3 shows the effects of other ions on the determination of sulphur in alunite by the hypophosphite–tin metal–CPA method. Such anions as halides, VO_3^-, WO_4^{2-}, MoO_4^{2-} and TeO_4^{2-}, which do not give H_2S-oxidizing compounds on decomposition or on reaction with hypophosphite, do not interfere. MnO_4^-, $Cr_2O_7^{2-}$, IO_4^-, BrO_3^- and NO_3^- undergo decomposition in CPA to give H_2S-oxidizing compounds, *e.g.* I_2, Br_2, Cl_2 and O_2. These ions, however, are reduced by hypophosphite, and therefore also do not interfere; ClO_4^- is preferentially reduced by hypophosphite (to chloride) and also does not interfere.

Marked interferences were observed only from ClO_3^-, NO_2^- and SeO_3^{2-}, the first two may be attributed to Cl_2 and NO_x, respectively, which are formed by their decomposition in CPA. The interference from

Table 3. Effect of diverse ions on the determination of sulphur in alunite by the hypophosphite–tin metal–CPA method

Ion*	Source	S content found† %
None		13.0
Bi^{3+}	Bi_2O_3	12.9
Hg^{2+}	$HgCl_2$	13.1
Pd^{2+}	$PdCl_2$	13.0
Sb^{3+}	$SbCl_3$	13.0
AsO_2^-	As_2O_3	11.7 (12.9)‡
BrO_3^-	$KBrO_3$	12.9
Cl^-	NaCl	13.0
ClO_3^-	$KClO_3$	12.7
ClO_4^-	$NaClO_4$	13.0
$Cr_2O_7^{2-}$	$K_2Cr_2O_7$	12.9
I^-	KI	13.0
IO_4^-	KIO_4	12.8
MnO_4^-	$KMnO_4$	13.0
MoO_4^{2-}	$Na_2MoO_4.2H_2O$	12.9
NO_2^-	$NaNO_2$	12.4
NO_3^-	$NaNO_3$	12.9
SeO_3^{2-}	SeO_2	32.6 (13.0)§
TeO_4^{2-}	$K_2TeO_4.3H_2O$	12.8
VO_3^-	NH_4VO_3	13.0
WO_4^{2-}	$Na_2WO_4.2H_2O$	13.0
CH_3COO^-	$CH_3COONa.3H_2O$	12.9
$C_2O_4^{2-}$	$Na_2C_2O_4$	12.8

* Amount added: 50 mg of the ion.
† S content (gravimetric) 12.9%.
‡ As_2O_3 added: 10 mg.
§ Pretreated with HBr–Br_2.[16]

selenium is easily eliminated by adding bromine and hydrobromic acid and heating to dryness.[16]

From the results in Tables 2 and 3, it is clear that such metal ions as Bi^{3+}, Pd^{2+} and Ag^+, which form stable sulphides in acidic solutions at ordinary temperatures, do not interfere with the determination of sulphur by the hypophosphite–tin metal–CPA method, which can also be applied to the determination of sulphur in samples containing iron, zinc, lead, cadmium, cobalt, nickel, manganese, barium, aluminium, calcium, *etc.*[17]

DISCUSSION

Role of hypophosphite and auxiliary reducing agents in the reduction of sulphate

When either tin metal or potassium iodide alone was used as a reducing agent for sulphate, elemental

Table 2. Effect of iodide on the reduction of sulphate in copper and silver sulphates

Sample	Iodide taken	Reduction system and S content found, %		
		Sn(II)	NaH_2PO_2	NaH_2PO_2–Sn metal
$CuSO_4.5H_2O$*	None	7.2	10.6	11.6
	PbI_2 0.2 g	12.7	12.7	12.7
	KI 0.2 g	12.5	12.6	12.7
Ag_2SO_4†	None	9.0	10.2	10.3
	PbI_2 0.2 g	9.8		
	KI 0.2 g	9.8		

* S content found (gravimetric), 12.8%.
† S content found (gravimetric), 10.3%.

sulphur or iodine was liberated together with hydrogen sulphide. These side-reactions, equations (1) and (2),

$$SO_4^{2-} + 3Sn + 8H^+ = S + 3Sn^{2+} + 4H_2O \quad (1)$$

$$SO_4^{2-} + 8I^- + 10H^+ = H_2S + 4I_2 + 4H_2O \quad (2)$$

can be prevented by the addition of hypophosphite. Consequently, it is presumed that the reduction of sulphate to sulphide is accompanied by intermediate formation of elemental sulphur, this step being accelerated by auxiliary reducing agents, *e.g.*, tin metal and iodide, and that elemental sulphur and iodine, if liberated, are rapidly reduced with hydrophosphite to hydrogen sulphide and iodide [equations (3) and (4)], respectively.

$$S + PO_2^{3-} + H_2O + H_2S + PO_3^{3-} \quad (3)$$

$$I_2 + PO_2^{3-} + H_2O = 2I^- + PO_3^{3-} + 2H^+ \quad (4)$$

Thus sulphate can only be reduced to hydrogen sulphide with hypophosphite if tin metal or iodide is also present. Low results were obtained if sodium phosphite was used as a reducing agent instead of sodium hypophosphite (Table 4).

Effect of iodide on the reduction of sulphate in copper and silver sulphates

The low recovery of sulphur from copper sulphate is probably due to the stability of cuprous sulphide [equation (5)].

$$2Cu^{2+} + 2SO_4^{2-} + 18H^+ + 18e^- = Cu_2S + H_2S + 8H_2O \quad (5)$$

Therefore, recovery of sulphur is enhanced by addition of iodide in the tin(II)–CPA and hypophosphite–tin metal–CPA methods. In the tin(II)–CPA method, however, the recovery of sulphur was somewhat low for both copper sulphate and other soluble sulphates, *e.g.*, alum, sodium sulphate and magnesium sulphate, when a soluble iodide such as the potassium salt was used. These results may be indicative of the fact that iodine is liberated not only in the reaction of cupric ion with iodide [equation (6)] but also in the reduction of sulphate with iodide [equation (2)] if the iodide to be added is easily soluble in CPA.

$$2Cu^{2+} + 4I^- = 2CuI + I_2 \quad (6)$$

The latter reaction may not be ignored in the tin(II)–CPA method, because it proceeds at lower temperatures than the reduction of sulphate with tin(II) does.

Table 5. Effect of several ions on the determination of sulphur in alunite by the hypophosphite–iodide method

Ion*	Source	S content found,† %
None	—	12.9
Hg^{2+}	$HgCl_2$	15.3
Sb^{3+}	$SbCl_3$	14.5
BrO_3^-	$KBrO_3$	12.7
ClO_3^-	$KClO_3$	12.9
NO_2^-	$NaNO_2$	12.2
MoO_4^{2-}	$Na_2MoO_4.2H_2O$	11.6

* Amount added: 50 mg of the ion.
† S content (gravimetric) 12.9%.

In this respect, the use of insoluble iodides such as lead iodide is recommended. In the presence of lead iodide, reactions (2) and (6) are almost completely suppressed, the cuprous sulphide formed being converted into cuprous iodide gradually but quantitatively.

$$Cu_2S + PbI_2 + 2H^+ = 2CuI + Pb^{2+} + H_2S \quad (7)$$

In the hypophosphite–iodide–CPA method, potassium iodide cannot always be replaced by lead iodide. Thus, the recovery of sulphur when lead iodide was used was quantitative for alum but not for barytes.

The low recovery of sulphur when silver sulphate was decomposed by the tin(II)–CPA method (Table 2) is probably due to the occlusion in the silver metal of the silver sulphide first formed:

$$2Ag^+ + SO_4^{2-} + 8H^+ + 8e^- = Ag_2S + 4H_2O \quad (8)$$

$$Ag_2S + 2H^+ + 2e^- = 2Ag + H_2S. \quad (9)$$

In the hypophosphite methods, the silver metal formed is porous because of the evolution of hydrogen and/or phosphine, resulting in the quantitative conversion of silver sulphide into hydrogen sulphide.

Effect of diverse ions

The interferences from foreign ions, especially those having the ability to oxidize iodide, are more serious in the hypophosphite–iodide–CPA method than the hypophosphite–tin metal–CPA method, since iodine is more or less liberated in the former case (Table 5). The interferences from Hg^{2+} and Sb^{3+} are attributed to the distillation of their iodides. The interferences from ClO_3^-, BrO_3^-, NO_2^- and NO_3^- were more serious in the tin(II)–CPA method than in the hypophosphite methods, for these ions are decomposed in the former

Table 4. Comparison of hypophosphite with phosphite as reducing agent

Reducing agent		$Na_2HPO_3.5H_2O$*		$NaH_2PO_2.H_2O$	
Auxiliary reducing agent		Sn	KI	Sn	KI
S content found, %	Alum	10.3	12.5	13.4	13.4
	Barite	12.8	13.5	13.8	13.8

* Samples were decomposed under the recommended conditions (Table 1). The only difference was that 1 g of $Na_2HPO_3.5H_2O$ was used instead of $NaH_2PO_2.H_2O$.

method to give H_2S-oxidizing compounds, *e.g.*, Cl_2, Br_2, NO_x. Consequently, the hypophosphite–tin metal–CPA method is superior to the tin(II)–CPA and hypophosphite–iodide–CPA methods, in respect of interferences from foreign ions.

CONCLUSION

Many variants of the hypophosphite–iodide method have been proposed for the determination of sulphur. The basic data concerning the reduction conditions, however, are very scanty, and the reduction yield is sometimes questionable.[1,4] The present investigation, therefore, may be useful for understanding the chemical behaviour of sulphate in its reduction to hydrogen sulphide.

The hypophosphite–tin metal–CPA method seems to be the best of the methods tested here, since the recovery of sulphur is quantitative at lower temperatures and the interferences are largely reduced. The application of the methods to the determination of sulphur in various kinds of sample will be discussed in a separate paper.[17]

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ANALYTICAL APPLICATIONS OF CONDENSED PHOSPHORIC ACID—IV*

IODOMETRIC DETERMINATION OF SULPHUR IN SULPHATE AND SULPHIDE ORES AND MINERALS AND OTHER COMPOUNDS AFTER REDUCTION WITH SODIUM HYPOPHOSPHITE AND TIN METAL IN CONDENSED PHOSPHORIC ACID

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Summary—Sulphate in sulphate ores, *e.g.*, alunite, anglesite, barytes, chalcanthite, gypsum, manganese sulphate ore, is reduced to hydrogen sulphide by the hypophosphite–tin metal–CPA method, if a slight modification is made. Sulphide ores, *e.g.*, galena, sphalerite, are quantitatively decomposed with CPA alone to give hydrogen sulphide. Suitable reducing agents must be used for the quantitative recovery of hydrogen sulphide from pyrite, nickel sulphide, cobalt sulphide and cadmium sulphide, or elemental sulphur is liberated. Iodide must be used in the decomposition of chalcopyrite; the copper sulphide is too stable to be decomposed by CPA alone. Molybdenite is not decomposed in CPA even if reducing agents are added. The pretreatment methods for the determination of sulphur in sulphur oxyacids and elemental sulphur have also been investigated.

The tin(II)–CPA method proposed by Kiba *et al.*[1] has been used for the determination of sulphur, especially in solid samples, partly because CPA is a very powerful agent for their dissolution. Earlier studies[2,3] were done on the basis that every sulphur compound could be reduced to hydrogen sulphide with the tin(II)–CPA reagent. However, we have shown in our previous study that the determination of sulphur by the tin(II)–CPA method suffers interference from copper and silver on account of the stability of their sulphides.[4] It is also questionable whether elemental sulphur, which is often contained in geological samples, is reduced quantitatively to hydrogen sulphide with tin(II)–CPA.

In the present study, we have investigated the decomposition behaviour of various sulphur compounds, *e.g.*, sulphate and sulphide ores and minerals, sulphur oxyacid compounds, elemental sulphur, in CPA alone and/or CPA containing some reducing agents, to obtain basic data concerning the application of the CPA methods, particularly the hypophosphite–tin metal–CPA method, to the determination of sulphur in as many kinds of sample as possible. The methods of pretreatment of the above-mentioned samples are also discussed.

EXPERIMENTAL

The apparatus and reagents have already been described.[4]

Samples

Sulphate and sulphide ores and minerals were supplied by the Department of Mining and Mineral Engineering, Faculty of Engineering, Tohoku University, and Research Institute of Mineral Dressing and Metallurgy, Tohoku University. Nickel, cobalt and cadmium sulphides were obtained from commercial sources. Sulphur oxyacid compounds, except for dithionate and tetrathionate, were also from commercial sources. Sodium dithionate and potassium tetrathionate were prepared by Pfanstiel's method[5] and Martin's method,[6] respectively.

Procedures

Sulphate and sulphide ores and minerals (general procedure for the determination of sulphur by the hypophosphite–tin metal–CPA method). See the preceding paper[4] for the detailed procedure. Transfer a suitable weight of powdered sample, containing 2–10 mg of sulphur, to the reduction vessel and reduce with 1 g of sodium dihydrogen hypophosphite monohydrate, 0.2 g of tin metal and 15 g of CPA, heating for 15 min at 300°. Determine the hydrogen sulphide by iodometry after its collection as zinc sulphide.

The procedures for the tin(II)–CPA and hypophosphite–iodide–CPA methods are also the same as those described previously.[4]

Sulphur oxyacid compounds. Transfer a suitable weight of sample, containing 2–10 mg of sulphur, to the reaction vessel. Add about 0.5 ml of 1*M* sodium hydroxide and swirl until the sample is dissolved. Add several drops of 6% hydrogen peroxide and evaporate the content to dryness by immersing the vessel in a boiling water-bath. Add about 0.5 ml of concentrated nitric acid and several drops of 6% hydrogen peroxide and then evaporate to dryness to oxidize any dithionate and polythionates quantitatively. Reduce the resulting sulphate at 200° by the procedure above.

Samples containing elemental sulphur. Transfer a suitable weight of powdered sample to the reaction vessel, add 0.1 g of potassium chlorate and dampen with a few drops of concentrated hydrobromic acid. Add about 0.5 ml of concentrated nitric acid and let stand at room temperature,

* Part III: T. Mizoguchi, H. Iwahori and H. Ishii, *Talanta*, 1980, **27**, 519.

Table 1. Determination of sulphur in sulphate ores

Sample	Reducing system and S content found, % Sn (II)	NaH_2PO_2–Sn metal	NaH_2PO_2–KI	Gravimetric S, %
Alunite	12.8	13.0	12.9	12.9
Anglesite	13.4	13.3	13.2	13.4
Barytes	13.8	13.8	13.8	13.8
Chalcanthite	9.2(11.7)*	10.9(11.7)*	11.6	11.7
Gypsum	18.0	18.4	18.3	18.5
Manganese sulphate ore	10.3	10.2	9.2	10.3

* Lead iodide added, 0.2 g.

with occasional swirling, until the droplet of bromine disappears. Evaporate to dryness in a boiling water-bath. Add several drops of concentrated hydrochloric acid and evaporate to dryness. Repeat the treatment with concentrated hydrochloric acid, then reduce the sulphate as described above.

Note 1. For sulphur compounds such as sulphite and dithionite, which are easily oxidized to sulphate with alkaline hydrogen peroxide, the oxidative treatment with nitric acid and hydrogen peroxide may be omitted.

Note 2. When the sample contains unidentified sulphur compounds, the following pretreatment method is recommended: oxidation with hydrogen peroxide in 1*M* sodium hydroxide, oxidation with potassium chlorate in an acid mixture of hydrobromic acid and nitric acid, followed by decomposition of chlorate with hydrochloric acid. This treatment takes only an hour or less.

Note 3. The interference from copper can be eliminated by addition of 0.2 g of lead iodide.

RESULTS AND DISCUSSION

Sulphate ores

The three types of CPA method have been applied to the determination of total sulphur in several kinds of natural sulphate ore. The results shown in Table 1 indicate that the hypophosphite–tin metal–CPA method gives the values nearest to those obtained by gravimetry,[7] and that the hypophosphite–tin metal–CPA method can be applied to the analysis not only of easily soluble sulphates, *e.g.*, alum, but also of insoluble sulphate ores, *e.g.*, anglesite and barytes, which conventionally must be opened out by alkaline fusion.[7]

The hypophosphite–tin metal–CPA and tin(II)–CPA methods gave somewhat lower results than the hypophosphite–iodide–CPA method or gravimetry for chalcanthite, which may be attributed to the copper in it.[4] The addition of iodide brought about more accurate results for such samples. The reason why the hypophosphite–iodide–CPA method gave a lower result for manganese sulphate ore may be that iodine is liberated by the action of higher oxidation states of manganese.

Sulphide ores and minerals

Of all the sulphide ores and minerals tested with CPA alone, complete recovery of sulphur was obtained only for galena and sphalerite (Table 2). Molybdenite was not decomposed in CPA even if reducing agents were added to it. For pyrrhotite the CPA method gave a slightly lower result than that obtained by gravimetry, probably because of contamination with pyrite.

A large amount of elemental sulphur was liberated in the decomposition of pyrite with CPA alone. However, the hypophosphite–tin metal–CPA method gave results for pyrite in close agreement with those by gravimetry. Therefore, it is assumed that pyrite is decomposed in CPA according to the reactions:[8–10]

$$FeS_2 = FeS + S \qquad (1)$$

Table 2. Determination of sulphur in sulphide ores and minerals

Sample	Decomposing agent and S content found, % CPA*	NaH_2PO_2–Sn metal–CPA	Gravimetric S, %
Pyrite	22.8	52.8	53.2
Chalcopyrite	21.9	29.2(34.7)†	34.9
Pyrrhotite	34.7	36.6	36.4
Galena	13.3	13.4	13.4
Sphalerite	33.4	33.5	33.6
Nickel sulphide	5.0	17.9(17.6)‡	17.9
Cobalt sulphide	2.0	11.8(11.4)‡	11.8
Cadmium sulphide	15.5	20.3(19.9)‡	20.3
Molybdenite	0.1	0.1	37.8

* Decomposition: 30 min at 300°.
† Lead iodide added 0.2 g.
‡ Tin(II)-CPA method.

Table 3. Determination of sulphur in sulphur oxyacid compounds

Sample	S content found, % NaH_2PO_2–Sn metal method A*	B*	Gravimetric
Sodium thiosulphate pentahydrate	25.6	25.8	25.8
Sodium dithionite	35.0		35.0
Potassium tetrathionate	42.1	42.3	42.3
Sodium sulphite	25.3		25.3
Sodium dithionate dihydrate	26.0	26.5	26.5
Potassium alum	13.4		13.4

* Pretreatment A: $NaOH$–H_2O_2, B: $NaOH$–H_2O_2 followed by HNO_3–H_2O_2 (see text for procedures).

$$FeS + 2H^+ = Fe^{2+} + H_2S \quad (2)$$

When chalcopyrite was decomposed with CPA alone, bulky and black residues were left. When reducing agents such as tin(II) and hypophosphite–tin metal were added, the recovery of sulphur was increased but still remained too low. The recovery of sulphur was quantitative only when iodide was present. From the chemical analysis of the residues, it seems that chalcopyrite is first decomposed to give copper(II) and iron(II) sulphides [equation (3)]. The iron(II) sulphide is easily decomposed with CPA to give hydrogen sulphide [equation (2)], but copper(II) sulphide is stable in the absence of reducing agent. However, when reducing agents such as tin(II) and hypophosphite–tin metal are present, copper(II) sulphide is reduced to copper(I) sulphide accompanied by the evolution of hydrogen sulphide [equation (4)].

$$CuFeS_2 = CuS + FeS \quad (3)$$

$$2CuS + 2H^+ + 2e^- = Cu_2S + H_2S \quad (4)$$

The recovery of sulphur from nickel, cobalt and cadmium sulphides was quantitative only when the hypophosphite–tin metal–CPA method was applied (Table 2, column 3). In the decomposition of these sulphides, no particular reducing or oxidizing agents were used except for CPA, so the liberation of elemental sulphur is presumably a consequence of stabilization of the sulphide by high lattice energy, so that simple protonation to yield hydrogen sulphide is not possible, and oxidation of the sulphide to sulphur, probably by interaction with the condensed phosphoric acid.

Nagashima *et al.*[3] have proposed a phase-analysis method for sulphide sulphur and non-sulphide (sulphate) sulphur, based on the principle that sulphide sulphur, including sulphur in pyrite and chalcopyrite, is evolved as hydrogen sulphide when the sample is heated with CPA alone, and both sulphide and sulphate sulphur are evolved as hydrogen sulphide with tin(II)–CPA. However, our results indicate that the method would not be applicable to cobalt, nickel or cadmium sulphide, and difficulty might be experienced with pyrite and silver or copper compounds.

Sulphur oxyacid compounds

Sulphur oxyacid compounds of low oxidation state must be oxidized to sulphate before the reduction to hydrogen sulphide, or they will undergo acid decomposition to liberate sulphur dioxide and/or elemental sulphur.[11–13]

When the samples were oxidized with hydrogen peroxide before the reduction, the results obtained for sodium sulphite and dithionite were in good agreement with those obtained by gravimetry (Table 3).

The sulphur content obtained for dithionate was wholly identical with that by gravimetry only when the sample was treated with nitric acid and hydrogen peroxide. The results for thiosulphate and tetrathionate were also somewhat higher when the samples were treated in this way. This indicates that these sulphur oxyacid compounds cannot be oxidized quantitatively to sulphate with hydrogen peroxide alone.[13,14]

Various oxidizing agents have been proposed for the oxidation of sulphur oxyacid compounds.[15–17] The hydrogen peroxide–nitric acid method proposed by us is considered to be superior to the others in the following respects: (1) dithionate is quantitatively oxidized to sulphate, (2) the residual hydrogen peroxide is easily removed by heating to dryness.

Samples containing elemental sulphur

Elemental sulphur may be present in greater or lesser degree in almost all of the sulphur-containing samples. The behaviour of elemental sulphur in the reduction by the CPA methods, however, has hardly been investigated.

When about 10 mg of elemental sulphur were reduced for 20 min at 200° with three kinds of reducing agent, the recoveries of hydrogen sulphide were as follows: tin(II)–CPA 0.5%, hypophosphite–tin metal–CPA 13% and hypophosphite–iodide–CPA 80%.

The recovery of hydrogen sulphide from elemental sulphur was 99% or higher only when elemental sulphur was oxidized to sulphate before the reduction.

The oxidation of elemental sulphur proceeded rapidly when the sulphur was dissolved in liquid bromine. This is the reason for adding hydrobromic acid in the pretreatment of elemental sulphur with chlorate.

CONCLUSION

From the results described, it may be concluded that sulphur in almost all of the samples can be determined by the CPA methods, especially the hypophosphite–tin metal–CPA method, if the samples are pretreated properly by the methods proposed.

We have used samples with sulphur contents of 10–53% to obtain the basic data concerning the chemical behaviour of the sulphur in these compounds, the hydrogen sulphide evolved being determined by iodometry. The hypophosphite–tin metal–CPA method may also be applicable to the microdetermination of sulphur; this possibility is being studied.

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THE INFLUENCE OF DISSOCIATION ON SIMULTANEOUS DETERMINATION OF ZIRCONIUM AND HAFNIUM WITH XYLENOL ORANGE

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Summary—The molar absorptivities of the zirconium and hafnium Xylenol Orange (1:1) complexes are said to be similar in the acidity range 0.1–2.0*M* HCl. However, the absorbances obtained for the zirconium–Xylenol Orange complex in the acidity range 0.5–2.0*M* HCl are much higher than those for the same concentrations of hafnium. The absorbance differences are generally due to the higher stability of the zirconium complex at such acidities. Calculations based on the conditional stability constants of these complexes show the influence of dissociation on the results of simultaneous determination of zirconium and hafnium with Xylenol Orange.

The determination of zirconium and hafnium individually when they occur together presents a difficult analytical problem because of their close chemical similarity.[1–3] The simultaneous determination of these elements was reported by Cheng[1,2] and Challis.[3] Cheng's attempts to determine hafnium in presence of zirconium by control of acidity were unsuccessful. His method for hafnium determination with use of hydrogen peroxide to mask zirconium gave results which were better but still not quantitative.

For simultaneous determination of zirconium and hafnium Challis[3] proposed a "three-point" method based on absorbances obtained at three levels of acidity: 0.35, 1.12 and 2.0*M*. His results were satisfactory for nickel-base alloys.

Initially, in the investigations reported below, the experiments were concerned with determination of conditional stability constants for 1:1 hafnium and zirconium complexes with Xylenol Orange at chosen acidity levels. The results obtained were compared with those given by other authors[2,4] and used to explain the conditions needed for simultaneous hafnium and zirconium determination with Xylenol Orange.

THEORETICAL

Hafnium and zirconium complexes with Xylenol Orange (HfXO, ZrXO) are only very slightly dissociated at pH 0.6–1.2 even when the components are present in stoichiometric ratio (1:1). If a sufficient excess of Xylenol Orange is present the dissociation can be neglected.

The absorbance measured against a reagent blank in this acidity range is:

$$A_1 = (\epsilon_{HfXO} C^0_{Hf} + \epsilon_{ZrXO} C^0_{Zr})l \qquad (1)$$

where ϵ_{HfXO} and ϵ_{ZrXO} are the molar absorptivities of HfXO and ZrXO complexes relative to that of XO, C^0_{Hf} and C^0_{Zr} are the total concentrations of hafnium and zirconium, and l is the path-length.

Equation (1) can be written:

$$\frac{A_1}{\epsilon_{ZrXO} l} = SC^0_{Hf} + C^0_{Zr} \qquad (1a)$$

where

$$S = \frac{\epsilon_{HfXO}}{\epsilon_{ZrXO}}$$

In more acidic solutions (pH < 0.5) the effect of dissociation cannot be neglected and then:

$$A_2 = [\epsilon_{HfXO}(1 - \alpha_{HfXO})C^0_{Hf} + \epsilon_{ZrXO}(1 - \alpha_{ZrXO})C^0_{Zr}]l \qquad (2)$$

where α_{HfXO} and α_{ZrXO} are the degrees of dissociation of the complexes, and

$$\frac{A_2}{\epsilon_{ZrXO} l} = SC^0_{Hf} + C^0_{Zr} - (S\alpha_{HfXO}C^0_{Hf} + \alpha_{ZrXO}C^0_{Zr}) \qquad (2a)$$

Subtraction of (2a) from (1a) gives

$$\frac{A_1 - A_2}{\epsilon_{ZrXO} l} = S\alpha_{HfXO}C^0_{Hf} + \alpha_{ZrXO}C^0_{Zr} \qquad (3)$$

Transformation of the equation for the stability constant of a 1:1 metal–Xylenol Orange complex (K_{MXO}) gives

$$K_{MXO} = \frac{1 - \alpha_{MXO}}{\alpha_{MXO} C_{XO}} \qquad (4)$$

where C_{XO} is the concentration of unbonded XO and α_{MXO} is the degree of dissociation of MXO.

Equations (2) and (4) yield

$$\frac{A_2}{\epsilon_{ZrXO}l} = SK_{HfXO}\alpha_{HfXO}C_{XO}C^0_{Hf} + K_{ZrXO}\alpha_{ZrXO}C_{XO}C^0_{Zr} \quad (5)$$

and

$$\frac{A_2}{K_{ZrXO}C_{XO}\epsilon_{ZrXO}l} = S\frac{K_{HfXO}}{K_{ZrXO}}\alpha_{HfXO}C^0_{Hf} + \alpha_{ZrXO}C^0_{Zr} \quad (5a)$$

Subtraction of (3) from (5a) gives:

$$\frac{A_2}{K_{ZrXO}C_{XO}\epsilon_{ZrXO}l} - \frac{A_1 - A_2}{\epsilon_{ZrXO}l} = S\left(\frac{K_{HfXO}}{K_{ZrXO}}\alpha_{HfXO}C^0_{Hf} - \alpha_{HfXO}C^0_{Hf}\right) \quad (6)$$

which allows calculation of the free hafnium concentration C_{Hf} if C_{XO} is known:

$$C_{Hf} = \alpha_{HfXO}C^0_{Hf} = \frac{C_{XO}K_{ZrXO}(A_1 - A_2) - A_2}{Sl\epsilon_{ZrXO}C_{XO}(K_{ZrXO} - K_{HfXO})} \quad (7)$$

and with equation (3) taken into account, the free zirconium concentration:

$$C_{Zr} = \alpha_{ZrXO}C^0_{Zr} = \frac{A_2 - C_{XO}K_{HfXO}(A_1 - A_2)}{l\epsilon_{ZrXO}C_{XO}(K_{ZrXO} - K_{HfXO})} \quad (8)$$

The concentration of free Xylenol Orange is:

$$C_{XO} = C^0_{XO} - [(1 - \alpha_{HfXO})C^0_{Hf} + (1 - \alpha_{ZrXO})C^0_{Zr}] \quad (9)$$

From equation (2):

$$(1 - \alpha_{HfXO})C^0_{Hf} + (1 - \alpha_{ZrXO})C^0_{Zr} = \frac{A_2}{\epsilon_{ZrXO}l} - (S - 1)(1 - \alpha_{HfXO})C^0_{Hf} \quad (2b)$$

Taking into account equations (2b), (4), (7) and (9), C_{XO} can be calculated as

$$C_{XO} = C^0_{XO} - \frac{A_2}{\epsilon_{ZrXO}l} + (S - 1)(1 - \alpha_{HfXO})C^0_{Hf} \quad (9a)$$

If $\epsilon_{ZrXO} = \epsilon_{HfXO}$ then $S = 1$ and

$$C_{XO} = C^0_{XO} - \frac{A_2}{\epsilon_{ZrXO}l} \quad (9b)$$

but if $S \neq 1$ then from equation (4):

$$C_{XO} = C^0_{XO} - \frac{A_2}{\epsilon_{ZrXO}l} + (S - 1)K_{HfXO}C_{XO}\alpha_{HfXO}C^0_{Hf} \quad (9c)$$

and from equation (7):

$$C_{XO} = C^0_{XO} - \frac{A_2}{\epsilon_{ZrXO}l} + (S - 1)K_{HfXO}\frac{C_{XO}K_{ZrXO}(A_1 - A_2) - A_2}{Sl\epsilon_{ZrXO}(K_{ZrXO} - K_{HfXO})} \quad (10)$$

Transformation of equation (10) gives:

$$C_{XO} = \frac{C^0_{XO}\epsilon_{HfXO}l(K_{ZrXO} - K_{HfXO}) - A_2(SK_{ZrXO} - K_{HfXO})}{\epsilon_{HfXO}l(K_{ZrXO} - K_{HfXO}) - (S - 1)K_{HfXO}K_{ZrXO}(A_1 - A_2)} \quad (11)$$

Rearranging equation (4) yields:

$$\frac{1}{\alpha_{MXO}} = 1 + K_{MXO}C_{XO} \quad (12)$$

Equations (7), (8) and (12) yield equations (13) and (14):

$$\frac{C_{Hf}}{\alpha_{HfXO}} = C^0_{Hf} = C_{Hf}(1 + K_{HfXO}C_{XO}) \quad (13)$$

$$\frac{C_{Zr}}{\alpha_{ZrXO}} = C^0_{Zr} = C_{Zr}(1 + K_{ZrXO}C_{XO}) \quad (14)$$

These equations, in conjunction with equation (11) or (9b) allow the total concentrations of hafnium (13) and zirconium (14) to be calculated.

EXPERIMENTAL

Reagents

Standard zirconium solution, 0.01M in 1M hydrochloric acid. Standardized gravimetrically (ZrO_2).

Standard hafnium solution. Hafnium dioxide (Johnson, Matthey spectral purity grade) was fused with a mixture of sodium carbonate and borax at 1100 ± 20°. The cooled melt was washed several times with water and dissolved in 6*M* hydrochloric acid. Hafnium hydroxide was precipitated with ammonia, filtered off, washed with water and dissolved in 1*M* hydrochloric acid to give an approx. 0.01*M* hafnium solution. This stock solution was standardized gravimetrically (HfO_2).

The standard zirconium and hafnium solutions were diluted with hydrochloric acid to give the working solutions.

Xylenol Orange solutions were prepared from reagents purified by the Yoshino method for Methylthymol Blue.[5]

Procedure

Solutions of zirconium and hafnium in hydrochloric acid were mixed with a solution of XO in the same concentration of hydrochloric acid and diluted to known volume with hydrochloric acid of the same concentration. The mixture was kept in a water-bath at 95 ± 1° for 15 min before spectral measurements were made against a reagent blank.

RESULTS AND DISCUSSION

Dependence of stability of HfXO and ZrXO on acidity

Preliminary investigations by the usual spectrophotometric methods[6–9] confirmed Chang's[1,2] and other authors'[4,9] statements that below pH = 1 a 1:1 hafnium or zirconium Xylenol Orange complex is formed.

To calculate the molar absorptivities and conditional stability constants of the HfXO and ZrXO complexes it was necessary to know the degrees of

dissociation at given initial metal and ligand concentrations.

The degrees of dissociation α_1 and α_2 obtained for two initial concentrations C_1^0 and C_2^0 of the metal (Hf or Zr) were calculated from the absorbances A' and A'' measured under the conditions:

$$C_M^0 = C_{XO}^0 = C_i^0$$

$C_i^0 l_i$ = constant (where l_i is the path-length)

$$C_1 = 2C_2$$

C_{HCl} = constant

where α_1, A', l_1 refer to C_1^0 and α_2, A'', l_2 to C_2^0.

Under these conditions

$$A_i = (1 - \alpha_i)\epsilon_{MXO} C_i^0 l_i \qquad (15)$$

and because

$$K_{MXO} = \frac{1 - \alpha_i}{\alpha_i^2 C_i^0} \qquad (16)$$

the ratio of the A_i values is

$$A_r = \frac{A'}{A''} = \frac{1 - \alpha_1}{1 - \alpha_2} = \frac{\alpha_1^2 C_1^0}{\alpha_2^2 C_2^0} = \frac{2\alpha_1^2}{\alpha_2^2} \qquad (17)$$

Transformation of (17) permits calculation of α_1 and α_2 from

$$\alpha_1 = \frac{A_r - 1}{(2A_r)^{0.5} - 1} \qquad (18)$$

$$\alpha_2 = \frac{A_r - 1}{A_r - (0.5A_r)^{0.5}} \qquad (19)$$

The values of α_i obtained were used to calculate the molar absorptivities ϵ_{MXO} for the HfXO and ZrXO complexes according to equation (15). The values are given in Table 1.

These values of α_i were introduced into equation (16) to calculate K_{HfXO} and K_{ZrXO} for particular hydrochloric acid concentrations. The results obtained are shown in Table 2.

Selection of acid concentration

The values of K_{HfXO} and K_{ZrXO} given in Table 2 allow the choice of the most advantageous acid concentrations for simultaneous determination of hafnium and zirconium. The difference between the ratios of the K_{MXO} values at the two acid concentrations should be as large as possible. The choice of the lower concentration is limited by the possibility of occurrence of $M(XO)_2$ complexes at $C_{HCl} < 0.1M$. The choice of the higher concentration is fixed by the maximum K_{MXO} ratio, which occurs with $1.2M$ hydrochloric acid.

Table 2. Effect of HCl concentration on conditional stability constants of HfXO and ZrXO complexes; mean values of 5 determinations

C_{HCl}, M	$\log K_{ZrXO}$	$\log K_{HfXO}$	$\log K_{ZrXO}/K_{HfXO}$
0.05	5.68	5.64	0.04
0.1	5.66	5.60	0.06
0.4	5.50	5.05	0.45
0.6	5.45	4.75	0.70
0.8	5.36	4.42	0.94
1.0	5.28	4.19	1.09
1.1	5.19	4.07	1.12
1.2	5.12	3.96	1.16
1.3	5.01	3.87	1.14
1.5	4.80	3.72	1.08
2.0	3.94	3.31	0.63

Selection of Xylenol Orange concentration

The absorbance differences between solutions of the HfXO and ZrXO complexes in $0.1M$ hydrochloric acid are small and practically independent of the Xylenol Orange concentration if this is more than 5 times the total concentration of hafnium and zirconium.

In contrast, the absorbance difference between HfXO and ZrXO solutions in $1.2M$ hydrochloric acid is large and strongly dependent on the XO concentration (Fig. 1). The absorbance differences for ZrXO and HfXO complexes as a function of XO concentration are shown in Fig. 2. It follows from Figs. 1 and 2 that the most advantageous XO concentrations are about 1.5–4 times the total concentration of the metals investigated.

Table 1. Effect of HCl concentration on molar absorptivities (XO as a blank λ = 535 nm) of HfXO and ZrXO complexes; mean values of 5 determinations

	HfXO		ZrXO	
C_{HCl}, M	Molar absorptivity, $l.mole^{-1}.cm^{-1}$	Standard deviation, $l.mole^{-1}.cm^{-1}$	Molar absorptivity, $l.mole^{-1}.cm^{-1}$	Standard deviation, $l.mole^{-1}.cm^{-1}$
0.1	4.10×10^4	7.5×10^2	3.51×10^4	6.5×10^2
0.3	4.12×10^4	90×10^2	3.52×10^4	8.5×10^2
0.5	4.08×10^4	9.5×10^2	3.50×10^4	8.0×10^2
1.0	3.99×10^4	1.20×10^3	3.52×10^4	9.0×10^2
1.2	4.07×10^4	1.30×10^3	3.49×10^4	9.5×10^2
1.5	4.06×10^4	1.30×10^3	3.48×10^4	1.10×10^3
2.0	3.98×10^4	1.60×10^3	3.49×10^4	1.30×10^3

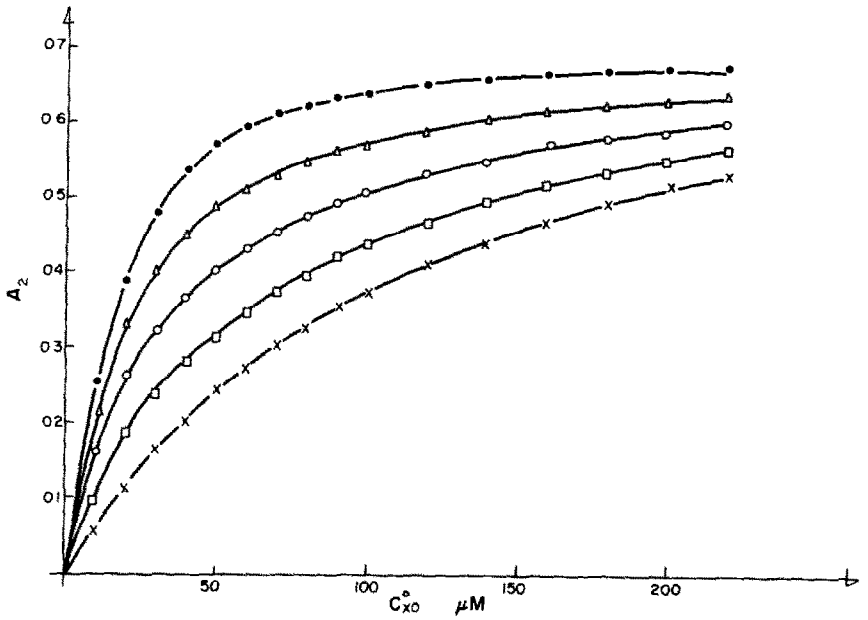

Fig. 1. Dependence of absorbance of HfXO and ZrXO complexes on Xylenol Orange concentration. λ = 535 nm, l = 1.000 cm, XO as blank, 1.2M HCl. Curves: ● 20 μM Zr; △ 15 μM Zr + 5 μM Hf; ○ 10 μM Zr + 10 μM Hf; □ 5 μM Zr + 15μM Hf; × 20 μM Hf.

Dependence of the absorbance on molar fraction of hafnium

To test the linearity between $(A_1 - A_2)$ and x_{Hf}, which could be predicted on the basis of the constant value of A_1 for a given C^0_M and the changes in A_2 (Fig. 1), the following transformation of equation (3) was necessary:

$$A_1 - A_2 = (S\alpha_{HfXO} C^0_{Hf} + \alpha_{ZrXO} C^0_{Zr})\epsilon_{ZrXO} l.$$

Introduction of the molar fractions $x_{Hf} = C^0_{Hf}/(C^0_{Hf} + C^0_{Zr})$ and $x_{Zr} = C^0_{Zr}/(C^0_{Hf} + C^0_{Zr})$ and use of the result $\alpha_{MXO} = 1/(1 + K_{MXO} C_{XO})$ from equation (12), gives

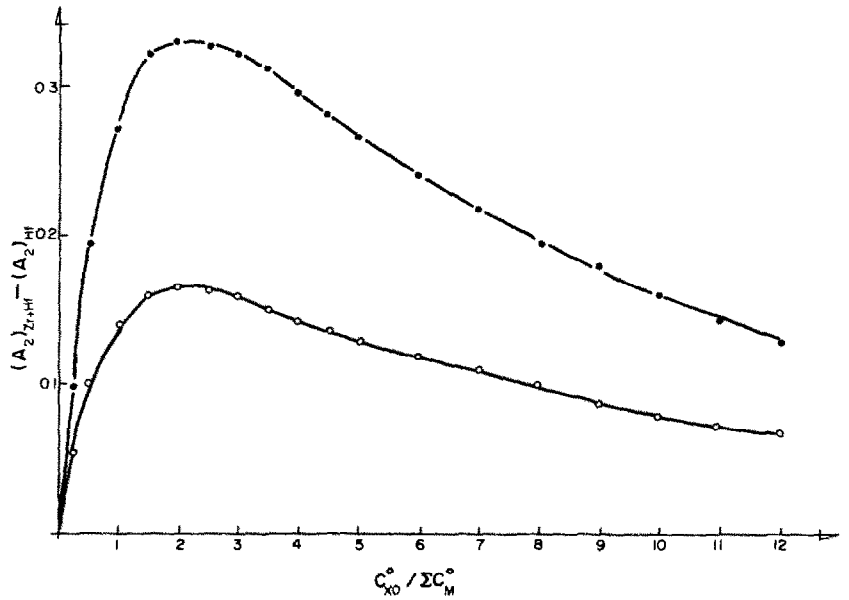

Fig. 2. Difference of ZrXO and HfXO absorbances *vs.* ratio of Xylenol Orange concentration to total concentration of zirconium and hafnium. λ = 535 nm, l = 1.000 cm, XO as blank, 1.2M HCl. Curves: ● $(A_2)_{ZrXO+HfXO}$ for 20 μM Zr; ○ $(A_2)_{ZrXO+HfXO}$ for 10 μM Zr + 10 μM Hf: $(A_2)_{HfXO}$ for 20 μM Hf (for both curves).

$$\frac{A_1 - A_2}{\epsilon_{ZrXO} l \Sigma C_M^0} = \frac{1}{1 + K_{ZrXO} C_{XO}} + x_{Hf} \frac{S}{1 + K_{HfXO} C_{XO}} - \frac{1}{1 + K_{ZrXO} C_{XO}} \quad (20)$$

where

$$\Sigma C_M^0 = C_{Hf}^0 + C_{Zr}^0.$$

From equation (20), at constant values of C_{XO} and ΣC_M^0 the dependence of $(A_1 - A_2)$ on x_{Hf} should be linear. This holds in practice if a relatively high C_{XO}^0 is used, since the relative changes in C_{XO} are then small and the values of $(1 + K_{HfXO} C_{XO})$ are much greater than those of $(1 + K_{ZrXO} C_{XO})$. The dependence of $(A_1 - A_2)$ on x_{Hf} is illustrated in Fig. 3 and is practically linear if C_{XO}^0/C_M^0 exceeds 2. However it is not advantageous to use very high concentrations of Xylenol Orange, because of the lower slope of the curves. The most advantageous range of C_{XO}^0/C_M^0 is 2–4, for ΣC_M^0 ranging from 2 to 20 μM.

The simultaneous determination

There are two methods of simultaneously determining hafnium and zirconium on the basis of the relations given in this paper.

1. Approximate determination of ΣC_M^0 based on equation (1), assuming that $\epsilon_{HfXO} = \epsilon_{ZrXO}$. Measurements are made of A_1 at $C_{HCl} = 0.1M$ and high excess of XO, and of A_2 at $C_{HCl} = 1.2M$ and low excess of XO, for several samples with the same ΣC_M^0 but different concentrations of hafnium and zirconium, to obtain the calibration curve. Determination of x_{Hf} for these solutions is based on the $(A_1 - A_2)$ values obtained from the calibration curve. The calculated x_{Hf} values can be used for more exact determinations of ΣC_M^0 [from equation (1)] and from this for more exact determinations of x_{Hf} by using the calibration curve.

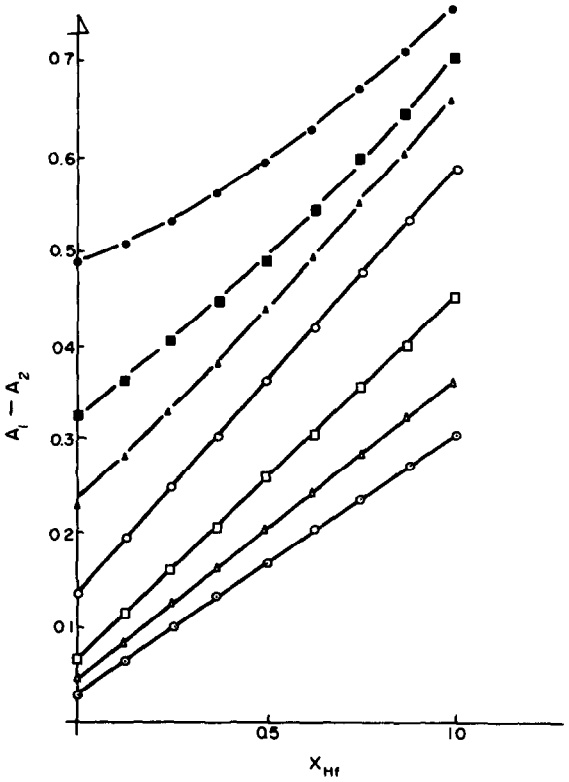

Fig. 3. Dependence of absorbance differences measured in 0.1M HCl (A_1) and 1.2M HCl (A_2) on molar fraction of hafnium (x_{Hf}). λ = 535 nm, l = 1.000 cm, XO as blank, $C_{Zr}^0 + C_{Hf}^0 = 20$ μM. Ratio of Xylenol Orange concentration to total concentration of zirconium and hafnium: ● 0.5; ■ 1.0; ▲ 1.5; ○ 2.5; □ 5.0; △ 7.5; ⊙ 10.0.

Table 3. Simultaneous spectrophotometric determination of Hf and Zr in hydrochloric acid solution, with Xylenol Orange: mean values of C_{Hf}^0, C_{Zr}^0, relative errors (E_r) and relative standard deviations (s) were calculated for 5 determinations

	Concentrations of hafnium and zirconium							
	Taken, μM		Found					
l, cm	C_{Hf}^0	C_{Zr}^0	C_{Hf}^0, μM	E_r, %	s, %	C_{Zr}^0 μM	E_r, %	s, %
1.000	10.00	10.00	10.08	+0.8	0.9	9.95	−0.5	0.6
	5.00	15.00	5.02	+0.4	1.2	15.01	+0.1	0.5
	15.00	5.00	15.11	+0.7	0.7	4.97	−0.6	1.0
	2.00	18.00	2.05	+2.5	2.0	17.96	−0.2	0.5
	18.00	2.00	17.94	−0.3	0.5	2.02	+1.0	2.4
2.000	5.00	5.00	5.07	+1.4	1.5	4.94	−1.2	1.4
	1.00	9.00	1.04	+4.0	4.6	8.95	−0.6	0.8
	9.00	1.00	9.07	+0.8	0.9	0.97	−3.0	3.7
5.000	2.00	2.00	2.08	+4.0	1.6	1.98	−1.0	1.5
	0.40	3.60	0.42	+5.0	6.8	3.56	−1.1	1.0
	3.60	0.40	3.62	+0.6	1.1	0.39	−2.5	4.9

This method gives results of similar accuracy to those obtained by the "three point" method of Challis.

2. Approximate determination of ΣC_M^0 as above, followed by calculation of C_{Hf}^0 and C_{Zr}^0 as in the following example.

Data required

Molar absorptivities: $\epsilon_{HfXO} = 4.08_5 \times 10^4$; $\epsilon_{ZrXO} = 3.50_5 \times 10^4$ l.mole^{-1}.cm^{-1}; $S = 1.166$.

Conditional stability constants ($C_{HCl} = 1.2M$):

$K_{HfXO} = 9.12 \times 10^3$; $K_{ZrXO} = 1.318 \times 10^5$.

Absorbances (XO as blank; $l = 1.000$ cm, $\lambda = 535$ nm):

at $C_{HCl} = 0.1M$, $C_{XO}^0 = 100\mu M$:

$$A_1 = 0.760;\ C_M^0 = \frac{2A_1}{(\epsilon_{HfXO} - \epsilon_{ZrXO})l} \sim 20\mu M;$$

at $C_{HCl} = 1.2M$, $C_{XO}^0 = 40\mu M$:

$A_2 = 0.365$; $A_1 - A_2 = 0.395$.

Calculations

Concentration of "free" Xylenol Orange $C_{XO} = 2.99 \times 10^{-5}M$ [equation (11)]. Concentration of "free" hafnium $C_{Hf} = 7.95 \times 10^{-6}M$ [equation (7)] and of zirconium $C_{Zr} = 2.00 \times 10^{-6}M$ [equation (8)]. Total concentration of hafnium $C_{Hf}^0 = 10.12 \times 10^{-6}M$ [equation (13)] and of zirconium $C_{Zr}^0 = 9.89 \times 10^{-6}M$ [equation (14)].

The metal concentrations actually used were both $10 \times 10^{-6}M$. Results obtained in this way are given in Table 3.

There are two conditions which should be fulfilled when using this second method: the Xylenol Orange should be of high purity, and all interfering species mentioned by Challis[3] should be removed.

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SHORT COMMUNICATIONS

DETERMINATION OF BORON IN SILICON-BEARING ALLOYS, STEEL, AND OTHER ALLOYS BY PYROHYDROLYSIS AND INDUCTIVELY-COUPLED ARGON-PLASMA SPECTROSCOPY

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Summary—Boron is quantitatively separated from silicon-bearing and other inorganic materials by pyrohydrolysis. Microgram amounts of boron are separated by passing oxygen-saturated steam over a sample mixed with vanadium oxide and copper oxide. The distillate is collected in dilute potassium hydroxide solution and determined by inductively-coupled argon-plasma spectroscopy.

The major difficulties in determining boron in trace quantities in silicon and other materials lie in the varied decomposition and separation techniques necessary. Silicon interferes in the spectrophotometric determination of boron when procedures requiring concentrated acid are used.[1] Boron is often lost during acid dissolution of silicon material, and although alkaline fusions (*e.g.*, with sodium peroxide and carbonate) will decompose the sample and retain the boron, the presence of silicate and large quantities of sodium salts interfere in the final determination.

The pyrohydrolysis method is a convenient means of isolating boron for analysis[2] and emission spectroscopy with the inductively-coupled argon plasma serves as a sensitive and rapid method for determining boron directly in the distillate. Use of separation and concentration techniques before the emission analysis minimizes or eliminates many troublesome spectral, physical and matrix problems.

It has been found necessary, however, to add a flux to ensure the complete recovery of boron from certain materials. To separate boron from magnesium hydroxide by pyropyrdrolysis, for example, vanadium oxide has been used. Our investigations have shown that for separation of boron from high-temperature alloys, stainless steel, silicon alloys and silicon metal by pyrohydrolysis it is necessary to add vanadium pentoxide and sometimes cupric oxide as well. Use of oxygen as carrier gas gives an oxidizing atmosphere which helps in the decomposition of the sample, shortens the time needed, and prevents suck-back of the distillate.

EXPERIMENTAL

Apparatus

The pyrohydrolysis apparatus described by Yoshimori *et al.*[2] is modified to include an oxygen inlet. All quartz parts are fabricated from high-purity low-boron quartz. The heat source consists of a Burrell model BT-1-9 "globar" electric furnace for the main combustion tube, a 500-W split-tube furnace for the preheater and a heating mantle for the 1-litre quartz flask.

Vitreosil quartz boats (4 in. long, $\frac{3}{4}$ in. wide, $\frac{1}{2}$ in. deep).
Nickel foil, 1 in. wide, 0.001 in. thick.

The induction-coupled argon-plasma system consists of a Jarrell-Ash Model 90-975 "Plasma Atom Comp" equipped with a Jarrell-Ash Model 90-555 spectrum shifter or background corrector. The direct-reading spectrometer system consists of a 0.75-m focal length spectrometer with a controller utilizing a DPSE central processing unit with 8K core memory for 23 channels, providing for all operating functions and computations.

The excitation source is a radiofrequency generator, plasma-therm type HFP 2000 D, which delivers up to 2 kW of power at 27.13 MHz through a water-cooled inductance coil.

Reagents

Reagent grade vanadium pentoxide, cupric oxide and potassium hydroxide are used as received. A stock boron solution (1000 ppm) is prepared from reagent grade boric acid and standardized titrimetrically. Dilute boron solutions are prepared from the stock solution as required.

Procedure

The apparatus is prepared for pyrohydrolysis by heating to 1200° and adjusting the boiling water and oxygen flow to deliver 3 ml of distillate per minute. A reagent blank is carried through all steps of the procedure.

A 0.25-g sample, accurately weighed, is transferred to a quartz boat lined with nickel foil and mixed with 1.0 g of vanadium pentoxide and 0.5 g of cupric oxide. The boat is placed in the centre of the heated zone of the combustion tube and the distillate is collected during 2 hr in a plastic beaker containing 40 ml of water and five drops of 10% potassium hydroxide solution.

The distillate is concentrated to appropriate volume, acidified with a few drops of hydrochloric acid, transferred to a plastic standard flask and diluted to volume. The wide linear range of the emission measurement allows flexibility in standardization and calibration. Two standards are

Table 1. Comparison of boron determination by different methods (% B)

	Argon plasma	Spectrophotometric*		
Sample	Pyrolysis	Pyrolysis†	Fusion§	Acid‡
50% FeSi				
J-5861	0.043	0.043	0.043; 0.045	
Std. A	0.14	0.14	0.14; 0.15	
Std. B	0.078	0.078	0.077	
NBS 59a	0.056			
(Cert-0.058)	0.058			
ASTM	0.0016	0.0018	0.002 ± 0.002	
(0.002)	0.0017			
Silicon briquet				
814787	0.056	0.056; 0.055		
814788	0.060	0.060; 0.061		
815109	0.071	0.070		
NBS-151 steel	0.0029	0.0027		0.0025–0.0029
(0.0027%)		0.0025		
		0.0029		
Chromium metal	0.0005	0.0004		

* The carminic acid spectrophotometric determination of boron is similar to the method used by Hatcher and Wilcox.[1]

† Ion-exchange is used to remove the vanadium before colour development.

§ The sample is fused in Na_2O_2 and Na_2CO_3, the cooled melt leached with water and the solution diluted to volume.

‡ The sample is dissolved under an air condenser, and the residue treated and combined with the main sample.

used; a low or zero concentration, and a high standard prepared from a stock solution of boric acid. The high standard, usually 1.0, 10.0, or 100.0 ppm boron, is chosen to match the expected boron level in the final volume of diluted distillate.

Plasma operating conditions

Forward r.f. power	1.1 kW
Reflected power	5 W by tuning
Argon coolant flow-rate	20 l./min
Auxiliary argon flow-rate	1 l./min
Sample solution uptake	1.0 ml/min
Wavelength	249.7 nm
Height of observation above coil	15 mm

RESULTS AND DISCUSSION

Boron is released in the form of boric acid by the action of steam and the vanadium pentoxide/cupric oxide flux. It was found necessary to use 100-mesh sample material and to pyrohydrolyse for 2 hr to remove the boron completely. It was also necessary to limit silicon-bearing samples to a maximum of 0.25 g for recovery of boron to be complete.

The method has been used to determine boron in various materials (*e.g.*, chromium, ductile iron, steel, vanadium alloys and ferrocolumbium).

Advantages of the plasma emission method are minimal matrix interference, clean background, and stability. Some vanadium and copper is carried over in the distillate but concentrations of 100–200 ppm have no effect on the boron determination.

The accuracy and precision are shown in Table 1.

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DETERMINATION OF NIOBIUM IN STEELS

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Summary—The determination of niobium at levels of 0.01% and below is required in certain specifications for stainless-steel welding electrodes, containing 2–3% molybdenum and 0.01% titanium. A method has been developed, based on initial extraction of niobium thiocyanate into butyl acetate followed by stripping with fluoride and re-extraction of niobium thiocyanate after masking of the fluoride by addition of boric acid. The absorbance of the extract is measured at 385 nm. Mo, Ti, V and W can be tolerated at 50 times the concentration of Nb. For higher amounts of Mo, corrections can also be applied. Ta should, however, be restricted to ten times the Nb level. Precision and accuracy of the method are satisfactory. The time taken for an individual determination is about an hour. The method is applicable to mild, low-alloy, stainless and niobium-stabilized steels.

Stainless-steel welding electrodes used for certain fabrication jobs have an upper limit of 0.01% for niobium and titanium. Molybdenum is present to the extent of 2–3% in these electrodes.[1] The methods available for determination of niobium in steels are for niobium-stabilized steels in which the niobium content is about 1%. They generally involve separation of niobium by hydrolysis, precipitation or ion-exchange,[2–6] followed by gravimetric or spectrophotometric determination. A few papers have also appeared on the direct spectrophotometric determination of niobium in steels. Emery[7] extracted niobium thiocyanate with acetone from an aqueous phase having a hydrogen-ion concentration $>14M$. Westland and Bezaire[8] extracted niobium thiocyanate into ether. These methods are for steels containing very little of the interfering elements Mo, Ti, V and W. A need therefore exists for a method for determination of niobium at the 0.01% level in presence of these elements.

A number of papers have described optimum conditions for formation of the niobium thiocyanate complex.[9–14] For the analysis of zircaloy for niobium Rodden[15] recommended $3.5M$ hydrochloric acid, $0.065M$ stannous chloride and $0.75M$ potassium thiocyanate and we have kept these conditions in the present studies, but have used butyl acetate for the extraction, instead of ether.

EXPERIMENTAL

Reagents

Standard niobium solution (20 μg/ml). Fuse 0.0715 g of ignited niobium pentoxide with 3 g of potassium pyrosulphate in a silica crucible; cool, and take up the melt in 10 ml of 10% tartaric acid solution. Transfer to a 100-ml standard flask and make up to volume with 10% tartaric acid solution. Dilute 4 ml of this stock solution to 100 ml with 10% tartaric acid solution.

Standard titanium solution (1 *mg/ml*). Fuse 0.416 g of ignited titanium dioxide with 4 g of potassium bisulphate. Take up the cooled fused mass in 50 ml of 10% sulphuric acid and make up to 250 ml with water.

Standard vanadium solution (1 *mg/ml*). Fume 0.180 g of vanadium pentoxide with 2 ml of concentrated sulphuric acid. Cool and dilute accurately to 100 ml.

Standard tungsten solution (1 *mg/ml*). Dissolve 0.179 g of sodium tungstate dihydrate in water and make up to 100 ml.

Iron solution (10 *mg/ml*). Dissolve 2.5 g of pure iron (tested to be niobium-free) in 50 ml of $6M$ hydrochloric acid and dilute to 250 ml.

Stannous chloride solution, 50%. Dissolve 50 g of stannous chloride dihydrate in 50 ml of concentrated hydrochloric acid and make up to 100 ml with water. Prepare fresh as required.

Iron, titanium, tungsten, vanadium and tantalum all interfere by being extracted as thiocyanate complexes which absorb at the wavelength of interest. A scheme was therefore worked out for the separation of niobium, taking advantage of the complexation of niobium with fluoride[16] and subsequent decomplexation with boric acid and re-extraction of the niobium as its thiocyanate complex.

Procedure

Determination of Nb at the 0.01% *level*. Dissolve 0.200 g of sample in 10 ml of concentrated hydrochloric acid and a few drops of nitric acid. Evaporate to dryness then take up with 5 ml of hydrochloric acid, and repeat this step twice more. Take up the residue in 10 ml of 10% tartaric acid solution with warming, cool, and transfer to a 100-ml separatory funnel. Add 10 ml of concentrated hydrochloric acid, 2 ml of stannous chloride solution and 5 ml of 30% potassium thiocyanate solution. After 5 min, shake with 10 ml of butyl acetate for 1 min. Reject the aqueous phase. Shake the organic phase for 30 sec with a solution containing 20 ml of $6M$ hydrochloric acid, 2 ml of stannous chloride solution and 0.8 ml of hydrofluoric acid (1 + 3). Transfer the aqueous phase to another 100-ml separatory funnel and wash it with 5 ml of butyl acetate. Transfer the aqueous layer to another 100-ml separatory funnel. Add 0.5 ml of iron solution and 5 ml of 30% potassium thiocyanate solution and mix. Within 1 min add 0.5 g of boric acid and shake to dissolve it. Immediately shake with 10 ml of butyl acetate for 1 min. Transfer the organic layer to a

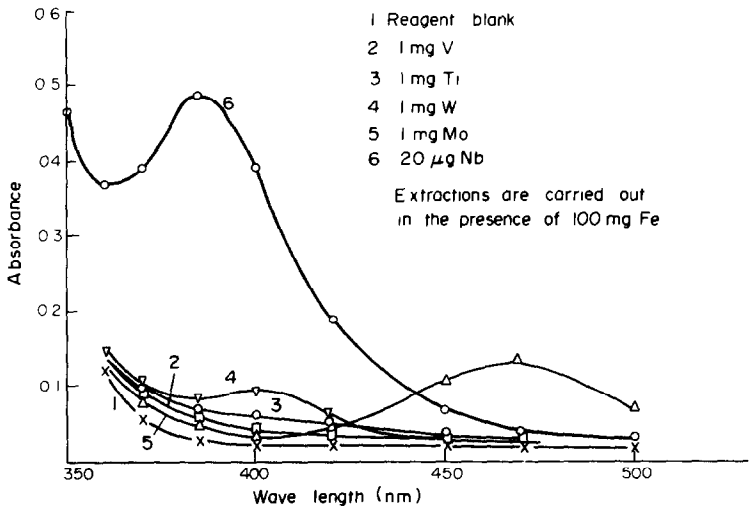

Fig. 1. Spectra of thiocyanate complexes of V, W, Ti, Mo and Nb in butyl acetate.

1.00-cm cell and measure the absorbance at 385 and 470 nm. If there is an absorbance signal at 470 nm indicating presence of molybdenum thiocyanate correct for the corresponding absorbance at 385 nm from a correction graph or by simple proportion. From the net absorbance at 385 nm calculate the amount of niobium with a calibration graph. The colour is stable for about 3 hr.

Determination of Nb at the 1% level. Fuse 0.100 g of sample with 4 g of potassium bisulphate and take up the cooled melt in 50 ml of 10% tartaric acid solution. Transfer to a 100-ml volumetric flask and make up to volume with 10% tartaric acid solution. Take an aliquot such that its niobium content falls within the calibration curve range and transfer it to a 100-ml separatory funnel. Add an aliquot of iron solution to bring the quantity of iron into the range 70–100 mg. Add 1 g of tartaric acid, and shake to dissolve it. Add 10 ml of concentrated hydrochloric acid, 2 ml of 50% stannous chloride solution and 5 ml of 30% potassium thiocyanate solution and proceed with the extraction as already described.

Calibration curve. Take portions of standard niobium solution equivalent to 0, 10, 15, 20 and 30 μg of Nb in 100-ml separatory funnels each containing 10 ml of iron solution and 1 g of tartaric acid. Add hydrochloric acid, stannous chloride solution and potassium thiocyanate solution and proceed as just described.

RESULTS AND DISCUSSION

Interferences

The elements which can interfere in the procedure are molybdenum, titanium, tantalum, vanadium and tungsten. The iron and any chromium and nickel present will not interfere as all these elements are left in the aqueous phase, the iron being reduced to iron(II) by the stannous chloride.

The five elements mentioned all interfere by forming extractable thiocyanate complexes which absorb at the wavelength of interest (Fig. 1, tantalum not shown). Niobium is therefore preferentially stripped (along with any tantalum) with fluoride,[16] and re-extracted after masking of the fluoride with boric acid. The necessary concentrations were found by trial and error. Table 1 shows that titanium, tungsten and vanadium can be tolerated in up to 50-fold ratio to niobium, and tantalum up to a 10-fold ratio.

However, when the molybdenum content is high (2–3%) compared to that of niobium (0.01%), as in the case of welding electrodes discussed in this paper, part of the molybdenum accompanies niobium even in the final extraction step and adds to the absorbance at

Table 1. Error in the determination of niobium, caused by interfering elements

A. *Pure solutions*

Interfering element	Ratio of interfering element to Nb	Nb taken, μg	Nb found, μg
Ti	50	20	20
V	50	20	21.5
W	50	20	20.2
Ta	3	20	20
Ta	5	20	20.2
Ta	10	20	19.4
Ta	50	20	15.0

B. *Synthetic solutions**

Interfering element	Ratio of interfering element to Nb	Nb present, %	Nb found, %
Ti	50	0.10	0.10
		0.40	0.38
V	50	0.10	0.11
		0.40	0.41
W	50	0.10	0.11
		0.40	0.41
Ta	10	0.10	0.09
		0.40	0.38

*(0.1% Nb) 0.100 g of NBS 160 b fused with $KHSO_4$, taken up in tartaric acid solution, 100 μg of Nb added, made up to 100 ml, 10 aliquot taken for determination, (0.4% Nb) 0.100 g of NBS 160 b fused with $KHSO_4$, taken up in tartaric acid solution, 400 μg of Nb added, made up to 100 ml, 25-ml aliquot taken for determination.

Table 2. Analysis of steels

Standard steel	Interfering elements, %	Niobium certified value, %	Niobium found, %	Relative standard deviation,* %	Remarks
NBS 123b	Mo 0.17 Ti 0.006 V 0.05 W 0.18	0.75	0.76	5.2	Stainless steel
NBS 345	Mo 0.122 V 0.041	0.23	0.23	1.7	16 Cr 4 Ni steel
NBS 101e	Mo 0.43 V 0.043 W 0.053	0.013	0.013	4.6	Stainless steel
NBS 160b	Mo 2.83 V 0.05	—	0.0035	8.8	19 Cr 14 Ni 3 Mo steel
		μg present	*μg found*		
Aliquots of NBS 160b + NBS 123b		18.5	18	2.6	
32E + 15 μg Nb	Mo 0.023 V 0.002	15	14.6		Low-alloy steel (Cr 068 Ni 1.19)
33d + 15 μg Nb	Mo 0.246	15	14.4		Low alloy steel (Ni 3.38)
Mild steel + 7.5 μg Nb	—	—	7.7		

* Calculated from six independent results.
Niobium content of samples 32E, 33D and mild steel was found to be <5 ppm.

385 nm. Fortunately, a correction can be applied, as the spectra of the two thiocyanates are different, as seen from Fig. 1. The correction graph is constructed as follows. Take 10 ml of 10-mg/ml iron solution in each of five 100-ml beakers. Add 0, 1, 3, 5 and 10 ml of 1-mg/ml molybdenum solution. Evaporate to dryness. Take up the residue in 10 ml of 10% tartaric acid solution and transfer to a separatory funnel. Add 10 ml of concentrated hydrochloric acid, followed by 2 ml of 50% stannous chloride solution and 5 ml of 30% potassium thiocyanate solution. Continue as for determination of niobium, but measure the absorbances at 385 nm and 470 nm. Plot the absorbance at 385 nm against that at 470 nm. From the absorbance at 470 nm a correction can be applied for the contribution at 385 nm. It is necessary to measure the absorbances of the sample solutions at both wavelengths of course.

Applications

The results obtained on stainless steel, low-alloy steel and mild steel samples are given in Table 2. In the case of stainless steels the range covered is from 0.0035% to 0.76% Nb. The values obtained by this method are comparable to the certified NBS values. In the case of NBS 160b, no value for niobium is certified. It was, however, found to contain 0.0035% Nb. To evaluate the recovery, an aliquot of a solution of 123b containing 15 μg of niobium was mixed with an aliquot of 160b containing 3.5 μg of niobium and the procedure followed. The recovery was found to be 98%.

The method has been extended to other types of steels. Known amounts of niobium were added to solutions of low-alloy standard samples NBS 32E and 33d and of a mild steel, and the procedure was followed. The results given in Table 2 show that the method is satisfactory for these types of steels as well.

The methods given in the literature are for niobium-stabilized steels, in which the niobium content is high (about 1%, with interfering elements practically absent). Almost all of them, except those of Emery and of Westland and Bezaire, involve long tedious separations. Compared to these, the advantages of the present method are (1) it can be applied to all types of steels, (2) interference of Mo, Ti, V, W and Ta is taken care of, (3) Nb can be determined at both high (up to 1%) and low concentrations (0.01%), (4) no prior separation is required, (5) the accuracy and precision are good, (6) the time taken for a single determination is about an hour.

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IODINE TRICHLORIDE AS AN ANALYTICAL REAGENT FOR DETERMINATION OF SOME ORGANIC COMPOUNDS

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Summary Procedures are described for the determination of organic compounds with iodine trichloride under Andrews's titration conditions. Samples are directly titrated with iodine trichloride or first reacted with an excess of iodine monochloride, with subsequent titration of the iodine formed. The direct titration is done initially in feebly acid medium, then the acidity is raised (biotin, methionine, cystine and thiomersal). Pre-oxidation with iodine monochloride is used if the organic compound reacts slowly [tryptophan and arsenic(III) compounds] or is determined in bicarbonate medium (hydroxylamine and thiosemicarbazide). The ferrocyanide formed by the reduction of ferricyanide (by thiourea and allylthiourea) can also be titrated. Arsenic(V) compounds are determined after reduction to arsenic(III), and iodine in organic compounds is converted into iodide by alkaline fusion into iodide and the iodide titrated.

Iodine trichloride is easy to prepare, stable and widely applicable.[1–3] It has been used for analysis of various organic compounds,[4–6] and further applications are described in the present work.

Iodine trichloride and iodine monochloride are reduced by certain organic substrates to iodine which can be extracted with carbon tetrachloride and titrated with iodine trichloride under Andrews's conditions, to form iodine monochloride:

$$I_2 + ICl_3 \rightarrow 3ICl$$

Iodine trichloride often reacts faster than iodate, especially when the hydrochloric acid concentration is low.

EXPERIMENTAL

Reagents

Solutions of iodine monochloride[7] (0.1M) *and iodine trichloride*[1] (0.02*M*). Prepared in dilute hydrochloric acid and standardized iodometrically.[7]

Test compounds. Most of the compounds were high-purity commercial chemicals. Solutions were prepared in suitable media, *e.g.*, 0.1*M* hydrochloric acid, water, methanol, ethanol or glacial acetic acid and standardized by previously checked independent methods. Organic compounds containing arsenic or iodine were either analytical-grade or suitably purified.

All other chemicals were of reagent grade.

Procedures

Determination of hydroxylamine or thiosemicarbazide. The sample is mixed with 25 ml of water, 5 ml of carbon tetrachloride and about 2 g of sodium bicarbonate in a 250-ml iodine flask. When the sample has dissolved, 10 ml of 0.1*M* iodine monochloride are added with vigorous swirling. After a minute, the mixture is cautiously acidified with 20 ml of 6*M* hydrochloric acid. When the evolution of carbon dioxide has ceased, the flask is stoppered and shaken. The liberated iodine is titrated with 0.02*M* iodine trichloride until the solution becomes pale brown. The flask is then stoppered and shaken vigorously, and the titration slowly continued until the organic layer becomes colourless.

Determination of biotin, methionine, thiomersal or cystine. A solution of methionine or thiomersal in water, of biotin in 1% sodium bicarbonate solution or of cystine in 0.1*M* hydrochloric acid is mixed with 20 ml of water and 5 ml of carbon tetrachloride. The mixture is titrated with 0.02*M* iodine trichloride until the colour of the organic layer reaches its maximum intensity. The flask is then stoppered and shaken well, about 20 ml of 6*M* hydrochloric acid are added and the solution is again shaken vigorously. The titration is continued until the organic layer becomes colourless.

Determination of tryptophan. A solution of tryptophan in 0.1*M* hydrochloric acid is shaken for 5 min with 20 ml of water, 5 ml of carbon tetrachloride and 10 ml of 0.1*M* iodine monochloride. Then 20 ml of 6*M* hydrochloric acid are added and the iodine is titrated with 0.02*M* iodine trichloride until the organic phase is colourless.

Determination of 2-mercaptobenzoic acid. A methanolic solution of the sample is mixed with 20 ml of methanol, 1 ml of acrylonitrile and 0.5 ml of 5% aqueous potassium hydroxide solution. After 2 min, 25 ml of water, 2 ml of 6*M* hydrochloric acid and 5 ml of carbon tetrachloride are added and the sulphide formed is titrated with 0.02*M* iodine trichloride as in the determination of biotin *etc.*

Determination of thiourea or allylthiourea. The sample is mixed with 20 ml of water, 2 g of potassium carbonate and about 100% excess of 0.1*M* potassium ferricyanide. The mixture is swirled for 5 min, then 5 ml of 6*M* hydrochloric acid are cautiously added. After addition of 5 ml of carbon tetrachloride, the ferrocyanide formed is titrated with 0.02*M* iodine trichloride as for biotin *etc.*

Determination of iodine in organic compounds. The sample, in a 50-ml porcelain crucible, is covered with about 10 g of anhydrous potassium carbonate. The crucible is heated first for 1 hr in an air-oven at 60°, then for 3 hr in a muffle furnace at 350°, then cooled to room temperature. The contents are dissolved in 100 ml of water to give a stock solution. A suitable portion is taken in a 250-ml iodine flask and treated cautiously with 6*M* hydrochloric acid till there is no further effervescence. Then 5 ml of carbon tetrachloride and 20 ml of 6*M* hydrochloric acid are added and the iodide is titrated with 0.02*M* iodine trichloride until the organic phase is colourless.

Determination of arsenic in organic compounds. A sample of an arsenic(III) compound is dissolved in about 10 ml of 2:1 v/v glacial acetic acid–ethanol mixture, with warming if

Table 1. Reactions of organic compounds with iodine monochloride or iodine trichloride

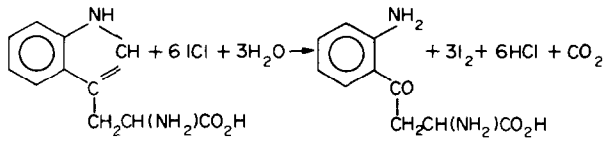
1. Hydroxylamine: $2NH_2OH + 4ICl \rightarrow N_2O + H_2O + 2I_2 + 4HCl$
2. Tryptophan: 
3. 2-Mercaptobenzoic acid: (a) $HO_2C.C_6H_4.SH + CH_2{=}CHCN \xrightarrow{OH^-} HO_2C.C_6H_4.SCH_2CH_2CN$
   (b) $HO_2C.C_6H_4.SCH_2CH_2CN + ICl_3 + H_2O \rightarrow HO_2C.C_6H_4.SCH_2CH_2\underset{\underset{O}{\|}}{C}N + 2HCl + ICl$
4. Thiomersal: $C_2H_5HgS.C_6H_5.CO_2^- + 3ICl_3 + 3H_2O \rightarrow HO_3S.C_6H_4.CO_2H + C_2H_5HgCl + 3ICl + 4HCl + Cl^-$
5. Thiosemicarbazide: $NH_2CSNHNH_2 + 10ICl + 6H_2O \rightarrow N_2 + NH_4Cl + H_2SO_4 + HCO_2H + 5I_2 + 9HCl$
6. Methionine and biotin: $RSR + ICl_3 + H_2O \rightarrow R\underset{\underset{O}{\|}}{S}R + ICl + 2HCl$
7. Cystine: $RSSR + 5ICl_3 + 6H_2O \rightarrow 2RSO_3H + 5ICl + 10HCl$
8. Thiourea and allylthiourea: (a) $RNHCSNH_2 + 8Fe(CN)_6^{3-} + 5CO_3^{2-} \rightarrow RNHCONH_2 + SO_4^{2-} + 8Fe(CN)_6^{4-} + 5CO_2$
   (b) $2Fe(CN)_6^{4-} + ICl_3 \rightarrow 2Fe(CN)_6^{3-} + ICl + 2Cl^-$
9. Arsenic(III) in organic compounds: $R_3As + ICl_3 + H_2O \rightarrow R_3AsO + ICl + 2HCl$
10. Iodine in organic compounds (after decomposition): $I^- + ICl_3 \rightarrow 2ICl + Cl^-$

Table 2. Determination of sulphur and nitrogen compounds

Compound	Range of sample weights, *mg*	Purity, % Present method[a] Range	Average	Comparison method
Thiosemicarbazide	0.98–6.38	99.4–99.7	99.6	99.8[b]
Thiourea	1.05–8.25	98.0–98.5	98.3	98.1[c]
Allylthiourea	2.12–13.62	97.5–98.0	97.8	97.5[d]
Biotin	4.90–24.56	99.7–100.1	99.9	99.7[e]
Methionine	5.99–20.72	98.7–99.0	98.9	98.8[f]
Cystine	2.13–15.84	99.2–99.5	99.3	99.5[g]
2-Mercaptobenzoic acid	1.98–10.22	99.5–99.8	99.6	99.4[h]
Thiomersal	5.21–21.70	99.6–99.9	99.8	100.0[i]
Hydroxylamine	1.65–8.25	99.7–100.2	99.9	99.7[j]
Tryptophan	3.40–15.66	99.5–99.9	99.7	99.8[k]

[a] Average of 8 determinations; [b] hypoiodite;[11] [c] hypoiodite;[21] [d] cerium(IV);[22] [e] periodate;[23] [f] chloramine-T;[24] [g] *N*-bromosuccinimide;[25] [h] lead(IV);[26] [i] AnalaR grade sample used; [j] ferricyanide;[27] [k] colorimetric determination.[28]

Table 3. Determination of arsenic(III), arsenic(V) and iodine in organic compounds

Compound	Range of sample weights, *mg*	As or I found, % Range	Average	As or I, theory, %
Triphenylarsine	14.67–38.25	24.4–24.5	24.4	24.51
2-Carboxyphenyldimethylarsine	10.28–40.69	33.3–33.4	33.3	33.19
2-Carboxyphenyldiphenylarsine	17.66–60.98	21.4–21.5	21.5	21.43
Triphenylarsine oxide	18.96–75.90	23.0–23.2	23.1	23.29
2-Carboxyphenylarsonic acid	11.61–55.29	30.5–30.8	30.7	30.49
Phenylarsonic acid	10.33–42.16	37.2–37.4	37.3	37.13
4-Chlorophenylarsonic acid	11.06–49.82	31.5–31.6	31.6	31.71
4-Tolylarsonic acid	10.92–48.25	34.7–34.9	34.9	34.72
4-Bromophenylarsonic acid	13.67–56.39	26.4–26.6	26.5	26.69
5,6-Di-iodo-8-hydroxyquinoline	8.33–38.74	63.7–63.9	63.8	63.97
Iodoform	6.21–36.08	96.4–96.7	96.6	96.76
2-Iodobenzoic acid	10.26–50.98	51.0–51.2	51.1	51.21
Sodium iodoacetate	9.67–41.26	61.0–61.2	61.1	61.05
2-Bromo-3,5-di-iodobenzoic acid	10.21–46.56	56.2–56.3	56.2	56.07

necessary. Water is added until a slight milkiness appears, then 5 ml of carbon tetrachloride and 10 ml of 0.1*M* iodine monochloride are added. The flask is stoppered and shaken for 1 min. The liberated iodine is titrated with 0.02*M* iodine trichloride after addition of 20 ml of 6*M* hydrochloric acid, as described for hydroxylamine.

Arsenic(V) compounds are dissolved in about 30 ml of the 2:1 acetic acid–ethanol mixture in a 150-ml long-necked flask and heated in a boiling water-bath. About 2 g of zinc are added to the hot solution in small portions during a period of 15 min. The mixture is then cooled to room temperature and the clear solution decanted. The residual zinc is washed 3 times with 5-ml portions of ethanol. The solution is titrated in a 250-ml iodine flask as above.

RESULTS AND DISCUSSION

The reactions used are given in Table 1. Results presented in Tables 2 and 3 show that the reactions are quantitative and that the Andrews two-phase indicator system can be used with iodine trichloride in moderately concentrated hydrochloric acid medium.

All determinations reported were done with 0.02*M* iodine trichloride except for biotin and 2-mercaptobenzoic acid (0.005*M* ICl_3). When the 0.005*M* reagent is used, it is best to oxidize the sample initially with 0.05*M* iodine monochloride and titrate the iodine liberated. In any case, the end-point detection is improved if, after each addition of titrant near the end-point, the flask is stoppered, shaken vigorously and inverted, the colour change being more easily seen when carbon tetrachloride has settled in the neck of the flask.

Iodine monochloride or trichloride will not react with the following substances under the conditions used (even when present in 10:1 molar ratio to the substance): acrylonitrile, serine, alanine, glucose, fructose, sucrose, glycine, formic, lactic, oxalic or citric acid, carbon disulphide, diphenyldisulphide, dimethylsulphoxide and urea. Unsaturated compounds, *e.g.*, cinnamic and maleic acids, that form addition compounds, or aromatic compounds, *e.g.*, phenol, aniline and sulphanilic acid, that undergo a substitution reaction with iodine monochloride, also do not interfere since the final determination involves an Andrews titration of the iodine liberated. The analytical interferences are due to iodide, sulphide, thiosulphate and thiocyanate. It is of course, necessary for only one oxidizable species to be present, and for its nature to be known so that the correct procedure can be chosen.

In the determination of cystine, biotin, methionine and thiomersal the oxidation is initially done in feebly acidic medium since at high concentrations of hydrochloric acid the reactions are slow and incomplete. Similarly, the oxidation of thiosemicarbazide takes about 30 min in acidic medium but only 1 min in a bicarbonate medium.

The proposed methods are convenient, rapid and precise. Solutions of iodine monochloride and iodine trichloride are stable for at least 6 months. Unlike bromate–bromide, iron(III), ferricyanide or permanganate, iodine monochloride reacts rapidly with hydroxylamine, in fixed stoichiometry, even in the presence of nitrite, which is a common interferent.[8]

In the determination of thiourea and allylthiourea a large excess of oxidant is used, to speed up an otherwise slow reaction. The reactions of biotin and methionine are interesting because, in contrast to bromine, iodine trichloride oxidizes these compounds only to sulphoxide, even when added in excess. There are several methods available for the determination of tryptophan, but they are tedious and the results are often uncertain.[9] Unlike other thiols, 2-mercaptobenzoic acid is oxidized beyond the disulphide stage by a number of oxidizing agents.[10] Methods for the determination of thiosemicarbazide involve oxidation with hypohalite,[11] chloramine-T or dichloramine-T,[12–14] lead tetra-acetate,[15,16] or dibromamine-T.[17] In most cases, the reaction is slow, taking 20–60 min. Barakat *et al.*[18] titrated iodide, formed by alkaline decomposition of organic iodine compounds, with *N*-bromosuccinimide, but iodine is intermediately formed in the titration and may volatilize, yielding low results. In the present method, the carbon tetrachloride dissolves the iodine and prevents loss. Methods for the determination of arsenic have been reviewed by Sandhu *et al.*[19] The titrations are done in glacial acetic acid or methanol to dissolve the test compound but the starch-iodine blue end-point is difficult to note.[20]

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A NEW SPECTROPHOTOMETRIC METHOD FOR DIFFERENTIATING MONONUCLEAR AND POLYNUCLEAR COMPLEXES AND FOR DETERMINING THEIR EXTRACTION CONSTANTS

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Summary—A new spectrophotometric method is proposed for differentiating mononuclear and polynuclear complexes as well as for determining the extraction constant K_e^* of any complex A_mB_n. The method is based on the effect of dilution on the degree of dissociation of the complex. The precision of log K_e^* is ± 0.03 when the degree of extraction of the metal is between 20 and 60%. The highest values of log K_e^* determinable by this method are 7, 19, 12 and 18 for 1:1, 2:2, 2:1 and 3:1 complexes, respectively.

In a previous work we proposed a graphical method for the differentiation of mononuclear and polynuclear complexes, and for determination of the stability constant of any complex A_mB_n.[1] The method was based on the effect of dilution on the degree of dissociation of the complex, and we now use the same principle for differentiating mononuclear and polynuclear complexes and determining their extraction constants.

The method is particularly useful in solvent extraction work because dimers and higher polymers are often formed in extractions. Also, although the method is developed for binary complexes, we think it could easily be extended to mixed-ligand complexes, which also frequently occur in extraction systems.

THEORY

Let us consider the formation and extraction of any complex by means of the equilibrium

$$mA_{(0)} + nB_{(w)} \rightleftharpoons A_mB_{n(0)}$$

where $m, n \geq 1$. For a particular value of pH, the conditional extraction constant is:

$$K'_e = \frac{K'P_e}{E_l^m} = \frac{[A_mB_n]_0}{[A]_0^m[B']_w^n} \quad (1)$$

where E_l, K' and P_e are respectively the extraction coefficient of the ligand, the conditional stability constant and the partition coefficient of the complex, given by the equations

$$E_l = \frac{[A]_0}{[A']_w} \quad (2)$$

$$K' = \frac{[A_mB_n]_w}{[A']_w^m[B']_w^n} \quad (3)$$

$$P_e = \frac{[A_mB_n]_0}{[A_mB_n]_w} \quad (4)$$

$[A']_w$ is the conditional concentration in the aqueous phase of the ligand not bound to the central ion B, in all its possible forms, molecular and ionic. Similarly, $[B']_w$ is the concentration of free metal ion in the aqueous phase that has not reacted with the complexant A.

Let a and b be the initial concentrations of ligand and cation in the organic phase and aqueous phase, respectively. Let us consider a 1:1 phase-volume ratio, and the condition $a/b = m/n$. Under these conditions, the degree of extraction of the metal, α_e, is

$$\alpha_e = \frac{[A_mB_n]_0}{[A_mB_n]_{max}} = \frac{[A_mB_n]_0}{a/m} = \frac{[A_mB_n]_0}{b/n} = \frac{A}{A_{0(b)}} \quad (5)$$

where A is the absorbance of the complex and $A_{0(b)}$ the absorbance when complexation and extraction are complete for cation concentration b, *i.e.*, when $\alpha_e = 1$.

By material balance from equations (2), (4) and (5), the concentrations of complex and reagents in equi-

librium are

$$[A_mB_n]_0 = \frac{b}{n}\alpha_c$$

$$[B']_w = b\left[1 - \left(1 + \frac{1}{P_c}\right)\alpha_c\right]$$

$$[A]_0 = \frac{m}{n} \cdot \frac{b\left[1 - \left(1 + \frac{1}{P_c}\right)\alpha_c\right]}{1 + \frac{1}{E_l}}.$$

Substituting these values in expression (1) gives

$$K'_c = \frac{K'P_c}{E_l^m} = \frac{n^{(m-1)}\alpha_c(E_l + 1)^m}{m^m b^{(m+n-1)}\left[1 - \left(1 + \frac{1}{P_c}\right)\alpha_c\right]^{(m+n)}} E_l^m$$

which can be rearranged to give

$$K_c^* = \frac{K'_c E_l^m}{(E_l + 1)^m} = \frac{n^{(m-1)}\alpha_c}{m^m b^{(m+n-1)}\left[1 - \left(1 + \frac{1}{P_c}\right)\alpha_c\right]^{(m+n)}} \quad (6)$$

By analogy with the previous work,[1] the following equation may be written:

$$\frac{(\beta A)^{1/(m+n)}}{(b_0/\beta)^{(m+n-1)/(m+n)}} = \left(\frac{K_c^* m^m A_{0(b_0)}}{n^{(m-1)}}\right)^{1/(m+n)} \times \left[1 - \left(1 + \frac{1}{P_c}\right)\frac{\beta A}{A_{0(b_0)}}\right]. \quad (7)$$

A straight line is obtained by plotting the left-hand side of equation (7) against βA.

When

$$\frac{b_0}{\beta} \to \infty; \quad \frac{(\beta A)^{1/(m+n)}}{(b_0/\beta)^{(m+n-1)/(m+n)}} \to 0 \text{ and } \beta A \to \frac{A_{0(b_0)}}{1 + \frac{1}{P_c}}$$

that is, the intersection of this straight line with the abscissa provides the value of $A_0(b_0)/(1 + 1/P_c)$. The slope of the straight line

$$\left(1 + \frac{1}{P_c}\right)\left(\frac{K_c^* m^m}{n^{(m-1)} A_{0(b_0)}^{(m+n-1)}}\right)^{1/(m+n)}$$

allows the calculation of $(1 + 1/P_c)K_c^*$. Metal chelates normally have high values of P_c, so $(1 + 1/P_c)K_c^* \sim K_c^*$.

If the method is to be used with ligands that have protolytic reactions, *e.g.*, a monoprotic ligand HA, is evident that the extraction coefficient of the ligand and the conditional stability constant of the complex depend on the pH in the aqueous phase:

$$E_l = \frac{[HA]_0}{[HA]_w\left(1 + \frac{K_a}{[H^+]}\right)} = \frac{P_l}{1 + \frac{K_a}{[H^+]}};$$

$$K' = \frac{K}{\left(1 + \frac{[H^+]}{K_a}\right)^m}$$

where P_l is the partition coefficient of the ligand, K_a the acidity constant of the ligand and K the stability constant of the complex. Under these conditions, neglecting possible side-reactions of the cation, equation (6) is transformed into

$$K_c^* = \frac{K'P_c}{(E_l + 1)^m} = \frac{KP_cK_a^m}{[K_a + (1 + P_l)[H^+]]^m}.$$

This equation describes the effect of pH on the conditional extraction constant.

The method proposed provides for the differentiation of mononuclear and polynuclear complexes. Suppose that the stoichiometry of a complex corresponds to a molar ratio equal to unity. If a plot of $(\beta A)^{\frac{1}{2}}/(b_0/\beta)^{\frac{1}{2}}$ *vs.* βA does not give a straight line then $(\beta A)^{\frac{1}{2}}/(b_0/\beta)^{\frac{1}{2}}$ can be plotted *vs.* βA and a straight line obtained in that case will confirm that the complex is 2:2 and not 1:1. The fact that the straight line and the curve have the same limit, $(\beta A)_{\lim.}$, aids in differentiating between them.

PRECISION AND FIELD OF APPLICATION OF THE METHOD

The precision of $\log K_c^*$ is ± 0.03 when α_c is between 20 and 60%. The highest values of $\log K_c^*$ determinable are 7, 19, 12 and 18, for 1:1, 2:2, 2:1 and 3:1 complexes, respectively.[1]

The approximation $(1 + 1/P_c)K_c^* \sim K_c^*$ introduces a new source of error, but it is easily evaluated as a function of P_c, with the following result:

P_c	10	20	30	40	50
$\Delta \log K_c^*$	+0.04	+0.02	+0.01	+0.01	+0.008

The error is practically negligible, even for quite small values of P_c.

EXPERIMENTAL

Procedure

Shake equal volumes of solutions of A and B (in immiscible solvents) at different concentrations, but in stoichiometric ratios so that in each the ratio $a/b = m/n$. Measure the absorbance of each extract against a reagent blank prepare with the same reagent concentration, but no cation. It is convenient to increase the cell path-length by a factor equal or proportional to the dilution factor. In this fashion, the value of βA is obtained directly.

Table 1.

b_0/β [Ni(II)], $10^{-5}M$	A (550 nm)	βA	$(\beta A)^{\frac{1}{2}}/(b_0/\beta)^{\frac{1}{2}}$	$(\beta A)^{\frac{1}{4}}/(b_0/\beta)^{\frac{1}{4}}$	α_c, %
1.0	0.060	0.480	219	4670	34.8
2.0	0.160	0.640	179	2990	46.4
4.5	0.470	0.835	134	1740	60.5
6.0	0.652	0.870	121	1420	63.0
8.0	0.935	0.935	108	1160	67.7

Reagents

Purpurin solution (1,2,4-trihydroxyanthraquinone). A $10^{-3}M$ solution was prepared by dissolving 0.256 g of the Merck product and diluting to 1 litre with methyl isobutyl ketone, A $2 \times 10^{-4}M$ solution was prepared by dilution.

Ni(II) solution. Prepared from $Ni(NO_3)_2.6H_2O$ and standardized with dimethylglyoxime. A $2 \times 10^{-4}M$ solution was prepared by dilution.

Ni(II)-Purpurin system

The stoichiometry of this complex was determined by the continuous variation method, maximal absorption being at 1:1 molar ratio. The extraction constant was determined for different values of pH and a b_0 value of $8 \times 10^{-5}M$. The results obtained at pH 8.48 are presented in Table 1.

The values of $(\beta A)^{\frac{1}{2}}/(b_0/\beta)^{\frac{1}{2}}$ with respect to βA (at several pH values) are presented graphically in Fig. 1, producing straight lines which intersect the abscissa to give a value of 1.38 for $A_{0(b_0)}/(1 + 1/P_c)$, which leads to a molar absorptivity of 1.72×10^4 l.mole^{-1}.cm^{-1}. The values of $(\beta A)^{\frac{1}{4}}/(b_0/\beta)^{\frac{1}{4}}$ (which would correspond to a 2:2 complex) are shown in the same figure, and give a curve. This clearly indicates that the complex is 1:1 and not 2:2. By substitution of the values found for $A_{0(b_0)}/(1 + 1/P_c)$ and

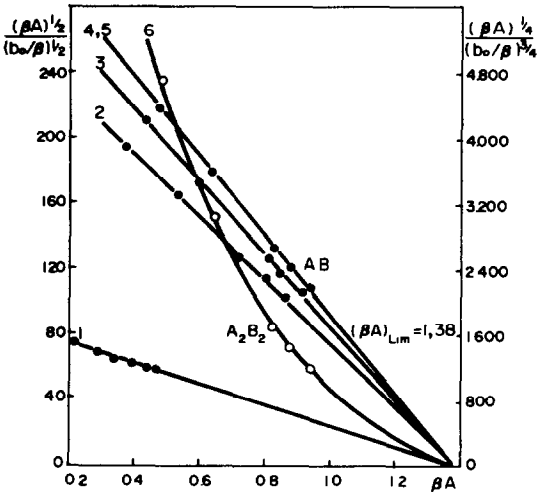

Fig. 1. Curve 1, pH 6.28; 2, 7.32; 3, 8.05; 4, 8.48; 5, 9.05; 6, 8.48 (A_2B_2).

the slopes of the straight lines into the expression for the slope, the following values for $(1 + 1/P_c)K_c^* \sim K_c^*$ can be calculated:

pH	6.28	7.32	8.05	8.48	9.05
$\log K_c^*$	3.61	4.71	4.83	4.91	4.91

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INSTRUMENTS IN ANALYSIS—CRITICAL REVIEWS

ABSORPTION SPECTROPHOTOMETRY

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Summary—A review is given of the development of spectrophotometers from the earliest models to the latest microprocessor instruments with photodiode detection systems.

Of the large numbers of determinations carried out by analytical chemists, an ever-increasing proportion is based on the application of Beer's law and involves the use of spectrophotometers. Though a wide range of the electromagnetic spectrum may be used for determining concentrations of atoms, radicals and molecules in the gas, liquid or solid phase from measurements of absorbance, most such measurements are made in the visible or ultraviolet region. This is partly because of the high efficiency and rapid response of the detectors developed for this part of the spectrum and partly because of the relative simplicity of the equipment required to resolve the characteristic absorptions produced by different molecules. The present review is therefore restricted to equipment which has been designed specifically for the measurement of absorbance in the visible and ultraviolet region of the electromagnetic spectrum. It is difficult to trace the earliest appearance of this equipment, for the spectrograph became changed imperceptibly into the spectrophotometer with the addition of the densitometer. However, it can be said that there has been a rapid increase in the sophistication of such instruments since the end of the war. The most recent development has been the incorporation of microprocessors, which is so far-reaching that a review of its implications is appropriate.

When an absorbance is measured the result is the sum of attenuations of the incident beam by a number of quite distinct and independent processes. If it is assumed that the incident beam experiences a large change in refractive index on passing into an isotropic medium which contains no discrete particles having a diameter of the same order as the wavelength of the incident radiation, then the following processes make significant contributions to the attenuation of the beam.

Reflection at the interface.
Rayleigh and Raman scattering within the medium.
Absorption of the radiation.

Each of these processes may have an angular dependence. The second will have an effect which varies with the size of the entrance aperture of the detector if it is assumed that scattering takes place over a solid angle of 4π. In addition the absorption process itself is complex and may involve radiation in the form of luminescence, photodissociation or other photochemical processes, external quenching or internal radiationless transitions. These processes may be dependent on the wavelength and intensity of the radiation and on temperature. It is important to remember that Beer's law is theoretically true only for one wavelength and one type of interaction, although perhaps somewhat surprisingly it can be used in many more complex systems. So far only the incident beam and the medium have been considered but the medium must be contained within some sort of vessel and the incident beam must be collimated, wavelength-selected and directed with the aid of optical components. Each of the optical surfaces may make some contribution, however small, to the selective absorption, dispersion and scattering of the beam. For these reasons alone it can be seen that the measurement of absorbance is similar to the measurement of activation energy for a chemical reaction. In both cases it is an essentially empirical property which is being measured. Further, it would not be surprising to find that in such measurements the result is sometimes sensitive to the style, age or mode of operation of the instrument. Later, some interesting comparisons will be made between instruments of different age and sophistication which illustrate this point.

In this discussion only a theoretical model has been considered; in practice the situation is even further complicated. In order to record a complete absorption spectrum, which may be considered as a graph of absorbance against wavelength, wavenumber or frequency, a source of continuous radiation is required. Such sources are readily available in the form of tungsten filament lamps or high-pressure gas discharge tubes, but their output is far from uniform over the whole region of the spectrum. Supposing that some dispersive element is used, then the radiation from such a source will be spread out in the form of an emission spectrum. If an absorber is then interposed, the resulting spectrum will differ in two ways. At every wavelength the intensity will be reduced because of the processes mentioned above. In addi-

tion, in some regions there will be a selective and significant reduction in intensity. These regions are the absorption bands which are characteristic of the absorbing species. It is within these bands that measurements of absorbance at specific wavelengths are made in order to determine concentration. The problem involved in making such measurements may be stated in its simplest form. Some means of determining the incident intensity in the absence of the absorber is required, together with some means of determining the attenuation in the presence of the absorber. Ideally the interposition of the absorber should have no effect upon the intensity of the beam entering the absorbing medium.

The earliest solution to this problem was the use of a photographic plate as the detector of both incident and transmitted radiation. The photographic plate has two great advantages as a detector: all wavelengths are recorded simultaneously, and the radiation falling upon the plate is integrated with time. As a result, variations in lamp intensity with time are only of secondary importance. Quite apart from the drawback of the time required for development, the fundamental disadvantage of photographic plates as detectors is their irreproducibility. For any given intensity and time of development the density of the photographic image depends on the coating weight of the photographic emulsion. This varies, not only from plate to plate, but across a single plate. However, before the 1940s most measurements of absorbance were made with photographic plates as detectors. The time-consuming task of comparing the intensity of incident and transmitted beams by densitometry was reduced with the aid of the Spekker photometer. This device is mentioned because it illustrates the principles used later in the development of photoelectric spectrophotometers. The radiation from the source was split into two approximately equal beams. One beam was allowed to pass through the cell containing the absorber and the second to pass through an identical cell containing only the solvent in which the absorber had been dissolved. While the radiation falling on the sample cell passed through a fixed aperture, that falling on the cell containing only the solvent passed through a variable aperture. The size of the aperture and hence the amount of radiation falling upon the cell could be adjusted mechanically and the ratio of the two apertures was indicated directly on a scale calibrated in absorbance. Pairs of spectra were photographed in juxtaposition at different settings of the variable aperture on the same photographic plate. From an inspection of the plate for areas of equal darkening, an absorption spectrum could be plotted. The error of the measurement of absorbance by photographic methods has been claimed to be as low as 2%. It is very unlikely that this can be achieved routinely and errors of up to ten times this may be experienced. There is a dramatic lowering of uncertainties in the measurement of absorbance when the photographic plate is replaced by some form of photoelectric device. There is an entirely different concept involved in the construction of an absorption spectrum by use of photoelectric detection. In the photographic method the whole spectrum was recorded, and the beam intensity was determined by the size of the variable aperture. A series of spectra was recorded at different aperture sizes, *i.e.*, different beam intensities, and an absorption spectrum was constructed from an inspection of the developed plate. In the photoelectric method it is necessary to make an instantaneous measurement of light intensity over a comparatively narrow wavelength range. A device such as a monochromator is required in order to isolate this narrow region of the spectrum. The absorption spectrum is then constructed by making successive measurements of intensity at different wavelength settings of the monochromator. It can be seen immediately that variations in lamp intensity are no longer acceptable and must be reduced to a minimum. At the same time the sensitivity of the detector must be invariant with time. Given such stability, it can be seen that photoelectric spectrophotometers are much more suitable for the measurement of absorbance at a given wavelength and it was this feature which prompted their rapid commercial development and general acceptance.

In the design of photoelectric spectrophotometers the choice of a suitable light-source was important mainly because of the need to obtain the greatest brightness possible per unit area. For the visible region the tungsten filament lamp provided the cheapest and most reliable source. However, to obtain the requisite brightness it was necessary to use tight coils of heavy-duty wire or ribbon filaments. The lamps selected were therefore low-voltage high-wattage bulbs of the car headlamp type. In order to gain brightness these lamps were sometimes run at a higher voltage, although this reduced lamp life. More recently lamp life has been extended by the inclusion within a quartz envelope of substances (halogens) which reduce the attrition of the lamp filament. Brightness per unit area has also been increased by the incorporation of built-in reflecting mirrors. The problem of obtaining short-term constant light output from such sources, in order to be able to measure absorbance reproducibly, was considerable. It was usually achieved by operating the lamps from a low-voltage storage cell which was continuously in a state of charge. This was soon replaced by solid-state low-voltage power supplies which had excellent short-term stability and without the tendency to drift exhibited by the storage cell. The light-source found most suitable for the ultraviolet region was the hydrogen discharge lamp and later, the deuterium discharge lamp. A continuous spectrum in the 150–500 nm region is obtained from a high-pressure discharge in molecular hydrogen and the lamp may be operated at a convenient low voltage if it is provided with a heated cathode. In some designs the cathode is surrounded by a tungsten anode provided with a slit

through which the radiation may pass to the monochromator.

Alternative light-sources for the ultraviolet region are provided by high-pressure discharges in the rare gases such as xenon. However, high-pressure xenon arc lamps are expensive and their use is restricted to equipment in which high light-intensities are essential, *e.g.*, spectrofluorimeters.

The early detectors of radiation were selenium photovoltaic cells. Their response was dependent upon both wavelength and light intensity and so they were most suitable for instruments employing the null method of measuring absorbance. The vacuum photocells which replaced them for measurements made in the ultraviolet region had a greatly improved linearity of response. Under favourable conditions this could be as good as $\pm 0.1\%$ non-linearity. As a result it became possible to design instruments in which the intensity of transmitted light-beams was measured directly from the photocell anode current. This was usually achieved by comparing the voltage developed across a high stability resistor in the anode circuit, by using a precision potentiometer.

The choice of the dispersive element in the optical system of a spectrophotometer for the visible and ultraviolet lies between quartz prisms and ruled diffraction gratings. All the early instruments used quartz prisms, but there has been a steady move to exploit the superior dispersive properties of diffraction gratings and to minimize their disadvantages.

A diffraction grating with the normally encountered dimensions of 50 × 50 mm and ruled with 600 lines/mm has a resolution of 30,000 (the resolution depending on grating size). The first-order resolving power of such a grating at a wavelength of 300 nm is thus 0.01 nm. The power of the dispersive system is however best expressed as the reciprocal linear dispersion. This quantity is defined as the wavelength difference between two lines, divided by the observed distance between them.

This quantity is very nearly constant (it varies as the cosine of the angle of diffraction) with wavelength for a grating, but varies for a prism. The reciprocal linear dispersion for a grating monochromator is commonly around 1.0 nm/mm for a collimator of focal length 1 m. The corresponding values for a quartz prism monochromator are 0.5 nm/mm at 200 nm in the ultraviolet, but 5 nm/mm at 450 nm in the visible region. Since there is a practical lower limit to the width of the exit slit, a large distance between it and the dispersive element is an advantage for obtaining the highest resolution. Alternatively, gratings of finer pitch, *i.e.*, 1200 and 1800 lines/mm, giving proportionately higher dispersion, may be used. However, increasing the size of the monochromator may introduce other disadvantages. In order to reduce stray light to a very low level the most expensive modern instruments make use of double monochromators in which two gratings, or a grating and prism are used in series; an increase in resolution is also obtained.

Until recently the gratings used in such combinations were high-quality plastic replicas cast from ruled gratings. However, a recent innovation has been the production of what have been called holographic gratings. These are produced photographically, by using the interference patterns created by laser light-sources, have better performance and are cheaper.

The first commercially successful design of photoelectric spectrophotometers was described by Hardy some fifty years ago. It was developed by the General Electric Company and was used most satisfactorily for making measurements of absorbance in the visible region of the spectrum. The light-source was a tungsten-ribbon filament lamp and a narrow wavelength band was isolated with the aid of a prism-type double monochromator. The radiation transmitted by the monochromator was passed to a beam-splitter which provided a pair of beams incident upon the faces of a pair of matched absorption cells. A photometric device of the kind described above adjusted the amount of radiation falling on the reference cell by control of an aperture. The beams transmitted by the sample and reference cells were incident upon the photocell detector alternately. In the first design this was achieved by the rotation of a sector which allowed one beam to pass through an unrestricted aperture, while reflecting the second from the rear of its opaque surface. In later modifications the same effect was produced by the aid of a polarizing system. This involved the use of a rotating polarizing prism and a second Wollaston double-image prism. Thus, as the intensity of one beam increased, the other was reduced, the angular dependence of intensity being $\cos^2\theta$. Finally, the beams were matched by the adjustment of a second polarizing prism. This system is not particularly efficient for the transmission of radiation, but it was used with success later, in studies of optical rotatory dispersion. In either system the beams are incident alternately upon the entrance of the photocell detector and if they are not matched the detector gives an a.c. signal. This signal is amplified and rectified and the resulting current allowed to activate a motor which drives the photometric system to its null point. At the null point the output signal falls to zero, the motor stops and the absorbance is read.

Some of the principles involved in the construction of this early instrument are still retained in modern designs.

The first commercial design of a manual photoelectric spectrophotometer was in 1941 by Cary and Beckman. The monochromator chosen was that modified from a mirror-collimated Littrow spectrograph incorporating a 30° aluminized quartz prism. This was chosen in preference to a grating monochromator because of the reduced intensity of stray light and was to become the standard optical system in a number of commercial instruments. Two photocells were used, one with a caesium oxide photosensi-

tive layer for the wavelengths longer than 625 nm and the second a photocell enclosed in ultraviolet-transmitting glass, suitable for operation at wavelengths down to 210 nm. The advantage of this duplicate detection system was that signals due to stray light were minimized because the photocell which was most sensitive to long-wavelength stray light was used only at monochromator settings where this was at a minimum. A 2000 MΩ resistor was included in the anode circuits of the photocells and the voltage developed across it was measured with a slide-wire potentiometer. The balance point was detected to within 0.2 mV with the aid of a valve voltmeter. The small dark-currents obtained with the photocells were compensated for by interposing a shutter and adjusting a second potentiometer to back off the signal. In practice the instrument was set to zero absorbance when the reference cell was in the beam and the scale sensitivity appropriate to the sample in the sample cell was selected by means of an additional potentiometer. This design took advantage of the linearity of response afforded by the vacuum photocell. It was the prototype of the first widely-used commercial photoelectric instrument, the Beckman D.U. Spectrophotometer. It set the pattern for manually operated single-beam spectrophotometers in which the wavelength was first selected by adjustment of the monochromator and the absorbance was then measured by comparing the signals obtained when the reference and sample cells were interposed between beam and detector. Later designs carried out this function automatically by the use of a beam-switching device. The monochromator was used in several commercial instruments. Light from either a tungsten filament lamp or a hydrogen discharge tube was focused on the entrance slit with the aid of a condensing mirror and a diagonal mirror. The light passing through the entrance slit was focused by a spherical mirror on to a 30° quartz prism aluminized on its back face. Thus after dispersion the light was internally reflected and returned along its original path but at a slightly higher position. It then left the monochromator through a second upper slit. The prism could be rotated mechanically, its position being indicated upon the wavelength scale. The light leaving the monochromator was then incident upon either the reference or sample cell, the positions of which could be interchanged. After passing through the cell the light was detected with the aid of a pair of photocells, or in later models a photomultiplier.

Two very similar instruments were developed later in this country. They were the Hilger Uvispek and the Unicam SP500. While in all three models the monochromators were similar, in the case of the Hilger Uvispek the entrance and exit slits were above and below the prism. It was claimed that this arrangement reduced the aberrational errors of the collimating mirror. In the Unicam SP500 the beams enter and emerge through the same slit at different heights. Other manufacturers who have produced manual photoelectric spectrophotometers are Bausch and Lomb, Cary, Coleman, Optica, Perkin–Elmer and Zeiss. The measurement of absorbance follows approximately the same pattern with all of these instruments. The appropriate wavelength is first chosen with the monochromator and the slit-width adjusted to give adequate sensitivity. With the reference cell in place the potentiometer is set to read zero absorbance or 100% transmission and the out-of-balance meter is set to zero. The sample cell is then exchanged for the reference cell, usually by the simple motion of a carriage and the potentiometer is adjusted until the out-of-balance meter again reads zero. The potentiometer is normally calibrated in absorbance or transmittance and is read directly.

While the manual photoelectric spectrophotometers readily permit the measurement of absorbance at a given wavelength to be made for a number of different samples in a comparatively short time, the manual plotting of an absorption spectrum is tedious. It requires separate absorbance readings at a large number of wavelengths. The procedure becomes more tedious the wider the wavelength range to be covered, or the smaller the wavelength interval between plotted points. One solution to this problem was provided by the Beckman Company (about 1943) who produced a design in which measurement of absorbance was automated. The absorbance appeared as a point on a graph with wavelength as ordinate and absorbance as abscissa. The time required for plotting a single point was 8 sec and the interval in wavelength between plotted points could be selected by the adjustment of a control. However, the absorption curve produced was in the form of a series of points and there was an increasing demand for an instrument which could provide an absorption spectrum in the form of a continuous curve. The initial requirements for such an instrument were that it should have continuous scanning of the spectrum by motor drive of the monochromator setting, a double-beam facility so that sample and reference cells would not have to be moved and that it should produce a written record, usually in the form of a graph of absorbance against wavelength. Because of the impossibility of matching pairs of light sources and maintaining their output identical, the double-beam facility must be achieved by switching the same monochromatic beam alternately through reference and sample cells with the aid of a moving mirror. To obtain high accuracy and to permit a rapid rate of scan for the whole spectrum this beam-switching must be carried out rapidly.

Making measurements of light intensity at this rapid repetition rate was extremely difficult with the photoemissive cells employed in the manual instruments. At the low intensities of light transmitted by both monochromator and absorption cell, very small photocurrents were developed. These small currents were usually measured with the aid of a high-value resistor of several thousand MΩ and a high-impedance input valve voltmeter. Such combinations have

a long time-constant and do not respond sufficiently rapidly. The need for a fast-response amplifier between the photoemissive cell and the read-out was made unnecessary by the introduction of the photomultiplier tube. In this device electrons from a photocathode similar to that in photoemissive cells are accelerated in a potential field and strike an electrode where they cause the emission of a shower of secondary electrons. These are in turn accelerated to a second electrode or dynode where a second, larger, shower is generated. The coupling of between 9 and 18 dynodes produces effective gains of about 10^6. The photomultiplier tube is thus equivalent to a photoemissive cell with a built-in high-gain fast-response amplifier. It was thus eminently suitable for use as a detector in the large automatic double-beam spectrophotometers which began to be developed in the 1960s. The first of these was designed by Cary, and this Company has marketed a range of double-beam instruments of which the Model 11 (and later 14 and 15) are early examples and the Model 219 the most recent. The Cary Model 14 was a double-beam photoelectric instrument employing a more sophisticated monochromator, consisting of a plane reflecting echelette grating and a 30° quartz prism. The light sources were, as in other designs, a tungsten lamp and a hydrogen discharge tube (water-cooled to provide high intensity). The light from one of these sources passed through the monochromator and fell upon a rotating mirror which sent the monochromatic radiation alternately through reference and sample cells at a frequency of 30 Hz. The synchronous motor driving the mirror also operated a chopper disc which provided a dark interval between each half-cycle. The beams passing through the cells were directed by mirrors to fall in turn upon a photomultiplier tube. The two signals provided by the pulses of radiation passing through the two cells were compared, and any difference activated a motor driving a potentiometer. This reduced the signal derived from the reference beam until it matched that from the sample beam. The motion of the potentiometer was coupled to the pen-drive and was plotted on a chart with linear absorbance and wavelength co-ordinates. It is interesting that the problem of comparing two signals which differ both in intensity and time of arrival has been solved in several ways. The method described above has been used in many models of spectrophotometer, the system used being different in method, rather than in principle. Modern electronic circuitry has provided comparison methods which are more stable and do not require heavy damping. One alternative method is to attenuate not the electrical signal but the light-beam itself. This involves the provision of an adjustable diaphragm (or comb-wedge) in the path of the beam incident upon the reference cell. Its aperture is adjusted through a servo-motor from the difference between the two electrical signals, and displayed on a recorder, usually with the aid of a mechanical linkage. The application of digital methods and of microprocessors (with their ability to store signals arriving at different times) has simplified this problem.

The accuracy of the manual instrument depends upon the linearity of response of the detector and this is good for vacuum photocells and photomultipliers. The accuracy of double-beam instruments on the other hand depends on the linearity of the beam attenuation (by diaphragms or comb-wedges).

One of the interesting features of the development of ultraviolet spectrophotometry has been the introduction of the low-cost double-beam instruments. These are sufficiently precise for all but the most exacting work and are capable of recording complex spectra in a few minutes. Typical of this type of instrument is the Pye Unicam SP800. In this model, radiation from one of the standard light-sources is selected by means of a solenoid-operated mirror, the position of which changes when the wavelength reaches 370 nm. The monochromator is of the modified Littrow type, the prism being rotated by a cam and lever mechanism to give a constant rate of change of wavelength with time. The light issuing from the exit slit is split into two beams, which pass alternately through attenuators and the sample and reference cells. The light-beams reach the photomultiplier and the output signals are used to adjust the attenuators, so that the intensity of light in the two beams is equal. The attenuators are coupled to the recorder pen so that absorbances between 0 and 2 are recorded linearly.

THE ADVENT OF THE MICROPROCESSOR

There are two fundamental pieces of information provided by all spectrophotometers, the absorbance or transmittance of the cell and the wavelength of the radiation at which the absorbance is being measured. The first is normally in the form of an analogue signal read on a meter or indicated upon a chart recorder. The second is normally obtained from a scale or the recorder chart. The first step in the introduction of microprocessor techniques to spectrophotometry was the provision of these two pieces of information in digital form. The absorbance was dealt with by the use of a digital voltmeter and the wavelength by setting the monochromator with a stepping motor, the condition of which could be read. It then became possible to use one of the many printers to accept the data in digital form and provide a permanent record. A further step forward was the direct processing of these data, that is, for example, the conversion of absorbance into concentration, or wavelength into wavenumber. The final step came when it was possible to use a microprocessor not merely to accept or process data, but to use them as feedback. Thus the microprocessor could accept instructions from a keyboard and transfer these to the spectrophotometer. With the provision of a suitable interface the microprocessor could control scan-rate, slit-width, and filter, lamp or sample-cell positions, while accepting

data and calibration constants.

Cell corrections for reflectance and path length, which may be neglected by the operator of a manual instrument, are automatically taken into account by the microprocessor-controlled spectrophotometers.

In 1976 a new interface was introduced for Varian Cary spectrophotometers. This allowed connection of the instrument to a computer terminal, a teletypewriter or a data set. The data which could be sampled included absorbance or transmittance, wavelength and time. Selection of any two of these parameters could be used for different applications. The variation of absorbance with time could be used to monitor kinetic reactions. The variation of absorbance with wavelength allowed ready tabulation of spectral data. A combination of all three parameters could be used when monitoring absorbance with time at two selected wavelengths, or over certain restricted wavelength regions. Thus, it was possible to follow the decay of a reactant and the appearance of a product in a reaction system. An alternative approach was the interfacing of the spectrophotometer with a programmable calculator. An example of this is given by the Macpherson Instruments series 700 spectrophotometers. These instruments incorporate a digital controller for digital-step wavelength scans with synchronized output data-processing. The chart-drive of the recorder is digitally coupled to the wavelength drive. This allows wavelength scales to be adjusted to between 0.1 and 50 nm/in. on the chart recorder. Photometric data are collected only during the stationary period of the digital stepwise wavelength scan. As soon as the signal averaging process has been completed, the wavelength is advanced by one unit and signal processing begins again. In this model the digital wavelength information is generated by an incremental encoder attached to the lead-screw in the monochromator. The encoder signals are processed and presented on a five-digit display, with a resolution of 0.01 nm. All transfers between the spectrophotometer and programmable calculator are buffered by an interface which matches calculator logic with interface logic and interface logic with the electrical inputs of the spectrometer. It also contains timing circuitry which may merely record elapsed time, or may control the time parameter in experiments. Thus the functions of the spectrometer may be controlled by signals originating from programs stored on magnetic cards, permitting the execution of a complex series of operations to be automated. At the same time, the data collected may also be processed by a number of stored programs and the final result of the experiments printed out on an associated printer. Other spectrophotometers which can be controlled in this way include the Cary Model 219.

The final logical step in the application of microprocessor techniques to spectrophotometry is the integration of a microcomputer into the spectrometer. The great advantage of such a combination is that the instrument can be programmed to be self-calibrating, that is, it automatically sets 0 and 100% transmission.

An example of this type of instrument is the Perkin–Elmer 554. This is a double-beam ratio-recording instrument, with computer control of both ordinate and abcissa on the recorder chart. As indicated, it has automatic setting of 100% transmission or zero absorbance and automatic concentration calibration. All operating parameters may be selected by keyboard entry and there is an automatic background correction. Thus a wavelength may be selected and the monochromator is automatically adjusted to the correct setting. Repetitive scans may be prescribed and the limits of each scan set. All values selected and stored may be recalled and displayed. This gives values of absorbance from 0.001 to 4 in steps of 0.001. The noise level is less than this. A further advantage of this type of instrument is that first and second derivative spectra may also be recorded, and these can sometimes be useful in the study of complex mixtures.

A modern version of the medium-priced double-beam spectrophotometer is the Pye Unicam SP8-100. In this design the monochromator is of the Ebert type, with a grating having 1200 lines/mm. In the early models this was one of the familiar high-quality replica gratings, but recently these have been replaced in the SP8-100 series by holographic gratings. The beam from the monochromator falls upon the beam-splitter, which is in the form of a pair of contra-rotating sector mirrors. As before, the beam-splitter produces a pair of pulsed beams, which pass alternately through sample and reference cells. After passing through the cells the two beams are combined on the surface of an end-window photomultiplier. One interesting feature of this instrument is that back-reflections from the surface of the photomultiplier and of the cells are rejected from the incident beams by ensuring that incidence to these surfaces is at a small angle. The output signals from the photomultiplier are amplified, compared to determine absorbance, and digitized to give a reading on an absorbance scale of 0–2, with a noise level of 0.005. The problem of keeping the recorder and monochromator drives synchronized, so that wavelength calibration of the chart remains constant, is overcome by driving the two stepping motors from a common train of pulses. Additional circuits allow the wavelength scan to be programmed and controlled. Upper and lower limits may be set for the scan, or the instrument can be adjusted to jump between four selected wavelengths and to record the absorbance at each. Control circuits can also determine cell position when up to four samples are being monitored in kinetic studies. The absorbance of each cell is set to zero and changes in absorbance with time are recorded on an absorbance scale appropriate to each sample. The instrument is compatible with teletypewriters, printers and programmable calculators for recording and processing of results.

In most of the instruments discussed so far a single

detector of radiation, sensitive to a wide wavelength band, was employed. As a result the process of recording a spectrum involved the presentation to the detector in turn of small wavelength bands selected from the incident polychromatic beam by some form of monochromator. Because of the finite response time of detectors and associated measuring circuits, the process of scanning and recording the spectrum over the full wavelength range of say 200–800 nm took an appreciable amount of time, in the range 1–10 min. Recently, Hewlett-Packard have overcome this time limitation by dispensing with the familiar photomultiplier tube and replacing it with an array of photodiodes. In practice these diodes are coupled with condensers in such a way that when the diode is illuminated the condenser is discharged. The current required to keep the condenser charged is then proportional to the amount of light falling on the diode. Because of the very small size of these detectors it is possible to produce an array of some 400 units, comparable in size with the space occupied by a spectrum, 200 units covering the range 200–400 nm and a further 200 units covering the range 400–800 nm. It is thus possible to have a detector with a resolution of 1 nm in the ultraviolet region and 2 nm in the visible region. Since all wavelengths are now detected simultaneously and each detector has a rapid rate of response, the array provides information about the whole of the spectrum in a time as short as 1 sec. This method of detection is particularly suitable for combination with a microprocessor and the Hewlett-Packard 8450A UV/VIS Spectrophotometer uses such a combination to provide an almost instantaneous presentation of a spectrum on a cathode-ray screen. The same screen acts as the visual display unit for the microprocessor so that instructions and the results of computations can also appear on the screen on demand from the instrument keyboard. Standard spectra can be stored for identification or for the determination of unknown concentrations, and their first and second derivatives obtained.

The flexibility of this system means that the stored spectra may be used in the rapid analysis of multicomponent mixtures and that the absorbance of a rapidly changing sample may be monitored at many wavelengths. The ability of the system to respond to and correct rapid calibration or baseline changes has allowed the manufacturers to use a greatly simplified optical system. The polychromatic light from the usual tungsten or deuterium lamp passes through the sample cell before it is dispersed by means of a holographic grating. It is claimed that a mirror which may direct the beam through several cell positions is the only moving optical member of the spectrometer. At present this design provides the most complete example of the integration of the microprocessor and the spectrophotometer.

ERRORS

Photoelectric spectrophotometers all rely for their measurements of absorbance on electric circuits and these may be prone to instability or error. In addition, false signals may be generated by stray light originating from reflections within the optics of the instrument. These errors and false signals may change with time both in intensity and frequency. For these reasons it has been common practice for manufacturers to prescribe photometric standards with which to check the calibrations of both wavelength and absorbance scales. Checking the calibration of the wavelength scale requires either the use of a low-pressure discharge lamp, giving emission lines at known wavelengths, or a sample with sharply defined absorption bands which can be readily identified. Some instruments have been fitted with mercury discharge lamps which can be used for calibration. Benzene vapour gives a number of sharp absorption bands which have been used for calibration, but it is more common for manufacturers to supply glass filters containing rare-earth ions, such as neodymium and praseodymium, or holmium. The provision of absorbance standards is more difficult. It requires the use of solutions of known concentrations of pure substances for which absorbance values have been measured precisely by many investigators. Substances which have been used are potassium nitrate, potassium chromate in alkaline solution and potassium dichromate in acid solution. Secondary standards such as neutral density filters or wire mesh screens are sometimes used. Equally important is the checking of stray-light levels within an instrument. Although claims are made that stray light may make only small differences to absorbance values (0.001 in the middle regions of the wavelength range) it may become very important at the shorter wavelengths where absorbance is high. A glass filter with total absorption at one region of the spectrum but with only weak absorption outside this region, is particularly useful. Measurement of absorbance beyond the cut-off point on the wavelength scale then gives an approximate measurement of the percentage of stray light. Checks for changes in overall instrumental sensitivity depend upon the nature of the instrument, but a common criterion is the smallest slit-width at which the instrument can be balanced.

In high-absorbance measurements, the energy reaching the detector is low and so signal-to-noise ratio is reduced. Digital voltmeters will collect counts over selected time intervals and will therefore present an average over the counting period. Noise with a low repetition rate will therefore cause flickering of the final digit. The round-up facility of digital voltmeters will tend to present a higher value than is sometimes justified.

Bandpass or slit-width is an important variable in spectrophotometry. It determines the resolution and affects the absorbance measurement. Because of the possibility of the lack of resolution in absorption bands some statement of slit-width is important, for comparison of results obtained with different instruments. Errors result when the absorbance varies with

Table 1. Comparison of measured absorbance with various spectrophotometers ($9.62 \times 10^{-4} M$ $K_2Cr_2O_7$)

Wavelength (λ_{max}, *nm*)	Absorbance	Absorbance ratio	Instrument
349, 350, 351 255, 256, 257, 258, 259	0.822 1.10	1.34	Pye Unicam SP 500
349, 350, 351, 352, 353 255, 256, 257, 258	0.802 1.07	1.33	Pye Unicam SP 6-400
350.5 256, 257, 258, 259	0.81 1.11	1.37	Pye Unicam SP 800
351.5 257	0.817 1.067	1.306	Pye Unicam SP 8-100
353 258.5	0.835 1.120	1.34	Pye Unicam SP 1800
352 257.5	0.863 1.149	1.33	Perkin–Elmer 552
351.5 258	0.90 1.174	1.304	Beckman DB
351, 352 257, 258	0.817 1.10	1.34	Gilford 2445
352 260	0.88 1.04	1.18	Cecil (CE) 505
352, 353	0.804	—	Cecil 393 (digital)

slit-width and may give lower values at absorption maxima and higher values at minima. In general it is best to record spectra at several slit-widths until further reduction produces no change in absorbance.

During the course of the development of spectrophotometry there have been many studies, both by manufacturers and other groups, to establish the reliability of commercial instruments. For example, the accuracy and precision of manual photoelectric spectrophotometry has been checked on 10 Beckman D.U. instruments and also on 38 Beckman D.U., 20 Unicam SP500 and 14 Hilger Uvispek spectrophotometers, with potassium hydrogen phthalate and potassium dichromate solutions. These trials showed that there was a greater divergence between results measured on different instruments than there was between replicate measurements made on the same instrument, a somewhat disturbing conclusion. These divergences were always greater in the shorter wavelength region of the spectrum, where the stray light due to reflection from the optical components becomes more important and forms a larger proportion of the total energy reaching the detector. It has also been found that it is advisable to check the calibration of an instrument many times during its life because of changes. These may be due to alteration in lamp intensity with wavelength, changes in stray-light intensity owing to aging of the surfaces of the optical components, variation in the photomultiplier gain and noise. Other studies have shown that there is a large variation in instrumental sensitivity between different instruments and the same instrument at different stages during its life. The general conclusion of this early work was that while most commercial instruments gave satisfactory readings of wavelength, values obtained for absorbance were at best reliable only to $\pm 0.5\%$. When different instruments were used it was suggested that no significance should be ascribed to differences in absorbance of less than $\pm 2.5\%$. Co-operative studies on recording double-beam spectrophotometers were first carried out with the Cary Model 11. Improved reliability was claimed and measurements of the absorbance of potassium chromate solutions at 371 nm showed an error of $\pm 0.2\%$. A later and more comprehensive study of recording spectrophotometers examined a sample of potassium nitrate solution with 94 instruments of 11 different types. The results of this study were less reassuring and gave a coefficient of variation of 2.75%. It has been pointed out, however, that some of this lack of precision may be due to the operator, rather than to the instrument. More consistent results have been obtained with careful measurements made on more restricted ranges of instruments. It has been suggested that with a double-beam recording spectrophotometer, used with care and in good working order, reproducible spectra may be obtained and a photometric error of less than $\pm 0.5\%$ achieved.

To indicate the variation which may be experienced with different types of spectrophotometers of widely different age, a single sample of potassium dichromate solution was examined, with the same pair of cells and on the same day, with a number of instruments. The results of this study are summarized in Table 1.

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THE USE OF OUTER-SPHERE COMPLEX FORMATION REACTIONS IN ION-EXCHANGE CHROMATOGRAPHY

SEPARATION OF OXALATE AND SULPHATE IONS

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Summary—It has been found that outer-sphere complex formation reactions can be used to increase the selectivity of ion-exchange separations. A method has been developed for the quantitative separation of sulphate and oxalate. The stability constants of the sulphate and oxalate 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 $I = 0$ have been obtained by extrapolation. They are $\log K[Co(en)_3^{3+} + SO_4^{2-}] = 3.38$; $\log K[Co(NH_3)_6^{3+} + SO_4^{2-}] = 3.60$; $\log K[Co(en)_3^{3+} + C_2O_4^{2-}] = 3.23$.

Inner-sphere complex formation reactions have been used in ion-exchange chromatography for quite a long time.[1–6] The purpose of the work reported in this paper has been the use of outer-sphere (ion-association) complex formation reactions for the enhancement of the selectivity of the separation of anions. Changes in the retention of bivalent sulphate and oxalate ions on an anion-exchanger column caused by the presence of cationic cobalt(III) complexes [such as tris(ethylenediamine)cobalt(III) and hexa-amminecobalt(III)] in the eluent have been studied. The selectivity of the sulphate–oxalate 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 crystalline potassium sulphate, potassium oxalate and potassium chloride were used for the eluents. Analytical grade crystalline barium chloride and calcium chloride were used for the determination of sulphate and oxalate, respectively. Tris(ethylenediamine)cobalt(III) chloride and hexa-amminecobalt(III) chloride were prepared from crystalline cobalt(II) chloride,[7] recrystallized from aqueous solution and dried, after washing with ethanol, at 105°. The column was packed with a 100–200 mesh strong anion-exchanger, Dowex AG 1 × 8, in chloride form.

Instruments

A universal pH-meter equipped with a combined glass–calomel electrode (for pH determinations) and a silver–silver chloride electrode (for argentometric titrations) was used. Spectrophotometric measurements were made with a Zeiss Specord UV-VIS spectrophotometer.

Elution experiments

A 132 × 5.5 mm column was packed with the anion-exchanger (bed volume 3.2 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, determined by argentometric titration, was maintained at 0.1*M*. The pH was adjusted to between 6.30 and 7.52. A 30-μl portion of 0.7*M* potassium sulphate or 1.8*M* potassium oxalate solution was injected with a Hamilton microsyringe for the elution experiments. Because of precipitation, oxalate could not be eluted with solutions containing hexa-amminecobalt(III) chloride. Fractions of 1.5–4.5 ml of the effluents were collected by fraction-collector. The retention volumes were determined as the average of 10 identical elution experiments.

The concentration of the oxalate and sulphate ions was determined turbidimetrically on the basis of their precipitation reactions with calcium and barium. For determination of the sulphate concentration 0.1 ml of 1*M* hydrochloric acid and 1.0 ml of 5% barium chloride solution were added to each fraction, and 1.0 ml of 5% calcium chloride solution was added to each fraction for the determination of the oxalate concentration. After the addition of the reagents the solutions were diluted to 5.0 ml and thoroughly mixed, and their absorbance at 667 nm was measured. The calibration plot was linear over the range studied.

Three solutions of different concentrations (0.1, 0.15 and 0.2*M chloride* concentration) were prepared from each of potassium chloride, tris(ethylenediamine)cobalt(III) chloride and hexa-amminecobalt(III) chloride. All elution experiments were repeated with all the eluents. From the results the retention volume was determined and distribution coefficients were calculated.

Separation of anions

Sulphate and oxalate were separated on a 250 × 7.0 mm column packed with the strong anion-exchanger, by the method used for the elution experiments: 0.10*M* potassium chloride (pH 7.52) and 0.033*M* tris(ethylenediamine)cobalt(III) chloride (pH 7.02; chloride concentration 0.10*M*) were used as the eluents. First, sulphate and oxalate were eluted separately with the potassium chloride solution, 75 μl of 0.7*M* potassium sulphate and 75 μl of 1.8*M* potassium oxalate being injected, and the elution diagrams were obtained. The retention volumes of the two anions were also determined from separate elution experiments with the tris(ethylenediamine)cobalt(III) chloride solution (50 μl

each of 0.7*M* potassium sulphate and 1.8*M* potassium oxalate were injected). Finally, 120 μl of a 1:1 mixture of the 0.7*M* potassium sulphate and 1.8*M* potassium oxalate were injected onto the column and eluted with tris(ethylenediamine)cobalt(III) chloride solution. The elution diagrams were plotted (*cf.* Fig. 2).

DISCUSSION

The distribution coefficient of an ion can be determined from the data obtained from elution experiments:[8]

$$D = \frac{V_{max}}{X} - a \qquad (1)$$

where a is the void volume fraction (0.4), D the volumetric distribution coefficient, V_{max} the elution volume, 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:

$$D = KQ^Z[B]^{-Z} \qquad (2)$$

where K is the concentration equilibrium constant of the ion-exchange reaction, Q 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

$$D' = \frac{K}{\alpha_L} Q^Z[B]^{-Z} \qquad (3)$$

where α_L is the function which accounts for the side-reaction.

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 ion-association complex can be derived from equations (1) and (3) as:

$$\log D - \log D' = \log \alpha_L \qquad (4)$$

$$\log \alpha_L = \log (1 + [M(x)_n]K_1) \qquad (5)$$

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 in the side-reaction.

The sulphate and oxalate ions were eluted with potassium chloride, tris(ethylenediamine)cobalt(III) chloride and hexa-amminecobalt(III) chloride solutions, each at three different chloride concentrations. The distribution coefficients were determined by means of equation (1) from the elution volumes (Tables 1 and 2). Their logarithms are plotted in Fig. 1 against the logarithms of the chloride concentration. The slopes of the lines are described by equations (2) and (3). It can be concluded from Fig. 1 that sulphate and oxalate cannot be separated with sufficient selectivity by the potassium chloride eluent, because the distribution coefficients are not sufficiently different. However, with tris(ethylenediamine)-cobalt(III) chloride solution as eluent the distribution coefficients of the two ions are sufficiently different for a selective separation. From the calculations above, the two ions should be separable on a 250 × 7 mm column of the strong anion-exchanger resin. The separation, effected with 0.1*M* tris(ethylenediamine)cobalt(III) chloride as eluent is shown in Fig. 2b.

Table 1. The distribution coefficient of the sulphate anion at various chloride concentrations

Eluent	$[Cl^-]$, *M*	$\log[Cl^-]$	V_{max}, *ml*	*D*	log *D*
KCl	0.099	−1.001	66.4	20.35	1.308
	0.148	−0.830	29.4	8.79	0.944
	0.195	−0.710	16.9	4.88	0.688
$[Co(en)_3]Cl_3$	0.096	−1.018	30.4	9.10	0.959
	0.138	−0.860	15.8	4.53	0.656
	0.193	−0.714	10.7	2.94	0.468
$[Co(NH_3)_6]Cl_3$	0.100	−1.000	24.0	7.11	0.851
	0.150	−0.824	11.4	3.16	0.499
	0.200	−0.699	7.5	1.94	0.287

Table 2. The distribution coefficient of the oxalate anion at various chloride concentrations

Eluent	$[Cl^-]$, *M*	$\log[Cl^-]$	V_{max}, *ml*	*D*	log *D*
KCl	0.099	−1.001	77.5	23.82	1.377
	0.148	−0.830	32.0	9.60	0.982
	0.195	−0.710	21.0	6.16	0.789
$[Co(en)_3]Cl_3$	0.096	−1.018	46.5	14.13	1.150
	0.138	−0.860	24.0	7.10	0.851
	0.193	−0.714	16.0	4.60	0.663

Table 3. Data for the calculation of the stability constants of the outer-sphere complexes of sulphate and various cobalt(III) complex cations

Complex	$[Cl^-]$, M	Cation, mM	I	log D	log D'	log α_L	log K_1	log K_1 (Ref. 9)
$([Co(en)_3]SO_4)^+$	0.05	16.7	0.1	1.910	1.455	0.455	2.06	1.78
	0.10	33.3	0.2	1.290	0.942	0.348	1.57	1.40
	0.15	50.0	0.3	0.935	0.643	0.292	1.28	1.18
	0.20	66.7	0.4	0.679	0.429	0.250	1.06	1.00
	0.25	83.3	0.5	0.475	0.261	0.214	0.88	0.89
$([Co(NH_3)_6]SO_4)^+$	0.05	16.7	0.1	1.910	1.315	0.595	2.24	2.00
	0.10	33.3	0.2	1.290	0.805	0.485	1.79	1.72
	0.15	50.0	0.3	0.935	0.500	0.435	1.54	1.51
	0.20	66.7	0.4	0.679	0.289	0.390	1.34	1.44
	0.25	83.3	0.5	0.475	0.122	0.353	1.18	1.35

Table 4. Data for the calculation of the stability constants of the outer-sphere complex of oxalate and tris(ethylenediamine)cobalt(III)

Complex	$[Cl^-]$, M	$[Co(en)_3^{3+}]$, mM	I	log D	log D'	log α_L	log K_1
$([Co(en)_3]C_2O_4)^+$	0.05	16.7	0.1	1.98	1.628	0.352	1.87
	0.10	33.3	0.2	1.378	1.116	0.262	1.39
	0.15	50.0	0.3	1.022	0.814	0.208	1.09
	0.20	66.7	0.4	0.769	0.600	0.169	0.85
	0.25	83.3	0.5	0.572	0.432	0.140	0.67

The results obtained with 0.1M potassium chloride as eluent are shown in Fig. 2a, and obviously the separation is not feasible (the elution volumes were determined in separate elution experiments).

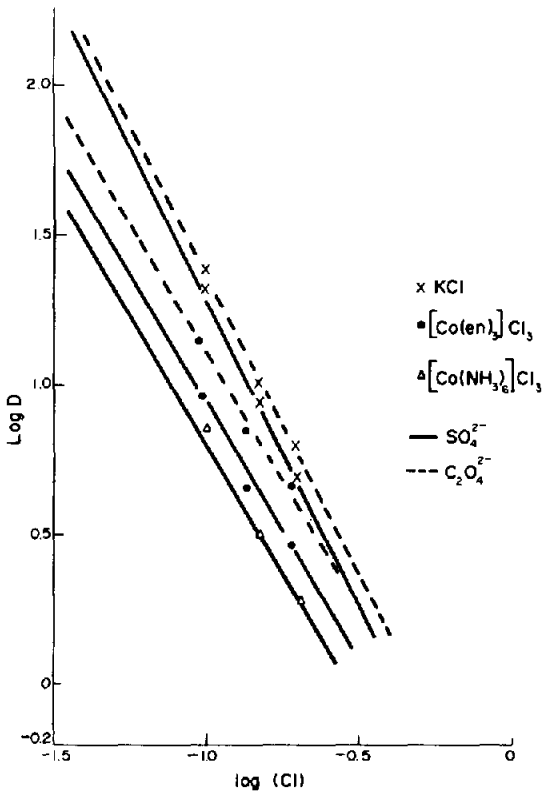

Fig. 1. Distribution coefficients of sulphate and oxalate as functions of the logarithm of the chloride concentration.

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 can be calculated by means of equations (4) and (5) from the elution volumes determined at identical chloride concentrations (Tables 3 and 4). The data determined by Mironov *et al.*[9] by potentiometry are also shown in the tables.

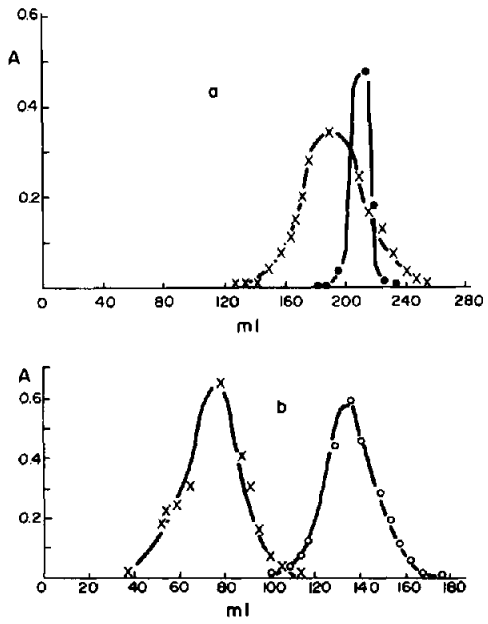

Fig. 2. Chromatograms of the separation of sulphate and oxalate with potassium chloride and tris(ethylenediamine)-cobalt(III) chloride as eluents. ○, $C_2O_4^{2-}$, ×, SP_4^{2-}; (*a*) $[Cl^-]$ 0.1 M, eluent KCl; (*b*) $[Cl^-]$ 0.1 M, eluent $[Co(en)_3]Cl_3$.

Table 5. Stability constants of the outer-sphere complex of tris(ethylenediamine)cobalt(III) and sulphate at $I = 0$

$\log K_1$	Method	Reference
3.45	Conductometry	11
2.72	Polarography	11
3.22	Chronopotentiometry	11
2.93, 3.10	Spectrophotometry	11
3.24	Potentiometry	12
3.30	Potentiometry	9
3.60	Conductometry	13
3.40	Conductometry	14

Table 6. Stability constants of the outer-sphere complex of hexa-amminecobalt(III) and sulphate at $I = 0$

$\log K_1$	Method	Reference
3.56	Conductometry	11
3.46, 3.21	Polarography	11
3.30	Chronopotentiometry	11
3.32, 2.95, 2.89, 3.26	Spectrophotometry	11
3.52, 3.60	Solubility measurement	11
3.41	Potentiometry	12
3.40	Potentiometry	9
3.50	Conductometry	13

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. 3. The stability constants at $I = 0$ can be obtained from the intercepts. They are:

$$\log K[\mathrm{Co(en)_3^{3+}} + \mathrm{SO_4^{2-}}] = 3.38$$

$$\log K[\mathrm{Co(NH_3)_6^{3+}} + \mathrm{SO_4^{2-}}] = 3.60$$

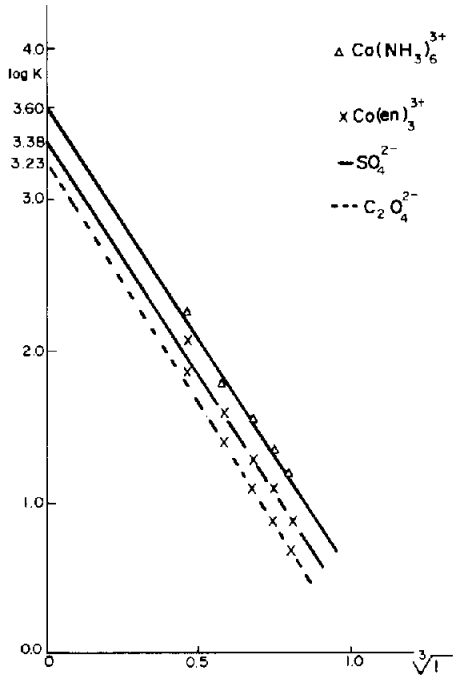

Fig. 3. Extrapolation of the stability constants to $I = 0$.

$$\log K[\mathrm{Co(en)_3^{3+}} + \mathrm{C_2O_4^{2-}}] = 3.23.$$

Figure 3 is in agreement with the cube-root law of ionic strength recently described in the literature.[10]

The stability constants of the outer-sphere complexes of sulphate with tris(ethylenediamine)cobalt(III) and hexa-amminecobalt(III), determined by different methods, are taken from the literature and summarized in Tables 5 and 6. It can be seen that the values determined here agree well with those in the literature. No stability constant could be found in the literature for the outer-sphere complex of oxalate with tris(ethylenediamine)cobalt(III).

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OXIDATION WITH PERMANGANATE IN PRESENCE OF FLUORIDE: DETERMINATION OF HYPOPHOSPHITE

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Summary—A rapid method is described for the determination of hypophosphite by oxidation with permanganate in acid medium in the presence of fluoride to prevent formation of insoluble oxides of manganese. The optimum conditions for visual and potentiometric end-point detection are given. Hypophosphite is oxidized to $H_2PO_3^-$. Under optimum conditions the titrations are fast and exhibit a reasonable potential change at the end-point. The method demonstrates the feasibility of determining amounts of $H_2PO_2^-$ corresponding to 0.08–7 mg of phosphorus.

Several titrimetric methods[1–7] have been reported for the determination of hypophosphorous acid and hypophosphite, based upon their tendency to be oxidized to phosphite or phosphate. Pound[7] described an indirect method for the determination of hypophosphite by oxidation with permanganate in acid solution, but the reaction was found to be markedly slow. The direct titration with permanganate was, however, found very difficult. Pound's method[7] includes the addition of a small amount of potassium bromide as catalyst and even then the hypoposphite is only determined indirectly after the addition of an excess of permanganate in sulphuric acid medium and in the presence of potassium bromide, whereby after 2–3 hr the manganese oxides are dissolved in the acid and the excess of permanganate is back-titrated with iron(II).

Hypophosphorous acid is known to reduce permanganate in alkaline medium but the reaction has not been studied quantitatively.[6]

In the present investigation we studied the oxidation of hypophosphite (or hypophosphorous acid) with permanganate in acid medium in the presence of fluoride ions in order to overcome the difficulties encountered by Pound[7] and to establish the conditions favouring quantitative and direct permanganometric determination of hypophosphite.

EXPERIMENTAL

Solutions

Potassium permanganate solutions were prepared by a method similar to that of Simon[6] and standardized with sodium oxalate.[8] Sodium hypophosphite, hypophosphorous acid and sodium phosphite solutions were prepared from analytical grade products and standardized according to recommended procedures.[3,7] Other solutions included 2% sodium fluoride, 2*M* and 4*M* sulphuric acid and 0.25*M* copper sulphate. Manganese(III) solution was prepared from equivalent amounts of Mn(II) and permanganate in the presence of fluoride under the optimum conditions given previously.[10,11] Doubly-distilled water was used for the preparation of all solutions.

Equipment

The potentiometric titration apparatus was similar to that described before.[9]

The ultraviolet and visible region spectra of the solutions were recorded with a Unicam SP 8000 spectrophotometer within the wavelength range 200–600 nm, using 1-cm matched silica cells. The electrochemical cells used for the redox potential measurements were as follows:

(a) $Hg|Hg_2Cl_2\|KCl$ (saturated) $\quad MnO_4^- + Mn(III)$ $\quad [NaF] = m_1,\ [H^+] = m_2$ $\quad Pt$

(b) $Hg|Hg_2Cl_2\|KCl$ (saturated) $\quad H_2PO_2^- + H_2PO_3^-$ $\quad [NaF] = m_1,\ [H^+] = m_2$ $\quad Pt$

In this study m_1 was kept constant, while m_2 was varied by adding different amounts of 2*M* sulphuric acid. The ratio [Ox]/[Red] in both systems was 1:1. The electrodes were immersed in the cell for sufficient time (~1 hr) before the E_H measurements were made.

Titration procedure

A volume of sodium hypophosphite (or hypophosphorous acid) solution containing 0.08–7 mg of phosphorus was placed in a titration vessel and mixed with the required volume of 4*M* sulphuric acid to give an acidity of 0.25–0.65*M* (for the sodium salt) or 0.04–0.1*M* (for the free acid) and also with 50 ml of 2% sodium fluoride solution. The mixture was diluted to 100 ml with doubly-distilled water and then titrated with potassium permanganate solution at room temperature (~25°C). For visual end-point detection an appropriate amount of 0.25*M* copper sulphate solution was added.

RESULTS AND DISCUSSION

The reaction of hypophosphite with permanganate in the presence of fluoride [by which Mn(III) is stabilized as MnF_4^-] proceeds rapidly, but as the end-point is approached the electrode equilibration becomes less rapid, needing ~3 min between additions of titrant. The results of the potentiometric titration are not affected by the addition of the Cu(II) salt which is essential for the detection of the end-point in the visual titration[10,12] (to mask the pink colour of the

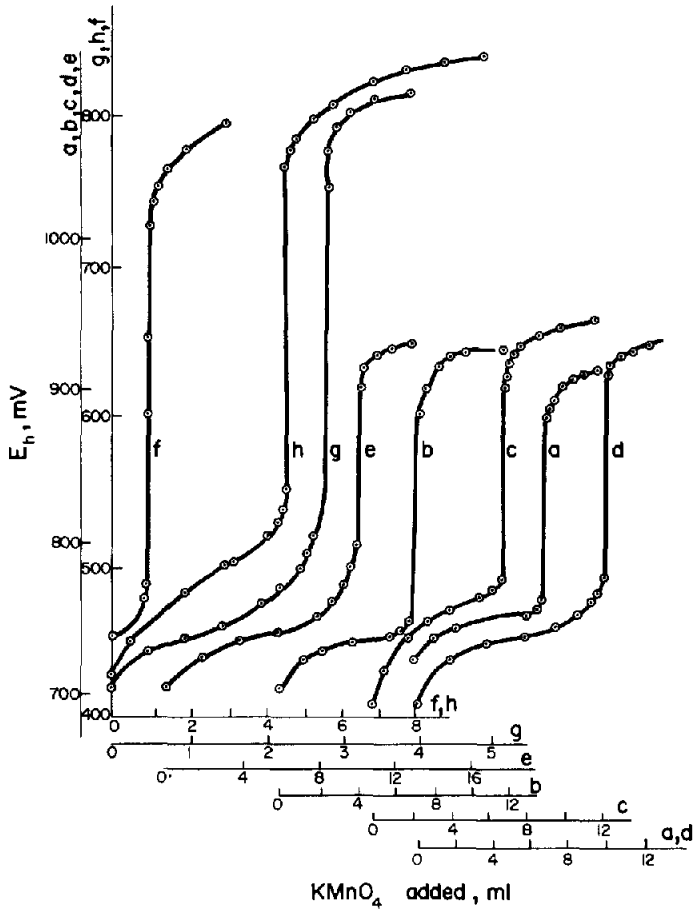

Fig. 1. Titration of $H_2PO_2^-$ with permanganate. (a) Titration of $2.295 \times 10^{-3}M$ NaH_2PO_2 with $8.05 \times 10^{-4}M$ $KMnO_4$ in the presence of $0.28M$ H_2SO_4 and $0.24M$ NaF. (b) Titration of 5 ml of $2.295 \times 10^{-3}M$ NaH_2PO_2 with $8.05 \times 10^{-4}M$ $KMnO_4$ in the presence of $0.48M$ H_2SO_4 and $0.24M$ NaF. (c) Titration of 5 ml of $2.295 \times 10^{-3}M$ NaH_2PO_2 with $8.05 \times 10^{-4}M$ $KMnO_4$ in the presence of $0.68M$ H_2SO_4 and $0.24M$ NaF. (d) Titration of 7.5 ml of $2.295 \times 10^{-3}M$ NaH_2PO_2 $8.09 \times 10^{-4}M$ $KMnO_4$ in the presence of $0.4M$ H_2SO_4 and $0.36M$ NaF. (e) Titration of 5 ml $0.574 \times 10^{-3}M$ NaH_2PO_2 with $2.02 \times 10^{-4}M$ $KMnO_4$ in the presence of $0.4M$ H_2SO_4 and $0.36M$ NaF. (f) Titration of 0.5 ml of $3.84 \times 10^{-2}M$ H_3PO_2 with $9.9 \times 10^{-3}M$ $KMnO_4$ in the presence of $0.05M$ H_2SO_4 and $0.24M$ NaF. (g) Titration of 2.5 ml of $3.84 \times 10^{-2}M$ H_3PO_2 with $2.02 \times 10^{-2}M$ $KMnO_4$ in the presence of $0.05M$ H_2SO_4 and $0.24M$ NaF. (h) Titration of 6 ml of $3.84 \times 10^{-2}M$ H_3PO_2 with $1.92 \times 10^{-2}M$ $KMnO_4$ in the presence of $0.05M$ H_2SO_4 and $0.24M$ NaF.

MnF_4^- complex). The visual end-point method is applicable in the presence of copper sulphate solutions containing 0.07–0.5 g of copper per 100 ml of solution to be titrated.

Titration of hypophosphite with permanganate

From the results obtained the reaction appears to proceed quantitatively in accordance with the equation

$$MnO_4^- + 2\,H_2PO_2^- + 2\,HF_2^- + 2\,H^+ \rightleftharpoons MnF_4^- + 2\,H_2PO_3^- + 2H_2O. \quad (1)$$

The potentiometric titration curves possess only one inflection (Fig. 1) due to the formation of Mn(III). The stoichiometry of reaction (1) can be obtained from the equations

$$MnO_4^- + 6\,H^+ + 2\,HF_2^- + 4e \rightleftharpoons MnF_4^- + 4\,H_2O \quad (2)$$

$$H_2PO_2^- + H_2O \rightleftharpoons H_2PO_3^- + 2\,H^+ + 2e. \quad (3)$$

The redox system in equation (2) was previously studied by Issa *et al.*[13] However, the experimental conditions applied in this investigation permit the existence of only $H_2PO_2^-$ and $H_2PO_3^-$ species since their existence in solution is pH-dependent. As shown in Table 1, the titration process was carried out at pH 1.7–4.85 and accordingly no HPO_3^{2-} should be present. This was confirmed by scanning the solution

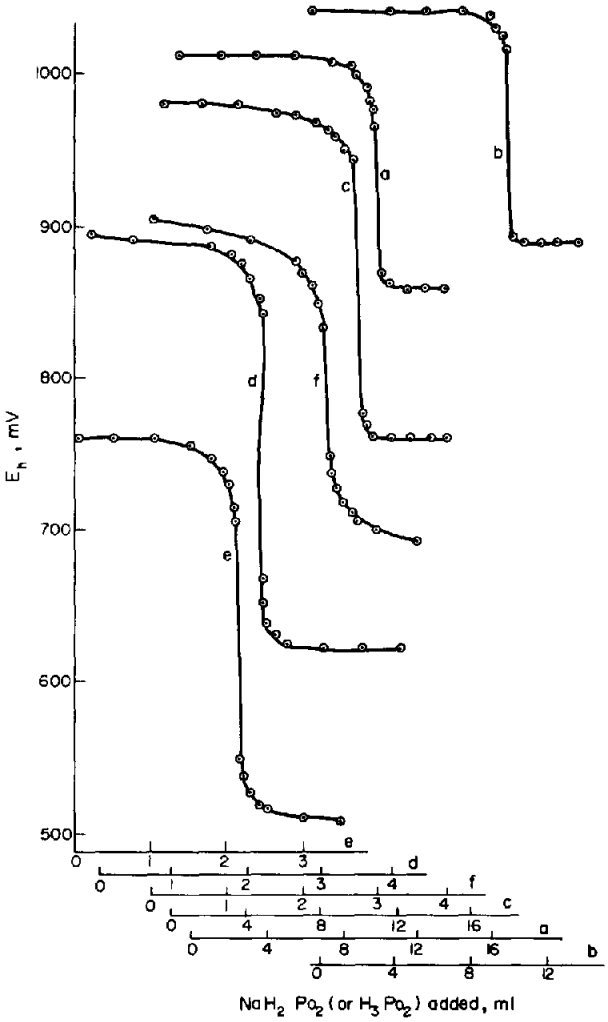

Fig. 2. Titration of $KMnO_4$ with NaH_2PO_2 (or H_3PO_2). (a) Titration of 15 ml of $4 \times 10^{-4}M$ $KMnO_4$ with $1.148 \times 10^{-3}M$ NaH_2PO_2 in the presence of $0.32M$ H_2SO_4 and $0.38M$ NaF. (b) Titration of 15 ml of $4 \times 10^{-4}M$ $KMnO_4$ with $1.148 \times 10^{-3}M$ NaH_2PO_2 in the presence of $0.44M$ H_2SO_4 and $0.38M$ NaF. (c) Titration of 15 ml of $2 \times 10^{-4}M$ $KMnO_4$ with $5.74 \times 10^{-4}M$ NaH_2PO_2 in presence of $0.4M$ H_2SO_4 and $0.38M$ NaF. (d) Titration of 2.5 ml of $2.003 \times 10^{-2}M$ $KMnO_4$ with $0.045M$ H_2SO_4 in presence of $0.1M$ H_2SO_4, and $0.24M$ NaF. (e) Titration of 2.5 ml of $6.65 \times 10^{-4}M$ $KMnO_4$ with $1.5 \times 10^{-3}M$ H_3PO_2 in presence of $0.075M$ H_2SO_4 and $0.38M$ NaF. (f) Titration of 2.5 ml of $0.02M$ $KMnO_4$ with $0.045M$ H_3PO_2 in presence of $0.075M$ H_2SO_4, and $0.38M$ NaF.

spectra at the end-point. The appearance of the absorption bands characterizing $H_2PO_3^-$ ($\lambda_{max} = 253$ nm) and MnF_4^- in the spectrum[14] provides support for equation (1).

Reaction (1) was also verified experimentally by finding the pH range within which hypophosphite solutions can be titrated successfully. The reaction is quantitative if the pH lies in the range 2–4.8, and is reasonably free from interferences. The spectra of the reaction mixtures (under the optimum conditions) at the end-point show no evidence for the formation of phosphate as an oxidation product. It is worth mentioning that the blank solution used in recording the spectra contained the same concentrations of sodium fluoride and sulphuric acid as the reaction mixture to be investigated.

Optimum experimental conditions

Reasonable accuracy in either the visual or potentiometric titration with permanganate as titrant, is attained at acidities ranging from 0.25 to $0.6M$ sulphuric acid in the presence of $0.24M$ sodium fluoride, while if permanganate is titrated with hypophosphorous acid the optimum acidity lies in the range 0.04–$0.1M$ sulphuric acid, in the presence of the same fluoride concentration. At low acidities the solution becomes turbid owing to partial production of higher oxides of manganese, but at higher acidities the end-point occurs too early, presumably due to the partial production of Mn(II).

The results obtained indicate that the fluoride concentration can be varied in the range 0.24–$0.38M$ at the optimum concentration of sulphuric acid, without

Table 1. Titration of hypophosphorous acid with permanganate in acid medium in the presence of sodium fluoride (volume made up to 100 ml with doubly-distilled water)

Composition of the reaction mixture	Measured pH	Volume of permanganate, ml Calculated	Found	Error, %	Max $\Delta E/\Delta V$, *mV*/0.05 *ml*
		Potentiometric method			
(*a*) Titration of 2.5 ml of 0.384*M* H_3PO_2 with 0.0202*M* $KMnO_4$ in presence of 50 ml of 2% NaF and various amounts of 2*M* H_2SO_4 (*X*)					
X, *ml* $[H_2SO_4]$, *M*					
1.25 0.025	4.95	Yellowish turbidity			
2.00 0.040	4.65	2.38	2.37	0.4	135
2.50 0.050	3.60	2.38	2.37	0.4	185
4.50 0.090	2.45	2.38	2.36	0.8	190
6.50 0.120	2.10	2.38	2.35	1.3	195
(*b*) Titration of 2.5 ml of 0.0384*M* H_3PO_2 with 0.0202*M* $KMnO_4$ in presence of 2.5 ml 2*M* H_2SO_4 and various amounts of 2% NaF (*Y*)					
Y, *ml*					
0.00		Slow reaction			
25.00	3.35	2.38	2.37	0.4	120
50.0	3.60	2.38	2.37	0.4	150
75.0	4.85	2.38	2.36	0.8	195
		*Visual method**			
Titration of various amounts of 0.038*M* H_3PO_2 (*Z*) with $KMnO_4$ in presence of 2.5 ml of 2*M* H_2SO_4 and 50 ml of 2% NaF					
	Z, *ml* P, *mg*				
1	10.00 11.78	9.89	9.70	1.9	
2	8.00 9.42	7.92	7.85	0.9	
3	4.00 4.71	3.95	3.95	nil	
4	2.00 2.36	1.979	1.97	0.5	
5	0.50 0.59	0.989	0.98	0.9	
6	0.25 0.29	0.989	0.97	1.9	

(1–4) 0.0192*M* $KMnO_4$; (5) 9.6 × 10^{-3}*M* $KMnO_4$; (6) 4.8 × 10^{-3}*M* $KMnO_4$.
* In presence of 5 ml of 0.25*M* $CuSO_4$.

causing appreciable errors. The visual titration was found to be favourable for the determination of amounts of hypophosphite containing 0.25–9.0 mg of phosphorus, whereas the potentiometric procedure is applicable for 0.08–7.5 mg of phosphorus. With higher hypophosphite concentrations the reaction becomes slower and the titration medium slightly turbid owing to hydrolysis of Mn(III) to Mn_2O_3.[13] The procedure is simpler than those previously published.

Precision and accuracy

To evaluate the reproducibility[15] for the determination of hypophosphite, 10 titrations at each of 15 different concentrations were done. The values obtained for the coefficient of variation varied from ±0.01 to 0.76%. The tabulated *t*-value for 18 degrees of freedom is much larger than the *t*-value calculated from the results (0.0024), for all given levels of significance, indicating that the practical and theoretical end-points are not significantly different.

Conditional redox potential of the $H_2PO_3^-/H_2PO_2^-$ *system*

Conditional redox potentials were determined from the potentials of the cells (a) and (b). The potentials were measured over the pH range 1.5–4.3. The values of E° for the two half-cells were determined from the Nernst equation, neglecting activity coefficients.

$$E_H = E^0 + \frac{RT}{nF} \ln \frac{[Ox]^a}{[Red]^b} + \frac{RT}{nF} \ln [H^+]^x$$

where x represents the number of H^+ ions and a and b are the number of ions of oxidant and reductant in the half-cell reaction. The equation can be written in the following forms for the two half-cell reactions at 25°C ($a = b = 1$):

$$_{I}E_H = {}_{I}E^0 + \frac{0.0591}{4} \log \frac{[MnO_4^-]}{[Mn(III)]} - \frac{0.0591}{4} x_I \, pH \quad (4)$$

$$_{II}E_H = {}_{II}E^0 + \frac{0.0591}{2} \log \frac{[H_2PO_3^-]}{[H_2PO_2^-]} - \frac{0.0591}{2} x_{II} \, pH \quad (5)$$

where x_I and x_{II} represent the number of H^+ ions involved in the $MnO_4^-/Mn(III)$ and $H_2PO_3^-/H_2PO_2^-$ half-cell reactions respectively.

Plots of E_H *vs.* pH are straight lines with slopes of −0.095 and −0.051 for the $MnO_4^-/Mn(III)$ and $H_2PO_3^-/H_2PO_2^-$ systems respectively, which on extra-

Table 2. Titration of sodium hypophosphite with permanganate in acid medium in the presence of sodium fluoride (volume made up to 100 ml with doubly-distilled water)

Composition of the reaction mixture		Measured pH	Volume of permanganate, *ml* Calculated	Found	Error, %	Max. $\Delta E/\Delta V$, *mV*/0.1 *ml*
			Potentiometric method			
(*a*) Titration of 5 ml of $2.295 \times 10^{-3}M$ NaH_2PO_2 with $8.05 \times 10^{-4}M$ $KMnO_4$ in presence of 75 ml of 2% NaF and various amounts of $4M$ H_2SO_4 (X)						
X, *ml*	$[H_2SO_4]$, *M*					
5.0	0.20	2.7	7.13	7.25	1.7	45
8.0	0.32	2.5	7.13	7.17	0.6	55
11.0	0.44	2.1	7.13	7.13	nil	62
15.0	0.60	1.8	7.13	7.07	0.8	72
18.0	0.72	1.7	7.13	7.00	1.8	40
(*b*) Titration of 5 ml of $2.295 \times 10^{-3}M$ NaH_2PO_2 with $8.08 \times 10^{-4}M$ $KMnO_4$ in presence of 10 ml $4M$ H_2SO_4 and various amounts of 2% NaF (Y) at 35°						
Y, *ml*						
00.0				Yellowish turbidity		
25.0		2.05	7.09	6.85	3.4	35
40.0		2.30	7.09	7.02	0.0	50
50.0		2.45	7.09	7.02	1.0	85
75.0		2.95	7.09	7.06	0.4	175
			Visual method			
Titration of various amounts of NaH_2PO_2 (Z) with $KMnO_4$ in presence of 10 ml of $4M$ H_2SO_4 and 75 ml of 2% NaF at 60°						
	ml	*M*	P, *mg*			
1	10.0	0.0529	16.40	13.15	12.90	1.9
2	7.0	0.0529	11.48	9.20	9.12	0.0
3	3.5	0.0529	5.74	4.60	4.57	0.7
4	3.5	0.0529	5.74	4.60	4.50	2.2
5	15.0	0.00529	2.46	19.72	19.60	0.6
6	15.0	0.000529	0.25	19.72	19.90	0.9
7	10.0	0.000529	0.16	13.15	13.50	2.7

(1–4) 0.02012M $KMnO_4$; (5) 0.002012M $KMnO_4$; (6–7) $2.012 \times 10^{-4}M$ $KMnO_4$.
* In presence of 5 ml of 0.25M $CuSO_4$.

polation to pH = 0 give E° values of 1.58 and 0.74 V respectively.

The conditional potential for the redox system of the phosphorus compound at any required pH can be deduced from equation (5) and the E° value at pH = 0.

Since the slope of the E_H *vs.* pH graph is equal to $-0.0591\ x/n$, then for the MnO_4^-/Mn(III) system $x_I = 6$ and for the $H_2PO_3^-/H_2PO_2^-$ system $x_{II} = 2$, which confirms equations (2) and (3) and consequently the net reaction will be represented by equation (1).

The validity of this equation is verified by determining the slope of the E_{cell} *vs.* pH curve for the overall cell reaction. According to equations (3) and (4) when $[MnO_4^-]/[Mn(III)] = [H_2PO_3^-]/[H_2PO_2^-] = 1$,

$$E_{cell} = E^0 + \frac{0.0591}{4}(4x_{II} - x_I)\,pH \qquad (6)$$

Since $x_I = 6$ and $x_{II} = 2$,

$$E_{cell} = E^0 + \frac{0.0591 \times 2}{4}pH = E^0 + 0.0295\,pH$$

The slope found experimentally was 0.030, in good accordance with that expected.

Titration of permanganate with hypophosphite

The titration of permanganate with hypophosphite in the absence of fluoride leads to the formation of

Table 3. Determination of phosphorus(I)

No.	Phosphorus, *mg* Taken	Found	Standard† deviation, *mg*	Inflection at end-point, *mV*/0.1 *ml*
1	7.13	7.10	0.012_5	150
2	3.571	3.557	0.006	200
3	0.595	0.594	0.005	262
4	3.208	3.19†	0.006	150
5	3.208	3.216	0.006	100
6	1.333	1.328	0.002	70
7	0.712	0.710	0.0003	75
8	0.178	0.178	0.0003	75
9	0.088	0.088	0.0003	60
10	0.356	0.354	0.0005	75

* (1–3) 0.0384M H_3PO_2, 0.0198M $KMnO_4$; (4,5) 0.0414M H_3PO_2, 0.0202M $KMnO_4$; (6) 0.0057M NaH_2PO_2, 0..002M $KMnO_4$; (7, 8, 10) $2.295 \times 10^{-3}M$ NaH_2PO_2, $0.81 \times 10^{-3}M$ $KMnO_4$; (9) $0.57 \times 10^{-3}M$ NaH_2PO_2, $0.0225 \times 10^{-4}M$ $KMnO_4$, determined in under optimum conditions of acidity and F^- concentration.
† Ten titrations for each concentration.

insoluble oxides of manganese which make it impossible to detect the end-point. In the presence of fluoride the reaction runs smoothly as permanganate is reduced quantitatively to Mn(III) according to equation (1). The titration curves have a single sharp inflection, varying in height with acidity. Since all manganese(III) salts are strong oxidizing agents, further reaction between the Mn(III) formed and more hypophosphite to produce Mn(II) and phosphite would be expected, but it was found difficult to continue the titration to the Mn(II) stage owing to the sluggishness of the reaction.

Titrations using sodium hypophosphite or hypophosphorous acid as titrant could be done successfully in the presence of 0.1–0.25*M* sulphuric acid and 0.24*M* sodium fluoride at 25°. Amounts of permanganate containing 0.1–4.5 mg of manganese could be titrated with fair accuracy under the optimum conditions, the error being generally $<0.7\%$.

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CHLORID-SPURENANALYSE IN SILIKATGESTEINEN DURCH MASSENSPEKTROMETRISCHE ISOTOPENVERDÜNNUNGSANALYSE

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Zusammenfassung—Ein Verfahren zur massenspektrometrischen Isotopenverdünnungsanalyse von Chloridspuren in Silikatgesteinen mit einem ^{37}Cl-Indikator wird beschrieben. Die dabei notwendigen Chlor-Isotopenverhältnismessungen werden mit Hilfe der Erzeugung negativer Thermionen an einem heißen Rheniumband im Massenspektrometer durchgeführt. In einer Teflon-Druckbombe wird die Probe mit Flußsäure aufgeschlossen sowie das Chlorid durch selektive Fällung als AgCl aus der HF-sauren Lösung abgetrennt. Die relative Standardabweichung liegt bei 1–4% für Chloridgehalte von 100 bis 200 ppm, was eine wesentliche Verbesserung der Genauigkeit gegenüber anderen bisher in der Literatur beschriebenen Verfahren bedeutet. Die Problematik des Blindwerts bei der Chlorid-Spurenanalyse in Gesteinen wird diskutiert.

Chlorid-Spurenanalysen in Gesteinen wurden bisher bevorzugt mit photometrischen[1–3] oder mit chemischen Methoden[3] durchgeführt. In der letzten Zeit wurde hierfür auch immer häufiger die ionenselektive Elektrode eingesetzt.[1,4,5] All diese Verfahren setzen voraus, daß das im Gestein enthaltene Chlorid nach dem Aufschluß quantitativ isoliert wird. Wegen der hohen Flüchtigkeit des Chloridions aus sauren Lösungen heraus, werden alkalische Schmelzaufschlüsse bei der genannten photometrischen Methode bzw. bei Verwendung der ionenselektiven Elektrode benutzt. Trotzdem schwanken die an gleichen Proben mit unterschiedlichen, analytischen Verfahren erhaltenen Chloridgehalte zum Teil beträchtlich. So hat Haynes[1] vor kurzem für ein Standard-Gestein (Granit, U.S.G.S. G2) eine Aufstellung gemacht, nach der die am weitesten auseinanderliegenden Ergebnisse mit der Neutronenaktivierungsanalyse zu 53 ppm[6] und mit einer photometrischen Methode zu 192 ppm Chlorid[7] analysiert wurden. Haynes selbst bestimmte den Chloridgehalt dieses Standard-Gesteins mit einer photometrischen Methode zu 84 ppm bei 13% relativer Standardabweichung und mit einer ionenselektiven Elektrode zu 101 ppm bei 14% relativer Standardabweichung. Dabei wurde die Probe in einem Platintiegel in einer Schmelze aus Natriumcarbonat und Zinkoxid aufgeschlossen.

Diese Unsicherheit in der Chlorid-Spurenanalyse geologischer Proben hat dazu geführt, daß für geologische Referenzmaterialien der Chloridgehalt meist nur als "empfohlener Wert" (recommended magnitude[8]) bzw. als "vorgeschlagener Wert" (simply proposed value[9]) angegeben wird.

Vom Prinzip her kann die massenspektrometrische Isotopenverdünnungsanalyse (MS–IVA) als Absolutmethode angesehen werden, so daß bei Anwendung dieser Methode besonders reproduzierbare und auch "richtige" Analysenergebnisse zu erwarten sind. Einer der großen Vorteile der MS–IVA gerade für die Chloridbestimmung in Festkörperproben besteht darin, daß nach eingetretener Isotopenverdünnung keine quantitative Isolierung des Chlorids mehr erfolgen muß. Deshalb haben wir eine MS-IVA zur Chlorid-Spurenbestimmung in geologischen Proben entwickelt. Eine der Voraussetzungen dazu war die genaue Isotopenverhältnismessung des Chlors im Massenspektrometer, was wir durch die Erzeugung negativer Thermionen an einem heißen Metallband erreichen konnten.

ISOTOPENVERDÜNNUNGSANALYSE MIT EINEM ^{37}Cl-INDIKATOR

Eine Voraussetzung für die Durchführung einer MS–IVA ist, daß das zu bestimmende Element mindestens zwei stabile oder sehr langlebige, radioaktive Isotope besitzt. Beim Chlor ist dies durch die beiden stabilen Isotope ^{35}Cl und ^{37}Cl gegeben. Für die Isotopenverdünnungsanalyse von Chlorid fügt man eine genau bekannte Menge eines ^{37}Cl-angereicherten Indikators zu der Probe. Anschließend muß durch eine chemische Operation (z.B. Aufschluß) sichergestellt werden, daß Indikator- und Probenchlorid vollständig miteinander vermischt werden. Bis zu diesem Zeitpunkt darf kein Chloridverlust, weder aus dem Indikator noch aus dem Probenmaterial, erfolgt sein. Danach haben allerdings Chloridverluste keinerlei Einfluß mehr auf das analytische Ergebnis. Damit ist auch keine quantitative Chloridisolation notwendig, was häufig ein Problem bei anderen analytischen Verfahren darstellt.

Im Massenspektrometer werden die Ionenintensitäten von ^{35}Cl und ^{37}Cl der isotopenverdünnten

Probe gemessen. Hierbei setzt sich die Gesamtintensität jedes Isotops aus der Summe der jeweiligen Anteile von Proben- und Indikatorchlorid zusammen. Damit gilt für das Isotopenverhältnis $R = {}^{37}Cl/{}^{35}Cl$ nach der Isotopenverdünnung:

$$R = \frac{N_{Pr}h_{Pr}^{37} + N_{Ind}h_{Ind}^{37}}{N_{Pr}h_{Pr}^{35} + N_{Ind}h_{Ind}^{35}} \quad (1)$$

N = Zahl der Chloratome; h = Chlor-Isotopenhäufigkeit [%]; Pr = Probe, Ind = Indikator, 35 = ^{35}Cl-Isotop, 37 = ^{37}Cl-Isotop.

Löst man Gleichung (1) nach dem gesuchten Chloridgehalt N_{Pr} in der Probe auf, so erhält man:

$$N_{Pr} = \frac{N_{Ind}(h_{Ind}^{37} - Rh_{Ind}^{35})}{Rh_{Pr}^{35} - h_{Pr}^{37}} \quad (2)$$

Bisher sind keine Isotopenvariationen des Chlors in geologischen Proben bekannt.[10] Deshalb können in Gleichung (2) die natürlichen Isotopenhäufigkeiten[11] für h_{Pr} eingesetzt werden: $h_{Pr}^{35} = 75{,}77\%$, $h_{Pr}^{37} = 24{,}23\%$. Um den Chloridgehalt x einer Gesteinsprobe in ppm zu ermitteln, kann unter Berücksichtigung der Proben-Einwaage G in g Gleichung (3) benutzt werden:

$$x = 5{,}887 \cdot 10^{-17} \cdot \frac{N_{Pr}}{G} \text{ ppm} \quad (3)$$

Aus Gleichung (2) folgt, daß sich Fehler bei der Gehaltsbestimmung des Indikators direkt auf das Analysenergebnis auswirken. Benutzt man bei der Chloridbestimmung des Indikators eine inverse Isotopenverdünnungstechnik (Zugabe einer Chloridlösung natürlicher Isotopenzusammensetzung), so kann der Chloridgehalt im Indikator mit relativen Standardabweichungen von besser als 0,5% ermittelt werden. Die Genauigkeit der MS–IVA steigt, je größer die Differenz im Zähler bzw. Nenner von Gleichung (2) wird. Dies kann durch einen hochangereicherten ^{37}Cl-Indikator erreicht werden. Außerdem sollte der Indikatorzusatz so gewählt werden, daß der Wert von R optimiert wird. Solche Optimierungsrechnungen wurden u.a. bereits für Bor-[12] und Calciumbestimmungen[13,14] durchgeführt. In Anlehnung daran kann man die Gleichungen (4) und (5) ableiten. Dabei wird vorausgesetzt, daß h_{Ind}^{35} und h_{Ind}^{37} keinen wesentlichen Beitrag zum statistischen Fehler des Analysenergebnisses liefern. Da– wie noch beschrieben wird—durch viele Wiederholungsmessungen diese Werte z.B. für einen etwa 98% ^{37}Cl-angereicherten Indikator mit relativen Standardabweichungen von $s_{rel}(h_{Ind}^{35}) = 0{,}5\%$ und $s_{rel}(h_{Ind}^{37}) = 0{,}01\%$ bestimmbar sind, kann diese Voraussetzung als gegeben angesehen werden.

$$\sigma_{N_{Pr}}^2 \approx \sigma_{N_{Ind}}^2 + f_R^2\sigma_R^2 \quad (4)$$

$$R_{opt} = [(^{37}Cl/^{35}Cl)_{Pr}(^{37}Cl/^{35}Cl)_{Ind}]^{\frac{1}{2}} \quad (5)$$

σ = Standardabweichung; f_R = Fehlerübertragungsfaktor.

Aus Gleichung (4) folgt, daß jeder Fehler bei der Isotopenverhältnismessung R noch durch einen Fehlerübertragungsfaktor verstärkt wird. Gleichung (5) gibt die Bedingung dafür an, wann der optimale R-Wert (R_{opt}) und damit das günstigste Mischungsverhältnis Probenchlorid/Indikatorchlorid bezüglich des Minimalwerts von f_R erreicht ist.

Das Ergebnis einer solchen Optimierungsrechnung in Abhängigkeit von verschiedenen ^{37}Cl-Indikatoranreicherungen ist in Abb. 1 wiedergegeben. Wie man erkennt, fällt der Betrag des f_R-Werts mit steigender ^{37}Cl-Anreicherung. Auch überstreichen die Kurven bei höherem ^{37}Cl-Gehalt in einem weiten Mischungsbereich Probenchlorid/Indikatorchlorid f_R-Werte, die nahe dem Minimum liegen. Um nun den statistischen Fehler des Analysenergebnisses nicht unnötig zu erhöhen, sollte bei der MS–IVA ein Mischungsverhältnis Probenchlorid/Indikatorchlorid gewählt werden, welches einen niedrigen f_R-Wert bedingt. Dies setzt allerdings die ungefähre Kenntnis des Probengehalts voraus.

In dieser Arbeit haben wir einen etwa 75% bzw. 98% angereicherten ^{37}Cl-Indikator verwendet. Für diese beiden Indikatoren liegt das Minimum des Betrags von f_R bei Mischungsverhältnissen Probenchlorid/Indikatorchlorid von 1,0 bzw. 0,3, wobei auch akzeptable Werte von $|f_R| < 3$ im Mischungsbereich 0.2–4,8 bzw. < 8,0 erreicht werden. Die beste Reproduzierbarkeit der massenspektrometrischen Isotopenverhältnismessung wird bei $R \approx 1$ erhalten, was bei einem 75% angereicherten Indikator für Mischungsverhältnisse von 1:1 gegeben ist. Dagegen hat ein höherer Indikatorzusatz den Vorteil, daß die Chloridabtrennung nach dem Aufschluß der Probe erleichtert wird. Aus diesem Grund haben wir bei den Analysen mit dem 75% ^{37}Cl-angereicherten Indikator Mischungsverhältnisse Probenchlorid/Indikatorchlorid von ungefähr 0,4 für Bestimmungen im 100–200-ppm Bereich, von 0,1–0,2 in Bereichen <100 ppm verwendet. Für die Analysenreihe mit dem 98% angereicherten Indikator wurde ein für den f_R-Wert optimales Mischungsverhältnis von 0,3 benutzt.

EXPERIMENTELLER TEIL

Massenspektrometrische Messungen

Es wurde ein einfachfokussierendes Massenspektrometer mit einer Zweiband-Thermionenquelle (Varian MAT, Typ CH5-TH) benutzt. Die Bänder der Thermionenquelle bestanden aus Rhenium. Ein Faradaykäfig mit angeschlossenem hochohmigen Widerstand von $10^{11}\ \Omega$ wurde als Ionenauffänger verwendet. Bei der Separation des Chlorids aus den Gesteinsproben fiel dieses stets als AgCl an. Mit 1 2 Tropfen 15%-iger Ammoniaklösung wurde kurz vor der Messung das Silberchlorid in lösliches $[Ag(NH_3)_2]Cl$ überführt. Ein Teil dieser Lösung, die etwa 10–30 μg Chlorid enthalten sollte, wurde mit einer Mikrolitерspritze auf das Verdampferband der Thermionenquelle aufgetragen und dort zur Trockne eingedampft. Im Massenspektrometer wurde dann das Ionisierungsband auf 1850° (Kontrolle mit einem optischen Pyrometer) aufgeheizt. Dabei war in der Regel die Strahlungswärme des Ionisierungsbandes ausreichend, um genügend Probenmaterial vom Verdampferband abzudampfen, ohne dieses selbst zu

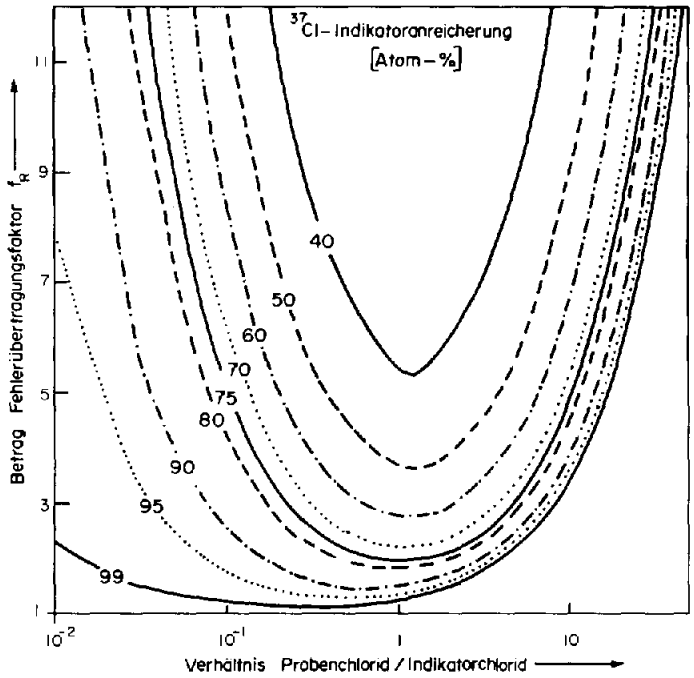

Abb. 1. Abhängigkeit des Fehlerübertragungsfaktors bei der Chloridbestimmung durch MS–IVA von Indikatoranreicherung und vom Mischungsverhältnis Probenchlorid/Indikatorchlorid.

heizen. Unter diesen Bedingungen konnte von der Rheniumbandoberfläche ein negativer Cl^--Ionenstrom emittiert werden, der am Auffänger einen Ionenstrom von etwa $3 \cdot 10^{-11}$ A erzeugte. Weitere Details der Messung von Chlorisotopenverhältnissen durch negative Thermionisation wurden bereits von Heumann und Hoffman beschrieben.[15] Von jeder Probe wurden die Ionenintensitäten für $^{35}Cl^-$ und $^{37}Cl^-$ 10mal hintereinander vermessen und aus den 10 Isotopenverhältnissen $R = {}^{37}Cl/{}^{35}Cl$ einer solchen Meßserie wurde der Mittelwert von R berechnet. Die relative Standardabweichung war dabei etwa 0,1%.

Herstellung des ^{37}Cl-Indikators

Der Indikator wurde aus Natriumchlorid, welches ^{37}Cl angereichert enthielt (Oak Ridge Nat. Lab.), durch Lösen in bidest. Wasser hergestellt. Der Chloridgehalt der Indikatorlösung wurde durch inverse Isotopenverdünnung mit einer Chlorid-Standardlösung natürlicher Isotopenzusammensetzung bestimmt. Dabei wurde etwa 1 g genau eingewogene Indikatorlösung mit jeweils soviel $10^{-2}M$ Chlorid-Standardlösung versetzt, daß die bezüglich des Fehlerübertragungsfaktors optimalen Mischungsverhältnisse erhalten wurden. Für den etwa 75% ^{37}Cl-angereicherten Indikator wurden drei, für denjenigen mit etwa 98% Anreicherung fünf Parallelanalysen durchgeführt. Der Chloridgehalt ergab sich für je 1 g der Indikatorlösungen zu $(1{,}017 \pm 0{,}005) \cdot 10^{19}$ Cl^--Ionen beim 75% angereicherten, zu $(1{,}005 \pm 0{,}004) \cdot 10^{19}$ Cl^--Ionen beim 98% angereicherten Indikator. Dies entspricht einer etwa $1{,}68 \cdot 10^{-2}M$ Chloridlösung, wobei die relativen Standardabweichungen der Analyse 0,5 bzw. 0,4% betragen. Zur Kontrolle wurde der Gehalt des 75% angereicherten Indikators auch mit einer ionenselektiven Chloridelektrode (Orion Research, Typ 96–17) bestimmt, was einen Wert von $(1{,}016 \pm 0{,}006) \cdot 10^{19}$ Cl^--Ionen/g Lösung ergab. Damit ergibt sich eine Abweichung der Analysenwerte beider Verfahren von weniger als 0,1%, wobei die relative Standardabweichung mit 0,6% bei dem Ergebnis mit der ionenselektiven Elektrode nur geringfügig höher als bei demjenigen der MS–IVA liegt.

Die Isotopenhäufigkeiten wurden nach der beschriebenen massenspektrometrischen Meßtechnik für den niedriger angereicherten Indikator aus 3 Meßserien zu $h^{35}_{Ind} = (24{,}63 \pm 0{,}05)\%$ und $h^{37}_{Ind} = (75{,}37 \pm 0{,}05)\%$, für den höher angereicherten aus 10 Meßserien zu $h^{35}_{Ind} = (1{,}85 \pm 0{,}01)\%$ und $h^{37}_{Ind} = (98{,}15 \pm 0{,}01)\%$ bestimmt.

Aufarbeitung der Gesteinsproben

Abbildung 2 zeigt schematisch die verschiedenen analytischen Teilschritte, welche für die Aufarbeitung von Silikatgesteinen zur Chlorid-Spurenanalyse durch MS–IVA notwendig sind. Ungefähr 1 g der zerkleinerten Probe (Korngröße etwa 80–180 μm) wird in einen Teflontiegel eingewogen. Eine sich an den Optimierungsrechnungen für den Indikatorzusatz orientierende Menge des Indikators wird hinzupipettiert und genau ausgewogen. Anschließend gibt man 17 g 40%-ige Flußsäure und 7 g conz. Schwefelsäure (beide Suprapur, Merck) hinzu, schließt den Tiegel mit einem Teflondeckel und erhitzt in einem Autoklaven für 1–3 Stunden auf 180°, indem der Autoklav in einen Trockenschrank gestellt wird. Die dabei benutzte Aufschlußapparatur lehnt sich an bereits früher beschriebene Modelle an.[16] Während des Aufschlusses findet eine vollständige Vermischung von Proben- und Indikatorchlorid statt, so daß sich danach Chloridversluste nicht mehr auf das Analysenergebnis auswirken. Nach Abkühlen des Autoklaven auf etwa Zimmertemperatur wird der Teflontiegel geöffnet und die gelöste Substanz mit einem Methanol/Wasser-Gemisch (1:1) in eine 50-ml Polyethylenflasche überführt. Anschließend füllt man die Flasche fast ganz mit der Methanol/Wasser-Mischung auf. Die Methanolzugabe ist wichtige Voraussetzung zur Durchführung des späteren Filtrationsschrittes, da der dann benutzte Teflonfilter nur von einem organischen Lösungsmittel benetzt wird. Weiterhin begünstigt der Methanolzusatz die Chloridausfällung als Silberchlorid. Zunächst aber werden Festkörperteilchen, die hauptsächlich aus Sulfatniederschlägen bestehen, in der Polyethylenflasche abzentrifugiert und die Lösung wird dann in ein Polyethylenbecherglas abdekantiert. Dort wird das Chlorid mit 0,5

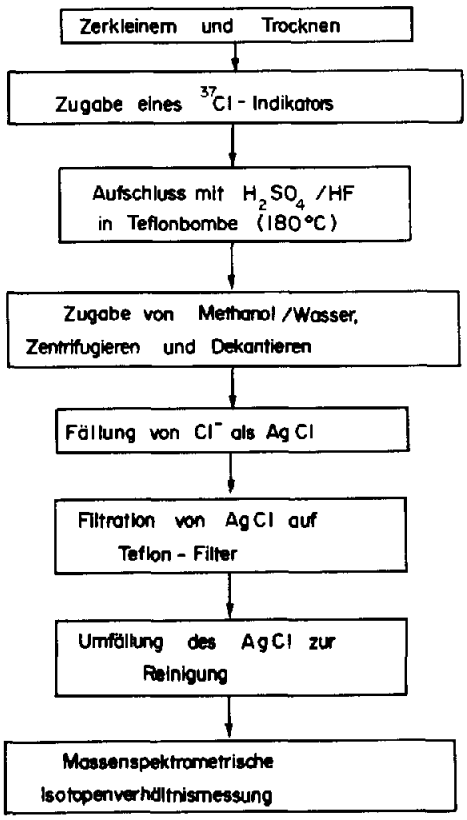

Abb. 2. Aufarbeitungsschema für die Chlorid-Spurenbestimmung in Silikatgesteinen durch MS–IVA.

ml einer 0,1*M* Silbernitratlösung gefällt. Nachdem das Teflonfilter (Schleicher und Schüll, Typ TE38, Porengröße 5 μm) in einer von uns dafür konstruierten Teflonnutsche benetzt wurde, wird das Silberchlorid von der flußsäurehaltigen Lösung abfiltriert. Die Teflonnutsche ist mit einer Teflonfilterplatte (Forschungsinstitut Berghof, Nr. 42, mittlerer Porendurchmesser 10 μm) von 12 mm Durchmesser versehen. Zur Reinigung des Silberchloridniederschlags wird einmal umgefällt, indem der Niederschlag in wenigen Tropfen Ammoniaklösung (1 + 1) gelöst und dann durch Zugabe von conz. Salpetersäure zum Filtrat wieder ausgefällt wird. Die Umfällung der Probe ist für die massenspektrometrische Isotopenverhältnismessung von Vorteil, da frühere Untersuchungen gezeigt haben, daß reine Substanzproben eine genauere Isotopenverhältnisbestimmung als unreine Proben ermöglichen.[15,17] Der umgefällte Silberchloridniederschlag wird auf einem Membranfilter aus Cellulosenitrat (Sartorius, Typ SM 11301, Porengröße 8 μm) abfiltriert, von dem er sich nach dem Waschen mit Wasser und Trocknen unter einem Oberflächenverdampfer gut ablösen läßt. Die Probe wird dann bis zur massenspektrometrischen Messung in einem dunklen Präparategläschen aufbewahrt. Das dunkle Aufbewahrungsgefäß ist notwendig, da sich Silberchlorid durch Licht natürlicher Wellenlängenverteilung unter Isotopenfraktionierung zersetzt.[18]

Wenn die Chloridmenge der isotopenverdünnten Probe so gering ist, daß die Fällung des Silberchlorids Schwierigkeiten bereitet, werden der flußsauren Lösung 1–2 Tropfen einer Natriumjodidlösung zugesetzt. Dies hat eine Mitfällung des Chlorids mit dem sich schnell bildenden Silberjodidniederschlag zur Folge. Bei der Umfällung mit Ammoniak bleibt dann wieder ein großer Teil des Silberjodids auf dem Teflonfilter zurück.

Bestimmung des Blindwerts

Eine Chloridspurenanalyse stellt—vor allem in einem Chemischen Institut—wegen des Blindwerts ein nicht geringes Problem dar. Neben den bei der Aufarbeitung verwendeten Chemikalien liefert hauptsächlich die Luft einen Blindwertbeitrag, wobei hier Chlorid sowohl in Staubteilchen enthalten ist, aber auch gasförmig u.a. als Chlorwasserstoff vorliegt. Um den Blindwert möglichst niedrig zu halten, wurden nur Chemikalien der Reinheitsklasse "Suprapur" verwendet, die bei der Aufarbeitung benutzten Kunststoffgefäße wurden vor dem Gebrauch mehrfach mit. Salpetersäure (1 + 1) ausgekocht. Die Proben wurden in einem Labor aufbereitet, in welchem bis dahin noch nicht chemisch gearbeitet worden war.

Das bei der Analyse verwendete Wasser wurde wie folgt gereinigt: nach einer Zweifachdestillation wurde das bidest. Wasser mit einigen Tropfen verd. Natronlauge in eine Quartzdestilliergerät gegeben und erneut zweimal destilliert. Damit konnte der Chloridgehalt des Wassers bis auf 1,5 ng Cl^-/ml H_2O verringert werden (Bestimmung mit MS–IVA). Da andere Reinigungsverfahren, wie z. B. weitere Destillationsstufen aber ohne Natrium hydroxidzugabe, stets schlechtere Werte lieferten, ist zu vermuten, daß der Hauptverunreinigungsanteil des Chlorids in dest. Wasser durch HCl hervorgerufen wird.

Unter Beachtung der genannten Vorsichtsmaßnahmen wurde der Blindwert der Analyse dadurch bestimmt, daß der beschriebene Aufarbeitungsprozess nach Einfügen der jeweiligen Menge an Indikatorlösung in den Teflontiegel ohne eine Gesteinsprobe durchgeführt wurde. Aus der Abweichung des $^{37}Cl/^{35}Cl$-Isotopenverhältnisses der dann isolierten Probe von demjenigen des Indikators konnte nach Gleichung (2) der Blindwert berechnet werden.

ERGEBNISSE UND DISKUSSION

In Tabelle 1 sind die Ergebnisse der Chloridanalysen verschiedener silikathaltiger Proben wiedergegeben. Betrachtet man die Ergebnisse, die im Bereich von 100–200 ppm Chloridgehalt liegen, so sind hier relative Standardabweichungen s_{rel} von 1,0 bis 4,4% erreicht worden. Diese Standardabweichungen beziehen sich auf jeweils drei bzw. fünf (Albtal-Granit) voneinander unabhängig durchgeführte Analysen derselben Probe. Vergleicht man dieses Ergebnis mit anderen in der Literatur beschriebenen Chlorid-Spurenbestimmungen in geologischen Proben, so wird mit der beschriebenen MS–IVA eine ganz wesentliche Verbesserung der Reproduzierbarkeit erhalten. So hat vor kurzem Haynes[1] Chloridanalysen in zwei Granitproben mit einer photometrischen Methode sowie mit einer ionenselektiven Elektrode durchgeführt. Bei Verwendung eines Schmelzaufschlusses mit Na_2CO_3/ZnO im Platintiegel ergaben sich relative Standardabweichungen von 13 bzw. 7% für die photometrische Methode, von 14 bzw. 10% bei Verwendung der ionenselektiven Elektrode, wobei der Chloridgehalt der beiden analysierten Granite bei etwa 100 bzw. 350 ppm lag. Auch in der von Akaiwa *et al.*[5] veröffentlichten Chloridbestimmung in Gesteinsproben durch Ionenaustauschchromatographie mit direkter potentiometrischer Messung durch ionenselektive Elektroden lag die relative Standardabweichung bei ungefähr 6%, wenn ein Chloridgehalt etwas über 200 ppm bestimmt wurde. Die günstigeren

Tabelle 1. Chlorid-Spurenanalysen in Silikatgesteinen durch MS–IVA

Probe	Fundort	^{37}Cl-Indikatoranreicherung, %	Cl^--Gehalt, *ppm*	s_{rel}, %
Granit	Albtal, Schwarzwald	98	197 ± 3	1,5
		75	197 ± 2	1,0
Metablastit	Kastel, Schwarzwald	75	141 ± 3	2,1
Hornblende	Hohemuttlen, Schwarzwald	75	114 ± 5	4,4
Amphibolit	Waldmatt, Schwarzwald	75	9 ± 3	33,0

Standardabweichungen bei Verwendung des Verfahrens mit MS–IVA führen wir vor allem darauf zurück, daß sich nach der Isotopenverdünnung Substanzverluste nicht mehr auf das Analysenergebnis auswirken. Auch ist in diesem Fall die massenspektrometrische Nachweismethode wesentlich selektiver, da die Massenzahlen 35 und 37 der Chlorisotope durch kein anderes Element gestört werden, dagegen sowohl das photometrische Verfahren als auch die ionenselektive Elektrode durch Fremdionen beeinflußt werden können.

Unabhängig von der Reproduzierbarkeit ist vor allem auch bei der Chlorid-Spurenanalyse in geologischen Proben das Problem des "richtigen" Analysenergebnisses vorhanden. Dies zeigt sich an den starken Diskrepanzen der Chloridbestimmung in geologischen Proben bei Verwendung unterschiedlicher Methoden, wie u.a. bei der Bestimmung des bereits erwähnten Standardgesteins (Granit, U.S.G.S. G2). Hier waren Werte zwischen 53 ppm[6] und 192 ppm[7] gefunden worden. Da die MS–IVA vom Prinzip her als Absolutmethode angesehen werden kann, ist damit ein relativ "richtiges" Analysenergebnis zu erwarten. Bei Vernachlässigung des vergleichsweise kleinen Fehlers durch die Isotopenverhältnismessung wird die Richtigkeit des Ergebnisses hauptsächlich durch den Blindwert beeinflußt.

Bei den Fehlerangaben in Tabelle 1 fällt auf, daß fast unabhängig vom Chloridgehalt in der Probe die Standardabweichung etwa ±3 ppm beträgt. In den Gehaltsangaben der Tabelle 1 ist bereits der jeweils parallel zu den Probenaufschlüssen ermittelte Blindwert berücksichtigt. Tabelle 2 gibt das Ergebnis von fünf Blindwertanalysen wieder. Mittelt man über diese fünf Werte, so ergibt sich eine Unsicherheit in der Blindwertbestimmung von ±3,2 µg pro Aufarbeitungsprozeß. Bei 1 g Einwaage für die Gesteinsanalyse entspricht dies einem Anteil des Blindwerts an der Standardabweichung von ±3,2 ppm. Etwa 1 g Einwaage war, bis auf die Hornblendenprobe, wo aus Materialmangel kleinere Mengen verwendet wurden, überall gegeben. Die etwas höhere Standardabweichung bei der Hornblendenbestimmung ist somit auch zu erklären. Damit folgt aus den Analysenergebnissen, daß die Standardabweichung des hier beschriebenen Verfahrens hauptsächlich von der Unsicherheit des Chlorid-Blindwerts beeinflußt und damit auch limitiert wird. Wie die Analyse des Amphiboliten zeigt, steigt dadurch die relative Standardabweichung im unteren ppm-Bereich stark an, während bei Chloridgehalten >300 ppm s_{rel}-Werte von <1% erreicht werden sollten. Auch die praktisch gleiche Standardabweichung bei der Granitanalyse mit einem etwa 75 bzw. 98% angereicherten ^{37}Cl-Indikator zeigt, daß unter den gegebenen Versuchsbedingungen sich der etwas kleinere Fehlerübertragungsfaktor bei Verwendung des 98% angereicherten Indikators nicht mehr erkennbar gegenüber dem Einfluß des Blindwerts auswirkt.

Nach Angaben von Haynes[1] bedingt die Verwendung einer ionenselektiven Elektrode allein schon einen internen Meßfehler bei dem von ihm verwendeten Verfahren, der ± 10 ppm Chlorid in der Gesteinsprobe entspricht. Während somit auf diese Weise keine Chloridanalysen im unteren ppm-Bereich möglich sind, können mit der MS–IVA grundsätzlich auch wenige ppm Cl^- bestimmt werden. Um den ppm-Bereich unterschreiten zu können, müßte der Blindwert allerdings gesenkt werden. Dabei käme als eine Möglichkeit die Verlagerung des gesamten Aufarbeitungsprozesses der Proben in geschlossene Boxen, deren Luft bezüglich Staub und HCl-Gas gefiltert ist, in Frage. Zusätzliche Untersuchungen haben gezeigt, daß ein großer Teil des Blindwerts durch die verwendete, "Suprapure" Flußsäure verursacht wird, so daß auch hier Ansatzpunkte für eine weitere Verringerung des Blindwerts gegeben sind.

Zusammenfassend kann man sagen, daß der Flußsäureaufschluß in einem geschlossenen System (Autoklav) und die sich nach der Isotopenverdünnung anschließende offene Aufarbeitung der Probe auch für die Bestimmung eines im sauren pH-Bereich so flüchtigen Anions, wie es das Chlorid ist, als praktikable und vergleichsweise schnelle Methode erwiesen

Tabelle 2. Blindwertanalyse bei der Chloridbestimmung mit MS–IVA

Blindwert Nr.	Cl^--Menge pro Aufarbeitungsprozeß, µg
1	22,4
2	19,0
3	15,0
4	16,2
5	21,5
Mittelwert	18,8 ± 3,2

hat. Die mit dem vorliegenden Verfahren der MS–IVA erreichten Reproduzierbarkeiten sind meist wesentlich besser als die bisher in der Literatur für vergleichbare Proben beschriebenen Werte. Außerdem dürfte es damit möglich sein, bezüglich der "Richtigkeit" günstige Ergebnisse bei der Standardisierung geologischer Referenzmaterialien zu erhalten.

Danksagungen—Herrn Dr. W. Luecke, Universität Karlsruhe, danken wir für die Überlassung der Gesteinsproben, der Deutschen Forschungsgemeinschaft sowie dem Fonds der Chemischen Industrie für die finanzielle Unterstützung.

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Summary—An analytical procedure for determining traces of chloride in silicate rocks, using mass spectrometric isotope dilution analysis with a ^{37}Cl-enriched spike, is described. The chlorine isotope ratios are measured with a double-filament thermal ion-source using the production of negative ions from a hot rhenium filament as the ionization method. The samples are decomposed with hydrofluoric acid in a Teflon bomb and chloride is separated from the acidic solution by selective precipitation as AgCl. The relative standard deviation for the chloride determination is 1–4% for chloride contents of 100–200 ppm. This precision is better than any described in the literature for chloride trace analysis. The problem of the blank in chloride trace determination in rock samples is discussed.

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STATISTICAL ADJUSTMENT OF PARAMETERS FOR POTENTIOMETRIC TITRATION DATA

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Summary—The paper deals with the statistical problem of adjusting parameters to the experimental data from potentiometric titrations. The parameters to be refined are, first, the value of the stability constant of the titration reaction, and, second, the equivalence volume. The paper discusses the selection of the error variable and the need for weighting in the optimization procedure. The case where both parameters are refined at the same time is also considered. Special reference is made to linearized titration curves for the determination of the equivalence volume.

In the last few years the introduction of automatic titration devices and electronic computers has made it possible to gather and process large amounts of experimental data. This approach has been used successfully in solution chemistry in the determination of stability constants.

It is quite reasonable to assume that the law of large numbers can be applied to *random* errors in the experimental data used to obtain the "best" values of the constants. The law states that for N independent observations (normally distributed) with *equal* weight the standard error of the mean will diminish by a factor of $1/\sqrt{N}$. Consequently, the larger the number of observations, the more confident we may be that the calculated means are good estimates of the "true" values of the parameters. By choosing a large N the influence of random errors on the values of the constants can be diminished. In potentiometry applied to the study of complex equilibria, data averaging combined with a refined experimental technique has yielded excellent results.

It may now be asked whether the analytical chemist too could make use of the law of large numbers for the determination of the equivalence point in potentiometric titrations. There is, indeed, a trend in analytical chemistry towards numerical calculation of equivalence volumes from many points of titration curves instead of utilizing only one point, *i.e.*, the point of inflection. The assumption of equal weights for the data points is, however, crucial.

The mathematical treatment of the data points can be done as a linear or a non-linear parameter-estimation. In the first case the potentiometric titration curve is transformed into a linear form, so that the parameters can be evaluated visually or by a linear least-squares fit. The application of a functional transformation to experimental data will necessitate transformation of the weights associated with each point, and the linear plot will usually involve data pairs with standard deviations varying from point to point by orders of magnitude. Rigorous statistics will call for a weighted least-squares procedure.[1–4] The simplicity of the unweighted treatment will be destroyed, as the weighting factors are often functions of the parameters to be refined and the optimization problem has to be solved by iteration.

In a non-linear least-squares fit the use of an unweighted procedure may be better justified. The situation is dependent on the problem involved, the magnitude of the errors in the recorded parameters, and the error variable chosen for the optimization. Since the error variable is not a linear function of the parameters, the non-linear case requires iterative schemes and good initial parameter estimates.

The transformation of the experimental data into linear form serves both visual and numerical aims. Deviations from linearity may indicate systematic errors, and the slope and the intercept can be used for parameter evaluation. For example, the transformed data pairs can be fitted to a straight line in order to determine the values of two stepwise stability constants,[4] or the parameters to be refined may be the equivalence volume and the standard potential of the electrode in an E^0-titration.

The numerical refinement of the parameters can be based on a standard least-squares procedure, by minimizing

$$\sum w[y(\text{exp}) - y(\text{calc})]^2 \qquad (1)$$

where w is the weight associated with each data point, y(calc) is the calculated value of the error variable, and y(exp) is the corresponding experimentally observed value. Mathematical considerations suggest that the data may be weighted according to the estimated variance. The weighting factor is then calculated as the reciprocal of the expected variance of y.

In potentiometric work systematic errors may be greater than random errors. Systematic errors are present in the standardization of the electrodes, in the values of the equilibrium constants used, and in the

analytical concentrations assumed. Furthermore, solutions may contain impurities and activity factors may change during titrations.

The effects of systematic and random errors can be calculated by using a Taylor-series expansion. Suppose $y = f(x_i)$; *i.e.*, y is a function of several variables $x_1, x_2, \ldots, x_n$, all independent of each other. If Δx_i is the systematic error of the variable x_i, the error-propagation law gives the systematic error in y as

$$\Delta y = \sum_{i=1}^{n} \frac{\partial y}{\partial x_i} \Delta x_i \qquad (2)$$

provided the errors Δx_i are small enough for higher-order derivatives to be discarded. For random errors, the variance of y can be calculated according to "the propagation of variance"

$$(s_y)^2 = \sum_{i=1}^{n} \left(\frac{\partial y}{\partial x_i}\right)^2 (s_{x_i})^2 \qquad (3)$$

where $(s_{x_i})^2$ is the variance of the component x_i.

Equations (2) and (3) show that the influence of systematic and random errors on the logarithm of the stability constants of the titration reaction or on the equivalence volume can be discussed on the basis of first-order partial derivatives. The magnitude of the derivatives will vary during the course of a titration. These variations will be illustrated graphically. The basis of the discussion is a protonation reaction, but the same principles apply for a metal-complexation reaction.

Potentiometric titrations provide us with two kinds of experimentally recorded parameters: the cell potential and the volume of titrant added. The effects of errors in these quantities on the parameters to be refined will be discussed, as will the influence of errors in some of the input parameters. In an introductory example we discuss briefly the weighting problem in a Gran titration.[5] We will not discuss the corresponding problem for determining two stability constants, as this has already been done in great detail.[4]

INTRODUCTORY EXAMPLE

The application of a functional transformation to experimental data will require transformation of the weights associated with each point.[1–4] This means that experimental data with the same weight can easily have unequal weights in the transformed form. This statement will be illustrated by the following example. Consider the reaction

$$M + A \rightleftharpoons MA; \quad K_{MA} = \frac{[MA]}{[M][A]} \qquad (4)$$

(all charges are omitted). Let the electrode have a Nernstian response for the M-ions and let the titrant solution contain the A-ions. If the potential E is plotted as a function of the volume of titrant added, V, the titration curve will have the familiar S-shape. A typical example is shown in Fig. 1. A high stability

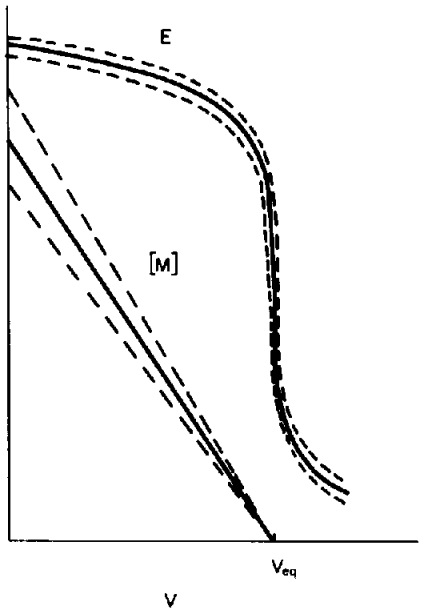

Fig. 1. The potential E and the concentration of M-ions as a function of the volume V.

constant for the titration reaction is assumed, so that the dissociation of the complex MA may be neglected. During the titration the concentration [M] (corrected for dilution) will decrease linearly with the titrant volume added until the equivalence point, V_{eq}, is reached. The linear function will represent a typical Gran plot[5] since the concentration of free M-ions is proportional to $V_{eq} - V$.

We may now ask how errors in the potential readings will affect the Gran plot. Let us assume a constant error in this parameter as illustrated in Fig. 1 by the broken lines on either side of the potential curve. The error may be a constant systematic error in the form of a wrong standardization of the electrode or a random error with a constant variance. The corresponding error in [M] can be calculated by differentiating the Nernst equation:

$$\frac{\text{Percentage error}}{\text{Millivolt units}} = \frac{100\,\Delta[M]}{[M]\,\Delta E} = \frac{zF}{RT}100 \qquad (5)$$

where z is the charge on the metal ion. As the concentration of free M-ions decreases linearly during the course of the titration, the corresponding errors in the Gran plot can be illustrated by the broken lines on either side of the linear function.

Figure 1 shows that the nearer we are to the equivalence point, the less will be the error in [M]. Hence for calculating the random error in the parameter the points on the curve that are close to the equivalence point should be given greater weights than the points at the beginning of the titration. In this way the need for a weighted procedure for the least-squares analysis may be justified. Figure 1 shows also that a constant systematic error in the potentials will alter the gradient of the linear function but will have no in-

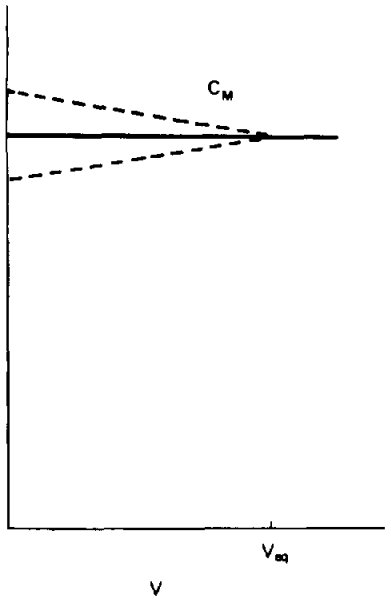

Fig. 2. The error in the calculated value of C_M as a function of V.

fluence on the location of the equivalence point, which is given by the intersection point between the linear function and the volume axis. This is of importance, as it is much easier to determine the potential with good precision than to evaluate the standard potential with good accuracy.[6,7]

In a titration, we wish to determine the total concentration of M-ions, C_M. Suppose the concentration of the titrant solution is known precisely, without any systematic error. Every point on the titration curve can then be used for the calculation of C_M as the sum of complexed M-ions, corresponding to the added amount of titrant, and free M-ions, obtainable from the electrode potential. An uncertainty in the potential readings will cause an uncertainty in [M], and hence also in C_M. Figure 2 shows C_M and the uncertainty in this concentration as a function of V.

The assumption of a constant variance in the potential readings may not be strictly valid. As is well known, it is difficult and time-consuming to get stable potential readings in the proximity of the equivalence point, perhaps because of the low buffer capacity of the solution. According to these considerations, the most reliable data points for the evaluation of the equivalence volume value will be the readings close to the potential jump.

The remainder of this section will discuss the so-called E^0-titration technique. This is commonly used in high-precision potentiometric work with glass electrodes.[8] The relationship between the potential E and the hydrogen-ion concentration is given by

$$E = E^0 + Q \log[H] + E_j \qquad (6)$$

where E^0 is the standard potential, including the pH-independent part of the activity coefficients, $Q = (RT \ln 10)/F$, and $E_j = j_H[H] + j_{OH}[OH]$ stands for the liquid-junction potential. The constants j_H and j_{OH} are characteristic of the ionic medium.

The value of E^0 is determined as a part of every titration. A known volume of a strong acid is titrated with strong base and the titration is discontinued at a hydrogen-ion concentration suitable for the subsequent experiment. If the concentrations of acid and base are precisely known, a plot of $E - Q \log[H]$ against [H] will yield E^0 as the intercept and j_H as the slope.[8] The inset in Fig. 3 shows such a plot. There is some scatter, as the main aim was to determine the E^0-value and the concentration of the titrant solution. The effect of an error in j_H was minimized by titrating a rather dilute solution of strong acid ($C_{HCl} = 5.00 \times 10^{-3} M$). The potentials were measured to ± 0.1 mV.

The main part of Fig. 3 shows a plot of the equivalence volume of the titrant solution (sodium hydroxide), analogous to Fig. 2. The curves were calculated for a value of the standard potential equal to that determined, and for values offset by 0.1 and 0.5 mV, respectively. The influence of the liquid-junction potential was neglected. Curved Gran plots result. Thus, it is not possible to neglect the E_j-term in equation (6) when a strong acid is titrated. As E_j is dependent on E^0, the E^0 and V_{eq} can most conveniently be evaluated iteratively. In the example, the ionic strength of the solution was made 0.5 with barium chloride and the value of j_H was -23 mV.l.mole^{-1}.

The error made in measuring the volume of titrant should also be considered. If the volume error can be assumed to be constant, the relative concentration error will be constant during the entire titration.[6]

Rigorous statistics show that a weighted procedure is preferable, and the use of properly weighted data has been proved superior to an unweighted least-squares treatment.[4,9] A weighted least-squares fit will emphasize the later part of the titration curve more than the unweighted method does, and mathematical analysis suggests that this treatment is the more correct. Of course, the weighting can be achieved by gathering more data points in the important region.

WEAK COMPLEXES

We now turn our interest to the study of weak complex formation. The expressions for the first-order derivatives can be used to illustrate the influence of errors in the experimental parameters during the course of the titration. The mathematical model presented can also serve as a basis for a discussion of the weighting functions in a linear or a non-linear least-squares refinement of the formation constant and of the equivalence volume.

Theory

In the following, the case of weak complex formation will be discussed. As an example, the titration of

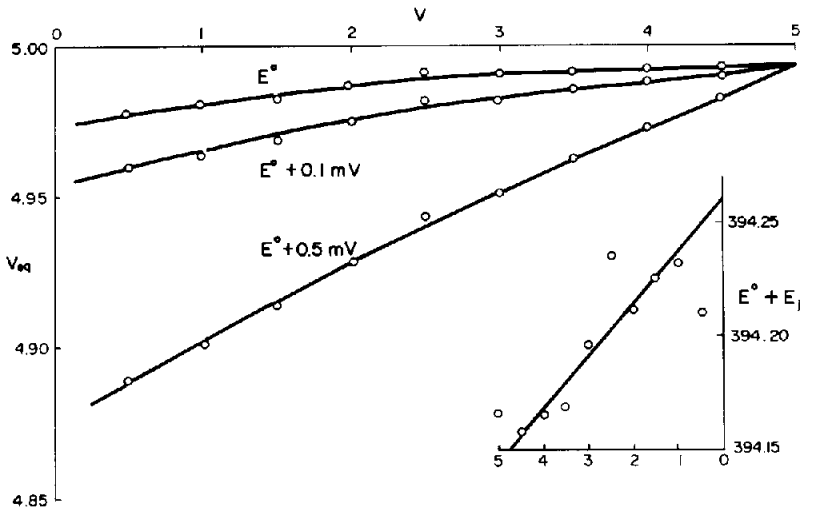

Fig. 3. The right-hand part of the figure shows a standard E^0-titration. The main part shows the calculated value of V_{eq} as a function of V with the standard potential equal to E^0, $E^0 + 0.1$ mV, and $E^0 + 0.5$ mV, respectively. The liquid junction potential, E_j, is neglected.

a weak acid with a strong base will be considered:

$$HA + OH \rightleftharpoons A + H_2O; \quad K_{HA} = \frac{[HA]}{[H][A]} \qquad (7)$$

The titration curve can be transformed into a linear form by means of the following equation[10] (V_0 = initial volume of solution):

$$V_{eq} - V = V[H]K_{HA} + \frac{V_0 + V}{C_{NaOH}}([H] - [OH])(1 + [H]K_{HA}) \qquad (8)$$

where C_{NaOH} is the concentration of the titrant solution. The equation can be put in an alternative form:

$$\frac{V_{eq}}{1 + [H]K_{HA}} = V + \frac{V_0 + V}{C_{NaOH}}([H] - [OH]) \qquad (9)$$

The linear equation requires a knowledge of the formation constant K_{HA}. Suppose we are interested in the error in the equivalence volume. The experimentally recorded parameters are pH (defined as $-\log[H]$) and V; $\log K_{HA}$ is an input parameter. Differentiation of equation (8) or (9) will give

$$-(\log e)\frac{\partial V_{eq}}{\partial pH} = V[H]K_{HA} + \frac{V_0 + V}{C_{NaOH}}([H] + 2[H]^2K_{HA} + [OH]) \qquad (10)$$

$$(\log e)\frac{\partial V_{eq}}{\partial \log K_{HA}} = [H]K_{HA}\left\{V + \frac{V_0 + V}{C_{NaOH}}([H] - [OH])\right\} \qquad (11)$$

$$\frac{\partial V_{eq}}{\partial V} = \{1 + [H]K_{HA}\}\left\{1 + \frac{[H] - [OH]}{C_{NaOH}}\right\} \qquad (12)$$

The discussion of the error in $\log K_{HA}$ can be based on the partial derivatives of this quantity with respect to pH, V, and V_{eq}. These derivatives can be calculated by differentiation or by combination of the derivatives given in equations (10)–(12).

The magnitude of the derivatives will be illustrated by the following titrations. A 100-ml sample of $5 \times 10^{-3}M$ weak acid is titrated with $5 \times 10^{-2}M$ sodium hydroxide. The titration curves in Fig. 4 were calculated[11] for $\log K_{HA}$ equal to 5, 8 and 10, respectively.

Figures 5 and 6 illustrate the partial derivatives, and how errors in the titration parameters will affect the calculated equivalence volume and the logarithm of the stability constant. The derivatives are calculated for $\log K_{HA} = 5.0$ in Fig. 5 and for $\log K_{HA} = 10.0$ in Fig. 6. A value of $\log K_{HA}$ equal to 8.0 gives curves for the derivatives that almost coincide with those in Fig. 5. The subsequent discussion will be focused on these derivatives and their implication for the parameter-refinement procedure.

Before turning to the general discussion we will make a brief investigation of the influence of impurities on the equivalence volume. In a strong acid–strong base titration the co-titration of impurities such as carbonic acid may be avoided by choosing data points sufficiently far away from the equivalence point for them not to be affected by the impurity. This is one of the main advantages[6] of Gran titrations. In the weak acid–strong base titrations discussed in this section, co-titrations may not be avoided. Let us assume that carbonic acid is present as an impurity in the solution to be titrated. Figure 7 shows the error in the calculated equivalence volume, denoted as ΔV, as a function of V. The following input data for carbonic acid were used: concentration $5 \times 10^{-5}M$, $\log K_{HB} = 10.1$, $\log K_{H_2B} = 6.3$. The curves were constructed for $\log K_{HA} = 5$, 8, and 10, respectively.

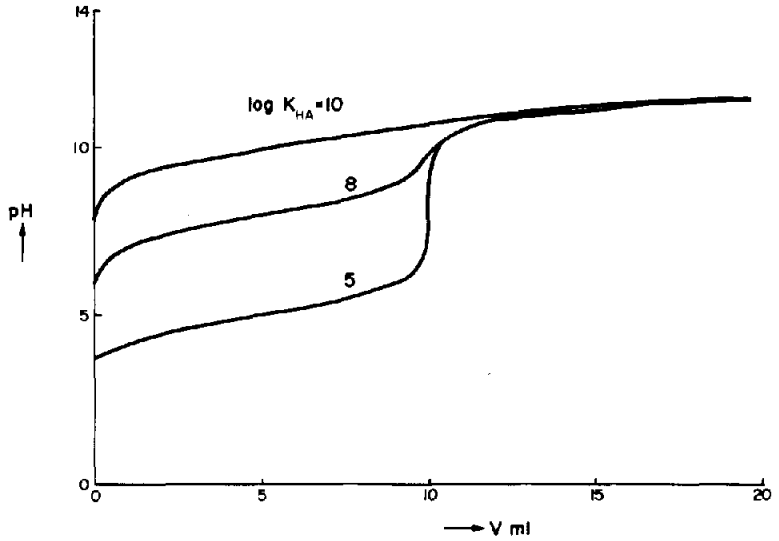

Fig. 4. The titration of 100 ml of $5 \times 10^{-3}M$ acid HA with V ml of $5 \times 10^{-2}M$ NaOH for different values of log K_{HA}.

If the carbonate impurity is instead in the titrant solution the corresponding curves can be obtained by multiplying the ΔV co-ordinate by the volume.

The curves shown in Fig. 7 can be interpreted as a perturbation of the linear function of equation (8). For an acid having log $K_{HA} = 5$, the influence of the impurity starts rather close to the equivalence volume, and the linear plot will have a gradient that changes its value in the vicinity of the equivalence point. For acids with log $K_{HA} = 8$ and 10 the impurity will be converted into bicarbonate early in the titration and the resulting Gran plot will be curved.

DISCUSSION

The method of least squares is the most widely used estimation procedure for parameter evaluation. In it, the sum of the squares of the residuals is minimized. In many cases we will, however, find that it is more correct to use a weighted sum as the objective function, with the weights given by the elements of the inverse of the covariance matrix.[1,2] A weighted least-squares procedure introduces a mathematical complication as the weighting factors are usually functions of the parameters to be refined. As pointed out earlier,

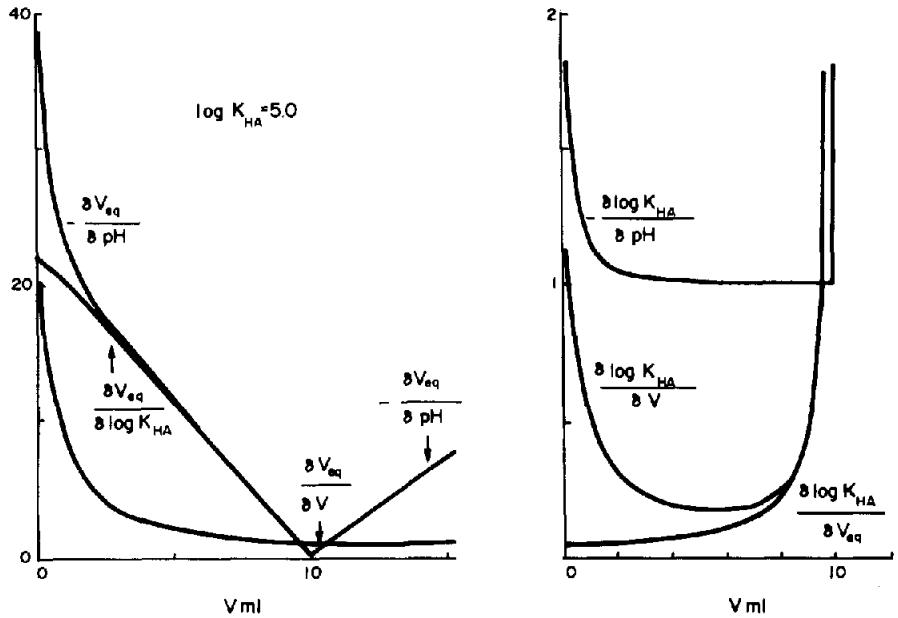

Fig. 5. The partial derivatives as a function of V if log $K_{HA} = 5.0$.

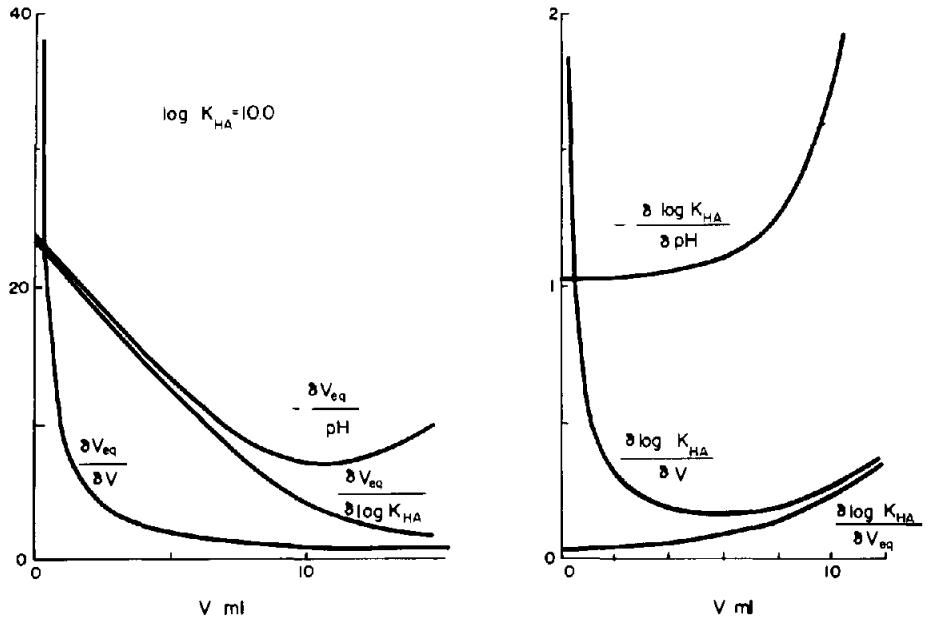

Fig. 6. The partial derivatives as a function of V if log K_{HA} = 10.0.

the situation is dependent on the model applied to the experimentally recorded data.

If the variables can be transformed in such a way that the model is a linear function of the parameters, the method of multiple linear regression can be used to calculate the parameters. The advantage gained derives from the fact that the estimate can be obtained by direct calculation and there is no need for an initial guess of the parameters. In the case of two parameters to be refined there is also a gain in the ease with which the problem can be visualized in a graph.

Non-linear regression requires iterative procedures and an initial guess for the parameters. A good initial guess is essential in order to obtain rapid convergence. Good initial guesses can be provided by graphical plots or by numerical calculations based on an unweighted linear least-squares fit. In the non-linear case the need for a weighted procedure becomes of minor importance.

In potentiometric titrations it is often assumed that the volume error is negligible compared with the error in the potential readings. The potential is a logarithmic function of the free-ion concentration. It follows that a weighted procedure is the more correct when a linear least-squares fit is used, whereas an unweighted procedure may be justified in the non-linear case with the potential readings (or pH) as the error variable.

The error made in measuring the volume of titrant should also be considered. An error made in this quantity may become dominant in the region close to the equivalence point.[6]

A further complication derives from the fact that systematic errors in pH-values are more important than random ones. In the introductory example it was stated that there is a need for a rigorous statistical treatment of the data in E^0-titrations in order to facilitate evaluation of the standard potential with good accuracy.

In addition, none of the input parameters is known exactly: all contain a more or less severe systematic error. Some of the input constants will be assumed to have a negligible influence on the parameters to be refined. We will restrict the discussion to the influence of an error in the equivalence volume on the determi-

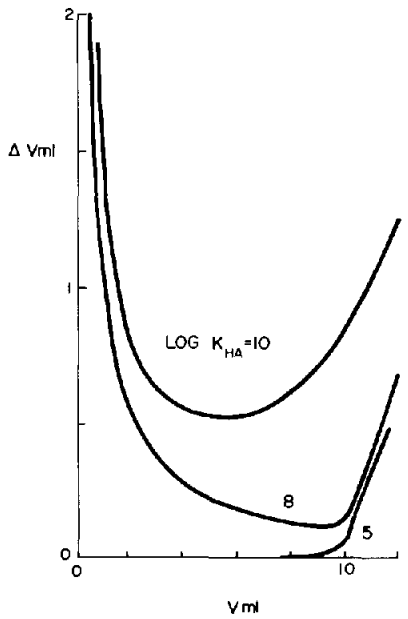

Fig. 7. The error in the calculated equivalence volume, ΔV, caused by a carbonic acid impurity as a function of the volume V for different values of log K_{HA}. The input data for the main reaction are those of Fig. 4, and the input data for the carbonic acid can be found in the text.

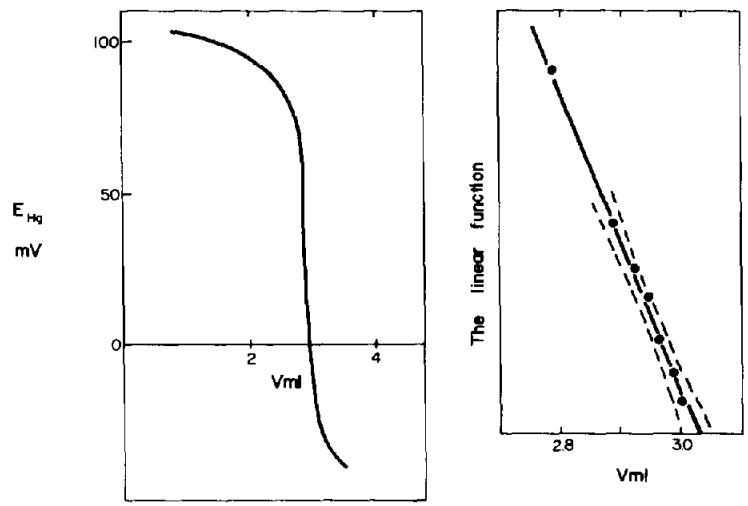

Fig. 8. The potentiometric titration of a calcium solution with EDTA, with a mercury indicator electrode, at pH 10. The corresponding linear function as a function of V. The broken lines indicate the confidence curves.

nation of the stability constant, and to the opposite problem of how an error in log K_{HA} will affect V_{eq}.

It has been advised that systematic errors, such as analytical errors and errors in E^0-values, should be treated as adjustable parameters, and that the final determination be done with an optimization procedure.[12] This approach is promising, but it should be borne in mind that the result will be slightly dependent on the choice of error variable, as will the amount of acidic impurity calculated. The explanation is that the experimental errors influence the error variable differently in different parts of the titration. The curves in Figs. 5 and 6 show how an error made in one of the titration parameters will be transferred to the corresponding error in the value of log K_{HA} and V_{eq}, respectively.

Evaluation of the stability constant

Linear-regression analysis has been used in the evaluation of stability constants, but rigorous statistics inevitably lead to a weighting procedure.[4] The weighting problem arises from the transformation of the potentials into ionic concentrations and will not be discussed here, since several articles have already been devoted to the subject.[13–15] The calculation of the weights is a rather tedious task, so it may be favourable to choose an error variable where weighting is not essential.

Weighting is often assumed to be unnecessary in non-linear optimization procedures. The situation depends to a certain degree on the selection of error variable. A discussion of the extent to which the use of constant weights is justified can be based on the simple examples in Fig. 4 and the curves given in the right-hand parts of Figs. 5 and 6.

Let us assume that the random errors in the potential readings are the errors that have the greatest effect on the value of the constant. Equation (3) then takes the form

$$(s_{\log K_{HA}})^2 \sim \left(\frac{\partial \log K_{HA}}{\partial \mathrm{pH}}\right)^2 (s_{pH})^2 \qquad (13)$$

This means that in minimizing the residuals in pH or potential readings the weighting function will be $1/(\partial \log K_{HA}/\partial \mathrm{pH})^2$. Figures 5 and 6 show the course of the derivative $\partial \log K_{HA}/\partial \mathrm{pH}$ as a function of the volume of titrant. The value of the derivative is approximately constant in the whole buffer region, and the choice of potential or pH as error variable is obvious. The introduction of weighting is superfluous, but care should be taken when dealing with data at the beginning of the titration, as in Fig. 5 (log $K_{HA} = 5.0$) or close to the equivalence point, as in Fig. 6 (weak complexation reaction).

The availability of potentiometers with a resolution of 0.1 mV or better and the more widespread use of E^0-titrations means that errors in volumes may have a significant influence on the value of the stability constant. In Figs. 5 and 6 a ΔE-value of 1 mV (Δ pH ~ 0.02) corresponds to a ΔV-value of ~0.02 ml and the potential readings may be the major source of errors. But if we are able to decrease the error in the potentials by one order of magnitude, a corresponding decrease in the error of the volume measurements may be hard to obtain with ordinary burettes.

In a situation where the errors in each observable parameter will contribute to the error in the stability constant we can rearrange equation (3) and minimize either[16]

$$\sum \frac{(E(\mathrm{exp}) - E(\mathrm{calc}))^2}{s_E^2 + (\partial E/\partial V)^2 s_V^2} \qquad (14)$$

or[17]

$$\sum \frac{(V(\mathrm{exp}) - V(\mathrm{calc}))^2}{s_V^2 + (\partial V/\partial \mathrm{pH})^2 s_{pH}^2} \qquad (15)$$

Reasonable values can be assigned to the magnitudes of the variances, which may be assumed to be constant for all titration points. (It is, however, well known that the actual variance of the pH or potential will increase in the steeper part of the titration curve.)

In the buffer region the derivative $\delta V/\delta$pH is almost constant. It follows that we can equally well calculate the residuals from the volume of titrant added and use constant weights. The favourable titration range is seen in Figs. 5 and 6. In many computer programs weighting is assumed to be unnecessary when calculating residuals from the volume.[12,18,19]

Figure 6 shows that for weak-complex formation $\delta \log K_{HA}/\delta V$ is approximately constant in the region close to the equivalence point. Such a situation prevails, for example, in the study of micelle formation, and the parameter V is demonstrably superior to the potential as the error variable.[20]

The concept of formation function and average ligand number has proved to be very useful in treating complex equilibria, and these functions have been widely used as error variables in non-linear regression analysis.[12] The formation function is closely related to the volume, but contains in addition an ionic concentration. The combination of the two observable parameters into the transformed one will result in correlated errors. Rigorous statistics can be applied,[4] but the mathematics are much more involved than for uncorrelated errors.

Figures 5 and 6 illustrate how an error in the analytical concentration will affect the value of the stability constant. A systematic error in the standardization of the solution will cause a drift in the value of log K_{HA},[21] as illustrated by the derivative $\delta \log K_{HA}/\delta V_{eq}$.

The left-hand parts of Figs. 5 and 6 illustrate how errors in some of the titration parameters will affect the equivalence volume, and we now turn our interest to a discussion of the analytical problem.

Evaluation of the equivalence volume

In the introductory example we discussed briefly the use of linear functions in the evaluation of equivalence volumes. In the example considered, the titration of a strong acid with strong base, it was shown that a systematic error in the potentials will alter the gradient of the linear plot, but give a correct value for the equivalence point. (It was assumed that only titration data before the equivalence point were used.)

For moderately weak or weak complex formation the linear plot based on equation (8) is suitable for the evaluation of V_{eq}.[10] The use of the equation requires a knowledge of the stability constant, and it is of interest to evaluate the titration error caused by an error in the value of log K_{HA}.

Figure 5 shows the derivatives for a titration of a moderately strong acid (log $K_{HA} = 5.0$). It is seen that a small systematic error in log K_{HA} is linear in $(V_{eq} - V)$ and will not affect the precision of equation (8) as long as data points are not used from both sides of the equivalence point (the function $\delta V_{eq}/\delta \log K_{HA}$ equals zero for volumes greater than 10 ml). In the opposite case, if the equation is applied to titration data from both sides of V_{eq}, a change in slope will be observed at the equivalence point.

Almost the same reasoning will apply to an error in pH or E^0. The differences are in the early part of the titration and in the region after the equivalence point. A systematic error in E^0 will not prevent us from getting good analytical results. The strategy is to use titration points either before or after the equivalence point, neglecting the data points at the beginning of the titration.

Doing an E^0-titration before every analysis is time-consuming. From an analytical point of view it may be advantageous to define E^0 or the pH-scale on the basis of the assumed value of log K_{HA}.[7] A systematic error in the value of the constant will mean a wrong definition of the pH-scale. The derivatives $\delta V_{eq}/\delta \log K_{HA}$ and $\delta V_{eq}/\delta$pH coincide in the region before the equivalence point and, accordingly, the errors in log K_{HA} and E^0 will cancel each other if the early part of the titration is discarded. The method can perfectly well be used for analytical purposes. In the region after the equivalence point the derivatives can still be approximated by straight lines but they have gradients differing from those before the equivalence point ($\delta V_{eq}/\delta \log K_{HA}$ coincides with the volume axis). This means that we have to calculate a new value for the ionic product of water if we want to construct a linear plot with data from both sides of V_{eq}.

Figure 6 shows the corresponding derivative curves for weak complex formation. We observe that a linear extrapolation of the $\delta V_{eq}/\delta \log K_{HA}$ and $\delta V_{eq}/\delta$pH curves will not intersect the volume axis at V_{eq}. Consequently, a systematic error in the value of the stability constant or in E^0 will mean a systematic error in V_{eq} as calculated from the linear plot. A systematic error in one of the parameters mentioned above will give a plot that has the wrong gradient and is curved.

In analytical applications we can use the assumed value for log K_{HA} as standard. Since the titration data are from the alkaline region we will preferentially calculate an "alkaline" E^0-value. Again, the systematic errors will cancel each other, so a correct value for the equivalence volume is obtained.

In the introductory example we used a linear plot to evaluate the equivalence volume. From error analysis we found that a weighted least-squares procedure is needed for the statistical refinement of the parameter value. The discussion was based on an assumption that the major source of error is in the potential readings. From Figs. 5 and 6 we see that the same situation prevails in titrations with log $K_{HA} = 5.0$ and 10.0.

In the discussion of the two titrations it was concluded that a small systematic error in E^0 and/or in

log K_{HA} can be tolerated under certain circumstances. From an analytical point of view the precision of the potential readings will be more important than the accuracy, and it follows that the errors made in measuring the volume of titrant may not be negligible, especially close to the equivalence point. As a result, a rigorous statistical treatment of the linear equation (8) must also take account of the correlation of errors in the variables.

The choice of error variable will be crucial in the application of non-linear least-squares methods to the evaluation of the equivalence volume. Figures 5 and 6 show that the choice of the potential as error variable will require a weighting function to be introduced, if we are not restricting ourselves to the region close to the equivalence point. The derivative $\delta V_{eq}/\delta V$ looks promising, because the curves tend to approach a constant value (equal to unity) soon after the half-titration point. This means that a constant weighting function may be applied if the residuals in the volume of titrant added are minimized. Some caution is necessary with data from the early part of the titration.

During recent years computer programs have been developed for the refinement of analytical concentrations.[22–24] These programs use the volume of titrant added as the error variable and assume constant weights. The programs have been applied to the quantitative analysis of mixtures of protolytes, but they can also be used for simultaneous refinement of stability constants and standardization of the electrodes. The general strategy has been to use all the data points. The course of the derivative curves in Figs. 5 and 6 shows that a weighted procedure may be more justified in the early part of the titration. Constant weighting will over-emphasize this region and the results may be that the determination of the concentration of strong acid is erroneous. (For example, see Table 1 in ref. 24.)

Figures 5 and 6 show that the volume of titrant added is a good choice as error variable for the simultaneous determination of concentrations and stability constants. If the values of the stability constants are known with good accuracy, the analytical concentrations can be evaluated by linear least-squares methods, since the volume is a linear function of these parameters. Accordingly, the analytical problem can in simple cases be solved with a programmable pocket calculator with a big enough memory.[11]

On the basis of the two titrations, we have discussed the choice of error variable and the favourable region for the evaluation of stability constants and equivalence volumes. It is possible to combine the features of the error variables in such a way that potential is used for the refinement of the stability constant on the basis of data from the buffer region, whereas volume is used for the calculation of the analytical concentration from data from the region around the equivalence point. It remains to be seen what advantages can be gained by such a strategy.

CONCLUDING EXAMPLE

In the concluding example we will show that errors in volume readings can easily be the limiting factor in the precision of the equivalence volume. The discussion is based on the concept of confidence limits.

The example considered is the titration of calcium ions with EDTA (H_4Y), with a mercury electrode as the indicator electrode.[25] The variation in potential of this electrode is given by

$$E_{Hg} = k + \frac{RT}{2F} \ln [Ca][HgY]/[CaY] \qquad (16)$$

where k is a constant. Consequently, a plot of

$$f(V) = (V_0 + V)\, V\, 10^{34.4\,(E_{Hg} - k)} \quad (25°C) \qquad (17)$$

vs. the volume of titrant added will yield a straight line intersecting the volume axis at the equivalence point.[5] The equation is valid for titration points before the equivalence point. In addition, data points in the immediate neighbourhood of V_{eq} are discarded, because dissociation of the complex CaY cannot be completely neglected.

Calcium-salt solutions of concentration 1×10^{-3}–$5 \times 10^{-3} M$ were titrated with $5.15 \times 10^{-2} M$ EDTA in ammonia–ammonium nitrate buffered solution (ionic strength = 0.1). A typical titration curve and the corresponding linear plot are presented in Fig. 8. A Metrohm E 274 piston burrette was used for the volume measurements. The uncertainty in the volume readings was estimated to be 0.1–0.2%. The precision in the potential readings was only 1 mV.

The precision of V_{eq} and the linear function will depend on the number of observations, on the extent of scatter about the straight line, and on the range of values of the independent variable. The situation is illustrated in the linear plot in Fig. 8. On either side of the straight line the confidence limits may be drawn with a previously chosen level of confidence, say (1–2 v), where the constant v has a preselected small value. The two confidence curves can be used to predict the confidence range of V_{eq}. This interval will be defined by the intersections of the curves with the V-axis. The interval has again a probability of (1–2 v).

It can be shown that the confidence limits of V_{eq} can be calculated from the expression[26]

$$V_{eq} \pm t_{n-2} \frac{s}{b} \sqrt{1 + \frac{1}{n} + (V_{eq} - \bar{V})^2 / \sum (\bar{V} - V)^2} \qquad (18)$$

where

$$s^2 = \frac{1}{n-1} \sum [a + bV - f(V)]^2 \qquad (19)$$

and t is the Student's t-value corresponding to $n - 2$ degrees of freedom and a confidence level of (1–2 v), n is the number of observations, s is the estimated value of the standard deviation of the residuals. $\bar{V}$ is the mean of the V-values, and $f(V) = a + bV$ is the equation of the regression line.

We assume that data points in the vicinity of the equivalence point have the same weights. Experimen-

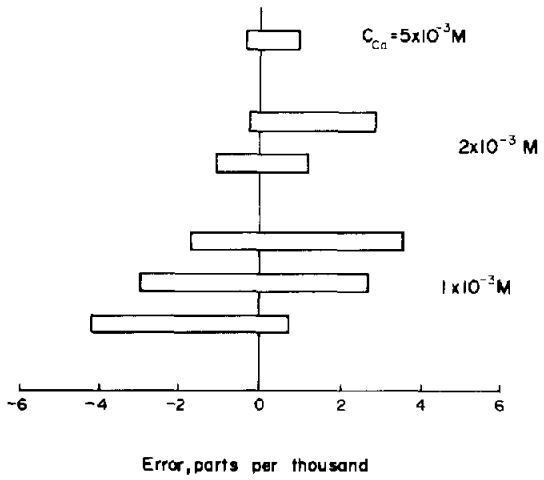

Fig. 9. The confidence intervals for some calcium titrations (confidence level = 95%).

tal points from this region can then be used for calculation of the confidence interval. The calculations are very approximate as they are based on a small number of experimental data points. Furthermore, the point of intersection with the V-axis is obtained by extrapolation.

In Fig. 9 the confidence intervals obtained are presented. The confidence level was 95% ($\pm 1.96\,s$). The figure shows that the width of the confidence interval is inversely proportional to the equivalence volume. This means that the precision in the delivery of titrant determines the limit of precision in the determination of the analytical concentration. The example can be interpreted on the basis of the curves in Fig. 5 with a precision of 0.1–0.2% in the volume and 1 mV in the potential readings. The experiments are in agreement with theory, in that the precision in the delivery of titrant is the limiting factor for the precision in the equivalence volume provided that data points close to the equivalence point are used for the evaluation. Further improvement in analytical precision might be obtained by using a top-loading balance and a weight-burette or gravimetric titration system. An alternative is to use coulometric titrations.

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COLORIMETRIC DETERMINATION OF ORGANIC COMPOUNDS BY FORMATION OF HYDROXAMIC ACIDS

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Summary—Carboxylic acids, their chlorides, anhydrides, esters, lactones, amides, lactams and imides react with hydroxylamine to give hydroxamic acids which are then treated with iron(III). Other compounds or groups of compounds can also be determined after a prior conversion into hydroxylamine-reactive derivatives. The colorimetric applications of these reactions are reviewed. The effect of various factors is discussed. A selective procedure for determination of acid chlorides and anhydrides and an improved procedure for determination of carboxylic esters and lactones are presented.

Colorimetric analysis of organic compounds remains of great value, in spite of the steadily growing resort to purely physical methods. Moreover, there is renewed interest in some colorimetric methods because of the development of fractionation techniques such as automated ion-exchange chromatography or HPLC. Although detection by use of ultraviolet light, which has been most widely used in liquid chromatography, can often lead to more sensitive measurements, methods based on functional-group colorimetry are more specific. Also, conventional ultraviolet detectors are insensitive to compounds such as saturated aliphatic acids and some of their derivatives. In such instances, recourse to colorimetric procedures which can be readily automated may often be a convenient answer to the problem. These considerations prompted us to present this general study on determinations based on the formation of hydroxamic acids.

Under alkaline conditions, carboxylic esters react with hydroxylamine to form hydroxamic acids, R–CO–NHOH, which yield red-violet complexes with iron(III) in acidic medium. Proposed at first as a spot test by Feigl *et al.*,[1] the reaction was later made quantitative and extended to a number of carboxylic acid derivatives which behaved in the same way, as well as to compounds or groups of compounds which could be converted into such derivatives by suitable chemical reactions.

Although the sensitivity is only moderate, the molar absorptivities of the ferric complexes of monocarboxylic acid hydroxamates being $1.0–1.7 \times 10^3$ $l.mole^{-1}.cm^{-1}$,* colorimetric methods based on the reaction have been the subject of intensive study. In 1973, Vejdělek and Kakáč[2] listed more than 400 compounds that had been estimated in this way, and various recent studies show that the reaction is still arousing sustained interest.

* All molar absorptivities quoted in this paper refer to the species actually measured.

In the following survey, only a limited number of references are quoted in order to illustrate the scope of the hydroxamation reaction.

DETERMINATION OF CARBOXYLIC ACIDS AND THEIR DERIVATIVES

Esters

The first applications to carboxylic esters through the hydroxylamine–iron(III) reaction were described by Hill[3] and Hestrin,[4] who used the method to study the enzymatic synthesis of acetylcholine. A method applicable to numerous esters of aliphatic and aromatic acids was then proposed by Goddu *et al.*[5] Esters of phenols, such as phenyl acetate,[5] 2-naphthyl benzoate and 2-naphthyl salicylate[6] were also determined.

These or similar procedures were applied to the estimation of triglycerides,[4,7–9] esters of α-amino-acids or peptides,[10–12] carboxylic esters of steroids,[13,14] esters of cardiac glycosides,[15–17] alkaloids bearing an ester group such as aconitine, cocaine, scopolamine[18] and homatropine,[19] and esters of chloramphenicol[20] and malathion.[21]

The kineties of reaction of hydroxylamine with ethyl acetate[22] and adiphene[23] have also been studied.

Lactones

Derivatives of γ-butyrolactone and δ-valerolactone can be considered as the internal esters of the corresponding γ- or δ-hydroxy-acids. They usually react with hydroxylamine under milder conditions than do other carboxylic esters and can therefore be determined in their presence.[5] Various procedures have been proposed for the estimation of gluconolactone,[24] glucuronolactone,[25] peltanins,[26] picropodophyllin,[26] pilocarpine,[27,28] santonin,[29] etc. A procedure has also been given for the determination of cardiac aglycones,[18] in which the butenolide ring is a lactone, but it has poor sensitivity.

If the medium is too alkaline, the hydrolysis of the lactone may compete with the hydroxamation reaction,[24] for which three mechanisms have been proposed on the basis of kinetic studies.[30]

Amides

Amides react more slowly than esters with hydroxylamine, so that the formation of the corresponding hydroxamates requires more drastic conditions. The most general procedure seems to be that of Bergmann.[31] Beside simple amides such as formamide, *N*,*N*-dimethylformamide, acetamide, *N*-methylacetamide and nicotinamide, this author determined α-amino-acid derivatives such as asparagine, glutamine, *N*-acetylglycine, glycylglycine and glutathione. Procedures were proposed for the estimation of *N*-[(4-aminophenyl)sulphonyl]acetamide[32] and of acetylsulphisoxazole in the presence of sulphisoxazole.[33] The rate of the hydroxamation reaction can be appreciably affected by the substituents attached to the acyl group or to the amido nitrogen atom.[10,31]

When performed in the presence of hydroxylamine, the enzymatic hydrolysis of various amides (including glutamine and asparagine) yields the corresponding hydroxamates, presumably through a direct reaction of hydroxylamine with the acyl–enzyme complex.[34–36]

Lactams

Lactams can be considered as the internal amides of the corresponding amino-acids but, with the exception of ε-caprolactam[31] and nitrazepam,[37] the hydroxylamine–iron(III) reaction was only applied to penicillins. Both manual and automated procedures have been used for several years to determine the penicillin content of process samples and formulations.[38] The fact that no colour is developed by the corresponding penicilloic acids establishes that, under the given conditions, hydroxylamine attacks the β-lactam ring.[39]

Imides

The hydroxamate reaction can be applied to cyclic imides. The kinetics and mechanisms of the reaction of hydroxylamine with succinimide were studied by Notari.[40] In alkaline medium at 40°, succinimide simultaneously hydrolyses to form succinamic acid (which then condenses very slowly with hydroxylamine) and reacts with hydroxylamine to form *N*-hydroxysuccinamide, which undergoes ring closure to give *N*-hydroxysuccinimide. This ring closure is much more rapid than the hydroxylaminolysis step. At pH 9.5, *N*-hydroxysuccinimide is the major derivative formed. At pH 12, it undergoes a further hydrolysis to yield *N*-hydroxysuccinamic acid. The first of these compounds also forms a ferric complex, but its molar absorptivity (0.53×10^3 l.mole^{-1}.cm^{-1}) is lower than that of the complex obtained with the second (of the order of 1.4×10^3 l.mole^{-1}.cm^{-1}).

The hydroxylamine–iron(III) reaction has been applied in particular to the determination of succinimide,[41] bemegride,[42] glutethimide[42,43] and cycloheximide,[44] and to the study of the enzymatic hydrolysis of cyclic imides.[45]

Acid chlorides and anhydrides

Acid chlorides and anhydrides react readily with hydroxylamine in a slightly alkaline medium. The method proposed by Goddu *et al.*[5] for lactones can be used for their determination, but it has given rise to only few applications.

Carboxylic acids

At one time it was considered that free carboxylic acids could not be directly hydroxamated. Consequently, a prior reaction yielding a hydroxylamine-reactive derivative was deemed necessary. Besides formation of the acid chloride with thionyl chloride[46] or esterification with diazomethane,[47] two more convenient reactions were proposed, both of which permit the use of aqueous samples: (*a*) esterification with ethylene glycol in the presence of sulphuric acid, which has been applied to the determination of carboxylic acids in sewage-sludge liquors,[48] (*b*) esterification with methanol in the presence of dicyclohexylcarbodi-imide.[49]

As a matter of fact, free carboxylic acids can react with hydroxylamine in slightly acidic medium, and two methods have been worked out.

Hydroxamation catalysed by nickel(II). At about pH 6, hydroxylamine condenses directly with carboxylic acids under the catalytic action of nickel(II).[50] An advantage is that the reaction is performed in aqueous medium. It has been applied by Mays *et al.*[51] to manual and automated determination of cephalosporins. These authors showed that the methods used for penicillins[38] could not be adopted because of the greater stability of the β-lactam ring.

Hydroxamation in the presence of dicyclohexylcarbodi-imide (DCC). Under the dehydrating action of DCC, carboxylic acids condense with hydroxylamine (used as its hydrochloride or perchlorate) without addition of any base. Two procedures have been proposed for acids in ethanolic solution.[52,53] Since esters do not react under the given conditions, acetic acid can be estimated in butyl acetate at a concentration of about 0.1%.[52] Another procedure permits the determination of carboxylic acids in aqueous solution.[53] It has been automated for their estimation by ion-exchange chromatography[54] and applied to their determination in beverages, blood serum and urine.[55]

It is worth mentioning that carboxylic acids also condense directly with hydroxylamine through a reaction involving ATP and coenzyme A.[56]

DETERMINATION OF MISCELLANEOUS COMPOUNDS

Some other compounds or groups of compounds have been estimated by the hydroxylamine–iron(III)

reaction, but few studies have been made, chiefly because more sensitive and convenient colorimetric methods exist. They are mentioned here for the sake of completeness.

Alcohols

These can be determined as acetates after acid-catalysed acetylation.[57] At least several hundred micrograms are required. With the exception of a study on various hydroxysteroids in neutral urinary extracts,[58] this method has not given rise to further investigation.

Chlorobutanol (1,1,1-trichloro-2-methyl-2-propanol) reacts directly with hydroxylamine, and affords 2-methyl-lactylhydroxamic acid.[59]

Aldehydes

Aldehydes will react directly with benzenesulphohydroxamic acid according to the equation:

$$R\text{–}CHO + C_6H_5SO_2NHOH \rightarrow C_6H_5SO_2H + R\text{–}CO\text{–}NHOH$$

They were estimated in this way by Bayer *et al.*[60] The method was turned to account by Exley *et al.*[61] for the selective estimation of 20,21-dihydroxysteroids. Only these derivatives afforded a 17-carboxaldehyde upon bismuthate oxidation;[62] determinations were made at levels of 10–100 μg.

Compounds yielding lactones

γ- and δ-Hydroxy-acids. By dehydration in acid medium, a γ- or a δ-hydroxy-acid yields the corresponding lactone, which can then be determined as already described. Gluconic and glucuronic acids,[25] and mevalonic acid[63] have been estimated in this way.

Reducing sugars. When treated with an acid, the cyanohydrin obtained by reacting the sugar with hydrocyanic acid is hydrolysed to the corresponding uronic acid, which then cyclizes to give a lactone.[25]

Glutamic acid. Glutamic acid reacts with nitrous acid to give the lactone of 2-hydroxyglutaric acid, and can be estimated in protein hydrolysates.[64] It can also be converted into the lactam of 2-aminoglutaric acid (2-pyrrolidone-5-carboxylic acid) by heating in acid medium.[65]

Pantothenic acid. By acid hydrolysis, pantothenic acid affords pantolactone,[66] which reacts more readily than the acid itself with hydroxylamine, through its amide group.[31]

FACTORS AFFECTING THE HYDROXYLAMINE–IRON(III) REACTION

When a given procedure is applied to different compounds bearing the same functional group (*e.g.*, esters), the yield of the hydroxamation reaction may vary with the compound tested, because the reactivity depends on the structure of the whole molecule. This was mentioned above in the section on amides.

Likewise, the molar absorptivity of the ferric complex may vary with the structure of the hydroxamic acid. For instance, under given conditions, Kasai *et al.*[53] found the following values for authentic ferric complexes of benzo-, caprylo- and *p*-nitrophenylacetohydroxamic acids: 1.45, 1.05 and 1.15 × 10^3 l.mole^{-1}.cm^{-1}, respectively. For a given complex, the absorptivity may also vary with the composition of the medium in which it is formed.[41,53] The wavelength of maximum absorption also depends on these factors but is usually between 505 and 550 nm. Further the different derivatives of a carboxylic acid (ester, amide, *etc.*) have different reactivities towards hydroxylamine, and conditions of varied severity are therefore required for the hydroxamation reaction to take place. Consequently, a suitable procedure may allow selective determinations, as already mentioned in the section on lactones. In what follows, the apparent molar absorptivity of the product will be the molar absorptivity calculated from the volume of final solution and the amount of parent substance that has given rise to the product. The reaction yield is then the apparent molar absorptivity divided by the true molar absorptivity of the product.

The hydroxamation reaction

The direct hydroxamation of free carboxylic acids was dealt with above. For the acid derivatives, the yield and selectivity of the reaction depend mostly upon the alkalinity and, to a lesser extent, upon the hydroxylamine concentration of the solution. The reaction time and temperature also vary according to the compound or class of compounds tested. The reactivity of the major derivatives of carboxylic acids decreases in the order acid chlorides ≥ acid anhydrides > lactones > esters > amides.

The hydroxamation reagent is usually prepared by adding a suitable amount of a methanolic solution of sodium hydroxide to a solution of hydroxylamine hydrochloride in the same solvent.

Acid anhydrides and chlorides. Goddu *et al.*[5] used a reagent that was neutral to phenolphthalein and performed the hydroxamation by heating at 70° for 10 min. The concentration of hydroxylamine in the reaction mixture was 0.43*M*. The apparent molar absorptivities ($\epsilon \times 10^{-3}$ l.mole^{-1}.cm^{-1}) found were: acetic anhydride, 1.20; propionic anhydride, 0.46; phthalic anhydride, 0.17.[67]

In our procedure (see Experimental) a less alkaline reagent is used and the concentration of hydroxylamine in the reaction mixture is only 0.0062*M*. Exception for phthalic anhydride no heating is required, and the condensation is instantaneous with acetic and propionic anhydrides. The apparent molar absorptivities ($\epsilon \times 10^{-3}$ l.mole^{-1}.cm^{-1}) are: acetic anhydride, 1.29; propionic anhydride, 1.03; phthalic anhydride, 1.59.

Comparison of these procedures shows that the concentration of hydroxylamine is far from critical; the lower sensitivity obtained for propionic and

phthalic anhydrides by the first method may result from a side-reaction. The reaction mixture contains 37.5% of methanol. Consequently, esterification may compete with hydroxamation under the alkaline effect of hydroxylamine, and esters do not react under the given conditions. In the second procedure, the mixture contains only 0.66% of methanol and it is less alkaline. The yield is nearly quantitative for acid chlorides and for acetic anhydrides (the molar absorptivity of pure acetohydroxamic acid under the same conditions is 1.34×10^3 $l.mole^{-1}.cm^{-1}$). Compared with the apparent molar absorptivity obtained with benzoyl chloride, that obtained with benzoyl anhydride evidences a yield of 70% from this latter compound.

Our method is also highly selective. No other carboxylic acid derivative reacts, whereas the procedure of Goddu *et al.* allows the determination of some lactones.

Esters and lactones. In the procedure of Goddu *et al.*,[5,68] the concentrations in the reaction mixture are approximately 0.34*M* hydroxylamine, 0.26*M* sodium hydroxide and it is heated at 70° for 5 min. We use (see Experimental) 0.133*M* hydroxylamine and 0.47*M* sodium hydroxide and no heating.

Whereas ethyl acetate, butyl acetate and diethyl malonate behave almost in the same way in both methods, the second procedure gives better sensitivity for dimethyl phthalate and benzyl benzoate as a result of the higher alkalinity, which also allows the omission of heating. The concentration of hydroxylamine is not critical, but concentrations lower than ~0.13*M* result in a loss of sensitivity.

Our method also allows the estimation of lactones. Almost the same apparent molar absorptivities are obtained with butyrolactone and santonin (1.15×10^3 and 1.30×10^3 $l.mole^{-1}.cm^{-1}$, respectively). These compounds can also be determined in the presence of esters by the method proposed by Goddu *et al.* for acid anhydrides, but the apparent molar absorptivities are lower (0.99×10^3 and 0.25×10^3 $l.mole^{-1}.cm^{-1}$, respectively). These differences evidence the effect of alkalinity. They also show that a higher concentration of hydroxylamine does not compensate for a lower alkalinity.

Amides. Amides are poorly reactive and require rather drastic conditions. In the method of Bergmann,[31] the concentrations in the reaction mixture are 0.4*M* hydroxylamine and 0.3*M* sodium hydroxide and several hours at 26 or 60° are required for completion of the reaction. The yields are rather moderate[69] and vary appreciably with the amide tested.

We tried increasing the reagent concentrations and improved the yield to about 75% (from 33% by Bergmann's method), but the colour was very unstable and release of fine bubbles from the final solution often impeded the reading, and this procedure cannot be recommended.

Formation of the ferric complex

The hydroxamic acid–iron(III) reaction is performed in acid medium. Ferric ammonium sulphate has been proposed as reagent,[45,51] but ferric chloride and especially ferric perchlorate are most commonly used. The formation of the complex and its stability depend on several factors.

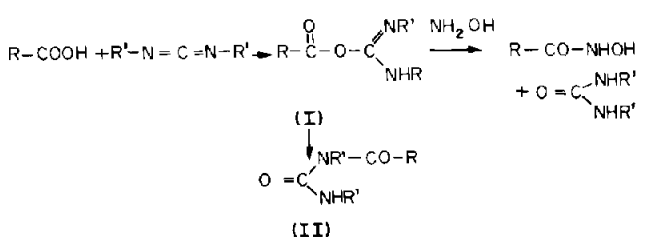
Hill[3] attributed the instability of the colour to reduction of iron(III) by hydroxylamine. The addition of hydrogen peroxide,[3,41] nitric acid[3] or acetone[57,66] was therefore proposed.

Goddu *et al.*[5] stated that the two important variables in the development of the colour were the iron(III) concentration in the final solution, which should be higher than 0.002*M*, and the acid concentration, which should be adjusted to 0.1*M*. Notari and Munson[41] showed that solutions containing a large excess of iron(III) relative to hydroxylamine were more stable, that little difference in stability was found for perchloric acid concentrations between 0.11 and 0.31*M* and that the water content should be kept as low as possible, to increase stability and yield.

Kasai *et al.*[53] found that, in aqueous solution, increasing the acidity reduced the formation of the ferric complex, but addition of ethanol counteracted the effect, which became negligible when the ethanol concentration was >80%. They also demonstrated that nitric acid gradually decomposes the hydroxamate and that hydrochloric acid interferes by competitive complexation of iron(III). They concluded it is best to use 0.1–1.0*M* perchloric acid with large excess of iron(III) and a high concentration of ethanol.

Comparison of different overall procedures is therefore difficult, unless the individual yields of the hydroxamation and complexation reactions are known, or the conditions for one of them are similar in the two methods. Thus the conclusion drawn earlier from the comparison of two methods of determination of acid anhydrides is valid because, in both procedures, conditions for complexation are similar, and the differences in sensitivity therefore most probably result from differences in the yields of hydroxamation; this is also the case for the two procedures for determination of esters.

Free carboxylic acids. Several parameters are important in the direct hydroxamation of carboxylic acids under the dehydrating action of dicyclohexylcarbodiimide (DCC). Kasai *et al.*[53] assumed the reaction sequence to be



In the absence of hydroxylamine, (I) is spontaneously transformed into (II). In order to avoid that reaction, hydroxylamine should be added to the carboxylic acid before the DCC.

A large amount of hydroxylamine decomposes DCC, and a smaller amount of it would leave surplus DCC, which would decompose the hydroxamic acid through a Lossen rearrangement followed by decarboxylation:

$$R\text{–}CO\text{–}NHOH \rightarrow R\text{–}NH_2 + CO_2$$

Kasai *et al.* conclude that both reagents should be used in large excess (>1000:1) with respect to the carboxylic acid, and a 10% excess of hydroxylamine relative to DCC should be used.

However, in the procedure of Pesez and Bartos[52] which affords comparable results, there is a 68% excess of DCC relative to hydroxylamine and the molar ratio of hydroxylamine to carboxylic acid is only about 20. It may be concluded that under these conditions, the Lossen rearrangement practically does not occur.

Since oxalic acid is decomposed by DCC, it cannot be estimated by procedures involving this reagent.

SENSITIVITIES OF DETERMINATIONS

In Table 1, we summarized the results found in our laboratory with several of the methods referred to in this paper.

Since the ratio of sample volume to final volume varies from procedure to procedure, comparison of sensitivity in terms of apparent molar absorptivity alone can be misleading. In practical terms, a better guide is the sample concentration required for an absorbance of 0.10 to be obtained and this value is given in Table 1 as the criterion of sensitivity (or performance).

Table 1

Compounds	References	Apparent ϵ, $l.mole^{-1}.cm^{-1} \times 10^{-3}$	Sample concentration for $A = 0.10$, $\mu g/ml$
Free carboxylic acids			
Acetic	48, 71	0.53	334
	49, 72	0.41	146
	50	0.99	72
	52	0.70	72
Benzoic	52	1.21	84
Butyric	49, 72	0.29	300
	52	0.68	108
Citric	48, 71	0.20	2840
	50	0.45	506
Formic	48, 71	0.30	454
	50	0.94	58
	52	0.97	40
Lactic	48, 71	0.30	894
	50	0.37	294
	52	0.65	116
Malic	48, 71	0.49	806
	50	0.21	766
	52	1.28	88
Mandelic	52	0.96	134
Oxalic	48, 71	0.34	776
	50	0.14	766
Palmitic	52	0.63	340
Propionic	48, 71	0.47	460
	49, 72	0.30	250
	50	0.89	100
	52	0.67	94
Succinic	48, 71	0.67	516
	50	1.06	134
	52	1.10	90
Tartaric	48, 71	0.31	1420
	50	0.13	1400
	52	0.91	138
Acid chlorides			
Benzoyl	*	1.74	36
Myristoyl	*	1.36	82
Palmitoyl	*	1.40	88
Pelargonyl	*	1.32	60
Undecanoyl	*	1.42	65

Continued overleaf

Table 1—*continued*

Compounds	References	Apparent ϵ, $l.mole^{-1}.cm^{-1} \times 10^{-3}$	Sample concentration for $A = 0.10$, $\mu g/ml$
Acid anhydrides			
Acetic	5, 70	1.20	84
	*	1.29	36
Benzoic	*	1.22	83
Maleic	*	1.10	40
Phthalic	5, 70	0.17	866
	*	1.59	42
Propionic	5, 70	0.46	284
	*	1.03	57
Lactones			
Butyrolactone	5, 70	0.99	86
	†	1.15	34
Santonin	5, 70	0.25	1000
	†	1.30	85
Esters			
Benzyl benzoate	5, 68	0.63	334
	†	0.81	118
Butyl acetate	5, 68	0.99	116
	†	1.14	46
Diethyl malonate	5, 68	1.78	90
	†	2.08	35
Dimethyl phthalate	5, 68	1.08	180
	†	1.82	48
Ethyl acetate	5, 68	0.88	100
	†	1.16	34
Ethyl lactate	†	1.18	45
Monoethyl malonate	†	1.30	46
Amides			
Acetamide	31, 69	0.79	17
Acetanilide	31, 69	0.45	71
Dimethylformamide	31, 69	0.41	42
Nicotinamide	31, 69	0.37	76
Succinimide	31, 69	0.69	33

* Procedure for acid anhydrides and chlorides given in this paper.
† Procedure for esters given in this paper.

EXPERIMENTAL

Determination of acid anhydrides and chlorides

Reagents. (*a*) Neutralize (to phenolphthalein) a 10% solution of hydroxylamine hydrochloride in methanol with a 10% solution of sodium hydroxide in the same solvent and just decolourize by dropwise addition of the hydroxylamine hydrochloride solution, and filter. Dilute 1 ml of filtrate to 50 ml with ethyl acetate and filter again. Prepare fresh before use. (*b*) A 0.3% solution of ferric chloric hexahydrate in a 1% v/v solution of 70% perchloric acid (s.g. 1.67) in ethanol.

Procedure. To 1.0 ml of sample solution in benzene or peroxide-free tetrahydrofuran, add 0.50 ml of reagent *a*. Let stand at $t°$ for T min (see table below), cool to room temperature if necessary and add 3.0 ml of reagent *b*. Mix and read at the wavelength of maximum absorption. Beer's law is obeyed up to at least $A = 0.8$.

Tetrahydrofuran is used for compounds insoluble in benzene.

	Solvent*	T, *min*	t, °C	λ_{max}, *nm*
Benzoyl chloride	B	0		535
Myristoyl chloride	B	0		525
Palmitoyl chloride	B	0		525
Pelargonyl chloride	B	0		525
Undecanoyl chloride	B	0		525
Acetic anhydride	B	0		525
Benzoic anhydride	B	5	20 ± 2	530
Maleic anhydride	THF	30	20 ± 2	525
Phthalic anhydride	THF	15	50	525
Propionic anhydride	B	0		535

* B = benzene, THF = tetrahydrofuran.

The sample concentrations affording $A = 0.10$ are given in Table 1. Carboxylic acids, esters, lactones and amides do not react.

Determination of esters and lactones

Reagents. (*a*) Neutralize (to phenolphthalein) 5 ml of a 10% solution of hydroxylamine hydrochloride in methanol with a 10% solution of sodium hydroxide in the same solvent. Then add 10 ml more of the sodium hydroxide solution and filter. Prepare fresh before use. (*b*) A 0.3% solution of ferric chloride hexahydrate in a 3% v/v solution of 70% perchloric acid (s.g. 1.67) in ethanol.

Procedure. To 1.0 ml of sample solution in ethanol, add 0.50 ml of reagent *a* and let stand at 20 ± 2° for *T* min (see table below). Add 3.0 ml of reagent *b*, mix, let stand at 20 ± 2° for 15 min and read at the wavelength of maximum absorption. Beer's law is obeyed up to at least $A = 0.8$.

	T, min	λ_{max}, nm
Benzyl benzoate	60	535
Butyl acetate	10	520
Diethyl malonate	30	515
Dimethyl phthalate	30	515
Ethyl acetate	10	520
Ethyl lactate	10	520
Monoethyl malonate	30	515
Butyrolactone	2	525
Santonin	5	525

The sample concentrations affording $A = 0.10$ are given in Table 1. Carboxylic acids do not react. Acid anhydrides and chloride react. Some amides can interfere, but react very weakly.

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SHORT COMMUNICATIONS

ESTIMATION OF PROPRANOLOL HYDROCHLORIDE

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Summary—A simple colorimetric method for the determination of propranolol hydrochloride has been worked out. The method involves nitration of the drug with a mixture of potassium nitrate and sulphuric acid. The coloured nitro-derivative has an absorption maxima at 360 nm. The working concentration range is 10–50 μg/ml. The method is applicable to analysis of pharmaceutical preparations.

Propranolol hydrochloride, a derivative of propanol, is used as an anti-adrenergic agent ("beta-blocker"). It has specific pharmacological activity on the heart by specific action on the beta-adrenergic receptors, and inhibition of the sympathetic stimulation of the heart. Its determination is described in various pharmacopoeias. The B.P.[1] describes a non-aqueous titrimetric method using perchloric acid as titrant and 1-naphtholbenzoin solution as indicator. Few methods[2–5] are available for the determination of propranolol hydrochloride. The present investigation deals with a spectrophotometric method based on nitration of the naphthalene ring with potassium nitrate and sulphuric acid, and measurement of the absorbance of the product at 360 nm. Any nitrous acid is removed with urea. The method has been successfully applied to pharmaceutical preparations and gives satisfactory results.

EXPERIMENTAL

Reagents

Standard solution. Weigh accurately 50 mg of the drug, dissolve it in distilled water, add 1 ml of 1*M* hydrochloric acid and make up to volume in a 50-ml standard flask with distilled water. The solution should be freshly prepared.

Blank stock solution. Dilute 1 ml of 1*M* hydrochloric acid to 50 ml with distilled water.

Preparation of calibration curve

Prepare a series of solutions of the drug with concentrations ranging from 100 to 500 μg/ml in distilled water as follows. Transfer x ml (x = 1, 2, 3, 4, 5) of the standard and $(5 - x)$ ml of blank stock solution to a series of 10-ml standard flasks and dilute to volume with distilled water. For each solution pipette 1 ml into a 10-ml standard flask, then add 1.5 ml of 2% potassium nitrate solution and 0.5 ml of concentrated sulphuric acid. After 10 min add 2 ml of 5% urea solution to remove any nitrous acid formed, leave for 5 min, dilute to volume with distilled water and measure the absorbance at 360 nm against a blank made by diluting 0.5 ml of blank stock solution to 10 ml. Plot the absorbances against concentration. The Beer–Lambert law is obeyed over the concentration range 10–50 μg/ml.

Analysis of pharmaceutical products

Bulk powder. Proceed as for the calibration curve, with dilution of 2 or 3 ml of the 50-mg/ml sample solution to 10 ml.

Tablets. Powder 20 tablets in a glass mortar. Accurately weigh a portion of the powder equivalent to about 50 mg of propranolol hydrochloride and extract with two 15-ml portions of distilled water, filtering into a dry flask through a porosity-4 sintered-glass filter and washing with three 5-ml portions of distilled water. Add 1 ml of 1*M* hydrochloric acid to the filtrate and dilute it accurately to 50 ml with distilled water. Treat this solution as for the bulk powder solution.

Interferences

Drugs such as diazepam and bendrofluazide were tested for interference in the method, as they may be administered in combination with propranolol hydrochloride. As such combinations are not produced commercially, mixtures were prepared in the laboratory. These drugs do not interfere, and in any case their solubility in water is very low.

Recovery

Pharmaceutical preparations and mixtures were analysed by the proposed method with and without addition of known amounts of propranolol hydrochloride.

RESULTS AND DISCUSSION

Propranolol hydrochloride can be nitrated easily with nitric acid (formed *in situ*) to give a yellow derivative with an absorption maximum at 360 nm. The reaction is quite fast at room temperature, and use of higher temperatures does not appear to affect the intensity of the colour, and offers no advantage.

The colour intensity is affected by the concentrations of potassium nitrate and sulphuric acid used. It was found that 1.5 ml of 2% potassium nitrate solution and 0.5 ml of concentrated sulphuric acid are the minimum volumes needed to give maximum absorbance, and larger amounts do not increase the absorbance.

Any nitrous acid formed *in situ* is removed by adding urea, 2 ml of 5% solution being more than

sufficient. The time between addition of the urea solution and dilution to volume should be at least 5 min (with shaking). The colour is stable for more than 120 min. The time required for a complete determination is about 40 min. A concentration of 40 μg/ml gives an absorbance of 0.81 at 360 nm (1-cm cell).

The method was found to give agreement within 1% with the B.P. results for propranolol hydrochloride preparations, and gave 99–100% recovery for known amounts added to diazepam or bendrofluazide or to commercial tablets containing the drug, showing that the method is suitable for control purposes. It is applicable to both isomers. Because the instruments used could be read to only ±0.01 absorbance unit, the results were rounded off to two significant figures.

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DETERMINATION OF NITRATE AND NITRITE WITH ASCORBIC ACID

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Summary—A direct titrimetric procedure has been developed for the determination of nitrate and nitrite with ascorbic acid in 9–12*M* phosphoric acid medium. Ferroin, *N*-phenylanthranilic acid, barium diphenylamine sulphonate and diphenylbenzidene can be used as the indicator. The method has also been applied for the assay of nitrate in fertilizers.

A literature survey reveals no determination of nitrate with ascorbic acid, and although nitrite has been titrated potentiometrically[1] and indirectly[2] with ascorbic acid, no titration with a visual end-point has been reported. We have therefore undertaken the study of the oxidation of ascorbic acid with nitrate and nitrate and the present paper describes a direct titrimetric determination of nitrate and nitrite with ascorbic acid, with a visual end-point.

EXPERIMENTAL

Reagents

Sodium nitrate solution, 0.1*N*. Prepared from analytical grade reagent and standardized.[3]

Sodium nitrite solution, 0.1*N*. Prepared from analytical grade reagent and standardized.[4]

Ascorbic acid solution, 0.1*N*. Prepared from guaranteed grade reagent, stabilized with formic acid and EDTA, and standardized.[5]

Manganese(II) sulphate solution, 5%.

Solutions of ferroin (0.025*M*), *N*-phenylanthranilic acid (NPA) (0.1%), barium diphenylamine sulphonate (BDAS) (0.1%) and diphenylbenzidine (DB) (0.1%) were prepared in the usual way.

Analytical grade reagents were used whenever possible.

Procedures

Determination of nitrate. A sample of nitrate solution is transferred to a titration vessel and treated with enough orthophosphoric acid to give 10*M* concentration of the acid at the end-point. To this solution are added 1 ml of manganous sulphate solution and 0.04 ml of ferroin or 0.2 ml of any of the other indicators, followed by suitable dilution with distilled water. The mixture is titrated with a standard solution of ascorbic acid to a sharp colour change from pale blue to orange red (ferroin), reddish pink to yellow (NPA), or bluish violet to light yellow (BDAS or DB).

Determination of nitrite. A known volume of standard ascorbic acid solution is taken in a titration vessel and treated with enough orthophosphoric acid to give 10*M* concentration of the acid at the end-point. To this is added 0.04 ml of ferroin or 0.2 ml of BDAS, followed by suitable dilution with distilled water. The mixture is then titrated with the nitrite solution, with the tip of the burette under the surface of the well-stirred solution, to a sharp colour change from orange red to pale blue (ferroin) or from yellow to brown (BDAS).

Determination of nitrate in fertilizers

Dissolve 1–2 g of sample (accurately weighed) in distilled water and dilute to give a nitrate concentration of 3–4 mg/ml in a known volume of solution, and take a 5-ml aliquot. Alternatively, dissolve a suitable weight of sample in the minimum quantity of distilled water. Add 1 ml of the manganese(II) solution and enough orthophosphoric acid to give a concentration of 10*M* at the end-point, followed by 0.04 ml of ferroin or 0.2 ml of any of the other indicators, and titrate as already described.

RESULTS AND DISCUSSION

In preliminary experiments it was observed that the titration of nitrate or nitrite with ascorbic acid in hydrochloric or sulphuric acid media to a visual end-point is not feasible because the reduction with ascorbic acid is slow, and the indicator reaction is also slow (or even non-existent). The titration is found to be possible in 9–12*M* phosphoric acid medium but in the case of nitrate a catalyst is needed. The catalysts commonly used for redox reactions are molybdate, osmic acid, manganese(II) *etc*. We found that manganese(II) at a minimum final concentration of $0.4 \times 10^{-3}M$ (and added before the indicator) is suitable. However, it is advisable to add the first 3 or 4 drops of ascorbic acid slowly (during 10–15 sec). The mechanism of the catalysis may be that Mn(II) is oxidized to Mn(III) by nitrate, followed by reduction back to Mn(II) by ascorbic acid. A large excess of manganese(II) has no adverse effect on the course of the reaction.

The reduction of ferroin by ascorbic acid is fairly fast whereas that of the oxidized forms of NPA, BDAS or DB is relatively slow. With the last three indicators quantitative results are obtained if the titrant is added slowly when the evolution of nitric oxide begins. Below 9*M* phosphoric acid concentration, manganese(II) is no longer effective as a catalyst.

If nitrite is titrated with ascorbic acid, low values are obtained because of loss of gaseous products from the acidified nitrite.[4] Hence a reverse titration is used, with the tip of the burette kept under the surface of

Table 1. Determination of nitrate and nitrite with ascorbic acid

	Taken, *mg*	Mean found, *mg* Ferroin	NPA	BDAS	DB
Nitrite	9.57	9.57	—	9.57	—
		(0.01)	—	(0.01)	
	23.92	23.97	—	23.90	
		(0.02)		(0.03)	
	62.13	62.2_3	—	62.2_3	
		(0.04)		(0.05)	
Nitrate	4.097	4.10	4.11	4.11	4.11
		(0.006)	(0.008)	(0.008)	(0.009)
	10.26	10.27	10.27	10.27	10.27
		(0.02)	(0.03)	(0.03)	(0.03)
	26.64	26.66	26.67	26.67	26.67
		(0.03)	(0.02)	(0.03)	(0.03)

Values in parantheses are standard deviations (5 variates).

the solution. Variation of the phosphoric acid concentration shows that a concentration below 9*M* is not suitable, because oxidation of the indicators is slow, although the oxidation of ascorbic acid is fast even at 7*M* phosphoric acid concentration. In 9–12*M* phosphoric acid medium nitrite oxidizes the indicators instantaneously. Ferroin and BDAS function satisfactorily, but not DB. NPA is destroyed and gives no colour change at all.

In both titrations the reduction product is nitric oxide and the oxidation product is dehydroascrobic acid. The indicator colour changes are sharp and stable. With nitrite the indicator correction is negligible and with nitrate it is zero. Results are presented in Table 1. Results for nitrate in fertilizer were the same ($\pm 0.1\%$) as those obtained by Leithe's method.[3]

Interferences

Ce(IV), V(V), Cr(VI) and higher oxidation states of manganese interfere. Fe(III), U(VI), Mo(VI), Al(III), fluoride, sulphate and chloride do not interfere.

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DETERMINATION OF LANTHANIDES WITH *m*-TOLUIDINYLOXAMIC ACID

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Summary—*m*-Toluidinyloxamic acid is shown to be about as efficient as the *para* isomer as a gravimetric reagent for La, Pr and Nd. Conditions have been established for the determination of these lanthanides in presence of a number of metal ions.

We have already described the synthesis of *p*-toluidinyloxamic acid and its utility for the gravimetric determination of some lanthanides.[1] Here we report the synthesis and use of its *meta* isomer.

EXPERIMENTAL

Synthesis of m-*toluidinyloxamic acid*

Heat an equimolar mixture of freshly distilled *m*-toluidine and anhydrous oxalic acid (heat the dihydrate at 100° overnight) on an oil-bath at 120–130° for 1 hr. Dissolve the solid product in boiling water, filter hot and cool. Collect the crystalline *m*-toluidinyl oxalate, wash it repeatedly with cold water, then boil it with an appropriate amount of dilute sulphuric acid, cool and extract with ether. Evaporate the ether on a water-bath to crystallize the *m*-toluidinyloxamic acid, and further purify it by repeated recrystallization from water.

The product (m.p. 140 ± 1°) is insoluble in benzene, chloroform and carbon tetrachloride, moderately soluble in water, and fairly soluble in ammonia, alcohol, ether and acetone. It is used as a 1% solution in alcohol for gravimetric work.

The stoichiometry of the chelates was established by potentiometric titration in the same way as for the *p*-isomer compounds,[1] the results being analogous in all respects.

The chelates prepared from pure solutions of the lanthanides were analysed, and the results confirmed the 1:3 metal:ligand composition found potentiometrically. The structure proposed is the same as for the *p*-isomer products and further evidence for it is provided by the changes in the infrared spectrum when complexation takes place.

The optimum pH range for precipitation of the lanthanides is 2–7, and there is a slight negative error (up to −0.5%) for precipitation of 17–90 mg of metal ion from a pure solution of the lanthanide. Excess of reagent can be washed out with 20% aqueous ethanol.

The *o*-isomer has also been prepared and tested; it gives lanthanide compounds which are moderately soluble.

Interferences

The complexes of the ligand with some transition[2] and non-transition metal ions are soluble in aqueous and non-aqueous media. Alkali metals, Mg(II), V(V), As(III), Se(VI), Mo(VI) and W(VI) do not interfere. Cu(II), Pb(II), Cd(II), Fe(III) and Co(II) can be masked with thiosulphate, and U(VI), Zn(II) and Ni(II) with thiocyanate. Ag(I) can be masked by precipitation as silver chloride which is then dissolved in ammonia to yield the diammine complex. Hg(II) is appreciably masked by addition of excess of potassium iodide.

High concentrations of thiocyanate, thiosulphate and iodide do not affect the quantitative separation of the lanthanides at room temperature, but Cu(II), Fe(III), Co(II), Cd(II) and Pb(II), when masked with an excess of thiosulphate at higher temperature (>60°), may be co-precipitated as sulphides. Similarly, a high concentration of ammonia precipitates the lanthanides as hydroxides at pH >8. In view of this, the temperature should not exceed 50° and the pH should be <7 when these two masking agents are used.

Procedure

Add appropriate amounts of masking agents to the sample solution containing the nitrates of the metals present, dilute to 100 ml, and precipitate the lanthanide with 1% alcoholic solution of the precipitant. Filter off, wash with water and alcohol, and dry at 100–110° to constant weight.

The masking agents used (20 ml of 1% solution of each) were $CN^-/SCN^-/S_2O_3^{2-}$ for Cu(II), Fe(III), Co(II), Cd(II); CN^-/SCN^- for Ni(II), Zn(II); CN^-/NH_3 for Ag(I); CN^-/I^- for Hg(II); SCN^-/H_2O_2 for U(VI); $S_2O_3^{2-}$ for Pb(II); CN^- for Mn(II), Tl(I); H_2O_2 for Ti(IV), Zr(IV). Positive errors of up to 0.4% were obtained for mixtures of 35–90 mg of a lanthanide (La, Pr, Nd) and up to 60 mg of an individual metal ion, so presumably there is a small compensatory amount of co-precipitation.

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ESTIMATION OF INDIVIDUAL THIO-SALTS AND SULPHATE IN FLOTATION MILL SOLUTIONS

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Summary—This paper presents methods found to be suitable for the determination of thio-salts and sulphate in flotation mill solutions. Thiosulphate, tetrathionate and trithionate are estimated spectrophotometrically after cyanolysis. A modified iodimetric procedure is used for sulphite and a titrimetric method for direct determination of sulphate. The observation that dithionate is not oxidized by hydrogen peroxide but is oxidized to sulphate by potassium chlorate–nitric acid mixture is the basis for estimation of dithionate.

The presence of thiosulphate and polythionates ($S_nO_6^{2-}$ where $n = 2, 3, 4 \ldots .$) in flotation mill effluents is undesirable because they eventually produce acidity in water streams. To study the generation of thio-salts during the processing of a pyritic Zn–Pb–Cu ore, it was necessary to find suitable analytical methods for individual thio-salts and sulphate, and those finally adopted for this purpose are given in this paper.

The photometric determination of thiosulphate, tetrathionate and trithionate in a mixture is adapted from the procedures of Mizoguchi and Okabe.[1] It is based on differential cyanolysis and photometric measurement of the thiocyanate formed. A modified iodimetric method is used for sulphite. A direct titrimetric method for sulphate has been developed because the gravimetric procedure gives high results in the presence of thio-salts. The procedure for dithionate is based on differential oxidation to sulphate.

Most mill solutions are neutral or basic, having been treated with sodium carbonate or lime. These solutions contain only traces of heavy metals, which do not interfere with the analysis. No separation is necessary other than the removal of solids. However, where SO_2 has been added in large quantities, the solutions are acid (pH ~ 5) and contain much ferrous ion which has to be removed. The sample preparation procedure developed precipitates iron or calcium (which interferes in the determination of sulphate) with a sodium bicarbonate–sodium carbonate buffer solution (pH 9.8).

EXPERIMENTAL

Reagents

Ferric nitrate–perchloric acid solution. This contains 606 g of $Fe(NO_3)_3 . 9H_2O$ and 372 ml of 70% perchloric acid in 1 litre.

Buffer solution A, pH 4.5. Contains 77 g of ammonium acetate, and 100 ml of glacial acetic acid in 1 litre.

Buffer solution B, pH 4.5. Contains 500 g of sodium acetate trihydrate and 200 ml of glacial acetic acid in 1 litre.

Buffer solution, pH 9.8. Contains 13.0 g of sodium bicarbonate and 16.0 g of sodium carbonate in 1 litre.

Barium chloride solution 0.02*M*. Contains 4.886 g of barium chloride dihydrate and a few drops of 1*M* hydrochloric acid in 1 litre.

Methanolic formaldehyde solution. To 50 ml of water add 10 ml of methanol and 40 ml of formaldehyde solution (37%).

EBT indicator solution. Dissolve 0.20 g of Eriochrome Black T and 0.02 g of Methyl Red in 100 ml of methanol.

Buffer solution, pH 10.0. Contains 70 g of ammonium chloride and 570 ml of concentrated ammonia solution in 1 litre.

Sample preparation

Allow some time for the pulp solids in a bulk sample to settle, then decant the liquid into a sample bottle. Pipette 20 ml of the decanted sample into a 40-ml centrifuge tube. Cool to ~17°, and add a measured volume of $NaHCO_3$–Na_2CO_3 buffer solution (pH 9.8) sufficient to precipitate the iron. Centrifuge for 5 min and take known fractions of the clear supernatant solution for analysis.

Spectrophotometric determination of thiosulphate

To a 50-ml standard volumetric flask add 10 ml of water followed by a known volume of sample (0.25–2.0 ml). Add 10 ml of acetate buffer *A*, 1 ml of 0.5*M* potassium cyanide, and 1.5 ml of 0.2*M* cupric chloride, in that order, mixing after each addition. Finally, add 5 ml of ferric nitrate–perchloric acid solution with continuous mixing. Make up to volume and measure the absorbance at 460 nm against a reagent blank within 30 min of colour development.

For the calibration graph apply the procedure to 0.25–2.0 ml volumes of standard 0.004*M* thiosulphate (1 ml ≡ 4 μmole of $S_2O_3^{2-}$).

Determination of tetrathionate

To a 50-ml standard flask add 15 ml of water and the same volume of sample as taken for the thiosulphate determination. Add 3 drops of thymolphathalein indicator (0.1% solution in ethanol) and 1*M* ammonia solution until the solution is blue (if necessary, first decolorize with a drop of 0.5*M* sulphuric acid). Add 10 ml of acetone, cool to ~17° and after 20 min add 1.5 ml of 0.2*M* cupric chloride and continue as for thiosulphate. Subtract the thiosulphate result from the total thiosulphate obtained from $S_2O_3^{2-}$ + $S_4O_6^{2-}$ and divide by 2 to obtain the tetrathionate content.

Determination of trithionate

To a 50-ml standard flask, add 15 ml of water and the

same volume of sample as taken for the thiosulphate determination. Add 3 drops of thymolphthalein indicator, 2 ml of 0.5*M* potassium cyanide and, if necessary, 1*M* ammonia until the solution is blue. Keep the flask in hot water ($\geq 85°$) for 30 min, cool to room temperature, add 1.5 ml of 0.2*M* cupric chloride and continue as above for thiosulphate.

To calculate the trithionate content, subtract the total thiosulphate found in the tetrathionate determination from the total thiosulphate obtained from all three thio-salts.

Titrimetric determination of thiosulphate

To 5 ml of sample add 50 ml of water, 5 ml of formaldehyde solution (37% HCHO), 5 ml of buffer solution *B*, 5 ml of 20% potassium iodide solution, and starch indicator. Titrate with 0.004*N* iodine to a blue end-point (1 ml ≡ 4 μmole of $S_2O_3^{2-}$).

Determination of sulphite

To 20 ml of water add 5 ml of buffer solution *B*, 5 ml of 20% potassium iodide solution and 20.0 ml of 0.004*N* iodine. Introduce the sample drop by drop from a 5-ml graduated pipette into the iodine solution until only a faint yellow colour remains. Titrate the excess of iodine with 0.004*M* sodium thiosulphate. The sulphite content is obtained from the difference between the net iodine consumption in this titration and the iodine consumption in the titrimetric determination of thiosulphate (or the iodine equivalent to the thiosulphate found spectrophotometrically). 1 ml of 0.004*N* I_2 ≡ 2 μmole of SO_3^{2-}.

Determination of sulphate in presence of thio-salts

To 10.0 ml of sample and 10 ml of water add a small piece of red litmus paper and make basic to litmus with 10% sodium carbonate solution. Add 5 ml of the methanolic formaldehyde solution and let stand for 5 min (to allow the sulphate to react with formaldehyde). Add 1*M* hydrochloric acid until the litmus paper is red and then 0.10*N* iodine until the solution is slightly yellow. Add enough 0.004*M* thiosulphate to remove the yellow colour. Transfer to a 50-ml standard flask containing 10.0 ml of 0.02*M* barium chloride, make up to volume with water, shake well and allow the precipitate to settle for 30 min. Transfer 25 ml of the supernatant solution to a 250-ml conical beaker, add 25 ml of water, 3.0 ml of 0.01*M* magnesium chloride, 10 ml of ammonia buffer (pH 10.0) and 1 ml of EBT indicator and titrate rapidly with 0.01*M* EDTA to a blue end-point. Add 2.0 ml of 0.01*M* magnesium chloride and titrate with EDTA to the blue end-point.

Repeat without the sample present, to obtain the volume of EDTA equivalent (~15 ml to 5.0 ml of the magnesium solution and 5.0 ml of the barium solution. Subtract the total volume of EDTA used for the sample solution (1 ml net consumption ≡ 2 mmole of SO_4^{2-} per liter on a 10-ml sample).

Estimation of total thio-salts

All thio-salts except dithionate are oxidized to sulphate by hydrogen peroxide in slightly acid solution. The difference between the true sulphate content and the total sulphate after oxidation with peroxide gives an estimate of the thio-salt content.

Make a 25-ml sample slightly acidic with 1*M* hydrochloric acid and add 2 ml of hydrogen peroxide (30%). Heat the sample at about 80° for 15 min and then boil it gently for 10 min. Make the hot solution basic with 1*M* ammonia, add 5 drops of 1% cobaltous acetate solution and boil gently for a few minutes until foaming ceases. Allow the precipitate to settle and decant the hot supernatant solution into a 100-ml standard flask. Filter the remainder through an 11-cm Whatman No. 40 paper into the flask and wash with hot water. Cool the combined filtrate and washings to room teemperature and make up to volume with water. Make a 20-ml aliquot of the oxidized solution acidic with 1*M* hydrochloric acid and transfer it to a 50-ml standard flask containing 10.0 ml of 0.02*M* barium chloride. Continue as for determination of sulphate.

Estimation of dithionate

Dithionate can be oxidized to sulphate with potassium chlorate in nitric acid solution. The difference between the total sulphate found by this method and found by peroxide oxidation is an estimate of the dithionate content.

Make a 25-ml aliquot of sample slightly acid with 1*M* hydrochloric acid and add 2 ml of hydrogen peroxide (30%). Heat at about 80° for 15 min and then boil gently for 10 min. Add 0.3 g of potassium chlorate and 10 ml of concentrated nitric acid and evaporate on a hot-plate just to dryness (do not bake). To the residue add 25 ml of hot water, a piece of red litmus paper and sufficient 1*M* ammonia to turn the paper blue. Boil the solution for a few minutes and filter through an 11-cm Whatman No. 40 paper into 100-ml standard flask, washing with hot water. Cool the combined filtrate and washings to room temperature, make up to volume with water. Complete the estimation as for thio-salts.

RESULTS AND DISCUSSION

Sample preparation

The stability of the different thio-salt species with respect to pH has to be considered. Sulphite, thiosulphate and trithionate are relatively stable in basic solution. However, tetrathionate is decomposed by sulphite in neutral solution and by hydroxide in basic solution according to the equations:

$$S_4O_6^{2-} + SO_3^{2-} \rightarrow S_3O_6^{2-} + S_2O_3^{2-} \quad (1)$$

$$4S_4O_6^{2-} + 6OH^- \rightarrow 2S_3O_6^{2-} + 5S_2O_3^{2-} + 3H_2O \quad (2)$$

It was found, however, that at pH 10.0 and room temperature, the rate of reaction with hydroxide [equation (2)] is very slow. From samples containing iron and tetrathionate, iron can be removed by the addition of buffer solution (pH 9.8) without, in the short term, significant decomposition of tetrathionate, Table 1. Sample 3 (Table 1) was an aged mill solution, which contained some tetrathionate and much ferrous ion. This sample was spiked with 1.40 mmole of tetrathionate.

Table 1. The effect of addition of $NaHCO_3$–Na_2CO_3 buffer solution (pH 9.8) on the determination of tetrathionate (all solutions at room temperature ~22°C)

Sample		Found, *mmole*		
		$S_2O_3^{2-}$	$S_4O_6^{2-}$	$S_3O_6^{2-}$
1	Without buffer	0.24	3.89	0.22
1	Without buffer	0.24	3.89	0.44
1	Buffer added	0.29	3.86	0.80
1	Buffer added	0.29	3.86	0.67
2	Without buffer	0	5.11	0
2	Buffer added	0	5.07	0.04
3	Mill sample + buffer	0	1.11	0
3	Mill sample + buffer	0	1.08	0
3*	Mill sample + buffer	0	2.47	0
3*	Mill sample + buffer	0	2.42	0

* Mill sample containing ferrous ion was spiked with 1.40 mmole of tetrathionate.

Table 2. Replicate determinations of 3.04 mmole of thiosulphate, 2.93 mmole of tetrathionate and 3.02 mmole of trithionate in a mixture

Found, *mmole*		
$S_2O_3^{2-}$	$S_4O_6^{2-}$	$S_3O_6^{2-}$
3.17	2.94	3.15
3.17	2.97	2.92
3.17	2.92	3.16
3.17	2.97	3.21
3.17	2.92	3.37
3.11	3.03	3.21

Spectrophotometric determination of thiosulphate, tetrathionate and trisulphate

A linear graph was obtained for the thiosulphate range 1–20 μmole/50 ml. Separate blanks were prepared for each procedure. The precision was found by making replicate determinations of thiosulphate, tetrathionate and trithionate in a mixture of the three, Table 2. The results show that the precision for thiosulphate and tetrathionate is about the same (coefficient of variation, CV, ~ 1%) but the variability is higher for trithionate (CV = 4%) because of the cumulative error involved in its determination.

Titrimetric determination of thiosulphate and sulphite

In slightly acid solution, ions such as thiosulphate and the species obtained from sulphur dioxide in solution ($SO_2.H_2O$, HSO_3^-, SO_3^{2-}) are quantitatively oxidized by iodine, whereas polythionates are not affected. Sulphide is not usually found with thiosalts in mill solutions, because it reacts with polythionates. Formation of the formaldehyde–sulphite complex in slightly acid solution allows titration of only the thiosulphate with iodine. The spectrophotometric method for thiosulphate is preferred, however.

Estimation of sulphate and thio-salts

After precipitation of the sulphate, the excess of barium is titrated with standard EDTA solution, at pH 10.0, to an Eriochrome Black T end-point. Precipitation is done at room temperature because at a higher temperature high results are caused by decomposition of trithionate. Sulphite is complexed with formaldehyde and thiosulphate is oxidized to tetrathionate with iodine before the precipitation.

Interference due to ferrous ion and/or calcium, which are also complexed with EDTA, is prevented by removing them by the carbonate treatment described in the sample preparation procedure. A sample can be analysed without the addition of barium chloride, to determine the EDTA consumed by any magnesium in the mill solution.

Table 3. Effect of thio-salts and sulphite on the determination of ~10 mmole of sulphate

Thio-salt	Added, *mmole*	No. of determinations	Average SO_4^{2-} found, *mmole*	Range, *mmole*
—	—	6	9.95	9.80–10.08
$S_2O_3^{2-}$	20	5	10.00	9.98–10.02
$S_3O_6^{2-}$	20	4	9.60	9.40–9.80
$S_4O_6^{2-}$	20	5	9.96	9.48–10.38
SO_3^{2-}	20	3	10.27	10.00–10.60

Table 4. Comparison of oxidation methods for the oxidation of thiosulphate to sulphate ($S_2O_3^{2-}$ taken ~15 mmole)

Sulphate found, *mmole*	
H_2O_2	$KClO_3$–HNO_3
28.4	29.2
27.6	29.2
28.4	28.8
28.4	28.8

Table 5. Oxidation of dithionate to sulphate ($S_2O_6^{2-}$ taken 15 mmole)

Sulphate found, *mmole*	
H_2O_2	$KClO_3$–HNO_3
0	29.6
1.2	30.0
1.2	29.6
0.4	30.0

An analytical method was needed to ascertain whether dithionate was present in flotation mill solutions. It was observed that all thio-salts except dithionate were oxidized to sulphate by hydrogen peroxide and that all thio-salts (including dithionate) are oxidized to sulphate by potassium chlorate–nitric acid reagent. The difference in the total sulphate produced by the two oxidation reagents gives an estimate of the dithionate present.[2,3]

The effect of thio-salts on the determination of free sulphate present is shown in Table 3. The first row shows the precision of replicate determinations in the absence of thio-salts. The succeeding rows show the effect of the addition of 20 mmole of individual thio-salts or sulphite. The results show that the procedures used to suppress interference by the individual species are successful.

Table 4 shows a comparison of peroxide and chlorate–nitric acid oxidation in the determination of the sulphate produced from ~15 mmole of thiosulphate. A slightly lower average value is obtained by peroxide oxidation. Table 5 shows that dithionate is quantitatively oxidized only by potassium chlorate–nitric acid reagent; hydrogen peroxide effects only a minor and variable degree of oxidation.

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ADSORPTION BEHAVIOUR OF SOME LANTHANIDES ON ZIRCONIUM PHOSPHATE SILICATE (ZPS) IN MINERAL ACIDS

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Summary—The distribution coefficients of tervalent eruopium, terbium, thulium and scandium between zirconium phosphate silicate and mineral acids have been determined. The distribution coefficients were found to change from one element to another and to depend on the acid used and its concentration. Separation factors were calculated and a separation scheme for these elements was worked out.

Zirconium phosphate (ZP) is one of the most thoroughly investigated inorganic ion-exchangers.[1,2] Several zirconium phosphate derivatives have been prepared. Titanium phosphate silicate (TPS) has been prepared and used for separation of plutonium.[3] Zirconium titanium phosphate (ZTP) has been prepared and used for separation of rare earths and some other fission products from mineral acids.[4,5] Zirconium phosphate silicate (ZPS) has been used for the isolation of plutonium from rare earths in nitric acid medium[6,7] and for separation of zirconium, ruthenium, neptunium and berkelium.[8–10]

The present investigation is part of a wider study planned to investigate the possible separation of the lanthanides and actinides from each other on zirconium phosphate derivatives. In this part the adsorption behaviour of tervalent Eu, Tb and Tm (as representatives of the light, middle and heavy lanthanides) on ZPS in hydrochloric, nitric and sulphuric acid media was investigated. Scandium, which resembles the lanthanides in many respects, was also included in the investigation.

EXPERIMENTAL

Apparatus

An ECKO scintillation counter, type N 664 C, with a well-type NaI(Tl) crystal, connected to an ECKO automatic scaler, type N 610 B, was used for gross activity measurements of the radioactive isotopes.

A Telefunken type M_s Str. 1104/1 gamma-ray spectrometer connected to an M_s Z_z 831/2 NaI(Tl) detector was used for checking the isotope purity.

Materials

Unless otherwise stated, all chemicals used were of analytical grade; no further purification was done.

The radioactive isotopes ^{152,154}Eu, ^{160}Tb, ^{170}Tm and ^{46}Sc were used as tracers. They were prepared by irradiating samples of the appropriate target materials, Eu_2O_3, Tb_4O_7, Tm_2O_3 and Sc_2O_3 in the Egyptian ET-RR-1 reactor (neutron flux 1×10^{13} n.cm^{-2}.sec^{-1}) for 48 hr. After a sufficient cooling period, the targets were dissolved in the desired media. The gamma spectra of all the isotopes prepared showed that they were radiochemically pure.

Zirconium phosphate silicate was prepared according to the procedure given by Naumann.[11] The 50–100 mesh fractions were used in this study.

Determination of the distribution coefficients

The distribution coefficients, D, were determined at room temperature ($20 \pm 2°$) by the batch technique as detailed in a previous paper.[5]

Each D value reported is an average of three measurements, the deviation of the individual values from the average being about 10% for the highest D values.

RESULTS AND DISCUSSION

The uptake of Eu^{3+}, Tb^{3+}, Tm^{3+} and Sc^{3+} on zirconium phosphate silicate (ZPS) as a function of acid concentration is shown in Figs. 1–3. Generally, the distribution coefficients of the lanthanides (Eu^{3+}, Tb^{3+} and Tm^{3+}) are high at low acid concentrations and decrease with increase in acid concentrations. Therefore it may be deduced that exchange-adsorption is the ruling process, the lanthanides being exchanged for the protons in the resin. The flattening of the curves in the dilute acid range may be attributed to polymerization of the ions. At higher acid concentration, the deviation from mass-action law behaviour shown by some ions can be attributed to increase of cation–anion interaction in both aqueous and resin phases, together with possible invasion of the resin by the acid. The cation-anion interaction and the resin invasion may also explain the variation in behaviour of the distribution coefficients from one element to another and from one acid to another.

The adsorption behaviour of Sc^{3+} on ZPS differs according to the acid used. The distribution coefficient for Sc^{3+} generally increases with increasing acid concentration, passes through a maximum and then decreases. The scandium ion, being smaller, has a

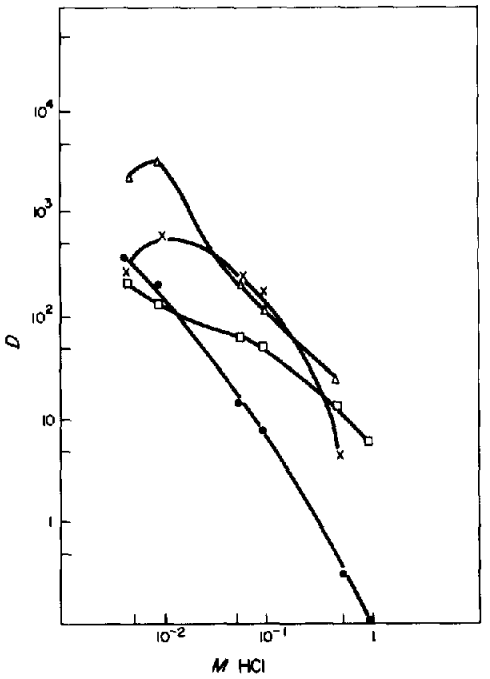

Fig. 1. Distribution of Eu, Tb, Tm and Sc between ZPS and hydrochloric acid ● Eu^{3+}, △ Tb^{3+}, □ Tm^{3+}, × Sc^{3+}.

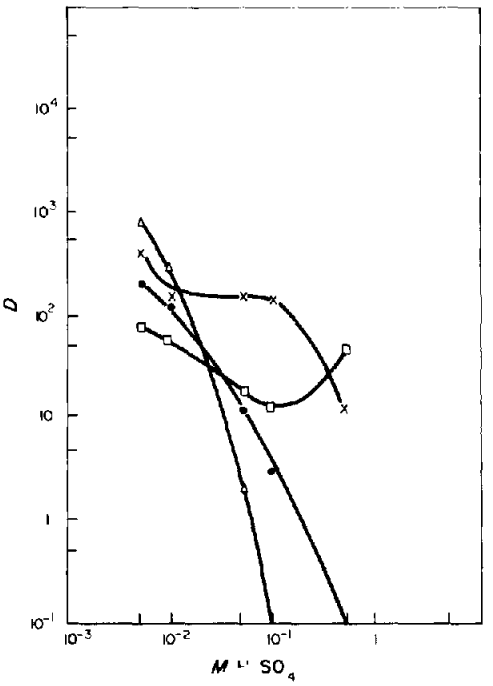

Fig. 3. Distribution of Eu, Tb, Tm and Sc between ZPS and sulphuric acid ● Eu^{3+}, △ Tb^{3+}, □ Tm^{3+}, × Sc^{3+}.

much greater tendency to hydrolysis than the lanthanide ions. Therefore, at low acid concentration the scandium ions are hydrolysed and the increase of acid concentration will decrease the hydrolysis, increase the percentage of free ions and consequently increase the adsorption of scandium on ZPS.

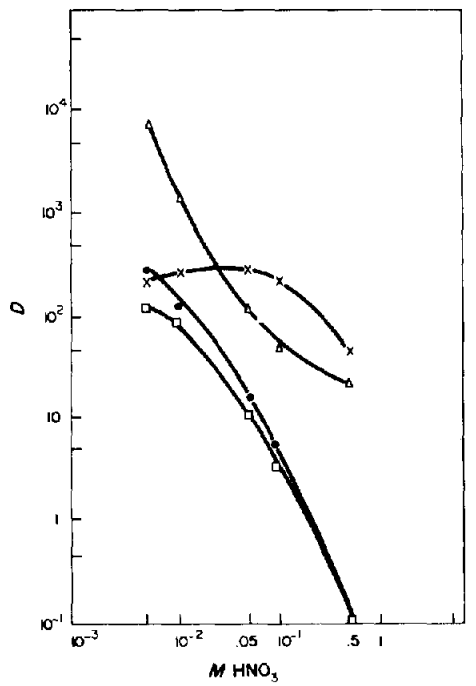

Fig. 2. Distribution of Eu, Tb, Tm and Sc between ZPS and nitric acid ● Eu^{3+}, △ Tb^{3+}, □ Tm^{3+}, × Sc^{3+}.

The difference in the adsorption behaviour of Sc^{3+} and the lanthanides on ZPS from different acid solutions may facilitate the separation of Sc^{3+} from the lanthanides. The separation factors (α_{Ln}^{Sc}) between Sc^{3+} and the lanthanides investigated are calculated and collected in Table 1. Sc^{3+} is strongly retained on ZPS from 0.1*M* sulphuric acid, whereas the three lanthanides are much less adsorbed. Many schemes for separating Sc^{3+} from the investigated lanthanides can be deduced from Table 1.

The separation of the lanthanides from each other with zirconium phosphate silicate may be achieved from different acid solutions. The separation factors were calculated from the distribution coefficients and are collected in Table 2. It is clear that they depend on the acid used and its concentration. Another useful feature seen from Table 2 is the variation of the separation factors from very high to very low values. This large variation illustrates the versatility of the ZPS. An element which is eluted first from one acid medium can be eluted last from another acid medium. Therefore, several separation schemes can be deduced from Table 2. An easy separation procedure is adsorption of the lanthanides at low acid concentration, then elution with 0.5*M* hydrochloric acid strips Eu^{3+}, elution with 0.5*M* nitric acid removes Tm^{3+} and finally Tb^{3+} can be collected by elution with 0.5*M* sulphuric acid. This is the simplest separation but many other schemes can be deduced from Table 2.

It can be concluded that although the distribution

Table 1. Separation factors between scandium and lanthanides on ZPS from different acids

Acid	Concentration, M	Separation factors α_{Eu}^{Sc}	α_{Tb}^{Sc}	α_{Tm}^{Sc}
HCl	0.005	0.7	0.1	1.1
	0.010	3.3	0.2	4.2
	0.050	13.3	0.7	3.3
	0.100	30.2	1.6	4.1
	0.500	13.3	0.2	0.3
HNO_3	0.005	0.7	0.0	1.7
	0.010	1.8	0.2	2.8
	0.050	20.0	2.7	30.0
	0.100	42.3	4.1	73.3
	0.500	400	1.8	400
H_2SO_4	0.005	2.0	0.5	5.3
	0.010	1.5	0.6	3.3
	0.050	11.7	82.4	8.2
	0.100	35.0	1400	11.7
	0.500	120	120	0.3

Table 2. Separation factors of lanthanides on ZPS from different acids

Acid	Concentration, M	Separation factors α_{Eu}^{Tb}	α_{Eu}^{Tm}	α_{Tm}^{Tb}
HCl	0.005	7.1	0.6	11.4
	0.010	21.3	0.8	26.7
	0.050	20.0	4.0	5.0
	0.100	18.3	7.3	2.5
	0.500	73.3	40.0	2.5
HNO_3	0.005	25.0	0.4	62.5
	0.010	13.7	1.0	14.5
	0.050	7.3	0.7	11.0
	0.100	10.4	0.6	18.0
	0.500	220	1.0	220
H_2SO_4	0.005	4.3	0.4	11.3
	0.010	2.3	0.5	5.0
	0.050	0.1	1.4	0.1
	0.100	0.0	3.0	0.0
	0.500	1.0	430	0.0

coefficients of the lanthanides on ZPS are smaller than on ZTP or ZP,[5] the separation factors are sufficiently high. Therefore, a good mutual separation of the lanthanides may be achieved on zirconium phosphate silicate. The practical application of the different separation schemes is in progress in our laboratory.

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POTENTIOMETRIC DETERMINATION OF D(+)GLUCOSE, D(+)MANNOSE OR D(−)FRUCTOSE IN A MIXTURE OF HEXOSES AND PENTOSES, BY USING *STREPTOCOCCUS MUTANS* FERMENTATION

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Summary—A potentiometric sensor has been developed, based on selective fermentation of carbohydrates by *Streptococcus mutans*. This combination of bacterial action and a glass electrode responds to β-D(+)glucose, D(+)mannose and β-D(−)fructose over a narrow concentration range, with a response time of 4 min, and has negligible response to other hexoses and pentoses.

Determination of carbohydrates in the presence of other reducing sugars is a frequent laboratory problem. The normal procedure is to separate the carbohydrates by partition chromatography[1,2] and then determine the individual sugars.[3–8] However, fermentation methods[3,9] for the determination of D-glucose in a mixture of sugars or of D(+)glucose, D(+)mannose, D(+)galactose and D(−)fructose in a mixture of hexoses and pentoses, are available.[10] The enzyme glucose dehydrogenase can be used for manometric estimations of glucose in the presence of carbohydrates and proteins.[11]

We now describe the development and use of a selective carbohydrate-sensing sensor in which living micro-organisms, such as *S. mutans*, are employed in suspension. The sensor gives a selective response to β-D(+)glucose, D(+)mannose and β-D(−)fructose, but negligible response to other hexoses and pentoses. The response is based on the measurement of hydronium ions produced during the bacterial fermentation of certain carbohydrates. The type of processes involved in the fermentation of glucose, fructose or mannose, resulting in the formation of lactic acid,[12] is shown in Scheme 1, where ADP stands for adenosine diphosphate, ATP for adenosine triphosphate and IPS for intracellular polysaccharides.

It has been shown that lactic acid is the main product during the fermentation of carbohydrates.[13,14] The acids produced in this manner cause a change in pH which is sensed by the glass electrode and a potentiometric steady-state response, proportional to the sugar concentration, is obtained.

EXPERIMENTAL

Apparatus

All potentiometric measurements were taken on a Corning model 12 pH-meter connected to a Heath-Schlumberger strip-chart recorder, model SR-225-B. Measurements were made with a Corning pH combination electrode (cat. no. 476216), in a cell controlled at 37.0 ± 0.2°. Cells were grown in a Torbal model AJ-3 jar.

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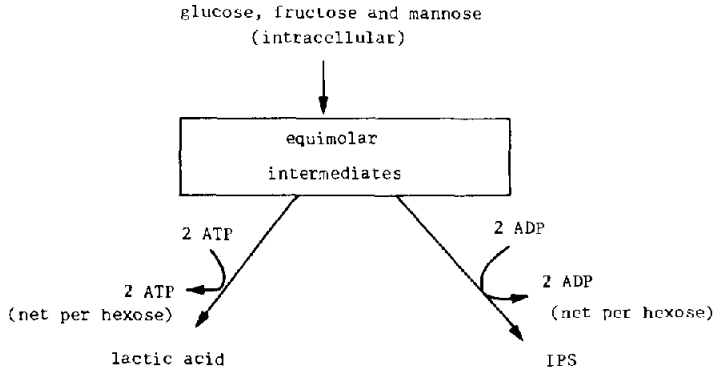

Scheme 1

Reagents

Streptococcus mutans No. 25175 was obtained from the American Type Culture Collection, Rockville, Maryland. Todd–Hewitt broth was purchased from Difco Lab., Michigan. The carbohydrates β-D(+)glucose, D(+)galactose, β-D-allose, L-mannose, D(+)mannose, D-idose, D-gulose, D-talose, D-altrose, β-D(−)fructose, L(−)sorbose, D(+)tagatose, L(+)arabinose, D(−)arabinose, L(−)xylose, D(+)xylose, D-ribose, L(−)ribose, D-lyxose, L-lyxose, D-ribulose and D-xylulose in purest form available, were obtained from Sigma Chemicals Co. and were used without further purification. All other chemicals used were of analytical grade. Distilled water was used in all the experimental work.

Procedure

The *S. mutans* strain was maintained (at 4°) in Todd–Hewitt broth with excess of calcium carbonate added.[15] Transfer to fresh media was made every 2 weeks. For experimental purposes, the organisms were cultivated in a medium of the following composition: trypticase, 2%; NaCl, 0.2%; KH_2PO_4, 0.4%; Na_2HPO_4, 0.2%; K_2CO_3, 0.1%; $MgSO_4$, 0.012%; $MnSO_4$, 0.0015%; D(+)glucose, 0.2%. The D(+)glucose was autoclaved separately and added aseptically to complete the medium. The culture was incubated anaerobically at 37° for 18 hr in a Torbal jar filled with a mixture of 95% nitrogen and 5% carbon dioxide. Cocci were harvested by centrifugation (4500 rpm) at room temperature, washed 3 times with 0.004*M* potassium phosphate buffer (pH 6.8) prepared in 0.050*M* potassium chloride, and stored at 4° for 24 hr in the phosphate buffer solution.[15]

A fraction of the cells (~ 2 mg air-dry weight) was washed three times with Ringer's solution, consisting of 0.015*M* NaCl, $3.1 \times 10^{-4}M$ KCl and $2.2 \times 10^{-4}M$ $CaCl_2$, and before use the pH was set at 6.95 with dilute sodium hydroxide solution. The washed cocci were suspended in 1.50 ml of Ringer's solution (pH = 6.95) in a temperature-controlled cell sealed from the atmosphere, and were kept in suspension by magnetic stirring. The glass electrode was immersed in this solution and the head-space was flushed with nitrogen. Calibration curves were then constructed by adding successive portions of the standard carbohydrate solutions, which had been standardized by conventional methods.[6,8] The largest volume added was 120 μl and a volume-correction was used in the calculations. After the cells had been used, they were harvested, washed and stored in the potassium chloride–phosphate buffer solution at 4°. Eight separate calibration curves for each of D(+)glucose, D(+)mannose and β-D(−)fructose were constructed. The selective response of *S. mutans* to all three carbohydrates was tested by the use of more or less equal numbers of cells (~ 1.5 mg air-dry weight).

RESULTS AND DISCUSSION

Typical calibration curves are shown in Fig. 1 for the response of the same cell crop at different ages to β-D(+)glucose. Similar calibration curves (*i.e.*, within ±5 mV) were obtained with D(+)mannose or D(−)fructose. Freshly cultivated cell crops exhibited a response slope of 148 mV/decade (±5 mV) in the linear range from 4.6×10^{-5} to $2.5 \times 10^{-4}M$ glucose (Fig. 1a). By the fourth day the response slope had dropped slightly to 124 mV/decade (Fig. 1c), for the same cell crop. *S. mutans* has less than 4 mV/decade response to other hexoses and pentoses, which we consider negligible, but high concentrations ($> 10^{-3}M$) of D-xylulose and D(+)galactose will interfere. The linear response is over a narrower range than that of the plaque electrode,[10] but starts at a lower concentration, and the slope is higher by ~ 45 mV/decade. The reason for the higher response may be the inhibition effect of various elements on the plaque fermentation process[16–18] because the plaque cells are not cultures. The response slope is greater than the theoretical Nernstian value because the yield of lactic acid increases while that of the volatile acids and alcohols decreases with decreasing pH, during the fermentation process.[10] The effective lifetime of the *S. mutans* electrode is also about a day longer than that of the plaque electrode, which contains some fermen-

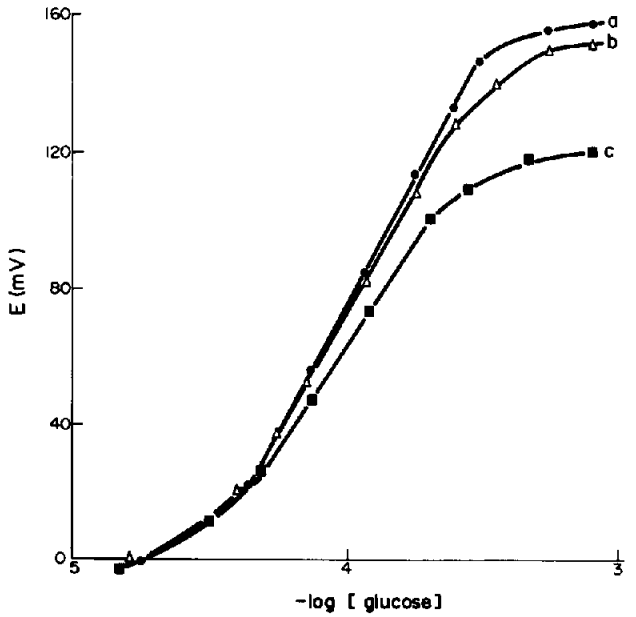

Fig. 1. Calibration curves for *S. mutans*–glass electrode combination for β-D(+)glucose in Ringer's solution on (*a*) the first day, (*b*) the fourth day and (*c*) the fifth day, at 37°C.

Table 1. Analysis of a known amount of (a) β-D(+)glucose, (b) D(+)mannose and (c) β-D(−)fructose in Ringer solution, in the presence of 1.7×10^{-4} M of each of the following: β-D-allose, L-mannose, D-idose, D-gulose, D-talose, D-altrose, L(−)sorbose, D(+)tagatose, L(+)arabinose, D(−)arabinose, L(−)xylose, D(+)xylose, D-ribose, L(−)ribose, D-lyxose, L-lyxose and D-ribulose. Five samples of each taken amount of each carbohydrate were analysed

Amount of carbohydrate taken (μg)	Mean absolute difference and SD	Mean percentage difference
(a) β-D(+)glucose		
20.21	1.11 (0.60)	−0.23
36.39	1.91 (0.56)	−0.50
(b) D(+)mannose		
18.75	0.96 (0.53)	−0.41
37.82	1.79 (0.49)	−0.11
(c) β-D(−)fructose		
20.61	1.20 (0.43)	−0.66
35.90	1.56 (0.59)	−0.23

tation species which become deactivated faster[19,20] than *S. mutans*.

The ideal amount of *S. mutans* cells to be used in 1.50 ml of solution is 1.0–2.0 mg (air-dry weight). Smaller amounts result in a decrease of the linear range, showing there is an optimum fermentation limit for each amount of cells, but larger amounts make no difference.

The time required for the potential reading to come within 2 mV of the steady-state value increased from 4 min to 6 as the carbohydrate concentration increased from 4.0×10^{-5}M to 1.0×10^{-3}. It also increased as the cells became older, showing a drop in the cell activity, possibly on exposure to various media.[19] The longer response time (20 min) of the plaque electrode[10] could have been due to the decrease of the cell contact area while the cells were being slightly squeezed between the membrane and the glass membrane of the electrode. The same long response time was observed if *S. mutans* was similarly held on the glass electrode.

Table 1 shows little or no interactions between the different carbohydrates during the determination of β-D(+)glucose or D(+)mannose or β-D(−)fructose.

As expected, *S. mutans* is slightly more selective than plaque for carbohydrate fermentation (*S. mutans* is a minor plaque component of which contains a number of other species[20]).

It is clear that the use of *S. mutans* offers some advantages over the use of plaque for the determination of the three carbohydrates in a mixture of their isomers and pentoses.

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DETECTION AND DETERMINATION OF CERTAIN UREAS WITH *o*-PHTHALALDEHYDE AND *N*-(1-NAPHTHYL)-*N'*-DIETHYLETHYLENEDIAMINE

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Summary—*o*-Phthalaldehyde and *N*-(1-naphthyl)-*N'*-diethylethylenediamine give red or orange colours with urea and certain substituted ureas in dilute hydrochloric acid at 37°. The reaction has good sensitivity and the colours are fairly stable. An individual urea can be detected at a concentration of *ca.* 0.05–0.2 μg/drop, and determined in the range of *ca.* 0.01–0.2 μmole.

Several colour tests are available for the detection of urea-type compounds in spot-tests, and paper and thin-layer chromatography,[1–10] but only a few are used for determination of the compounds,[11] probably because of lack of sensitivity and selectivity. Urea-type compounds are now usually determined by a thermal detection method,[12] gas–liquid chromatography[13–17] and a ^{14}C-labelling technique,[18] but a sensitive colour reaction could still be useful for some laboratories, because these compounds constitute a widespread group of pharmaceutical and agricultural chemicals, and are of biochemical interest. It could also be important in functional group analysis. Jung *et al.*[19] have reported a new colorimetric method for determination of urea with *o*-phthalaldehyde and *N*-(1-naphthyl)ethylenediamine in moderately concentrated sulphuric acid. Levinson[20] has modified the method by using more dilute sulphuric acid, but the colour developed was unstable, the absorbance of the reagent blank increased with time, and strictly controlled conditions were required to obtain reproducible results.

In the present method the reagent amine is replaced by *N*-(1-naphthyl)-*N'*-diethylethylenediamine, which in combination with *o*-phthalaldehyde reacts not only with urea but also with certain substituted ureas in dilute hydrochloric acid on incubation at 37°. The colours produced are fairly stable at room temperature, the blank is a faint yellow, and the reaction is especially suitable for the detection and determination of monosubstituted ureas.

EXPERIMENTAL

Reagents

N-(1-Naphthyl)-N'-diethylethylenediamine reagent, 5m*M*. Prepared by dissolving 0.854 g of the amine oxalate $C_{16}H_{22}N_2 \cdot C_2H_2O_4 \cdot \frac{1}{2}H_2O$ in 500 ml of water by gentle warming, followed by addition of 1.0 g of Brij 35. Brij 35 is a surfactant and prevents the precipitation of a certain substance (of unknown composition) which occurs in the colour reaction. Stable for at least 8 months when stored in a refrigerator.

o-Phthalaldehyde reagent, 20m*M*. Prepared by dissolving 1.341 g in 500 ml of 2*M* hydrochloric acid by gentle warming. Stable for at least 8 months at room temperature.

Table 1. Limits of detection and data for colorimetric determination

Ureas	Limits of detection, *μg/drop*	Absorption maxima, *nm*	Times to develop maximum intensity, *min*	Beer's law limits, *μmole/0.5 ml*
Urea	0.1	520 → 450	50	0.02–0.3
Methylurea	0.05	520	20	0.01–0.2
Ethylurea	0.05	520	20	0.01–0.2
Allylurea	0.05	520	20	0.01–0.2
Citrulline	0.05	520	20	0.01–0.2
1,1-Dimethylurea	0.2	520	120*	0.02–0.3
Benzylurea	0.1	520	10	0.01–0.2
Phenylurea	0.1	460	40	0.01–0.2
o-Tolylurea	0.1	450	50	0.01–0.2
m-Tolyurea	0.1	460	40	0.01–0.2
p-Tolylurea	0.1	460	50	0.01–0.2

* Time needed to reach plateau.

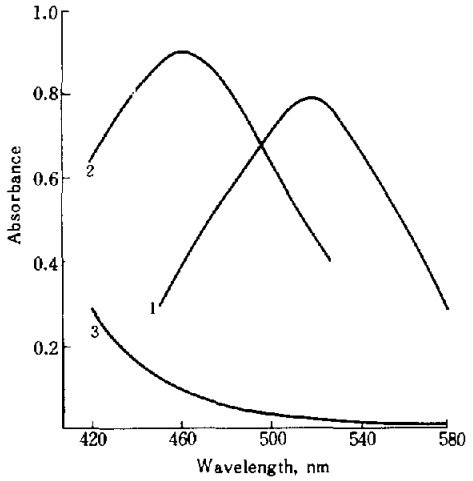

Fig. 1. Absorption spectra for sample and blank solutions: 0.2 μmole of (1) methylurea, (2) phenylurea; (3) reagent blank.

Spot-test

Take 1 drop of aqueous sample solution in a small test-tube and add 1 drop each of 5m*M* *N*-(1-naphthyl)-*N'*-diethylethylenediamine, 20m*M* *o*-phthalaldehyde and concentrated hydrochloric acid. A violet, red or orange colour appears instantly or after several minutes, according to the amount of the urea. The blank is a faint yellow.

Determination of ureas

Take 0.50 ml of aqueous sample containing the amount of a urea shown in Table 1, add successively 2.0 ml of 5m*M* *N*-(1-naphthyl)*N'*-diethylethylenediamine and 2.0 ml of 20m*M* *o*-phthalaldehyde, and incubate at 37° for the time shown in Table 1. Cool in running water, and read the absorbance against the reagent blank at the wavelength given in Table 1. The colour, once developed, is stable for at least 1 hr at room temperature. The calibration curve is linear for a given urea and passes through the origin.

RESULTS AND DISCUSSION

The absorption spectra obtained by applying the procedure to methylurea, phenylurea and a reagent blank are shown in Fig. 1. The rate of colour development and the absorbance are related to the reagent concentration and the reaction temperature. The temperature was first fixed at 37°, which is conveniently obtainable in any laboratory. The optimum conditions for the determination of substituted ureas were investigated with 0.2 μmole of methylurea (as model) measured at the absorption maximum of 520 nm.

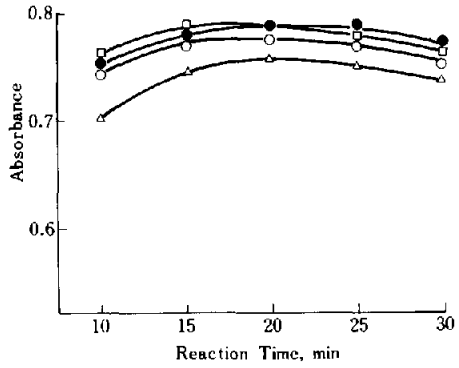

Fig. 3. Effect of the concentration of *N*-(1-naphthyl)-*N'*-diethylethylenediamine on the absorbance. △—3m*M*, ○—4m*M*, ●—5m*M*, □—6m*M*.

The effect of the concentration of the hydrochloric acid used to dissolve the *o*-phthalaldehyde is shown in Fig. 2. A higher absorbance is obtained with increasing concentration of the acid, but the absorbance of the blank also increases in the shorter wavelength region. The concentration of the acid was fixed at 2*M* because acid of that concentration is commercially available and is suitable for the determination of most ureas.

N-(1-Naphthyl)-*N'*-diethylethylenediamine was found to decompose gradually in hydrochloric acid in the presence of Brij 35, and hence was dissolved in water. The concentration of 5m*M* is enough to develop the colour in 15–20 min as shown in Fig. 3, and a large excess is used a substance of unknown nature tends to separate from the reaction mixture. *o*-Phthalaldehyde is needed in large excess (Fig. 4), so a 20m*M* solution is used.

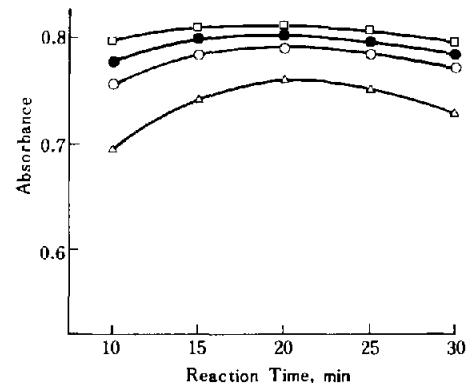

Fig. 2. Effect of the concentration of hydrochloric acid on the absorbance. △—1.5*M*, ○—2*M*, ●—2.5*M*, □—3*M*.

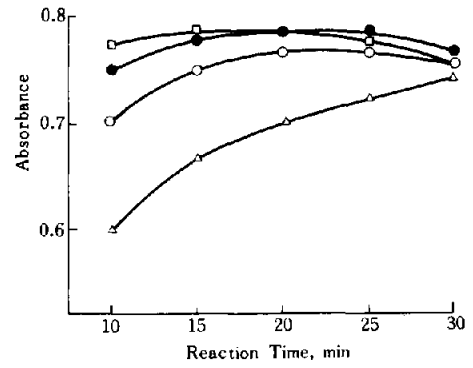

Fig. 4. Effect of the concentration of *o*-phthalaldehyde on the absorbance. △—10m*M*, ○—15m*M*, ●—20m*M*, □—25m*M*.

Table 2. Non-interfering substances

Compound	Tolerance level, *μmole*/0.5 *ml*	Compound	Tolerance level, *μmole*/0.5 *ml*
Glucose	20	L-Leucine	10
Fructose	20	L-Lysine	10
Lactose	20	L-Ornithine	10
Glucuronic acid	20	L-Phenylalanine	10
Ascorbic acid	10	L-Proline	10
Acetone	10	L-Tryptophan	1
Acetophenone	10	*N*-Methylaniline	5
Formaldehyde	1	Citric acid	10
Acetaldehyde	10	Pyruvic acid	10
Benzaldehyde	10	2-Ketoglutaric acid	10
Salicylaldehyde	10	Salicylic acid	10
Methylamine	10	Acetamide	2
Benzylamine	10	Creatine	5
L-α-Alanine	10	Creatinine	5
L-Arginine	5	Uric acid	2
L-Aspartic acid	10	Benzamide	2
Cysteine	5	Salicylamide	5
Glutamic acid	10	Phenol	10
Glycine	2	Hydroquinone	10

The absorption maxima of the urea derivatives and the heating times needed to give maximum intensity are shown in Table 1. Urea initially gives a red colour, which turns to orange.

The detection limits in the spot-test are also shown in Table 1. This test was carried out with the same reagents but with concentrated hydrochloric acid to develop the colour at room temperature.

The precision was tested with methylurea. Three separate runs with 10 aliquots of 0.2m*M* methylurea gave a coefficient of variation of 0.5%.

Examinations of various substituted ureas showed that acetylurea and allantoin gave a less sensitive reaction, and 1,3-dimethylurea, 1,3-diethylurea, biuret, semicarbazide and thiourea gave negative response. These results show that the colour reaction needs a non-substituted amino-group in the molecule. With urea itself, one of the amino-groups might give the first red colour, and the second be responsible for the colour change to orange.

Interfering substances

Several types of compound were tested by adding them to 0.2m*M* methylurea to see whether they interfered. The mixture and the methylurea solution alone were treated as in the procedure, and the compound tested was considered to cause no interference if the difference in the absorbances was below 1% of that for the methylurea solution (Table 2).

Aromatic primary amines give a similar colour reaction and interfere in both the detection and determination.

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ANALYTICAL DATA

SPECTROPHOTOMETRIC STUDY OF THE EQUILIBRIA IN AQUEOUS COPPER(II) AZIDE SOLUTIONS

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Summary—The reaction between copper (II) and azide has been studied spectrophotometrically at four wavelengths, at 25°, and ionic strength 4.00*M* (sodium perchlorate). The formation constants β_2 and β_3 found are $2.90 \pm 0.08 \times 10^4$ and $3.02 \pm 0.07 \times 10^6$ respectively. The results obtained from potentiometric measurements with a solid-state electrode disagree with those calculated from the spectrophotometric data. Causes of the discrepancy are discussed.

The reaction between copper(II) and azide is of analytical interest by virtue of its application to the spectrophotometric determination of azide.[1] A number of azide complexes can be formed[2–7] and it is the purpose of this paper to present improved values for the stability constants of those complexes, especially that for $Cu(N_3)_3^-$.

The complex CuN_3^+ was studied spectrophotometrically at low ionic strength by Saini and Ostacoli.[9] Estimation of the activity coefficients enabled the thermodynamic formation constants β_1°–β_4° to be calculated, from solubility measurements, at low ionic strength.[5,6] At high ionic strength, 4.0*M*, maintained with perchlorate, the formation constant of CuN_3^+ has been determined by spectrophotometry and solubility measurements have been used for the determination of other constants,[8] except β_3, which was only estimated. However, very good agreement was found for the β_4 values from polarographic[4] and solubility measurements.[8]

In this study the copper(II)/azide system has been studied spectrophotometrically at an ionic strength of 4.0*M* (sodium perchlorate) and 25°. Attempts to perform analogous potentiometric measurements in the copper(II)/azide system with a CuS, Ag_2S/Ag solid-state electrode were also made but the results were not consistent with those obtained by spectrophotometry.

EXPERIMENTAL

Reagents

All reagents were of analytical grade unless otherwise specified.

Sodium azide. Recrystallized from aqueous solution by adding ethanol, washed with pure ethanol to remove traces of hydroxide, dried under vacuum and finally left overnight in an oven at 100°.

Standard copper(II) solution, 1M. Prepared from copper nitrate and standardized electrolytically. The solution was made 0.0100*M* in perchloric acid to prevent hydrolysis.

Sodium perchlorate solution, 7M. Standardized gravimetrically by evaporation of 2–5 ml to dryness in an oven at 110–120° overnight, cooling in a cacuum desiccator and weighing. This procedure has been found to be more reproducible and reliable than titration after cation-exchange.

Apparatus

Spectrophotometry. A Zeiss PMQ II spectrophotometer with 1-cm quartz cuvettes was used at $25.0 \pm 0.2°$.

Potentiometry. An Orion 801 A instrument was used with an Orion 94–29 electrode at $25.0 \pm 0.1°$. The reproducibility was best when the electrode surface was freshly polished with calcium carbonate. At the time of measurement a mobile salt bridge with a small flux of one drop per hour, and filled with saturated sodium chloride solution, was inserted in the test solution to make contact with a reference electrode. Thus, the potentiometric measurements refer to the cell:

Ag	Ag_2S, CuS	Cu(II) = 0.1m*M* ($NaClO_4$ + NaN_3) = 4.00*M*	NaCl satd.	NaCl satd. Hg_2Cl_2	Hg

RESULTS AND DISCUSSIONS

Spectrophotometric measurements

Copper(II) in azide solutions has very favourable spectral characteristics for equilibrium studies. A

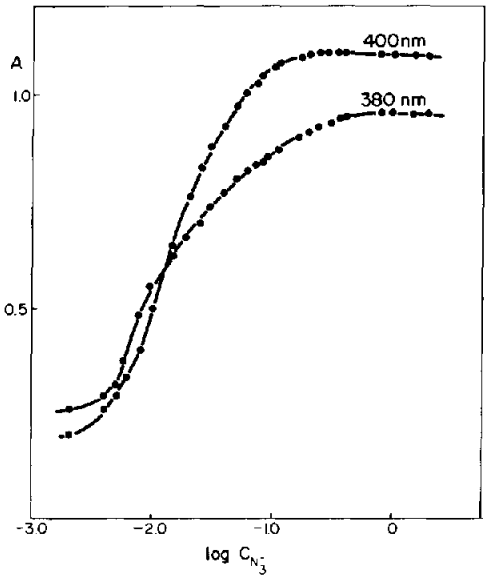

Fig. 1. Change of absorbance of the copper (II) complexes (0.1m*M*) with the analytical azide concentration at two wavelengths.

broad absorption band, typical of charge transfer, is observed in the region 350–450 nm, quite separated from the absorption spectra of azide and copper(II) ions. The average molar absorptivity, $\bar{\epsilon}$, is 7×10^3 $l.mole^{-1}.cm^{-1}$ at azide concentration $> 0.5M$. The absorption maximum varies from 375 nm for CuN_3^+ species to 417 nm for $Cu(N_3)_4^{2-}$.

Figure 1 shows how the absorbance changes with the analytical azide concentration, $C_{N_3^-}$ at two wavelengths: $\bar{\epsilon}$ increases and then tends to a constant value at higher azide concentrations. $Cu(N_3)_4^{2-}$ predominates at azide concentrations $>1M$, and its molar absorptivity, ϵ_4, can be determined accurately.

In the region where all species co-exist, $\bar{\epsilon}$ at each wavelength can be taken as a function of the azide concentration, the formation constants of the complexes, β_n, and the molar absorptivities, ϵ_n, provided the absorbances, A, of the complexes are additive,[10] with no contribution from azide and uncomplexed copper(II):

$$\bar{\epsilon} = \frac{A}{C_{Cu(II)}} = \frac{\epsilon_1\beta_1[N_3^-] + \cdots + \epsilon_4\beta_4[N_3^-]^4}{1 + \beta_1[N_3^-] + \cdots + \beta_4[N_3^-]^4}. \quad (1)$$

The equilibrium concentration of azide can be easily evaluated from $\bar{n}$,[10,11] calculated from the approximate values of the constants by iteration:

$$[N_3^-] = C_{N_3^-} - \bar{n}C_{Cu(II)} \quad (2)$$

Graphical treatment of data, by Yatsimirskii's method,[10] with a function derived from $\bar{\epsilon}$ [equation (1)] was quite unreliable, as extrapolations of steep curves were required. Thus, another calculation procedure was applied.

The first approach was to diminish the number of unknowns in equation (1), as β_1, ϵ_1 and β_4 had been previously determined.[7,8] In addition, ϵ_4 was also determined from measurements at several wavelengths and $2.0M$ azide concentration, at which there is experimental evidence that $Cu(N_3)_4^{2-}$ is virtually the only complex (Fig. 1).

From equation (1), the following expressions are derived:

$$\phi = \bar{\epsilon} + \beta_1[N_3^-](\bar{\epsilon} - \epsilon_1) + \beta_4[N_3^-]^4(\bar{\epsilon} - \epsilon_4) \quad (3)$$

$$\phi = \beta_2[N_3^-]^2(\epsilon_2 - \bar{\epsilon}) + \beta_3[N_3^-]^3(\epsilon_3 - \bar{\epsilon}) \quad (4)$$

$$\frac{\phi}{[N_3^-]^2} = \beta_2(\epsilon_2 - \bar{\epsilon}) + \beta_3(\epsilon_3 - \bar{\epsilon})[N_3^-]. \quad (5)$$

As a preliminary solution to obtain data for ϵ_2, ϵ_3 and β_2 and β_3, this last equation was initially used with ϕ calculated from known $\bar{\epsilon}$, ϵ_1, ϵ_4, β_1, β_4 and $[N_3^-]$ data. It was inferred from such studies that ϵ_2 and ϵ_3 were intermediate between ϵ_1 and ϵ_4. It was then assumed that ϵ_2 and ϵ_3 could be linearly interpolated between ϵ_1 and ϵ_4, in order to decrease the number of unknowns in the equations:[8]

$$\epsilon_2 = \frac{\epsilon_4 - \epsilon_1}{3} + \epsilon_1 \quad (6)$$

$$\epsilon_3 = \frac{2(\epsilon_4 - \epsilon_1)}{3} + \epsilon_1. \quad (7)$$

The $\phi/[N_3^-]^2$ data were then treated further by introducing ϵ_2 and ϵ_3 into the equations, to give a system of simultaneous equations with only two unknowns. From the spectrophotometric data for the copper(II) azide system, taken at four wavelengths (370, 380, 390 and 400 nm), sets of simultaneous equations were used to solve for β_2 and β_3. The results are shown in Table 1. Very good agreement was found for the β_2 and β_3 values from the four wavelengths. The

Table 1. Final data for ϵ_n, β_2 and β_3 at the four wavelengths used.

λ(nm)	ϵ_4*, $l.mole^{-1}.cm^{-1}$	ϵ_1†, $l.mole^{-1}.cm^{-1}$	β_2	β_3
370	5.33×10^3	1.94×10^3	2.90×10^4	2.94×10^6
380	6.26×10^3	1.82×10^3	2.80×10^4	3.10×10^6
390	6.83×10^3	1.52×10^3	3.00×10^4	2.99×10^6
400	7.24×10^3	1.17×10^3	2.89×10^4	3.05×10^6

Mean $\beta_2 = (2.90 \pm 0.08) \times 10^4$
Mean $\beta_3 = (3.02 \pm 0.07) \times 10^6$
*Beer's plot.
†Calculated with data from reference 8.

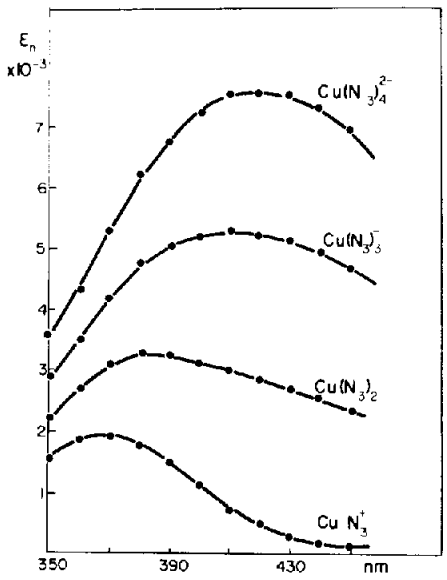

Fig. 2. Individual spectra determined for CuN_3^+ and $Cu(N_3)_4^{2-}$ and calculated [equations (6) and (7)] for the intermediate species.

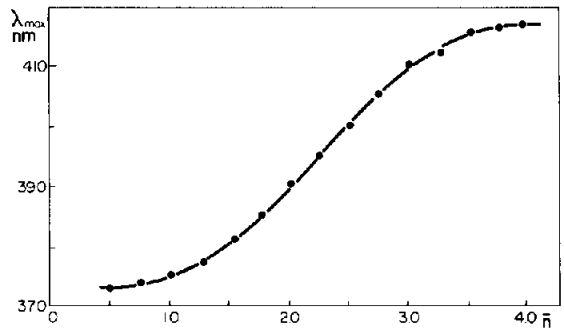

Fig. 4. Plot of λ_{max} for several azide concentrations *vs.* the average ligand number, $\bar{n}$, calculated from the formation constants.

β_2 value was also in good agreement with that from the solubility measurements,[8] 3.0×10^4 (see below). A small disagreement was found between the more reliable value presented in this paper for log β_3 (6.48) and an earlier estimate of 6.11 made from a linear plot of log β_n *vs.* $(n-1)$.[8]

It should be added that the comparison between the experimentally measured absorbance and that calculated from equation (1) and the known parameters, leads to a relative average deviation of $\pm$ 0.3% at 370 nm, $\pm$ 0.6% at 390 nm and $\pm$ 0.1% at 400 nm.

The results suggest that the assumption for estimation of ϵ_2 and ϵ_3 by equations (6) and (7) was reasonable. Thus the individual spectra can be calculated for the intermediate complexes on the basis of measurements of solutions with large excess of copper(II) (*i.e.*, CuN_3^+ is the only complex) or large excess of azide [only the $Cu(N_3)_4^{2-}$ complex]. Figure 2 shows the individual spectra. A plot of $\bar{\epsilon}_{max}$ *vs* the calculated $\bar{n}$, (Fig. 3) shows that there is a significant increase in oscillator strength when the third ligand is introduced. The relationship between λ_{max} and $\bar{n}$ is shown in Fig. 4.

Potentiometric measurements

Membrane ion-selective electrodes are now widely used for many purposes.[12] However, it is questionable whether they are accurate enough for measurements of equilibrium constants.[13] There has been some dispute about the advantages and disadvantages of the use of the $CuS,Ag_2S/Ag$ selective electrode for the determination of copper(II) ions in complexing media.[14–17] This suggested that a comparison should be made between the results obtained by spectropho-

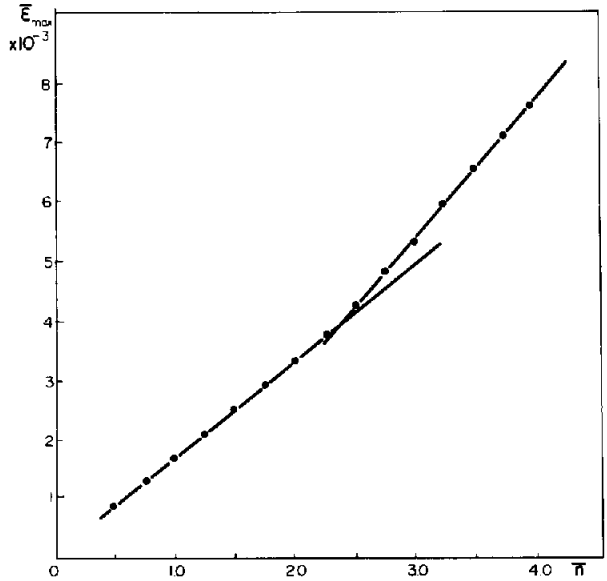

Fig. 3. Plot of $\bar{\epsilon}_{max}$ (l.mole^{-1}.cm^{-1}) experimentally determined for several azide concentrations *vs.* the average ligand number, $\bar{n}$, calculated from the formation constants.

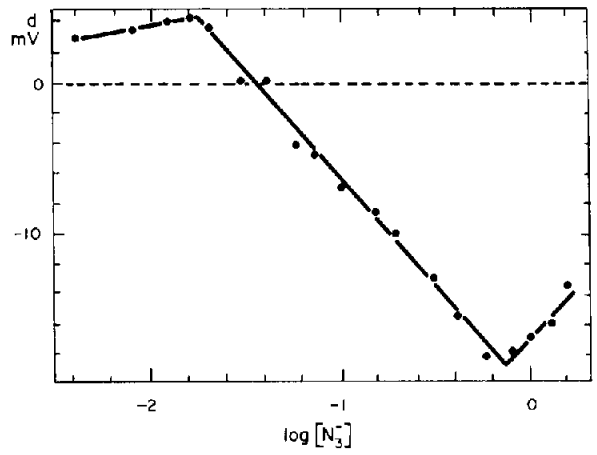

Fig. 5. Deviation, d, between the calculated $\Delta E^{o'}_{x,s}$ [equation (9)] and the experimental value (taken with the solid membrane copper electrode).

tometry and potentiometry. Thus, the shift of potential on changing from non-complexing to complexing medium, $\Delta E^{o'}_{x,s}$ (see Experimental), calculated from

$$\Delta E^{o'}_{x,s} = \frac{0.05916}{2} \log(1 + \beta_1[N_3^-] + \cdots + \beta_4[N_3^-]^4) \quad (8)$$

was compared with the experimental value for the same azide concentration. Figure 5 shows how the error changes with the azide concentration.

As azide has a tendency to stabilize copper(I) by complexation[4] the conditional potential of the copper(II)/copper(I) system should change when the azide concentration is increased. The errors observed for the measurements in such solutions could well be due to a mixed potential, which probably results from a response of the solid membrane to the copper(II)/copper(I) system, superimposed on the normal response for the copper(II)/copper system. It is interesting that less uncertainty in the measurements with the $CuS,Ag_2S/Ag$ electrode can result when complexones are used,[17] probably because these ligands preferentially complex copper(II), with no special tendency to stabilize copper(I).

Another explanation for the abnormal electrode response would be the oxidation of the metal sulphide to sulphate, by hydrazoic acid or the copper(II) complexes. In fact it was observed that there was light corrosion of the electrode surface after its immersion in the working solutions.

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DERIVATIVES OF 2-THIOHYDANTOIN AS SPECTROPHOTOMETRIC ANALYTICAL REAGENTS—II*

CONDENSATION AT C-5 WITH AROMATIC NON-PYRIDINE ALDEHYDES

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Summary—The synthesis, characteristics, properties and reactions with metallic ions of six aromatic derivatives of 2-thiohydantoin have been studied. The reagents exhibit two pK values in aqueous solution, and with Pd(II), Au(III), Ag(I) and Hg(I) and (II), especially with the first, form stable complexes with molar absorptivities adequate for spectrophotometric measurements.

The derivatives of 2-thiohydantoin formed by condensation with aromatic non-pyridine aldehydes at the C-5 position have scarcely been studied as spectrophotometric reagents apart from a few proposals for use in qualitative[1–4] and colorimetric[5] analysis. In a previous paper[6] the analytical possibilities of four analogous pyridine derivatives were considered and the importance of these compounds for physiological, biochemical and pharmaceutical purposes was emphasized.

This paper is concerned with the spectrophotometric application of the non-pyridine derivatives, those prepared being the 5-benzal (*A*), 5-(*o*-hydroxy)benzal (*B*), 5-(*m*-hydroxy)benzal (*C*), 5-anisal (*D*), 5-naphthal (*E*) and 5-(*p*-demethylamino)benzal (*F*) 2-thiohydantoins.

EXPERIMENTAL

Reagents

Chemicals of analytical-reagent grade or better were used throughout. All metal ion solutions were standardized.

Buffer solution, pH 4.7. Dissolve 56.0 g of sodium acetate trihydrate and 25.0 ml of glacial acetic acid in water and dilute to 1 litre.

Procedure

The reagents tested were prepared and examined as described earlier.[6] The elemental analyses agreed with the values calculated for the empirical formulae.

RESULTS AND DISCUSSION

Properties of the reagents

The solutions of the reagents ($4 \times 10^{-5} M$) in ethanol (dimethylformamide for the anisaldehyde derivative) are stable for at least a month. In aqueous solution they are stable below pH 10 but in strongly alkaline medium undergo conversion into the corresponding thiohydantoic acid.[7] Reducing agents do not perceptibly affect the spectra of the reagents; oxidizing agents decrease the absorbance rapidly. The measurements were made at 360 nm for *A* and *C*, 370 nm for *B*, 380 nm for *E* and *D*, and 450 nm for *F*.

Infrared spectra were obtained (KBr discs), and the bands assigned to the "thioureide band"[8,9]—coupling between the ν^{C-N} and ν^{N-H} modes—at about 1500 cm^{-1} and to the antisymmetric and symmetric stretching modes of NCS bonds[10] (designated as *C* and *D* bands in Jensen's paper) at about 1450 and 1245 cm^{-1}, respectively.

The ultraviolet spectra of the aqueous solutions of the reagents, in neutral medium, show an absorbance maximum between 360 and 380 nm (at 450 nm for *F*). In aqueous 4% ethanol medium the reagents show at least two pK values, due to dissociation of the thioimidol and imidol groups of the thiohydantoin molecule.[6] The values found [11,12] were: *A*, $pK_1 = 7.8$, $pK_2 = 12.6$; *B*, $pK_1 = 7.0$, $pK_2 = 13.2$, $pK_{phenol} = 9.5$; *C*, $pK_1 = 7.2$, $pK_2 = 12.3$; *D*, $pK_1 = 7.9$, $pK_2 = 12.6$; *E*, $pK_1 = 7.8$, $pK_2 = 13.0$ and *F*, $pK_{amine} = 3.3$, $pK_1 = 8.1$, $pK_2 = 12.7$.

Reactions with metal ions

The reactions of metal ions with the reagents were tested in acetate buffer and at pH 1, and the results are summarized in Table 1. Generally, the chelates are yellow or orange-yellow in aqueous solution and the absorption spectra show a band with a maximum at 390–450 nm. The behaviour of *F* is different since the spectra undergo bathochromic shifts in acetate media and the absorption maximum is situated between 500 and 550 nm.

The reagents react well with Pd(II), Au(III), Ag(I) and Hg(I) and (II), especially with the first of these. Other ions of the platinum group, Os(VIII), Rh(III) and Ru(IV), do not give appreciable reaction, even if the solutions are heated. It is obvious that strongly acid medium increases the selectivity of the reagents with regard to some cations, such as Pd(II) and Au(III).

* Part I—*Talanta*, 1978, **25**, 331.

Table 1. Photometric characteristics of the complexes in solution*

Cation	*A*				*B*			
	λ_{max} pH 5	ϵ_{max} pH 5	λ_{max} pH 1	ϵ_{max} pH 1	λ_{max} pH 5	ϵ_{max} pH 5	λ_{max} pH 1	ϵ_{max} pH 1
Pd(II)	406	2.4×10^4	406	2.4×10^4	426	2.2×10^4	426	2.2×10^4
Pt(IV)	406	9.4×10^2	402	5.4×10^2	430	9.0×10^2	422	3.9×10^2
Os(VIII)	404	2.3×10^2	410	1.5×10^2	428	1.9×10^2	438	0.9×10^2
Rh(III)	420	0.8×10^2	420	0.8×10^2	440	1.0×10^2	446	0.8×10^2
Au(III)	406	1.9×10^4	420	3.0×10^3	426	2.6×10^4	426	2.5×10^3
Ag(I)	410	3.2×10^3	420	3.2×10^2	430	2.1×10^3	430	1.3×10^3
Cu(II)	406	0.7×10^2	406	0.4×10^2	422	3.8×10^2		
Hg(I)					418	1.5×10^4		
Hg(II)	402	3.1×10^3			420	9.0×10^3	420	6.4×10^2
Fe(III)	402	2.2×10^2	398	1.6×10^2	450	0.6×10^2	418	0.4×10^2
V(V)	402	3.8×10^2	404	0.3×10^2	422	3.2×10^2		

	C				*D*			
	λ_{max} pH 5	ϵ_{max} pH 5	λ_{max} pH 1	ϵ_{max} pH 1	λ_{max} pH 5	ϵ_{max} pH 5	λ_{max} pH 1	ϵ_{max} pH 1
Pd(II)	412	2.3×10^4	412	2.3×10^4	428	2.5×10^4	431	2.5×10^4
Pt(IV)	410	7.8×10^2	406	4.7×10^2	428	2.1×10^3	436	1.0×10^3
Os(VIII)	414	1.9×10^2	420	1.5×10^2	436	1.9×10^2	430	1.9×10^2
Rh(III)	440	1.0×10^2	440	1.0×10^2			420	1.0×10^2
Au(III)	408	1.8×10^4	431	2.2×10^3	430	1.2×10^4	430	1.2×10^2
Ag(I)	412	1.6×10^3	416	1.4×10^3	428	2.1×10^3	430	1.6×10^3
Cu(II)	420	0.4×10^2			432	0.9×10^2		
Hg(II)	409	2.6×10^3	404	2.4×10^2	442	1.2×10^3		
Fe(III)	400	0.7×10^2			450	0.5×10^2		
V(V)	407	4.2×10^2			425	0.2×10^3		

	E				*F*			
	λ_{max} pH 5	ϵ_{max} pH 5	λ_{max} pH 1	ϵ_{max} pH 1	λ_{max} pH 5	ϵ_{max} pH 5	λ_{max} pH 1	ϵ_{max} pH 1
Pd(II)	434	2.1×10^4	440	2.1×10^4	512	2.6×10^4	396	2.4×10^4
Pt(IV)	434	9.0×10^2	424	2.7×10^2			395	7.4×10^2
Os(VIII)							404	1.5×10^2
Au(III)			440	3.8×10^3	526	1.2×10^4	470	3.1×10^3
Ag(I)	434	2.7×10^3		ppte.			494	1.0×10^3
Cu(II)	440	5.0×10^2			530	4.0×10^3		
Hg(I)	427	1.2×10^4		ppte.				
Hg(II)	427	2.4×10^4		ppte.	560	5.6×10^3	500	1.6×10^2
Fe(III)	450	0.9×10^2	424	0.7×10^2			404	0.6×10^2
V(V)	428	1.8×10^2					510	0.5×10^2

* λ_{max}, *nm*; ϵ_{max}, $l.mole^{-1}.cm^{-1}$.

Influence of solvent extraction and EDTA on the reactivity

Several solvent systems have been tested: water–benzene, water–toluene, water–chloroform and water–MIBK. Of these the last is the best. EDTA has been employed as masking agent. The reactions are more affected by solvent extraction than by EDTA, but the palladium complexes were not generally influenced by either these systems. Consequently, the compounds studied should be good spectrophotometric reagents for Pd(II), since the interferences of other ions can be decreased or eliminated.

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DIPHENYLGLYOXAL AND DIPYRIDYLGLYOXAL BIS(BENZOYLHYDRAZONE) AS ANALYTICAL REAGENTS

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Summary—The synthesis, characteristics and analytical properties of diphenylglyoxal and dipyridylglyoxal bis(benzoylhydrazone)s are described. The solubility, spectral characteristics, p*K* values, effect of oxidizing and reducing agents, hydrolysis resistance, and reactions with common cations are reported.

Bis-aroylhydrazones have recently been proposed as analytical reagents. The photometric and fluorimetric properties of the bis(4-hydroxybenzoylhydrazone)s of glyoxal, methylglyoxal and dimethylglyoxal have been reported by Lever.[1] The photometric reactions with Ca(II),[2] In(III),[3] Ti(IV)[4] and Sn(II)[5] of the diphenylglyoxal and dipyridylglyoxal bis(2-hydroxybenzoylhydrazone)s have been investigated by us. Monoaroylhydrazones have been also studied by us.[6–8]

In this paper studies on the synthesis, properties and applications of diphenylglyoxal (A) and dipyridylglyoxal bis(benzoylhydrazone) (B) are reported.

EXPERIMENTAL

Synthesis

The appropriate amount of diphenylglyoxal or dipyridylglyoxal with the stoichiometric amount of benzoylhydrazone was dissolved in ethanol–water mixture (1 + 1), several drops of concentrated hydrochloric acid were added and the mixture was refluxed for 30 min. It was then allowed to cool to room temperature. The products were filtered off and washed with hot ethanol–water (1 + 1).

Compound (A) had m.p. 194–195° and analysis gave C 75.0%, H 5.1%, N 12.5%: $C_{28}H_{22}N_4O_2$ requires C 75.33%, H 4.93%, N 12.55%. Compound (B) had m.p. 198–199° and analysis gave C 69.4%, H 4.5%, N 18.6%: $C_{26}H_{20}N_6O_2$ requires 69.64%, H 4.46%, N 18.75%.

Properties of the reagents

The solubilities (Table 1) were measured by the method of Wittenberger.[9] The absorption spectra at various pH values are shown in Fig. 1.

Solutions 0.1% of the reagents in dimethylformamide were stable for at least one week.

The Phillips and Merritt method[10] was used for the determination of the p*K* values, which were calculated by the methods of Strenstom and Goldsmith[11] and Sommer.[12] The average values obtained from measurements at two wavelengths are $pK_1 = 9.07 \pm 0.05$ for reagent A and $pK_1 = 2.60 \pm 0.05$ and $pK_2 = 8.63 \pm 0.05$ for reagent B.

Analytical reactions

The reactions of these reagents with 30 cations at various pH values were investigated. The solutions were prepared in 25-ml standard flasks from appropriate amounts of the cation, 5 ml of 0.1% reagent solution in dimethylformamide, 10 ml of dimethylformamide and 5 ml of pH 4.75 or 9.75 buffer, and diluted to volume with distilled water. The absorbances were measured between 350 and 700 nm against a blank solution. The characteristics of the most important chelates are shown in Table 2.

RESULTS AND DISCUSSION

These reagents have an absorption maximum at about 300 nm in acidic and neutral media, with a bathochromic shift in alkaline medium.

Reagent A has only one p*K* value, corresponding to the ionization of the hydroxy group. This behaviour is

Table 1. Characteristics of both compounds in several solvents

Solvent	Reagent A Solubility, *g/l.*	λ_{max} *nm*	Molar absorptivity, 10^3 *l. mole*$^{-1}$ *cm*$^{-1}$	Reagent B Solubility, *g/l*	λ_{max} *nm*	Molar absorptivity, 10^3 *l. mole*$^{-1}$ *cm*$^{-1}$
Water	0.09	—	—	0.16	—	—
Ethanol	0.56	305	31.8	1.24	310	34.2
Acetone	2.12	335	8.5	1.08	340	6.4
Dimethylformamide	> 33.81	310	25.5	5.62	310	27.3
Chloroform	> 21.16	305	43.0	2.32	310	42.7
Benzyl alcohol	3.18	317	31.8	1.56	325	30.0
Benzene	0.91	305	40.6	0.34	310	36.0

Table 2. Characteristics of complexes in solution

Reagent	Metal ion	Buffer solution, pH 4.7			Buffer solution, pH 9.8		
		λ_{max}, *nm*	Molar absorptivity, 10^3 . $mole^{-1}$, cm^{-1}	Colour of complex	λ^{max}, *nm*	Molar absorptivity, 10^3 *l.* $mole^{-1}$, cm^{-1}	Colour of complex
A	Cu(II)	415	4.51	yellow	415	1.20	yellow
	Ni(II)	—	—	—	440	0.99	yellow
	Co(II)	—	—	—	—	—	—
	Fe(II)	400	0.45	yellow	400	2.80	yellow
	Fe(III)	410	0.39	yellow	400	2.30	yellow
	U(VI)	500	4.76	orange	400	7.40	orange-yellow
	Ti(IV)	520	0.81	orange	480	0.19	orange
	In(III)	500	1.38	orange-yellow	460	5.05	orange-yellow
B	Pb(II)	395	17.6	yellow	395	12.9	yellow
	Cu(II)	395	39.4	yellow	405	24.1	yellow
	Ni(II)	395	34.4	yellow	405	21.4	yellow
	Co(II)	385	29.0	yellow	400	20.0	orange-yellow
	Fe(II)	665	12.3	green	665	9.2	green
	Fe(III)	370	7.5	yellow	470	6.2	orange-yellow
	U(VI)	395–460	6.5–6.8	orange-yellow	450	16.9	orange
	Ti(IV)	395–470	8.9–2.4	orange	470	1.2	orange-yellow
	In(III)	395	22.9	yellow	470	23.5	yellow

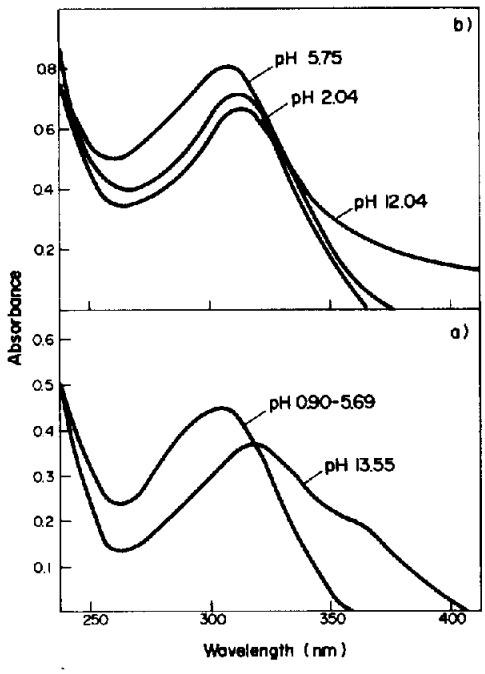
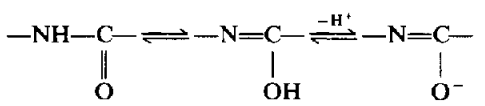


Fig. 1. Absorption spectra of the reagents in ethanol-water (2 + 3) solution at various pH values. (a) A reagent, $1.4 \times 10^{-5} M$; (b) B reagent, $2.6 \times 10^{-5} M$.

in accord with the keto–enol equilibrium:



Reagent B has two ionization constants, one from ionization of the hydroxyl group and the other from the protonated pyridine nitrogen atoms.

Oxidizing agents (hydrogen peroxide, potassium peroxodisulphate) slightly alter the absorption spectra of both reagents, especially in ammonium chloride–ammonia and basic medium but reducing agents (ascorbic acid, sodium sulphite, hydroxylammonium chloride) are without effect at any pH.

These bis-aroylhydrazones are resistant to hydrolysis of the >C=N– group at any pH.

As an analytical reagent B is better than A, its colour-forming reactions being much more sensitive.

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STUDIES ON THE BEHAVIOUR OF β-ETHYLTHIOETHYLENETHIOGLYCOLLATE IN AQUEOUS Ni(II) AND Cu(II) SOLUTIONS

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Summary—The stability constants of the 1:1 and 1:2 complexes of nickel and copper(II) with β-ethylthioethylenethioglycollic acid have been determined at 25° at ionic strength 1.0 ($NaClO_4$). The values for the nickel complexes are $K_1 = 1.11 \pm 0.06 \times 10^2$ (spectrophotometrically) or $1.25 \pm 0.11 \times 10^2$ (potentiometrically) and $K_2 = 3.04 \pm 0.24 \times 10^2$ (potentiometrically). The corresponding values for the copper complexes are $K_1 = 1.27 \pm 0.02 \times 10^3$ or $1.28 \pm 0.03 \times 10^3$ and $K_2 = 7.29 \pm 0.30 \times 10^2$.

β-Ethylthioethylenethioglycollic acid (β-ETETGA) is known to form complexes with palladium(II),[1] nickel[2] and copper(II),[3] but only the first has had its stability constant determined.[4] We have now determined the constants for the complexes of nickel and copper.

EXPERIMENTAL

Apparatus

The spectrophotometers used were a Zeiss PM QII (1.0 and 4.0 cm silica cells), a Beckman DB-G and a Cary 14. A Metrohm E388 potentiometer with combined glass and silver/silver chloride electrode was used for pH measurements, with perchloric acid at ionic strength 1.0 ($NaClO_4$) as reference.[5] All measurements were made at 25° and ionic strength 1.0 ($NaClO_4$). A Perkin–Elmer 337 infrared spectrometer and a Dupont thermoanalytical system 330 apparatus and 351 thermogravimetric analyser were also used.

Reagents

β-ETETGA and its sodium salt (Na-ETETG) were prepared and standardized as previously reported.[1,4] The nickel and copper perchlorates were prepared from a slight excess of the metal carbonate and 20% perchloric acid, and recrystallized several times from water. Their solutions were standardized electrogravimetrically. Sodium perchlorate was purified by recrystallization and its stock solutions standardized by titration after conversion into the acid by cation exchange.

Preparation of the complexes

A mixture of 4 ml of 1*M* nickel sulphate and 20 ml of 0.4*M* Na-ETETG at pH 5.0 was kept in the refrigerator for several days. The pale green product was then filtered off, and washed with water and then ethanol. The m.p. was 100–104°. Elemental analysis was in agreement with the formula $Ni(C_6H_{11}O_2S_2)_2.2H_2O$. The magnetic moment (Gouy balance) was 3.4 B.M. at 23°.

A mixture of 4.4 ml of 0.9*M* copper perchlorate and 20 ml of 0.4*M* Na-ETETG at pH 5.0 gave a dark green solid after 1 day in the refrigerator. The m.p. was 105–110°, and the analysis in agreement with $Cu(C_6H_{11}O_2S_2)_2$. The magnetic moment was 1.9 B.M. at 25°.

The compositions were confirmed by thermogravimetric analysis; the m.p. and the presence of water were also confirmed by DTA analysis.

Spectrophotometry

Na-ETETG was added to nickel perchlorate solution in molar ratios ranging from 0 to 20. As the ratio of ligand to metal (L:M) increased, the absorption maximum at about 615 nm shifted slightly to shorter wavelengths, and the absorbance became practically constant when L:M exceeded 10 (Fig. 1). The effect of pH was also examined (Fig. 2), and the optimum for complex formation was about 5.0. Figure 3 shows the spectrum obtained with a large excess of nickel present, and indicates the existence of a 1:1 complex, with an absorption maximum at 650 nm.

The colour was stable for at least 40 hr. The Vosburgh and Cooper method,[6] applied at several wavelengths and at pH 4.95 ± 0.09, indicated the existence of ML_3 (585 and 600 nm plots), ML_2 (605 and 630 nm) and ML (655 and 660 nm) complexes. These were confirmed by the mole-ratio method.

Similar studies were made for the copper complexes at 330, 340, 350 and 370 mm and pH = 4.97 ± 0.40 (Figs. 4–6) and showed only ML_2 and ML complexes, the optimum pH for formation being 5.0. The colour was stable for at least 2 hr if the solution was kept in the dark and a large excess of ligand was not present, but faded more rapidly in daylight.

Potentiometric titration

Eleven buffer solutions were prepared with Na-ETETG concentrations ranging from 2.53×10^{-3} to $7.06 \times 10^{-2}M$ and perchloric acid concentrations from 1.0×10^{-3} to $2.8 \times 10^{-2}M$, so that the reagent stoichiometric concentration:acid concentrations ratio (C'_L/C'_{HL}) was always 32. The hydrogen-ion concentrations $[H^+]_1$ were obtained from pH measurements.

Another set of the same buffers was prepared containing nickel at five concentrations (C_M) ranging from 3.597×10^{-3} to $11.99 \times 10^{-3}M$. The free perchloric acid concentration of the metal stock solution (C_H) was determined from C_M, since it had been found from pH measurements at constant ionic strength (1.0*M*, $NaClO_4$) that $[H^+]/C_M$ was constant. The pH of the metal–buffer mixtures was determined and the corresponding hydrogen-ion concentrations $[H^+]_2$ were deduced.

Similar experiments were performed for the copper system, with six buffer solutions with [Na-ETETG] ranging from 1.01×10^{-3} to $1.0 \times 10^{-2}M$ and $[HClO_4]$ from 4.04×10^{-4} to $4.04 \times 10^{-3}M$ ($C'_L \cdot C'_{HL} = 3.2$): the copper concentration ranged from 4.34 to 10^{-4} to $6.51 \times 10^{-4}M$.

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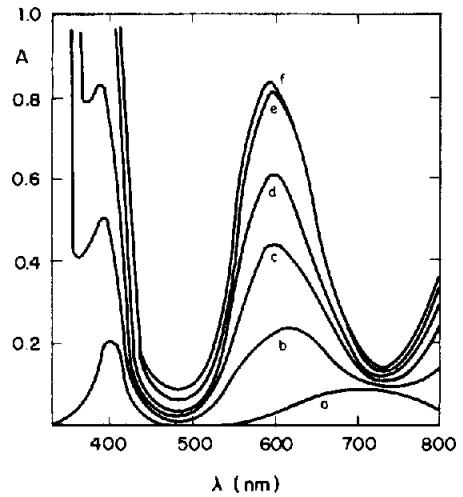

Fig. 1. Absorption spectra for $1.00 \times 10^{-2}M$ $Ni(ClO_4)_2$ with ligand concentrations of (*a*) zero, (*b*) $1.00 \times 10^{-2}M$, (*c*) $2.00 \times 10^{-2}M$, (*d*) $3.00 \times 10^{-2}M$, (*e*) $1.00 \times 10^{-1}M$, (*f*) $2.00 \times 10^{-1}M$. $I = 1.0M$; pH = 4.95 ± 0.06; $T = 25.0 \pm 0.1°$; optical path = 4.00 cm. Measurements against reagent blank.

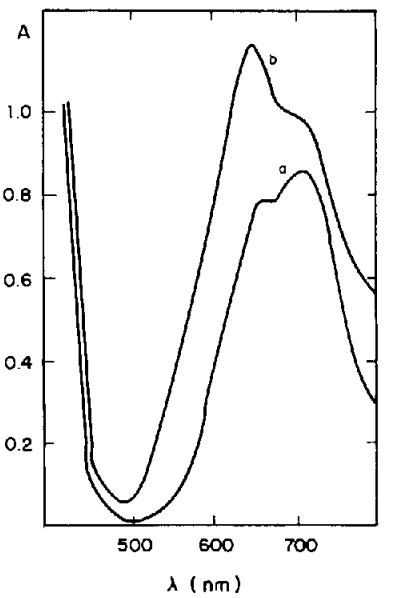

Fig. 3. Absorption spectra $1.09 \times 10^{-1}M$ $Ni(ClO_4)_2$ (*a*) without and (*b*) with $2.03 \times 10^{-2}M$ ligand; pH = 5.0; $I = 1.0M$; $T = 25.0 \pm 0.1°$; optical path = 4.00 cm. Measurements against reagent blank.

RESULTS AND DISCUSSION

The McConnell and Davidson method[7] was modified on the basis of the views of Saini and Ostacoli[8] and of Senise and Neves,[5] to give the equation

$$\frac{C_M^0 C_L^0}{(A - A')\left(1 + \frac{[H^+]}{K_a}\right)} = \frac{1}{K_1(\epsilon_1 - \epsilon_0)} + \frac{C_M^0 + C_L^0 - \left(\frac{A - A'}{\epsilon_1 - \epsilon_0}\right)}{(\epsilon_1 - \epsilon_0)\left(1 + \frac{[H^+]}{K_a}\right)}$$

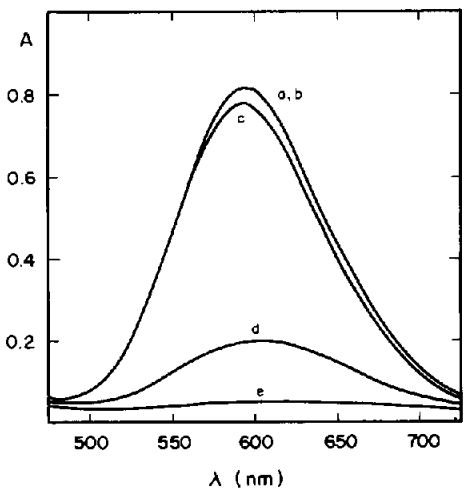

Fig. 2. Absorption spectra for $1.00 \times 10^{-2}M$ $Ni(ClO_4)_2$ and $1.00 \times 10^{-1}M$ ligand at pH, (*a*) 6.39, (*b*) 4.87, (*c*) 3.49, (*d*) 2.22, (*e*) 1.49; $I = 1.0M$; $T = 25.0 \pm 0.1°$; optical path = 4.00 cm. Measurements against reagent blank.

where C_M^0 and C_L^0 are the analytical concentrations of the metal ion and ligand, A is the absorbance of a solution containing metal ion (M) and ligand (L), A' is that of a solution containing the same analytical concentration of metal ion, ϵ_1 and ϵ_0 are the molar absorptivities of the ML and M species, K_1 is the stability constant of ML_1 and K_a is the dissociation constant of HL.

To determine both K_1 and K_2 (the stepwise constant for ML_2) the Rossotti and Rossotti equation:[9]

$$\frac{\bar{n}}{(1 - \bar{n})[L]} = K_1 + \frac{K_1 K_2 (2 - \bar{n})[L]}{(1 - \bar{n})}$$

was used, [L] and $\bar{n}$ being evaluated from the potentiometric data by use of the similar Sandell equations:[10]

$$[L] = \frac{[H^+]_1(C'_L + [H^+]_1)(C'_{HL} + C_H - [H^+]_2)}{[H^+]_2(C_{HL} - [H^+]_1)}$$

$$\bar{n} = \frac{C_L + [H^+]_2 - C_H - [L]}{C_M}$$

and the Irving and Rossotti criteria for $\bar{n}$ values.[11]

The value of K_a, previously determined,[3] was taken as $2.46 \pm 0.03 \times 10^{-4}$ under the conditions used. For K_1 determined spectrophotometrically an L:M ratio of 1:5 was used for the nickel system and 1:10 for the copper system, and measurements were made at five wavelengths for six solutions.

Equation (1) was solved approximately for K_1 and $(\epsilon_1 - \epsilon_0)$ by neglecting the term $(A - A')/(\epsilon_1 - \epsilon_0)$ and then refined solving the complete equation by successive approximation. The least-squares method was used for all calculations. The values found, sum-

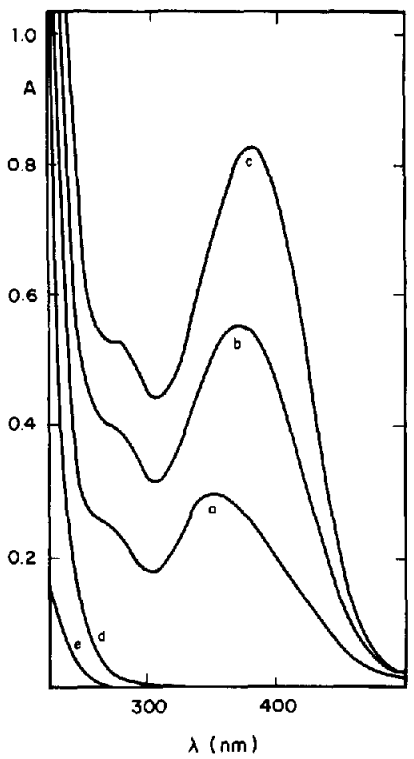

Fig. 4. Absorption spectra for (*a*), (*b*), (*c*), (*e*) $4.56 \times 10^{-4}M$ $Cu(ClO_4)_2$; (*a*) $4.56 \times 10^{-4}M$ ligand, (b) $9.12 \times 10^{-4}M$ ligand, (*c*) $1.37 \times 10^{-3}M$ ligand, (*d*) $1.37 \times 10^{-3}M$ ligand; $I = 1.0M$; pH = 4.9 ± 0.3; $T = 25.0 \pm 0.1°$; optical path = 1.00 cm. Measurements against reagent blank.

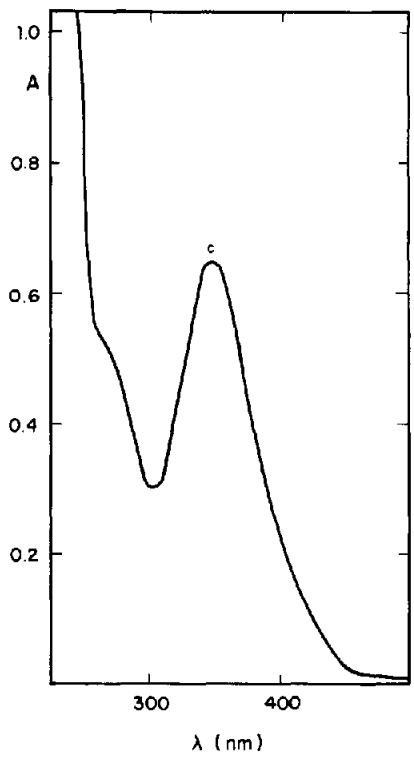

Fig. 6. Absorption spectrum for $4.20 \times 10^{-3}M$ $Cu(ClO_4)_2$ and $4.20 \times 10^{-4}M$ ligand; $I = 1.0M$; pH = 4.88; $T = 25.0 \pm 0.1°$; optical path = 1.00 cm; $Cu(ClO_4)_2$ and ligand do not absorb in this region. Measurements against reagent blank.

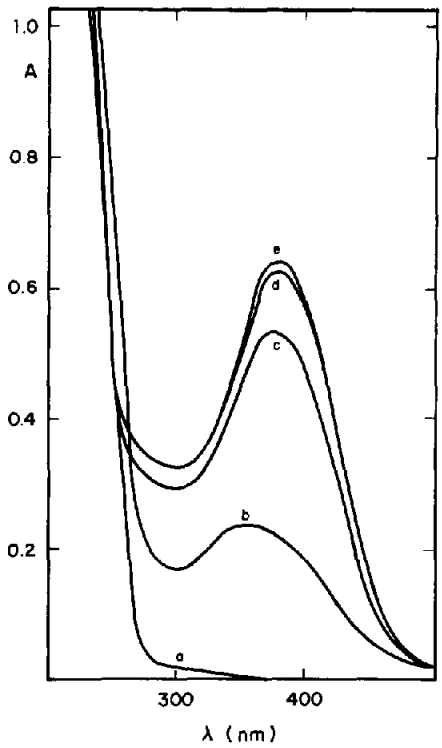

Fig. 5. Absorption spectra for $2.0 \times 10^{-4}M$ $Cu(ClO_4)_2$ and $2.10 \times 10^{-3}M$ ligand at pH; (*a*) 1.34, (*b*) 3.13, (*c*) 4.03, (*d*) 5.03, (*e*) 5.68; $I = 1.0M$; $T = 25.0 \pm 0.1°$; optical path = 1.00 cm. Measurements against reagent blank.

marized in Table 1, show reasonable agreement and are in accord with the Irving–Williams rule.[12]

All the points on the formation curves fell on the same line, irrespective of the metal-ion concentration, indicating that no polynuclear complexes were formed.

From Sandell's K_a values for alkoxyacetic and alkylthioglycollic acids,[13] we conclude that these acids are stronger than acetic acid, and that the ethoxy derivatives are stronger than the alkylthio derivatives, in agreement with the suggestion[14] that the alkoxy groups have the greater electron-withdrawing inductive effect. In β-ETETGA the group C_2H_5–S–C_2H_4– seems to have a similar inductive effect.

At the working pH the ligand will almost all be in the anionic form, so the sulphur atoms will be in β-position to $-CH_3$ and $-COO^-$ groups, which are electron donors. Thus the two sulphur atoms could behave as "soft" donors and the carboxylate group as a "hard" donor, so the ligand could be either bidentate or terdentate. Sandell[13] considers that the ethoxyacetate and ethylthioglycollate complexes of Cu(II) and Ni(II) are more stable than the acetate complexes because these two anions can act as bidentate ligands. The greater stability for the β-ETETG complexes might similarly be attributed to terdentate behaviour of the ligand, though the presence of two

Table 1. Stepwise stability constants [25.0 ± 0.1°, ionic strength 0.1 ($NaClO_4$)]

System	Spectrophotometry K_1	Potentiometry K_1	Potentiometry K_2
Ni-ETETG	$1.11 \pm 0.06 \times 10^2$	$1.25 \pm 0.11 \times 10^2$	$3.04 \pm 0.13 \times 10^2$
Cu-ETETG	$1.27 \pm 0.02 \times 10^3$	$1.28 \pm 0.03 \times 10^3$	$7.29 \pm 0.03 \times 10^2$

Table 2. Selected infrared bands (cm^{-1})

Compound	Reference	ν_{C-S}	$\nu_{s(COO)}$	$\nu_{as(COO)}$
Na β-ETETG	This work	680	1.415(m)	1.575(s)
Na β-ETETG	4	680m	1.410(m)	1.570(s)
Ni(β-ETETG)$_2$.2H$_2$O	This work	655(w) 630(w)	1.370(m)	1.595(s)
Cu(β-ETETG)$_2$	This work	645(w) 638(w)	1.375(m)	1.640(s)

molecules of water in the formula for the solid nickel complex might be regarded as an argument against this. From the shift in ν_s and ν_a in the infrared spectra, however, it may be concluded that the carboxylate group participates in the complexation (Table 2), and as a shift of the C–S stretching frequency to lower wave-number is considered as evidence of metal–sulphur co-ordination,[1,4,16,17] at least one of the sulphur atoms is also bound to the metal. The magnetic moment indicates an octahedral structure, as in similar compounds,[17–19] and suggests that in the solid state the complexes are mononuclear.

For the Cu(II) system K_1 is greater than K_2, as is usual, but the reverse is the case for the Ni(II) system. Rossotti[20] has suggested various interpretations of such anomalies, but also points out that if the complexes are weak, caution should be observed in basing interpretations on the relative values of successive constants.

From the analytical point of view the results strengthen the ideas reported previously[1] that this and similar ligands (with "soft" sulphur atoms) will complex strongly with typically "soft" metal ions such as Pd(II),[4] Hg(II) and Cu(I), and with borderline ions with a tendency towards "soft" character, such as Cu(II).[10] Furthermore, in strongly acidic medium this ligand will not complex Ni(II) or Cu(II), confirming that these species will not interfere in the determination of palladium.[21,22] The earlier assumption[1] that the ligand could be a potential spectrophotometric reagent for Cu(II) was confirmed, but there are evidently limitations arising from the properties of the system, notably the fading of the colour of the complex, which seems to be associated with reduction, as Cu(I) was detected in the decolourized solution by the cuproin test.

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LETTER TO THE EDITOR

ELECTROACTIVE PRODUCT FROM AMPICILLIN

SIR,

We recently reported a polarographic method for determination of ampicillin, and its application to capsules and tablets.[1] It was based on the polarographic behaviour of an electroactive degradation product of the antibiotic. This product was not identified, but was suggested to be a 2,5-diketopiperazine derivate.

This letter reports the isolation and identification of the product. The hydrolysed ampicillin solution[1] was adjusted to pH 4 with sodium hydroxide, buffered with Sørensen's pH-5 citrate solution (42 g of citric acid and 204 ml of 2M sodium hydroxide diluted to 1 litre), and extracted repeatedly with ethyl acetate. The combined organic extract was evaporated to dryness and the crude product obtained was recrystallized from ethyl acetate. The product isolated had an uncorrected m.p. of 206–208°. Elemental analysis gave C 70.7%, H 5.5%, N 15.2%; $C_{11}H_{10}N_2O$ requires C 70.95%, H 5.41%, N 15.04%.

The spectroscopic characteristics were as follows: λ_{max} (in 0.3M hydrochloric acid) 380 nm; infrared bands (KBr discs) at 2800 cm^{-1} (OH); 1650 cm^{-1} (amide); 1617, 1295, 750 and 690 cm^{-1} (unassigned). The NMR spectrum (deuterochloroform; TMS external standard) gave δ 2.36 (3Hs), 7.42 (4Hm) 13.45 (1Hs).

The TLC R_f value on silica-gel G was 0.51 with chloroform:acetone (1:1) as solvent. These characteristics demonstrate that the electroactive product is 2-hydroxy-3-phenyl-6-methylpyrazine. A similar product was found in fluorescence studies by Barbhaiya *et al.*[2]

2-Hydroxy-3-phenyl-6-methylpyrazine exhibits a well-define pH-dependent reduction wave, with a half-wave potential of -0.55 V *vs.* SCE in 0.3M hydrochloric acid. The wave is irreversible and diffusion-controlled.

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24 December 1979

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Note. During consideration of this letter a paper appeared (A.G. Fogg and N.M. Fayad, Anal. Chim. Acta, 1980 113, 91) in which a similar concluaion was reached.

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TRACE ELEMENT DETERMINATION–I

USE OF 2,9-DIMETHYL-1,10-PHENANTHROLINE IN DETERMINATION OF COPPER IN HEAVY MATRICES BY CARBON FURNACE ATOMIC-ABSORPTION SPECTROMETRY

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(*Received* 2 *July* 1979. *Revised* 7 *January* 1980. *Accepted* 25 *February* 1980)

Summary—A method for the determination of copper in complex matrices by electrothermal atomic-absorption spectrometry has been developed. It uses neocuproine as complexing agent. The detection limit is 0.2 ng/ml, and interferences are minimized.

Atomic-absorption spectrometry has been found a useful and easy technique for the determination of trace elements, owing to its extremely low detection limit and high selectivity, but some problems arise in the analysis of heavy matrices. When the deuterium background corrector is used in such cases the determination limits are not as low as those obtained for light matrices. However, the standard addition method, widely used in connection with background correction to improve precision, appears to be reliable but time-consuming: see, for example, trace element determinations in fish tissues,[1] sea-water,[2,3] low alloys.[4] Owing to the increasing need for trace metal determination in connection with contamination, the problem of determining very low concentrations (often in the presence of complex matrices) frequently arises; this is the case for detection of trace metals in water. Determination by direct comparison with aqueous standards has been found impractical because of matrix interference.[5] Difficulties in analytical technique have been blamed for the lack of information on trace metals in the biochemical and geochemical cycles of the oceans.[2]

The present paper deals with trace copper determination in complex matrices: two samples from the harbour of Ancona (Italy) and Portonovo beach (a resort area near Ancona), and two BCS standard alloys of aluminium and iron with known copper content. 2,9-Dimethyl-1,10-phenanthroline (neocuproine)[6] is a highly selective complexing agent for copper(I)[7] and has frequently been used for its spectrophotometric determination, *e.g.*, in silicon and germanium,[8] aluminium and lead–tin alloys,[9] mineral oils,[10,11] water,[12] steels and alloys,[13] and biological matrices.[14] Owing to the limited sensitivity of colorimetric determinations, the lowest concentration of copper determined is about 25 ng/ml in sea-water.[12] The method we propose in this paper simply combines complexation with neocuproine, selective extraction, and electrothermal atomic-absorption analysis. The method offers two advantages over earlier procedures: first neocuproine is highly selective in comparison with APDC,[15] dithizone[16] or DDTC,[17,18] which require use of the standard addition method; secondly the procedure is very simple and the detection limit is 0.2 ng/ml, a remarkable improvement on the limits previously attainable (according to Cruz and Van Loon[19] the lowest achievable limit for direct copper determination in heavy matrices by the standard addition method is not less than 30 ng/ml by flame photometry and 12 ng/ml when electrothermal atomic absorption is used.

EXPERIMENTAL

Apparatus

Separatory funnels of borosilicate glass with Teflon plugs and polypropylene stoppers were used for extractions. A Perkin–Elmer model 300 atomic-absorption spectrometer, equipped with a Perkin–Elmer model 56 chart recorder, deuterium background corrector and HGA-2200 heated graphite atomizer, was used at the following settings: resonance line, 324.7 nm; bandpass, 0.7 nm; lamp current, 15 mA; scale expansion, 1 mV range for lowest concentrations; carbonization cycle, 800° for 30 sec; atomization cycle, 2400° for 5 sec. Argon was used as purge gas, at a flow-rate of 30 division on the flowmeter.

Reagents

Distilled water was passed through a suitable cation-exchange column (Merck Ion-Exchanger I; flow-rate 1 $ml.cm^{-1}.min^{-1}$): no detectable copper was found in control determinations with the general procedure described below. The chloroform and methyl isobutyl ketone were Merck "analysis grade" stock solvents with 10^{-6}% analysed copper content. The pH-4.7 buffer solution was 1*M* in acetic acid and sodium acetate. The reducing agent was 10% hydroxylamine sulphate solution. Both solutions were rendered copper-free by the extraction technique described in the general procedure. Sodium hydroxide solution (10%), and 10% and 30% v/v hydrochloric acid were prepared from the Merck "suprapur" compounds. A 0.1%

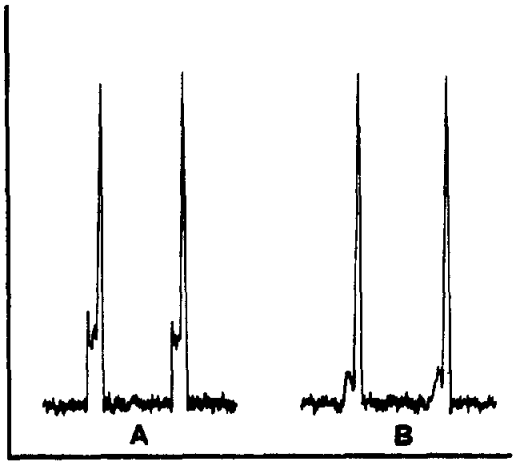

Fig. 1. Comparison between determinations of 7.5 ng/ml copper solution in organic phase with (B) and without (A) deuterium background correction.

neocuproine solution in alcohol was prepared from the Merck reagent. This solution is suitable for a few days but must be kept in the cold. Stock 100-ppm copper in Merck "suprapur" nitric acid: more dilute solutions were prepared fresh daily from the stock solution.

General procedure

A volume of 100 ml of aqueous copper solution of the appropriate concentration was exactly measured and placed in a separatory funnel and 1 ml of the hydroxylamine sulphate solution was added and the pH adjusted to 4.7 with buffer solution. The solution was vigorously shaken for a few seconds to ensure complete mixing and then 1 ml of neocuproine solution and 10 ml of solvent (chloroform or methyl isobutyl ketone) were added. The funnel was then vigorously shaken for about 1 min and the phases were allowed to separate completely (with gentle intermittent swirling). The organic phase was run off and the copper content of a 20-μl aliquot determined by atomic absorption. The detection limit was calculated as 0.14 ng/ml, this being twice the standard deviation of the background signal (10 replicates).

Copper extraction from sea-water

Samples were drawn with polyethylene bottles, immediately acidified with 5 ml of 30% hydrochloric acid per litre and filtered through 0.8-μm pore-size "Millipore" discs. A 100-ml portion of sample was placed in a separatory funnel, 5 ml of buffer solution and 1 ml of 10% hydroxylamine sulphate solution were added and the pH was adjusted to 5 with a few drops of 10% sodium hydroxide solution or hydrochloric acid. The solution was vigorously shaken to ensure complete reaction and then treated with 1 ml of 0.1% neocuproine solution and extracted with 10 ml of chloroform as already described.

Copper extraction from aluminium alloy (BCS 182/2)

A 50 mg sample was dissolved in 50 ml of *aqua regia*, and the solution was evaporated till fuming, after addition of 5 ml of concentrated sulphuric acid: the solution was cooled, diluted, and filtered free from any undissolved silicon–aluminium compounds and diluted to a volume of 1.00 litre. A 100-ml aliquot of this solution was then treated according to the general procedure.

Copper extration from low-alloy steel (BCS 403)

The procedure for the aluminium alloy was followed except that an additional tenfold dilution step was introduced before the final 100-ml fraction was taken, and three times as much hydroxylamine sulphate was used, because of the presence of large amounts of tervalent iron.

RESULTS AND DISCUSSION

The method proposed has four advantages: (*i*) the possibility of operating without background correction (Fig. 1); (*ii*) the high selectivity of the complexing agent; (*iii*) complete extraction of the copper-neocuproine complex by common solvents; (*iv*) ions forming coloured complexes (*e.g.*, nitrites) do not interfere. The completeness of separation in a single extraction and the possibility of using aqueous solutions for calibration (Fig. 2) greatly simplifies the procedure. Another advantage is the use of solvents such as chloroform or methyl isobutyl ketone which can be obtained with extremely low copper content, unlike propylene carbonate, and no oxidative impurities, unlike isoamyl alcohol; this avoids the need for complicated and lengthy purification. For the same reason, hydroxylamine sulphate, which can easily be freed from copper, is better than hydroquinone, which is necessary in certain colorimetric determinations.[12] The aqueous/organic phase-volume ratio of 10 gives a good concentration factor and is largely responsible for the low detection limits: Fig. 3 clearly shows the sensitivity. Table 1 shows the effect of some common cations on the copper determination: the small posi-

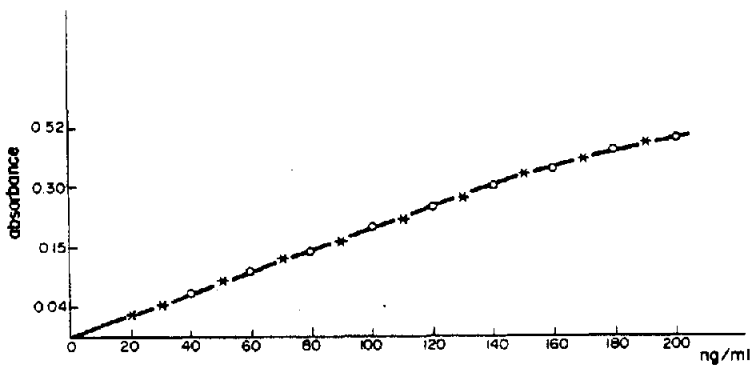

Fig. 2. Calibration curve drawn with results from aqueous (*) and organic (O) solutions. The identity of the two curves clearly shows the possibility of using aqueous standards.

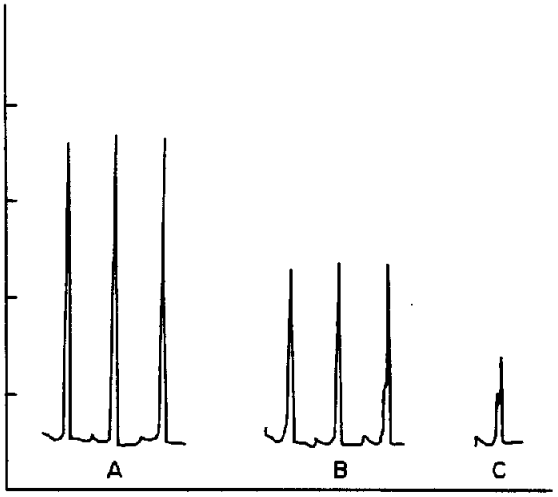

Fig. 3. Typical electrothermal atomic-absorption determination of copper. A: 1 ng/ml (10 ng/ml in the $CHCl_3$ phase); B: 0.5 ng/ml (5 ng/ml in the $CHCl_3$ phase); C: reagent blank to be subtracted.

tive differences are considered to be more likely to be due to traces of copper in the added salt solution than to interference. This view is supported by the evident lack of appreciable interference in the determination of copper in alloys: despite the high concentration ratio between the matrix and copper, no negative effect appears (Table 2). In the case of sea-water (Table 3) there is no interference from the matrix salts (which compel the use of the standard addition method in other procedures and preclude such low detection limits from being reached) or from nitrite (which can cause up to 80% error in spectrophotometric methods[7,12]).

Table 1. Effect of cations on determination of copper (10 ng/ml)

Cation	Added, *ppm*	Cu found, *ng/ml*	Error, %
Al^{3+}	20	9.8	−2
As^{3+}	20	10.0	0
Cd^{2+}	20	10.3	+3
Co^{2+}	20	10.1	+1
Cr^{3+}	20	9.9	−1
Pb^{2+}	20	10.1	+1
Mn^{2+}	20	10.3	+3
Ni^{2+}	20	10.5	+5
Sn^{2+}	20	10.2	+2
Zn^{2+}	20	9.6	−4
Fe^{2+}	20	10.1	+1
Fe^{2+}	200	10.2	+2
Al^{3+}	200	10.2	+2
Na^{+}	200	10.2	+2
K^{+}	200	10.4	+4
Ca^{2+}	200	10.1	+1
Mg^{2+}	200	9.8	−2

As chloride, sulphate, nitrate, nitrite or acetate, as convenient.

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Table 2. Determination of copper in BCS standard 182/2 (Al alloy with 11% Si: Cu content, 0.045%) and BCS standard 403 (low alloy steel: Cu content, 0.170%)

Sample	Cu found, %	Cu found, *ng/ml*	SD, *ng/ml* (10 replicates)
BCS 182/2	0.0454	22.7	0.9
	0.0452	22.6	1.1
	0.0454	22.7	0.9
BCS 403	0.176	88.2	1.6
	0.176	87.9	1.5
	0.176	88.0	2.1

Table 3. Determination of copper in sea-water (A, Porto Novo; B, Ancona harbour)

Sample	Cu added, *ng/ml*	Cu found,* *ng/ml* Present method	AAS†	Spectrophotometry§
A	—	9.8 (1.3)	9.6 (1.1)	7
A	10	19.5 (1.1)	19.6 (1.4)	17
A	20	29.4 (1.4)	29.6 (1.1)	27
B	—	22.7 (2.5)	22.6 (1.6)	22
B	10	32.5 (1.4)	32.6 (1.0)	31

* Standard deviations (10 replicates) shown in brackets.

† Copper extracted with APDC into MIBK at pH 3–4 and determined by electrothermal AAS with deuterium-lamp background correction and use of the standard addition method.[2]

§ Measurement at 457 nm.

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PREPARATION AND PROPERTIES OF MACRORETICULAR RESINS CONTAINING THIAZOLE AND THIAZOLINE GROUPS

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Summary—Three macroreticular polystyrene-based resins with amino- or imino-thiazole and thiazoline groups as the functional groups have been prepared. The resins are highly stable in acid and alkaline solutions and have high selectivity for mercury(II). In the presence of hydrochloric acid, sorption of mercury attains equilibrium fairly rapidly, the time for 50% uptake of mercury being 3–6 min. There are practically no interferences. In a column operation, mercury is quantitatively recovered by elution with 0.1*M* hydrochloric acid containing 5% thiourea. The thiazoline resin column can be used to concentrate mercury from sea-water.

Thiourea, which forms metal complexes with heavy metal ions in acidic media has been utilized as a masking agent in complexometric titrations.[1] Some chelating resins containing thiourea derivatives as the functional groups have been prepared and their ion-exchange behaviour toward several heavy metal ions explored.[2,3] However, thiourea groups on the resins gradually decompose in strongly acidic media.[3] This is a serious disadvantage in long-term use because the resins are sometimes treated with strong acids to desorb metal ions. On the other hand, Koster and Schmuckler[4] have prepared a chelating resin containing thiouronium salt groups, and have applied it for the separation and recovery of noble metals. It seems that this group is more unstable than thiourea itself to acid and alkaline solutions.

It would be of interest to prepare more stable resins which still possess the thiourea function. In this paper, new macroreticular (MR) resins containing thiazole or thiazoline groups are presented. These resins were prepared by the reaction of chloroacetyl groups on styrene–divinylbenzene copolymer beads with thiourea or its methyl derivatives. As expected, the resins show good stability and high efficiency for sorption of mercury(II).

EXPERIMENTAL

Apparatus and reagents

The infrared spectra were measured on a JASCO model DS-701G spectrophotometer, with potassium bromide discs. The surface properties of the resins were measured by the BET method with a Sorptomatic-1800 (Carlo Erba). The radioactivity was measured with a scintillation counter (Aloka Co., Ltd., model TDC-501) equipped with a well-type sodium iodide crystal detector. The metal ion solutions (0.1–1*M*) were prepared from reagent-grade nitrates, except for iron(III) (chloride) and copper(II) (sulphate), and standardized by complexometric titration. The radioisotope ^{203}Hg was supplied by the New England Nuclear Corp. and was used as the tracer.

Preparation of resins

Resin III. Twenty-five g of resin II[5] (Cl, 16.7%) were added to 150 ml of dimethylformamide and stirred for 1 hr at room temperature, then 100 ml of 20% aqueous thiourea solution were added, and the mixture was heated at 80° for 6 hr with stirring. The product was filtered off, then washed with 1*M* sodium hydroxide, water and methanol, successively, and dried in a vacuum desiccator. A light brown resin (*ca.* 28 g) was obtained.

Resin IV. This was prepared from resin II (25 g) and 100 ml of 20% aqueous monomethylthiourea solution according to the procedure described above. A light brown resin (*ca.* 29 g) was obtained.

Resin V. Resin II (25 g) and *N,N'*-dimethylthiourea (20 g) were treated according to the procedure described for resin III. Yield, 30 g.

Stability of resins

A 1-g portion of each resin was shaken with 50 ml of acid or alkaline solution of various concentrations for 7 days, then filtered off and washed with water. After the acid treatment, the resins were washed first with 1*M* sodium hydroxide and then with water until the washings became neutral. After drying, the nitrogen and sulphur contents and sorption capacity for mercury were determined.

Sorption of metal ions on resins

Unless otherwise stated, the batch method was applied, as follows. To a glass-stoppered test-tube containing 100 mg of dry resin, 9 ml of 0.25*M* hydrochloric acid–0.25*M* sodium acetate solution (adjusted to pH 1–5) were added. When this mixture had equilibrated, 1 ml of 0.1*M* 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 by complexometric titration.

Recovery of mercury

Resins III, IV and V on which mercury(II) containing ^{203}Hg had been sorbed were shaken with various desorbents (hydrochloric acid, nitric acid, *etc.*) for 2 hr. After filtration, the amount of mercury in the filtrate was determined by scintillation counting.

Column operation with resin V

A column (1 × 10 cm) containing 2 g of resin V was

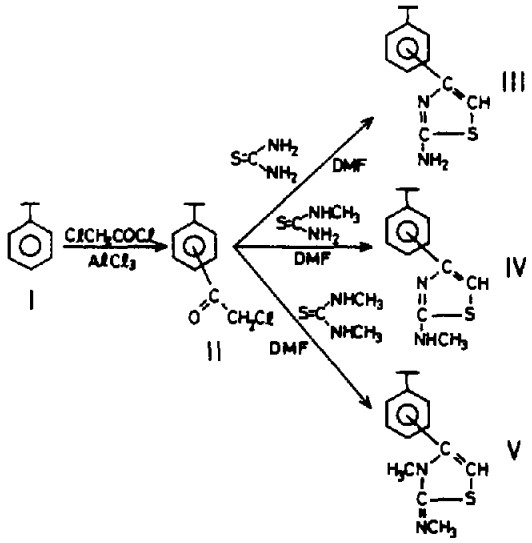
equilibrated with 0.1*M* hydrochloric acid and then 1 litre of mercury(II) solution, prepared by adding a trace amount of mercury(II) containing ^{203}Hg to sea-water adjusted to pH 1 with hydrochloric acid, was passed through the sorbent at a flow-rate of 3 ml/min. After the column had been washed with 50 ml of sea-water adjusted to pH 1, the sorbed mercury was eluted with 0.1*M* hydrochloric acid containing 5% thiourea.

RESULTS AND DISCUSSION

Preparation and characterization of resins

The resins used in this study were prepared from 35–100 mesh MR styrene–divinylbenzene copolymer beads[6] by the steps shown in Scheme 1. The elemental analyses and infrared spectra of resins I–V are shown in Table 1 and Fig. 1, respectively.

The absorption bands at 1670 cm^{-1} ($\nu_{C=O}$) and 650 cm^{-1} (λ_{C-Cl}), which are observed for resin II, are absent for resins III, IV and V. Absorption bands at



Scheme 1

Table 1. Analytical data for resins

Resin	Analysis, %*		
	Cl	N	S
II	16.7	—	—
III	0	10.8 (11.9)	12.5 (13.6)
IV	0	10.5 (11.2)	12.6 (12.8)
V	0	10.3 (10.6)	12.1 (12.2)

* Values in parentheses indicate the calculated values based on the Cl content of resin II.

3420 cm^{-1} and 3340 cm^{-1} (ν_{NH_2}) are observed for resin III. Resin IV shows an absorption band at 3400 cm^{-1} (ν_{NH}) and resin V shows bands at 2760 cm^{-1} (ν_{N-CH_3}) and 1620 cm^{-1} ($\nu_{C=N}$). The sulphur and nitrogen contents agree with the calculated values based on the chlorine content of resin III, and the molar ratio of sulphur to nitrogen was 1:2 in resins III, IV and V. These spectral changes and the elemental analyses correspond to the reaction scheme presented.

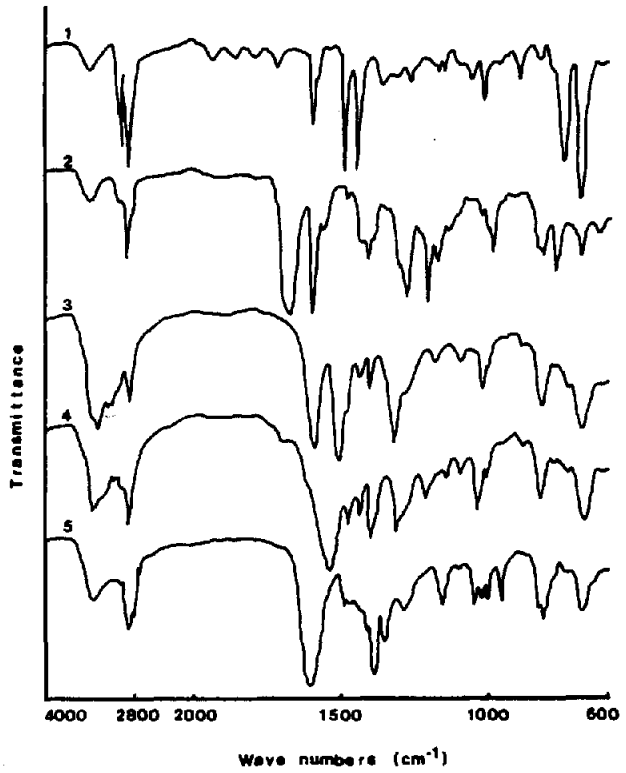


Fig. 1. Infrared spectra of resins in KBr disks. 1, resin I; 2, resin II; 3, resin III; 4, resin IV; 5, resin V.

Table 2. Physical properties of resins

Resin	Specific surface area, m^2/g	Pore volume, cm^3/g	Average pore diameter, nm	Water regain, g/g
I	19	0.43	135.8	—
II	29	0.073	15.5	—
III	46	0.068	8.9	1.10
IV	41	0.073	10.7	1.13
V	36	0.080	13.3	1.14

The chemical stability of the resins in 1–5*M* hydrochloric acid, nitric acid, perchloric acid and sodium hydroxide solution was examined. No significant changes in nitrogen and sulphur contents were observed and the sorption capacities for mercury(II) were not reduced, even by the treatment with strongly acid solution. From these facts, it is clear that the resins are sufficiently stable.

Physical properties of the resins are listed in Table 2. The surface area increases with chemical modification, while the pore volumes and average pore diameters become smaller than those of the original beads. In addition, the hydrophilicity of resins III, IV and V is increased by the introduction of thiazole or thiazoline groups, and a large water regain is obtained.

Sorption and desorption of metal ions

The sorption behaviour of metal ions such as manganese(II), iron(III), cobalt(II), nickel, copper(II), zinc, cadmium and mercury(II) on resins III, IV and V in the batch method is shown in Figs. 2–4. Most chelating resins containing sulphur and nitrogen atoms in their functional groups are liable to sorb both copper(II) and mercury(II).[2,6,7] It is found that resins III, IV and V have higher selectivity only for mercury. However, in the case of resin V, cadmium is also sorbed at low pH. This behaviour may be attributed to anion-exchange of negatively charged cadmium chloro-complexes, because the resin seems to have higher basicity than resins III and IV. The maximum sorption capacities of resins III, IV and V for mercury were 1.8, 1.9 and 2.8 mmole/g, respectively, when 100 mg of each resin and 10 ml of 0.1*M* mercury solution in 0.1*M* hydrochloric acid were used.

The effect of shaking time on the sorption of mercury is shown in Fig. 5. The time required for 50% uptake of mercury from 0.01*M* mercuric nitrate in hydrochloric acid and in an acetate buffer is found to be 3–6 min and 8–10 hr, respectively. When rapid sorption of mercury is required, the presence of hydrochloric acid is desirable. It can be assumed that mercury is mainly sorbed by anion-exchange in hydrochloric acid solution and is sorbed by complexation from the acetate buffer. Since it has already been found that the presence of chloride ion strongly reduces the amount of mercury sorbed on MR polymer beads by a physical mechanism,[8] the sorption of mercury on resins III, IV and V, as shown in Figs. 2–4, seems not to be physical in nature.

In the batch method, the presence of diverse metal ions such as cobalt, nickel, copper, zinc, strontium, cadmium, barium, lead and uranium(VI) did not interfere with the sorption of mercury. Similar results

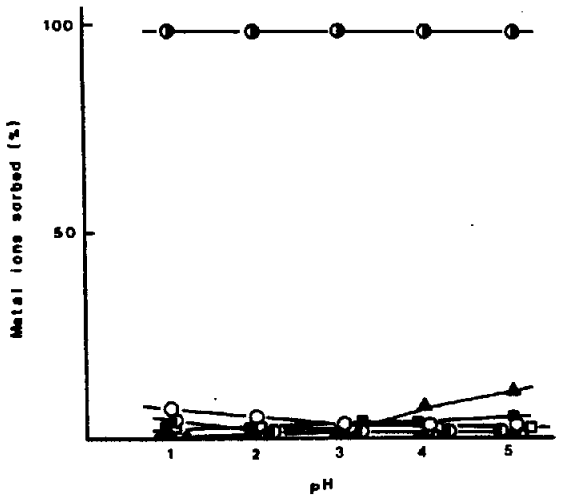


Fig. 2. Effect of pH on the sorption of metal ions with resin III. Shaking time 24 hr. ■: Mn(II); ◐: Fe(III); □: Co(II); ●: Ni(II); ▲: Cu(II); △: Zn(II); ○: Cd(II); ◑: Hg(II).

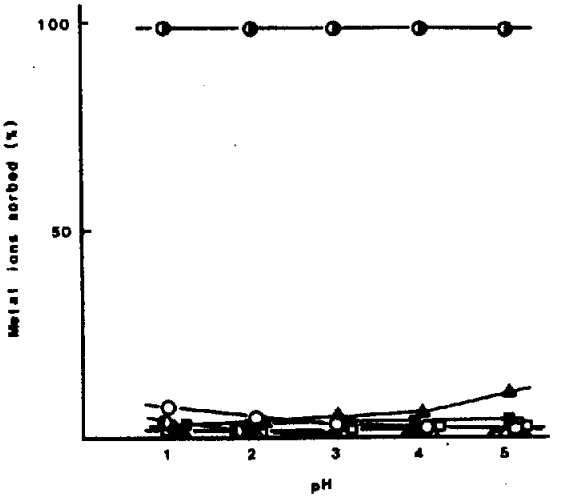


Fig. 3. Effect of pH on the sorption of metal ions with resin IV. Conditions and symbols are the same as those in Fig. 2.

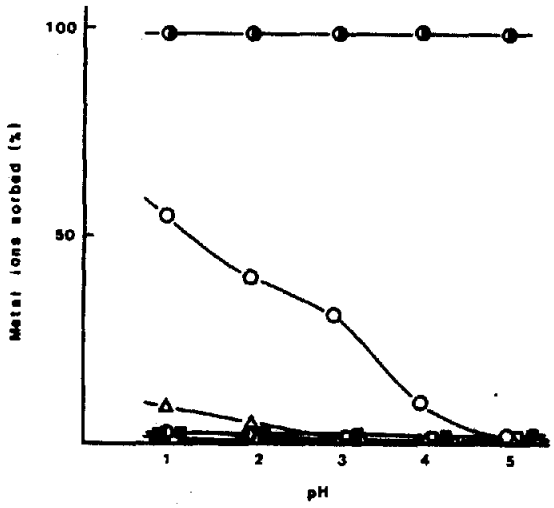


Fig. 4. Effect of pH on the sorption of metal ions with resin V. Conditions and symbols are the same as those in Fig. 2.

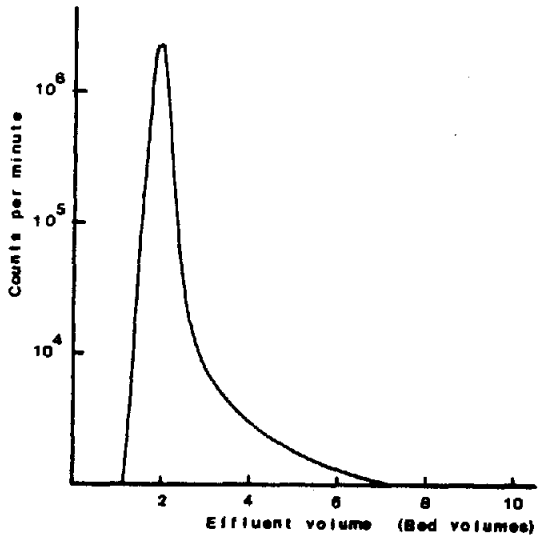


Fig. 6. Typical elution curve for mercury. Column, 1 × 10 cm; resin V, 2 g; flow-rate, 0.5 ml/min.

were obtained in the presence of neutral salts (sodium chloride, nitrate, sulphate and thiocyanate). The non-interference of sodium chloride is of interest for application of the results in the concentration of mercury from highly saline solutions.

The breakthrough experiments were carried out by the column method, on sea-water at pH 1, containing 20 ppm mercury and ^{203}Hg tracer. In the case of resin III, about 120 bed-volumes of feed solution could be passed through without any leakage of mercury into the effluent, and in the case of resin V, up to about 640 bed-volumes. On the basis of these results, resin V can be useful for preconcentration of trace amounts of mercury from highly saline medium.

Mercury was recovered from resins III, IV and V by the batch method, and the results are listed in Table 3. They indicate that mercury can be recovered with 0.1*M* hydrochloric acid or perchloric acid con-

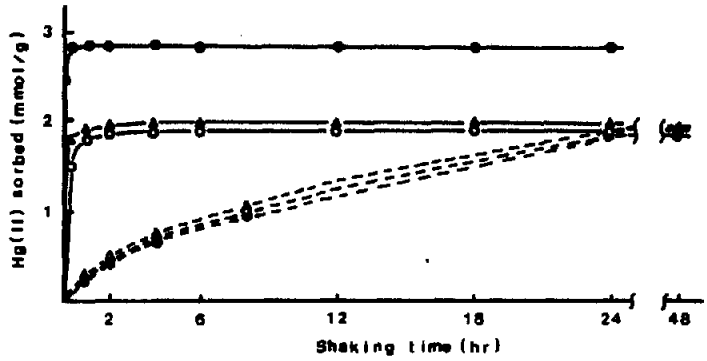


Fig. 5. Effect of shaking time on the sorption of mercury with resins III, IV and V. Hg(II) solution, 0.01*M*, 100 ml; resin, 100 mg ——, 0.1*M* HCl; -----, acetate buffer, 0.2*M* acetic acid–0.2*M* sodium acetate (pH 3.5); ○, resin III; △, resin IV; ●, resin V.

Table 3. Recovery of mercury from resins III, IV and V

Resin	Recovery of mercury, %				
	5*M* HCl	5*M* HNO_3	5*M* $HClO_4$	5% Thiourea in 0.1*M* $HClO_4$	5% Thiourea in 0.1*M* HCl
III	40.8	61.7	59.8	95.1	94.1
IV	27.1	54.6	56.5	94.8	91.0
V	27.7	70.8	83.4	95.6	94.0

taining 5% thiourea. The concentration of mercury from sea-water was examined by the column method. One litre of sea-water containing a tracer amount of mercury(II) and adjusted to pH 1 with hydrochloric acid was passed through the column under the same conditions as in the break-through experiments, and the sorbed mercury was recovered with 0.1 *M* hydrochloric acid containing 5% thiourea. As shown in Fig. 6, mercury was completely eluted with less than 8 bed-volumes of eluent.

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OPTIMIZATION OF THE DETERMINATION OF SELENIUM BY ATOMIC-ABSORPTION SPECTROMETRY: COMPARISON OF TWO HYDRIDE GENERATION SYSTEMS

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Summary—Two commercially available systems for the determination of selenium by hydride-generation and atomic-absorption spectrometry were compared. Chemical and physical parameters were optimized both for an electrothermally heated closed atom-cell method and a flame-heated open-cell technique. Both systems were evaluated with respect to performance and applicability to determination of traces of selenium.

The role of selenium in man has aroused increasing interest concerning the presence of this element in human tissues and other biological materials, in environmental matrices (air, water, soil, plants) and human diet.

In recent years the determination of selenium as the gaseous hydride, H_2Se, by atomic-absorption techniques has gained widespread application. Various techniques have been described with regard to the generation, storage, injection and decomposition of hydrides. The hydrides can be decomposed in a flame or tube furnace. A comprehensive review has been presented by Ihnat.[1]

The present paper describes a comparison of two techniques for the determination of selenium at nanogram levels, based on hydride generation and atomic-absorption spectrometry (AAS). One system is semi-automated and uses an electrically heated quartz tube instead of the flame or graphite furnace in conventional AAS. The other also uses a quartz tube as the atomization cell, but it is heated by an air–acetylene flame.

The same chemical reaction is used in both systems. The volatile hydrides are produced (along with hydrogen) by reaction with sodium tetrahydroborate in acid medium, after the system has been purged with an inert gas to expel all air from the reaction vessel and the atomization cell. This purging is of great importance in the case of selenium, which has its resonance line in the far ultraviolet region of the spectrum, where absorption by air is considerable. A second reason for purging air from the system is the risk of explosive combustion of hydrogen–air mixtures.

The two systems differ chiefly in the way atomization is achieved, and by the fact that the semi-automated system is completely enclosed whereas the other, which is manually operated, is open. The influence of variation of the chemical and physical parameters on the sensitivity and precision is discussed.

EXPERIMENTAL

Apparatus

The hydride-generation assemblies. The two hydride-generation devices were the Perkin–Elmer MHS-1 electrothermal system and the MHS-10 flame-heated tube system.

The MHS-1 uses a high-purity quartz tube (120 × 12 mm) closed at both ends by quartz windows; it can be heated to 1000° and is easily replaceable. This atomization device is mounted in the sample compartment of the spectrometer and is carefully aligned in the light-beam to give maximum light transmission. The control module allows the selection of four different analytical programmes, which differ only in the duration of the purge and of the analytical measurement cycle. The sample and reagent solution are mixed by an electronically operated magnetic stirrer.

The MHS-10 system has no electronics and consists of a very simple analyser device that is operated pneumatically. The quartz tube (165 × 12 mm) is placed in an air–acetylene flame, about 5 mm above the slot of a 10-cm single-slot burner head. Both ends are provided with graphite rings that cool them to avoid ignition of the hydrogen–air mixture formed by diffusion of the hydrogen into the ambient air. Addition of reductant solution to the sample is initiated manually with a plunger. The initial gas flow is divided and gas passes into both the reductant reservoir and the sample vessel. The pressure thus built up in the reductant vessel forces the sodium tetrahydroborate solution through a connecting Teflon tube into the sample solution. The conical shape of the sample container, the deep immersion of the reductant inlet tube in the sample solution, and the violent generation of hydrogen, ensure efficient mixing of sample solution and reagent.

The AAS systems. The MHS-10 system was combined with a Perkin–Elmer model 360 atomic-absorption spectrometer, with a standard air–acetylene single-slot burner and UDR-3 digital read-out system. A slightly blue flame

* Aspirant of the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek.

was used for heating the atomization cell, which was held by a metal frame with hooks so that the ends of the cell were not heated directly by the flame.

The MHS-1 system was operated in a Perkin–Elmer model 372 atomic-absorption spectrometer with the quartz cell assembly mounted in place of the burner. The cell position was optimized with the quartz windows removed. The quartz windows can be cleaned when necessary by boiling in nitric acid for 5 min and then rinsing with doubly distilled and demineralized water, drying with hot air, and finally polishing with lens tissue. Light-absorption by clean windows should not exceed 0.15 absorbance unit. Selenium electrodeless discharge lamps were used, with a Perkin–Elmer dual EDL power supply. Background correction proved unnecessary in most cases. Time constants were chosen so as to give a satisfactory compromise between peak shape and sensitivity.

The recording systems. The PE 360 spectrometer was fitted with a Bryans 28,000 *y,t* recorder. Its response time of approximately 0.5 sec made it suitable for recording transient signals. The PE 372 spectrometer was used with a Perkin–Elmer model 023 strip-chart recorder adapted for external drive by the MHS-1 control module. Only the analytical phase of the determination was recorded. Table 1 gives the main instrumental settings used.

Reagents and gases

All acid solutions except hydrochloric acid were prepared from extremely pure concentrated acids by dilution with doubly distilled and demineralized water. They were stored in either dark glass bottles or polyethylene flasks. Merck suprapur sulphuric, perchloric and nitric acids and *pro analysi* hydrochloric acid were used.

A 1000-ppm selenium standard (Pierce Inorganics) was diluted with 6*M* hydrochloric acid to give a 2.0-ppm working standard. The working solution (prepared daily) was stored in 10-ml polystyrene tubes, sealed with polyethylene stoppers. Aliquots (50 μl) were taken with Eppendorf or Finnpipette micropipettes. They contained 100 ng of Se^{4+}, with which all parameters were optimized. Occasionally a 0.2-ppm standard solution was prepared.

Sodium tetrahydroborate solutions were prepared from powder from the 96% analytical grade solid (Pierce Inorganics), weighed into a polypropylene beaker, on top of sodium hydroxide pellets (used for stabilization) and dissolved in doubly distilled demineralized water. Care was taken to avoid contact with metal, a porcelain spatula and polyethylene stirring bars being used. The solution was filtered into a polyethylene bottle and stored under refrigeration until use. High-purity argon containing less than 0.1 ppm oxygen (L'Air Liquide) was used to purge the atomization cells.

Procedure

The parameters were varied one at a time. The standard procedure consists of the addition of 100 ng of Se^{4+} to 10 ml of acid. This order of addition results in better sensitivity and precision than the reverse sequence of addition. The vessel is then connected to the hydride-generation manifold, air is purged from the system, the reductant is added and stirring started (in the MHS-1 system), and the transient absorption signal is recorded on the *y, t* recorder.

Reagent blank solutions are also measured and their absorbance is subtracted from the Se signal.

RESULTS AND DISCUSSION

Effect of various reductant and hydrochloric acid concentrations

Solutions of sodium tetrahydroborate need stabilization to prevent hydrolysis, and sodium hydroxide can be used for this purpose. It was found that the concentration of stabilizing agent is not critical, but too high a concentration increases the solution viscosity unfavourably and gives rise to unreproducible and less sensitive determination because it reduces the acid concentration to below the optimum level for the reduction. Solutions stabilized with 2% w/v sodium hydroxide can be stored for at least 8 weeks without change in the reductant activity. Figures 1a and 1b represent the average peak heights for 100 ng of Se^{4+}, as a function of acid concentration at various concentrations of the sodium tetrahydroborate solution. Table 2 gives the precisions obtained. In hydrochloric acid the sodium tetrahydroborate reacts according to the equation

$$BH_4^- + H^+ + 3H_2O \rightleftharpoons H_3BO_3 + 4H_2.$$

The generation of hydrogen selenide proceeds according to

$$4H_2SeO_3 + 3BH_4^- + 3H^+ \rightleftharpoons 4H_2Se + 3H_3BO_3 + 3H_2O.$$

The acid concentrations required for both the neutralization of the stabilizing agent and for the quantitative generation of hydrogen were calculated and agree fairly well with the results obtained as can be seen

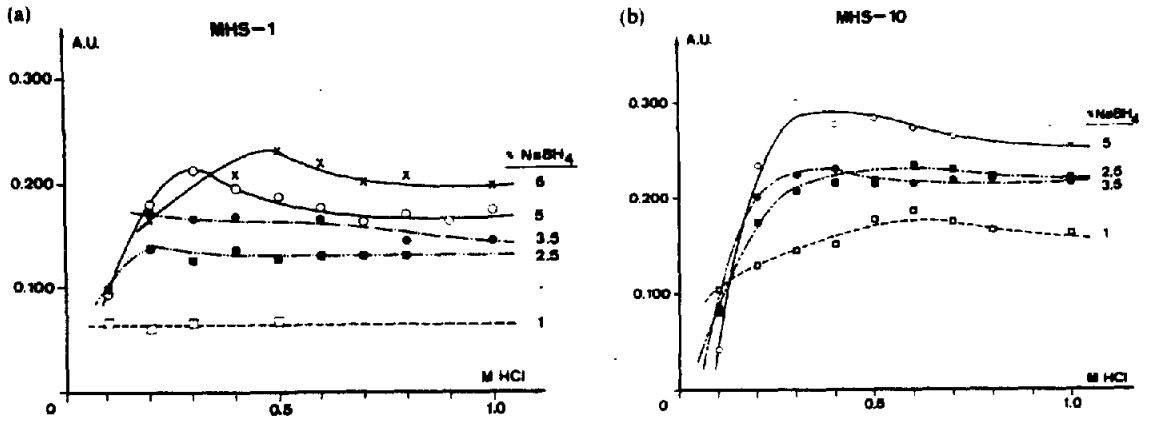

Fig. 1. Influence of increasing concentration of $NaBH_4$ on the signal from 100 ng of Se, at different concentrations of hydrochloric acid. Volume 10 ml, 5 replicates. (a) MHS-1; (b) MHS-10.

Table 1. Instrumental parameters

Lamp	EDL
Lamp power	6.5 W
Wavelength	196.0 nm
Band-pass	2.0 nm
Mode	absorbance
Signal	continuous
Damping	1 sec
Background correction	none
Flow-rates (MHS-10)	air 11.5 l./min acetylene 2.3 l./min

from Figs. 1a and 1b and Table 2. Acid concentrations below the calculated optimum give rise to lower sensitivity and poor reproducibility, owing to incomplete and unreproducible reaction of the sodium tetrahydroborate. The hydrogen acts as an additional carrier gas and its vigorous generation sweeps the hydride into and out of the atomization cell without undue diffusion. Figure 2 shows the influence of the generation rate. Slow evolution of hydrogen (low concentrations of acid and tetrahydroborate) cause the peak to tail and also to fail to return to the base-line in the case of the MHS-10. Tailing can be considered as resulting from dilution of the atom population within the atomization cell and this effect is more pronounced in the open-ended system. On the other hand, the generation of hydrogen must be controlled. Too vigorous an evolution causes extremely sharp but unreproducible signals, as can be demonstrated by the use of 10% sodium tetrahydroborate solution. In this case the rate of generation of hydrogen and its transfer to the atomization cell probably exceeds the rate of formation of hydrogen selenide considerably. Hydrochloric acid concentrations higher than the optimum for the generation of hydrogen exert little influence on the sensitivity provided the sodium tetrahydroborate concentration does not exceed 3.5%. At higher concentrations of the reductant there is a distinct optimal acid concentration, and more acid than this depresses the selenium signal. This effect is most pronounced in the MHS-1 system which does not allow diffusion of the acid vapours into the atmosphere. In some instances acid vapours condense in the exhaust hoses. The deposition of these heavy drops may impair removal of the atomized hydride and thus explain some of the unreproducible results (see Table 2). The choice of appropriate concentrations of both acid and reductant is generally determined by the precision required and to a lesser extent by the sensitivity. In the closed system (MHS-1) the use of a 5% sodium tetrahydroborate solution and 10 ml of 0.4–0.5*M* hydrochloric acid yields satisfactory results. Signals obtained by use of 0.3*M* hydrochloric acid were much less reproducible. The use of 6% tetrahydroborate solution may be advantageous when working near the detection limit, where a small gain in sensitivity may be important. The fact that the flame-heated furnace system is an open one limits the concentration of sodium tetrahydroborate that can be used. If this concentration exceeds 4%, the excess of hydrogen diffuses into the ambient air, yielding an inflammable hydrogen–oxygen mixture. The graphite rings cannot prevent ignition at very high concentrations of hydrogen, flames form at the ends of the quartz tube and erroneous measurements occur. A 3.5% solution was chosen for further work with the MHS-10 system.

Effect of different acids on the signal from 100 ng of Se^{4+}

To select the most suitable acid with respect to peak height, reproducibility and blank values, three commonly used acids were tested and compared with hydrochloric acid. Sodium tetrahydroborate concentrations of 5 and 3.5% were used with the MHS-1 and MHS-10 systems respectively. Figures 3a and 3b show the results, and Table 3 gives the relative standard deviations.

The use of acids other than hydrochloric offers no advantages. The sensitivity and precision remain good throughout a wide concentration range for sulphuric acid, but ultrapure acid needs to be used to minimize the blank values. High concentrations of nitric or perchloric acid strongly suppress the selenium signal, the effect being most pronounced in the closed MHS-1 system. This could indicate that interference mainly takes place during the atomization step of the analysis and is less likely to be due to oxidation of selenium to the sexivalent state, which cannot be determined by HGAAS.

The use of hydrochloric acid deserves recommendation both from an analytical and from an economical point of view.

Effect of temperature

The energy needed for dissociation of hydrogen selenide to yield an atomic vapour of selenium is provided by an air–acetylene flame in the MHS-10 system and by electric heating in the MHS-1 system. The choice of the flow-rates of air and acetylene is determined by the physical characteristics of the quartz tube. A lean blue oxidizing flame produces temperatures approaching the melting point of quartz and causes rapid deterioration of the tube. The gas flows need to be adjusted to provide a flame that is only

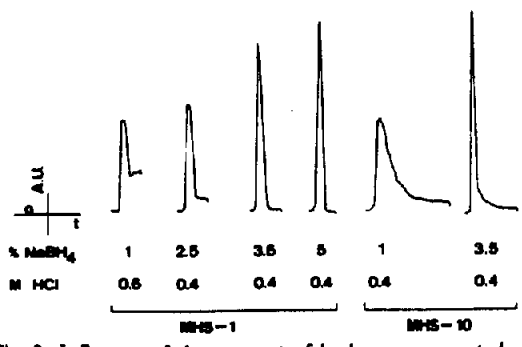

Fig. 2. Influence of the amount of hydrogen generated on the shape of the signal from 100 ng of Se.

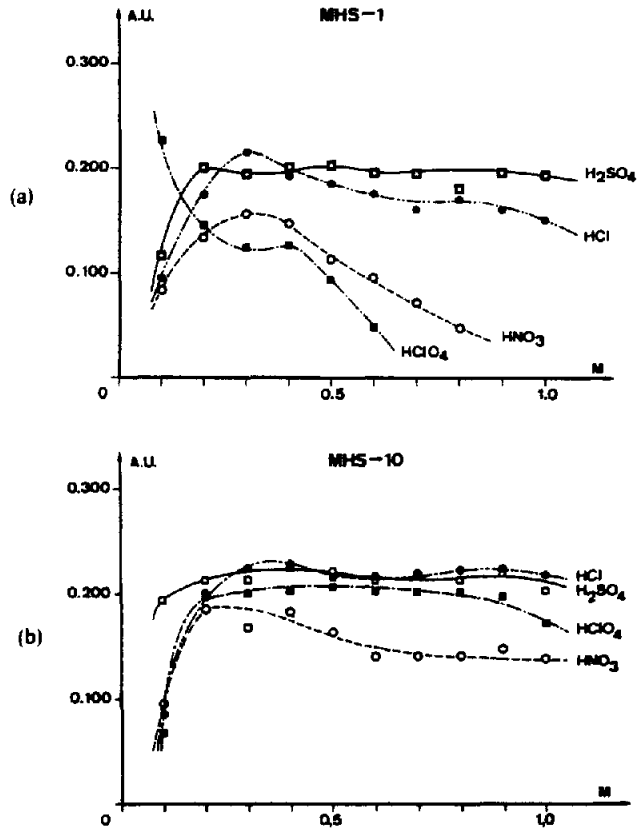

Fig. 3. Influence of the concentration of H_2SO_4, $HClO_4$, HCl, and HNO_3 on the signal from 100 ng of Se. V_{acid} = 10 ml. (a) MHS-1; c_{red} = 5%. (b) MHS-10 c_{red} = 3.5%.

slightly oxidizing and a temperature of about 830° within the cell. The temperature in the MHS-1 system was measured with a Cr/Al thermocouple, and differed from those specified by the manufacturer, especially in the low temperature range. Signals obtained for 100 ng of selenium with the MHS-1 at different temperature settings can be judged from Fig. 4. Below approximately 700° insufficient energy is supplied to break down the hydrogen selenide molecules. Between 700° and 800° only partial atomization is achieved, and temperatures higher than 950° reduce the peak heights. This last effect could be due to dilution effect caused by expansion of the argon–hydrogen carrier-gas mixture.[2] The temperature selected for further work with the MHS-1 system was approximately 850°.

Effect of gas flow-rates

Both systems use an inert gas such as argon or nitrogen to expel air, thus allowing measurements to be made in the far ultraviolet region. During the analytical phase of the procedure, the purge gas flow is reduced by a built-in pressure regulator to about half its initial value, for sweeping the hydride into the atomization cell.

Figure 5 shows dependence of the absorption signal from 100 ng of selenite on argon flow during the determination step. The two systems differ remarkably with regard to the dependence on flow-rate, the MHS-1 system being unaffected whereas the flow-rate is critical in the MHS-10 system. The optimum argon flow-rate is $\geqslant$400 ml/min. Peak-shape remains unaltered in the MHS-1 system whereas tailing increases slightly with decreasing flow-rate in the MHS-10 system. The difference is presumably due to the design of the systems, dilution effects being more likely to occur in the open system, thus causing peaks to tail.

The fact that even very low flow-rates suffice for optimal absorption signals to be obtained in the closed (MHS-1) system illustrates the importance of the hydrogen generated as an additional carrier-gas that adds to the efficient transfer of the hydride into the quartz tube. However, too low a flow-rate may be inadequate to remove the decomposition products from the tube and could thus give rise to memory effects. Therefore an analytical flow of 250 ml/min was chosen for further work.

Effect of sample volume

Both systems exhibit strong dependence on the total volume of solution in which the analyte is present. Figures 6a and 6b show the average peak-heights as a function of the solution volume, for concentrations of selenium situated in the linear part of the calibration curve (100 ng) and near the detection

Table 2. Relative standard deviations for 100-ng Se signals at different concentrations of $NaBH_4$ and of HCl (5 replicates)

[HCl] *M* \ [$NABH_4$], % *w/v*	1		2.5		3.5		5		6
	MHS-1	MHS-10	MHS-1	MHS-10	MHS-1	MHS-10	MHS-1	MHS-10	MHS-1
0.1	5.2*	5.0*	51.0†	17.1	—	25.3	36.9†	50.7	—
0.2	4.7*	3.9*	2.9	5.9	7.2†	2.7	15.5†	6.1‡	19.5†
0.3	5.1*	4.1*	4.4	4.9	4.2†	5.5	2.7†	2.9†	—
0.4	—	1.5*	4.9	4.1	1.2	5.0	4.1	1.9‡	15.3†
0.5	5.1*	5.9*	5.5	2.7	—	4.7	3.5	3.6‡	1.4
0.6	—	1.0*	2.4	4.3	2.8	1.9	1.0	5.2‡	2.3
0.7	—	4.1*	7.7§	3.8	—	2.1	2.2	—	3.5
0.8	—	—	4.3§	2.7	6.0	4.5	3.0	6.8	7.2§
0.9	—	—	—	1.1	—	5.7	2.5	—	—
1.0	—	14.0*	—	4.6	5.5	2.4	6.6	4.4	10.0§

* Abnormal tailing of the peak or inability to return to zero absorbance after atomization.
† Additional evolution of hydrogen on addition of acid to the solution after analysis.
‡ Flame formed at the ends of the atomization cells; such measurements were not included in the calculation of the precision.
§ Condensation of acid in Teflon exhaust hoses.

Table 3. Relative standard deviation for 100-ng Se signals at different concentration levels of HCl, HNO_3, $HClO_4$, H_2SO_4. Volume 10 ml

acid \ conc *M*	HCl		H_2SO_4		HNO_3		$HClO_4$	
	MHS-1	MHS-10	MHS-1	MHS-10	MHS-1	MHS-10	MHS-1	MHS-10
0.1	36.9†	*25.3*	45.0†	*9.9*	32.6†	*7.2*	24.1†	*8.6*
0.2	15.5†	*2.7*	2.0†	*3.1*	36.5†	*5.2*	3.3†	*3.6*
0.3	2.7†	*5.5*	4.0	*2.7*	7.4†	*9.3*	13.2†	*5.7*
0.4	4.1	*5.0*	3.5	*6.6**	6.7	*4.3*	5.4	*5.4**
0.5	3.5	*4.7*	3.2	*3.6**	3.4	*7.1*	14.6*	*4.4*
0.6	1.0	*1.9*	4.1	*2.8**	12.8	*3.0*	12.8*	*2.4*
0.7	2.2	*2.1*	3.4	*4.8**	18.1	*7.6*	—	*4.0*
0.8	3.0	*4.5*	1.6	*3.2**	29.3	—	—	*2.6*
0.9	2.5	*5.7*	5.2	*5.7**	—	*6.2*	—	*3.5*
1.0	6.6	*2.4*	3.9	*5.5**	—	—	—	*6.0*

* Abnormal tailing of the peak.
† Additional evolution of hydrogen on addition of more acid to the solution after analysis.

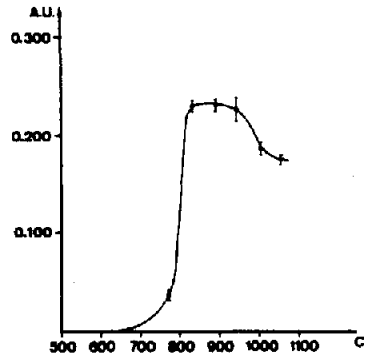

Fig. 4. Effect of cell temperature on the sensitivity (MHS-1); 100 ng of Se.

limit (5 ng), where instrumental scale-expansion is necessary.

The minimum volume that can be used is determined by the ability of the system to provide thorough mixing of the solution with the reductant, and this depends mainly on the shape of the reaction vessel. It can be seen that increasing the volume will cause a sharp fall in sensitivity, when aqueous standard solutions are measured. The optimal volumes are 10 ml for the MHS-1 system and 5 ml for the MHS-10. Use of 15 ml in the first case and 10 ml in the second will cause the signal to decrease by approximately 15%, whereas increasing the volume to 60 ml and 40 ml respectively will yield a signal that amounts to only 40–50% of the optimal signal. The peaks tend to broaden with increasing solution volume, indicating a delayed release of the hydride from the solution. It is clear that the loss in sensitivity may be due to inadequate and inhomogeneous mixing of the reductant solution with the acid and to partial dissolution of the gaseous hydride in the solution on its way to the atomization cell.

Although the precision of the measurements remains satisfactory throughout the whole volume range (RSD $< 5\%$) it is most important to keep the volumes as constant as possible for replicate analyses, but a variation of ± 1 ml is permissible.

In view of the fact that dilution of a "real" sample may affect the peak height beneficially by reducing matrix interferences, it should be stressed that comparison with aqueous standards is only permissible when the total volumes for samples and standards are the same.

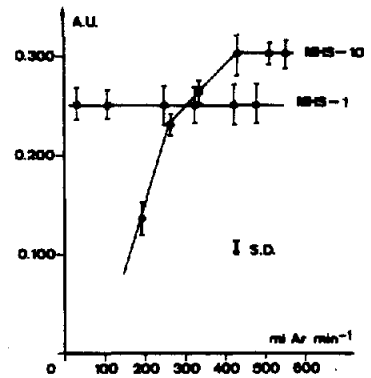

Fig. 5. Effect of analytical gas flow-rate on the sensitivity for 100 ng of Se.

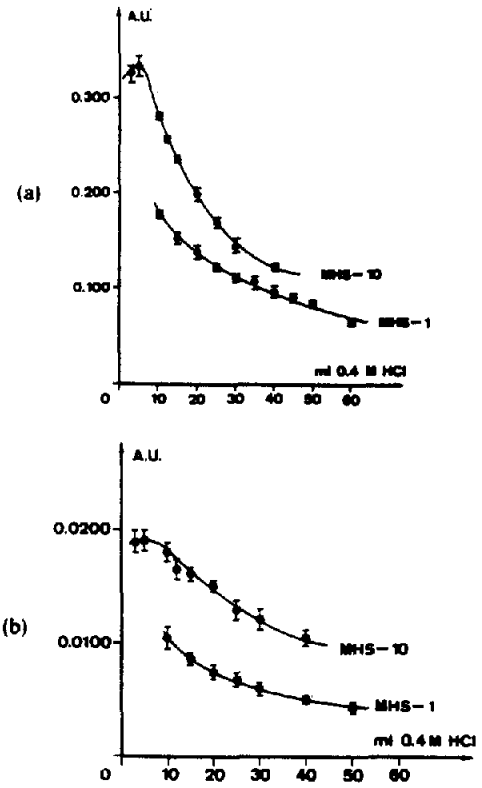

Fig. 6. Effect of increasing sample volume on the sensitivity. (a) For 100 ng of Se; (b) for 5 ng of Se.

Analytical characteristics

The sensitivity, defined as the slope of the calibration curve, is approximately 2.50 absorbance units per μg of Se for the MHS-1 system and about 3.0 for the MHS-10. As can be seen from Fig. 7, the linear re-

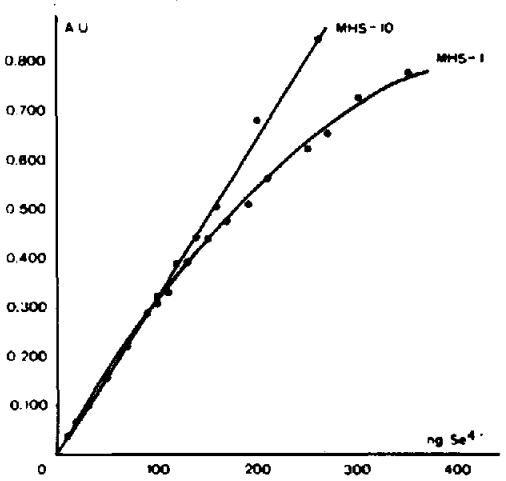

Fig. 7. Calibration curves for the two systems.

sponse range of the open (MHS-10) system is at least twice that of the closed system.

In optimal conditions both systems generate equally precise results. Typical values for the relative standard deviations (10 replicates) are 3–5% in the 10–250 ng concentration range, and approximately 12–16% near the detection limit. It should be stressed, however, that whereas sensitivity and precision are relatively constant from day to day for the MHS-10, this is certainly not the case with the MHS-1 system. Both depend to a large extent upon the condition of the quartz tube. When a particular quartz tube is used continuously in the MHS-1 system solely for the determination of selenium, it is noticed that the atomization cell suffers from a rapid deterioration which proceeds from the inner towards the outer surface of the wall. Investigations by X-ray diffraction and electron microscopy have revealed that physical transformations take place within the quartz material, leading to a very much shortened life for the atomization cell. These changes are presumably related to a reaction of hydrogen selenide with heated quartz. Although the alterations are perceptible macroscopically, loss of precision (*i.e.*, RSD > 3–4%) for a 100-ng aqueous standard indicates the beginning of the cell's break-down. Further investigations of this problem are being made and will be reported later.

The detection limits can be calculated from the equation:[3]

$$q_L = (\bar{x}_L - \bar{x}_{bl})/S = ks_{bl}/S$$

where $\bar{x}_L$ is the average value of the smallest signal that can be distinguished from the blank signal with reasonable certainty, $\bar{x}_{bl}$ is the mean of the blank signal, s_{bl} is the standard deviation of the blank signal, S is the sensitivity of the method determined at low levels of the analyte, $k = 3$ for a probability of about 0.90.

The sensitivity S was determined from series of measurements of 1, 2 and 4 ng of Se. Application of the equation yields limits of detection between 0.5 and 1 ng for both systems. However, figures obtained in this way underestimate the contribution of various sources of noise to the overall base-line and electronic noise and practical detection limits of between 1 and 2 ng are more realistic. Figure 8 shows recorder tracings of 1-ng and 2-ng signals, measured with the

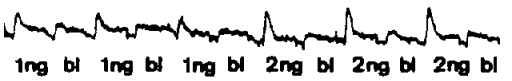

Fig. 8. Determination of the limit of detection of Se by HGAAS (MHS-1).

MHS-1 system. For "real" samples the relative limit of detection may give a more meaningful indication of performance. The relative detection limit, defined as the smallest quantity of analyte that can be measured per unit of weight of sample, will depend on the maximum aliquot of mineralized sample that can be used. Excessive foaming on addition of sodium tetrahydroborate solution to a decomposed sample often prevents the use of large aliquots, and the presence of matrix interferences also reduces the amount of decomposed sample that can be taken for analysis. The accuracy of the optimized method was evaluated by measuring NBS standard reference material 1577, bovine liver. The certified selenium content is 1.1 ± 0.1 μg/g. An average value of 1.2 ± 0.2 μg/g was found for 9 replicate decompositions followed by the determination of the selenium concentration by the standard addition method.

CONCLUSION

Both techniques are sufficiently sensitive and precise for the determination of nanogram quantities of selenium. The differences between the optimum settings of several parameters can mainly be related to the design (closed or open) of the systems. Although the MHS-10 system is substantially the cheaper, the MHS-1 system gives slightly faster analysis. The MHS-10 system is also slightly more sensitive, has a wider linear response range and is less affected by choice of acid.

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A RAPID DETERMINATION OF BRASS COMPOSITION AND PLATING WEIGHT ON BRASS-PLATED STEEL WIRE AND CORD BY X-RAY FLUORESCENCE SPECTROMETRY

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Summary—A rapid and simple means for determination of the brass composition and plating weight on brass-plated steel wire and cord is described. The sample preparation procedure is very simple; wires can be mounted as such, and cords can be mounted either as such or as unstranded single wires. The copper content of the brass and the plating weight are determined by measuring the intensities of the different elements by sequential X-ray fluorescence spectrometry (XRF). There is good agreement between the results obtained by XRF and those obtained by differential pulse polarography or spectrophotometry/complexometry; the precision is even better.

The composition and thickness of the brass plating on steel wire are important parameters in the adhesion between steel cord and rubber as used in steel-belted radial tyres. The analysis of the brass layer, which is done in order to determine the copper content of the brass and the plating weight of the layer, mostly comprises dissolution of the plating, followed by analysis with wet chemical or atomic-absorption methods.

In our plants, wet chemical analysis by spectrophotometry/complexometry or by differential pulse polarography has been used. The time required for this analysis, including sample preparation and calculation, varies between 1 and 2 hr. During this period the brass-plating production unit runs uncontrolled; quality control of intermediate as well as end products takes too much time.

For the efficiency and quality assurance of production units, and for the acceptance control of intermediate as well as end products and incoming shipments, it seemed worthwhile to investigate whether X-ray fluorescence spectrometry (XRF) would be applicable as a rapid (< 15 min) and simple means for the determination of brass composition and plating weight on steel wire and cord.

The principle of this method (the determination of different metal coatings on different substrates with flat surfaces) is mentioned by several authors.[1–7]

Gianelos[8] has described an XRF-method for the direct determination of the brass composition and the plating weight for brass-plated steel wire and cord. However, his method needs separate plating-weight curves for each wire size and has not been tested for all different cord constructions. We have critically examined his method and optimized the sample preparation and experimental conditions. A mathematical basis has been found for universal calibration curves for all kinds of wire and cord.

EXPERIMENTAL

A Philips PW 1410 X-ray spectrometer provided with a dual sample-changer was used. The samples were mounted in a specially designed Dural (DIN 1725) aluminium sample holder (Fig. 1A) in which a number of pieces of wire or cord, each between 42 and 44 mm long, were arranged side by side in the grooves, which were 1.2 mm wide (2.0 mm for larger diameter wire or cord), and covered with a lid (Fig. 1B) to keep them in place.

It was not necessary to place the wires in close contact to present as regular a surface as possible because the determination is based on intensity ratios.

The holder containing the wires or cords was placed in a Philips PW 1427/10 aluminium sample holder and the samples were analysed under the following conditions: X-ray tube: chromium target, 50 kV, 25 mA; counter: argon–methane filled flow-counter with 6-μm window; collimator: fine; crystal: lithium fluoride 200; path: vacuum; spinner: on, 60 rpm; counting time: 30 sec; analytical lines: ZnK_α = 41.80°, 2θ = 0.1437 nm, background = 43.50° 2θ, CuK_α = 45.02°, 2θ = 0.1542 nm, FeK_β = 51.76°, 2θ = 0.1757 nm.

RESULTS AND DISCUSSION

Calibration

Since XRF is essentially a comparative method of

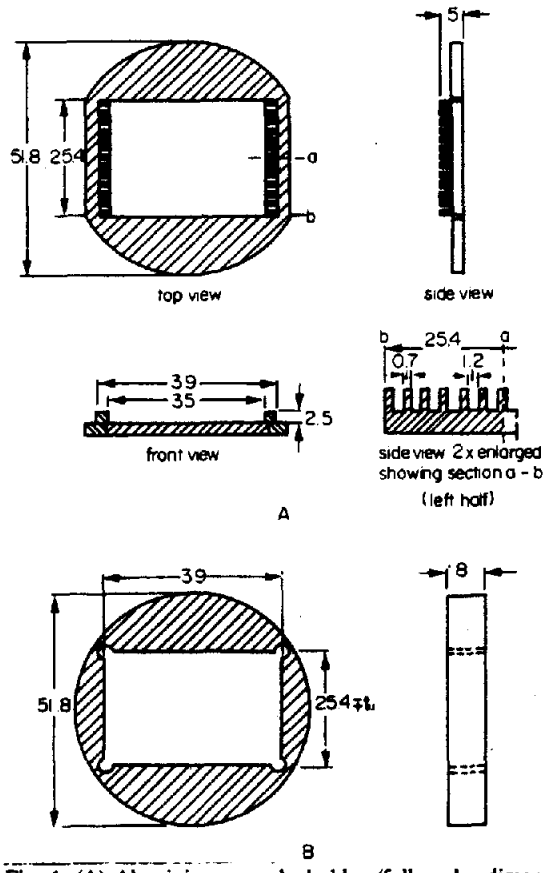

Fig. 1. (A) Aluminium sample holder (full scale, dimensions in mm). (B) Aluminium lid for sample holder.

analysis, quantitative analysis by XRF can only be performed if standards are available which have the same physical properties as the samples, including mass absorption coefficients, density, diameter and construction, and in this case have the same range of plating thickness and copper content of the brass layer.

Therefore, a series of samples covering the range of diameters (0.25–1.35 mm), constructions, copper content (59–78%) and plating thickness (0.27–2.7 μm) was analysed polarographically (differential pulse mode) for Cu and Zn (relative standard deviation 0.5%). The plating weights and the copper content of the brass layers were calculated; from the plating weights the plating thicknesses were calculated; in the formula used, $\bar{D}$ is the diameter of the steel wire or of the brass-plated wire, or the mean diameter of the individual strands of the steel cord.

A calibration curve for determining the copper content in the brass layer was constructed by plotting the intensity ratios $I_{Cu}/(I_{Cu} + I_{Zn})$ (all corrected for background and dead-time) of these standard samples against the copper contents determined polarographically. A least-squares calculation of a linear relation gave

$$\% \text{ copper} = -8.12 + 108.2 \frac{I_{Cu}}{I_{Cu} + I_{Zn}}$$

with a correlation coefficient of 0.990 (see Fig. 2).

A calibration curve for the determination of the brass plating thickness on steel (in μm) was obtained by plotting the intensity ratios $(I_{Cu} + I_{Zn})/I_{Fe}$ (all corrected for background and dead-time) of the standard samples against the calculated plating thickness of wires and the calculated mean plating thickness of the cord filaments.

Figure 3 shows a calibration curve for the brass plating thickness of the complete series of standard samples. By regression analysis a quadratic relationship can be calculated:

$$d(\mu m) = 0.0203 + 0.5767\left(\frac{I_{Cu} + I_{Zn}}{I_{Fe}}\right) - 0.016\left(\frac{I_{Cu} + I_{Zn}}{I_{Fe}}\right)^2$$

By means of a computer program based on the Sherman equation,[9] Schuck[10] calculated the expected calibration curves for the copper content in the brass layer and for the brass plating thickness.

Figures 2 and 3 show the agreement of the theoretical curves (dotted lines) and the experimental curves (full line); it proves that the assumption of a mean plating thickness for cords gives a reliable calibration curve for all kinds of wires and cords and that there are no positive or negative effects although the measured intensities are obtained from round, sometimes from stranded, and occasionally from flattened samples.

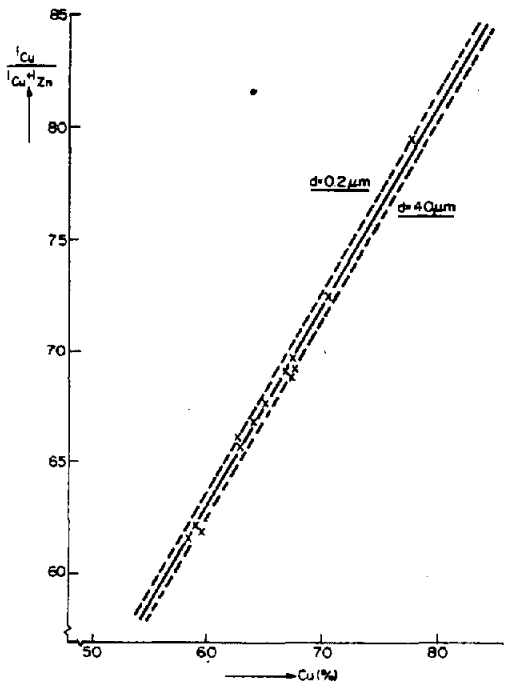

Fig. 2. Relation between the copper content of the brass layer on steel cord and the measured and theoretically calculated intensity ratios: × = measured; solid line = experimental calibration curve (coincides with theoretical curve for $d = 1.8$ μm); dashed lines = theoretically calculated calibration curves for extreme d-values.

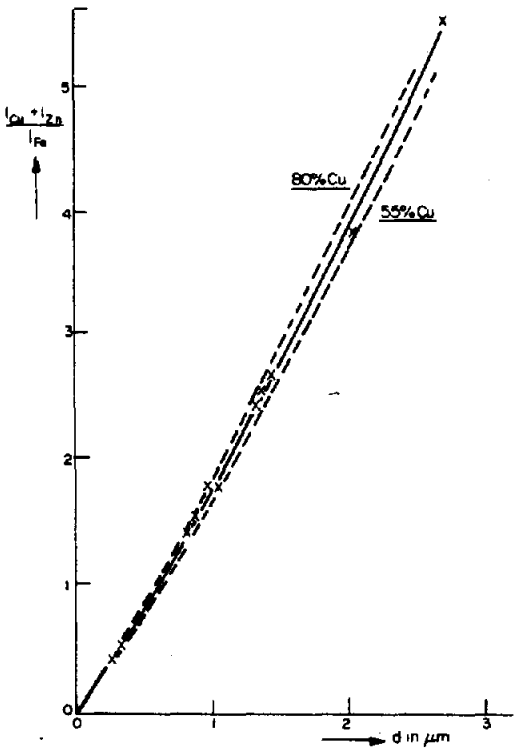

Fig. 3. Relation between the thickness of the brass layer on steel cord and the measured and theoretically calculated intensity ratios: × = measured; solid line = experimental calibration curve (coincides with theoretical curve for 70% Cu); dashed lines = theoretically calculated calibration curves for extreme Cu-contents.

The plating weight (in g/kg) of brass on steel wire and cord can now be calculated from the plating thickness by using an appropriate factor (f), which depends on the diameter of the wire or the mean diameter of the single filaments of the cord. A calibration curve for this factor was constructed by plotting the ratio of plating thickness to plating weight against the mean diameter of the standard sample wires. Figure 4 shows such a calibration curve.

By least-squares the following linear relationship can be calculated:

$$f = -9.4 \times 10^{-4} + 0.237\bar{D}$$

where, $\bar{D}$ the mean wire diameter, and f are both in

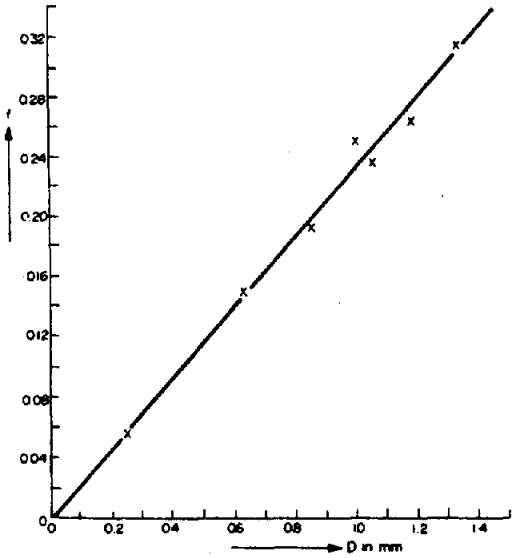

Fig. 4. Relation between conversion factor f and mean diameter of brass-plated wires and cords.

mm. The plating weight (g/kg) can then be calculated with the following formula:

$$\text{g of brass per kg} = \frac{d}{f} = \frac{d}{-9.4 \times 10^{-4} + 0.237\bar{D}}$$

A program for the HP 9815 desk-top calculator, containing all necessary equations, was written and used for routine calculations. This program is available upon request.

Analysis of samples

To assess the precision and accuracy, 10 samples from two different production batches were analysed according to the procedure described.

Table 1 shows the good agreement between the results of XRF-analysis and those of complexometric/spectrophotometric analysis; the standard deviations of the copper content and the plating weight are 0.10% Cu and 0.025 g/kg, respectively.

Furthermore, a number of samples of different diameters and constructions were analysed by XRF, differential pulse polarography and complexometry/spectrophotometry. From these results, listed in Table 2, it can be seen that the mean differences for the determination of the copper content are very

Table 1. Comparison of analysis results obtained by XRF and the complexometric/spectrophotometric method

Sample	Cu, %		Plating weight, g/kg	
	XRF	Chemical analysis	XRF	Chemical analysis
Wire	$\bar{x} = 70.7$	$\bar{x} = 70.4$	$\bar{x} = 5.49$	$\bar{x} = 5.36$
1.16 mm	$n = 10$	$n = 10$	$n = 10$	$n = 10$
	$s = 0.1$	$s = 0.8$	$s = 0.03$	$s = 0.14$
Cord	$\bar{x} = 67.9$	$\bar{x} = 67.7$	$\bar{x} = 4.87$	$\bar{x} = 4.88$
7 × 4 × 0.22	$n = 10$	$n = 10$	$n = 10$	$n = 10$
+0.15 mm	$s = 0.1$	$s = 0.8$	$s = 0.02$	$s = 0.13$

Table 2. Analysis of brass-plated steel wires and cords by XRF, differential pulse polarography and spectrometry/complexometry

Sample	Construction (diameter), *mm*	Cu, %			Plating weight, *g/kg*		
		XRF	Diff. pulse pol.	Spectr. compl.	XRF	Diff. pulse pol.	Spectr. compl.
1	1.16	68.4	69.9	69.0	2.81	2.98	3.11
2*	1.16	53.2	49.5	48.2	4.25	4.37	4.38
3	1.16	83.6	83.7	82.9	6.12	6.46	6.35
4	1.16	65.6	64.9	66.2	7.63	7.94	7.98
5	1.16	64.9	63.3	65.8	11.38	12.02	11.93
6	1.16	57.8	58.7	57.8	3.71	3.94	3.93
7	1.16	72.8	72.3	72.6	5.54	5.79	5.83
8	1.16	77.5	77.9	77.7	3.52	3.71	3.87
9	1.16	83.8	83.2	84.0	6.40	6.70	6.75
10	1.16	66.4	67.3	66.7	4.80	4.94	4.87
11	1.08	66.6	67.4	66.4	5.61	5.57	5.59
12	0.86	66.5	66.6	67.6	5.27	5.56	5.74
13	0.75	68.9	68.9	66.7	5.05	5.04	4.92
14	0.75	68.2	69.1	66.3	5.32	5.22	5.11
15	0.65	64.3	65.0	64.8	5.20	5.65	5.43
16	4 × 0.38	67.7	68.2	66.8	4.75	4.82	4.77
17	5 × 0.25	70.6	70.6	70.3	5.74	5.76	5.70
18	4 × 0.25	66.5	67.6	67.8	4.59	4.48	4.47
	4 × 0.25	67.3	67.8	67.6	4.68	4.56	4.49
19	7 × 4 × 0.22 + 0.15	64.9	64.4	64.5	4.15	3.91	3.97
20	7 × 4 × 0.22 + 0.15	68.5	69.0	67.9	5.04	4.83	4.79
21	7 × 4 × 0.22 + 0.15	67.0	67.1	67.8	4.74	4.55	4.56
22	2 + 7 × 0.22 + 0.15	67.1	67.9	67.4	4.92	4.83	4.77
23	2 + 7 × 0.22 + 0.15	67.8	67.2	66.9	5.26	4.98	4.96
24	7 × 4 × 0.175	67.0	68.0	69.8	3.93	3.96	3.80

* Cu content out of the range of the calibration curve.

small; the mean differences for the plating weight are larger but acceptable.

Conclusions

In conclusion, XRF is found to be a good means for the direct and rapid determination of brass composition and plating weight of a wide range of wire and cord. The method developed permits all constructions of wire and cord, single filaments as well as unstranded cords, to be analysed.

The method saves much time; one analysis takes about 10 min, including sample preparation and calculation, compared with 60–90 min for the wet chemical methods, thus reducing the time needed by the customer for acceptance control and the manufacturer for production control.

The method has now been used by Enka on a routine basis for about a year without giving rise to any complaints.

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DIRECT AND KINETIC FLUORIMETRIC METHODS FOR DETERMINATION OF MICRO AMOUNTS OF Ti(IV), BASED IN ITS CATALYTIC EFFECT ON AERIAL OXIDATION OF PICOLINALDEHYDE NICOTINOYLHYDRAZONE

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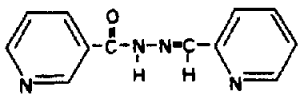
Summary—The oxidation of the new reagent picolinaldehyde nicotinoylhydrazone by atmospheric and/or dissolved oxygen is catalysed by traces of Ti(IV), and produces a fluorescent product. Monitoring the fluorescence provides one non-kinetic and three kinetic (initial-rate, fixed-time and fixed-intensity) methods for determination of Ti(IV) at the 60–400 ng/ml level. The variables affecting these methods have been optimized and the tolerance levels for foreign ions determined.

Monitoring of the development of fluorescence in a catalysed reaction can provide highly sensitive and selective kinetic techniques for the determination of trace metals. Valcárcel *et al.* have used the catalytic effect of certain metals on the mild oxidation of hydrazones and azines to compounds which show intense fluorescence, to establish kinetic and non-kinetic methods for the determination of traces of copper,[1] cobalt,[2] mercury,[3] gold[3,4] and platinum.[5]

The analytical properties of a new compound, picolinaldehyde nicotinoylhydrazone (PANH) are described for the first time in this paper, and its aerial



oxidation is used for the kinetic and non-kinetic fluorimetric determination of traces of Ti(IV) by means of their catalytic effect. Similar compounds (salicylaldehyde and acetylpyridine nicotinoylhydrazones) are under investigation.

Very few kinetic methods for the determination of Ti(IV) have been described. Most are based on formation of polarographic catalytic waves.[6–11] Two methods have been based on the iodide–hydrogen peroxide indicator reaction in acid medium,[12,13] and a chemiluminescent kinetic method for titanium and hafnium based on the luminol–hydrogen peroxide indicator reaction has also been proposed.[14] Only one indirect fluorescent determination, based on a mixed-metal complex and using an extraction technique, has been found in the literature.[15]

EXPERIMENTAL

Synthesis of the reagent

Nicotinoylhydrazine (1.00 g) was dissolved in 25 ml of distilled water and mixed with 0.78 g of picolinaldehyde dissolved in 5 ml of ethanol. The mixture was shaken for 1 hr, and a yellowish-white precipitate appeared. The product, recrystallized from 1:1 ethanol:water, had m.p. 162°. Analysis gave C 63.6%, H 4.5%, N 24.6%; $C_{12}H_{10}N_4O$ requires C 63.71%; H 4.42%; N 24.77%.

Reagents

All reagents used were of analytical grade. Ethanolic solutions of PANH (0.1 and 0.2%) were used. A standard solution of Ti(IV) was prepared by drying titanium metal at 110°, dissolving appropriate amounts of it in 100 ml of 6*M* hydrochloric acid and making up to 1 litre with distilled water. Further dilutions were made daily as required. Triethanolamine–hydrochloric acid buffer solution, pH 6.5, was used.

Apparatus

A Perkin–Elmer MPF-43A spectrofluorimeter, fitted with a unit for kinetic measurement and with 1-cm quartz cells, a Perkin–Elmer 402 recording spectrophotometer and a Pye Unicam SP6-500 digital spectrophotometer, (both with 1-cm quartz cells) were used.

Non-kinetic Ti(IV) determination

In 25-ml standard flasks 5 ml of 0.1*M* potassium nitrate, 5 ml of the pH–6.5 buffer, 2 ml of 0.1% PANH solution, 1.85 ml of 0.2*M* sodium hydroxide and appropriate volumes of Ti(IV) solution [to give a final concentration of Ti(IV) between 60 and 300 ng/ml] were mixed in that order and made up to volume with distilled water. The fluorescence intensity was measured 15 min later (excitation λ_{max} = 365 nm, emission λ_{max} = 445 nm).

Kinetic Ti(IV) determination

In a 25-ml standard flask 5 ml of 0.1*M* potassium nitrate, 5 ml of the pH-6.5 buffer, 2 ml of 0.1% PANH solution and 1.85 ml of 0.2*M* sodium hydroxide were mixed in that order and made up to volume with distilled water. Then 3.5 ml of this solution were placed in the quartz spectrofluorimeter cell and when the required temperature was reached, 100 µl of Ti(IV) solution were injected from a microsyringe. The fluorescence intensity was monitored, starting 20 sec after addition of the titanium.

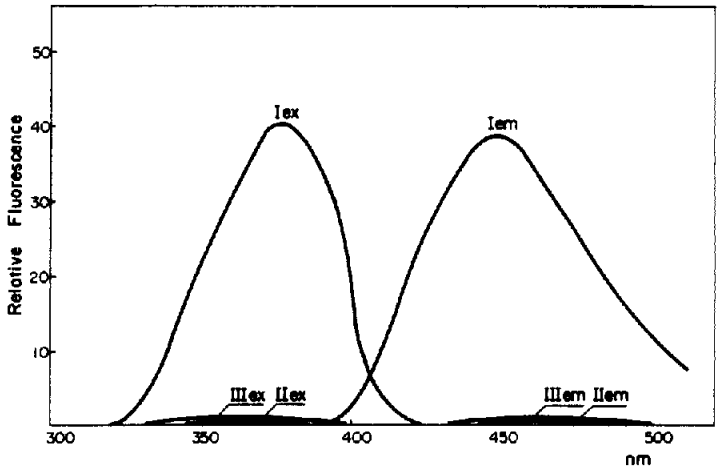


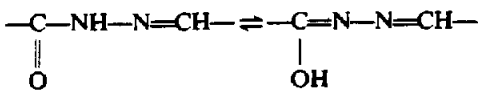
Fig. 1. Excitation and emission spectra of $3.5 \times 10^{-4}M$ PANH (pH = 6.5). Sensitivity × 30 (λ_{ex} = 365 nm, λ_{em} = 445 nm). I, In the presence of Ti(IV) (100 ng/ml) and oxygen. II, In the absence of Ti(IV) and presence of oxygen. III, In the presence of Ti(IV) and absence of oxygen.

RESULTS AND DISCUSSION

Characteristics of the reagent

Aqueous and ethanolic solutions of PANH do not fluoresce, so the characteristics of the reagent were studied spectrophotometrically. Two absorption maxima appear at pH-values close to neutrality; one at 220 nm, which is unaltered by change in pH, and the other at 305 nm, which shifts to longer wavelengths with change in pH (λ_{max} = 340 nm at pH 1.0 or 10.0). A third maximum appears at 260 nm at low pH. Aqueous solutions of the reagent are stable at pH 1–10. Oxidizing agents such as persulphate or hydrogen peroxide and reducing agents such as ascorbic acid or hydroxylamine change the absorption spectrum, the rate being faster in basic medium.

PANH exhibits two dissociation constants, pK_1 = 4.1, attributed to dissociation of protons from the protonated pyridine nitrogen atoms, and pK_2 = 10.1, corresponding to the ionization of the enolic hydroxyl group:



Its reactivity with metal ions at various pH values has been studied. Colour reactions of low sensitivity ($pD < 4$) are given by Cu(II), Fe(II), Ni(II) and Co(II).

It gives fluorescent compounds with Al(III), Ga(III) and Zr(IV) (λ_{ex} = 380 nm, λ_{em} = 445 nm) and with Ti(IV) (λ_{ex} = 365 nm, λ_{em} = 445 nm), the fluorescence intensity in this case being five times greater than in the others and thus more interesting from an analytical point of view.

Study of the Ti(IV)–PANH system

Nature of the reaction. Figure 1 shows the spectra obtained in presence and absence of Ti(IV). All attempts to find a stoichiometric relation between Ti(IV) and PANH have failed, implying that the fluorescence is not due to formation of a chelate. Further experiments have shown that aerial oxidation of the reagent occurs and is catalysed by traces of Ti(IV).

Mild oxidizing agents in moderate concentrations do not produce the fluorescence, which is due solely to the presence of atmospheric and/or dissolved oxygen. Solutions made from oxygen-free reagents and measured in an inert atmosphere do not show fluorescence (Fig. 1, curve III). When the same systems are exposed to air for 5 min, they show a similar fluorescence to those prepared in contact with the atmosphere (Fig. 1, curve I). Solutions prepared in an inert atmosphere and in the presence of certain oxidizing agents (iodate or periodate) also develop a fluorescence similar to that obtained by contact with air. Other oxidizing agents, such as persulphate, hydrogen peroxide or bromate do not develop the fluorescence due to this oxidized form of the reagent.

The mechanism of the catalytic reaction has not been completely elucidated but, since the reaction is first-order with respect to both Ti(IV) and PANH, the reaction steps are probably:

$$\text{HNPA} + \text{Ti(IV)} \rightarrow \text{Ti(III)} + \text{HNPA}_{(ox)}$$

$$\text{Ti(III)} = \tfrac{1}{2}O_2 \rightarrow \text{Ti(IV)}$$

with Ti(IV) serving as mediator in the electron transfer. The direct interaction of PANH with aerial oxygen is thermodynamically possible but kinetically hindered. This proposed mechanism is supported by the known reductive character of the hydrazones.

Influence of pH. The fluorescent product shows maximum fluorescence at pH 6–8 (Fig. 2). Various buffers were tested [phosphate; triethanolamine; Britton and Robinson (phosphate/boric acid/diethylbarbituric acid; sodium diethylbarbiturate)]

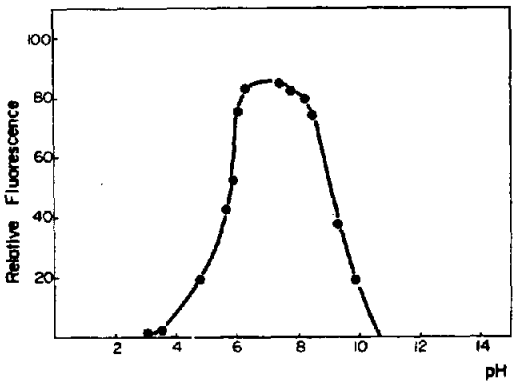


Fig. 2. Influence of pH on the fluorescence of the oxidation product. (PANH) = $3.5 \times 10^{-4}M$, Ti(IV) = 100 ng/ml. Sensitivity × 30 (λ_{ex} = 365 nm, λ_{em} = 445 nm).

and it was observed that those containing phosphate produced a total loss of fluorescence (this was predictable from the insolubility of titanium phosphate). The other buffers allowed rapid development of the fluorescence, which reached its maximum in 3–4 min and remained constant for at least 4 hr. The use of the triethanolamine buffer produces greater fluorescence intensity than the Britton and Robinson buffer, and was therefore chosen for further work, at pH 6.5.

Non-kinetic determination

Optimization of the method. The optimum order of addition is Ti(IV) after the reagent; this is logical because the cation would be precipitated as the hydroxide in an almost neutral medium. The order of addition of the other reagents is immaterial provided the Ti(IV) is added after the reagent.

Variation of the ionic strength (and of the concentration of the electrolytes used to control it) is without influence on the fluorescence.

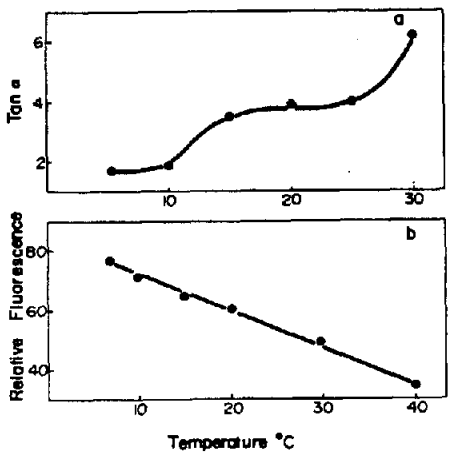


Fig. 3. Influence of temperature. PANH = $3.5 \times 10^{-4}M$, Ti(IV) = 100 ng/ml. Sensitivity × 30 (λ_{ex} = 365 nm, λ_{em} = 445 nm). (a) Kinetic method. (b) Non-kinetic method.

Increase in the ethanol concentration decreases the fluorescence intensity. An 8% ethanol concentration is used to prevent precipitation of the reagent.

Increase in the temperature decreases the fluorescence intensity (Fig. 3). A temperature of 20° is recommended and must be rigorously controlled (±0.1°) during preparation and measurement of the samples.

Doubling the PANH concentration (from 0.1 to 0.2%) increases the fluorescence by only 2.1%. The calibration curves obtained with both reagent concentrations, with measurement after 20 min to ensure total development of the fluorescence, are linear for titanium concentrations from 60 to 300 ng/ml. The error is ±1.1% (for $P = 0.05$ and $n = 11$ samples).

Interferences. The interferences are listed in Table 1, and are not only few in number, but are also minimized by using the higher reagent concentration (0.2%). The most important interference is caused by Ce(IV) at above 3-fold ratio to Ti(IV).

Kinetic determination

The influence of the order of addition, ionic strength and ethanol concentration is the same as for the non-kinetic method. The temperature effect is not so pronounced, however, especially in the range 15–25°; thus a temperature of 20° is again recommended.

Figure 4 shows typical fluorescence intensity *vs.* time curves for different reagent concentrations. A graph of the logarithm of the initial rate *vs.* the logarithm of the reagent concentration gives a straight line with a slope equal to the reaction order with respect to reagent.

The optimum reagent concentration is that at which the relative standard deviation is minimal for the initial rate measurements in a region in which the reaction order with respect to the reagent is zero or as close to it as possible, because when this condition is satisfied, small variations in the reagent concentration will not affect the initial rate of the reaction, since the initial rate is independent of the concentration of a zero-order reagent. The results imply that the reaction is first-order with respect to PANH.

Calibration curves were obtained for the two reagent concentrations (0.1 and 0.2%) used in optimization of the non-kinetic method.

Initial-rate method. The calibration curve is linear for the titanium concentration range between 60 and 400 ng/ml. The higher reagent concentration gives a greater slope. The error is 0.8% ($P = 0.05$ and $n = 11$). Comparison of the calibration curves for the initial-rate method and the non-kinetic method affirms that although increasing the reagent concentration gives a greater rate of development of fluorescence, the final fluorescence intensity is the same and depends solely on the Ti(IV) concentration.

Fixed-time method. In this method, a set time of 50

Table 1. Interference levels of foreign ions on the determination of titanium traces

Tolerance ratio [ion]/[Ti(IV)]	Non-kinetic method [Ti(IV) 140 ng/ml]	Kinetic metbods [Ti(IV) 100 ng/ml]
100	Mg, Ca, Sr, Ba, Al, Ga, In, Bi, U(VI), VO_3^-, NO_3^-, SO_4^{2-}, WO_4^{2-}	Mg, Ca, Sr, Ba, Al, Ga, In(III), Bi, U(VI), Cd, Mn, Be, VO_3^-, NO_3^-, SO_4^{2-}, WO_4^{2-},
50	Cd, Mn(II), Be, Fe(II) and (III), Cr(III), Ni, Zn, Hg(II), Pb	Fe(II) and (III), Cr(III), Ni(II), Zn(II), Hg(II), Pb(II)
20		Cu(II)
10	Cu(II), Th, Zr	Th(IV), Zr(IV)
3	Ce(IV)	Ce(IV)

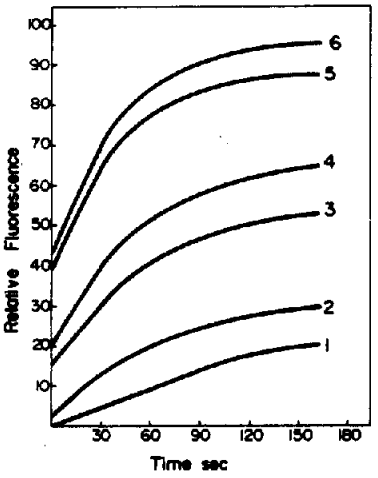


Fig. 4. Typical fluorescence intensity *vs* time curves at various PANH concentrations: (1) $8.7 \times 10^{-5}M$; (2) $1.7 \times 10^{-4}M$; (3) $3.5 \times 10^{-4}M$; (4) $5.25 \times 10^{-4}M$; (5) $7.0 \times 10^{-4}M$; (6) $8.7 \times 10^{-4}M$.

sec is recommended; the calibration curve is linear for Ti(IV) concentrations from 60 to 700 ng/ml because the fluorescence develops quickly. The error in this method is 2.4% ($P = 0.05$ and $n = 11$), which is greater than that of the initial-rate method, possibly because of the difficulty in measuring with accuracy the small time interval.

Fixed-intensity method. A fixed fluorescence intensity signal of 40 units is recommended, because the rapid development of the fluorescence does not permit the measurement of a small signal without a large error; thus very low Ti(IV) concentrations cannot be measured, because they do not produce enough fluorescence. The time needed to reach this fluorescence intensity is a linear function of Ti(IV) concentration between 200 and 800 ng/ml. The error of the method is 3.1% ($P = 0.05$, $n = 11$).

Interferences. The interferences are given in Table 1, and though again they are few, especially when the more concentrated reagent is used, the tolerance level is lower in the kinetic methods.

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REDOX POTENTIALS OF TUNGSTEN AND ITS ALLOYING PARTNERS—FUNDAMENTALS OF TUNGSTEN DETERMINATION BY REDOX TITRATION

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Summary—The fundamentals of tungsten determination by redox titration in hydrochloric acid medium are described. Conditional redox potentials are given for W, Cr, V, Fe, Ti, Mo, Cu and Sn as a function of HCl concentration. The basic experimental conditions and the difference in conditional potentials required for consecutive titrations are discussed. Any mixture of tungsten with other metals can be analysed without separation if it does not contain both Cr and Ti or both Mo and V.

Remarkably few methods have been developed for the titrimetric determination of tungsten. An exhaustive review was given recently.[1] Precipitation and complexation titrations of tungsten lack specificity, as do those of many other hard A-group metals. Usually a previous separation as $WO_3.H_2O$ will be necessary. Redox titrations are possible by making use of W(V) or W(III). The redox potentials of these oxidation states are very low and hitherto known only with uncertainty. With reductants in solution, *i.e.*, by reductimetric titrations, only W(V) can be obtained quantitatively. W(III) can be obtained by means of metal reductors and titrated with oxidants.

In minerals and technical products tungsten usually occurs along with other transition metals. Under the conditions required for its redox titration, many of these can also change their oxidation state and thus may interfere in the determination of tungsten. A table of redox potentials would provide information about any possible interference, but it is not the normal E_0 values that are required, because they only apply to idealized reactions, such as $Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$. In practice the solutions almost inevitably contain other substances which react with the species to be considered.The competing equilibria may exert a drastic influence on the redox reaction. W(VI) and W(V) are sufficiently soluble only in hydrochloric acid that is at least 7*M*. At lower acidity, tungstic acid or tungsten blue precipitates. For practical purposes a 9–11*M* hydrochloric acid medium is advisable. The conditional redox potentials in concentrated hydrochloric acid are known for hardly any of the elements in question. The only previous attempt to determine W(VI) in presence of Mo(VI) and Fe(III) was made at an undefined acidity and led to a titration curve showing no separation of Mo and Fe.[2] Therefore the potentials of the redox couples tungsten(VI)/(V), tungsten(V)/(III), chromium(III)/(II), iron(III)/(II), titanium(IV)/(III), molybdenum(VI)/(V), molybdenum(V)/(III), vanadium(IV)/(III), vanadium(III)/(II), copper(II)/(I), and tin(IV)/(II) in fairly concentrated hydrochloric acid media have been determined. These values E_0' apply only to the acid concentrations specified; they are conditional potentials, *i.e.* effective constants analogous to those used for the description of complexation equilibria. From the data given in Table 1 it can be predicted which mixtures of these species can be analysed without interference. The analytical possibilities for the determination of tungsten are discussed subsequently.

It had to be ensured that these potentials would be valid under the conditions of analytical practice. Therefore, they were determined not by using prepared mixtures of the two oxidation states, but by redox titrations. According to the Nernst equation, E_0 or E_0' is the potential when the concentration ratio (strictly speaking, the activity ratio) of the oxidized and reduced forms is unity. This condition is fulfilled to a sufficiently good approximation at that point of the titration curve at which just half the total equivalent amount of titrant has been consumed.[3] To test whether the potentials measured depended on experimental conditions, the electrode material, temperature, speed and direction of titration (oxidizing or reducing) were varied within the analytically useful range. No effort was made, however, to use highly purified substances (*i.e.*, to ensure the absence of any catalytically active trace metals) or extremely long waiting times for the equilibration of potential, as used for Cr(III)/Cr(II) in weakly acid solution.[4]

EXPERIMENTAL

Apparatus and procedure

Metrohm EA 880-50 controlled-temperature titration cells, an E 510 precision pH-meter, E536 recording titrator and motor burettes, were used. Pure, deaerated solutions of known hydrochloric acid concentration were titrated with stepwise addition of reagent and waiting times up to 5 min to ensure stable electrode response. One year later, control

Table 1. Conditional redox potentials E_0 (*V*) *vs.* NHE at 20°

[HCl], *M*	Cr(III) Cr(II)	W(V) W(III)	V(III) V(II)	Sn(IV) Sn(II)	Ti(IV) Ti(III)	W(VI) W(V)	Mo(V) Mo(III)	Fe(III) Fe(II)	Cu(II) Cu(I)	V(IV) V(III)	Mo(VI) Mo(V)
3	−0.40	(−0.35)	−0.18								
3.5		−0.33									
5		−0.30									
7		−0.25	−0.19	+0.14	+0.22	+0.21	+0.26	+0.59	+0.62	+0.63	+0.68 (Au)
	ca. −0.22										+0.72 (Pt)
9	(Hg)	−0.22	−0.21	+0.13	+0.29	+0.30	+0.30	+0.53	+0.60	+0.67	+0.62
11	−0.1	−0.20	−0.22	+0.12	+0.35	+0.38	+0.34	+0.51	+0.59	+0.68	+0.69
13.5	(Au)	−0.19				+0.48	+0.48	+0.49			(+0.69)
15					+0.39	+0.55	+0.56	+0.49	+0.61	+0.67	(+0.7)

Remarks 1. Potentials below +0.1 V were measured with Hg, all others with Au, except for Sn(IV)/Sn(II) which was measured with Pt.
2. In parentheses: uncertain values.
3. Values for Cr(III)/Cr(II) and V(IV)/V(III) are uncertain by about 0.05 V, all others by 0.02 V or less if not otherwise stated in the text.

measurements were run at various temperatures with continuous addition of titrant at speeds automatically reduced in ranges of sharp potential change. Given good pretreatment of the indicator electrodes, the results matched within the uncertainty limits specified below.

Indicator electrodes

Gold wire (47 mm²), Metrohm EA 261; combined electrode with gold cap (315 mm²), Metrohm EA 278; platinum wire (13 mm²), Metrohm EA 202; tungsten wire (25 mm²), Metrohm EA 249; mercury cup (4–6 mm²), Metrohm EA 256. The electrodes should be kept in acidified Cr(II) solution to avoid any passivation by aerial oxygen and to provide for a well-defined surface state. Gold is most suitable for both high and moderately low potentials, but in the range of Cr(III)/Cr(II) (−0.2 V) the values indicated are too high, and in the range of Cl_2/Cl^- (+1 V) they are too low. At potentials above about +0.1 V platinum also suits well; for the measurement of negative potentials its hydrogen overvoltage is insufficient in concentrated hydrochloric acid. A tungsten wire serves well in the negative range, but for highly positive potentials gives values which are generally too low. Unfortunately, it is difficult to keep the surface of tungsten in a reproducible state (blackening on use). Mercury is inapplicable in hydrochloric acid in the positive range, because its potential is then mainly determined by the couple Hg_2Cl_2/Hg, as is shown by the consecutive titration of Fe(III) and W(VI) with Cr(II), see Fig. 2. The potential jump of more than 0.2 V at the equivalence point for iron is hardly indicated at all by mercury, because it lies at about +0.4 V. The equivalence potential for the reduction of W(VI) at about +0.1 V is just well enough indicated.

The importance of kinetic effects in the measurement of low potentials can be seen from the influence of temperature, Fig. 1. Whereas the potentials indicated by gold or platinum are scarcely affected by temperature in the highly positive range, negative potentials are considerably more positive at 90 or 56° than at 20°, owing to diminished kinetic hindrance of evolution of hydrogen. The higher the temperature and the acid concentration, the faster the electrode response and the better the fit of the titration curves to the theoretical shape. This effect is more pronounced, the more difficult the response to a redox couple. For Fe(III)/Fe(II) undistorted curves are obtained at 20° in 7*M* hydrochloric acid, and for W(VI)/W(V) at 20° in the 11*M* acid but in 7*M* acid only at 90°, and for Mo(VI)/Mo(V), the electrode equilibration is considerably hindered even in 11*M* hydrochloric acid and at the highest possible temperature of 60°, and hence the titration curve is distorted.

Reference electrodes

All measurements were made *vs.* the Ag/AgCl electrode with 3.5*M* lithium chloride as electrolyte (potassium chloride precipitates in the diaphragm in concentrated hydrochloric acid). The types used were the Metrohm EA 437 with a diaphragm vessel and two ceramic diaphragms, or a combined electrode with one ceramic diaphragm, like the EA 278. The electrode potentials were checked with hydroquinone. The measured values were referred to the normal hydrogen electrode (NHE) at 20° by using reported potentials.[5]

Titrants

Cr(II) solution, 0.1*M*, was prepared in 2*M* sulphuric acid from potassium dichromate and zinc, and stored under nitrogen. The conditional potentials of all the systems investigated are not affected by a sulphuric acid concentration up to at least 0.5*M*. Fe(III) solution, 0.1*M*, was made in 9*M* hydrochloric acid from ferric oxide. Chlorine water, 0.1*M*, was also prepared.

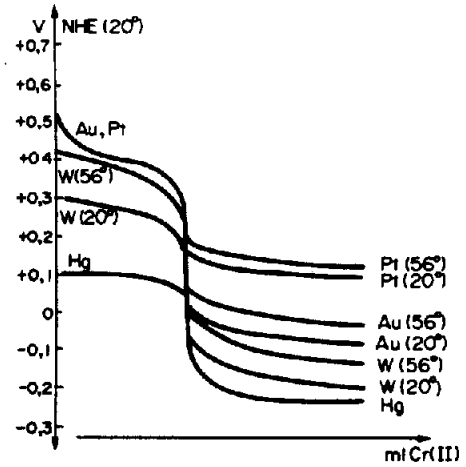

Fig. 1. Influence of temperature and electrode material on the titration of W(VI) with Cr(II) (in 1*M* HCl).

RESULTS AND DISCUSSION

Tungsten(VI)/(V)

The conditional potential increases almost linearly with hydrochloric acid concentration. The repeatability is better than 0.01 V over some days; in the course of a year deviations up to 0.05 V may occur if the electrode is not pretreated with Cr(II) solution. The electrode equilibration is fast enough for titrations, 9*M* hydrochloric acid and 55° being favourable conditions in practice. In titrations with Cr(II) in 15*M* hydrochloric acid (at −3°) noticeable evolution of hydrogen occurs at the gold electrode near the end-point. Potentials agreeing to better than 0.02 V are obtained from titrations of W(VI) with Cr(II) and of W(V) with chlorine water. Using a platinum wire electrode, Geyer and Henze[6] obtained potentials about 0.1 V more positive than those quoted here; they used mixtures of W(VI) and W(V) in 9–12.5*M* hydrochloric acid. Phosphoric acid concentrations up to 6*M* in 7*M* hydrochloric acid at 90° do not influence E'_0, the accuracy of the end-point or the speed of electrode response. Up to 0.2*M* hydrofluoric or 0.5*M* sulphuric acid in 9*M* hydrochloric acid at 90° is also without effect; higher concentrations were not tested.

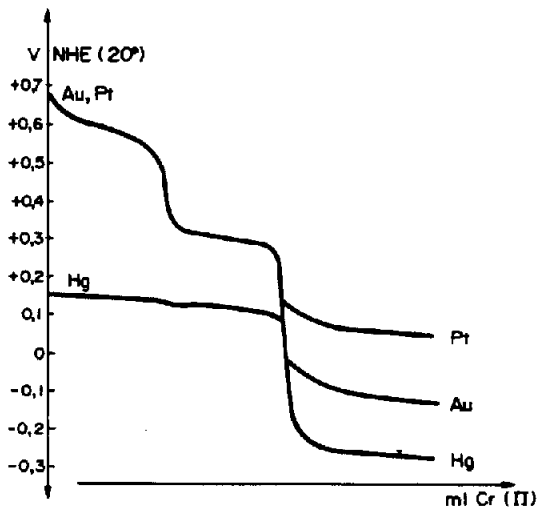

Fig. 2. Consecutive titration of Fe(III) + W(VI) with Cr(II), measured with different electrodes (in 9*M* HCl at 20°C).

Tungsten(V)/(III)

Tungsten(III) was generated from W(VI) with zinc dust in concentrated hydrochloric acid and titrated with Fe(III) or chlorine water at ambient temperature. At a platinum electrode E'_0 values of about 0 V were obtained, similar to those reported earlier by Geyer and Henze.[6] A gold electrode shows about −0.2 V. The values given in Table 1 were measured with mercury, the response of which is fast. At below 3.5*M* hydrochloric acid concentration the electrode response is very uncertain, probably owing to W(V) settling on its surface. The potential of W(V)/W(III) is much lower than previously thought. Tungsten(III) is a stronger reductant than V(II) and about as strong as Cr(II). This explains why the titration of W(VI) with Cr(II) cannot lead to quantitative reduction of W(V) and to a second potential jump. Like Cr(II), tungsten(III) is very unstable in strongly acid medium. It is doubtful with either system whether the potentiometrically measurable potentials are unbiased. It must be suspected that at least at higher acidities the hydrogen overvoltage of mercury is insufficient and the indicated values are largely determined by the reduction of H^+ at the metal surface. Therefore, the increase of the potential of W(V)/W(III) with acidity is possibly an artefact, though it corresponds to the well-ascertained behaviour of the system Mo(V)/Mo(III).

It cannot be decided from potentiometric measurements whether Cr(II) or W(III) is the stronger reductant. An answer may be found from photometric investigation of the equilibrium

$$W(V) + 2\ Cr(II) = W(III) + 2\ Cr(III).$$

Chromium(III)/(II)

The accepted value $E'_0 = -0.41$ V was obtained with mixtures of highly purified salts of Cr(III) and Cr(II) in 0.1*M* hydrochloric acid; difficulties arose when the 1*M* acid was used.[4] At the acidities necessary for the titration of tungsten, chromium(II) is rapidly oxidized by H^+, the reaction being additionally catalysed by the metal electrode. An unbiased potential measurement is impossible. The values obtainable become more positive with increasing acidity and temperature, owing to increased reduction of H^+.

Vanadium(III)/(II)

Vanadium(II) is very slowly decomposed at a mercury surface even in 1*M* hydrochloric acid. At Pt or Au considerable evolution of hydrogen occurs and the

potentials obtained are too high. Titrations of V(II) with Fe(III) or chlorine water give results in agreement. Despite its strongly negative range the potential further decreases with increasing acidity. This gives hope that it truly describes the redox couple V(III)/V(II) and not just the increasing reduction rate of H^+. The decrease of potential with increase in hydrochloric acid concentration corresponds to the behaviour of Fe(III)/(II). Transition metals usually form more stable chloro-complexes in the tervalent than in the bivalent state. A conditional potential of $E'_0 = -0.26$ V has been reported for 0.05–1M sulphuric acid solutions.[7]

Iron(III)/(II)

The decrease of potential with increasing hydrochloric acid concentration meets expectation. The response of Pt and Au electrodes is fast in concentrated hydrochloric acid. The presence of iron even improves the indication of systems with difficult response, such as Mo(VI)/Mo(V) and V(IV)/V(III). In such cases iron is evidently involved in deciding the potential at the electrode even in a range much higher than E'_0 for Fe(III)/Fe(II).

Titanium(IV)/(III)

The increase of potential with hydrochloric acid concentration proves Ti(III) to be a softer electron-pair acceptor than Ti(IV). Gold or tungsten electrodes respond quickly to the titanium potential. Identical values result from titrations of Ti(III) with Fe(III) at a Pt electrode and of Ti(IV) with Cr(II) at Au or W electrodes. Potentials of +0.22 V in 7M hydrochloric acid and +0.29 V in the 9M acid were found with mixtures of Ti(IV) + Ti(III) at a Pt electrode after long waiting times.[8] The potentials of Ti(IV)/Ti(III) and W(VI)/W(V) differ by 0.16 V in 15M hydrochloric acid. The titration curve of a mixture shows a corresponding potential step, but it is too flat for quantitative work.

Molybdenum(V)/(III)

The gold electrode quickly responds to the couple Mo(V)/Mo(III) even at ambient temperature, unlike the case for Mo(VI)/Mo(V). The potential is close to that of tungsten(VI)/(V) over all the investigated range of acidity, so a redox titration always determines their sum.

Molybdenum(VI)/(V)

The potential equilibration at Au or Pt is very slow and unreliable even at elevated temperature. A reductometric titration curve is considerably distorted, but the end-point is correctly indicated. Presence of iron improves the indication of the Mo(VI)/Mo(V) potential. The values listed in Table 1 are uncertain to about 0.05 V. There seems to be no distinct dependence on the concentration of hydrochloric acid. The Mo(VI)/Mo(V) potential is reported as +0.60 V in a solution 6M in hydrochloric acid and 50% in acetone.[8]

Vanadium(IV)/(III)

This redox couple shows properties similar to those of the Mo(VI)/Mo(V) couple. A potential of +0.34 V was found in 1M sulphuric acid.[7]

On the importance of $\Delta E'_0$ for an accurate end-point

In the course of a redox titration we have three decisive potential values, $E'_{0(a)}$ of the analyte, the equivalence potential E_{vp} and $E'_{0(r)}$ of the reagent, the first and second of which are approximately marked by inflection points. With the simplifying assumption of a very large difference $\Delta E'_0$ between the two conditional potentials, the titration curve can be split into two independent parts, before and after the equivalence point, to calculate them separately. At low values of $\Delta E'_0$ this is no longer feasible. A practicable mathematical expression for the entire course of the titration has been developed.[10] If $\Delta E'_0$ is decreased, the equilibrium constant and the slope of the potential jump also decrease. The minimum values of $\Delta E'_0$ for an equivalence inflection point to be obtained at all have been derived.[11]

In the evaluation of a titration curve, we may distinguish three points: the equivalence point determined by stoichiometry, the geometrically defined inflection point of the potential jump, and the "end-point", *i.e.*, the point actually evaluated by the analyst. For an accurate result the fraction f of determinand titrated at the end-point has to attain a reasonably high value. For simplicity, textbooks usually deal with the question of how f depends on $\Delta E'_0$ at the equivalence point. For a pair of one-electron reactants, $\Delta E'_0 = 0.35$ V is required for f to reach 0.999. In practical analysis, however, the equivalence point remains unknown and the inflection-point in the equivalence region is taken as the end-point. Therefore, the systematic error is determined by f at this point. For two one-electron reactants, a rigorous approach was made by Goldman.[12,13] In our investigations, the equivalence-region inflection point could be unambiguously located for systems having a $\Delta E'_0$ of only 0.18 V. This corresponds to $f = 0.996$ or an error of −0.4%. For $\Delta E'_0 = 0.20$ V the error is only −0.17%, which in many cases forms but a minor part of the total error.

The situation is even more favourable in consecutive titrations of two components A and B, see Fig. 2. An important case is that where the conditional potentials of the determinands differ but slightly, whereas the titrant couple has a very different potential. For example, at the first end-point f_A may be say 0.99 and f_B is already 0.01. At the second end-point not only has all the rest of A reacted but B has also been titrated almost quantitatively. If the initial normal concentrations c_A and c_B are equal, no error occurs at all. In practice they will hardly differ by a factor of more than 2 or 3, since otherwise the burette

reading error becomes too large for the minor component and consecutive titration will be inapplicable.

It has not yet been investigated how the fractions titrated at the inflection points of a consecutive titration depend on the ratio c_A/c_B. Nevertheless it is evident that accurate determinations can be made of mixtures having much smaller values of $\Delta E_0'$ than those indicated by conventional textbook statements. The actual limit is largely given by the precision of locating the inflection point of a flat potential jump. This depends, among other factors, on the evaluation procedure applied.

It should be realized, however, that such considerations are merely theoretical. The accuracy of a titration procedure is determined by several factors, among which the degree of completion of the reaction may not be the most important. Deviations from stoichiometry, decomposition of reagent, time lag of electrode response, and more or less biased procedures of end-point location contribute to the total error. The degree of accuracy of a complete procedure can only be found from regression analysis and significance tests.[14] This is commonly done in spectroscopy and other physico-chemical methods, but in titrimetry equivalent weights and equilibrium constants are still often regarded as the sole links between volume consumed and amount of sample present.

Determination of tungsten alone in a mixture

By use of the oxidation state change W(VI)/W(V), tungsten can be directly determined in presence of Cr, V, Sn, Fe, and Cu, by either oxidation or reduction. If molybdenum is present, the titration curve shows two steps: one indicates Mo(VI)/Mo(V), the other the sum Mo(V)/Mo(III) + W(VI)/W(V). Tungsten can be determined from the difference if the ratio Mo/W is not too unfavourable. The sample must then not contain, however, any elements having potentials similar to that of Mo(VI)/Mo(V). These are vanadium and copper. The latter is also undesirable because it is finally reduced to the metal, which soils the titration cell or the reductor. Titanium interferes.

The oxidation of tervalent to quinquevalent tungsten allows the determination in presence of titanium, and the direct determination in samples containing molybdenum. There is now interference, however, by Cr and V, the measurable conditional potentials of which are close to that of W(V)/W(III).

In principle, the determination of tungsten is possible in any mixture which does not contain either Mo + V or Ti + Cr, because these two combinations cause interference with both oxidation steps of tungsten. In these two cases a separation is necessary.

COMPLETE ANALYSIS OF TUNGSTEN-CONTAINING MIXTURES

Determination by titration with chromium(II)

Mixture Mo + Fe + W. This most important system shows the pronounced influence of hydrochloric acid concentration on conditional potentials and the separation of redox steps. In 7*M* hydrochloric acid, Mo(VI)/Mo(V) and Fe(III)/Fe(II) are not sufficiently separated. There is only a very flat potential step between them, but their complete reduction gives rise to a fairly steep jump, after which Mo(V) and W(VI) are reduced simultanuously. In 9–11*M* hydrochloric acid three distinct steps occur, indicating Mo(VI)/Mo(V), Fe(III)/Fe(II) and the sum Mo(V)/Mo(III) + W(VI)/W(V). In 13.5–15*M* hydrochloric acid again only two steps are formed, the first of them due to Mo(VI)/Mo(V) and the second indicating the sum Fe(III)/Fe(II) + Mo(V)/Mo(III) + W(VI)/W(V). These variations are caused by the strong decrease in the conditional potential of Fe(III)/Fe(II) with increasing hydrochloric acid concentration, whereas the potentials of both Mo(V)/Mo(III) and W(VI)/W(V) increase sharply and that of Mo(VI)/Mo(V) is hardly influenced at all.

Mixture V + Fe + W. In 9–11*M* hydrochloric acid there are three steps, which are almost too flat for reliable evaluation. In 7*M* hydrochloric acid the sum V(IV)/V(III) + Fe(III)/Fe(II) is indicated by a clear jump, which is followed by a separate step for W(VI)/W(V). In 13.5*M* hydrochloric acid, on the contrary, vanadium forms a distinct step of its own, followed by a potential jump showing the sum Fe(III)/Fe(II) + W(VI)/W(V).

Mixtures Cu + W + Ti and V + W + Ti. The behaviour of these two systems is similar. At moderate acidities, V or Cu gives a separate step, but only the sum W(VI)/W(V) + Ti(IV)/Ti(III) is indicated. In 15*M* hydrochloric acid the potential of titanium is low enough to give a separate step after the reduction of tungsten, but on the other hand the tungsten potential has increased so much that there is no further separation from copper or vanadium.

Determination after reduction with zinc

Mixture W + Ti + Fe + Mo. In principle, all four components can be evaluated from four distinctly separated steps: W(V)/W(III), the sum Ti(IV)/Ti(III) + W(VI)/W(V) + Mo(V)/Mo(III), then Fe(III)/Fe(II) and Mo(VI)/Mo(V).

Mixture W + Cr + Fe + Mo. In principle, all four components can be evaluated from four distinctly separated steps: the sum W(V)/W(III) + Cr(III)/Cr(II), the sum W(VI)/W(V) + Mo(V)/Mo(III), then Fe(III)/Fe(II) and Mo(VI)/Mo(V).

In practice, the accuracy will hardly suffice in either of these cases, but simpler mixtures can readily be analysed.

Practical applications of the new possibilities shown by this work will be published later.[14]

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DETERMINATION OF GOLD IN SILVER, COPPER, LEAD, SELENIUM AND ANODE SLIME BY ATOMIC-ABSORPTION SPECTROMETRY

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Summary—A simple and sensitive combined solvent-extraction and atomic-absorption spectrometric method has been developed for the determination of gold in silver, copper, lead, selenium and anode slime. Samples are decomposed with hydrochloric and nitric acids, and gold is extracted as the trioctylmethylammonium–gold bromide complex and determined by atomic-absorption spectrometry by direct spraying of the extract into the flame. Optimal conditions for the extraction and determination of gold have been established. As little as 0.5 μg of gold in a sample can be determined. The extraction of gold from hydrochloric or hydrobromic acid solution with trioctylamine or trioctylmethylammonium chloride (or bromide) has also been investigated.

The determination of gold in silver, copper, lead or selenium is often needed for their metallurgical evaluation. Gold contents of these metals are extremely low and methods for the determination of gold at the 1-ppm level are required. Atomic-absorption spectrometry provides an excellent means for the determination of trace amounts of gold, usually in combination with a prior separation or concentration technique. However, very few practical applications of atomic-absorption spectrometry to the determination of gold in the above-named metals have been reported. Pollock and Andersen,[1] Kallmann and Hobart[2] and Sen Gupta[3] have determined gold in copper[1,2] and native silver[3] by atomic-absorption spectrometry, after separation of gold from the matrices by solvent extraction with ferroin[1] and by the fire-assay technique[2], and after the removal of silver as silver chloride.[3]

The solvent extraction technique, combined with atomic-absorption spectrometry, provides a simple and sensitive determination of gold. Previous investigations have indicated that high molecular-weight amines and ammonium salts are good extractants for gold. They are effective for the separation of gold from other elements. The extraction of gold from various media with high molecular-weight amines and ammonium salts, such as trioctylamine,[4–7] tetrabutylammonium perchlorate[8] or trioctylmethylammonium chloride[4,9,10] has been investigated.

In the present work, the extraction of gold with trioctylamine (TOA), trioctylmethylammonium chloride [TOMA(Cl)] and trioctylmethylammonium bromide [TOMA(Br)] from hydrochloric or hydrobromic acid solution has been investigated. The TOMA(Br) extraction system has been applied to the determination of trace amounts of gold in silver, copper, lead, selenium, blister copper and anode slime from electrolytic copper refining.

EXPERIMENTAL

Apparatus

A Hitachi 208 atomic-absorption spectrophotometer with a three-slot 100-mm long burner, a Hitachi HLA-3 gold hollow-cathode lamp and a Hitachi 056 recorder were used with the following operating conditions: wavelength 242.8 nm; slit-widths 0.18 mm (entrance) and 1.0 mm (exit); burner height position 2; lamp current 15 mA; air flow-rate 14 l./min; acetylene flow-rate 1.5 l./min.

Reagents

Hydrochloric acid, (1 + 1), (2 + 1) and concentrated.
Hydrobromic acid, 0.2*M* and 3*M*.
Nitric acid, (1 + 3), (1 + 1) and concentrated.
TOA solution. Dilute 6 ml of TOA to 200 ml with n-butyl acetate.
TOMA(Cl) solution. Dilute 6 ml of TOMA(Cl) to 200 ml with n-butyl acetate.
TOMA(Br) solution. Dilute 6 ml of TOMA(Cl) to 20 ml with n-butyl acetate. Transfer this solution to a separatory funnel and shake with two 40-ml portions of 3*M* hydrobromic acid for 10 min each time (discard the aqueous layers). Dilute the organic layer to 200 ml with n-butyl acetate.
Standard gold solution. Dissolve 0.500 g of gold metal in 10 ml of *aqua regia*. Add 40 ml of hydrochloric acid (1 + 1) and make up to 500 ml with water. Dilute the solution to the desired concentration immediately before use.

Procedure

Copper and selenium. Decompose 5 g of sample with 15 ml of hydrochloric acid (1 + 1) and 20 ml of nitric acid (1 + 1) (for copper), or with 30 ml of hydrochloric acid (2 + 1) and 20 ml of concentrated nitric acid (for selenium); heat gently to assist decomposition. Transfer the solution to a 200-ml separatory funnel, add 10.0 ml of 3*M* hydrobromic acid and dilute to 150 ml with water. Shake vigorously with 10.0 ml of the TOMA(Br) solution for 5 min. Discard the aqueous layer. Wash the organic layer by shaking with 50 ml of 0.2*M* hydrobromic acid for 3 min. Discard the aqueous layer. Spray the organic layer into the flame and measure the atomic-absorption signal.

Silver and lead. Decompose 2 g (for silver) or 5 g (for lead) of sample with 10 ml of nitric acid (1 + 1) (for silver), or with 40 ml of nitric acid (1 + 3) (for lead); heat gently

to assist decomposition. Add 20 ml of hydrochloric acid (1 + 1) and heat on a steam-bath for 1 hr. Transfer the solution, together with the silver chloride or lead chloride precipitate, to a 100-ml standard flask and dilute to the mark with water. Transfer a 50-ml aliquot of the supernatant solution to a 200-ml separatory funnel and dilute to about 120 ml with water. Add 10.0 ml (for silver) or 2.5 ml (for lead) of 3*M* hydrobromic acid and make up to 150 ml with water. Complete the determination as for copper and selenium samples.

Blister copper. Decompose 5 g of sample with 15 ml of hydrochloric acid (1 + 1) and 20 ml of nitric acid (1 + 1); heat gently to assist decomposition. Transfer the solution to a 100-ml standard flask and dilute to the mark with water. Transfer an aliquot (containing less than 15 μg of Au) of the solution to a 200-ml separatory funnel. Add 10.0 ml of 3*M* hydrobromic acid and dilute to 150 ml with water. Complete the determination as for copper and selenium samples.

Anode slime Decompose 0.1–1 g of the sample, previously dried at 250° for 10 hr, with 15 ml of concentrated hydrochloric acid and 5 ml of concentrated nitric acid; heat on a sand-bath to assist decomposition. Evaporate the solution nearly to dryness, add 20 ml of hydrochloric acid (1 + 1) and 10 ml of nitric acid (1 + 1) and heat gently to dissolve the salts. Transfer the solution together with the residue to a 100-ml standard flask and dilute to the mark with water. Transfer an aliquot (containing less than 15 μg of Au) of the supernatant solution to a 200-ml separatory funnel. Add 10.0 ml of 3*M* hydrobromic acid and dilute to 150 ml with water. Complete the determination as described above.

Table 1. Effect of hydrochloric acid, hydrobromic acid, TOA and TOMA concentration on extraction of gold

HCl or HBr concentration, *M*	TOA or TOMA concentration, %v/v	Extraction system and scale reading*			
		TOA–HCl	TOA–HBr	TOMA(Cl)–HCl	TOMA(Br)–HBr
0.02†	3.0	40.3	40.0	40.0	40.1
0.10†	3.0	40.0	40.2	39.7	39.9
0.20	3.0	40.0	40.0	40.0	40.0
1.0	3.0	40.0	40.3	40.0	40.0
3.0	3.0	40.2	40.0	40.0	40.3
0.20	0.2	41.3	42.3	43.4	42.7
0.20	1.0	40.9	42.2	41.7	40.8
0.20	2.0	40.5	41.3	41.1	40.4
0.20	3.0	40.0	40.0	40.0	40.0
0.20	4.0	39.6	39.2	39.0	40.0
0.20	5.0	38.4	37.5	37.5	39.0
0.20	7.0	37.7	37.3	36.1	37.9
0.20	10	35.3	35.3	33.9	36.6

* Gold taken 10.0 μg.

† Five ml of 3*M* sulphuric acid were added to the aqueous phase to avoid formation of an emulsion or a turbidity.

Table 2. Effect of other acids on the extraction of gold

	Concentration of acid, *M*	Extraction system* and scale reading			
		TOA–HCl	TOA–HBr	TOMA(Cl)–HCl	TOMA(Br)–HBr
No addition		40.0	40.0	40.0	40.0
H_2SO_4	1.0	40.2	40.2	39.8	40.0
	2.0	40.3	40.0	40.0	40.3
	3.0	40.4	40.4	40.4	40.0
HNO_3	1.0	40.2	40.0	40.0	40.2
	1.5	40.0	40.4	40.0	39.8
	2.0	37.7	40.0	37.7	40.0
	2.5	35.7	40.2	37.3	40.0
	3.0	34.9	39.8	36.1	40.0
	4.0	30.6	40.2	31.6	40.2
$HClO_4$	0.01	40.0	40.0	40.0	40.0
	0.10	36.6	39.7	38.9	40.0
	0.20	35.9	39.3	35.9	40.0
	0.40	33.9	38.6	34.7	39.8
	1.0	24.7	37.6	26.2	39.6
	2.0	31.2	35.8	29.1	37.2
	3.0	28.5	32.0	20.4	25.7
HCl	1.0		40.0		40.0
	3.0		40.0		40.0
	6.0		40.4		40.0

* Gold taken 10.0 μg; HCl or HBr concentration 0.2*M*; TOA or TOMA concentration in n-butyl acetate 3%v/v.

Preparation of calibration curve. Transfer portions (containing up to 150 μg of Au) of standard gold solution to 200-ml separatory funnels. To each solution and a blank (water), add 5 ml of hydrochloric acid (1 + 1), 5 ml of nitric acid (1 + 1) and 10.0 ml of 3*M* hydrobromic acid, and dilute to 150 ml with water. Treat these solutions as described above and plot a curve of the atomic-absorption signal against amount of gold.

RESULTS AND DISCUSSION

Extraction of gold chloride and bromide TOA and TOMA ion-association complexes

The extraction of gold with TOA and TOMA(Cl) from hydrochloric acid solution, and with TOA and TOMA(Br) from hydrobromic acid solution has been

Table 3. Analytical results for various samples

Sample	Sample taken, *g*	Au added, *μg*	Total Au found, *μg*	Au in sample (average), *ppm*	Au in sample (spectrophotometric method), *ppm*
Copper metal (electrolytic)	5.0	0	<0.5	<0.1	<0.1
	5.0	0	<0.5		
	5.0	3.0*	2.97		
	5.0	3.0*	2.94		
Copper metal (tough pitch)	5.0	0	0.68	0.14	0.14
	5.0	0	0.70		
	5.0	2.0*	2.70		
	5.0	2.0*	2.67		
Copper metal (V.M.C.)	5.0	0	0.94	0.19	0.21
	5.0	0	0.90		
	5.0	2.0*	2.95		
	5.0	2.0*	2.95		
Blister copper	5.0	0	3.42	0.67	0.70
	5.0	0	3.32		
	5.0	3.0*	6.32		
	5.0	3.0*	6.32		
Blister copper	5.0	0	19.9	4.1	4.6
	5.0	0	20.1		
	5.0	20.0*	40.6		
	5.0	20.0*	40.4		
Blister copper	5.0	0	98.0	20	20
	5.0	0	99.6		
	5.0	100*	200.0		
	5.0	100*	198.0		
Selenium metal	5.0	0	<0.5	<0.1	
	5.0	0	<0.5		
	5.0	5.0*	5.04		
	5.0	5.0*	4.96		
Silver metal	2.0	0	4.43	2.2	2.0
	2.0	0	4.26		
	2.0	5.0*	9.40		
	2.0	5.0*	9.32		
Silver metal	2.0	0	13.3	6.6	6.7
	2.0	0	13.1		
	2.0	10.0*	23.7		
	2.0	10.0*	23.2		
Lead metal	5.0	0	<1.0	<0.2	<0.2
	5.0	0	<1.0		
	5.0	5.0*	4.92		
	5.0	5.0*	5.04		
Anode slime	0.20	0	142	0.068%	0.065%
	0.20	0	138		
	0.20	120*	248		
	0.20	120*	253		
Anode slime	0.10	0	188	0.19%	0.17%
	0.10	0	200		
	0.10	200*	386		
	0.10	200*	394		

* Gold, in the form of aliquots of standard gold solution, was added to the solid samples before decomposition.

investigated. To a portion (10 μg of Au) of standard gold solution, hydrochloric or hydrobromic acid was added to give the desired final concentration and the solution diluted to 75 ml with water. Gold was then extracted by shaking with 10.0 ml of an n-butyl acetate solution of TOA, TOMA(Cl) or TOMA(Br) and the atomic-absorption of the organic layer measured.

Examination of the extraction of gold from 0.02–3.0*M* hydrochloric and hydrobromic acid solutions showed that the atomic-absorption signals (scale readings) were independent of the hydrochloric and hydrobromic acid concentrations in all the TOA–HCl, TOMA(Cl)–HCl, TOA–HBr and TOMA(Br)–HBr systems. Differences in the signals among the four extraction systems were negligible (Table 1).

The concentrations of TOA, TOMA(Cl) and TOMA(Br) in n-butyl acetate were varied; the atomic-absorption signals decreased gradually with increasing TOA and TOMA concentrations over the whole concentration range tested (Table 1). The decrease in signal may not be due to a lowering of the degree of extraction of gold, but is probably due to a lowering of the aspiration rate of the extract into the flame owing to an increase in viscosity of the solvent with increase in the TOA or TOMA concentration.

Shaking time for the extraction was varied from 1 to 10 min and it was found that the absorption signals were independent of shaking time.

Effects of other acids

The effects of sulphuric, nitric, perchloric and hydrochloric acids on the extraction of the gold chloride and bromide TOA and TOMA complexes were investigated (Table 2); the experimental procedure was as described above. In the bromide extraction systems, sulphuric, nitric and hydrochloric acids had no effect on the extraction of gold over the whole concentration range, but perchloric acid at concentrations above 0.1*M* in the TOA–HBr system and above 1.0*M* in the TOMA(Br)–HBr system interfered. In the chloride extraction systems, sulphuric acid did not interfere, but nitric acid above 1.5*M* and perchloric acid above 0.01*M* decreased the absorption signals. These results show that the TOMA(Br)–HBr system is superior to the others.

Effects of various elements

On the basis of the results above, the TOMA(Br)–HBr system with 0.2*M* hydrobromic acid, 3% TOMA(Br) in n-butyl acetate and a 5-min extraction time was adopted for the determination of gold in the materials mentioned. The effects of various elements on the extraction and determination of gold were then examined. The procedure was as described above with the addition of a 3-min wash of the organic layer with 50 ml of 0.2*M* hydrobromic acid.

There was no interference from 1 mg of Pd(II), Pt(IV); 5 mg of Al, Be, Ce(III), Cr(III) Hg(II), La, Mg, Mo(VI), Ti(IV), Tl(III), V(V), Zr; 10 mg of In; 50 mg of Bi, Ca, Cd, Co, Mn(II), Sb(III); 100 mg of As(III), As(V), Fe(III), Sn(IV), Te(IV); 1 g of Zn and 5 g of Cu, Ni and Se(IV).

Up to 100 mg of lead did not interfere , but 1 g of lead formed a lead bromide precipitate on the addition of hydrobromic acid and interfered with the extraction of gold. To avoid precipitation of lead bromide, a hydrobromic acid concentration of 0.05*M* was effective: even so amounts of lead as large as 5 g did not interfere if gold was extracted from 0.05*M* hydrobromic acid solution.

Up to 10 mg of silver did not interfere though a silver bromide precipitate was formed; it is, however, desirable to separate silver before the extraction of gold. Up to 1-mg of thallium(I) did not interfere, but larger amounts caused negative errors.

Applications

Gold in silver, copper, lead, selenium, blister copper and anode slime has been determined by the proposed method (Table 3); amounts of gold added to samples before decomposition were recovered quantitatively. As little as 0.5 μg of gold in a sample or in an aliquot of the sample solution could be determined. The gold contents determined by the proposed method are in good agreement with those determined by the spectrophotometric method[6] using 4,4′-bis (dimethylamino)thiobenzophenone (thio-Michler's ketone) as reagent. The proposed method is simple, rapid and sensitive.

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SEPARATION CHROMATOGRAPHIQUE DES AZEPINES

NANOCHROMATOGRAPHIE SUR COUCHE MINCE ET CHROMATOGRAPHIE EN PHASE LIQUIDE SOUS PRESSION

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Résumé—Deux méthodes chromatographiques sont proposées pour séparer, identifier et éventuellement doser des psychotropes appartenant soit à la série des dibenzoazépines, soit à celle des benzodiazépines. Dans chacune des séries, une méthode sur nanocouche-mince a été codifiée, puis sa transposition en HPLC a été tentée. Cette transposition a nécessité une légère modification du solvant éluant. Les R_f et temps de rétention sont présentés et deux applications quantitatives sont décrites.

Nous nous sommes intéressés à l'analyse de médicaments psychotropes, c'est à dire agissant sur le système nerveux central, soit comme stimulants (psychoanaleptiques), soit comme inhibiteurs (psycholeptiques), soit même comme antiépileptiques.

Le groupe des psychoanaleptiques comprend les dibenzoazépines que nous avons classées en deux sous-groupes chimiques.

—Les dibenzoazépines proprement dites: Carbamazépine, Opipramol.

—Les dibenzodihydroazépines: Chlorimipramine, Imipramine, Désipramine, Trimipramine.

Le groupe des psycholeptiques comprend entre autres molécules les benzodiazépines-1,4 que nous classerons en quatre sous-groupes.

—Groupe des aminobenzodiazépines-1,4: Chlordiazépoxide.

—Groupe des benzodiazépines-1,4: Médazépam.

—Groupe des benzodiazépines-1,4 ones-2: Diazépam, Nitrazépam.

—Groupe des hydroxybenzodiazépines-1,4: Oxazépam, Lorazépam.

Parallèlement à ces douze azépines, nous avons également étudié une dibenzodiazépine (la dibenzépine) et une molécule comportant trois atomes d'azote, le chlorhydrate de propizépine, qui est un thymoanaleptique. Toutes ces molécules sont présentées dans les tableaux 1 et 2.

Ces diverses molécules sont utilisées en thérapeutique soit seules, soit en association, ce qui peut poser différents types de problèmes analytiques:

—contrôle du médicament lui-même,

—dosage dans les liquides biologiques, en particulier détermination spécifique des taux sanguins,

—étude des métabolismes.

Ces différents aspects de l'analyse impliquent des séparations et identifications sélectives ce qui justifie une étude chromatographique de ces molécules.

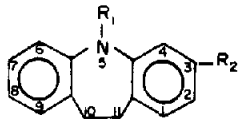
Les travaux portant sur la séparation des dibenzoazépines ou des benzodiazépines par chromatographie sur couche mince sont très nombreux, mais nous n'avons jamais rencontré de publications comportant une étude des quatorze molécules précédemment énumérées. Toutes les séparations chromatographiques décrites ont été réalisées sur couche mince de gel de silice, avec des éluants variés.

En ce qui concerne les dibenzoazépines, Castagnou et Artiges[1] proposent les deux systèmes de solvants suivants: benzène–acétone–NH_4OH 25% (50:10:10) et benzène–acétone–acide acétique (50:50:0,5) pour séparer désipramine, imipramine, chlorimipramine, trimipramine, opipramol et carbamazépine. Bourdon et Nicaise[2] modifient très légèrement ce solvant et avec un mélange de benzène–acétone–NH_4OH 25% (50:10:5) parviennent à séparer désipramine, imipramine, chlorimipramine et trimipramine. Marca et Huehlemann[3] apportent eux aussi un léger changement au solvant de migration de Bourdon et Nicaise et propose le mélange benzène–acétone–diéthylamine (50:10:5) pour séparer deux molécules supplémentaires, l'opipramol et la dibenzépine. Un autre système de solvant fréquemment cité est le mélange éther–acétone–diéthylamine (90:10:1), que Viala *et al.*,[4] Vignoli *et al.*[5] et Marca et Huehlemann[3] utilisent pour séparer diverses dibenzoazépines en association.

En ce qui concerne les benzodiazépines, le nombre de systèmes de solvants proposé est encore plus vaste que dans la série précédente. Lafargue *et al.*[6,7] ont choisi les mélanges benzène–chloroforme (3:1) ainsi que chloroforme–éthanol (29:1) pour séparer diverses benzodiazépines et leurs métabolites en mélange. Viala *et al.*[8] ont obtenu des résultats intéressants avec le système dichloroéthane–méthanol–NH_4OH 25% (95:5:1). Schutz *et al.*[9] réalisent quant à eux une chromatographie bidimentionnelle avec le solvant

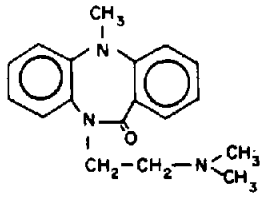
Tableau 1

Dibenzoazépines



	R_1	R_2	10–11
Carbamazépine	$CO-NH_2$	H	Δ
Chlorimipramine	$CH_2-CH_2-CH_2-N(CH_3)_2$	Cl	H
Désipramine	$CH_2-CH_2-CH_2-NH-CH_3$	H	H
Imipramine	$CH_2-CH_2-CH_2-N(CH_3)_2$	H	H
Opipramol	$CH_2-CH_2-CH_2-N$ (pipérazine) $-CH_2-CH_2OH$	H	Δ
Trimipramine	$CH_2-CH(CH_3)-CH_2-N(CH_3)_2$	H	H

Dibenzépine

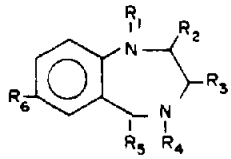


n heptane–acétate d'éthyle–éthanol–NH_4OH 25% (70:70:10:5).

La révélation des spots se fait par lecture directe sous ultra violet, soit après pulvérisation de divers réactifs (réactif de Forrest, réactif phosphocérique pour les dibenzoazépines et réactif de Bratton–Marshall ou réactif de Draggendorf pour les benzodiazépines).

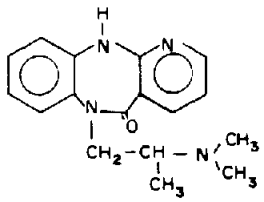
Tableau 2

Benzodiazépines



	R_1	R_2	R_3	R_4	R_5	R_6
Chlordiazépoxide		$NH-CH_3$	H_2	→ O	C_6H_5	Cl
Diazépam	CH_3	O	H_2		C_6H_5	Cl
Lorazépam	H	O	OH			Cl
Medazépam	CH_3	H_2	H_2		C_6H_5	Cl
Nitrazépam	H	O	H_2		C_6H_5	NO_2
Oxazépam	H	O	OH		C_6H_5	Cl

Propizépine



La chromatographie en phase liquide sous pression (HPLC) est une technique plus récente, ce qui explique le nombre plus restreint de publications. Elle est en général conduite sur une phase stationnaire de silice. De Tavernier *et al.*[10] fut l'un des premiers à séparer imipramine, chlorimipramine, trimipramine et désipramine sur une colonne de Micropak Si 10, avec un mélange éluant constitué de dichlorométhane–n heptane (1:1) comportant 0,2% de propanol-2 et 0,13% de n propylamine. Dixon et Stoll[11] séparent sur Corasil 2 avec différents systèmes de solvants, certains mélanges de carbamazépine, trimipramine, chlorimipramine, imipramine, désipramine, diazépam, nitrazépam et chlordiazépoxide. Knox et Jurand[12] séparent aussi l'imipramine et la trimipramine d'autres dérivés tricycliques non azépiniques, sur Merckosorb Si 100, avec un système éluant constitué de chlorure de méthylène–acide acétique (10%)–pentane ou hexane (de 0 à 25%) et eau (de 0 à 0,1%). Mais c'est le travail de Gonnet et Rocca[13] qui nous semble le plus intéressant, car il est l'un des premiers à réaliser une transposition chromatographie sur couche-mince classique–HPLC pour différentes séries de molécules, dont les benzodiazépines. Il effectue également une étude en chromatographie en phase liquide sous pression des dibenzoazépines, qui sont séparées sur Lichrosorb Si 60 ou Partisil 5, avec un système éluant constitué de dichlorométhane–méthanol (90:10) saturé par 10 ml de NH_4OH à 28% pour 250 ml d'eau. Cependant, dans cette série, à notre connaissance, une transposition CCM–HPLC n'a pas été réalisée.

PARTIE EXPERIMENTALE

Nanochromatographie sur couche mince

La chromatographie sur couche mince à haute performance ou nanochromatographie, est une technique récente qui se différencie de la chromatographie sur couche mince classique par différents points qui sont:

—la régularité et la finesse de la granulométrie des adsorbants (5 μm pour la nano-CCM contre 150 à 200 μm pour la chromatographie classique);

—l'épaisseur des couches (15 μm pour la nano-CCM contre 250 à 300 μm). Ces conditions impliquent le dépôt de très petites quantités de produits, afin de ne pas surcharger les plaques. La résolution est ainsi très nettement améliorée et il est possible de diminuer les distances de migration, donc de gagner du temps.

Nous avons utilisé des cuves Camag mesurant 9 × 5 × 3,5 cm et des plaques de Kieselgel 60 F 254 Merck, sur support aluminium, redécoupées aux dimensions intérieures de la cuve (7 × 3,5 cm). Les échantillons à étudier ont été solubilisés dans le système éluant utilisé en nano-CCM et en HPLC, à raison de $10^{-3}M$. Les dépôts sont effectués avec une seringue de 1 μl qui nous a permis de déposer de 0,2 à 0,5 μl de solution.

Le système de migration est réalisé avec des produits répondant aux normes maximales de pureté nécessaires pour la chromatographie. Il est constitué du mélange cyclohexane–éthanol–n butanol–NH_4OH 25% (80:20:10:1); 4 à 5 ml de ce solvant suffisent. La migration est très rapide, elle dure environ 5 à 10 mn. Le front du solvant est à 6,5 cm, ce qui correspond pratiquement au sommet de la plaque. Les solvants constituant le mélange éluant ont été choisis non seulement en fonction de leur polarité, mais également de leur transparence en ultraviolet, notre objectif étant de tenter par la suite une transposition en HPLC avec un détecteur ultraviolet.

La mise en évidence des spots a été faite par lecture directe sous ultraviolet, après séchage à l'air de la plaque.

HPLC

La séparation envisagée, après avoir été mise au point sur CCM, a été transposée en HPLC.

Nous avons utilisé une pompe Pye Unicam LC 20, nous permettant de travailler à une pression d'environ 80 bars, ce qui correspondait à un débit de 1,7 ml/mn. La colonne en acier inoxydable (15 × 0,4 cm) a été remplie au laboratoire, par voie humide, avec du Lichrosorb Si 60 Merck 5 μ, en utilisant un poste de remplissage Chromatem. Nous avons suivi l'élution de nos produits à l'aide d'un détecteur spectrophotométrique Philips à 254 nm, le détecteur étant relié à un enregistreur monocanal. L'injection des solutions a été réalisée par l'intermédiaire d'une boucle de 10 μl.

Le solvant mis au point précédemment sur couche mince a dû être légèrement modifi afin d'obtenir une meilleure séparation. Il est constitué du mélange cyclohexane–éthanol–n butanol–NH_4OH 25% (80:20:10:0,4). D'autre part, il est nécessaire de thermostater à 20° afin de toujours conserver la même concentration en ammoniaque.

La mesure de la hauteur des pics a été mise à profit pour tracer certaines courbes d'étalonnage dans un but quantitatif.

RESULTATS

La technique de nanochromatographie sur couche mince proposée a été appliquée d'une part aux six dibenzoazépines et à la dibenzépine et d'autre part aux six benzodiazépines et à la propizépine.

Dans la première série, la séparation chromatographique est totale et les migrations se font dans l'ordre présenté dans le tableau 3.

Dans la deuxième série, il n'a pas été possible de séparer oxazépam et lorazépam, molécules très proches qui ne se différencient que par un atome de

Tableau 3. Série des dibenzoazépines + dibenzépine et des benzodiazépines + propizépine

Molécules étudiées	R_f*	Temps de rétention en HPLC† (en *mn*)
Trimipramine	0,61	3,1
Chlorimipramine	0,54	4,1
Imipramine	0,47	4,6
Carbamazépine	0,38	5,8
Dibenzépine	0,33	9,2
Opipramol	0,20	13,2
Desipramine	0,13	23,7
Médazépam	0,57	28
Diazépam	0,50	30
Chlordiazépoxide	0,40	36
Nitrazépam	0,35	41
Oxazépam	0,24	48
Lorazépam	0,24	46
Propizépine	0,18	104

* Nano-CCM = HPTLC: Kieselgel 60 F 254; solvant cyclohexane–éthanol–butanol–NH_4OH 25% (80:20:10:0,4).

† HPLC: colonne Si 60; solvant cyclohexane–éthanol–butanol–NH_4OH 25% (80:20:10:0,4).

Débit 1,75 ml/mn.

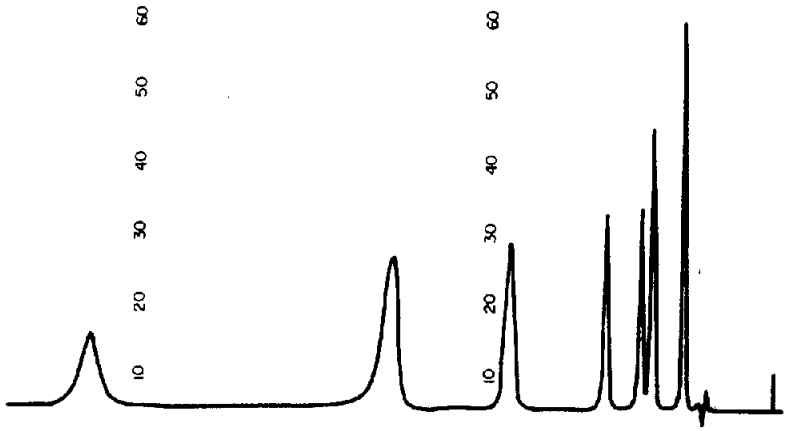


Fig. 1. Séparation des dibenzoazépines et de la dibenzépine. Colonne Si.60; solvant cyclohexane–éthanol–butanol–NH_4OH 25% (80:20:10:0,4); débit 1,75 ml/mn.

chlore sur le cycle benzénique substitué en 5. L'ordre de migration est présenté dans le tableau 3.

En HPLC, les mêmes résultats ont été obtenus, c'est à dire séparation complète pour la première série et impossibilité de séparation de l'oxazépam et du lorazépam dans la deuxième série. Les temps de rétention sont présentés respectivement dans le tableau 3. Les enregistrements sont présentés sur les figures 1 et 2. L'obention de pics étroits et symétriques pour un grand nombre de molécules nous a incités à tenter des essais quantitatifs. L'expérience a été réalisée sur deux des molécules, la chlorimipramine et la trimipramine, avec des solutions allant de $2.10^{-6}M$ à $10^{-5}M$, après injection de 10 μl de solutions. Les droites d'etalonnage sont linéaires.

DISCUSSION

Les résultats présentés montrent que la transposition nanocouche mince–HPLC est possible dans la série étudiée, sous réserve d'une très légère modification de la concentration en ammoniaque du solvant d'élution. L'ordre des R_f est tout à fait comparable à celui des temps de rétention.

L'examen de ces R_f et temps de rétention en fonction de la structure chimique permet de tirer un certain nombre de conclusions concernant l'influence de cette dernière sur l'affinité pour la phase stationnaire polaire (donc sur la polarité).

Pour les dibenzoazépines, l'augmentation de la masse molaire par l'introduction d'une ramification sur la chaine alkylaminée, diminue l'affinité pour la phase stationnaire. Si elle est dûe à l'introduction d'un atome de chlore, ce dernier semble avoir une double influence en sens opposé: augmenter la polarité par sa nature électronégative, la diminuer par suite de l'augmentation de la masse. L'introduction de groupements polaires favorise la fixation dans l'ordre croissant suivant: amide–carbonyle–alcool–amine secondaire, la molécule la plus fixée étant celle qui possède une fonction amine secondaire à la place de l'amine tertiaire.

Pour les benzodiazépines, il est facile de montrer que l'affinité pour la phase stationnaire croît avec la nature et le nombre des groupements fonctionnels polaires introduits par rapport au cycle de base.

Si l'on prend comme module de base le médazépam, molécule la moins fixée sur la phase stationnaire, il est logique que le diazépam possédant un groupement carbonylé supplémentaire soit plus retenu, que le chlordiazépoxide le soit encore davantage puisqu'il possède une double liaison supplémentaire, une fonction amine secondaire et un groupement aminoxyde. Le nitrazépam est nitré sur le cycle au lieu d'avoir un atome de chlore, ce qui justifie son affinité supérieure à celle du diazépam, affinité qui est encore augmentée par le fait que l'azote cyclique en 1 est secondaire et non plus tertiaire. Enfin, pour oxazépam et lorazépam, ces molécules bénéficient de deux substituants polaires influençant fortement l'affinité et ce sont donc les molécules les plus fixées. Comme

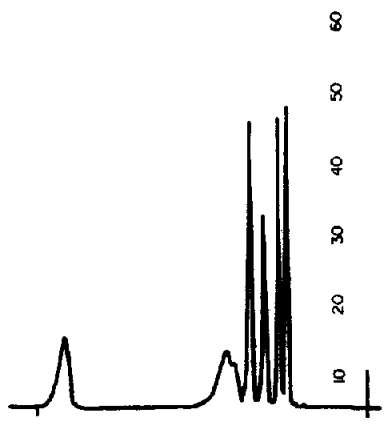


Fig. 2. Séparation des benzodiazépines et de la propizépine. Colonne Si.60; solvant cyclohexane–éthanol–butanol–NH_4OH 25% (80:20:10:0,4); débit 1,75 ml/mn.

nous l'avons déja dit, la présence d'un atome de chlore supplémentaire sur le cycle en 5, n'apporte pas d'affinité différentielle et ne permet donc pas de les séparer.

Les différences de structure entre les molécules appartenant soit à la série des dibenzoazépines, soit à celles des benzodiazépines ont donc permis de séparer les principaux représentants de ces séries, utilisés en thérapeutique, à l'exception près signalée plus haut.

Il reste cependant à distinguer ces deux classes de molécules entre elles, ce qui semble possible en jouant sur leurs solubilités différentielles. Ce travail qui est en cours permettrait de résoudre les problèmes posés par l'administration concomitante de dibenzoazépines et benzodiazépines pouvant dès lors se retrouver ensemble dans les liquides biologiques.

En ce qui concerne les essais quantitatifs, nous avons montré que pour certaines molécules donnant des pics étroits et symétriques, la mesure de la hauteur des pics pourrait permettre une bonne détermination quantitative. Nous l'avons d'ailleurs appliquée avec succès au contrôle de comprimés et solutés injectables renfermant ces principes actifs.

CONCLUSION

Il est possible de séparer entre elles, soit par nanochromatographie sur couche mince, soit par HPLC, les principales dibenzoazépines et benzodiazépines utilisées en thérapeutique. Il semble en outre que pour ces deux séries, la transposition d'une technique de séparation sur couche mince en HPLC soit possible avec le minimum de modifications. Enfin la HPLC présente l'avantage de permettre à la fois la séparation, l'identification et le dosage, ce qui a été montré en particulier pour chlorimipramine et trimipramine.

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Summary—Two chromatographic methods are proposed for separation, identification and determination of dibenzoazepines and benzodiazepines. A micro thin-layer method has been developed and transposed to give an HPLC system, which requires a slight modification of the solvent. Retention data are given, and two quantitative applications are described.

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FIELD EXPERIENCE WITH AMBIENT-LEVEL FLAME-PHOTOMETRIC SULPHUR DETECTORS*

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Summary—Carbon dioxide exhaled by the operators produces increased levels of this gas inside the trailers used for housing monitoring equipment for use in the field. These levels can be high enough to produce serious calibration errors in flame photometric detectors used for measuring ambient concentrations of gaseous sulphur products. Quantitative measurement of the carbon dioxide interference in the sulphur emission is reported, as well as a method of estimating the extent of quenching agent pollution.

A field programme is in progress in Montana conducted by the Environmental Protection Agency (EPA) to identify and measure the environmental effect of coal-fired power plants. Part of it is devoted to monitoring air-quality several miles downwind from the Colstrip power plants in a rather remote area of the state. Four monitoring stations with different degrees of instrumentation have been established downwind in the most prevalent direction of air-mass flow. They are located in semi-arid rangeland with access essentially limited to 4-wheel drive vehicles. Each station basically consists of a small trailer which serves to provide shelter and a controlled environment for the instruments. Three of them are completely self-contained, generating and regulating their own power systems. Each is equipped with a sulphur analyser for continuous monitoring of the atmosphere. At present these analysers are all flame photometric devices (FPD). This report covers experiments conducted at our major monitoring location (Hay Coulee). Here we have gained experience with several manufacturers' instruments as well as various analytical monitoring techniques for atmospheric sulphur determination.

In a major effort to improve the accuracy of the data being collected under sponsorship of the EPA, an independent contractor (Rockwell International) has been assigned the task of ensuring quality and routinely inspects and tests all equipment utilized for data collection in programmes operated by or for this Federal agency. The results of these routine audits are used to suggest methods of improving accuracy and defining the limits of error for a particular batch of data. In general, this system has fulfilled its purpose. We have been responsible for operation of the Colstrip (Montana) stations during the past year, and have found unexplained differences between the Rockwell calibrations of the sulphur monitors and ours, even though standard NBS permeation tube calibration sources and other accepted dilution techniques were presumably used throughout. This paper is a report of the magnitude and cause of these errors, of conditions conducive to their occurrence, and of their elimination.

Extensive use has been made of the flame spectrometric method for the determination of sulphur-containing compounds.[1,2] It is based on the light emitted from the excited S_2 band in a hydrogen-rich flame. The emission is highly dependent on the nature and temperature of the flame. Precise gas-metering and mixing make high accuracy and reproducibility attainable. The effect of other gaseous substances on the molecular emission of the S_2 species has been reported,[3,4] that of organic compounds and carbon dioxide being a decrease in response. The interference is a function of the concentration of the compounds concerned, but is often independent of the sulphur-compound level.[5]

At the Hay Coulee site it was noted from time to time that unexplained zero shifts would occur when the internal trailer air was used as the carrier-gas. These shifts were not constant from day to day. Small zero shift effects which may not have been observed or considered important by other workers were critical to the validity of our monitoring data since all values measured in the clean environment were below 50 ppM (parts per milliard)†. For this reason we made

*This work was performed for the U.S. Environmental Protection Agency under Interagency Agreement EPA-IAG-D7-0473.

† All concentrations are expressed as v/v.

it a practice always to use ambient air from outside the trailer as the primary source of "zero" air, and it was always passed through appropriate clean-up beds. At the time of the last audit we noted that air from inside the trailer was being used as the carrier-gas. It seemed reasonable to assume that interfering materials which were not scrubbed from the carrier gas might be causing the discrepancies. Although the temperature within the trailers was controlled for the benefit of the instruments, there was no provision for ventilation with air from outside. Indeed some effort had been made in the past to reduce draughts on account of the harsh climate. The most obvious cause of flame interference was increased atmospheric concentrations of carbon dioxide from human breath, and the effect would be greater on audit days when 3 or 4 adults would be present.

EXPERIMENTAL

To examine and measure the effects of changes in CO_2 concentration in the carrier-gas on sulphur response, an experimental arrangement for gas-mixture control was built. This consisted of a supply of pure CO_2 gas fed into a double-dilution air system. The dilution air was passed through "Ascarite" to remove CO_2. Standardized concentrations of CO_2 in air could then be prepared, including the range typical of normal air. This mixture was purified over silica gel and charcoal before entering an SO_2 permeation system calibrated by NBS. The response to changing mixtures of SO_2 and CO_2 was measured in a standard FPD sulphur analyser.* This instrument was initially calibrated with normal outside air and an SO_2 permeation tube. In this manner, deviations in SO_2 response, caused by changes in CO_2 concentration, could be observed and related to the auditing problems. Four concentrations of SO_2 gas were used, in the range 20—100 ppM. At each concentration, the sulphur emission intensity was observed when 4 standard carbon dioxide mixtures and normal outside air were used in turn as carrier gas compositions of these mixtures were chosen to include the CO_2 concentrations to be expected under such confined conditions. A series of similar experiments was conducted with a second ambient-air monitor.† Both monitors were utilized on this project for sulphur analysis.

DISCUSSION

The results of carbon dioxide interference in the sulphur emission are shown as a log–log plot in Fig. 1. Included for comparison is the effect of carrier air containing the CO_2 concentration produced during a typical day with two adult workers inside the trailer. It is apparent that the slopes of these curves are essentially constant. That is, the interference with the emission is independent of the concentration of the sulphur dioxide. This is in agreement with the findings of Sugiyama *et al.* for other quenching agents.[5]

A plot of the instrumental response to low concentrations of SO_2 in the presence of varying CO_2 levels is shown in Fig. 2. The response is expressed as net photomultiplier tube (PMT) current in nA, which is

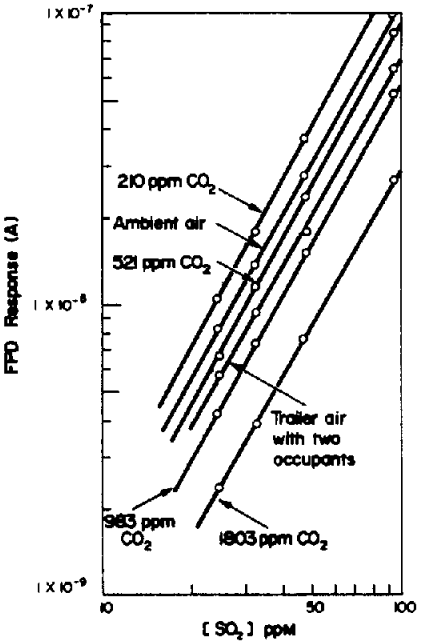

Fig. 1. Quenching of low-level sulphur emission by CO_2.

the same as the percentage shown on the 1E − 7 scale of the Meloy SA160 analyser. The net PMT current is computed by subtracting from the gross PMT current the dark current and the current obtained for "zero air". Extrapolation of the instrumental output to higher concentrations of carbon dioxide indicates that the sulphur response should be completely quenched by about 3000 ppm of CO_2. The slopes of the curves are a function of the SO_2 levels. Figure 3 is a curve generated by log–log regression analysis, illustrating the empirical relationship between changing instrumental response and changing interference level. The slope of the carbon dioxide *vs.* response curve may be expressed as a function of the SO_2 levels,

$$\frac{\Delta \ln[CO_2]}{\Delta \text{response}} = f[SO_2] \quad (1)$$

where $[CO_2]$ and $[SO_2]$ are expressed in ppm and ppM respectively. This curve is the basis of an empirical relationship showing how the analyser response varies as the CO_2 level changes.

The curve was analysed with respect to the actual variations produced by changes in SO_2 level. It was found that

$$f[SO_2] = K[SO_2]^x \quad (2)$$

where K and x are -140 and -2.0, respectively, and therefore the change in instrumental output is

$$\Delta \text{response} = \frac{\Delta \ln[CO_2]}{-140[SO_2]^{-2.0}}. \quad (3)$$

*Meloy Laboratories Model SA 160.
† Meloy Laboratories Model 285E.

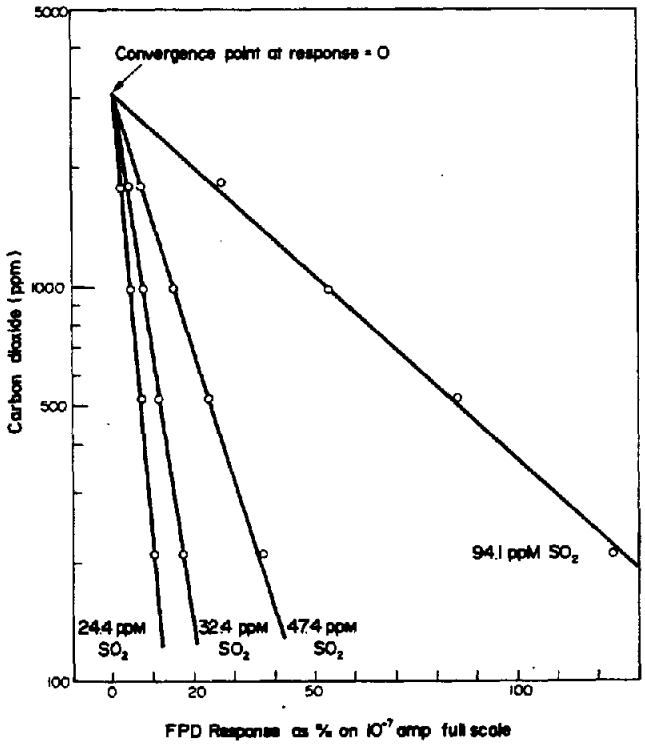

Fig. 2. Effect of CO_2 on FPD response to varying $[SO_2]$.

Therefore:

$$\Delta(\text{response}) = \frac{(\ln[CO_2]_{final} - \ln[CO_2]_{initial})[SO_2]^{2.0}}{-140} \quad (4)$$

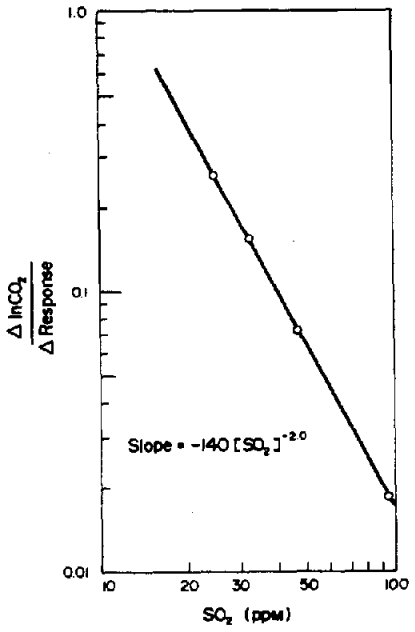

Fig. 3. Relationship between CO_2 concentration, FPD response and SO_2 concentration.

The standard deviations for K and x were derived from 3 regression lines. The relative standard deviations were 18.9% for K and 0.04% for x. It is apparent that increasing concentrations of CO_2 will decrease the response. Equation (4) can then be used to predict the response from the expected changes in carbon dioxide level and observations on standards. By using the response changes from Fig. 1 for particular concentrations of sulphur dioxide and substituting into equation (4) we can also calculate the level of CO_2 present when the trailer had two occupants. This was found to be 750 ppm.

Other estimates can be made of the carbon dioxide concentration within the trailer when it is occupied. The respiratory rate for a man in light activity is about 1200 litres of air per hr, with an oxygen to carbon dioxide conversion of about 4%.[6] The net carbon dioxide input is, therefore, 48 l./man-hr and thus 96 l./hr when two men are present. Our trailer volume is approximately 40,000 litres of which 13.2 will be CO_2 under standard conditions. Assuming that sufficient time had elapsed for CO_2 equilibrium to have been achieved, then the value of 750 ppm measured represents the resultant input from the two occupants. This net carbon dioxide gain of 171./hr is the resultant of the production rate (96 l./hr) and the loss-rate from the trailer. The rate of carbon dioxide loss is, therefore, 79 l./hr. If we assume that complete mixing occurs between the incoming air containing 330 ppm of carbon dioxide and the polluted air before escape

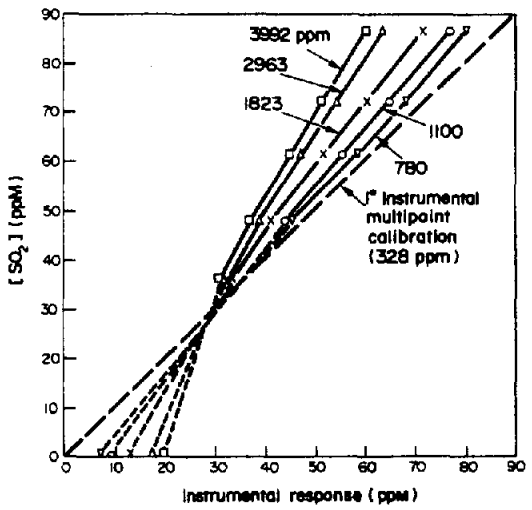

Fig. 4. Interfering effects of CO_2 on the SO_2 response of a Meloy 285E ambient sulphur analyser.

of the latter, then some 2.7 air-changes occur per hour within the trailer. Less complete mixing would allow the observed carbon dioxide concentrations with less air turnover. From this, the carbon dioxide concentrations to be expected from additional occupants can be estimated. From human respiration rates, it is apparent that without some ventilation the 8-hr exposure limit of 5000 ppm (American Institute of Industrial Hygiene) could be exceeded within the trailer during an 8-hr day.

Experimental results from testing of the second type of instrument were sufficiently different from those for the first to be of interest. In this case, lower interference levels were observed in the presence of CO_2; however, certain peculiarities were manifest. Figure 4 illustrates the change in sulphur response with varying CO_2 concentrations. Both positive and negative effects were observed when elevated CO_2 levels were present, when the results were compared with those obtained with instruments calibrated to operate in air containing ambient CO_2 concentrations. The combination of the various effects produced a fortuitously correct response in the region of about 30 ppM SO_2. At higher SO_2 levels, increasing concentrations of CO_2 provide more quenching of the sulphur emission. This is in agreement with the observations in our first case study. At lower levels of SO_2, unexpectedly high instrumental responses were obtained, which increased with increasing CO_2 concentration. Discussions with the manufacturer suggest this to be the result of instrumental bias built in to minimize the effect of changing CO_2 concentrations upon the response curve. This analyser is adjustable to cope with almost any background CO_2 level (by means of the zero offset control, which is usually set by the factory for the normal ambient CO_2 concentration of about 330 ppm).

Obviously, analysts must be on guard concerning the possibility of build-up of carbon dioxide or organic material in the carrier air. This can result not only from respiration, but from many other sources, including natural features (swamps, biota), factories (downwind from chimney stacks), fire *etc.* It is important either to be aware of the effect of changing concentrations of interfering components present, or to make measurements after calibration under the conditions to be used for the tests themselves.

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SHORT COMMUNICATIONS

BROMAMINE-B AS A NEW OXIDIMETRIC TITRANT

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Summary—A new oxidimetric titrant, bromamine-B (sodium salt of *N*-bromobenzenesulphonamide) is introduced for use in aqueous medium. Direct potentiometric and visual end-point titrations and back-titration procedures have been developed for the determination of typical reductants.

Although organic bromamines such as dibromamine-T (DBT),[1-3] dibromamine-B (DBB)[4] and bromamine-T (BAT)[5] are well established as oxidimetric titrants, only bromamine-T is used in aqueous medium. In this communication, we report the introduction of bromamine-B (sodium salt of *N*-bromobenzene sulphonamide, abbreviated to BAB) which also has the advantage of being usable in aqueous medium. A wide variety of reductants such as hydrazine, ascorbic acid, oxine, As(III), Sb(III), I^-, NO_2^-, $C_2O_4^{2-}$, $HCOO^-$, and thiourea and its metal complexes have been estimated with this new titrant.

EXPERIMENTAL

Preparation of bromamine-B

BAB was prepared by the partial debromination of dibromamine-B (DBB),[4] which in turn was prepared by the bromination of chloramine-B (CAB). DBB (31.5 g) was added in small quantities at a time and with constant stirring to 50 ml of 4*M* sodium hydroxide. The mass was cooled in ice, filtered under suction and the product dried over anhydrous calcium chloride. The yield was about 25.6 g (90%). The compound was recrystallized from hot water (50°) and stored in brown bottles. The available bromine was determined by iodometry: found 27.9%; $Na^+(C_6H_5SO_2NBr)^-$. 1.5 H_2O requires 28.04%.

BAB was further characterized by infrared (KBr) and pulse Fourier-transform ^{13}C NMR spectroscopy. The latter provides clear evidence that replacement of the chlorine atom in CAB by bromine to form BAB increases the electron density around C-1 (the carbon atom attached to the hetero atom) and it is likely that BAB may function as a better oxidizing agent than CAB.

BAB is highly soluble in water (374.3 g/kg at 29°). Approximately 0.1*N* (0.05*M*) stock solution was prepared by dissolving 14.3 g in 1 litre of water and kept in amber-coloured bottles. It was standardized by addition of 2*N* sulphuric acid and a slight excess of potassium iodide solution and titration of the liberated iodine with thiosulphate. The solution is moderately stable for a fortnight, but should be standardized on the day of use.

The pH of 0.05*M* BAB is 9.25. The conditional redox potential determined by the extrapolation procedure is +0.80 V at 24° and pH 7.9.

Reductant solutions

Thiourea (tu) complexes of zinc and cadmium were prepared by literature methods. Solutions of sodium oxalate, sodium formate and $Cdtu_2(HCOO)_2$ (2–3 mg/ml) were prepared in 1*M* acetic acid. Oxine solution (~2 mg/ml) was prepared in 4*N* sulphuric acid, and $SbCl_3$ solution (~2.5 mg/ml) in 3*M* hydrochloric acid. Aqueous solutions (~2–5 mg/ml) of other compounds were employed.

Direct potentiometric titrations

A platinum indicator electrode and calomel reference electrode were used. For hydrazine sulphate, potassium iodide, sodium arsenite and ascorbic acid, the procedures were similar to those used with chloramine-B (CAB)[6,9] and BAT,[5] the titrations being done at 25 ± 2° in 1*M* hydrochloric acid, but a small crystal of potassium iodide was added in the case of ascorbic acid. Thiourea was titrated in the presence of about 1 g of potassium bromide and 5–10 ml of glacial acetic acid; otherwise the reaction was slow.

Direct visual end-point titration

For hydrazine sulphate, a simple Andrews type of titration, with 1 ml of carbon tetrachloride, was found suitable, even in the absence of a catalyst. The indicator blank was negligible.

For ascorbic acid starch–iodide was used as indicator, as with BAT.[5] For sodium arsenite and antimonious chloride Methyl Red (2 drops of 0.1% ethanol solution) was used, in presence of 10 ml of concentrated hydrochloric acid, with dilution of the titrand to 100 ml. The indicator blank was negligible.

Back-titrations

The reductants determined were hydrazine sulphate, ascorbic acid, oxine, nitrite, oxalate, thiourea and its complexes.

To the reductant in an iodine flask, 50% excess of standard BAB solution was added. The mixture was left for 5 min in the case of hydrazine, oxine, $Zntu_2(OAc)_2$, $Cdtu_2(OAc)_2$, $Zntu_2Cl_2$, $Zntu_2SO_4$, 10 min for thiourea and formate, 30 min for ascorbic acid, 45 min for $Cdtu_2(HCOO)_2$ and 50 min for oxalate. In the oxidation of sodium nitrate about 10 ml of 1*M* acetic acid were added before adding the oxidant and the mixture was left for 5 min. For $Cdtu_2(SCN)_2$, sodium hydroxide solution was added until its overall concentration was 0.25*M*, and the mixture was left for 5 min. Unreacted BAB was determined by iodometry and blanks were run for all back-titrations, under the titration conditions.

Table 1. Oxidimetric estimation with bromamine-B

Reductant*	Reductant taken, *mmole*	Reductant found, *mmole*	Coefficient of variance,† %	Range studied, *mg*	Error, %
Direct potentiometric titrations					
Hydrazine sulphate	0.3847	0.3877	0.15	100.1–5.0	0.1–0.8
Iodide	0.1235	0.1248	0.60	51.2–10.2	0.0–0.8
Sodium arsenite	0.3848	0.3826	0.33	98.9–9.9	0.2–1.0
Ascorbic acid	0.7102	0.7075	0.32	125.1–25.0	0.0–1.0
Thiourea	0.3284	0.3260	0.20	50.0–10.0	0.3–0.9
Direct visual end-point titrations					
Hydrazine sulphate	0.3847	0.3850	0.43	100.1–5.0	0.0–1.0
Ascorbic acid	0.7102	0.7086	0.00	125.1–10.0	0.0–0.6
Sodium arsenite	0.3848	0.3793	0.00	98.0–4.9	0.2–1.0
Antimony(III)	0.5446	0.5471	0.21	132.6–5.3	0.2–0.9
Back-titrations					
Hydrazine sulphate	0.1924	0.1922	0.42	100.1–2.5	0.0–1.0
Ascorbic acid	0.2839	0.2857	0.59	100.0–10.0	0.2–1.0
Oxine	0.3446	0.3455	0.99	50.0–10.0	0.0–1.0
Sodium nitrite	0.7147	0.7136	0.54	49.3–9.9	0.1–1.0
Sodium oxalate	0.3770	0.3764	0.22	50.5–10.1	0.0–1.0
Sodium formate	0.7830	0.7773	0.23	53.3–10.7	0.4–1.0
Thiourea	0.1314	0.1318	0.51	50.0–2.5	0.0–0.8
$Zntu_2(OAc)_2$	0.0745	0.0747	0.00	50.0–10.0	0.0–0.8
$Cdtu_2(OAc)_2$	0.0662	0.0524	0.35	50.7–2.5	0.0–0.9
$Zntu_2Cl_2$	0.0867	0.0869	0.12	50.0–2.5	0.0–0.8
$Zntu_2SO_4$	0.0257	0.0258	0.93	50.0–2.0	0.0–1.0
$Cdtu_2(HCOO)_2$	0.0565	0.0567	0.83	50.1–2.0	0.0–0.9
$Cdtu_2(NCS)_2$	0.0263	0.0264	0.67	25.1–2.0	0.0–1.0

* tu = NH_2CSNH_2.
† Four replicates.

RESULTS AND DISCUSSION

The results obtained are presented in Table 1. The potential jump at the equivalence points ranged from 150 mV per 0.05 ml of 0.1*N* BAB for ascorbic acid to 600 for hydrazine sulphate, and for iodide was ~40 mV per 0.05 ml of 0.01*N* BAB. Near the end-point the potential reached a steady value in under 1 min.

Hydrazine is oxidized to nitrogen and ascorbic acid to dehydroascorbic acid, while oxine undergoes bromination involving a four-electron change. As(III) and Sb(III) are oxidized to As(V) and Sb(V) respectively while nitrite, oxalate and formate undergo a two-electron change. Thiourea either alone or in its metal complexes reacts with the oxidant as follows:

$$NH_2CSNH_2 + 4RNBrNa + 5H_2O \rightarrow$$
$$NH_2CONH_2 + SO_4^{2-} + 4RNH_2$$
$$+ 4Na^+ + 4Br^- + 2H^+.$$

Formate and thiocyanate in the complexes are also oxidized under these conditions with a 2- and 8-electron change respectively. The urea formed in the oxidation of thiourea was detected by the diphenylcarbohydrazide[10] test and the cyanate from oxidation of thiocyanate was detected by spot-test.[11] In all these oxidations, BAB is reduced in accordance with the half-reaction:

$$RNBrNa + 2H^+ + 2e^- \rightarrow RNH_2 + Na^+ + Br^-.$$

The benzenesulphonamide formed was detected by TLC with a mixture of petroleum ether, chloroform and *n*-butanol (2:2:1 v/v) as the mobile phase and iodine as the detection agent (R_F = 0.88).

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AMPEROMETRIC DETERMINATION OF 2-BENZOTHIAZOLE SULPHENAMIDE ACCELERATORS

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Summary—A simple method for the amperometric estimation of *N*-cyclohexyl-2-benzothiazole sulphenamide, *N*-dicyclohexyl-2-benzothiazole sulphenamide, 2-(morpholinothio)benzothiazole and *N*-tert.-butyl-2-benzothiazole sulphenamide is reported. The results are obtained within 5 min and are correct within ±2.0%.

Sulphenamides are widely used as rubber accelerators in industry. The titrimetric methods for their estimation are usually based on cleavage of the S–N bond either by a reductive method[1,2] or by electrophilic attack.[3,4] The first procedure, involving the use of stannous chloride and hydrochloric acid mixture for the cleavage of the S–N bond and subsequent amperometric estimation of the resulting 2-mercaptobenzothiazole, though precise, entails a number of operations. On the other hand, the earlier methods based on attack of electrophiles on sulphenamides are also time-consuming and applicable only on the macro scale. We report here a micro method for estimation of various benzothiazole sulphenamides.

EXPERIMENTAL

Apparatus

The apparatus[5] used for the amperometric titration consists of a rotating platinum-wire indicator electrode, a microammeter and a mercuric iodide reference electrode. The electrode is rotated at a speed of 600 rpm. Thiosulphate is added from a microburette, and the titration is carried out in a 100-ml beaker.

Reagents

The accelerators *N*-cyclohexyl-2-benzothiazole sulphenamide (CBS), *N*-dicyclohexyl-2-benzothiazole sulphenamide (DCBS), 2-(morpholinothio)benzothiazole (MOR) and *N*-tert.butyl-2-benzothiazole sulphenamide (TBBS) were purified by crystallizing the commercial samples from ethanol. All other reagents were of analytical grade.

Procedure

One ml of 0.01*M* methanolic solution of the sulphenamide was taken in a 100-ml flat-bottomed vessel fitted with a B50 stopper and 1.5 g of potassium iodide, 2 ml of water and 5 ml of methanol were added. After dissolution of the potassium iodide in the mixture, 0.3 ml of 1*M* hydrochloric acid was added, and the mixture allowed to stand in an ice-bath for about 5 min in dark. Then 45 ml of water were added to the mixture and the iodine liberated was titrated amperometrically with 0.01*M* sodium thiosulphate previously standardized by amperometric titration with potassium iodate. A reagent blank was determined and a correction applied.

RESULTS AND DISCUSSION

The instability of the sulphenamides towards acid is believed to be due to co-ordination of the electrophile with the nitrogen lone-pair of electrons followed by nucleophilic attack on the activated S–N bond as depicted below.

$$RSNHR + \overset{\delta^+\delta^-}{XY} \rightarrow R{-}S{-}\overset{+}{N}HR \xrightarrow{Y^-} RSY + RNHX$$
$$\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\quad X$$

(X = electrophile)

Various alkyl sulphenamides undergoing reactions with electrophiles were reported by Heimer and Field.[3] The electrophilic attack of methyl iodide on *N*-(n-butylthio)piperidine or *N*-(ethylthio)piperidine resulted in the formation of n-butyl or ethyl disulphide, iodine *etc.* according to the equations below.

$$R^1SNR^2R^3 + CH_3I \rightarrow [R^1SN(CH_3)R^2R^3]^+I^-$$

$$[R^1SN(CH_3)R^2R^3]^+I^- \rightarrow R^1SI + CH_3NR^2R^3$$

$$2R^1SI \rightarrow R^1{-}S{-}S{-}R^1 + I_2$$

$$CH_3NR^2R^3 + CH_3I \rightarrow (CH_3)_2N^+R^2R^3I^-$$

The reaction was carried out in acetonitrile and after 1 hr the liberated iodine was estimated. The results corresponded to 102% recovery.

Groebel[4] investigated the reaction of ice-cold acetic acid and potassium iodide with *N*-phenylmercaptosuccinimide in acetonitrile. He isolated the diphenyl disulphide and quantitatively estimated the iodine set free after 1 hr of reaction.

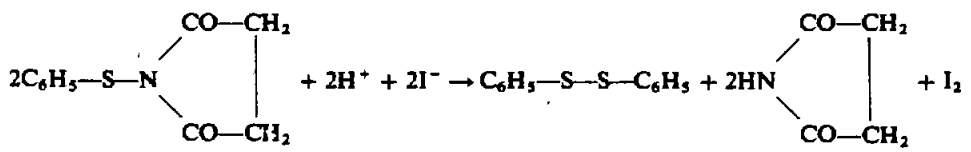
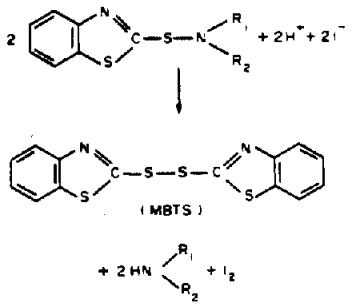


Table 1. Estimation of benzothiazole sulphenamide rubber accelerators

Accelerator	Amount taken, mg	Amount found, mg	Deviation, %
CBS	2.64	2.64	0.0
		2.59	−1.9
		2.59	−1.9
DCBS	3.46	3.39	−2.0
		3.39	−2.0
		3.46	0.0
MOR	2.52	2.56	+1.6
		2.52	0.0
		2.47	−2.0
TBBS	2.38	2.33	−2.1
		2.33	−2.1
		2.38	0.0
CBS	0.53	0.54	+1.9

We have observed that thiazole sulphenamides react almost instantaneously with potassium iodide and dilute hydrochloric acid in methanol–water mixture at room temperature. Presumably dibenzothiazyl disulphide is formed according to the equation.



A white precipitate forms during the reaction. It has been identified as dibenzothiazyl disulphide from its m.p. (180°) and comparison of its infrared spectrum with that of an authentic specimen.

It is known that like peroxides, disulphides easily oxidize hydriodic acid to free iodine. So, it can reasonably be expected that dibenzothiazyl disulphide should react with hydriodic acid and we have observed that it does. Under the circumstances it is evident that the concentration of hydrochloric acid used in the estimation of sulphenamides is crucial. If too large a concentration is used, the reaction becomes faster, but at the same time hydriodic acid is also produced in excess, which is liable to attack the dibenzothiazyl disulphide formed, especially if the reaction time is prolonged. Thus the results will be erroneous, and this is found in practice. We have found that by proper adjustment of the ratio of hydrochloric acid to accelerator, a balance can be attained and the further reaction made negligible. Under these conditions the results are fairly accurate (Table 1) and constant for reaction times of up to 15 min. A control experiment with a comparable amount of dibenzothiazyl disulphide under otherwise identical conditions produced no significant amount of iodine even at room temperature. The reaction rate can be reduced by lowering the reaction temperature to about 0°. The method gives rapid estimation of the four thiazole sulphenamides tested.

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METHOD FOR MEASUREMENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN PARTICULATE MATTER IN AMBIENT AIR

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Summary—A method for measuring polycyclic aromatic hydrocarbons (PAHs) in ambient air is described. The particulate matter is collected on fibre-glass filters. The loaded filters are placed in tetrahydrofuran and the PAHs dissolved with the aid of ultrasonics and analysed by high-performance liquid chromatography on a reversed-phase column with a methanol–water mixture as mobile phase. The PAHs are detected by use of a UV-detector and the 254-nm mercury emission line. The method is rapid and adequate for measuring about 15 PAH-components in ambient air.

Polycyclic aromatic hydrocarbons (PAHs) are emitted into the atmosphere mainly from combustion of fossil fuels. On account of their molecular weight (120–300) PAHs are preferentially bound in airborne particulate matter. The boiling points range from 330° for phenanthrene to 525° for coronene. Along with nitrosamines, aflatoxins and various heavy metals the PAHs are carcinogenic substances in our environment.[1] The PAHs with 4 or 5 rings such as benzo[*a*]-pyrene, benzofluoranthene and dibenzoanthracene have been recognized as directly carcinogenic.[2] Another group of PAHs *e.g.*, chrysene, pyrene and fluoranthene, seem to have a potentiating rather than a direct effect.[3]

It is therefore considered important to survey the emission levels and main sources of PAHs, and a simple and reliable method for doing this is required. Emphasis is placed on measuring the concentration profile of the PAHs, *i.e.*, their relative proportions, measured at sites of different air quality. Additionally, information about the diurnal and seasonal variations in concentration profile is desired.

EXPERIMENTAL

Sampling

The particulate matter is collected from ambient air on fibre-glass filters (Schleicher and Schüll, No. 9) 4.7 cm in diameter. Investigations with different filter materials have shown that fibre-glass and Teflon filters are adequate for the purpose, but membrane filters are not.[4] We decided to use fibre-glass filters because their air-resistance is lower than that of Teflon filters, and they are better suited for obtaining a measurable quantity of PAHs within a short sampling period.

Before sampling, the filters are heated in a muffle furnace at 300° for 24 hr to minimize the organic background of the filter. Various types of filter impregnation do not yield higher PAH recovery during the sampling.[5]

The filters (both before and after sampling) were wrapped air-tight in aluminium foil and stored at −25° to avoid chemical reactions of the collected PAHs.

For measurements at urban sites volumes of 50 m^3 of air sampled on fibre-glass filters 4.7 cm in diameter are sufficient for analysis. The small sampling volume makes it possible to use a silent small-volume sampler.

Preanalysis preparation

The used filter is cut into small strips, put into 50 ml of tetrahydrofuran (Lichrosorb, Merck) and exposed to an ultrasonic source at 70 W for 10 min. This treatment disintegrates the filters and the particulates are thus suspended in the solvent. The organic constituents, including PAHs, are dissolved in the tetrahydrofuran (THF). To dissipate the heat induced during the ultrasonic treatment the container is placed in a water-bath at 20°.

This procedure has several advantages over treatment in a Soxhlet extractor. It requires only a few minutes whereas the Soxhlet treatment takes hours, with the attendant risk of thermal decomposition of PAHs at the elevated temperatures used, and consequent distortion of the original PAH-profile.

After dissolution of the PAHs the solution is filtered through a fibre-glass filter that has been thermally treated beforehand (24 hr at 300°). The filter is washed with tetrahydrofuran to give a total filtrate volume of 100 ml. This solution is reduced to about 1.5 ml in volume (at 20° on a water-bath) by means of a rotary-evaporator. A specially designed flask terminating in a graduated tube is used. The solution is finally made up to exactly 2 ml. For determination of blanks unused filters are submitted to the same procedure.

Analysis

Up to the present PAH-analysis has preferentially been done by gas chromatography,[6–8] but this requires several time-consuming preliminary separation and extraction processes.[9] It is possible, however, to determine up to 150 components in the PAH-profile. For special investigations the gas chromatography method is therefore indispensable.

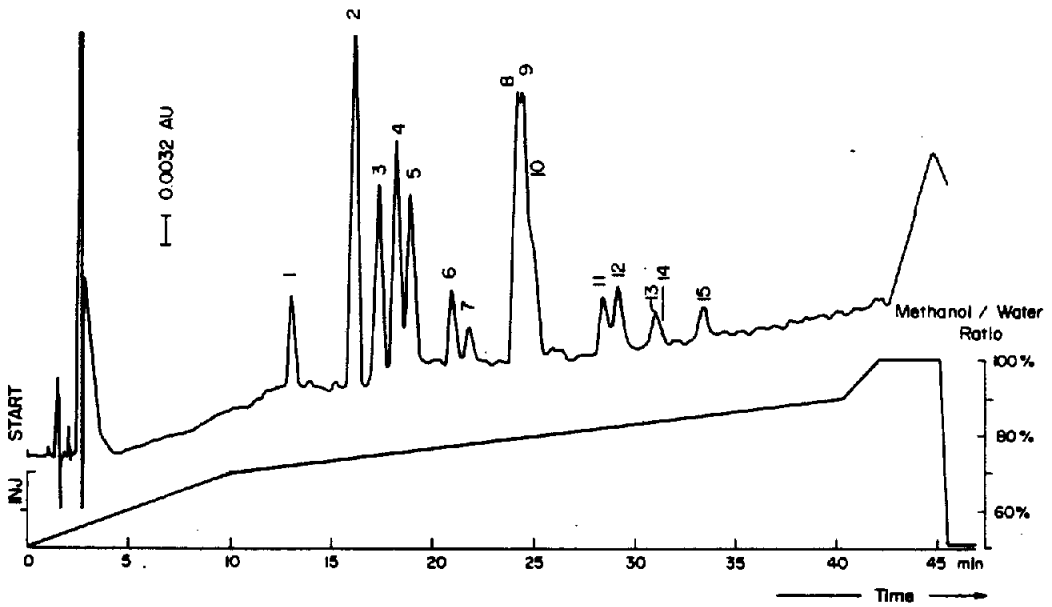

Fig. 1. Chromatogram of the calibration solutions: 1, naphthalene; 2, biphenyl; 3, acenaphthene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, triphenylene; 9, benzo[*a*]anthracene; 10, chrysene; 11, perylene; 12, benzo[*a*]pyrene; 13, dibenzo[*ah*]anthracene; 14, dibenzo[*ac*]anthracene, 15, benzo[*ghi*]perylene. Chromatographic conditions: flow-rate 1.50 ml/min; column pressure 232 bar; oven temperature 30°; solvent temperature 25° (water and methanol).

For routine analysis of large numbers of samples, as in quality control of urban air, a rapid and reliable measuring method is needed which should give an adequate survey of the PAH-profile.

For rapid analysis of the PAH-profile high-pressure liquid chromatography (HPLC) has proved to be adequate, especially for emission measurements. The methods previously used for PAH-measurements in air have been more time-consuming than the one described in this paper. Tetrahydrofuran (THF) has also proved to be a more effective solvent than cyclohexane, which is often recommended in the literature.[10]

Table 1. Correlation coefficients for measured PAH-quantities and calculated peak areas, determination limits and smallest detectable concentration in air

	Correlation coefficient	*a*, *ng*	*d*, *ng/m*^{3}
1. Coronene	0.973	36.4	6.1
2. Naphthalene	0.998	15.0	2.5
3. Acenaphthene	0.999	7.2	1.2
4. Dibenzo[*ah*]anthracene	0.984	5.4	0.9
5. Benzo[*ghi*]perylene	0.954	14.2	2.4
6. 7,12-Dimethylanthracene	0.961	4.7	0.8
7. Pyrene	0.976	12.2	2.0
8. Fluoranthene	0.963	5.8	1.0
9. Biphenyl	0.994	13.8	2.3
10. *p*-Terphenyl	0.999	4.2	0.7
11. Perylene	0.992	14.4	2.4
12. Dibenzo[*ac*]anthracene	0.996	6.5	1.1
13. Benzo[*a*]pyrene	0.999	11.0	1.8
14. Chrysene	0.991	8.6	1.4
15. Phenanthrene	0.992	2.9	0.5
16. Triphenylene	0.991	8.9	1.5
17. Benzo[*a*]anthracene	0.997	1.5	0.3
18. Anthracene	0.998	3.6	0.6
19. 9-Methylanthracene	0.985	1.1	0.2
20. 2-Methylanthracene	0.997	0.8	0.1

Calculation of determination limits d from detection limit a:

$$d = \frac{a \times \text{sample volume}}{\text{injection volume} \times \text{air volume}}$$

amount injected = 0.2 ml; volume of sample = 2 ml.

After the evaporation the samples are analysed by HPLC, a 50-μl portion of the THF-solution being injected into the column by an automatic injector (Hewlett-Packard 1084A). Larger samples (up to 200 μl) can be injected if desired. The reversed-phase separation is performed on a 250 × 4.2 mm column filled with Lichrosorb RB 8 (7 μm particle size) (Knauer, Berlin). A methanol–water mixture is used as mobile phase for gradient elution. During the first 10 min the analysis is run with a slow change from 50% to 70% methanol in order to eliminate the background of interfering polar substances. The PAHs (up to coronene) are separated by use of a linear increase in methanol concentration from 70% to 90% in 30 min. The gradient is continued up to 100% methanol in order to rinse out the remaining substances of the background. The separated PAH-compounds are detected with an ultraviolet detector operating with the 254-nm mercury emission line. For further investigations a variable-wavelength detector and a fluorescence detector were used. To detect specially selected components the wavelength giving the highest sensitivity can be picked out, but information about other components of the PAH-profile may then be partly or completely lost.[11]

To a first approximation the components can be identified from their characteristic retention times and measured by means of calibration curves obtained by use of standard solutions of each component at different concentrations. The variable-wavelength detector allows complete identification of the different compounds by use of the peak-height ratios for each component at two different wavelengths (which also allows detection of the presence of unresolved components which could interfere in the quantification).

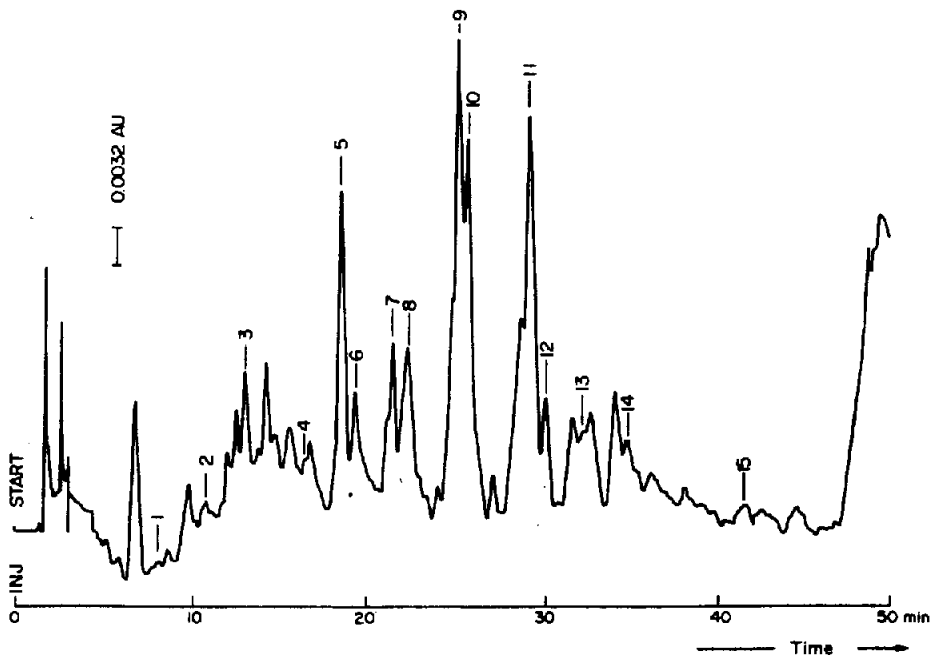

Fig. 2. Chromatogram of PAHs in particulate matter of urban air (Frankfurt/Main, 4 January, 1979): 1, benzene; 2, toluol; 3, naphthalene; 4, biphenyl; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene; 9, triphenylene, benzo[*a*]anthracene; 10, chrysene; 11, perylene; 12, benzo[*a*]pyrene; 13, dibenzo[*ah*]anthracene; 14, benzo[*ghi*]perylene; 15, coronene.

The procedure described makes it possible to determine the PAH-profile almost completely by use of the 254 nm Hg-line, with a single run, in contrast to specific detection at special wavelengths, which requires several separation runs. One complete analysis (including preparation) requires about 90 min.

RESULTS

Figure 1 shows the chromatogram of a calibration solution containing 15 PAHs. The calibration curves (peak area *vs.* concentration) for individual compounds were linear. The calibration solutions were prepared by dissolving known weights of commercially available PAHs in THF. The correlation coefficients varied between 0.954 for benzo[*ghi*]perylene and 0.999 for benzo[*a*]pyrene. The lower determination limits ranged from 36.4 ng for coronene to 0.8 ng for 2-methylanthracene (Table 1), the limit being defined as twice the standard deviation of the peak area for a sample at a concentration level near the determination limit. The determination limits for urban air concentrations are calculated on the basis of a sampling volume of about 60 m^3 (Table 1).

The chromatogram for particulate matter in Frankfurt urban air (Fig. 2) shows several different PAHs. Those identified are listed in the caption. By use of the calibration curves and the sample volume the PAH concentrations in the particulate matter of ambient air can be determined. However, biphenyl and dibenzo[*ah*]anthracene are not determinable from the profile chromatogram.

The method described provides a rapid and sufficiently reliable assessment of the PAH-profile in ambient air.

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SPECTROPHOTOMETRIC DETERMINATION OF MICRO AMOUNTS OF CADMIUM IN WASTE WATER WITH CADION AND TRITON X-100

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Summary—A spectrophotometric method for determination of micro amounts of cadmium in waste water with Cadion and Triton X-100 is described. The interference of foreign ions can be eliminated by masking with an ascorbic acid–Rochelle salt–potassium cyanide–potassium fluoride mixture. After demasking with formalin, cadmium is determined directly in aqueous solution without separation. Beer's law is obeyed for 0–8 μg of Cd in 25 ml of solution. The method is more sensitive than the dithizone method, its apparent molar absorptivity at 480 nm being 1.19×10^5 l.mole^{-1}. cm^{-1}. Results obtained by using the proposed method on waste water samples agree well with those obtained by atomic-absorption spectrophotometry.

The methods usually used for determination of micro amounts of cadmium in waste water are atomic-absorption spectrophotometry and extraction photometry with dithizone as chromogenic reagent. The dithizone method, which requires extraction and back-extraction, is tedious and time-consuming.

p-Nitrobenzenediazoaminobenzene-*p*-azobenzene (Cadion) was synthesized by Dwyer.[1] The dye is purple in alkaline alcoholic solution and forms an orange-red complex with cadmium in presence of polyvinylpyrrolidinone[2] or gelatine.[3] The colour reaction has been used to determine cadmium spectrophotometrically, but many ions such as Fe(III), Cu(II), Mg, Ni, Co(II), Ag and Hg(II) interfere, so its application is limited. Chavanne *et al.*[2] determined cadmium in the presence of many metal ions but prior extraction with dithizone was required.

In the present paper a spectrophotometric method for determining micro amounts of cadmium in waste water with Cadion is proposed. Triton X-100 is used as solubilizing agent. Foreign ions are masked together with cadmium with an ascorbic acid–Rochelle salt–potassium cyanide–potassium fluoride mixture, and finally cadmium is demasked with formalin, and cadmium can thereby be determined directly in aqueous solution without separation.

The method is convenient, highly selective and more sensitive than the dithizone method. Optimum conditions for the colour reaction have been studied, and cadmium contents in samples of waste water determined with satisfactory results.

EXPERIMENTAL

Apparatus

Model 721 spectrophotometer (Shanghai Analytical Instruments Factory).

Reagents

Cadion solutions. Stock solution of Cadion (0.02%) was prepared by dissolving Cadion in 0.02*M* potassium hydroxide in alcohol. Mixed Cadion solution was prepared by mixing the following in a 50-ml standard flask just before use and making up to the mark with distilled water: 1 ml of 20% Rochelle salt solution, 12 ml of 4*M* potassium hydroxide, 20 ml of 95% ethyl alcohol, 1 ml of 10% Triton X-100 solution and 10 ml of 0.02% Cadion solution.

Standard solution of cadmium (0.1 *mg/ml*). Cadmium powder was dissolved in nitric acid and the solution was evaporated to dryness after addition of hydrochloric acid. The evaporation step was repeated and the residue was taken up in enough conc. hydrochloric acid to give a final acid concentration of 5 ml/l. A 2-ppm solution of cadmium was prepared by dilution.

Triton X-100 solution (10%).

The other reagents were prepared from reagent grade chemicals.

General procedure

To the test solution containing not more than 8 μg of cadmium, in a 25-ml standard flask, add the following reagents in the order given, mixing between additions: *ca.* 50 mg of ascorbic acid, 2 ml of 20% Rochelle salt solution, 3 drops of saturated potassium hydroxide solution (more if the test solution is too acidic), 1 ml of 1*M* potassium cyanide, 1 ml of 1*M* potassium fluoride, 5 ml of the mixed solution of Cadion and then 1 ml of formalin solution (36–38% solution diluted with an equal volume of water). Dilute to the mark with water and measure absorbance at 480 nm in a 2-cm cell against a reagent blank.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of the cadmium–Cadion complex and the reagent blank were measured against water in the range 400–640 nm in 1-cm cells. The absorption maximum of the complex was at 485 nm and that of the reagent blank at 560 nm (Fig. 1). In the following experiments, the absorbances were measured at 480 nm against a reagent blank.

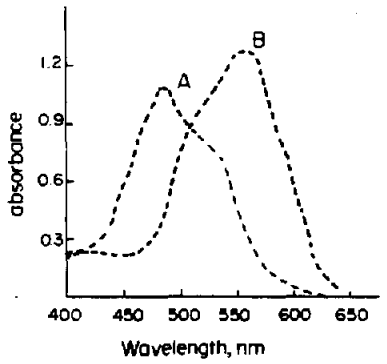

Fig. 1. Absorption spectra of A, cadmium–Cadion complex and B, reagent blank against water. With addition of all the reagents given in general procedure. (Cd taken, 50 μg; 1-cm cell.)

Effect of the concentration of potassium hydroxide

When the concentration of potassium hydroxide was less than 0.05*M*, the solution was slightly turbid; the absorbance of both the complex and reagent blank decreased slightly as the concentration of alkali was increased (Fig. 2). However, in the alkali range 0.05–0.5*M*, the difference in absorbance between the complex and the reagent blank varied by less than 5%. In our experiments, the concentration of potassium hydroxide was usually maintained at 0.2–0.3*M*.

Effects of amounts of reagents

In 25 ml of solution, 0.4–1.4 ml of 0.02% Cadion solution gave maximum and constant absorbance with 4 μg of cadmium, so 1 ml of 0.02% Cadion solution was added for determinations (this corresponded to the amount of Cadion in the mixed Cadion solution). The optimum concentration of Triton X-100 was in the range 0.02–0.08%. Below 0.02% the solution became turbid and had low absorbance. Therefore a concentration of 0.04% Triton X-100 was chosen for the determination (corresponding to the amount in the mixed Cadion solution). Other reagents in the amounts given in parentheses did not influence the results: ascorbic acid (300 mg), 20% Rochelle salt solution (5 ml), 1*M* potassium fluoride (5 ml). Formalin was added in excess for demasking the cadmium–cyanide complex and removing excess of cyanide; usually 1 ml of formalin (1 + 1) was introduced per ml of 1*M* potassium cyanide added. The zinc–cyanide complex was also demasked, but tetrahydroxozincate was formed under the experimental conditions, and did not interfere.

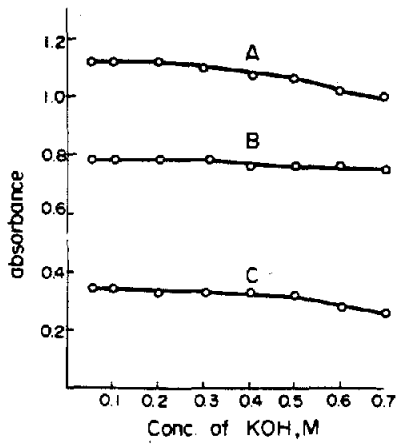

Fig. 2. Effect of KOH concentration. A, cadmium–Cadion complex; B, reagent blank against water; C, complex against reagent blank. With addition of all the reagents given in general procedure. (Cd taken, 4 μg; 2-cm cell.)

Time for complex formation

The complex was formed instantaneously and the absorbance remained stable for at least 5 hr.

Calibration curve, sensitivity and repeatability

A calibration curve was constructed in the usual way according to the general procedure. Beer's law was obeyed for 0–8 μg of Cd in 25 ml of solution, at 480 nm. The apparent molar absorptivity at 480 nm was found to be 1.19×10^5 $l.mole^{-1}.cm^{-1}$. The method is more sensitive than the dithizone method ($\epsilon = 8.56 \times 10^4$).[4] Ten parallel determinations of 4 μg of Cd gave a standard deviation of 0.024 μg.

Effect of foreign ions

The effect of various ions on the determination of cadmium was examined. The results are listed in Tables 1 and 2. Most of the ions did not interfere but only 10 and 30 μg of sulphide and EDTA respectively

Table 1. Effect of cations on determination of 4 μg of Cd

Cation, *mg*		Cation/Cd w/w	Cd found, μg	Error, μg
Cu(II)	4.0	1000	4.11	+0.11
Fe(III)	1.6	400	4.10	+0.10
Ni(II)	3.2	800	3.99	−0.01
Co(II)	0.5	125	4.05	+0.05
Zn(II)	10.0	2500	3.97*	−0.03
	0.4	100	4.09	+0.09
Ca(II)	5.0	1250	4.03	+0.03
Mg(II)	5.0	1250	3.85†	−0.15
	0.3	75	4.01	+0.01
Cr(III)	0.8	200	4.20	+0.20
Ag(I)	0.4	100	4.09§	+0.09
Pb(II)	10.0	2500	3.97	−0.03
Hg(II)	0.04	10	3.92	−0.08
NH_4(I)	10.0	2500	3.82	−0.18
Ti(IV)	0.08	20	4.01	+0.01
Al(III)	2.0	500	3.94	−0.06
Sb(III)	1.0	250	4.00	0.00
Sn(IV)	0.8	200	3.99	−0.01
Sn(II)	1.0	250	4.19	+0.19
K(I)	10.0	2500	3.94	−0.06
Na(I)	10.0	2500	3.91	−0.09
Bi(III)	0.6	150	3.95	−0.05
Ba(II)	5.0	1250	4.19	+0.19
Mn(II)	1.0	250	4.17	+0.17

* Concentration of KOH 0.4*M*.

† After addition of KF, stood for 30 min, then filtered and washed with small amount of water.

§ KOH and KCN were added together.

Table 2. Effect of anions on determination of 4 μg of Cd

Anion, *mg*		Anion/Cd *w/w*	Cd found, *μg*	Error, *μg*
Dichromate	1.2	300	4.17	+0.17
Nitrate	10.0	2500	3.94	−0.06
Chloride	10.0	2500	3.91	−0.09
Sulphate	10.0	2500	3.82	−0.18
Phosphate	10.0	2500	3.96	−0.04
Pyrophosphate	10.0	2500	4.07	+0.07
Carbonate	10.0	2500	3.95	−0.05
Oxalate	10.0	2500	4.00	0.00
Fluoride	10.0	2500	3.99	−0.01
Cyanide	10.0	2500	3.92	−0.08
Sulphide	0.01	2.5	4.12	+0.12
	1.0	250	3.92*	−0.08
Arsenate	10.0	2500	4.01	−0.01
Citrate	10.0	2500	3.94	−0.06
Tartrate	10.0	2500	3.93	−0.07
Nitrilotriacetate	0.5	125	4.00	0.00
Ethylenediaminetetra-acetate	0.03	7.5	4.01	+0.01
	1.0	250	3.94*	−0.06

* Digested with H_2SO_4–HNO_3 mixture before the determination.

Table 3. Results for determination of cadmium in waste water

Sample	Atomic-absorption spectrophotometry, *ppm*	Present method, *ppm*	Relative standard deviation, %
1	5.06	5.11	1.4
2	8.50	8.30	1.1
3	16.6	16.5	1.3
4	3.75	3.72	1.8
5	0.041	0.044	6.6
6	0.24	0.25	2.9

* Mean of 5 determinations.

could be tolerated. Interference of larger amounts of sulphide, EDTA and other organic compounds could be easily eliminated by digestion of the sample with sulphuric acid–nitric acid before the determination.

Determination of cadmium in waste water

The cadmium content of certain waste water samples was determined by the proposed method. The results shown in Table 3 are in reasonable agreement with those obtained by atomic-absorption spectrophotometry.

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PMR ASSAY OF NATURAL PRODUCTS IN PHARMACEUTICALS—III

ASSAY OF GRISEOFULVIN

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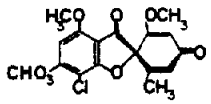
Summary—A rapid, accurate and specific PMR method for the determination of griseofulvin in the bulk drug, tablets and dry suspension powder has been developed. The determination is based on the integration of the 4- and 6-methoxy protons of griseofulvin, relative to that of the methyl protons of acetanilide (internal standard).

Griseofulvin [7-chloro-4,6-dimethoxycoumaran-3-one-2-spiro-1′-(2′-methoxy-6′-methylcyclohex-2′-en-4′-one)] is a systemic antifungal antibiotic commonly used in the treatment of dermatophyte infections in humans[1–4] and domestic animals.[5]



It is produced by *Penicillium grisofulvum Dierckx* and by *P. Janczewskii Zal.* [= *P. nigricans* (Banier) Thom]. Several synthetic routes to griseofulvin have been reported.[6–9]

The lack of analytical functional groups has greatly limited the possibility of an accurate and precise assay for griseofulvin. Most of the reported procedures are based on the carbonyl functions. A colorimetric method involving the coupling of griseofulvin with different diazo compounds and measuring the colour produced has been described. The colour developed with the stabilized diazonium salt of 4′-amino-2′,5′-dimethoxybenzanilide is measured at 495 nm.[10]

Ultraviolet spectrophotometric methods of assay of griseofulvin have been reported,[11–14] based on measuring the absorbance at 291 nm. This method is that officially adopted by B.P. 1973[13] and U.S.P. XIX 1975.[14] An indirect ultraviolet spectrophotometric method involving the conversion of griseofulvin into isogriseofulvin has been reported.[15] Spectrofluorimetric methods have been used for the determination of griseofulvin in biological fluids.[16–20] A combined TLC and fluorimetric method has been developed for the estimation of griseofulvin and griseofulvin 4′-alcohol in plasma.[21] A time-resolved phosphorimetric assay was used for determination of griseofulvin in mixtures with deschlorogriseofulvin.[22] GLC procedures have been applied for the determination of griseofulvin and its analogues in pharmaceutical formulations and biological fluids.[19,23–27] Recently, HPLC methods have been developed for the estimation of griseofulvin in human plasma.[28] Isotope dilution methods using ^{36}Cl-labelled griseofulvin[29] and tritium-labelled griseofulvin[30,31] have been reported.

The aim of this work is to establish the feasibility of using PMR spectroscopy for the determination of griseofulvin in bulk drug and dosage forms.

EXPERIMENTAL

Apparatus

All spectra were recorded at 37° on a Varian T 60 A, 60-MHz spectrometer, with ethanol-free chloroform as the solvent.[32] Chemical shifts were measured relative to tetramethylsilane at 0 ppm.

Assay of griseofulvin in tablets and dry suspension powder

Weigh accurately a portion of the powder (powdered tablets or dry suspension powder), equivalent to 25 mg of griseofulvin, into a glass-stoppered centrifuge tube. Add an accurately weighed amount of acetanilide as internal standard (20–25 mg), followed by 2 ml of ethanol-free chloroform. Stopper and shake for 3 min and then centrifuge. Transfer about 0.5 ml of the clear supernatant solution into an NMR tube and obtain the spectrum, adjusting the spin-rate to reduce the spinning side-bands as much as possible. Integrate the peaks of interest (the six protons of the 4- and 6-OCH_3 groups of griseofulvin appearing at 4.00 and 4.17 ppm and the three protons of the $-CH_3$ of acetanilide appearing at 2.20 ppm) at least three times and determine the average integrals.

The amount of griseofulvin is then calculated as follows:

$$\text{mg of griseofulvin} = \frac{A_g}{A_a} \times \frac{\text{E.W}_g}{\text{E.W}_a} \times \text{mg of acetanilide}$$

where A_g is the integrated value of the griseofulvin signal, A_a that of the acetanilide signal, E.W_g is a sixth of the formula weight of griseofulvin (= 58.8) and E.W_a is a third of the formula weight of acetanilide (= 45.05).

RESULTS AND DISCUSSION

From Fig. 1, it is evident that the PMR spectrum of griseofulvin exhibits, among other peaks, two singlets

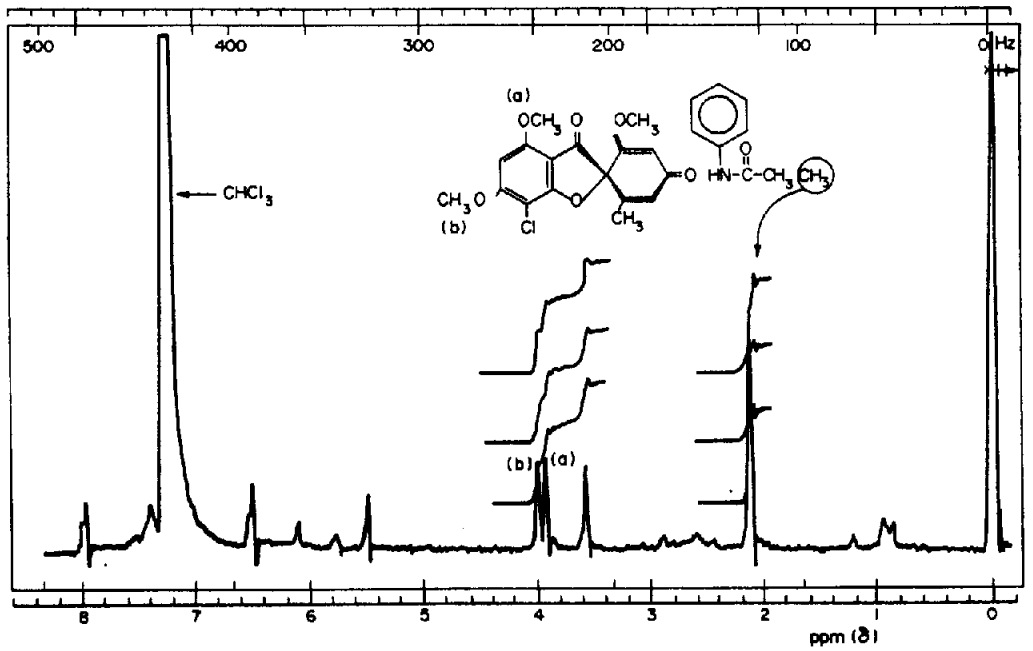


Fig. 1. PMR spectrum of griseofulvin I, acetanilide II and TMS in ethanol-free chloroform.

at 3.9 and 4.0 ppm (in deuterochloroform) assigned to the 4- and 6-methoxy protons, respectively. Since the integration of these two peaks gives the largest area for measurement, they are chosen for quantitative purposes.

Acetanilide, the PMR spectrum of which exhibits a three-proton singlet at 2.3 ppm (in deuterochloroform) assigned to its methyl group, is employed as internal standard.[33] Accurate determination is achieved, since the griseofulvin signals are widely separated from the acetanilide signal. Ethanol-free chloroform is used as the solvent. Its proton singlet at 7.25 ppm does not interfere with the upfield protons of both compounds.

Assay of a series of known mixtures of griseofulvin and acetanilide by this PMR technique showed the relative standard deviation to be 1.8%. Although the relative proportions of griseofulvin and internal standard has no significant bearing on the accuracy of the determination, it is preferable to use approximately equal amounts of the two. In this way, the error in integration measurements is minimized.

The results of estimation of griseofulvin in tablets and dry suspension powder by the PMR method and the B.P. 1973 method, are compiled in Table 1. Higher results were consistently obtained by the spectrophotometric method than by the PMR method. This can be attributed to the alcohol-soluble and ultraviolet-absorbing excipients, binders, sweeteners and flavouring agents. In the PMR method, such interference could not be observed. Moreover, very satisfactory recoveries of known amounts of griseofulvin added to commercial tablets and dry suspension powder were obtained.

Being accurate (relative standard deviation = 0.5%), rapid, reproducible and specific, the PMR method studied in this work has distinct advantages over previous methods.

Table 1. Assay of griseofulvin and formulations by PMR and spectrophotometric methods

Preparation	Amount found*, mg		Amount added, mg	Amount found*, mg		Recovery, %	
	PMR method	UV method		PMR method	UV method	PMR method	UV method
Grisovin, 125-mg tablets Glaxo	111.1	124.6	30	141.2	155.5	100	103
Grifulvin V® 500-mg tablets McNeil	445	510	100	544	611	99	101
Griseofulvin 125 mg/ml dry syrup (G. Buchmann AG)	113.6	153	25	138.5	178.4	99	102

* Average of 5 experiments.

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INDUCTIVELY-COUPLED PLASMA–OPTICAL EMISSION SPECTROMETRY

APPLICATION TO THE DETERMINATION OF MOLYBDENUM, COBALT AND BORON IN SOIL EXTRACTS

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Summary—An inductively-coupled radiofrequency plasma method applicable to the determination of Mo, Co and B in soil extracts has been developed. The interferences caused by some common metals and acids have been investigated. The detection limits observed at the chosen wavelengths were 0.01, 0.05 and 0.05 ppm for Mo, Co and B respectively. The mean relative standard deviation determined over an extended period of time was 0.9%.

Various analytical procedures have been described for the determination of trace elements in soil extracts, and generally employ colorimetric and arc or spark optical emission methods. These procedures involve the possible contamination of the sample by oxidizing acids and often employ preconcentration techniques. Atomic-absorption methods have been proposed, but their sensitivity is too poor for some elements such as Mo. The inductively coupled plasma (ICP), being a fairly new tool, has not yet been widely used for the analysis of soil samples[1,2] though a number of application papers have been published[3–8]. The ICP possesses certain unique properties and operational characteristics not found in spark or flame atomization systems.[9–11] This has led us to apply it in the determination os some trace elements in soil extracts. In this paper a rapid and convenient procedure for the determination of Mo, Co, and B is reported.

EXPERIMENTAL

Instrumental description

The ICP system used in this work utilized a Radyne 2.5-kW free-running valve oscillator operating at 36 MHz and a Techtron grating scanning monochromator. The plasma torch was a demountable Greenfield type and a dual-tube aerosol chamber similar to the one used by Fassel *et al.*[12] combined with a pneumatic nebulizer was employed for sample introduction. Details of the instrumental system are presented in Table 1.

Standard solutions

All chemicals were analytical reagent grade. The Mo and Co stock solutions (1000 ppm) were prepared by dissolving ammonium molybdate and cobalt chloride in distilled water. Boric acid was used to prepare boron stock solutions (1000 ppm).

Sample preparation

The standard extraction methods employed at the Macaulay Institute were used to extract Mo, Co, and B from soil saamples.

Mo extraction

A 50-g sample of soil was shaken overnight with 800 ml of neutral 1*M* ammonium acetate. The whole extract was filtered through an 18.5-cm Whatman No. 540 filter paper, as much of the soil as possible being transferred to the paper in order to minimize the passage of clay particles. The paper was washed several times with distilled water, and the filtrate evaporated to dryness, partially on a hot-plate and then to completion on a steam-bath. The residue was transferred to a 100-ml standard flask and made up to the mark. No oxidation with nitric acid was applied, as organic matter at the concentration levels generally occurring in the soil extracts did not cause any disturbance in the plasma. However, some extracted solutions contained solid particles which made the nebulization process unstable. In these cases a single-step oxidation with nitric acid was used, in order to obtain clear solutions (1 ml of conc. nitric acid was added to 10 ml of solution, and the mixture was boiled to low volume and then diluted to 10 ml again).

Co extraction

A 20-g sample of air-dried soil was shaken overnight on an end-over-end shaker with 800 ml of 0.5% acetic acid solution. The whole extract was filtered in the same manner as the ammonium acetate extracts and evaporated to dryness. The residue was then taken up in 100 ml of 0.1*M* hydrochloric acid.

B extraction

A 50-g sample of air-dried soil was boiled under reflux with 100 ml of water for 10 min. After partial cooling, the extract was filtered through an 18.5-cm Whatman No. 3 filter paper into a conical beaker and a 40-ml aliquot of the filtrate transferred to a silica beaker. The solution was taken to dryness and the residue was oxidized twice with 10 ml of 6% hydrogen peroxide solution. The residue was then diluted to 50 ml.

RESULTS AND DISCUSSION

Choice of analytical line, detection limits and calibration curves

The most sensitive Mo line (379.8 nm) was used although there is some interference from the Fe line at 379.8 nm. The Mo 390.3-nm line was found to be as

sensitive but the Fe 390.3-nm line, which is very intense, strongly interfered. The emission spectrum in the region of the Mo 317.0-nm line was complex and of high intensity, so this line was not used even though it is generally free from interferences. The most sensitive Co line (345.3 nm) was used. This line was found to be free from spectral interferences, whereas the Co 340.5-nm line sometimes suffers interference from the Ti line at 340.5 nm (for certain rocks, soils and minerals). Boron has a very simple spectrum with the sensitive doublet at 249.7 and 249.8 nm being the only useful analytical lines.

The detection limits observed at the chosen wavelengths were 0.01, 0.05 and 0.05 ppm for Mo, Co and B respectively. These were obtained under the optimum operating conditions, shown in Table 2. All the experiments reported were done under these optimized conditions. The detection limits for this work were in all cases worse than those reported by Fassel *et al.*[9] This can probably be attributed to the relatively poor spectral resolution of our monochromator.

Calibration curves were established by measuring the relative intensity of the appropriate emitted line when standard solutions were run. The calibration was linear over 4–5 orders of magnitude.

Interference effects

Effect of Al on Mo. The effect of aluminium on the intensity of the Mo 379.8-nm emission line was studied in detail. A 20% enhancement was observed to be caused by a 50-fold ratio of aluminium to molybdenum. Furthermore, as shown in Fig. 1, the maximum of the Mo line emission intensity was shifted to a position higher in the plasma. It is believed that the enhancement is due mainly to ionization suppression by the aluminium, although whether the shift in position of the maximum implies a change in the free atom distribution because of the presence of aluminium, it is not possible to tell without further work.

It was found that aluminium could be used as an interferent suppressor in our ICP. The presence of

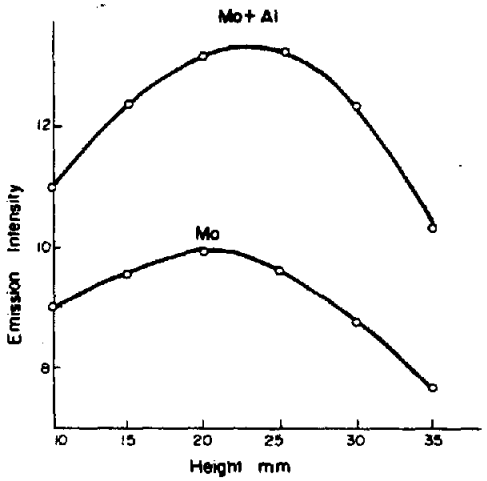

Fig. 1. Variation of relative emission intensity at 379.8 nm with height of observation observed from solutions containing 10 ppm Mo and 10 ppm Mo + 5000 ppm Al.

Table 1. Instrumentation

Plasma power supply	SC15; operating frequency 36 MHz; power output 0–2.5 kW, continuously variable; load coil 2½ turns, 6.3 mm o.d., internal diameter of coil 32 mm
Plasma torch	Demountable silica torch with brass base; coolant tube 25 mm bore, 1.5 mm wall; plasma tube 22 mm bore., 1 mm wall; injector tube 11 mm o.d., 9 mm i.d., and 2 mm orifice diameter
Nebulizer	Pneumatic nebulizer and spray chamber, uptake rate 1.5 ml/min at argon flow-rate of 2 l./min, efficiency ~ 7%
Spectrometer	Techtron AA4 grating monochromator
Slit-width	25 μm
Read-out	Signal from PMT, after amplification, was displayed on a Servoscribe chart-recorder model RE 511-20

Table 2. Plasma operating conditions

Net forward radiofrequency power	1200 W
Spectrometer slit	25 μm (entrance)
Argon coolant-gas flow-rate	14 L/min
Argon plasma-gas flow-rate	5 L/min
Injector gas flow-rate	1.6 L/min
Height of observation	25 mm above work coil

2000 ppm of calcium had no effect on the signal from 10 ppm of molybdenum when 5000 ppm of aluminium were present in the solution.

Other interferences. The effect of Na, K, Fe, Mg, Mn, and Ca on the Mo 379.8-nm, Co 345.3-nm and B 249.8-nm lines was investigated with 500 ppm of these elements present in a 10-ppm solution of the appropriate analyte (Table 3). A maximum of 25% enhancement of the intensity of the analyte line was observed in the case of calcium, which gave the most severe interference. The nature of the interferences is not definitely known, but it is suspected that they are most probably due to ionization suppression.

The presence of acetic acid at a concentration of 2.5*M* was found to enhance the Mo signal by approximately 10%. The presence of hydrochloric, nitric or sulphuric acid had no significant effect on the signals of any analyte.

The lines employed were all those of the neutral atoms, and it is possible that the interferences might be smaller if ionic lines were used.

Analysis of soil extracts

Mo. The soil samples examined contained 0.2–3 ppm of molybdenum. The extraction with ammonium acetate gave solutions containing 0.04–0.6 ppm. The method of standard additions, being found to be a convenient way to compensate for matrix effects, was used to determine molybdenum in the extracts. In this

Table 3. Effect of concomitant elements on emission from 10 ppm Mo, Co and B

Concomitant (500 ppm)	Emission intensity (arbitrary units)		
	Mo	Co	B
None	100	100	100
Na	119	115	118
K	107	104	107
Fe	115	103	108
Mg	119	110	108
Mn	110	102	100
Ca	125	101	106
Al	120	120	100

Table 4. Determination of Mo, Co, and B in soil extracts

Element	Sample	Concentration, *ppm*	
		ICP	Macaulay
Mo	D 41152	3.1	3.1
	D 63405	0.30	0.31
	D 63403	0.20	0.19
Co	D 46753	0.18	0.18
	D 41148	0.28	0.28
	D 44193	0.37	0.36
	D 40458	0.50	0.50
	D 88787	1.00	0.95
	D 44187	1.10	1.10
B	D 18668	0.37	0.46
	D 18669	0.50	0.51
	D 18670	0.67	0.71
	D 18671	0.58	0.69
	D 18672	0.44	0.45
	D 18673	0.45	0.47

method the effects of decreased flow rate due to increased viscosity of the sample, interelement effects, and other physical and chemical interferences apply to the standard in the same way as to the sample.

In the method of standard additions it is assumed that the entire emission by the sample is due only to the element being determined. It is therefore necessary to make background corrections for all elements (*e.g.*, iron and other species) emitting at the analytical wavelength.

The results of the analyses are shown in Table 4. Each value is the mean of three results. The results obtained at the Macaulay Institute are also shown for comparison.

Co and B. Similar methods were applied to the analysis of Co and B extracts. The results are shown in Table 4.

Precision

The reproducibility of the line intensities is here expressed as the relative standard deviation (RSD) at concentration levels 100 times the detection limit in aqueous solutions. The values obtained were 0.9% for all the lines studied (B 249.8 nm, Co 345.3 nm, Mo 390.3 and 379.8 nm).

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ANALYTICAL DATA

PHOTOMETRIC DETERMINATION OF THE STABILITY CONSTANT OF THE PALLADIUM(II)–PYRIDINE-2,6-DICARBOXYLATE COMPLEX

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Summary—From the absorption spectra it has been concluded that Pd(II) forms a 1:1 complex with pyridine-2,6-dicarboxylic acid in aqueous solution at pH 2. The influence of various concentrations of chloride on the spectra has been examined. Ternary complex formation with chloride does not occur to a measurable extent. The conditional stability constant has been determined from data collected near the equivalence point of the titration curve. With allowance for the different side-reactions in this medium at an ionic strength of 0.2, a value of $\log K = 16.0 \pm 0.2$ has been found for the stability constant.

Palladium(II) can be determined accurately with EDTA by back-titration with bismuth at low pH and 4-(2-pyridylazo)resorcinol (PAR) as indicator. However, in the presence of gold(III) the method is unsuitable because the gold slowly oxidizes EDTA. Although gold can be removed quantitatively as $HAuCl_4$ from 3*M* hydrochloric acid by extraction with ether, we envisaged the possibility of titrating palladium in the presence of gold with pyridine-2,6-dicarboxylic acid (PDC), which is less sensitive to oxidation. The investigation began with the determination of the composition and stability constant of the complex. PDC forms complexes (ML_1, ML_2, ML_3) with several metals. The stability constants of the complexes with Mn(II), Cd, Zn, Co(II), Cu(II), Fe(II), Ni and Pb have been determined.[1–4] A considerably higher value would be expected for the Pd(II) complex because of the strong affinity of Pd(II) for amine groups. From the absorption spectra (350–550 nm) no indication was found of occurrence of complexes other than the 1:1 species. The investigation was then extended to media containing chloride, which is usually present from dissolution procedures and sometimes present because of the need to keep other noble metals in a defined state in solution.

It turned out that PDC is not very suitable as a titrant for Pd because the reaction is slow, but the stability constant was determined and is reported here.

EXPERIMENTAL

A stock 0.01*M* palladium solution was prepared by heating the nitrate with concentrated nitric acid for some hours, removal of excess of acid by evaporation to about 2 ml, and dilution with dilute nitric acid (pH 0.8).

Pyridine-2,6-dicarboxylic acid is only slightly soluble in acidic media; a stock 0.01*M* solution was prepared at pH 8.5. Monochloroacetic acid buffer (0.05*M*) was used to keep the pH at 2.0. The solutions were mixed in the order Pd–HCl(KCl)–buffer–PDC, at high chloride levels HCl being partly replaced by KCl to avoid addition of too large an amount of acid. To 5-ml portions of $10^{-3}M$ Pd(II) (pH 0.8) in 100-ml standard flasks enough potassium chloride and monochloroacetic acid were added to give final concentrations of 0.15*M* and 0.025*M* respectively, followed by various amounts (0–20 ml) of $10^{-2}M$ PDC, and the solution was made up to the mark with 0.01*M* nitric acid. The pH was 2.01 ± 0.05 for all solutions. The absorbances at 367 nm were measured in 4-cm cells after 1 hr, and were constant for at least 3 days.

RESULTS AND DISCUSSION

With increasing average co-ordination number $\bar{n}$, the absorption maximum of $PdCl_n$ increases and shifts to longer wavelengths (Fig. 1). The Pd–PDC complex has an absorption maximum at 367 nm (Fig. 2); Pd(II) has a small absorption maximum at 388 nm. The addition of chloride changes the absorption pattern considerably (Figs. 3 and 4). At chloride concentrations $> 0.25M$ Pd–PDC is no longer detectable. In the wavelength of range of interest, 350–500 nm, PDC does not absorb. As in all cases the absorp-

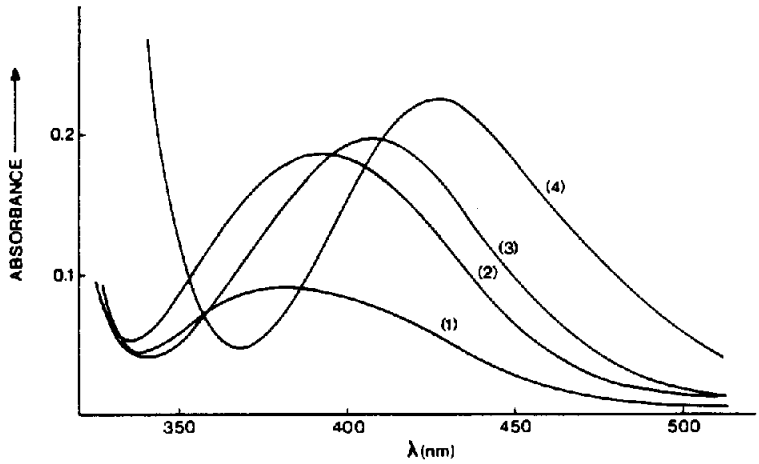

Fig. 1. Absorption spectra of $10^{-3}M$ Pd(II) with variable concentrations of chloride, measured in 1-cm cells. (1) No chloride, (2) $10^{-4}M$ Cl^-, (3) $10^{-3}M$ Cl^-, (4) $10^{-2}M$ Cl^-. The numbers refer to the line numbers. Solutions were buffered with $0.05M$ monochloroacetic acid (except No. 1), pH = 1.6.

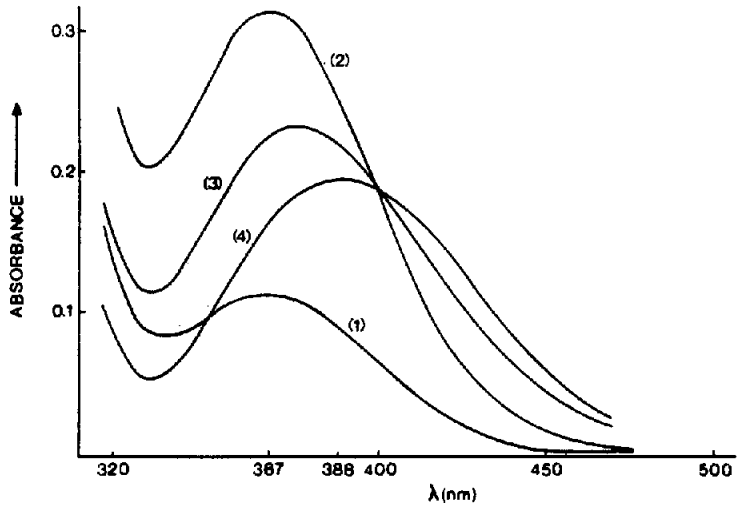

Fig. 2. Absorption spectra of the Pd–PDC system measured in 1-cm cells, $C_{Pd} + C_{PDC} = 10^{-3}M$. PDC has no absorption [see Fig. 3, line (1)]. Cl^- ions are not present. (1) $2 \times 10^{-4}M$ Pd, (2) $5 \times 10^{-4}M$ Pd, (3) $8 \times 10^{-4}M$ Pd, and (4) $10^{-3}M$ Pd. The numbers refer to the line numbers. Solutions were buffered with $0.05M$ monochloroacetic acid, pH = 2.0.

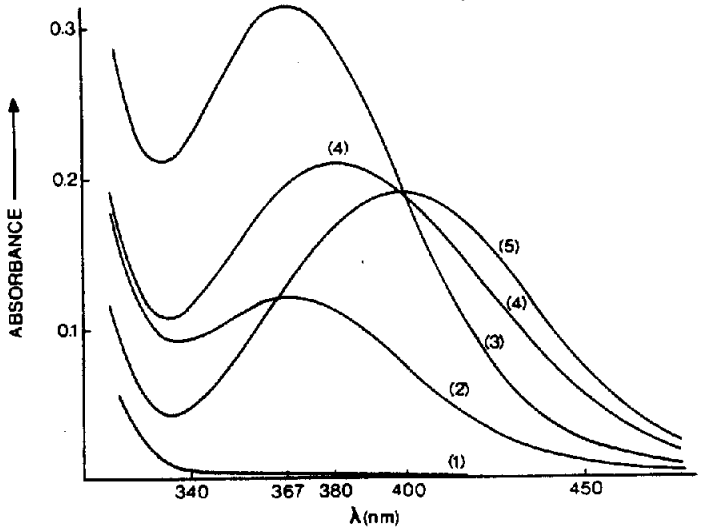

Fig. 3. Absorption spectra of Pd–PDC in the presence of $5 \times 10^{-4}M$ Cl^- (1-cm cells), $C_{Pd} + C_{PDC} = 10^{-3}$M. (1) No Pd, (2) $2 \times 10^{-4}M$ Pd, (3) $5 \times 10^{-4}M$ Pd, (4) $8 \times 10^{-4}M$ Pd, (5) $10^{-3}M$ Pd. The numbers refer to the line numbers. Solutions were buffered with $0.05M$ monochloroacetic acid, pH = 2.0.

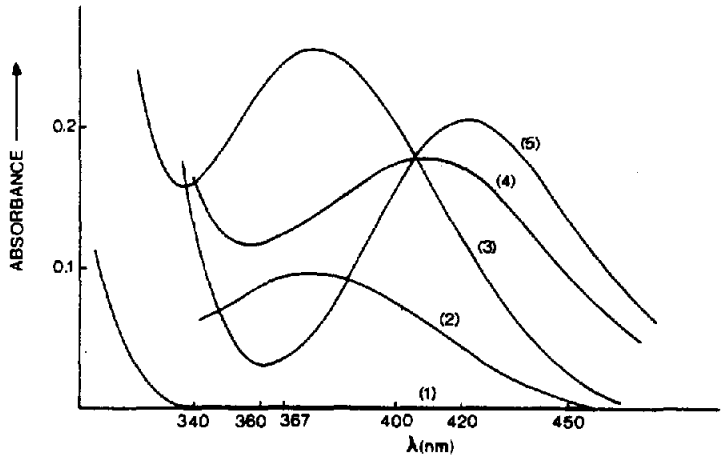

Fig. 4. Absorption spectra of Pd–PDC in the presence of $5 \times 10^{-3}M$ Cl^- (1-cm cells), $C_{Pd} + C_{PDC} = 10^{-3}M$. (1) No Pd, (2) $2 \times 10^{-4}M$ Pd, (3) $5 \times 10^{-4}M$ Pd, (4) $8 \times 10^{-4}M$ Pd, (5) $10^{-3}M$ Pd. The numbers refer to the line numbers. Solutions were buffered with $0.05M$ monochloroacetic acid, pH = 2.0

tion spectrum was the sum of the spectra for the appropriate concentrations of Pd–PDC and $PdCl_n$, it was concluded that there is no ternary complex formation.

A Job plot at 367 nm for Pd–PDC (from the data in Fig. 5) clearly indicated the formation of a 1:1 complex, with no indication that other complexes are formed.[5] As the nitrogen atom of PDC is not protonated at pH > 1, and Pd will be bound to it, protonation of the complex is not probable at the pH (2) used in our experiments. As dimerization is also unlikely the complex is taken to be $Pd–C_5H_5N(COO)_2$.

The stability constant can be determined in principle from data near the equivalence point of a spectrophotometric titration, performed as described in the experimental section.

Chloride was added to the system to decrease the conditional stability constant sufficiently for the titration curve to be considerably rounded. Its value was determined as follows. Defining C_{Pd} and C_L as the analytical concentrations of Pd and PDC, f as the titration parameter C_L/C_{Pd} and the formation coefficient γ as $[PdL']/C_{Pd}$ (the fraction of Pd transformed into Pd–PDC), the conditional constant[6] can be written as

$$K' = \frac{[PdL']}{[Pd'][L']} = \frac{\gamma}{(1-\gamma)(f-\gamma)C_{Pd}}. \qquad (1)$$

In this equation C_{Pd} and f are known from the conditions, and γ can be read from the titration curve (Fig. 6). Also γ is equal to the increase in absorbance ΔA (at degree of titration f) divided by the maximum

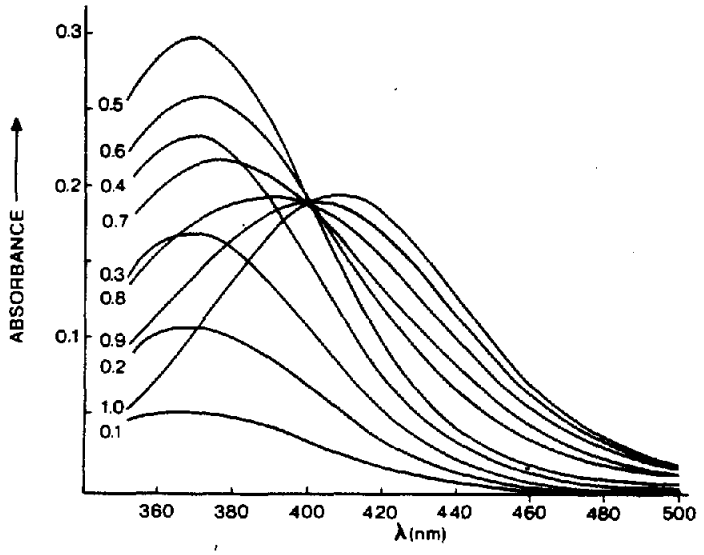

Fig. 5. Absorption spectra for mixtures of Pd(II) and PDC (1-cm cells), $C_{Pd} + C_{PDC} = 10^{-3}M$; $C_{Cl^-} = 10^{-3}M$, pH = 2, $x = C_{Pd}/(C_{Pd} + C_{PDC})$: numbers on the curves refer to the fraction x.

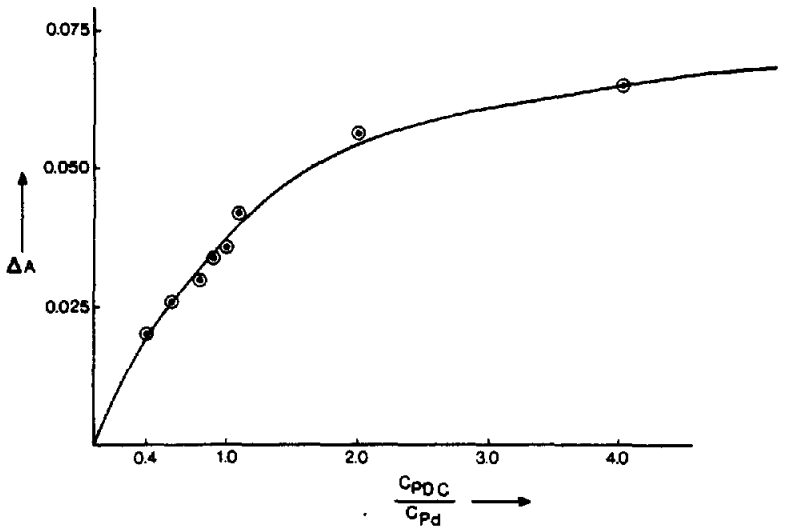

Fig. 6. Titration curve of $5 \times 10^{-3}M$ Pd(II) with $10^{-2}M$ PDC in a constant volume of 100 ml, measured at $\lambda = 367$ nm (4-cm cells). $C_{Cl^-} = 0.15M$, $0.025M$ buffer, pH = 2.0.

increase in absorbance ΔA_∞. In equation (1) and the relation $\gamma = \Delta A/\Delta A_\infty$ there are two unknown parameters, K' and ΔA_∞, which need to be adjusted to give a good fit of the ΔA as a function of f to the experimental points. The best fit was given by $\Delta A_\infty = 0.075$ (± 0.002) and $\log K' = 4.6 \pm 0.1$.

For calculation of the stability constant the side-reaction coefficients[6] $\alpha_{Pd(Cl)}$ and $\alpha_{PDC(H)}$ are required. The dissociation constants of PDC are practically independent of the type of supporting electrolyte and the ionic strength if $I < 0.2$.[7-9] The values $\log K_1 = 4.68$ and $\log K_2 = 2.10$ gave $\log \alpha_{PDC(H)} = 3.02$ at pH = 2.01.

On the assumption that chloride and perchlorate behave similarly as supporting electrolytes, the equation recently developed for evaluating $\log \beta_n$ for $PdCl_n$ complexes[10] was used to obtain $\log \beta_1 = 4.42$, $\log \beta_2 = 7.89$, $\log \beta_3 = 10.31$ and $\log \beta_4 = 11.52$. At a chloride concentration of $0.15M$ these lead to $\log \alpha_{Pd(Cl)} = 8.38 \pm 0.10$, which is sufficiently greater than other side-reaction coefficients of Pd and so be used as the overall α_{Pd}. If protonation of the complex and ternary complex formation are taken as absent, the stability constant for Pd–PDC is

$$\log K = 4.6 + 3.02 + 8.38 = 16.0 \pm 0.2.$$

It must be emphasized that the stability constant has been found from measurements performed at only one pH and one chloride concentration.

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EVALUATION OF THE GRAVIMETRIC TETRAPHENYLARSONIUM METHOD FOR THE DETERMINATION OF Tc(VII)

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Summary—Solubility losses in the gravimetric tetraphenylarsonium method for determining pertechnetate have been evaluated. Liquid scintillation counting was used to measure the β^- activity of ^{99}Tc in the filtrate, and indicated solubility losses of about 1% in analyses yielding 40–50 mg of precipitate. The solubility product of $(C_6H_5)_4AsTcO_4$ is estimated to be $(8.6 \pm 0.2) \times 10^{-10}$ at room temperature (24–25°), and K_{sp} for $(C_6H_5)_4AsReO_4$ at ~21–23° is estimated at $(2.6 \pm 0.3) \times 10^{-9}$. Both values are satisfactory for gravimetric purposes, but to keep solubility losses within 1% at least 40 mg of $(C_6H_5)_4AsTcO_4$ or 80 mg of $(C_6H_5)_4AsReO_4$ should be obtained (assuming 20 ml of solution, 20–30% excess of precipitant, and 6 or 7 washes with 5-ml portions of distilled water).

A wide variety of analytical methods is available for the determination of technetium,[1–6] including radioactivity, gravimetric analysis, spectrophotometry, polarography, cyclic voltammetry, coulometry, and atomic-absorption. However, relatively few of these are absolute methods. Most are relative methods, requiring one or more standard samples or standard solutions so that the analytical response factor under a prescribed set of conditions may be accurately determined. Of the *absolute* methods, gravimetric analysis is perhaps most widely used. Both tetraphenylarsonium chloride and nitron have been reported[7–15] as useful analytical reagents for the gravimetric determination and/or extraction (into chloroform) of perrhenate and pertechnetate. To a lesser extent, sulphide has also been used as a precipitant for these ions. Jasim *et al.*[16] compared these three precipitants and concluded that tetraphenylarsonium chloride was the best gravimetric reagent for both ReO_4^- and TcO_4^-, since in every case results with it were more reproducible.

Though the tetraphenylarsonium gravimetric method may be preferred, the literature contains little information on the solubility losses and other sources of determinate error, and from what is available there is some reason for concern. Souka and Ali,[14] for example, in studying the extractability of tetraphenylarsonium pertechnetate into chloroform define an equilibrium constant, K_{Tc}, for the reaction

$$(C_6H_5)_4As^+ + TcO_4^- \rightleftharpoons (C_6H_5)_4AsTcO_4,$$

and by using their extraction data in conjunction with data published by Bock *et al.*,[11,17,18] calculate a value of 5×10^5 for it. This suggests that the solubility product for tetraphenylarsonium pertechnetate may be as large as 2×10^{-6}, a value large enough to present some concern in gravimetric analysis.

An excellent analytical tool for investigating solubility losses in the tetraphenylarsonium gravimetric method for technetium is liquid scintillation counting. Goldstein[19] has reported that it is possible to count β^- emitters which have an E_{max} of not less than ~0.2 MeV with efficiencies approaching 100% (^{99}Tc is a β^- emitter with an E_{max} of 0.292 MeV). Taking the half-life of ^{99}Tc as 2.12×10^5 years, we may calculate the response factor for 100% counting efficiency as 3.74 dpm/pmole.

In this study, the filtrate recovered in the gravimetric determination of Tc(VII) by the tetraphenylarsonium method was counted. By combining the results with a careful evaluation of the detection efficiency for the measurement of ^{99}Tc with the liquid scintillation counter used, it was possible to calculate the error due to solubility losses. Samples of tetraphenylarsonium pertechnetate and perrhenate were also equilibrated with doubly distilled water and the resulting solutions were assayed, by liquid scintillation counting for ^{99}Tc in the first case and by spectrophotometry for $(C_6H_5)_4As^+$ in the second. The solubility products were then calculated for $(C_6H_5)_4AsTcO_4$ and $(C_6H_5)_4AsReO_4$ from the data obtained.

EXPERIMENTAL

Apparatus

All counting was done with a Beckman LS 7000 Liquid Scintillation System. Ultraviolet spectra were taken with a Beckman Acta M VI UV–visible spectrophotometer.

Reagents

Ammonium pertechnetate. NH_4TcO_4 was obtained in aqueous solution from the Amersham Corporation, Arlington Heights, Illinois, U.S.A. It was standardized by the combined gravimetric tetraphenylarsonium/liquid scintillation counting methods described in this paper.

Potassium perrhenate. $KReO_4$ (reagent grade) was obtained from Alfa Inorganics, Inc. Aqueous solutions were prepared with doubly distilled water.

Tetraphenylarsonium chloride. $(C_6H_5)_4AsCl$ (reagent grade) was also obtained from Alfa Inorganics, Inc. and aqueous solutions were prepared with doubly distilled water.

Procedure for gravimetric determination of pertechnetate

The determination was conducted in triplicate, with 10.00-ml aliquots of $7.69 \times 10^{-3}M$ ammonium pertechnetate (standardized by conductometric titration with tetraphenylarsonium chloride). Sufficient solid sodium chloride was added to each for the final solutions to be $0.5M$ in this salt. Next 10.00 ml of $1.00 \times 10^{-2}M$ tetraphenylarsonium chloride were added to each, followed by an overnight digestion. The solutions were then chilled in ice for at least 10 min and tested for completeness of precipitation, then the precipitates were filtered off on previously weighed medium porosity sintered-glass crucibles. The filtrate was recovered and diluted to 100.0 ml for Tc assay by liquid scintillation counting. Finally, the crucibles (and precipitates) were dried for 2 hr at 105°, allowed to cool, and weighed.

Liquid scintillation procedure

The counting was standardized with solutions made by adding 1.00 ml of ammonium pertechnetate solution to 10.00 ml of Beckman Ready-Solv HP scintillation cocktail. A response factor of 3.52 cpm/pmole was obtained, which corresponds to a 94.2% counting efficiency. From this response factor and the measured net count rate for "unknown" solution, such as the filtrate from the gravimetric tetraphenylarsonium method, the molarity of the test solution was calculated.

Procedure for solubility product determination

Portions of tetraphenylarsonium pertechnetate were scraped from the crucibles and added to 25 ml of doubly distilled water in small, stoppered Erlenmeyer flasks. The flasks were then shaken, intermittently, at room temperature for a period of at least one week to permit saturation to be reached. They were then opened and the temperature was noted. The samples were filtered and the filtrate assayed for ^{99}Tc by liquid scintillation counting.

Samples of tetraphenylarsonium perrhenate were prepared in triplicate by mixing 5.00 ml of $1.00 \times 10^{-2}M$ potassium perrhenate and 5.00 ml of $1.00 \times 10^{-2}M$ tetraphenylarsonium chloride. The product in each case was filtered off, washed thoroughly, dried, and transferred to a small stoppered Erlenmeyer flask containing 25 ml of redistilled water. The remaining procedure was the same as for the pertechnetate, except that the solubility was determined by comparing the ultraviolet spectrum of the filtrate with the spectra of standard solutions of the two reactants.

RESULTS AND DISCUSSION

Evaluation of the gravimetric method for pertechnetate

The results for the procedure described are shown in Table 1. Triplicate analysis was conducted on a solution standardized several years earlier and labelled as "$7.69 \times 10^{-3}M$ NH_4TcO_4". A 10.00-ml aliquot was taken for each run.

Thus in the gravimetric tetraphenylarsonium method, results will be about 1% low owing to solubility of the precipitate (for analyses yielding 40–50 mg of precipitate in 20 ml of solution, with about 25% excess of precipitant and washing with about 30 ml of water).

If the number of moles of precipitate is combined with the number of moles of technetium in the filtrate, the mean molarity of the restandardized solution may be calculated as follows:

$$\frac{\left(\frac{7.83 + 7.65 + 7.64}{3}\right) \times 10^{-5}\ \text{mole}}{1.00 \times 10^{-2}\ \text{litre}} = 7.71 \times 10^{-3}M.$$

This value supports the contention that there are no major sources of determinate error other than solubility losses in the gravimetric tetraphenylarsonium method for pertechnetate (assuming that other ions precipitated by the tetraphenylarsonium ion[10,11] are absent). The long-term stability of dilute aqueous solutions of ammonium pertechnetate is also confirmed by the results. The counting statistics were taken into consideration and found to contribute negligibly (coefficient of variation 0.2%) to the standard deviation of the amount of Tc found in the filtrate.

Evaluation of the solubility product of $(C_6H_5)_4AsTcO_4$

The procedure described was carried out in triplicate. Results are shown in Table 2.

The data show that a 7-day period, with intermittent shaking, is adequate for solubility equilibrium to be attained. The solubility at room temperature appears to be unaffected by minor temperature fluctuation. The counting statistics were again responsible for only a negligible error.

Table 1. Evaluation of solubility loss in the gravimetric tetraphenylarsonium method for TcO_4^-

Sample	Precipitate, *g*	Precipitate, *mole*	Tc in filtrate, *mole*	Fraction of total Tc in filtrate, %
1	0.0424	7.76×10^{-5}	7.1×10^{-7}	0.9
2	0.0413	7.56×10^{-5}	8.6×10^{-7}	1.1
3	0.0412	7.54×10^{-5}	9.5×10^{-7}	1.2
Mean	0.0416	7.62×10^{-5}	8.4×10^{-7}	1.1
Std. devn.	0.0007	0.13×10^{-5}	1.4×10^{-7}	0.2

Table 2. Evaluation of the solubility product of tetraphenylarsonium pertechnetate at room temperature (24–25°C)

Sample	Equilibrium time, *days*	Temperature of saturated solution, °C	Molarity of saturated solution	K_{sp}
1	7	24.2	3.02×10^{-5}	9.12×10^{-10}
2	10	25.1	2.89×10^{-5}	8.35×10^{-10}
3	12	24.1	2.90×10^{-5}	8.41×10^{-10}
Mean	10	24.5	2.94×10^{-5}	8.63×10^{-10}

Table 3. Evaluation of the solubility product of tetraphenylarsonium perrhenate at room temperature (21–23°C)

Sample	Equilibrium time, *days*	Temperature of saturated solution, °C	Molarity of saturated solution	K_{sp}
1	8	22.9	5.65×10^{-5}	3.19×10^{-9}
2	12	21.6	4.75×10^{-5}	2.26×10^{-9}
3	14	20.9	4.78×10^{-5}	2.28×10^{-9}
Mean	11	21.8	5.06×10^{-5}	2.58×10^{-9}

The K_{sp} of $(C_6H_5)_4AsTcO_4$ at 24.5 ± 0.5° is equal to $(8.6 \pm 0.3) \times 10^{-10}$ (uncertainty expressed in terms of standard deviation of the mean). This value is small enough for the tetraphenylarsonium method to be a satisfactory gravimetric method for pertechnetate. However, washing of the precipitate should be kept to a minimum, especially when determining small quantities of pertechnetate (< 50 mg of precipitate). Nevertheless, washing should be adequate enough to remove the sodium chloride present (it is added to give a salting-out effect[10,16]). It is estimated that ~0.4 mg of precipitate will be lost in the supernatant liquid and during the washing. Therefore, to ensure that the relative error does not exceed 1%, at least 40 mg of precipitate should be present for the final weighing.

Evaluation of the solubility product of $(C_6H_5)_4AsReO_4$

The K_{sp} of $(C_6H_5)_4AsReO_4$ at room temperature was determined as described. Results are presented in Table 3.

The ultraviolet spectrum of $(C_6H_5)_4AsCl$ (Fig. 1) is characterized by maxima at 270.5 nm ($\epsilon = 2.55 \times 10^3$ l.mole^{-1}.cm^{-1}), 263.5 nm ($\epsilon = 3.06 \times 10^3$) and 257.7 nm ($\epsilon = 2.42 \times 10^3$), and a shoulder at 220 nm ($\epsilon = 3.21 \times 10^4$). The spectrum of $KReO_4$, on the other hand, has a broad maximum at 220 nm ($\epsilon = 3.50 \times 10^3$) and a maximum at 197 nm

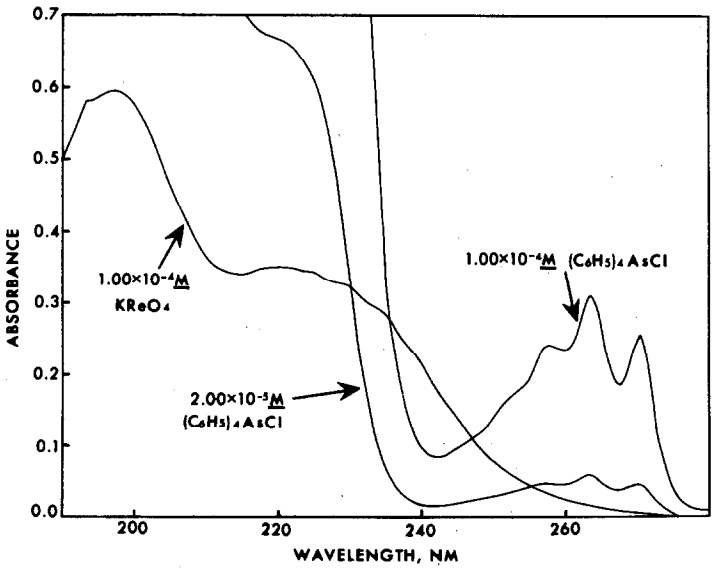

Fig. 1. Ultraviolet spectra of $1.00 \times 10^{-4}M$ $KReO_4$ and $1.00 \times 10^{-4}M$ $(C_6H_5)_4AsCl$.

($\epsilon = 5.71 \times 10^3$), Fig. 1. Thus there is a choice of several wavelengths for analytical purposes. A wavelength of 263.5 nm was selected, because this permitted direct measurement of the supernatant liquid, without any need for dilution. Assuming that the absorbances of the tetraphenylarsonium ion and the perrhenate ion are additive at 263.5 nm (ϵ for ReO_4^- at 263.5 nm is 371 $l.mole^{-1}.cm^{-1}$), the molarity of the saturated solution may be readily calculated from the total absorbance at this wavelength by using Beer's law. The $(C_6H_5)_4As^+$ ion (89.2% of the total absorbance at 263.5 nm is due to this ion) obeys Beer's law quite well at this wavelength.

The K_{sp} of $(C_6H_5)_4AsReO_4$ at 22 ± 1° is thus equal to $(2.6 \pm 0.3) \times 10^{-9}$.

Most of the general conclusions stated for the gravimetric tetraphenylarsonium method for TcO_4^- apply equally well to ReO_4^-. However, because of the greater formula weight, the solubility losses in a similar gravimetric procedure are estimated at ~0.8 mg.

Therefore, if an *absolute* analytical method is needed for pertechnetate or perrhenate, the gravimetric tetraphenylarsonium method is satisfactory, with the reservations noted. A simple ultraviolet spectrum (in the case of either ion) or a liquid scintillation count (TcO_4^- only) will indicate whether or not solubility losses are indeed negligible in a given situation. Of course, the gravimetric procedure would not be recommended for determining trace amounts of either ion. If a 1% error is considered tolerable, at least 40 mg of $(C_6H_5)_4AsTcO_4$ or 80 mg of $(C_6H_5)_4AsReO_4$ must be obtained in the gravimetric procedure, performed as described.

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ION-EXCHANGER COLORIMETRY—VI MICRODETERMINATION OF NICKEL IN NATURAL WATER

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Summary—Ion-exchanger colorimetry with 1-(2-pyridylazo)-2-naphthol (PAN) has been developed for the determination of nickel at the μg/l. level in natural water. With 1 litre of sample the detection limits are $1.3 \times 10^{-9}M$, *i.e.*, 0.077 μg/l. for fresh water and $5.8 \times 10^{-9}M$, *i.e.*, 0.34 μg/l. for sea-water. The distribution ratio is 5×10^4. Copper and zinc, which form coloured species with PAN in the resin phase, can be completely eluted with a masking solution composed of EDTA and thioglycollic acid (pH 7.8). Cobalt can be determined simultaneously by measurement at 628 nm.

Microdetermination methods for chromium(III, VI), iron(II, III), cobalt(II), copper(II), zinc, bismuth and cadmium in water have been developed.[1–5] These are based on measurement of the absorbance of an ion-exchange resin (ion-exchanger colorimetry) and take advantage of resin properties such as concentration, selective sorption and simultaneous colour development in the resin phase. There are three modes of colour development in the resin phase, depending on the properties of the reagent.[3]

PAN [1-(2-pyridylazo)-2-naphthol] is known to form complexes of very high absorptivity with many kinds of metal ions and has been used for spectrophotometric analysis in combination with solvent extraction.[6] This reagent is irreversibly sorbed on a cation-exchange resin and develops a coloured complex with a minute amount of nickel in the resin phase (the second mode of colour development). The resin behaves as a chelating resin, analogously to the previously described anion-exchange resin with Zincon sorbed on it.[1]

The concentration of nickel in natural waters is reported to be at the μg/l. level or lower.[7,8] The present paper describes the ion-exchanger colorimetry of nickel with PAN and its application to the determination of nickel in natural waters.

EXPERIMENTAL

Reagents

Standard nickel(II) solution. An appropriate amount of nickel chloride was dissolved in water and diluted to the required volume with demineralized water. The solution was standardized with EDTA, Cu–EDTA–PAN complex being used as indicator.

PAN resins. Dowex 50W-X2 (100–200 mesh) and -X4 (200–400 mesh) cation-exchange resins in the hydrogen form were used. To about 100 ml of a solution containing 24 ml of 0.1% PAN in methyl alcohol, 30 g of resin were added and the mixture was stirred. After 1 hr, the PAN-resin was converted into the sodium form by addition of sodium hydroxide solution. The resins were washed with demineralized water, air-dried and stored in glass containers in a refrigerator.

Masking solution for copper and zinc. The solution used was 0.1*M* in thioglycollic acid and 0.001*M* in EDTA. The pH of the solution was adjusted to 7.8 with ammonia.

All chemicals used were of analytical grade.

Apparatus

Absorbances were measured with a Hitachi recording spectrophotometer, Model EPS-3T, a perforated metal plate of absorbance 1.0 or 2.0 being used in the reference beam to balance the light intensities.

The pH-measurements were done with a Hitachi–Horiba pH-meter, Model M-5.

Procedure for the determination of nickel

To a 200-ml sample containing 0.005–0.2 μmole of nickel, 1 ml of 0.1*M* sodium pyrophosphate and 0.50 g of the PAN-Dowex 50W-X2 (100–200 mesh) resin were added. The pH of the solution was adjusted to 6.0 with hydrochloric acid or ammonia. The mixture was stirred for 20 min, the coloured resin filtered off and transferred to 25 ml of the masking solution. After stirring for 10 min the mixture was allowed to settle for 1–2 min and the resin slurry transferred to a 1-mm quartz cell with the aid of a pipette. The absorbances at 566 nm (the absorption maximum of the nickel–PAN complex species in the resin phase), $A_{(566)}$, and at 700 nm (in the range where only the resin absorbs light), $A_{(700)}$, were measured. The net absorbance of the complex species in the resin phase at 566 nm, $A_{RC(566)}$, is obtained from the difference between $A_{(566)}$ and $A_{(700)}$, minus the corresponding difference for the blank. Other procedural details were as described earlier.[1–5]

For the determination of lower levels of nickel in water a 1-litre sample can be used. To a 1-litre sample of fresh water containing 0.005–0.15 μmole of nickel or of sea-water containing 0.02–0.75 μmole of nickel, 5 ml of 0.1*M* sodium pyrophosphate and 0.50 g of the PAN-Dowex 50W-X4 (200–400 mesh) resin were added. After stirring for 1 hr, the mixture was treated as described above.

When natural waters were sampled, 10 ml of concentrated hydrochloric acid per litre of sample solution was added immediately after filtration of the sample through a Whatman glass fibre filter, GF/D. The sample solutions were stored in polyethylene containers.

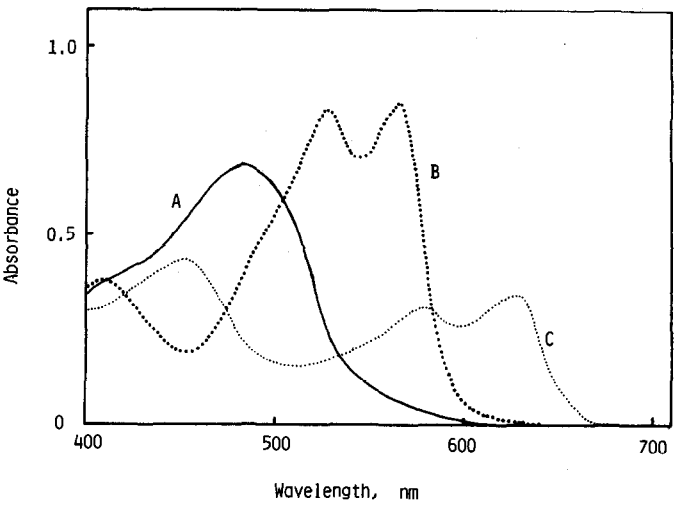

Fig. 1. Absorption spectra of PAN complexes in the cation-exchange resin phase (pH 7.8). Resin Dowex 50W-X2, 100–200 mesh, Na^+ form; cell path length 1 mm, A: 0.54 μmole of PAN/g of resin; B: 0.27 μmole of Ni–PAN/g of resin; C: 0.21 μmole of Co–PAN/g of resin.

Distribution measurements

To a 200-ml water sample containing 0.19 μmole of nickel, 1 ml of 0.1*M* sodium pyrophosphate and 0.50 g of Dowex 50W-X2 (100–200 mesh) resin were added as described above. This procedure was repeated 5 times. After the equilibrated solutions had been separated from the resin and collected, the nickel concentration in the solution was determined by ion-exchanger colorimetry. The distribution ratio, D = (μmole of Ni sorbed per g of resin)/(μmole of Ni per ml of solution), was calculated.

RESULTS AND DISCUSSION

Absorption spectrum of the resin

Figure 1 shows the absorption spectra of PAN and its nickel and cobalt complexes in the resin phase. Maximum absorbance of the nickel–PAN complex is at 566 nm, where the absorbance of the reagent blank is fairly small. In order to check the reproducibility of packing of the resin beads in the cell, the absorbance in the range where only the resin absorbs light (700 nm) is used in conjunction with that at 566 nm.

Each spectrum is similar to that observed in chloroform solution. The PAN:metal ratio in the complex in chloroform solution was found to be 2:1 by both the mole-ratio and the continuous variation methods.[6] The composition of the complex formed by nickel and PAN in the resin phase was determined by the mole-ratio method. The reagent concentration was kept constant by prior sorption on the resin. PAN sorbed on the resin was not desorbed during the measurements. Because of the very high distribution ratio, 5×10^4, almost all of the nickel can be sorbed on the resin so the mole-ratio method can be applied.[9] The results obtained also indicated a 2:1 ratio. The functional groups of the reagent may be free in the resin phase despite some interaction of PAN with the resin.

Dependence of colour development on pH

As shown in Fig. 2, the colour of the nickel complex has maximum intensity in the pH range 5.5–8, whereas the colour of iron(III), copper and zinc with PAN is developed in different pH-ranges; pH 6 is satisfactory for minimizing the contribution from other metal-ion complexes.

Time-dependence of colour development

The use of resins having a larger particle size results in slow sorption rates. It is desirable that the nickel in the sample solution is sorbed as rapidly as possible. In addition, the separation of the resin from an equilibrated bulk solution and its packing into a sample cell should be simple. For this reason, the 100–200 mesh resin was used for a 200-ml sample system. The colour development was almost complete within 20 min. For a 1-litre sample system, however, even the use of the finer 200–400 mesh resin required a longer equilibration time (1 hr). The presence of sodium chloride in 0.6*M* concentration retarded the colour development. The results of these experiments are shown in Fig. 3.

For rapid analysis it is possible to use a fixed stirring time, at which equilibration is incomplete but the calibration curve is still linear. To obtain higher sensitivity, however, the stirring time was fixed at 20 min for a 200-ml sample and at 1 hr for a 1-litre sample.

Masking of other metal ions

It is known that formation of the nickel–PAN complex is slow,[10] but once formed, the complex is very stable,[11] ($\beta_2 > 10^{25}$), and of low solubility.[10] The iron(III) complex with PAN behaves in the same manner and it is therefore necessary to carry out a prior masking of iron(III). The effects of a number of masking agents on the formation of the iron(III)–

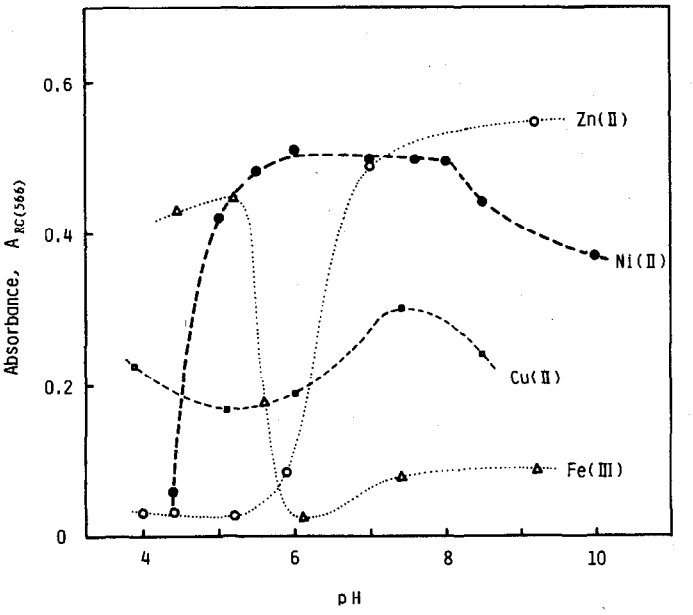

Fig. 2. pH-dependence of colour development. Solution: 200 ml. Ni, Cu, Zn: $5 \times 10^{-7}M$; Fe(III): $1 \times 10^{-6}M$. Resin: 0.50 g of Dowex 50W-X2, 100–200 mesh, Na^+ form, 0.40 mg PAN presorbed; stirring time: 20 min.

PAN complex in the resin phase was investigated. The presence of sodium pyrophosphate at concentrations higher than $5 \times 10^{-4}M$ prevented the reaction of $5 \times 10^{-5}M$ iron(III) with PAN (Fig. 4). To eliminate the interference of iron(III) completely, sodium pyrophosphate was used as the masking agent at the cost of a reduction in the intensity of the nickel–PAN colour.

Metals such as copper and zinc cannot be masked with sodium pyrophosphate. The presence of thioglycollic acid or EDTA prevented the reaction of nickel, as well as that of copper and zinc with PAN. However, once the complex of nickel with PAN has been formed in the resin phase the colour is not affected by addition of thioglycollic acid or EDTA, whereas almost all the copper (at the $5 \times 10^{-6}M$ level) may be eluted with thioglycollic acid solution at pH 7.8, as shown in Fig. 5. Zinc cannot be masked completely with this masking reagent, but complete removal of zinc (at the $5 \times 10^{-6}M$ level) was accomplished by washing with 0.001M EDTA solution (pH 7.8). In the standard procedure, the resin loaded with PAN is added to a water sample containing $5 \times 10^{-4}M$ sodium pyrophosphate at pH 6. After sorption of the

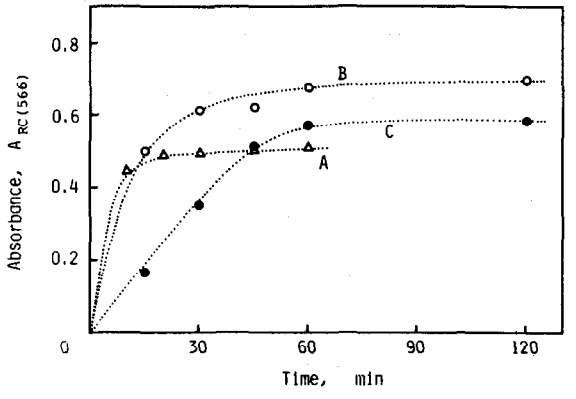

Fig. 3. Time-dependence of colour development. Solutions: A, 200 ml, $5 \times 10^{-7}M$ Ni; B, 1 litre, $1 \times 10^{-7}M$ Ni; C, 1 litre, 0.6M NaCl, $4 \times 10^{-7}M$ Ni. Resin: A, 0.50 g of Dowex 50W-X2, 100–200 mesh, Na^+ form, 0.40 mg of PAN presorbed; B and C, 0.50 g of Dowex 50W-X4, 200–400 mesh, Na^+ form, 0.40 mg of PAN presorbed.

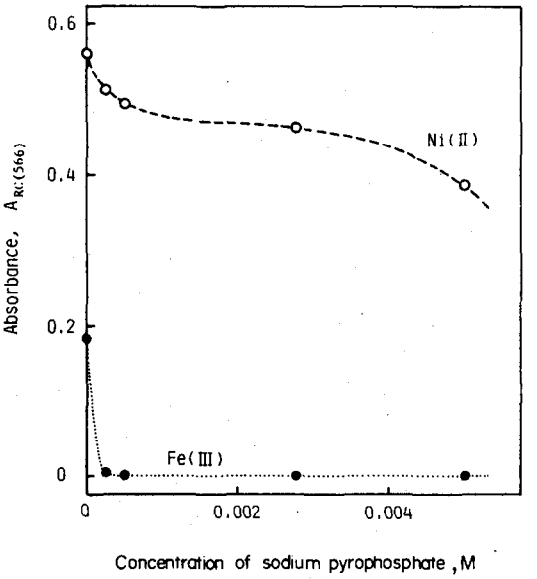

Fig. 4. Masking of iron(III) with sodium pyrophosphate. Solution: 200 ml, pH 6; Ni $5 \times 10^{-7}M$, Fe(III) $5 \times 10^{-5}M$. Resin: 0.50 g of Dowex 50W-X2, 100–200 mesh, Na^+ form, 0.4 mg of PAN presorbed; stirring time 20 min.

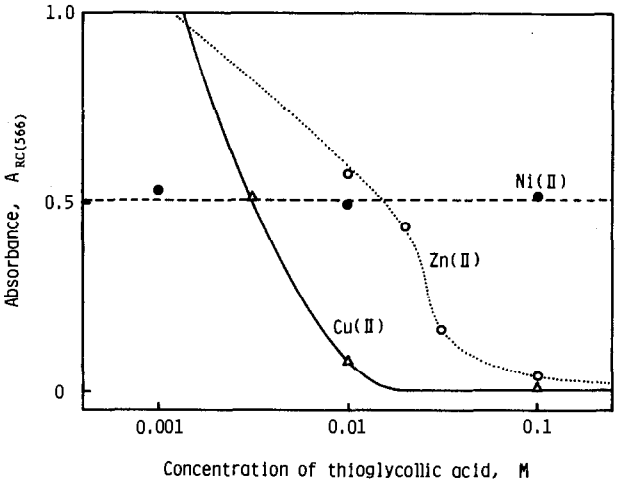

Fig. 5. Masking of copper and zinc with thioglycollic acid. Solution: 200 ml, pH 6; Ni $5 \times 10^{-7}M$, Cu and Zn $5 \times 10^{-6}M$. Resin: 0.50 g of Dowex 50W-X2, 100–200 mesh, Na^+ form, 0.4 mg of PAN presorbed. Masking solution: 25 ml, pH 7.8, stirring time for masking: 10 min.

nickel, the resin is washed with $0.1M$ thioglycollic acid and $0.001M$ EDTA (pH 7.8).

Calibration

The calibration curves are reasonably linear and may be expressed by the equations:

$$A_{RC(566)} = (1.01 \times 10^6)C$$

for 200-ml samples,

$$A_{RC(566)} = (6.02 \times 10^6)C$$

for 1-litre samples of fresh water, and

$$A_{RC(566)} = (1.39 \times 10^6)C$$

for 1-litre samples containing sodium chloride at the $0.6M$ level, where C is the concentration of nickel in the sample solutions, in mole/l.

Sensitivity

The sensitivity for each system has been compared with that of the conventional colorimetric method based on the extraction of the nickel–PAN complex into chloroform. The sensitivity is twice as high with a 200-ml sample and 12.5 times as high with a 1-litre sample.

The increase in sensitivity can be estimated from the distribution ratio, D.[2,9] In the case of the present system, D was found to be 5×10^4, suggesting the possibility of a much higher sensitivity if a larger amount of sample solution is used.

The background absorbance, $A^* = A_{(566)} - A_{(700)}$ (for the blank), was 0.048 ± 0.002 for the 1-litre sample system (10 determinations). The relative detection limit, defined as the concentration that produces an absorbance equal to twice the magnitude of the fluctuation in A^*, was $1.3 \times 10^{-9}M$, *i.e.*, 0.077 μg/l. for fresh water and $5.8 \times 10^{-9}M$, *i.e.*, 0.34 μg/l. for sea-water.

Effects of foreign ions

The effects of foreign ions were examined by the standard procedure for the 200-ml sample system and the results obtained are shown in Table 1. Metals, except for cobalt and copper, did not interfere when present at up to 100 times the concentration of nickel.

It is possible to determine cobalt simultaneously by taking advantage of the difference in wavelength of the absorption maxima of the nickel and cobalt complexes of PAN (Fig. 1). The calibration curves for cobalt in a 200-ml sample system may be expressed by the equations: $A_{RC(566)} = (2.39 \times 10^5)C$ at 566 nm and $A_{RC(628)} = (3.12 \times 10^5)C$ at 628 nm. After the concentration of cobalt has been determined by use of the curve at 628 nm, the contribution of the cobalt–PAN absorbance at 566 nm is calculated from the equations and the nickel is determined after deduction of cobalt absorbance.

Table 1. Effect of foreign ions on the determination of $4.85 \times 10^{-7}M$ nickel

Foreign ions	Mole-ratio of foreign ion to nickel	$A_{RC(566)}$	Ni found, $10^{-7}M$	Relative error, %
		0.490	4.85	0
Fe(II)	10	0.502	4.97	+2.5
	100	0.505	5.00	+3.1
Fe(III)	10	0.485	4.80	−1.0
	100	0.501	4.96	+2.3
Mn(II)	10	0.503	4.98	+2.6
	100	0.481	4.76	−1.9
Co(II)	0.1	0.501	4.96	+2.3
	1	0.662	6.55	+35.1
Cu(II)	10	0.485	4.80	−1.0
	100	0.196	1.93	−60.2
Zn(II)	10	0.497	4.92	+1.4
	100	0.511	5.06	+4.3
NaCl				
($0.01M$)		0.496	4.91	+1.2
($0.1M$)		0.409	4.05	−16.5
($0.6M$)		0.200	1.98	−59.2

Table 2. Determination of nickel in natural waters*

River water: Ohno River, Oita Pref.					
Ni added, μg	0	0.63	1.25	1.88	2.50
$A_{RC(566)}$	0.040	0.098	0.165	0.229	0.294
Ni found, μg	0.39	0.96	1.68	2.25	2.89
Sea-water: Tsuyazaki, Fukuoka Pref.					
Ni added, μg	0	2.9	5.7	8.5	11.4
$A_{RC(566)}$	0.025	0.100	0.166	0.243	0.288
Ni found, μg	1.1	4.3	7.1	10.4	12.2

* Sample: 1 litre. Resin: Dowex 50W-X4-Na^+, 200–400 mesh, PAN presorbed, 0.50 g.

The presence of copper at a concentration 100 times that of nickel reduces the absorbance due to nickel–PAN by lowering the amount of complex formed. However, the amounts of ions found in river and sea-water are generally much smaller than those listed in Table 1.

Alkali and alkaline-earth metal ions, which produce no colour with PAN, do not interfere. The presence of these metal salts at high concentrations, however, results in the production of a lower absorbance than that observed in their absence because of the lower distribution ratio. In this case, it is recommended to use a calibration curve prepared at the same concentration of background electrolyte as that in the sample solution. Alternatively, the method of standard additions can be applied.

Determination of nickel in river and sea-water

The method was applied to the determination of nickel in natural water samples. The results of some analyses are shown in Table 2. For sea-water, the calibration curve was prepared by using sample solutions from which trace elements had been stripped by passage of the sample through a column of chelating resin.[12] Recovery of the added nickel was almost complete. The concentrations found were $(6.4 \pm 0.7) \times 10^{-9} M$, *i.e.*, 0.38 ± 0.04 μg/l. for river water and $(2.2 \pm 0.7) \times 10^{-8} M$, *i.e.*, 1.3 ± 0.4 μg/l. for sea-water. The concentration of cobalt in both samples was less than the detection limit.

Comparison with other methods

Nickel has been determined in natural waters by the use of a column of chelating resin[12] in conjunction with atomic-absorption spectrophotometry. This method is somewhat time-consuming because of the need to use low flow-rates.

The solvent extraction method[6] has high sensitivity and selectivity comparable with those of our method, but the flexibility of the sample-to-solvent volume ratio is not so large as that for ion-exchanger colorimetry. The solubility of the solvent in the sample solution must also always be taken into consideration.

The advantages of ion-exchanger colorimetry are that concentration of the metal concerned and the colour development take place simultaneously and that the sensitivity and selectivity are high. As a result, low concentrations of the metal can be determined in a conveniently short time. Furthermore, our method can be directly applied to the determination of nickel in a turbid sample solution.

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GENERAL TREATMENT OF CONJUGATE ACID–BASE, REDOX AND COMPLEXATION EQUILIBRIA

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Summary—Acid–base, redox and complexation reactions are treated uniformly in terms of particle transfer. As a result, a single set of mathematical equations can be used to describe all three types of reaction and to perform the usual calculations for these systems.

By tradition, in analytical textbooks, the Brønsted–Lowry concept, oxidation–reduction and complex equilibria are discussed separately. Although an analogy between oxidation–reduction reactions and acid–base reactions was presented early on by Hazlehurst[1] and the conjugate reductant–oxidant concept was also accepted,[2–5] Pacer [6] has pointed out that usage of this terminology is not very widespread. He illustrated how relative acid–base strength is closely parallel to relative oxidizing–reducing strength. In some analytical chemistry texts, such that of Charlot,[7] many similarities between acid–base, redox and complexation reactions are given. The aim of this paper is to show the advantages of a new treatment based on the similarity between these equilibria. The many numerical examples are meant to show how to solve different analytical problems by means of this approach.

RELATIVE DONOR-ACCEPTOR STRENGTH

Let us consider acids, reductants and complexes are particle-donors, the particles being protons, electrons and ligands respectively. Bases, oxidants and metal ions should be considered as acceptors.

Thus the equilibrium

$$\text{Don} \rightleftharpoons \text{Acc} + n \text{ particles (p)} \qquad (1)$$

describes the transfer of particles between conjugate donor–acceptor pairs.

The equilibrium for such a system may be expressed as

$$K_{acc} = \frac{[\text{Acc}]\,[\text{p}]^n}{[\text{Don}]} = \frac{1}{K_{don}} \qquad (2)$$

where K_{acc} and K_{don} are the formation constants of the acceptor and the donor respectively.

The magnitude of the acceptor formation constant indicates the strength of the donor and its inverse indicates the strength of the acceptor. The greater the value of K_{acc}, the greater the donor strength. But the strength of a donor can also be defined by its formation constant, K_{don}, which is the reciprocal of the acceptor formation constant. Thus if the value K_{don} is small, the particle donor is strong, and *vice versa*. We prefer the latter way of expressing the donor strength since it permits us to introduce general principles in describing the three classes of chemical reactions.

Absolute strength is very difficult to measure and it is customary to use a second couple as a point of reference. Thus the strength of an acid–base pair in aqueous medium is measured relative to the system H_3O^+/H_2O, in terms of the equilibrium

$$HB + H_2O \rightleftharpoons H_3O^+ + B \qquad (3)$$

where B is the base and HB its conjugate acid.

The equilibrium constant of the reaction is, if the concentration of water is taken as constant and incorporated in the equilibrium constant:

$$K_B = \frac{[H_3O^+]\,[B]}{[HB]} = \frac{1}{K_{HB}} \qquad (4)$$

or

$$K_{HB}K_B = 1 \qquad (4a)$$

where K_{HB} and K_B are the donor and acceptor formation constants respectively.

Thus the stronger an acid, the weaker its conjugate. All acids with a greater tendency than H_3O^+ to lose a proton will have a relative formation constant, K_{HB}, smaller than 1. Relative acid base strength is usually listed in tabular form, showing acids and their conjugate bases in order of decreasing strength of the conjugate acid (Table 1). Those acids at the top of the table lose their protons most easily and are, therefore, the strongest acids. The conjugate pair H_2O^+/H_2O is taken as reference and arbitrarily assigned a log $K_{H_3O^+}$ value of zero. At the bottom of the table is the log K_{H_2O} value of the couple H_2O/OH^-, since the water molecules may also react as a weak acid, while OH^- is the strongest base in water as a solvent.

We can represent this reaction by

$$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-. \qquad (5)$$

Table 1. Acids and bases

	Acid–base couple	$\log K_{HB}$	
increasing strength of conjugate acid ↑	$HClO_4/ClO_4^-$	−8	↓ increasing strength of conjugate base
	HCl/Cl^-	−5	
	H_3O^+/H_2O	0	
	$H_3PO_4/H_2PO_4^-$	2.12	
	$H_2PO_4^-/HPO_4^{2-}$	7.12	
	HPO_4^{2-}/PO_4^{3-}	12.36	
	H_2O/OH^-	14.0	

The equilibrium expression for the protolysis of water is

$$K_e = \frac{[H_3O^+][OH^-]}{[H_2O]^2} = \frac{K_{H_3O^+}}{K_{H_2O}} \qquad (6)$$

where $K_{H_3O^+}$ and K_{H_2O} are the formation constants of the proton donors H_3O^+ and H_2O respectively.

Since the concentration of water can be considered as a constant, as well as $K_{H_3O^+} = 1$, the following expression is obtained:

$$[H_3O^+][OH^-] = K_e[H_2O]^2 = K_w = \frac{1}{K_{H_2O}} \qquad (7)$$

where K_w is the ion-product of water.

K_{H_2O} is a formation constant for the acid H_2O, comparable to K_{HB} but differing in that it is a product in which the concentration of water is treated as a constant.

Applying the same approach to the relative strengths of oxidants and reductants, electron transfer between members of a redox couple (Red/Ox) is compared with the transfer between the members of the reference couple H_2/H^+: $K_{red} = K_{don}$

$$\tfrac{1}{2}H_2 \rightleftharpoons H^+ + e \qquad K_{ox} = \frac{[H^+][e]}{p_{H_2}^{1/2}} = \frac{1}{K_{red}} \qquad (8)$$

The reference couple is arbitrarily assigned a constant, K_{red}, equal to unity. From the thermodynamic relationship

$$E^0_{red} = \frac{RT}{nF} \ln K^0_{red} \qquad (9)$$

it follows that the standard potential of the reference couple H_2/H^+ should be 0.0 V. Species with a greater tendency than H_2 to lose electrons will have $K_{red} < 1$ and consequently should be able to reduce H^+ to H_2 and thus have negative standard reduction potentials. Species with a smaller tendency than H_2 to lose electrons should have $K_{red} > 1$ and $E^0_{red} > 0$ V. Thus Table 2 indicates the relative strength of oxidants and reductants in terms of tendency to accept or donate electrons, with the H_2/H^+ couple as reference. At the top of the table are the best electron donors, the best reductants with the largest negative standard potentials, while the reductants poorer than H_2 have $E^0_{red} > 0$ V, and their conjugates are better oxidants than H^+.

Similarly, the relative donor strength of complexes can be measured in terms of the formation constants. The smaller the value of a formation constant, K_{ML}, the greater the tendency to lose ligands. On the other hand, a large formation constant indicates a poor donor of ligands. For example, for the system BaY^{2-}/Ba^{2+} and CaY^{2-}/Ca^{2+} we have $\log K_{BaY} = 7.8$ and $\log K_{CaY} = 10.7$; the constants show, therefore, that BaY^{2-} is a much better ligand donor than CaY, while Ca^{2+} is a much better acceptor than Ba^{2+} (Y^{4-} is used to represent the ethylenediaminetetraacetate ion). A number of formation constants of some metal chelates are tabulated in summary form in Table 3, indicating the relative strengths in terms of tendency to accept and donate Y^{4-}.

In all these cases, the magnitude of the donor formation constant indicates the relative strength of the donor and the conjugate acceptor.

POSITION OF EQUILIBRIUM

A particle-transfer reaction

$$n_2\,Don_1 + n_1\,Acc_2 \rightleftharpoons n_1\,Don_2 + n_2\,Acc_1 \qquad (10)$$

involves two conjugate pairs

$$Don_1 \rightleftharpoons n_1 p + Acc_1 \quad \text{and} \quad Don_2 \rightleftharpoons n_2 p + Acc_2 \qquad (11)$$

$$K_{don_1} = \frac{[Don_1]}{[p]^{n_1}[Acc_1]}; \quad K_{don_2} = \frac{[Don_2]}{[p]^{n_2}[Acc_2]}$$

In terms of these equilibria the overall equilibrium constant $K_e = K^{n_1}_{don_2}/K^{n_2}_{don_1}$. Hence the magnitude of the constant depends on the relative strengths of Don_2 and Don_1. With $K_{don_1} \sim K_{don_2}$ the reaction is

Table 2. Oxidants and reductants

	Redox couple	E^0_{red}, V	
increasing strength of conjugate reductant ↑	Li/Li^+	−3.045	↓ increasing strength of conjugate oxidant
	Ti^{2+}/Ti^{3+}	−0.37	
	Sn/Sn^{2+}	−0.136	
	$H_2/2H^+$	−0.000	
	$V^{3+} + H_2O/VO^{2+} + 2H^+$	+0.361	
	$2I^-/I_2$	+0.54	
	Pd/Pd^{2+}	+0.987	
	Co^{2+}/Co^{3+}	+1.84	

Table 3. Complexes of EDTA

	MY–M couple	$\log K_{MY}$	
increasing donor strength of conjugate complex ↑	LiY^{3-}/Li^{+}	2.8	↓ increasing acceptor strength of conjugate metal ions
	AgY^{3-}/Ag^{+}	7.3	
	MgY^{2-}/Mg^{2+}	8.7	
	CaY^{2-}/Ca^{2+}	10.7	
	ZnY^{2-}/Zn^{2+}	16.3	
	CuY^{2-}/Cu^{2+}	18.8	
	FeY^{-}/Fe^{3+}	25.1	

incomplete, while with $K_{don_2} \gg K_{don_1}$ the position of equilibrium lies far to the right. Thus in a particle transfer reaction the position of equilibrium favours the formation of the weaker donor and weaker acceptor.

For example, $\log K_{H_2PO_4} = pK_2 = 7.2$ and $\log K_{HPO_4} = pK_3 = 12.7$. The difference in magnitude between these constants shows that $H_2PO_4^-$ is a stronger acid and PO_4^{3-} is a stronger base, and the position of equilibrium

$$H_2PO_4^- + PO_4^{3-} \rightleftharpoons HPO_4^{2-} + HPO_4^{2-} \quad (12)$$

should lie to the right. The equilibrium constant should be $K_e = K_{HPO_4}/K_{H_2PO_4} = 10^{5.5}$.

Similar considerations show that the stronger donor BaY^{2-} will react with the stronger acceptor Ca^{2+} to be transformed into the weaker acceptor Ba^{2+} and weaker donor CaY^{2-}

$$BaY^{2-} + Ca^{2+} \rightleftharpoons CaY^{2-} + Ba^{2+}. \quad (13)$$

The same rule applied to the redox reaction

$$n_2Red_1 + n_1Ox_2 \rightleftharpoons n_1Red_2 + n_2Ox_1 \quad (14)$$

results in the equilibrium constant $K_e = K_{red_2}^{n_1}/K_{red_1}^{n_2}$. If this expression is put into logarithmic form and combined with equation (9), we obtain the relation

$$\log K_e = n_1n_2 \frac{E^0_{red_2} - E^0_{red_1}}{0.059} \quad (15)$$

Thus the position of equilibrium and the magnitude of the constant K_e depend on the relative strengths of Red_2 and Red_1. Thus the transfer of electrons will always proceed so that the stronger reductant and oxidant are converted into the weaker reductant and oxidant.

CALCULATIONS INVOLVING DONOR–ACCEPTOR CONSTANTS

Conditional constants

The application of equilibrium calculations to analytical problems is complicated and awkward since side-reactions very often interfere. But the effect of any of the interfering factors can be taken into account by appropriate coefficients.[8] Introducing this concept for use with donor–acceptor pairs we can write

$$K'_{don} = \frac{[Don']}{[p]^n[Acc']} = K_{don}\frac{\alpha_{don}}{\alpha_{acc}} \quad (16)$$

where K'_{don} is the effective or conditional donor constant, α_{don} and α_{acc} are the side-reaction coefficients, and [Don'], [Acc'] denote the total concentrations of the species, including the products of the side-reactions.

Similarly, if a reaction between two donor–acceptor pairs is accompanied by side-reactions, its conditional equilibrium constant will be

$$K_e = \frac{[Don'_2]^{n_1}[Acc'_1]^{n_2}}{[Acc'_2]^{n_1}[Don'_1]^{n_2}} = \frac{K'^{n_1}_{don_2}}{K'^{n_2}_{don_1}} \quad (17)$$

On the basis of the expressions above, the conditional constants can be calculated for different conditions. The calculation of conditional constants is illustrated in the following examples[8]

Example 1. The thermodynamic stability constant of the zinc–EDTA complex ($\log K_{ZnY} = 16.5$) is much larger than that of the calcium complex ($\log K_{CaY} = 10.7$).[8] Because of protonation of the EDTA anion and formation of hydroxo-complexes of the cations, the conditional constant will be a function of pH and at pH 9 the log K values are 15.0 for ZnY^{2-} and 9.4 for CaY^{2-}, so Zn^{2+} will displace Ca^{2+} from CaY^{2-} at this pH. If ammonia is also present, however, the formation of zinc ammine complexes decreases the conditional stability constant of ZnY^{2-} to $\log K'_{ZnY} = 7.8$ at pH 9 and $[NH_3] = 1M$, whereas that for CaY^{2-} remains at 9.4.[8] Thus under these conditions the reaction is reversed and Ca^{2+} can displace Zn^{2+} from ZnY^{2-}, the conditional equilibrium constant $K'_e = K'_{ZnY}/K'_{CaY}$ being less than 1.

Example 2. Find the relation between the conditional equilibrium constant of a redox reaction and the conditional standard potentials.

From equation (16) it follows that

$$K'_{red} = K_{red}\frac{\alpha_{red}}{\alpha_{ox}} \quad (18)$$

or in logarithmic form

$$\log K'_{red} = E^0_{red} + \frac{0.059}{n}\log\frac{\alpha_{red}}{\alpha_{ox}} \quad (19)$$

This equation can be modified to give

$$E'^0_{red} = E^0_{red} + \frac{0.059}{n}\log\frac{\alpha_{red}}{\alpha_{ox}} \quad (20)$$

where E'^0_{red} is the new conditional standard potential. Further, from the conditional equilibrium constant $K'_e = K'^{n_1}_{red_2}/K'^{n_2}_{red_1}$ it follows that

$$\log K'_e = n_1 \log K'_{red_2} - n_2 \log K'_{red_1} \quad (21)$$

or

$$\log K'_e = n_1n_2 \frac{E'^0_{red_2} - E'^0_{red_1}}{0.059} \quad (22)$$

Example 3. Is the reaction $2Fe^{3+} + 3I^- \rightleftharpoons 2Fe^{2+} + I_3^-$ practically completely masked by the addition

of enough of a soluble fluoride to give $[F^-] = 0.1M$ (pH > 3)?

The conditional constant of the reaction can be calculated from the redox potentials $E'^{\circ}_{red_1} = E'^{\circ}_{Fe(III)} = 0.41$ V and $E'^{\circ}_{red_2} = E'^{\circ}_{I^-} = 0.536$ V; $\log K'_c = 2(0.41 - 0.536)/0.059 = -4.3$. That is, about 98% will remain in the Fe(III) form.

QUANTITATIVE TREATMENT OF AQUEOUS DONOR–ACCEPTOR EQUILIBRIA

The application of the donor–acceptor concept allows a much more general and simplified treatment not only of acid–base, redox and complex equilibria, but also of titrimetric procedures.

Consider the case of a strong donor, when reaction (1) is complete so that then the following relations hold:

$$[p] = n\,C_{don} \quad \text{and} \quad [Acc] = C_{don} \tag{23}$$

where C_{don} represents the analytical concentration of the donor.

In the case of a weak donor equation (2) yields

$$-n \log [p] = \log K_{don} + \log \frac{[Acc]}{[Don]} \tag{24}$$

where [Acc], and [Don] are the equilibrium concentrations.

These equations can equally well be applied to the changes in concentration which occur during the course of acid–base, complex formation and oxidation–reduction titrations. They can also be used to deduce criteria for quantitativeness of titrations, as well as expressions for calculating the relative titration errors.

First we will illustrate their application to calculation of titration curves. A donor must of necessity be titrated with an acceptor or *vice versa*. To be suitable for use in a titrimetric procedure, Don_1 and Acc_2, *e.g.*, in equation (10), must react rapidly and the chemical equilibrium should lie to the right. If this condition is met, then in the vicinity of the equivalence point there is usually a marked change in the particle concentration in the solution. Then the log [p]-values before and after the equivalence point can be calculated from equations (23) and (24), according to the type of donor–acceptor couple and which of them is in excess.

At the equivalence point stoichiometric considerations dictate that

$$n_1[Don_1] = n_2[Acc_2]$$

and

$$n_2[Don_2] = n_1[Acc_1]. \tag{25}$$

Substitution in the equilibrium equations

$$-n_1 \log [p] = \log K_{don_1} + \log \frac{[Acc_1]}{[Don_1]} \tag{26}$$

$$-n_2 \log [p] = \log K_{don_2} + \log \frac{[Acc_2]}{[Don_2]} \tag{27}$$

gives

$$-(n_1 + n_2) \log [p] = \log K_{don_1} + \log K_{don_2} \tag{28}$$

In the titration of a strong donor, for example Don_1, substitution of equations (23) and (25) into (27) yields

$$-(n_2 + 1) \log [p] = \log K_{don_2} - \log n_1 C_{O_1} \tag{29}$$

where C_{o_1} is the analytical concentration of Don_1. By means of these relations it is possible to calculate the log [p]-values at any point on a titration curve. As an example we shall consider the titration curve for a weak acid at an analytical concentration C_0 titrated with a strong base (*e.g.*, sodium hydroxide). It is assumed that the concentration of the titrant is so great that volume changes during the titration can be neglected. The equation for the acid–base reaction may thus be written

$$HB + OH^- \rightleftharpoons H_2O + B^-. \tag{30}$$

Before the equivalence point the solution contains excess of acid, HB, and equation (24) gives

$$pH = \log K_{HB} + \log \frac{[B]}{[HB]} = \log K_{HB} + \log \frac{x}{1 - x} \tag{31}$$

where x is the degree of titration ($x = 1$ at the equivalence point).

At the equivalence point, the system is equivalent to dissolution of NaB in water, so equal amounts of HB and OH^- are present and therefore $[HB] = [OH^-]$ and $[B] \sim C_0$. So, using

$$pH = \log K_{HB} + \log \frac{C_0}{[HB]} \tag{32}$$

and

$$pH = \log K_{H_2O} + \log [OH^-] \tag{33}$$

we obtain

$$2pH = 14 + \log K_{HB} + \log C_0 \tag{34}$$

After the equivalence point there is an excess of OH^-, so that

$$[OH^-] = (x - 1)\,C_0. \tag{35}$$

According to equations (7), (31), we can write

$$pH = 14 + \log (x - 1)C_0. \tag{36}$$

Thus in a titration the pH of the solution becomes a function of the degree of titration, x, and the magnitude of K_{HB} and C_0.

Similarly, in titration of a reducing agent with an oxidizing agent the proportion of the reductant that is oxidized continually increases during the titration and equation (24) can again be used. In this case, it yields

$$-n \log [e] = \log K_{red} + \log \frac{[Ox]}{[Red]} \tag{37}$$

Consider the titration of 0.1M iron(II) sulphate with 0.1M cerium(IV) sulphate in 1M sulphuric acid. Before the equivalence point, both iron(II) and iron(III) ions are present and because $n = 1$ we have

$$\text{pe} = \log K_{\text{Fe(II)}} + \log \frac{[\text{Fe}^{3+}]}{[\text{Fe}^{2+}]}$$
$$= \log K_{\text{Fe(II)}} + \log \frac{x}{1-x} \qquad (38)$$

as the equivalence point is approached.

After the equivalence point the solution contains both cerium(III) and cerium(IV) ions and we have

$$\text{pe} = \log K_{\text{Ce(III)}} + \log \frac{x-1}{x} \qquad (39)$$

From the equilibrium equation for the reaction

$$\text{Fe}^{2+} + \text{Ce}^{4+} \rightleftharpoons \text{Ce}^{3+} + \text{Fe}^{3+}$$

it can be observed that at the equivalence point, since each Fe^{3+} produced must be accompanied by one Ce^{3+}, $[Fe^{3+}] = [Ce^{3+}]$ and $[Fe^{2+}] = [Ce^{4+}]$ and substitution of these values in the sum of the equations

$$\text{pe} = \log K_{\text{Ce(III)}} + \log \frac{[\text{Ce}^{4+}]}{[\text{Ce}^{3+}]} \qquad (40)$$

$$\text{pe} = \log K_{\text{Fe(II)}} + \log \frac{[\text{Fe}^{3+}]}{[\text{Fe}^{2+}]} \qquad (41)$$

leads to a zero value for the logarithmic term, so

$$2\text{pe} = \log K_{\text{Fe(II)}} + \log K_{\text{Ce(III)}}$$

For a general redox reaction from equations (25–27), it follows directly that

$$(n_1 + n_2)\text{pe} = \log K_{\text{red}_1} + \log K_{\text{red}_2}. \qquad (42)$$

By analogy with the preceding example, an oxidimetric titration curve will be a plot of pe *vs.* x and the shape of the curve will depend on the K_{red}-values of the pairs involved and on the number of electrons transferred for each pair. If pe is multiplied by the factor $2.3RT/nF$, the curve will show the change in potential during the titration and is known as a potentiometric titration curve.

CRITERIA OF QUANTITATIVE TITRATION

For all categories, the basic reaction in titrimetry should satisfy a number of general requirements, the most important being quantitativeness of the reaction between the two couples and an extremely fast reaction rate. Criteria for quantitative titration can be used to estimate the utility of a particular reaction.

Let us assume that a donor (or acceptor) is to be titrated to form its conjugate and that the magnitude of the error, caused by under- or over-titration, should not exceed $\pm\ \delta x$.

Thus reaction (9) will give an error not exceeding δx if

$$\left(\frac{1}{\delta x}\right)^{(n_1+n_2)} \leqslant K'_c = K'^{n_1}_{\text{don}_2}/K'^{n_2}_{\text{don}_1} \qquad (43)$$

or

$$n_1 \log K'_{\text{don}_2} - n_2 \log K'_{\text{don}_1} \geqslant (n_1 + n_2) \log \frac{1}{\delta x} \qquad (44)$$

If one of the donors is strong, e.g. Don_1, the criterion for calculation purposes becomes

$$\log K_{\text{don}_2} \geqslant 2 \log \frac{1}{\delta x} - \log C_{0_1} \qquad (45)$$

where C_{0_1} represents the analytical concentration of the strong donor.

We may generalize by stating that if these criteria are not satisfied, the resultant titration curve would have no vertical sequent: selection of a suitable visual indicator would be impossible; instrumental detection of the equivalence point would be difficult and liable to be in error.

Let us give some examples. For a weak acid reacting with a weak base, equation (44) is

$$\log K_{\text{HB}_2} - \log K_{\text{HB}_1} \geqslant 2 \log \frac{1}{\delta x} \qquad (46)$$

For titration of monochloroacetic acid ($\log K_{\text{HB}_1} = 2.9$) with ammonia ($\log K_{\text{HB}_2} = 9.2$) the difference $\log K_{\text{NH}_4^+} - \log K_{\text{ClHAc}}$ is 6.3, so δx does not exceed 0.001 and the titration reaction is therefore complete and gives an error $<0.1\%$.

For an oxidation reduction we have

$$E'^0_{\text{red}_2} - E'^0_{\text{red}_1} > \frac{n_1 + n_2}{n_1 n_2} 0.059 \log \frac{1}{\delta x} \qquad (47)$$

where $E'^0_{\text{red}_2}$ and $E'^0_{\text{red}_1}$ are the conditional standard potentials.

Thus for titration of iron(II) with cerium(IV) sulphate in 1M sulphuric acid we have $E^0_{\text{Fe(II)}} = 0.68$ V and $E^0_{\text{Ce(III)}} = 1.44$ V.

Then

$$E^0_{\text{Ce(III)}} - E^0_{\text{Fe(II)}} = 0.76 > \frac{n_1 + n_2}{n_1 n_2} 0.059 \log \frac{1}{\delta x}$$

Hence, $\log 1/\delta x < 0.76/0.188$, *i.e.*, < 6, so δx is again less than 0.001.

Further, suppose it is desired to titrate 0.5M Cu^{2+} with EDTA, using a visual indicator. What is the lowest pH at which the titration could be performed?

In this case, equation (45) is used in the form

$$\log K'_{\text{CuY}} \geqslant 2 \log \frac{1}{\delta x} - \log C_0$$

where K'_{CuY} represents the conditional formation constant of the complex of Cu^{2+} with EDTA. For $\delta x = 0.001$, $2 \log 1/\delta x - \log C_{\text{Cu}}$ will be 7.7, which means that $\log K'_{\text{CuY}}$ must be $\geqslant 7.7$. From a table of conditional constants[8] we conclude that the lowest permissible pH is 3.

Hence, in all cases the feasibility of a titration can be estimated without calculating the titration curve. The same approach can also be used to calculate the titration errors.

CONCLUSION

The aim of this paper was to show that the transfer of particles between two couples (acid–base, reductant–oxidant, complex–metal ion) obeys one and the same rule and may be described mathematically with identical equations. The practical application of this approach is limited by the tendency of some transfer reactions to proceed slowly or in steps through unstable intermediates.

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SPECTROPHOTOMETRIC STUDY OF THE INTERACTION OF SOME TRIPHENYLMETHANE DYES AND 1-CARBETHOXYPENTADECYLTRIMETHYLAMMONIUM BROMIDE

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Summary—The effect of the cationic surfactant 1-carbethoxypentadecyltrimethylammonium bromide at above and below its critical micelle concentration on the absorption spectra and the values of the apparent dissociation constant pK_2 of the dyes Phenol Red, Bromophenol Blue and Bromocresol Green has been studied, along with the influence of a strong electrolyte (NaCl) on the effects produced by the presence of surfactant micelles in the dye solution.

Recently, the utilization of the chelating properties of triphenylmethane dyes in the presence of various cationic surfactants for the spectrophotometric determination of metal ions has received a great deal of attention. The principle of these reactions, which are generally much more useful than similar reactions in the absence of surfactant, has not yet been satisfactorily elucidated.[1] Interactions between the dye and the surfactant also occur. These have now been studied with the simplest triphenylmethane dyes, Phenol Red (I), Bromophenol Blue (II) and Bromocresol Green (III) (Fig. 1), which do not have chelating properties but do have suitable structures for study of the interactions described.

The dissociation of the dyes is characterized by the pK values:

$$H_2X \overset{pK_1}{\rightleftharpoons} HX^- \overset{pK_2}{\rightleftharpoons} X^{2-}$$

The first dissociation step is that of the sulphonic acid and thus is that for a strong acid; pK_2 corresponds to dissociation of the –OH group. The pK_2 values (concentration constants) are 4.10 for (II),[2] 4.66 for (III);[2] the values of the thermodynamic constants are 8.035 for (I),[3] 4.22 for (II),[4] and 5.033 for (III).[3]

1-Carbethoxypentadecyltrimethylammonium bromide[5,6] is used as the cationic surfactant.

Kohara and co-workers[2,7] have studied the effect of the surfactant tetradecyldimethylbenzylammonium chloride (zephiramine) on Bromophenol Blue and Bromocresol Green and given pK_2 values of 2.68 for (II) and 3.95 for (III) in $5 \times 10^{-4}M$ surfactant and $0.1M$ potassium chloride medium. However, they did not study changes in the spectra over the whole surfactant concentration range, nor the effect of varying the strong electrolyte concentration. Bromophenol Blue and Bromocresol Green dyes have also been used for determining the pH-value at the micelle surface in 0.2–0.4% solutions of surfactants with various lengths of alkyl chains, which serve as model substances for general study of the individual ionized micelle layers. The effect of potassium bromide and chloride has also been studied.[8]

EXPERIMENTAL

Reagents

Solutions of 1-carbethoxypentadecyltrimethylammonium bromide (CPTB) with concentrations of 0.05, 0.03 and $0.005M$ were prepared by dissolving the appropriate amount of Septonex (Slovakofarma, Hlohovec, Czechoslovakia) in distilled water. The purity of the CPTB was in accordance with the Czechoslovak pharmacopoeia; a bromide content of 18.65% (theory 19.91%) was found by argentometric titration.

The stock $10^{-3}M$ dye solutions were prepared by dissolving the appropriate amounts of the pure substances (Lachema, Brno, Czechoslovakia) in distilled water, to which 1.5 ml of $0.1M$ sodium hydroxide had been added, and diluting to volume with distilled water. The dyes were purified by precipitation as the acid and by repeated recrystallization from benzene,[9,10] then stored in a vacuum desiccator over phosphorus pentoxide. The purity was checked by measuring the absorption spectra and by elemental analysis. The absorption spectra did not differ from

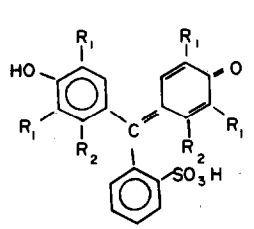

Fig. 1. Structural formula of Phenol Red (I), Bromophenol Blue (II) and Bromocresol Green (III). (I): $R_1 = H$; $R_2 = H$; (II): $R_1 = Br$, $R_2 = H$; (III): $R_1 = Br$, $R_2 = CH_3$.

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the spectra of the unpurified dyes, except for a higher molar absorptivity. For (I), $C_{19}H_{14}O_5S$ requires C 64.4%, H 4.0%, and the values found were C 63.6% and H 4.3%; for (II), $C_{19}H_{10}O_5SBr_4$ requires C 34.0%, H 1.5%, S 4.8%, Br 47.7%, and analysis gave C 33.0%, H 1.6%, S 4.6% and Br 43.4%; for (III), $C_{21}H_{14}O_5SBr_4$ requires C 36.1%, H 2.0%, S 4.4%, Br 45.8% and the results obtained were C 34.4%, H 2.1%, S 4.8: and Br 42.1%. TLC on Silutol R UV 254 plates with ethyl acetate–methanol–5*M* ammonia (6:3:1) showed the dye purities were about 95% for (I), 90% for (II) and 92% for (III); dyes (II) and (III) contained derivatives brominated to a lower degree.

All the chemicals employed were of analytical grade.

Procedure

In the study of the dye spectra, the pH of the solutions was adjusted with Britton–Robinson universal buffers; the ionic strength was adjusted to constant value with sodium chloride. In measurement of the dye dissociation constants, except for (I) (at $I = 0.015M$), the pH of the solution was adjusted by adding the calculated amount of 0.1*M* (or 0.01 or 0.001*M*) hydrochloric acid or sodium hydroxide and the ionic strength was adjusted by adding the required amount of 1*M* sodium chloride. For (I) it was necessary at low sodium chloride concentrations ($I = 0.015M$) to adjust the pH of the solution with Britton–Robinson buffer with added sodium chloride; in the absence of buffer the measurements in the pH region 5–8 were irreproducible.

The apparent dissociation constants were determined spectrophotometrically and evaluated from the absorbance as a function of pH, at the wavelengths of the absorption maxima for both forms of the indicator. The average value and the standard deviation were calculated from 8–10 values from the $A = f(\text{pH})$ plots.

RESULTS

In the study of the effect of the cationic surfactant CPTB, the absorption spectra of the dyes were measured at various pH values in the presence of various amounts of surfactant and compared with the spectra of the dyes alone. In $2 \times 10^{-5}M$ solutions of (II) and (III) in the presence of low concentrations of CPTB in the acid and alkaline regions turbidity was observed [for (II) up to $c_{CPTB} = 6 \times 10^{-5}M$, for (III) up to $c_{CPTB} = 8 \times 10^{-5}M$, 0.2*M* sodium chloride]. No turbidity was observed for (I) even at lower surfactant concentrations. At higher surfactant concentrations no turbidity is formed; its presence for all dyes in the acid region produces a shift of the absorption maximum for the HX^- form to shorter wavelengths and a decrease in the value of the apparent molar absorptivity and, in the alkaline region, a shift of the maxima to longer wavelengths and an unimportant change in the molar absorptivity. These changes occur as c_{CPTB} is increased up c_K (the critical micelle concentration at the given ionic strength)[11], after which there is no further change with increasing c_{CPTB}. Table 1 gives the values characteristic of these changes.

The changes in the spectra can be interpreted in terms of increased dissociation of the protons of the –OH groups from the HX^- dye anions. The spectra of (II) at various pH values in the presence and absence of surfactant are given in Fig. 2 as an example. The values of the mixed dissociation constants* corresponding to dissociation of the –OH groups of the dyes at various surfactant concentrations are given in Table 2.

Table 1. Effect of CPTB on the absorption maxima of the dyes ($c_{dye} = 2 \times 10^{-5}M$, $c_{CPTB} = 1 \times 10^{-3}M$)

Dye	Dye form	pH	λ_{max}*, nm	λ_{max}†, nm	$\Delta\lambda$, nm
(I)	HX^-	5.0	433	424	−9
	X^{2-}	10.5	558	572	+14
(II)	HX^-	1.8	438	428	−10
	X^{2-}	8.8	590	603	+13
(III)	HX^-	1.8	444	425	−19
	X^{2-}	8.8	615	627	+12

* For dye alone.
† For dye in presence of CPTB.

It follows from Table 2 that the sodium ions and the ions of the tetra-alkylammonium micelles compete in the formation of a solvation shell around the –OH and $-SO_3^-$ groups, *i.e.*, in deciding the degree to which the effect of c_{CPTB} is important with increasing sodium chloride concentration. The pK_2 values for (II), (III), and (I) (at $I = 0.015$) decrease with increase in surfactant concentration almost up to the critical micelle concentration[11] for the given sodium chloride concentration ($c_K = 2.4 \times 10^{-4}M$ for $c_{NaCl} = 0.015M$, $1.05 \times 10^{-4}M$ for $c_{NaCl} = 0.2M$ and $1.0 \times 10^{-4}M$ for $c_{NaCl} = 0.5M$). At c_{CPTB} above c_K the pK_2 values remain constant until $c_{CPTB} = 6 \times 10^{-3}M$, at which the pK_2 values of all the dyes increase somewhat. The dependence of pK_2 on c_{CPTB} in the concentration range 0–1 $\times 10^{-3}M$ is given in Fig. 3.

Strong electrolytes have a marked effect on the dye–surfactant system. With all the dyes the deproto-

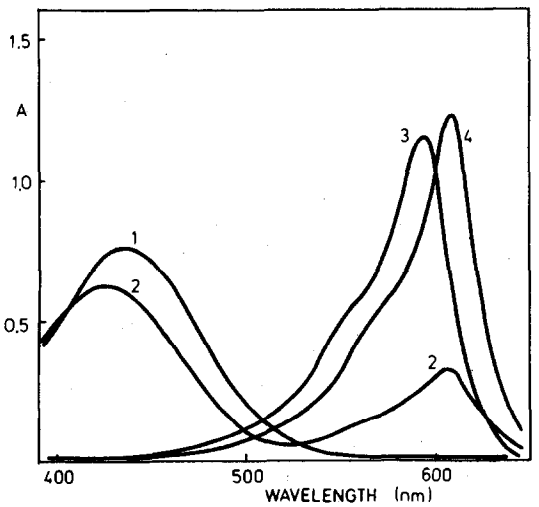

Fig. 2. The effect of CPTB on the absorption spectra of Bromophenol Blue ($c_{(II)} = 2 \times 10^{-5}M$, $c_{CPTB} = 5 \times 10^{-3}M$, $I = 0.015$). *1*: (II), pH 1.8; *2*: (II)–CPTB, pH 1.8; *3*: (II), pH 8.0; *4*: (II)–CPTB, pH 8.0.

* That is, derived from the activity of the hydrogen-ion and the concentrations of the other components.

Table 2. pK_2 values of the dyes at various values of c_{CPTB} and c_{NaCl} ($c_{dye} = 2 \times 10^{-5}M$; $20 \pm 0.5°$)

c_{CPTB}, M \ c_{NaCl}, M	0.015*	(I) 0.2	(I) 0.5	(II) 0.2	(III) 0.2
0.00	7.88 ± 0.01	7.68 ± 0.02	7.63 ± 0.03	3.89 ± 0.02	4.58 ± 0.02
8×10^{-5}	7.80 ± 0.05	7.71 ± 0.01	7.67 ± 0.02	3.37 ± 0.06	(4.35 ± 0.11)†
1×10^{-4}	7.57 ± 0.05	7.69 ± 0.02	7.72 ± 0.01	3.20 ± 0.05	4.25 ± 0.04
2×10^{-4}	7.08 ± 0.05	—	—	—	—
6×10^{-4}	6.93 ± 0.05	7.68 ± 0.02	7.88 ± 0.02	3.03 ± 0.03	4.09 ± 0.02
1×10^{-3}	6.90 ± 0.05	7.66 ± 0.03	7.91 ± 0.01	3.02 ± 0.02	4.08 ± 0.01
6×10^{-3}	7.02 ± 0.03	7.80 ± 0.02	8.00 ± 0.03	3.09 ± 0.03	4.13 ± 0.02

* Britton–Robinson buffer with a constant $I = 0.015$ value adjusted by additions of NaCl.
† Almost invisible opalescence appears in the solution.

nation caused by the presence of the surfactant is suppressed by increasing the sodium chloride concentration. This effect is most marked for Phenol Red, the weakest acid of the three,[3] $pK_2 = 8.0$ (Table 2, Fig. 3). At $c_{NaCl} = 0.2M$ the dissociation constant practically does not change except for the already mentioned increase at $c_{CPTB} = 6 \times 10^{-3}M$. At $c_{NaCl} = 0.5M$, addition of surfactant makes pK_2 higher than that for the dye alone under the given conditions. With (I) an increase in c_{NaCl} also decreases the wavelength shift of the absorbance maximum for the surfactant–X^{2-} system. For (II) and (III) the positions of the maxima for the surfactant–HX^- and surfactant–X^{2-} forms are not affected by increasing the concentration of sodium chloride.

CONCLUSIONS AND DISCUSSION

It follows from the results that the presence of cationic surfactant affects the anionic forms of the dyes studied. At surfactant concentrations much lower than the critical concentration, where the concentrations of the dye and surfactant are of the same order, ion-association species are apparently formed,[12] which can produce opalescence or turbidity of the solution, depending on the character of the dyes and the concentration ratio of the two components. It is assumed that at higher surfactant concentrations in the presence of the dye, larger submicelle aggregates are formed[13] and subsequently micelles. The dye can participate in the formation of these aggregates and micelles through its negatively charged groups ($-SO_3^-$, $-O^-$), which compensate the repulsion forces between the cationic groups of the surfactant and thus stabilize the growing micelle forms.

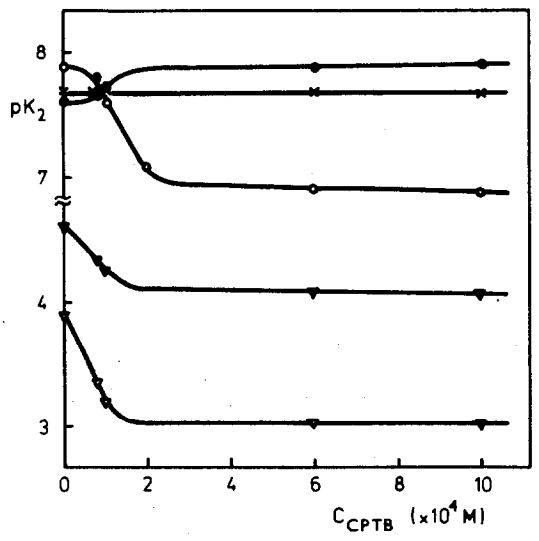

Fig. 3. The dependence of pK_2 on c_{CPTB} ($c_{dye} = 2 \times 10^{-5}M$). ▽ (II), $c_{NaCl} = 0.2M$; ▼ (III), $c_{NaCl} = 0.2M$; ○ (I), $I = 0.015$; × (I), $c_{NaCl} = 0.2M$; ● (I), $c_{NaCl} = 0.5M$.

The interaction between the dye and the surfactant affects the electronic structure of the dyes, which accounts for the hypsochromic shift in the HX^- spectrum and the bathochromic shift in the X^{2-} spectrum. In the first case, the surfactant–HX^- interaction apparently leads to changes in the charge distribution on the surface of the dye molecule; in the second the effect occurs through increased delocalization of the π-electrons of the system; the dye can also be bound to the micelles through two negatively charged sites.

The effect of CPTB on the HX^- form of the dye is analogous to that of other surfactants[2,8] in decreasing the pK_2 value. The increase in pK_2 at very high c_{CPTB} values, which appeared for all the dyes studied and for all c_{NaCl} values, cannot yet be completely explained. A dependence on the micelle structure[14] or negative absorption[8] may be involved. An increase in the concentration of the strong electrolyte in solutions containing mixed micelles of the surfactant and the dye apparently leads to a decrease in the relative permittivity around the micelle surface,[8] resulting in the decrease in the dissociation of the –OH group, caused by the surfactant.

For Phenol Red an increase in the concentration of strong electrolyte leads to suppression of the bathochromic shift and strongly affects the deprotonation of the HX^- anions. This is probably a result of the different molecular geometry and of the absence of bromine in the *ortho*-position and the consequent decrease in the acidity of the –OH group. The weaker interaction of Phenol Red with the surfactant becomes apparent even in solutions with low ionic strength where a decrease in pK_2 does occur, but only

at higher surfactant concentrations than for Bromophenol Blue and Bromocresol Green.

Because with more complicated triphenylmethane dyes (*e.g.*, Chromazurol S, Eriochrome Cyanine R, Pyrogallol Red[5,6,15,16]) in the presence of cationic surfactants (CPTB, cetylpyridinium bromide) the optimal c_{CPTB} region approaches the c_K value for the surfactant (for c_{CPTB} this is generally the region from $2 \times 10^{-4}M$ to $7 \times 10^{-4}M$), it can be assumed that the micelles play a key role in the formation of ternary metal–triphenylmethane dye–cationic surfactant complexes. At lower c_{CPTB} values, turbidity sometimes appears or the absorbance of the complex is low. At higher c_{CPTB} values the absorbance of the ternary complex again decreases, which can be explained by suggesting that the increase in the micelle concentration leads to a decrease in the dye concentration per micelle unit and thus to competition between the metal ions and the micellar dye ions.[17]

In conclusion, it is necessary to emphasize the important effect of the presence of a strong electrolyte on the formation of micelles in the ternary system; all these factors lead to the necessity of maintaining the optimal experimental conditions for obtaining the highest sensitivity in photometric determination of the metal ions when surfactants are added.[18]

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METAL CHELATES OF PHOSPHONATE-CONTAINING LIGANDS—IV

STABILITY OF SOME 1,6-HEXAMETHYLENEDIAMINE-*N*,*N*,*N*′,*N*′-TETRA(METHYLENEPHOSPHONIC) ACID METAL CHELATES

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Summary—The stability constants of the complexes formed between the anion of 1,6-hexamethylenediamine-*N*,*N*,*N*′,*N*′-tetra(methylenephosphonic) acid and some transition and non-transition metal ions have been measured potentiometrically at a temperature of 25° and an ionic strength of 0.1*M* (KNO_3). The acid dissociation constants of the ligand and stability constants of the protonated complexes are also reported. Comparisons are made with the acid dissociation constants of the analogous compounds EDTA, ENTMP and HDTA, and possible structural formulae are given.

In recent publications from this laboratory, the formation of the protonated and unprotonated chelates of ethylenediaminetetra(methylenephosphonic) acid (ENTMP) with copper, cadmium, calcium, magnesium and barium ions were described[1–3] and the corresponding equilibrium constants were calculated together with the acid dissociation constants of the ligand.

Because of the importance of this family of ligands as analytical reagents for the determination of some transition elements[1] and the unusual properties of the phosphonic group compared to the carboxylic group in inhibiting precipitation of sparingly soluble inorganic salts,[4] the chelating power of 1,6-hexamethylenediamine-*N*,*N*,*N*′,*N*′-tetra(methylenephosphonic) acid (TENTMP) has been examined.

The effect on the stability constants when the two iminodimethylenephosphonic acid groups are moved further apart by introduction of extra methylene groups is of importance in understanding the nature of chemical bonding. Similar studies have been reported by Schwarzenbach and co-workers.[5,6] They showed that moving the two iminodimethylenecarboxylic acid groups apart increases the binding of the last two protons, as revealed by the increase in the p*K* values, and that the stability constants of the ligands with a given metal ion generally decrease as the chain length increases, except for mercury(II). Anderegg[7,8] has reported a thermochemical study of this effect. Exothermic effects accompanied by a decrease in the entropies along the reaction series (especially for the smaller metal ions) were attributed respectively to reduction of the ring strain and to the considerable configurational freedom which remains in the polymethylene portion of the chelate.

In the present paper, the values of the equilibrium constants of TENTMP with manganese(II), cobalt(II), nickel, copper(II), palladium(II), cadmium and mercury(II) and the protonation constants of the free ligand and the complex species are reported.

EXPERIMENTAL

Reagents

Pure (99%) recrystallized TENTMP was used. A stock solution was prepared by dissolving a known weight in potassium hydroxide solution (4:1 molar ratio of base to acid) and diluted with doubly distilled water as required. Stock solutions of metal nitrates and chlorides were standardized with EDTA.[9] Alkalis were carbonate-free and all reagents were analytical-grade.

Potentiometric measurements

A Radiometer pH M62 digital pH-meter, fitted with a combined glass–calomel electrode was used. TENTMP, acidified with nitric acid, was titrated in the absence and presence of manganese, cobalt, nickel, copper, palladium, cadmium and mercuric ions in 1:1 molar ratio of metal to ligand. The final concentration of the ligand was approximately $1 \times 10^{-3}M$ and the volume of titrand was 50 ml. The ionic strength was kept at 0.1*M* with potassium nitrate. The activity coefficient for hydrogen-ion was taken as 0.769 at this ionic strength. A value of 13.79 was used for pK_w.

RESULTS AND DISCUSSION

Determination of the protonation constants

The titration curves are shown in Figs. 1 and 2. The curve for the free ligand is characterized by the two distinct inflections at $a = 2$ and 6, where a is the number of moles of base added per mole of TENTMP.

The protonation constants were calculated by Schwarzenbach's graphical method[5] with a small modification based on the assumption that a limited

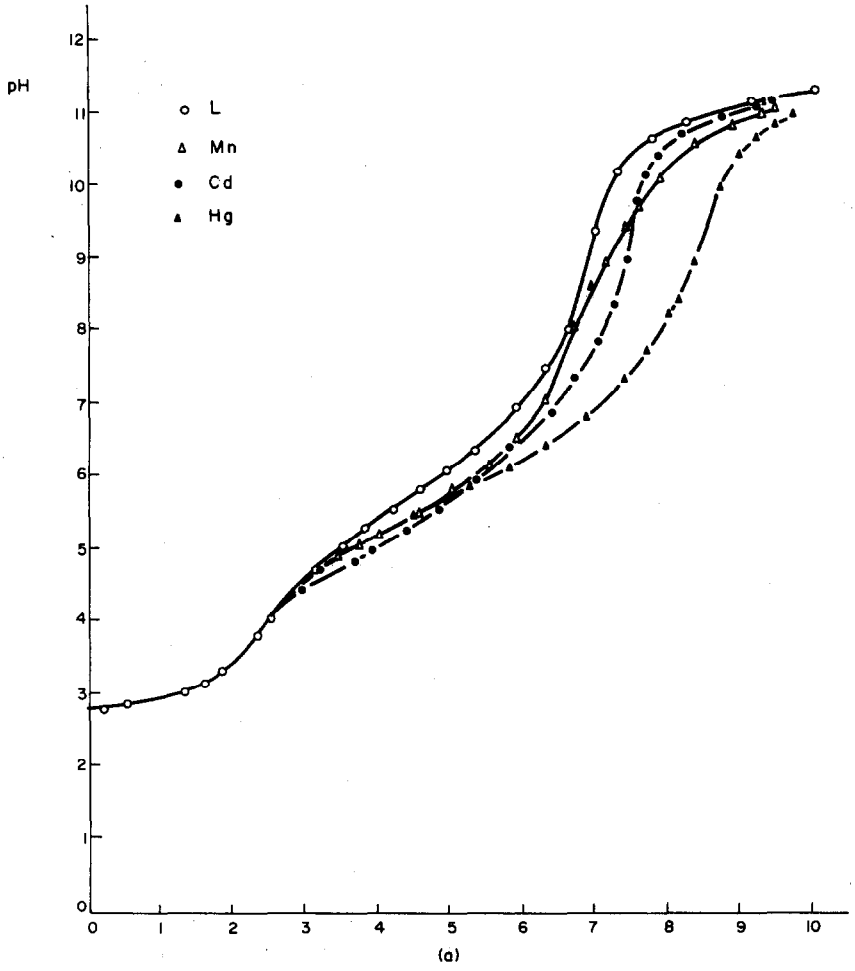


Fig. 1. Potentiometric titration curves of manganese, cadmium and mercuric ions in the presence of an equimolar amount of TENTMP (0.1*M* KNO_3, 25°).

number of protonated species is dominant within a buffer region. The details of the calculations were reported previously.[2] To check the values, the average number of protons bound to the ligand was calculated from experimentally measured quantities and the protonation constants, by means of equations (1) and (2) respectively.

$$\bar{n}_H = \frac{(8 - a)C_L + [OH] - [H]}{C_L} \quad (1)$$

and

$$\bar{n}_H = \sum_{i=1}^{i=N} \beta_i[H]^i \Big/ \sum_{i=0}^{i=N} i\beta_i[H]^i \quad (2)$$

where C_L is the total ligand concentration, β_i is the ith overall protonation constant of the ligand, and $\beta_0 = 1$.

The protonation constants were then adjusted to give the best agreement between both sets of $\bar{n}_H$ values. Table 1 summarizes the results and Table 2 lists the values of the protonation constants together with the values reported for ENTMP,[2] EDTA and HDTA (hexamethylenediaminetetra-acetic acid).[5]

Under the conditions used and probably because of the highly acidic nature of the 7th and 8th protons, it was not possible to determine β_7 and β_8 with accuracy and they are not listed here. The dissociation steps are illustrated by structures I–V in Fig. 3.

Table 1. Values of $\bar{n}_H$ calculated from equations (1) and (2)

a	$\bar{n}_H$ calculated from equation (1)	$\bar{n}_H$ calculated from equation (2)
1.85	5.46	5.53
3.14	4.83	4.78
3.48	4.51	4.51
3.82	4.17	4.20
4.58	3.42	3.39
4.95	3.04	3.00
5.33	2.67	2.67
5.86	2.13	2.11
6.28	1.72	1.74
6.62	1.38	1.39
7.26	0.98	0.98
7.74	0.95	0.95
8.23	0.89	0.91

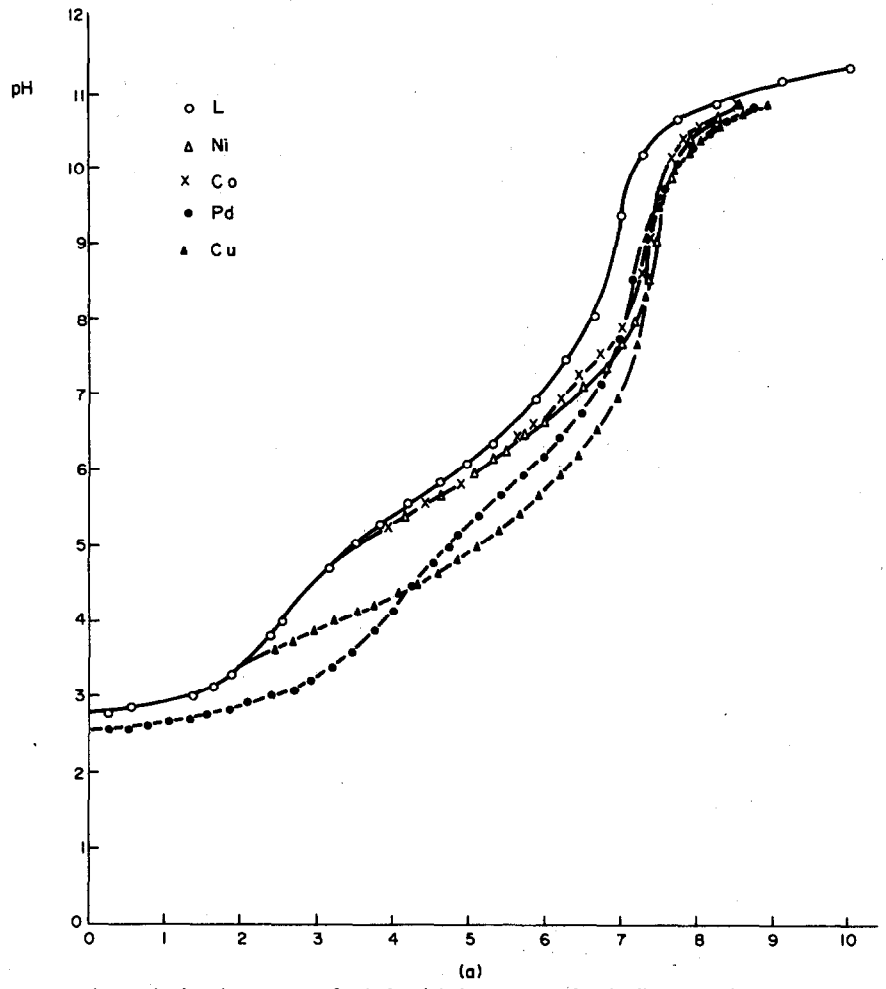
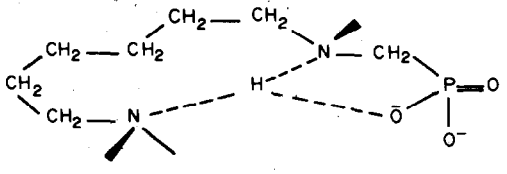


Fig. 2. Potentiometric titration curves of cobalt, nickel, copper and palladium ions in the presence of an equimolar amount of TENTMP.

A comparison between the first and second protonation constants for TENTMP, ENTMP, EDTA and HDTA shows that the difference between log K_1 and log K_2 decreases with increasing chain length in the polycarboxylate ligands whereas for the polyphosphonate ligands it increases with increasing separation distance between the two basic nitrogen atoms. It has been suggested that increasing the chain length decreases the repulsive forces between the positively charged nitrogen atoms and consequently increases their basicities. In ENTMP, the charge can be reduced through formation of hydrogen bonds between the two phosphonic groups and the imino group (structure III) which is not possible in the polycarboxylate ligands for stereochemical reasons and charge delocalization. In TENTMP, removal of one of these protons probably leads to the formation of the highly stable cyclic structure (VI):



(VI)

Table 2. Protonation constants of TENTMP, ENTMP,[2] EDTA[5] and HDTA[5]

	TENTMP	ENTMP	EDTA	HDTA
log K_1	11.82	10.60	10.26	10.65
log K_2	7.71	10.48	6.16	9.75
log K_3	6.23	9.27	2.67	2.70
log K_4	5.68	7.39	1.99	2.20
log K_5	5.12	5.63		
log K_6	3.25	3.80		

Determination of the stability constants

At relatively low pH values, all the curves for the metal–ligand mixtures exhibit a buffer region indicating the formation of protonated metal chelates. Calculation of the $\bar{n}_H$ values from equation (1) showed that tetraprotonated metal chelates are first formed, which then dissociate with increasing pH to give the unprotonated species. Interaction of the ligand with Cu^{2+} and Pd^{2+} ions is greater than with the other

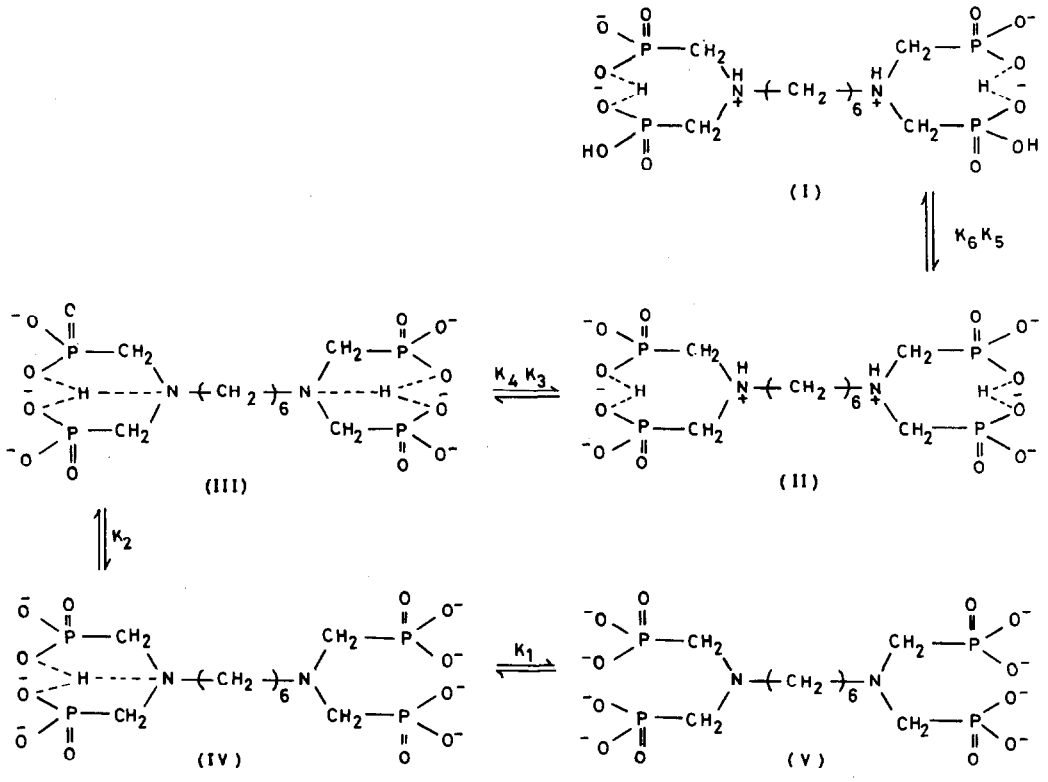


Fig. 3.

metal ions and appreciable complexation occurs even in strongly acidic medium. In the case of mercury, stable complexes are formed only at high pH. The buffer region at pH 8–10 suggests the presence of hydroxo-complex species of the type $HgL(OH)^{7-}$ since only one mole of base is consumed per mole of HgL complex. The equilibrium constant for the reaction,

$$HgL^{6-} + OH^- \rightleftharpoons HgL(OH)^{7-};$$

$$K_{HgL(OH)} = \frac{[HgL(OH)^{7-}]}{[HgL^{6-}][OH^-]} \tag{3}$$

is calculated from

$$\log K_{HgL(OH)} = \log \frac{a'C_{HgL} + [H^+] - [OH^-]}{(1-a')C_{HgL} - [H^+] + [OH^-]} - \log[OH^-] \tag{4}$$

where a' is the excess of base consumed after complete neutralization of the ligand.

From the mass balance for total metal (C_M) and total ligand (C_L) and the electroneutrality condition, the following relationships can be readily deduced:

$$C_L = A[L] + B[ML] \tag{5}$$

$$C_M = [M] + B[ML] \tag{6}$$

$$T_H = X[L] + Y[ML] \tag{7}$$

where

$$A = \sum_{i=0}^{8} [H]^i\beta_i; \quad B = \sum_{i=0}^{N} [H]^i\beta_i^H; \quad (\beta_0^H = 1)$$

$$X = \sum_{i=1}^{8} i[H]^i\beta_i; \quad Y = \sum_{i=1}^{N} i[H]^i\beta_i^H$$

and the total acid concentration

$$T_H = (8-a)C_L - [H^+] + [OH^-].$$

From equations (5–7), it can be shown that

$$K_{ML}^M = \frac{[ML]}{[M][L]} = \frac{(BX - AY)(XC_L - AT_H)}{(BT_H - YC_L)(ABT_H + BXC_M - AYC_M - BXC_L)} \tag{8}$$

As a first approximation, the values of the overall protonation constants, β_i^H for the metal chelates were obtained by numerical solution of equation (2) and fed into a FORTRAN IV program run on an ECLIPSE S/230 computer and based on the listed set of equations, to calculate the constant K_{ML}^M from different points on the titration curve. Iterative calculations were continued to get the set of β_i^H values which gave the best agreement of the K_{ML}^M values.

Table 3 summarizes the equilibrium constants determined for the different systems studied. These con-

Table 3. Formation constants of TENTMP with manganese, cobalt, nickel, copper, palladium, mercury and cadmium ions (25°, 0.1*M* KNO_3)

	$\log K^{M}_{ML}$	$\log K^{H}_{MHL}$	$\log K^{H}_{MH_2L}$	$\log K^{H}_{MH_3L}$	$\log K^{H}_{MH_4L}$	$\log K^{M}_{ML(OH)}$
Mn^{2+}	6.69	9.66	7.52	6.12	5.43	
Co^{2+}	5.90	10.18	7.26	6.28	5.59	
Ni^{2+}	5.40	9.52	7.58	6.25	5.61	
Cu^{2+}	10.08	9.05	6.10	5.70	4.73	
Pd^{2+}	10.83	9.56	6.71	5.73	4.65	
Cd^{2+}	6.88	10.00	7.13	6.07	5.28	
Hg^{2+}	9.51	7.50	6.52	5.97	5.46	4.61

stants are, in general, smaller than those for ENTMP as ligand. This can be explained in terms of the structural differences between the ligands. The structure of TENTMP is such that steric hindrance may prevent simultaneous co-ordination of all six donor groups to the metal ion. The only probable structure obtained is that in which the metal ion is co-ordinated to the two nitrogen atoms and one phosphonic group (structure VI, with M^{2+} replacing H^{+}). Attempts to measure the stability constants of the alkaline-earth complexes with TENTMP were unsuccessful (only very small pH shifts were obtained for the equimolar metal to ligand mixtures). This is in accord with the conclusions above, because of the low tendency of the alkaline earth metal ions to accept nitrogen atoms as donors.

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METAL CHELATES OF PHOSPHONATE-CONTAINING LIGANDS—V

STABILITY OF SOME 1-HYDROXYETHANE-1,1-DIPHOSPHONIC ACID METAL CHELATES

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Summary—A detailed study of the complexes formed between 1-hydroxyethane-1,1-diphosphonic acid and twelve metal ions, including the alkaline earth and transition and non-transition metal ions, is reported. The formation constants of the protonated and unprotonated complexes are measured from potentiometric data and possible structural formulae are given. The results reveal that only mononuclear (1:1) di-, mono- and unprotonated metal chelates are formed and that the general order of stability for the unprotonated complexes is Zn > Mn > Ca > Cu > Cd > Pd > Ni > Co ≃ Sr > Mg > Ag > Ba.

The importance of metal–phosphonate interactions in water desalination has long been recognized.[1] It is not surprising, therefore, that a great deal of work is devoted to understanding these interactions. Much of the work published has been restricted to the effect of phosphonate ligands on the precipitation and dissolution of sparingly soluble inorganic salts, especially those which are biologically important such as hydroxyapatite and oxalates.[2,3]

Many of the problems met in these studies are associated with the role of the phosphonate group. Some authors[1] attribute the inhibition of precipitation partly to the high tendency of this group to co-ordinate metal ions. They also correlate the magnitude of inhibition with the number of phosphonate groups in the ligand. A measure of the stability constants of these complexes should, therefore, shed some light on the problem.

In other work,[4–7] the stability constants of various phosphonic acids with some transition and non-transition metal ions were measured potentiometrically, and the chelating tendencies compared with those of the carboxylic acid analogues. Increasing the chain length between the two basic nitrogen atoms decreases the stability constants and it may be concluded that only one of the iminobis(methylenephosphonic) acid groups in hexamethylenediamine-*N*,*N*,*N*′,*N*′-tetra(methylenephosphonic) acid participates in co-ordination, while the other group remains free, possibly for steric reasons.

In the present paper, we report a detailed study of the complexes formed between 1-hydroxyethane-1,1-diphosphonic acid (HEDP) and twelve metal ions, including the alkaline-earth, transition and non-transition metal ions. Both protonated and unprotonated species are considered.

EXPERIMENTAL

Reagents

An approximately 60% (w/w) aqueous solution of 1-hydroxyethane-1,1-diphosphonic acid was used. Stock solutions were prepared by dilution and standardized by potentiometric titration.

Metal salt solutions were standardized with EDTA.[8] Alkalis were carbonate-free and all reagents analytical-grade.

Potentiometric measurements

The equilibrium constants were calculated from the data obtained by potentiometric titration at 1:2 metal:ligand molar ratio. The apparatus and general conditions were the same as in the previous work.[7]

RESULTS AND DISCUSSION

Protonation constants

The potentiometric titration curve of the free ligand is shown in Fig. 1. It is characterized by the two sharp inflections at $a = 2$ and 3 where a is the number of moles of base added per mole of HEDP.

The average number of protons bound to the ligand, $\bar{n}_H$, calculated for the points on the titration curve, are listed in Table 1. A maximum value of 2.53 was obtained in the most acidic region (*ca.* pH = 2.65 at $a = 0$). The first buffer region at $0 < a < 2$ corresponds to dissociation of the most acidic proton to yield the dibasic species H_2L^{2-} (structure II). The second buffer region in the pH range 5–8 is accompanied by the formation of the mono-protonated species ($\bar{n}_H = 1.00$ at $a = 3.00$). Further addition of base leads to complete deprotonation of the ligand to give L^{4-}.

It was inferred from the $\bar{n}_H$ values that the ligand hydroxyl group retains its proton even after the addi-

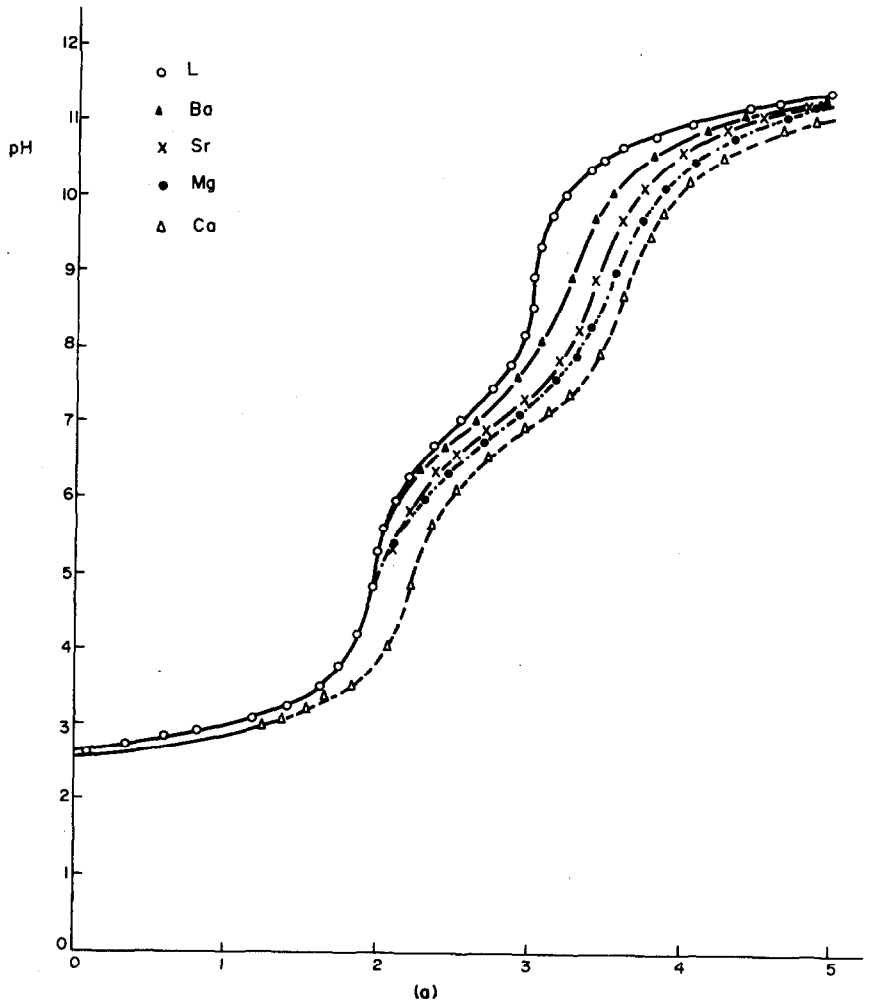


Fig. 1. Potentiometric titration curves of Mg, Ca, Sr and Ba chelates of HEDP at 25° (0.1*M* KNO_3).

tion of six equivalents of base. The different reactions and the possible structural formulae may be illustrated by the following scheme:

The calculated protonation constants of the ligand are given in Table 2, together with the values reported in the literature. Values of $\bar{n}_H$ back-calculated from

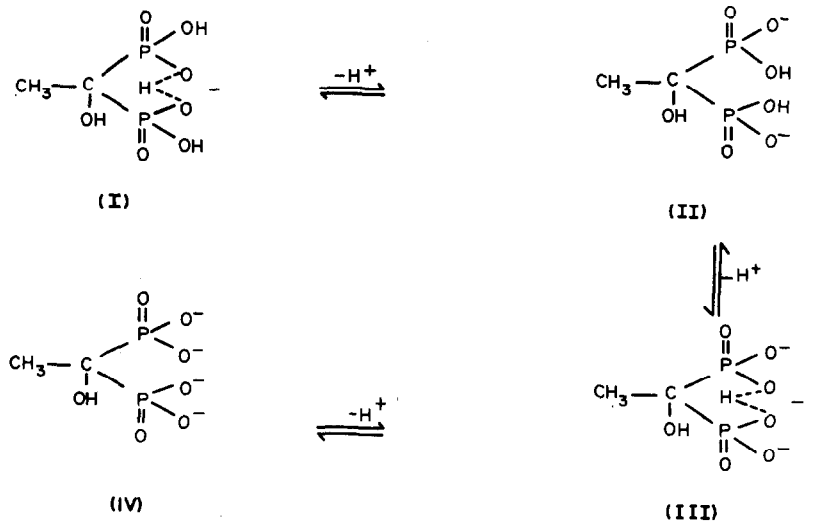


Table 1. Values of $\bar{n}_H$ calculated from the protonation constants and experimentally determined quantities

a	$\bar{n}_H$ experimental	$\bar{n}_H$ calculated
0.075	2.527	2.531
0.208	2.513	2.508
0.508	2.445	2.456
0.725	2.400	2.411
0.925	2.375	2.357
1.267	2.278	2.264
1.542	2.206	2.164
1.758	2.136	2.075
2.025	1.975	1.960
2.242	1.758	1.761
2.533	1.467	1.479
2.750	1.250	1.246
3.042	0.972	0.982
3.258	0.846	0.885
3.575	0.721	0.730
3.775	0.654	0.654
4.025	0.598	0.565

these constants are listed in Table 1 for comparison, to demonstrate the validity of the present constants. The extra stability conferred by the first proton (approximately 4 log units) may be interpreted in terms

Table 2. Protonation constants of HEDP (25°, 0.1M KNO_3)

Reaction	log K Present work	Ref. 9	Ref. 10
$H_{-1}L^{5-} + H^+ \rightleftharpoons L^{4-}$		11.13	
$L^{4-} + H^+ \rightleftharpoons HL^{3-}$	10.98	10.29	11.41
$HL^{3-} + H^+ \rightleftharpoons H_2L^{2-}$	6.87	7.28	6.97
$H_2L^{2-} + H^+ \rightleftharpoons H_3L^-$	2.59	2.47	2.54
$H_3L^- + H^+ \rightleftharpoons H_4L$		1.70	

of the formation of the hydrogen-bonded intermediate represented by structure III, which reduces considerably the repulsive forces between the two phosphonic groups.

Stability constants of the complex species

The potentiometric titration curves of HEDP in the presence of metal ions are illustrated in Figs 1–3. The shape of the curves shows clearly the different types of reactions observed, involving the formation of the simple mononuclear chelates, ML, and the various protonated species.

Estimates of the $\bar{n}_H$ values for the various metal–

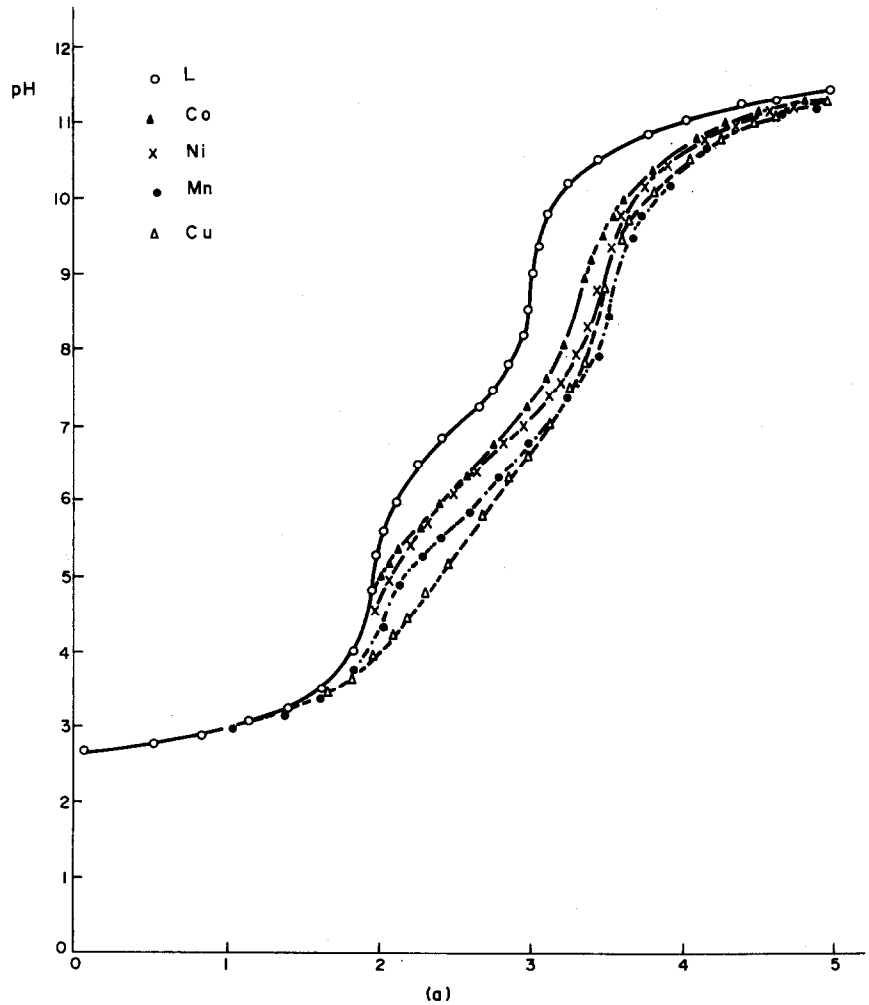


Fig. 2. Potentiometric titration curves of Mn(II), Co(II), Ni and Cu(II) chelates of HEDP.

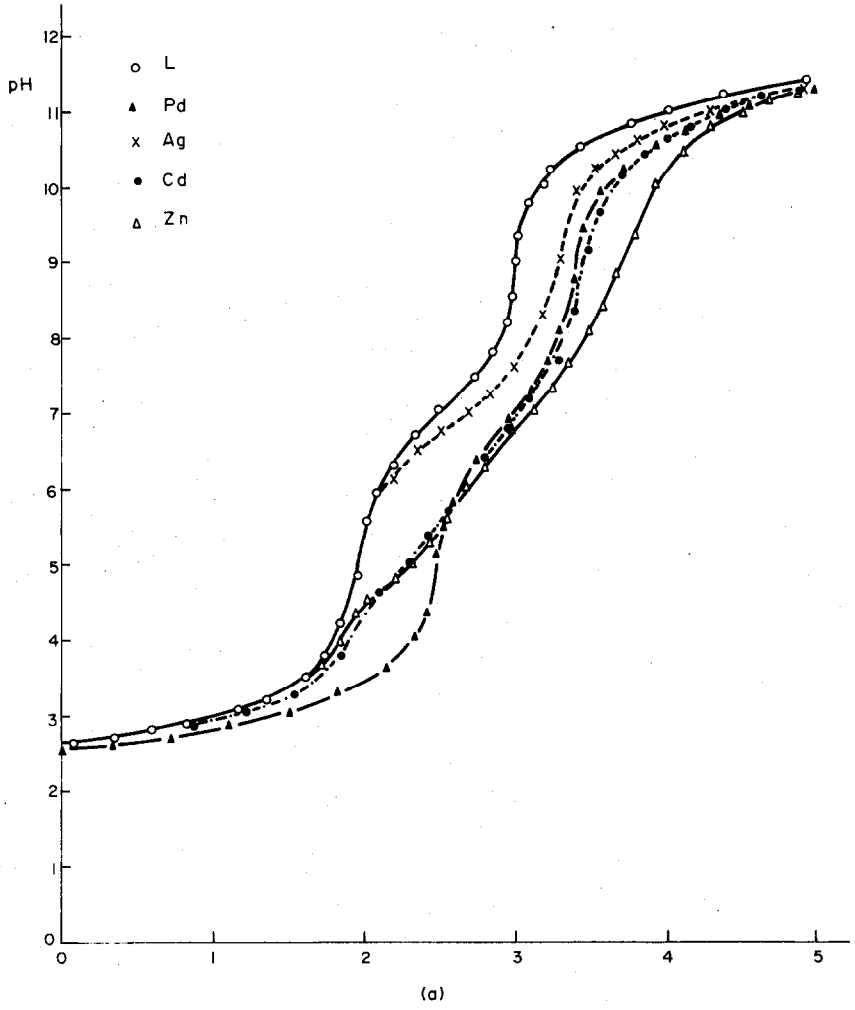


Fig. 3. Potentiometric titration curves of Ag, Pd(II), Zn and Cd chelates of HEDP.

ligand systems reveal that formation of the diprotonated complex species begins with the displacement of one proton from the ligand:

$$M^{2+} + H_3L^- \rightleftharpoons MH_2L + H^+$$

Further addition of base results in the successive dissociation of the remaining two protons to give the monoprotonated and unprotonated complex species, as indicated by the presence of two buffer regions in each case, and supported by the calculated $\bar{n}_H$ values. Although the hydroxyl group oxygen atom is stereochemically available for co-ordination with the metal ions, no evidence was gained from $\bar{n}_H$ calculations to account for the formation of $MH_{-1}L$ species. However, the hydroxyl group might participate in co-ordination, with retention of its proton. This could occur partly because of the negative charge accumulation exerted upon the loss of this proton, in addition to the high basicity of the oxygen atom. Analysis of the equilibrium data obtained from the potentiometric curves for tervalent aluminium, iron and gold ions[11] provides support for this conclusion. The data definitely show the presence of $M(H_{-1}L)_n$ species.

The values of log $K^M_{MH_nL}$ for the general reaction

$$M^{z+} + H_nL^{n-4} \rightleftharpoons MH_nL^{n+z-4};$$

$$K^M_{MH_nL} = \frac{[MH_nL]}{[M][H_nL]}$$

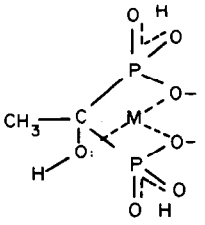
were calculated as described previously[7] and are listed in Table 3.

Table 3. Formation constants of HEDP–metal complexes (25°, 0.1*M* KNO_3)

	log $K^M_{MH_nL}$		
	$n = 0$	$n = 1$	$n = 2$
Mg^{2+}	4.49	3.31	1.39
Ca^{2+}	6.48	4.20	2.63
Sr^{2+}	4.80	3.18	1.52
Ba^{2+}	3.35	2.72	
Mn^{2+}	6.94	4.42	3.18
Co^{2+}	4.83	3.53	2.78
Ni^{2+}	5.64	3.80	3.01
Cu^{2+}	6.38	4.45	2.84
Zn^{2+}	7.36	4.51	3.10
Cd^{2+}	5.98	4.33	3.04
Pd^{2+}	5.74	4.44	2.41
Ag^+	4.17	3.39	3.13

It can be concluded from the data obtained that the behaviour of HEDP as a chelating ligand is invariably different from that reported for ethylenediamine-*N*,*N*,*N*′,*N*′-tetra(methylene-phosphonic) acid (ENTMP) and TENTMP and is more likely to be similar to that reported for ortho-, pyro-, and tripolyphosphate.[12]

The possible arrangement of the ligand donor groups in MH_nL (n = 0, 1 or 2) chelates may be illustrated by structure (V):



(V)

The metal ion is probably located between the two negatively charged phosphonate oxygen atoms and the hydroxyl group oxygen atom in approximately a tetrahedral position, with water acting as the fourth ligand. In electrostatic terms, it would be expected that the smaller the size of the metal ion, the greater the stability of the complex species. This concept holds fairly well for the triad calcium, strontium and barium. The low value of K_{ML}^{M} for magnesium may be satisfactorily explained on the basis that its small ionic radius leads to crowding of the donor groups on the surface of the metal ion, which in turn, increases the steric and repulsive coulombic forces involved. This explanation finds further support with ligands of high negative charges. Thus, in the case of ENTMP–alkaline earth metal chelates, the stability constants were found to decrease with ionic radius of the metal ion.[4]

The relatively low stabilities of the cobalt and nickel chelates compared to those of the manganese and zinc complexes can be rationalized in terms of the high stability of the tetrahedral arrangement relative to the octahedral or square-planar structure. In the case of copper, tetragonal distortion decreases the repulsive forces between the phosphonate groups and accounts for the stability being higher than in the case of cobalt and nickel complexes.

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DETERMINATION OF COMPONENT CONCENTRATIONS IN MIXTURES OF WEAK AND STRONG ACIDS AND BASES BY LINEAR ALGEBRAIC METHODS

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Summary—A general expression for transforming potentiometric titration curves of mixtures of weak acids into a system of linear equations is derived. The solution of the linear equations gives directly the concentrations of the components. This linear transformation method is illustrated by the analysis of mixtures of weak acids with overlapping dissociation equilibria. The possible presence of a strong acid or strong base in the mixture can also be detected and its concentration simultaneously determined. The method can also be used for analysis of an ampholyte and solutions containing a weak acid and its conjugate base. For example a mixture of hydroxyacetic acid (pK ~ 3.6), acetic acid (pK ~ 4.6) and hydroxylamine hydrochloride (pK ~ 6) was analysed in the presence of strong acid with an average relative error of ~2%.

The determination of concentrations of the individual components in mixtures of several acids is quite commonly required in practical analytical work in industrial, research and clinical laboratories. It is not an easy task to determine the concentrations of acids with overlapping dissociation equilibria, so chromatographic methods are often employed. In such methods, signals related to the amounts of the components are measured by detectors, and a calibration curve is needed. In titrimetry, however, the amount of substance is measured directly and the accuracy is far better than in methods employing calibration plots. Potentiometric acid–base titration is the most common and straightforward method for the analysis of mixtures of acids. Several different mathematical and graphical methods for evaluation of titration data have been given.[1–14] These methods have been summarized and discussed in a separate work[15] where a graphical method for the determination of a weak acid and its conjugate base in the same solution was also described. Midgley and McCallum have described a method for the analysis of solutions containing an acid and a weak base[16] and a weak and a strong acid.[17] Persson *et al.*[18] derived a general equation to describe the titration of a mixture of acids in the presence of weak bases. In their method only a rough calibration of the electrode system is needed and in some cases approximate values for the stability constants are found to be sufficient. Bos[19] applied a multiparametric curve-fitting method to the potentiometric data from titration of mixtures of a strong acid and a weak acid or a weak base. Seymour *et al.*[20] analysed mixtures of weak acids by linearizing the potentiometric titration curve. Different linear sections were obtained for each of the acids in the resulting plot. Johansson and Johansson[21] described a computational procedure to be used for potentiometric data from titration of complex mixtures of acids. Arena *et al.*[22] described a non-linear least-squares computer program for the refinement of all the parameters involved in potentiometric acid–base titrations. They were able to determine the concentrations of the components even in rather complicated mixtures. There are also methods for determination of small amounts of acidic and basic impurities in acid–base solutions.[9,10,23–27]

It has been shown that the potentiometric (pH) titration curves of mixtures of weak and strong acids and bases can be transformed into linear forms.[7,12,13,15,18,28] The titration curve for binary mixtures of weak acids may be transformed into a system of linear equations with two unknowns,[12,13,15] which are the equivalence volumes of the strong base consumed for the neutralization of the individual acids. A graphical method was also suggested for solution of these equations.

The aim of the present work is to generalize the considerations given in the preceding papers,[12,13,15] to provide a computer program to solve the generalized equations, and to illustrate the applicability of the method for the determination of concentrations of components in mixtures of weak acids.

THEORY

In the titration of a mixture of weak and strong acids the total, titratable hydrogen-ion concentration, T_H, at each experimental point can be expressed in two different ways.

(*i*) In terms of the analytical concentrations of the weak and strong acids, and the concentration of

strong base added to the solution:

$$T_{k,H} = \sum_{i=1}^{n} N_i T_{k,i} + [SA]_k - [SB]_k \quad (1)$$

where

$T_{k,H}$ = total titratable hydrogen-ion concentration
n = number of weak acids
$T_{k,i}$ = concentration of the ith weak acid
N_i = total number of dissociable protons of the ith weak acid
$[SA]_k$ = concentration of the strong acid
$[SB]_k$ = concentration of strong base added to the solution

The subscript k denotes that the quantity refers to the kth experimental point.

In the titration, T_i and [SA] are changed only by dilution. If the initial volume of the solution is V_0 and the volume of added strong base is v_k, equation (1) may be rewritten as

$$T_{k,H} = \sum_{i=1}^{n} N_i \frac{V_0}{V_0 + v_k} T_{i,0} + \frac{V_0}{V_0 + v_k}[SA]_0 - \frac{C_B v_k}{V_0 + v_k} \quad (2)$$

where the concentration of the strong base (the titrant) is denoted by C_B. The subscript 0 refers to the quantities at the beginning of the titration, *i.e.*, $k = 0$.

(*ii*) If there is no interaction between the differently protonated forms of one acid or between the different acids, *i.e.*, only mononuclear stepwise protonation takes place, then the sum of the bound and free hydrogen-ion concentrations can be expressed by the equation:

$$T_{k,H} = \sum_{i=1}^{n} \bar{n}_{k,i} \frac{V_0}{V_0 + v_k} T_{0,i} + [H^+]_k - \frac{K_w}{[H^+]_k} \quad (3)$$

where

$\bar{n}_{k,i}$ = average number of protons bound to the conjugate base of the ith weak acid
K_w = ionic product of water

Thus, when stepwise dissociation is assumed, $\bar{n}_{k,i}$ is determined by the hydrogen-ion concentration and the protonation constants of the conjugate base of the weak acid, $\beta_{j,i}$:

$$\bar{n}_{k,i} = \frac{\sum_{j=0}^{N_i} j\beta_{j,i}[H^+]_k^j}{\sum_{j=0}^{N_i} \beta_{j,i}[H^+]_k^j} \quad (4)$$

where

$$\beta_{j,i} = \frac{[H_jA]_i}{[A]_i[H^+]^j} = \prod_{m=N_i-j}^{N_i} \frac{1}{K_{m,i}} \quad (5)$$

$\beta_{0,i} = 1$
$K_{m,i}$ = mth dissociation constant of the ith weak acid

Combination of equations (2), (3) and (4) gives:

$$\sum_{i=1}^{n} \frac{\sum_{j=0}^{N_i} (N_i - j)\beta_{j,i}[H^+]_k^j}{\sum_{j=0}^{N_i} \beta_{j,i}[H^+]_k^j} T_{0,i} + [SA]_0 = C_B \frac{v_k}{V_0} + \frac{V_0 + v_k}{V_0}\left([H^+]_k - \frac{K_w}{[H^+]_k}\right) \quad (6)$$

Equation (6) can be written for each experimental point and if p points are taken, the following system of equations is obtained:

$$a_{1,1} T_{0,1} + a_{1,2} T_{0,2} + \ldots + a_{1,n} T_{0,n} + [SA]_0 = b_1$$
$$a_{2,1} T_{0,1} + a_{2,2} T_{0,2} + \ldots + a_{2,n} T_{0,n} + [SA]_0 = b_2$$
$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$
$$a_{p,1} T_{0,1} + a_{p,2} T_{0,2} + \ldots + a_{p,n} T_{0,n} + [SA]_0 = b_p$$

These equations are linear in $T_{0,i}$ and $[SA]_0$, so the following matrix expression can be written

$$\begin{bmatrix} a_{1,1}\, a_{1,2} \ldots a_{1,n} & 1 \\ a_{2,1}\, a_{2,2} \ldots a_{2,n} & 1 \\ \ldots\ldots\ldots & \\ a_{p,1}\, a_{p,2} \ldots a_{p,n} & 1 \end{bmatrix} \begin{bmatrix} T_{0,1} \\ \ldots \\ T_{0,n} \\ [SA] \end{bmatrix} = \begin{bmatrix} b_1 \\ b_2 \\ \ldots \\ b_p \end{bmatrix}$$

or in general:

$$\mathbf{A}.X = B \quad (7)$$

where the elements in the matrix **A** and in the vectors X and B are:

$$a_{k,i} = \frac{\sum_{j=0}^{N} (N_i - j)\beta_{j,i}[H^+]_k^j}{\sum_{j=0}^{N_i} \beta_{j,i}[H^+]_k^j}; \quad a_{k,n+1} = 1$$

$$x_i = T_{0,i}; \quad x_{n+1} = [SA]_0 \quad (8)$$

$$b_k = C_B \frac{v_k}{V_0} + \frac{V_0 + v_k}{V_0}\left([H^+]_k - \frac{K_w}{[H^+]_k}\right)$$

There is a straightforward method for solution of this matrix equation only if the number of experimental points is the same as the number of acids, *i.e.*, $p = n + 1$. In general, however, many more experimental points are taken, so it is more appropriate to use a least-squares treatment which involves the solution of the equation

$$(\mathbf{A}^T.\mathbf{A}).X = \mathbf{A}^T.B \quad (9)$$

where $\mathbf{A}^T$ is the transpose of **A**. Equation (9) can be solved by standard methods for the X-vector and the standard deviation, σx, of its components:[29]

$$X = (\mathbf{A}^T.\mathbf{A})^{-1}(\mathbf{A}^T.B)$$

$$\sigma x_i = \sqrt{\frac{s}{p - (n+1)}}\sqrt{q_{i,i}} \quad (10)$$

where

$$s = \sum_{k=1}^{p} (b_k^{exp} - b_k^{calc})^2$$

and $q_{i,i}$ = i,ith element of the matrix $(\mathbf{A}^T.\mathbf{A})^{-1}$

The values of b_k^{exp} are obtained with equation (8) and b_k^{calc} is calculated by equation (7) after the x_i-values are obtained from equation (10).

This approach for calculation of the concentrations is crucially dependent on the $\beta_{j,i}$ values. Thus it is essential to know these values under the same conditions of ionic strength, temperature, etc., used in titration of the samples. It is recommended that the $\beta_{j,i}$ or $K_{m,i}$ values for all of the components in the sample are redetermined by potentiometric titrations. These titrations may be regarded as calibration titrations, since the concentrations of the acids are known. The values of β_j obtained can be used in all future analyses for the acids. The unknown β values are evaluated from equation (6), with $n = 1$. This equation can be linearized for the β values by appropriate rearrangements:

$$\sum_{j=1}^{N} (j - \bar{n}_{k,H})[H^+]_k^j \beta_j = \bar{n}_{k,H} \qquad (11)$$

where

$$\bar{n}_{k,H} = \frac{NT_0 + [SA]_0 - C_B \frac{v_k}{V_0} - \frac{V_0 + v_k}{V_0}\left([H^+]_k - \frac{K_w}{[H^+]_k}\right)}{T_0}$$

This equation may also be solved as discussed above. The β_j values given by this linearization, however, are not always acceptable without further refinement, because they are *not* linear functions of the base added. The linearization distorts the measured experimental data differently in different parts of the titration curve. The $\bar{n}_{k,H}$ values, however, apart from those in the highly acidic and basic ranges of the titration curve, are linearly related to the amount of base added. Thus a non-linear Newton–Raphson refinement to minimize the function

$$u = \sum_{k=1}^{P} \left(\bar{n}_{k,H} - \frac{\sum_{j=1}^{N} j\beta_j[H^+]_k^j}{\sum_{j=0}^{N} \beta_j[H^+]_k^j} \right)^2 \qquad (12)$$

is also included in the program. Starting values for the Newton–Raphson refinement are found by solving equation (11). The problem of non-linearity does not arise when $T_{0,i}$ and $[SA]_0$ are calculated with equation (10) since these parameters are strictly linear functions of the amount of base added.

EXPERIMENTAL

Apparatus

All potentials were measured with a potentiometer based on a Newport digital voltmeter, resolution 0.01 mV. The meter was tested thoroughly and showed good long-term stability. A Metrohm glass electrode was used as the indicator electrode and a Beckman saturated calomel electrode and an Orion double-junction reference electrode were used as reference electrodes. The slope of the glass electrode response was determined several times and found to be near the theoretical value of 59.16 mV/pH at 25°. This value was used in the calculations. Titrations were performed in a titration vessel maintained at 25 ± 0.1°. A magnetic stirrer was used to mix the solution. The solutions were measured from calibrated Metrohm piston burettes (accurate to 5 μl).

Reagents

Stock solutions of hydroxyacetic, (+)-tartaric, succinic and citric acids and hydroxylamine hydrochloride (hydroxylammonium ion) were prepared from Merck reagents of analytical-reagent grade. Doubly distilled water was used throughout. The standard hydrochloric acid used in the E'_0-titrations and the standard potassium hydroxide solution used as the titrant were prepared by dilution from Merck ampoules. The titration medium in all cases was a 0.1*M* solution of Merck analytical-reagent grade potassium nitrate. All stock solutions and the standard hydrochloric acid and potassium hydroxide were prepared in 0.1*M* potassium nitrate, in order to keep a constant ionic medium. All the solutions were standardized by normal analytical methods. The concentration of the standard base was 0.04958*M*.

Procedure

Nitrogen was passed through the solution for 15 min before the titration, and continuously during the titration. The standard potential of the potentiometric cell was measured by doing an E'_0-titration[8] of 100 ml of hydrochloric acid of known concentration in 0.1*M* potassium nitrate. After the E'_0-titration was completed the sample was added and the titration continued. The electrodes were kept in the solution all the time. The standard base was added in increments of 0.5 or 1.0 ml. The potentials were measured after each addition of base and $-\log[H^+]$ values were calculated from the Nernst equation. The glass electrode may also be calibrated in the usual way with standard buffer solutions, but the dissociation constants then determined are mixed constants (activity of hydrogen ion is used instead of concentration as in this work).

A computer program to do the calculations has been written in both FORTRAN IV and BASIC version 4.00. The first input parameter decides whether the task is the evaluation of data from a calibration titration, *i.e.*, calculation of β values, or determination of the concentrations in a sample. The data input depends on the actual task.

The main program sets up the elements of the matrix A and vector *B*. A subroutine is used to solve the matrix equations for *X*, *i.e.*, for the concentrations of the acids or the unknown β values. One input parameter decides whether a strong acid or base is assumed to be present or not. Calculations are first done without assuming the presence of strong acid or base. If the deviation in the least-squares treatment of the data is much higher than the conceivable experimental error, the calculations are repeated with assumption of the presence of strong acid or base. The output also depends on the actual task. The program is available from the authors upon request.

RESULTS AND DISCUSSIONS

The proposed method was tested with data from the titration of different mixtures of hydroxyacetic acid (OHAc), acetic acid (HAc), hydroxylamine hydrochloride (NH_3OH^+), succinic acid (H_2Su), tartaric acid (H_2Tr) and citric acid (H_3Ci). One of the mixtures contained an excess of strong acid and one an excess of strong base. The method is also useful in the titration of ampholytes and of solutions containing a weak acid and its conjugate base. These cases were

Table 1. Titration of various mixtures of hydroxyacetic acid (OHAc), acetic acid (HAc), acetate (Ac^-), hydroxylamine hydrochloride (NH_3OH^+), tartaric acid (H_2Tr), hydrogen tartrate (HTr^-) succinic acid (H_2Su), citric acid (H_3Ci), excess of strong acid (SA) and excess of strong base (SB); temperature = 25° and $\mu = 0.1M$ (KNO_3)

	Mixture	pK_1	pK_2	pK_3	Number of points	Concentration, $10^{-3}M$ Taken	Found	s.d.	Error %
I	OHAc	3.639			15	1.264	1.275	0.004	+0.9
	HAc	4.569				1.430	1.469	0.008	+2.2
	NH_3OH^+	5.993				0.877	0.876	0.007	−0.1
II	OHAc	3.639			25	1.101	1.080	0.021	−1.9
	HAc	4.569				1.073	1.124	0.019	+4.8
	NH_3OH^+	5.993				1.221	1.220	0.012	−0.1
	SA					0.627	0.644	0.009	+2.7
III	H_2Tr	2.847	3.390		16	0.877	0.891	0.007	+1.6
	HAc	4.569				1.735	1.671	0.008	−3.7
	SB					0.437	0.428	0.009	−2.0
IV	H_2Su	4.002	5.231		23	1.544	1.465	0.033	−5.1
	HAc	4.569				2.008	2.112	0.063	+5.2
V	H_2Su	4.002	5.231		27	1.335	1.334	0.004	−0.1
	H_3Ci	2.924	4.346	5.690		0.993	0.985	0.003	−0.8
VI	H_2Tr	2.847	3.930		27	1.299	1.289	0.004	−0.8
	HAc	4.569				2.264	2.253	0.014	−0.5
	NH_3OH^+	5.993				1.301	1.327	0.017	+2.0
VII	HTr^-	2.847	3.930		8	2.120	2.115	0.003	−0.4
VIII	HAc	4.569			4	1.092	1.088	0.004	−0.4
	Ac					1.702	1.688	0.003	−0.8

tested by titration of potassium hydrogen tartrate (HTr^-) and a mixture of acetic acid and sodium acetate (Ac^-). The presence of strong base initially in the solution was indicated by the negative sign of [SA] in the print-out. In the case of a weak acid and its conjugate base, the print-out gives the sum of the concentrations of the acid and base, and the conjugate base alone is given as the concentration of strong base. The concentration of ampholyte is also given as the concentration of strong base. The acids used were chosen to give a complex mixture with overlapping dissociation equilibria. The values of the dissociation constants of the acids were determined in pure solution and with known concentrations of the acids. The dissociation constants of the acids and the results of the analyses are given in Table 1, from which it can be seen that the average error is 1–2% which can be regarded as quite satisfactory considering the complexity of the mixtures. In titration II, there are four different protonating species and in titration V, five overlapping dissociation steps. In most of the titrations many experimental points are used because the titrant base was added in 0.5-ml amounts and all the points were used in the calculations. The number of points can be reduced (*e.g.*, to 10) without losing much accuracy. It is important, however, to have experimental points in the dissociation ranges of all the acids, *i.e.*, in the pH range $pK \pm \sim 0.5$ for all of the dissociation steps. In titration VIII even four points give a good result, but in this case the statistical assessment of the data is questionable.

In this method, the total equivalence point is not needed in the calculations. This can be regarded as an advantage, because in the case of weak acids the overall inflection point of the titration curve might be difficult to determine, owing to the small pH change at the total equivalence point. Even in cases where the inflection point should be very distinct it might be distorted by the presence of small amounts of impurities, such as carbonate in the titrant. If the equivalence point of the whole titration can be determined accurately it naturally gives additional and valuable information on the concentrations of the acids and can be used as a constraint in the calculations.[3,7]

The matrix treatment of the unknown concentrations may hide the chemical principles of the procedure. It should be emphasized, however, that the basis of the method is the difference between the protonation behaviour of the acids. The protonation ranges of the acids in this work are shown in Fig. 1, where the $\bar{n}/N$ values for the conjugate bases of the weak acids are plotted against pH. The difference between the protonation pH ranges, in the case of two monobasic acids, is simply the difference between

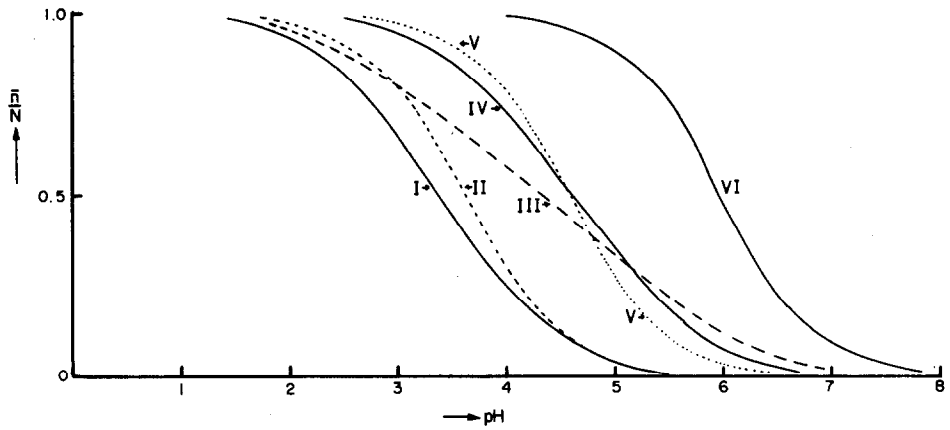

Fig. 1. $\bar{n}/N = f(\text{pH})$ curves for the acids studied; I = tartaric acid, II = hydroxyacetic acid, III = citric acid, IV = succinic acid, V = acetic acid, VI = NH_3OH^+.

their dissociation constants, *i.e.*, ΔpK. Small values of ΔpK indicate overlapping dissociation equilibria; in a binary mixture of weak acids, if $\Delta pK \geq 4$, two well-separated pH jumps can be obtained in the titration curve. In the matrix representation the two distinct dissociation steps mean that the linear system of equations, $(\mathbf{A}^T.\mathbf{A}).X = \mathbf{A}^T.B$, can be divided into two subsystems which are almost independent of each other. The degree of interdependence of the equations increases with decreasing ΔpK, which also results in the increase of the standard deviation of the calculated x_i values.

With polybasic acids, the comparison of pK values is not straightforward. In such cases the whole of the curves of $\bar{n}/N$ *vs.* pH should be compared. The larger the difference between these curves the more accurate are the results that may be expected. The difference of the $\bar{n}/N = f(\text{pH})$ curves of two polybasic acids may be represented by the area between the curves. This area may be transformed into an "apparent ΔpK", which is defined as the ΔpK which would give the same area between the curves if two monobasic acids were present.

Figure 1 shows that the area between the protonation curves of succinic and acetic acids is very small. In terms of "apparent ΔpK", it is only about 0.2–0.3. The high degree of overlapping dissociation of these acids explains the larger errors in the individual concentrations than in the other titrations given in Table 1. The total amount of the acids, however, is determined with good accuracy.

CONCLUSIONS

This study indicates that by presenting the titration as sets of linear equations, quite complex mixtures of acids can be analysed. Any excess of strong acid or base can also be determined. The apparent presence of strong base indicates the concentration of the conjugate base when a single acid is present, and when an ampholyte is present, the concentration of the ampholyte. When mixtures of acids are analysed the concentration of strong base gives only the total amount of conjugate bases, or the degree of titration.

The method of linear equations can be extended to other types of titrations. Work on such applications is presently in progress.

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IMPROVED SEPARATION OF IRON FROM COPPER AND OTHER ELEMENTS BY ANION-EXCHANGE CHROMATOGRAPHY ON A 4% CROSS-LINKED RESIN WITH HIGH CONCENTRATIONS OF HYDROCHLORIC ACID

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Summary—Iron(III) can be separated from copper(II) and many other elements by eluting these from a column of AG1-X4 anion-exchange resin with 8*M* hydrochloric acid, while iron(III) is retained and can be eluted with 0.1*M* hydrochloric acid. The separation is much better than the customary one with 3.5*M* hydrochloric acid. Columns containing only 8.8 ml (3 g) of resin can separate traces or up to more than 1 mmole of iron(III) from more than 1 g of copper. Mn(II), Ni, Al, Mg and Ca are quantitatively eluted together with copper(II). Lead, the alkali metals, Be, Sr, Ba, Ra, Sc, Y and the lanthanides, Ti(IV), Zr, Hf, Th and Cr(III) have not been investigated in detail but should be separated according to their known distribution coefficients. Separations are sharp and quantitative, less than 1 μg of copper remaining in the iron fraction when more than 1 g was present originally. Relevant elution curves and results of the quantitative analysis of synthetic mixtures are presented.

Iron(III) can be separated from copper(II), cobalt(II) and many other species with lesser tendency towards chloride-complex formation, by anion-exchange chromatography in hydrochloric acid on a strongly basic anion-exchange resin such as Dowex 1, AG1 or Amberlite IRA-400. The conditions for the separation from copper(II) are critical because this element is complexed by chloride almost as strongly as iron(III). Kraus *et al.*[1] in their original work eluted copper(II) with 2.5*M* hydrochloric acid while iron(III) was retained and then eluted with 0.5*M* hydrochloric acid. Unfortunately the original paper gives only an elution curve. No quantitative work seems to have been carried out. Schrenk *et al.*[2,3] also eluted copper(II) with 2.5*M* hydrochloric acid and apparently encountered difficulties even when separating only microgram amounts of copper(II) and iron(III). A column 25 cm in length was required for complete separation while a column 15 cm in length was found to be unsatisfactory. Because the copper(II)–iron(III) separation is so marginal and presents problems, Wilkins *et al.*[4] preferred to remove copper electrolytically and eluted cobalt(II) with 4*M* hydrochloric acid while iron(III) was retained, when analysing Alnico alloys. Gerdes *et al.*[5] have used 3.4*M* hydrochloric acid to elute copper(I), but they employed only 20-mg amounts of a brass sample which contained microgram amounts of iron(III). De *et al.*[6] have separated the components of manganese bronze and aluminium bronze by anion-exchange chromatography by applying the method of Gerdes *et al.*[5] Relatively large columns containing about 80 ml of resin were used, and the amounts of iron separated were always below 1 mg. Grossmann *et al.* have separated the trace elements in molybdenum,[7] tungsten[7] and niobium[8] by anion-exchange, eluting copper(II) with 4*M* and iron(III) with 0.5*M* hydrochloric acid. Again only microgram amounts of iron were present. Our own experiments showed that iron(III) tends to spread on the column seriously in 4*M* and somewhat in 6*M* hydrochloric acid and appears early in the eluate when larger amounts are present.

The work of Kraus *et al.*[9] on the distribution of elements with a strongly basic anion-exchange resin in hydrochloric acid shows that the sorption of iron(III) increases very steeply with hydrochloric acid concentration and reaches a maximum from ~10*M* hydrochloric acid, with a distribution coefficient larger than 2×10^4. The sorption curve for copper(II) has a very flat maximum at about 6*M* hydrochloric acid, with a distribution coefficient of about 20. Therefore a much higher hydrochloric acid concentration than that used normally[1–8] should have very distinct advantages for the separation of iron(III) from copper (II) and many other elements. Mazzucotelli *et al.*[10] have used 12*M* hydrochloric acid recently to elute copper(II) and manganese(II) in a silicate rock analysis scheme, iron(III) being retained. No details of the separation were given. The separation factor [$D_{Fe(III)}/D_{Cu(II)} > 500$] when 12*M* hydrochloric acid is used is very favourable, but the eluting agent and resin have nearly the same density and the resin tends to float, especially when a fine particle size is used. In 8*M* hydrochloric acid the resin tends to float somewhat less and the separation factor for the iron(III)–copper(II) pair is still >500. Because iron(III) is

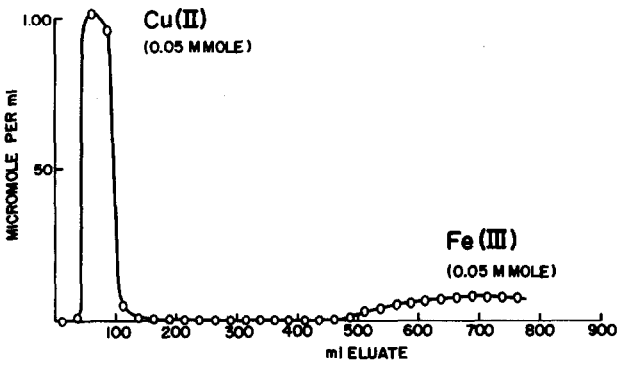

Fig. 1. Elution curve of Cu(II)–Fe(III) with 3.5*M* HCl; 27.6 ml (12 g) of AG1-X8 resin, 200–400 mesh, length 15.5 cm and diameter 1.5 cm; flow-rate 1.5 ± 0.3 ml/min; 0.05 mmole of each element.

sorbed so strongly [$D_{Fe(III)} > 10^4$] it should be possible to use small columns for the separation. Furthermore, fairly serious tailing of copper(II) is experienced with 8% cross-linked resins and a 4% cross-linked resin would offer further chances of improvement. The elution behaviour of copper(II) and iron(III) with both resins and various concentrations of hydrochloric acid as eluent was investigated and compared. From this an improved method for the separation of iron(III) from copper(II) and other elements was developed and will be described.

EXPERIMENTAL

Reagents

The resins used were the AG1-X8 and AG1-X4 strongly basic polystyrene-based anion-exchangers marketed by

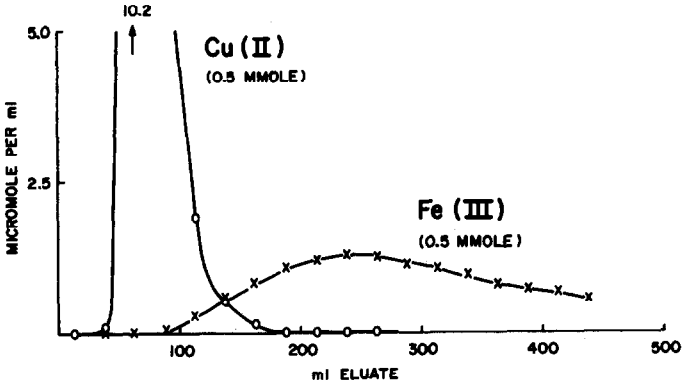

Fig. 2. Elution curve of Cu(II)–Fe(III) with 3.5*M* HCl. Conditions as for Fig. 1, but 0.50 mmole of each element.

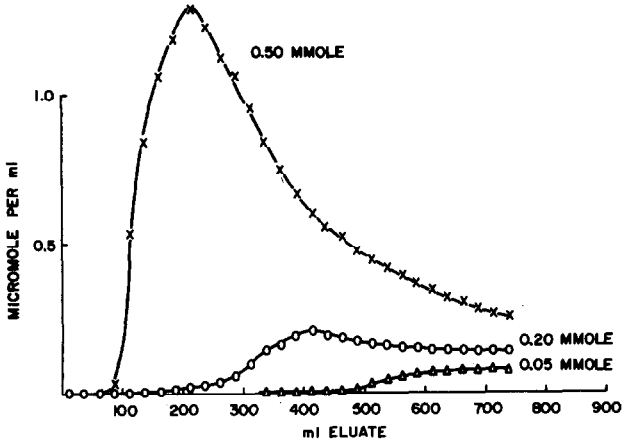

Fig. 3. Elution curves for various amounts of iron(III), with 3.5*M* HCl. Column and experimental conditions as for Fig. 1.

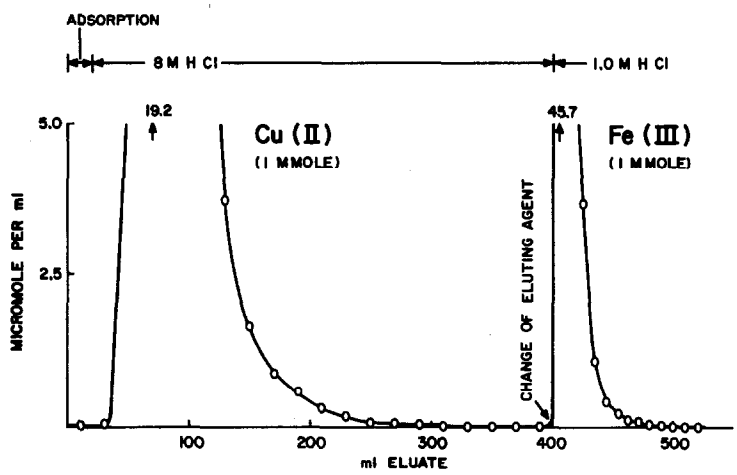

Fig. 4. Elution curve of Cu(II)–Fe(III) with 8.0*M* HCl; 6.9 ml (3 g) of AG1-X8 resin, 200–400 mesh; length 4.0 cm and diameter 1.5 cm; flow-rate 3.0 ± 0.5 ml/min; 1.0 mmole of each element.

Bio-Rad Laboratories of Richmond, California, USA. The 200–400 mesh fraction was used for column work. All reagents used were of analytical reagent quality and the water was distilled and then passed through an Elgastat demineralizer.

Apparatus

Borosilicate glass tubes of 15 mm bore and 30 cm length, fitted with a B14 ground-glass joint at the top and a porosity-2 sintered-glass plate and a tap at the bottom were used as columns. Atomic-absorption measurements were made with a Varian–Techtron AA-5 instrument.

Elution curves

*Elution with 3.5*M *HCl.* A column containing 27.6 ml (12 g dry weight) of AG1-X8 resin of 200–400 mesh particle size was equilibrated by passage of about 80 ml of 8*M* hydrochloric acid through it. The resin column in water was 15.5 cm long and 1.5 cm in diameter. A solution containing 0.05 mmole each of iron(III) and copper(II) in 1 ml of 8*M* hydrochloric acid was placed on top of the resin with a pipette and the ions were washed onto the resin with a few ml of acid of the same concentration. Copper(II), followed by iron(III), was then eluted with 3.50*M* hydrochloric acid at a flow rate of 1.5 ± 0.3 ml/min. Fractions 25 ml in volume were taken with an automatic fractionator, starting from the beginning of the sorption step and the concentrations of copper and iron in the fractions were determined by atomic-absorption spectrometry after evaporation and suitable dilution. The experimental elution curve is shown in Fig. 1.

Figure 2 shows an elution curve for 0.5 mmole of both elements, with the ions sorbed from 10 ml of 8*M* hydrochloric acid, and the other experimental conditions the same as for Fig. 1.

In Fig. 3 elution curves for various amounts of iron(III) with 3.5*M* hydrochloric acid are drawn on the same scale to give a quantitative indication of the elution behaviour.

*Elution with 8.0*M *HCl.* Figure 4 shows an elution curve for 1 mmole each of copper(II) and iron(III) on a small column containing only 6.9 ml (3 g dry weight) of AG1-X8 resin of 200–400 mesh particle size. The resin column was 4.0 cm long and 1.5 cm in diameter and had been equilibrated by passage of 30 ml of 8*M* hydrochloric acid. The ions were introduced on to the resin in 20 ml of 8*M* hydrochloric acid; they were washed onto it and copper(II) was eluted, with 400 ml of acid of the same concentration. Iron(III) was finally eluted with 1.0*M* hydrochloric acid. Fractions 10 ml in volume were taken and the flow-rate was 3.0 ± 0.5 ml/min.

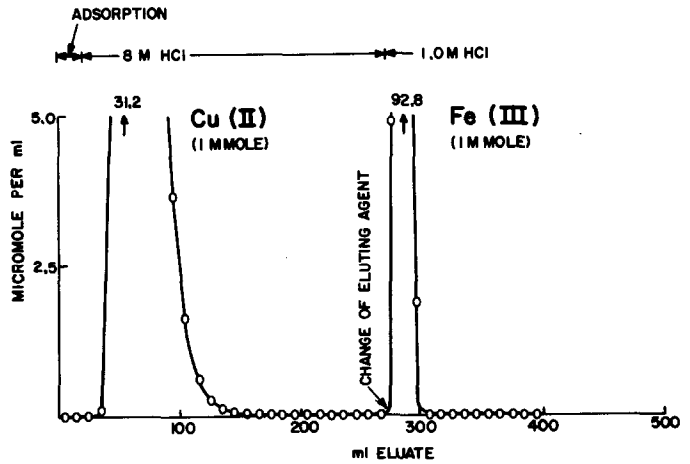

Fig. 5. Elution curve Cu(II)–Fe(III) with 8.0*M* HCl; 8.8 ml (3 g) of AG1-X4 resin, 200–400 mesh; length 5.0 cm and diameter 1.5 cm; flow-rate 3.5 ± 0.5 ml/min; 1.0 mmole of each element.

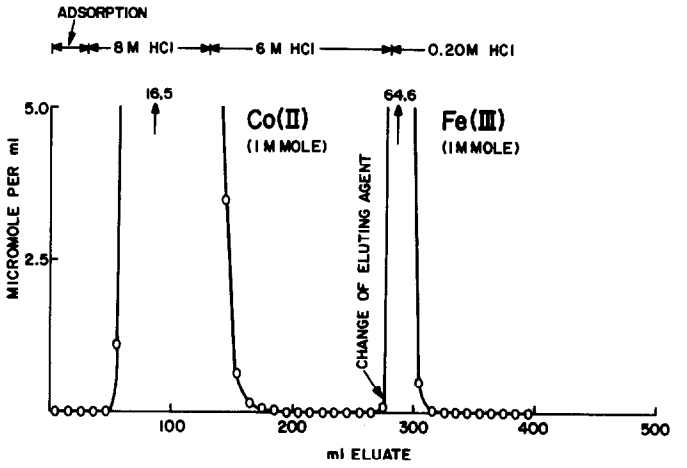

Fig. 6. Elution curve of Co(II)–Fe(III) with 8.0 and 6.0*M* HCl. Resin column and flow-rate as for Fig. 5; 1.0 mmole of each element.

Figure 5 shows an elution curve also for 1-mmole amounts of both elements but for a column containing 8.8 ml (3 g dry weight) of AG1-X4 resin of 200–400 mesh particle size. Sorption again took place from 20 ml of 8*M* hydrochloric acid, but copper(II) was eluted with only 250 ml of the same acid. The flow-rate was 3.5 ± 0.5 ml/min in this case.

Finally, Fig. 6 shows an elution curve for 1 mmole each of cobalt(II) and iron(III) on an 8.8-ml column of AG1-X4 resin as for Fig. 5. The ions were sorbed from 20 ml of 8*M* hydrochloric acid and cobalt(II) was eluted with 100 ml of the same acid followed by 150 ml of 6*M* hydrochloric acid. Iron(III) was eluted with 0.20*M* hydrochloric acid.

Quantitative separations

Appropriate volumes of standard solutions of iron(III) and one other element in dilute hydrochloric acid were measured out in triplicate, mixed, and evaporated to a volume of about 5 ml. About 20 ml of 10*M* hydrochloric acid were added and the solutions were passed through columns containing 8.8 g of AG1-X4 anion-exchange resin of 200–400 mesh particle size. The columns were 5.5 cm long in water and 1.5 cm in diameter and had been equilibrated by passage of about 30 ml of 8*M* hydrochloric acid. The ions were washed onto the resin with a few small portions of 8*M* hydrochloric acid and the ion other than iron(III) was eluted with the same acid concentration, a total volume of 200 ml being used. The eluate was collected, starting from the beginning of the sorption step. Iron(III) was then eluted with 60 ml of 0.10*M* hydrochloric acid when larger amounts were present and with 30 ml of 0.10*M* hydrochloric acid when trace amounts (<1 mg) were present. For cobalt(II), however, 100 ml of 8*M* hydrochloric acid followed by 100 ml of 6*M* hydrochloric acid were used for its elution. Iron(III) was then eluted as above. The eluates were evaporated almost to dryness, made up to convenient volumes and the elements determined by appropriate analytical procedures, on suitable fractions when required. Three blank runs were carried through the procedure and the results corrected accordingly. The results are presented in Table 1 and the analytical procedures are summarized in Table 2.

DISCUSSION

Figures 1–3 show the reason why the classical anion-exchange method[1–8] for the separation of iron(III) from copper(II) fails completely when larger amounts of iron(III) have to be separated. Iron(III) forms a very wide peak and shows serious heading

Table 1. Results of quantitative separations

Taken, *mg*		Found, *mg**	
Fe(III)	Other species	Fe(III)	Other species
56.67	Cu(II) 65.13	56.65 ± 0.03	65.14 ± 0.04
5.67	Cu(II) 130.3	5.66 ± 0.01	130.3 ± 0.1
0.099	Cu(II) 65.13	0.100 ± 0.001	65.12 ± 0.02
nil	3.00 g of $CuCl_2.2H_2O$ Merck "*pro analysi*"	3.1 ± 0.3 μg	not determined
56.67	Mn(II) 55.11	56.66 ± 0.03	55.12 ± 0.03
56.67	Ni(II) 58.62	56.68 ± 0.04	58.61 ± 0.03
56.67	Al 27.04	56.65 ± 0.04	27.04 ± 0.01
56.67	Mg 24.38	56.67 ± 0.03	24.39 ± 0.02
56.67	Ca 40.21	56.66 ± 0.03	40.22 ± 0.02
56.67†	Co(II) 59.12	56.62 ± 0.04	59.10 ± 0.04
11.30†	Co(II) 59.12	11.30 ± 0.01	59.09 ± 0.04
56.67†	Cu(II) 65.13	56.59 ± 0.04	65.15 ± 0.04

* Averages of triplicate determinations.

† Other species eluted with 100 ml of 8*M* HCl followed by 100 ml of 6*M* HCl.

Table 2. Analytical procedures

Element	Method
Fe(III), Cu(II)	Complexometrically by addition of excess of DCTA and back-titration with zinc sulphate at pH 5.5 (hexamethylenetetramine buffer), Xylenol Orange as indicator. Small amounts by AAS.
Mg, Mn(II)	Complexometry with EDTA at pH 10 (ammonia–ammonium chloride buffer), Eriochrome Black 6B as indicator [$NH_2OH.HCl$ present for Mn(II)].
Ni	Complexometry with EDTA in slightly ammoniacal solution, murexide as indicator.
Ca	Complexometry with DCTA in excess of ammonia, Methylthymol Blue as indicator.
Co(II)	Complexometry with EDTA at pH 6 (pyridine buffer), Naphthyl Azoxine S as indicator.

even at relatively slow flow-rates. When 0.2 mmole of iron(III) is present, the first traces of iron(III) above the background level are observed at an eluate volume of about 100 ml, long before larger amounts of copper(II) are completely eluted (Fig. 2). With 0.5 mmole of iron(III) the first traces are eluted after passage of 50 ml of eluent. According to the work of Kraus *et al.*[9] the distribution coefficient of iron(III) in 3.5*M* hydrochloric acid should be at least about 100. Apparently this is true for trace amounts of iron(III) as used by Kraus *et al.* in their work. It also seems from Fig. 3 that the distribution coefficient of iron(III) in 3.5*M* hydrochloric acid could very much depend on and decrease with increasing concentration of iron(III). Thus the working distribution coefficient for larger amounts is probably around a value of 20 or 30, which is much too low for the separation of large amounts of iron(III).

The situation is completely different in 8*M* hydrochloric acid. The distribution coefficient for iron(III) is considerably larger than 10^4 and, even with a 10-fold decrease in this at higher concentration, iron(III) should be safely retained. The high distribution coefficient makes it possible to use considerably smaller columns even for fairly large amounts of iron(III). Furthermore, with a 8% cross-linked resin copper(II) shows fairly strong tailing (Fig. 4) and the elution of copper is not quite complete even after 300 ml of eluting agent have been passed, about 30 μg of copper remaining on the column from 65 mg originally present. Considerable improvement is obtained by using a 4% cross-linked resin. Only about 2–3 μg out of 65 mg of copper then remain on the column after elution with 150 ml of 8*M* hydrochloric acid, but in our procedure 200 ml were used intentionally, to give a safety margin.

The proposed method provides an excellent means for the separation of iron(III) from copper(II) and many other ions. Traces or up to more than 50 mg of iron can safely be separated from up to more than 1 g of copper by using a column containing only 8.8 ml (3 g) of AG1-X4 resin of 200–400 mesh particle size. Iron is retained in a narrow band at the top of the column and even smaller columns can be used when only trace amounts of iron are present. Elution of iron is very fast and from 0.1 to 1.0*M* acid can be used. When only trace amounts are present about 98% will be eluted with the first 12 ml of eluent. Separations are sharp and quantitative. Only between 0.1 and 0.2 μg of copper are found in the iron fraction when 1 mmole was originally present and between 0.4 and 0.8 μg in separation from more than 1 g of copper. Manganese(II), nickel, aluminium, magnesium and calcium are separated even more easily than copper(II) and require only 50 ml of eluting agent. Many other ions with little or no tendency to chloride-complex formation such as those of the alkali metals, Be, Sr, Ba, Sc, Y and the lanthanides, Zr, Hf, Th, Ti(IV), and Cr(III) have not been investigated, but should present no problems and be easily separated according to their known distribution coefficients.[9] Lead is also not retained and should be separable. Cobalt(II) is very slowly eluted with 8*M* hydrochloric acid and requires more than 300 ml for complete elution from a 3-g resin column. An alternative is to elute cobalt with 100 ml of 8*M* followed by 100 ml of 6*M* hydrochloric acid (Fig. 6) but the iron(III) band spreads visibly on the column in 6*M* acid. When 1 mmole of iron(III) is present the first traces of iron(III) already appear in the eluate after 100 ml of 6*M* acid have been passed through the column. About 40–70 μg of iron (0.1% of the total) is found in the cobalt fraction. No iron is lost when smaller amounts (0.2 mmole) are present. In addition, cobalt(II) shows a slight amount of tailing and 20–40 μg of it appear in the iron fraction when 1 mmole of cobalt is initially present. A more complete separation of mmole amounts of iron(III) and cobalt(II) requires a larger column containing at least 5 or 6 g of resin.

Chloride-complex forming species such as Ga, U(VI), Bi(III), Cd, Zn, Hg(II) and Au(III) accompany iron(III) quantitatively or partially. Indium behaves very similarly to copper(II). The method should be very useful, especially for the separation of small amounts or traces of iron from large amounts of copper or other elements which have lesser tendency to chloride-complex formation.

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CATALYTIC AMPEROMETRIC AND CATALYTIC CONSTANT-CURRENT POTENTIOMETRIC TITRATIONS OF SILVER(I), PALLADIUM(II) AND MERCURY(II)

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Summary—Amperometry and constant-current potentiometry were used to follow the course of catalytic titrations of silver(I), palladium(II), and mercury(II) with potassium iodide. The Ce(IV)–As(III) and Ce(IV)–Sb(III) systems in the presence of sulphuric acid were used as indicator reactions. The possibilities of application of platinum, palladium, gold, graphite, and glassy-carbon indicator electrodes were investigated. Graphite appeared to be somewhat more advantageous than the other electrode materials. The effect of concentration of the components of the indicator reactions, and the presence of organic solvents and acids on the shape of the catalytic titration curves was studied. Amounts of 30–3000 μg of silver(I) nitrate, 90–900 μg of palladium(II) chloride, 130–1300 μg of mercury(II) chloride, and 150–1500 μg of mercury(II) nitrate were determined with a relative standard deviation less than 1.0%. The results obtained were in good agreement with those of comparable methods. The catalytic titration method developed was applied to determination of mercury in *Unguentum Hydrargyri*.

Catalytic precipitation titrations can be followed by various methods: visually, or by photometry or spectrophotometry, amperometry, potentiometry, constant-current potentiometry and thermometry.[1–7] The possibilities of application of constant-current potentiometric methods and amperometric methods for determination of the end-point of catalytic titrations have not been widely investigated, however, and only platinum has been used as the indicator electrode. It is well known that the nature of the indicator electrode can influence the results. The investigation of a palladium or gold electrode instead of platinum in biamperometric and bipotentiometric end-point determination was reported previously from our laboratory.[8,9] Furthermore, graphite and glassy-carbon electrodes have proved to be very suitable for various voltammetric and potentiometric determinations.

Continuing our previous investigations[10–12] on the possibilities of using constant-current potentiometry and amperometry in catalytic titrations, in this paper we describe some aspects of catalytic titrations of silver(I), palladium(II), and mercury(II) with potassium iodide, with various systems of indicator electrodes. The indicator reactions, which were catalysed by the first excess of titrant, were Ce(IV)–As(III) and Ce(IV)–Sb(III), both in the presence of sulphuric acid.

EXPERIMENTAL

Chemicals and solutions

All chemicals used were of analytical purity. Solutions were prepared with doubly distilled water.

Stock standard potassium iodide solutions, $6.8 \times 10^{-3}M$ and $1.0 \times 10^{-2}M$. Standardized against a known amount of silver nitrate by potentiometric titration, with the iodide-selective electrode as the end-point detector. Other standard solutions, $1.0 \times 10^{-4}M$ and $6.8 \times 10^{-4}M$, were prepared by dilution of the stock solutions.

Stock silver nitrate solution, $3.8 \times 10^{-3}M$. Prepared by dissolving the necessary amount of the solid. Diluted 100-fold as required.

Stock mercury(II) nitrate and chloride solutions, $1.0 \times 10^{-3}M$. Prepared by dissolving the necessary amounts of the solids. Diluted 10-fold as required.

Stock palladium(II) chloride solution, $1.0 \times 10^{-3}M$. Prepared by dissolving the necessary amount of the solid substance in 0.1*M* hydrochloric acid. Diluted 10-fold with 0.1*M* hydrochloric acid as required.

Arsenic(III) solution, 0.15*M*. Prepared by dissolving the necessary amount of arsenic trioxide in 2.0*M* sodium hydroxide, and acidifying to pH 8 with 2.0*M* sulphuric acid.

Antimony(III) solution, $3.8 \times 10^{-2}M$. Prepared by dissolving the necessary amount of potassium antimonyl tartrate, or as follows: 10.875 g of Sb_2O_3 were dissolved in 15 ml of hydrofluoric acid (38–40%) and diluted with distilled water to about 250 ml, then 5.644 g of tartaric acid were added and fluoride was partially precipitated with 5.000 g of $Th(NO_3)_4 \cdot 5H_2O$; after filtration, the solution was diluted to 500 ml with distilled water.

Cerium(IV) solution, $8.0 \times 10^{-2}M$. Prepared by dissolving ceric sulphate in 2.0*M* sulphuric acid.

Solutions necessary for determination of mercury in *Unguentum Hydrargyri* were prepared and standardized according to the procedure described in Farmakopeja FNRJ 1951 (II).

Instrumentation

The polarization voltages applied in the amperometric monitoring of catalytic titrations with two identical indicator electrodes, or with one indicator electrode (polarized anodically or cathodically) and coupled with an SCE, are given in Table 1. In constant-current potentiometric titrations a current of 1 μA was applied.

The platinum, palladium, gold and glassy-carbon (GC) electrodes were home-made. The metal electrodes were square plates with a surface area of about 0.80 cm^2. The

Table 1. Optimal reagent composition

Method	Titrand	Ce(IV), *ml*	As(III), *ml*	Sb(III), *ml*	4*M* H_2SO_4, *ml*
Amperometry, constant-current potentiometry	Ag	0.5	2.0	—	0.75
	Pd, Hg	0.5	1.0	—	1.0
Amperometry	Ag, Pd, Hg	0.1	—	1.0	2.5
Constant-current potentiometry	Ag, Pd, Hg	0.1	—	0.1	2.5

glassy carbon was "Sigri Elektrographit" prepared at 2400° in the form of a disc with one contact surface of 0.07 cm^2 area. The graphite electrode (C), 0.33 cm^2 area, was a Radelkis type OP-C-7112-D.

The influence of the surface finish of the indicator electrodes was investigated. Some common methods of surface activation were unsuitable because they resulted in poor end-points or reproducibility; they included treatment of the metal electrodes with chromic–sulphuric acid mixture or dilute nitric acid or by heating in an alcohol flame; polishing glassy-carbon electrodes with alumina powder or anodically polarizing them for 10 min (at 1.5 V *vs.* SCE) in 2.0*M* sulphuric acid; polarizing graphite indicator electrodes for 10 min anodically (1.5 V *vs.* SCE) or cathodically (−1.0 V *vs.* SCE) in 2.0*M* sulphuric acid.

The best pretreatment procedure was found to be as follows: after each measurement, metal electrodes are washed with distilled water, and graphite electrodes are polished with alumina powder wetted with water; before each measurement, glassy-carbon electrodes are polarized cathodically (−1.0 V *vs.* SCE) for about 10 min in 2.0*M* sulphuric acid and then washed with distilled water.

For potentiometric end-point detection, an iodide-selective ("Radiometer" F-1032J) or a platinum electrode, coupled with an SCE ("Iskra") or mercury sulphate electrode (MSE) respectively, were used, with a "Radiometer" pHM 26 pH-meter. Derivative titration curves were determined with the iodide-selective electrode and use of a suitable RC circuit. Conductivity measurements were made with a "Radiometer" CDM 3 conductivity meter. The reaction vessel and electrodes were washed with doubly distilled water.

Except where mentioned, the titrand was added continuously from a "Radiometer" ABU 12 automatic piston burette at 0.710 ml/min.

For current *vs.* potential measurements, the three-electrode system used consisted of the working electrode under investigation, an SCE reference electrode, and a square platinum counter-electrode. A "Tacussel" GSTP function generator, "Amel" 551 potentiostat and "Amel" 560 amplifier were used.

Procedure

Current vs. *potential curves.* These were scanned from −0.80 to 1.60 V *vs.* SCE at a rate of 20 mV/sec, with all components at concentrations corresponding to those present at different stages of a titration.

Catalytic potentiometric and amperometric titrations of silver(I), palladium(II) and mercury(II). A 5.00-ml portion of the metal solution to be investigated was mixed with the optimal volumes of the indicator reaction components (Table 1) and diluted with water to 30 ml. The reaction mixture was titrated with potassium iodide solution of appropriate concentration [$1.0 \times 10^{-2}M$ for $3.8 \times 10^{-3}M$ Ag(I); $6.8 \times 10^{-3}M$ for $1.0 \times 10^{-3}M$ Hg(II) or Pd(II); $1.0 \times 10^{-4}M$ for $3.8 \times 10^{-5}M$ Ag(I); $6.8 \times 10^{-4}M$ for $1.0 \times 10^{-4}M$ Hg(II) or Pd(II)].

The procedure for catalytic zero-current potentiometric titrations with the Ce(IV)–As(III) indicator reaction was similar to that of Hadjiioannou and Piperaki[13] except for the concentrations of the indicator reagents.

The zero-current potentiometric titrations using the iodide-selective electrode were done without the indicator reagents present; in conductometric titrations even the sulphuric acid was omitted.

The end-point of the catalytic titration was determined graphically from the intersection of straight-line extrapolations before and after the equivalence point or from the maximum of the derivative titration curve. The results were corrected for the blank.

Catalytic titration of mercury in Unguentum Hydrargyri. A sample of the ointment was prepared according to Farmakopeja FNRJ 1951 (II). About 0.20 g was weighed (to ±0.01 mg) into a 50-ml beaker and heated on a water-bath with 20 ml of conc. nitric acid. The solution was evaporated to about 5 ml, 20 ml of water were added, and the volume was again reduced to 5 ml. This evaporation procedure was repeated once more. After cooling for 30 min, the residue was filtered off on cotton and washed with water, the filtrate and washing being collected in a 100-ml standard flask, where they were oxidized with permanganate, the excess of which was then reduced with iron(II) sulphate. The solution was made up to volume and 5.00-ml aliquots were titrated by the catalytic procedure for mercury(II).

A comparison determination was done by titration with thiocyanate, with ferric ammonium sulphate as visual indicator, as suggested by Farmakopeja FNRJ 1951 (II), an ordinary burette with 0.05-ml divisions being used.

RESULTS AND DISCUSSION

The electrochemical properties of the Ce(IV)/Ce(III) system used in the indicator reactions allow the end-points to be determined both potentiometrically at constant current, and amperometrically.

Current vs. *potential curves*

To establish the amperometric and constant-current potentiometric titration conditions, current *vs.* potential curves were obtained for reaction mixtures simulating conditions before and after the equivalence point.

The curves obtained with graphite and glassy-carbon working electrodes were very similar, but those obtained with the metal electrodes had maxima due to metal oxide formation or reduction, and to oxidation evolution of adsorbed hydrogen atoms. For catalytic amperometric titrations with one anodically polarized indicator electrode the potential of the limiting diffusion current of Ce(III) oxidation was

Table 2. Working conditions applied in catalytic amperometric titrations

Indicator reaction	Ion determined	Indicator electrode, *I*	Polarization voltage, *mV*		
			I(+)/*I*(−)	*I*(+)/SCE(−)	*I*(−)/SCE(+)
Ce(IV)–As(III)	Ag(I)	Pt	100	1000	800
		Au	—	1200	300
		Pd	—	1200	800
		GC	30	1200	800
		C	100	1200	500
	Pd(II), Hg(II)	C	100	1000	600
Ce(IV)–Sb(III)	Ag(I)	Pt	—	1200	300
		Au	—	1000	800
		Pd	—	1000	800
		GC	100	1000	—
		C	60	1000	—
	Pd(II), Hg(II)	C	60	1000	600

selected. When the indicator electrode was cathodically polarized, the potential for the limiting diffusion current of Ce(IV) reduction was chosen (Table 2).

For catalytic constant-current potentiometric titrations a current of 1 μA was chosen. A higher current produces characteristic breaks beyond the equivalence point, which make the end-point determination more difficult.

Optimization of conditions

The influence of solvents, acids, and concentration of indicator reaction components was studied by varying one factor at a time.

Effect of organic solvents. It was found that the presence of certain organic solvents (propan-2-ol, acetone, ethanol) in 50% concentration at the equivalence point almost completely eliminated the catalytic effect. Therefore aqueous media were used.

Effect of acids. Sulphuric, perchloric and nitric acids were tested and only in sulphuric acid was the catalytic effect pronounced, probably because the Ce(IV) must be in sulphato-complexes for the reaction to be catalysed by iodide, which is in agreement with the literature.[14]

All authors agree that there is an optimal concentration of sulphuric acid for catalysis of the Ce(IV)–As(III) and Ce(IV)–Sb(III) indicator reactions by iodide but disagree on its value. We investigated the acid concentration range 7.3×10^{-2}–$1.34M$ and found that the change in signal at the end-point first increased with concentration then decreased. The optimal concentration of sulphuric acid in the Ce(IV)–Sb(III) reaction was $0.34M$, but in the Ce(IV)–As(III) reaction was $0.57M$ for determination of palladium(II) and mercury(II), and $0.10M$ for silver(I).

Effect of Ce(IV) concentration. In the mercury titration with the Ce(IV)–As(III) indicator reaction, the end-point signal increases with Ce(IV) concentration from 2.7×10^{-4} to $1.3 \times 10^{-2}M$, then decreases at higher concentration. For the palladium and silver titrations, however, the optimal Ce(IV) concentration was $0.0013M$, so this value was chosen for further use.

For the Ce(IV)–Sb(III) reaction, however, increasing the Ce(IV) concentration decreases the rate of signal change at the end-point, and a Ce(IV) concentration of $2.7 \times 10^{-4}M$ was chosen.

Effect of As(III) concentration. Increasing the concentration of As(III) from 5.0×10^{-4} to $2.5 \times 10^{-2}M$ increases the signal change at the end-point but further increase up to $0.1M$ decreases it [probably due to an inhibiting effect of As(V)], in agreement with the results of Rodriguez and Pardue.[15] The optimal concentration was $0.005M$ for silver and $0.01M$ for palladium and mercury.

Effect of Sb(III) concentration. If the Sb(III) solution is made from potassium antimonyl tartrate, the end-point signal change is considerably smaller than when the solution is made from the oxide, as described. Increasing the Sb(III) concentration from 1.3×10^{-4} to $1.3 \times 10^{-3}M$ does not appreciably affect the catalytic curves, but a further increase to $0.013M$ markedly decreases the signal change, probably because of the inhibiting effect of fluoride on this indicator reaction.[16] For constant-current potentiometric determinations, the optimal Sb(III) concentration was $1.3 \times 10^{-4}M$, but for amperometric titration $0.0013M$ was better.

Effect of tartrate concentration. Increasing the tartrate concentration increases the indicator reaction rate, and causes a signal change before the equivalence point. For this reason, the minimal tartrate concentration needed to keep Sb(III) in solution ($2.2 \times 10^{-4}M$) was used.

Choice of indicator electrodes. From Fig. 1 it can be seen that all the indicator electrodes tested are suitable but the graphite indicator electrodes (curve 2) are somewhat more advantageous for the constant-current potentiometric determinations, and the graphite and glassy-carbon electrodes are superior for the amperometric determinations. This was the reason for choosing graphite electrodes for determination of palladium, mercury, and lower amounts of silver.

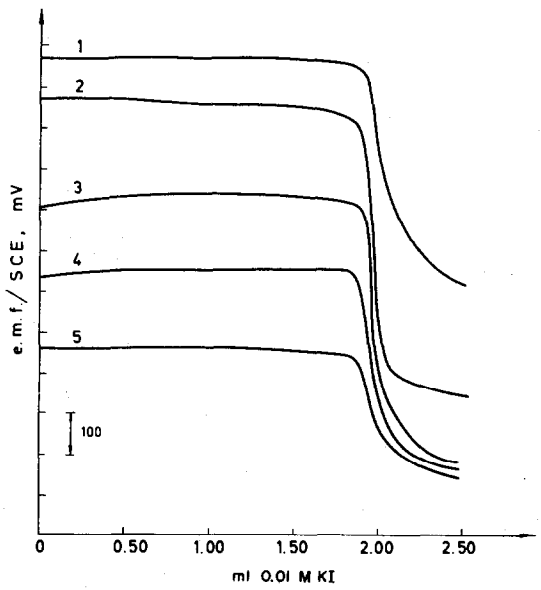

Fig. 1. Effect of nature of indicator electrodes, I, on the shapes of potentiometric [I(+)/SCE(−)] (i = 1 μA) titration curves for 3.23 mg of $AgNO_3$ titrated with 0.01M KI in the presence of Ce(IV), As(III), and H_2SO_4. Electrodes: 1—glassy-carbon; 2—graphite; 3—platinum; 4—gold; 5—palladium.

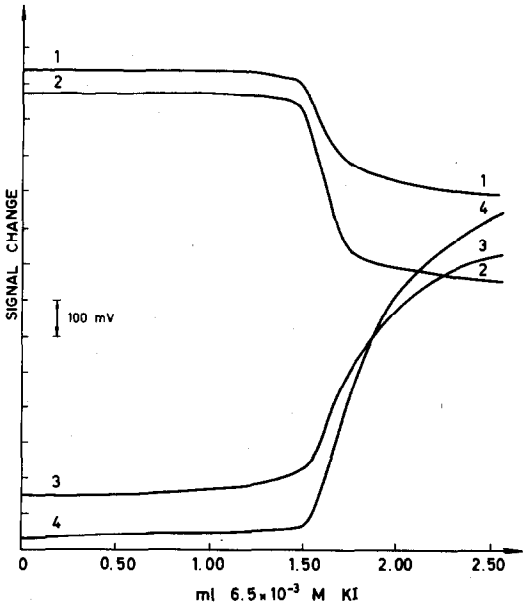

Fig. 3. Catalytic potentiometric titration curves for 850.4 μg of $PdCl_2$ titrated with 6.5 × $10^{-3}M$ KI in the presence of Ce(IV), As(III) and H_2SO_4: 1—(Pt/MSE), (i = 0), 5.7 × $10^{-4}M$ Ce(IV), 1.2 × $10^{-2}M$ As(III) and 2.1 × $10^{-1}M$ H_2SO_4; 2—(Pt/MSE), (i = 0), 1.3 × $10^{-3}M$ Ce(IV), 5.0 × $10^{-3}M$ As(III) and 3.0 × $10^{-1}M$ H_2SO_4; 3—[C(−)/SCE(+)], (i = 1 μA), 5.7 × $10^{-4}M$ Ce(IV), 1.2 × $10^{-2}M$ As(III) and 2.1 × $10^{-1}M$ H_2SO_4; 4—[C(−)/SCE(+)], (i = 1 μA), 1.3 × $10^{-3}M$ Ce(IV), 5.0 × $10^{-3}M$ As(III) and 3.0 × $10^{-1}M$ H_2SO_4.

Applicability of methods

Figure 2 shows the curves for catalytic titration of palladium(II) with iodide, with use of the Ce(IV)–As(III) reaction. All the methods are suitable, although amperometric titration using the cathodically polarized electrode (curve 1) is not nearly as good as the other methods. The same is found for the Ce(IV)–Sb(III) reaction, but the resulting signal change at the end-point is less pronounced. When mercury(II) is titrated as the chloride, the Ce(IV)–Sb(III) system is superior to the As(III) system, and the reverse holds for mercury(II) nitrate.

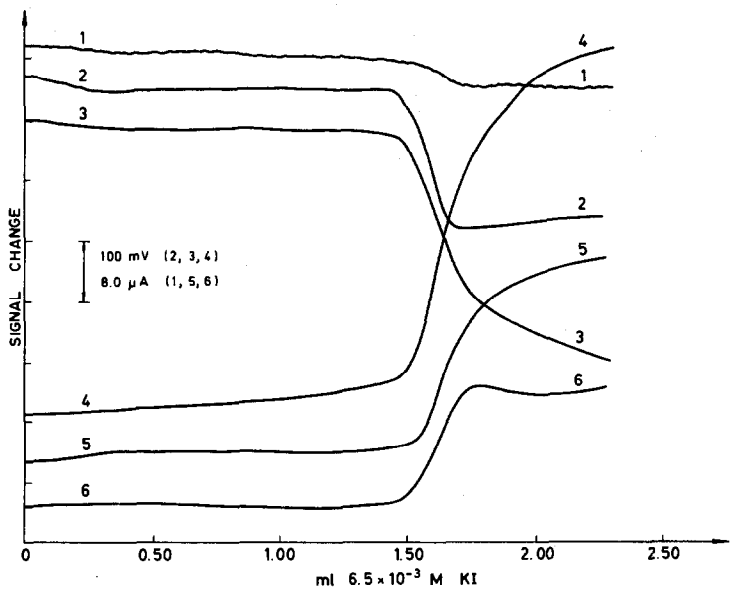

Fig. 2. Catalytic titration curves for 850.4 μg of $PdCl_2$ titrated with 6.5 × $10^{-3}M$ KI in the presence of Ce(IV), As(III), and H_2SO_4: 1—amperometry [C(−)/SCE(+)]; 2—constant-current potentiometry [C(+)/C(−)]; 3—constant-current potentiometry [C(+)/SCE(−)]; 4—constant-current potentiometry [C(−)/SCE(+)]; 5—amperometry [C(+)/SCE(−)]; 6—amperometry [C(+)/C(−)].

Table 3. Determination of milligram amounts of silver nitrate by various methods (6 titrations in each case)

Method of end-point determination		Indicator reaction Ce(IV)–As(III)		Ce(IV)–Sb(III)	
		a	*b*	*a*	*b*
Catalytic amperometric titration	Pt(+)/Pt(−)	3.21	0.0	—	—
	Pt(+)/SCE(−)	3.21	0.3	3.20	0.6
	Pt(−)/SCE(+)	3.20	0.1	3.20	0.8
	Au(+)/Au(−)	—	—	—	—
	Au(+)/SCE(−)	3.24	0.5	3.21	0.2
	Au(−)/SCE(+)	3.23	0.3	3.21	0.8
	Pd(+)/Pd(−)	—	—	—	—
	Pd(+)/SCE(−)	3.20	0.2	3.24	0.4
	Pd(−)/SCE(+)	3.23	0.6	3.23	0.6
	GC(+)/GC(−)	3.20	0.6	3.19	0.6
	GC(+)/SCE(−)	3.23	0.6	3.24	0.4
	GC(−)/SCE(+)	3.21	0.1	—	—
	C(+)/C(−)	3.22	0.5	3.23	0.6
	C(+)/SCE(−)	3.22	0.4	3.23	0.7
	C(−)/SCE(+)	3.20	0.1	—	—
Catalytic constant-current potentiometric titration	Pt(+)/Pt(−)	3.23	0.7	—	—
	Pt(+)/SCE(−)	3.21	0.3	3.21	1.0
	Pt(−)/SCE(+)	3.19	0.3	3.19	0.5
	Au(+)/Au(−)	—	—	3.22	0.9
	Au(+)/SCE(−)	3.21	0.2	3.22	0.6
	Au(−)/SCE(+)	3.21	0.1	3.23	0.6
	Pd(+)/Pd(−)	3.22	0.5	3.24	0.2
	Pd(+)/SCE(−)	3.22	0.3	3.19	0.8
	Pd(−)/SCE(+)	3.21	0.3	3.22	0.7
	GC(+)/GC(−)	3.24	0.1	3.21	0.4
	GC(+)/SCE(−)	3.21	0.3	3.20	0.9
	GC(−)/SCE(+)	3.21	0.6	3.20	0.4
	C(+)/C(−)	3.20	0.8	3.19	0.7
	C(+)/SCE(−)	3.20	0.1	3.23	0.4
	C(−)/SCE(+)	3.21	0.2	3.22	0.3
Potentiometric titration*	I^-/SCE	3.23	0.4	3.23	0.4
Conductometric titration*		3.20	0.5	3.20	0.5
Catalytic potentiometric titration ($i = 0$)	Pt/MSE	3.21	0.6	3.21	0.6

a, Quantity found (mg); *b*, relative standard deviation (%).
* In the absence of components of the indicator reaction.

Figure 3 shows the advantage of the constant-current potentiometric titration with the graphite electrode over the potentiometric method with the platinum electrode, and that our conditions (curves 2 and 4) are superior to those reported in the literature[13] (curves 1 and 3). Some results are given in Tables 3–6. The maximum relative standard deviation was 1.0%, and the results were in good agreement with those of potentiometry. Conductometry was not used as the comparative method in determination of palladium since the palladium solution had a high concentration of free hydrochloric acid and the conductivity change during titration was too small to be detected.

Empty spaces in the tables correspond to titrations giving unsatisfactory results (mainly the methods with two identical metal electrodes).

Effect of foreign ions

The influence of foreign ions on constant-current potentiometric titration [C(+)/SCE(−); Ce(IV)–As(III)] was investigated. The ions chosen were those which form precipitates or complexes with the reactive components of the system. The tolerance limit was taken as the level which causes a titration error of ±1% (Table 7). Silver, palladium and mercury give mutual interference, of course.

Sulphides, periodates and oxalates cause premature

Table 4. Determination of microgram amounts of silver nitrate by various methods (6 titrations in each case)

Method of end-point determination		Indicator reaction: Ce(IV)–As(III)		Ce(IV)–Sb(III)	
		a	*b*	*a*	*b*
Catalytic amperometric titration	C(+)/C(−)	32.3	1.0	32.3	0.7
	C(+)/SCE(−)	32.3	0.3	32.4	0.3
	C(−)/SCE(+)	—	—	—	—
Catalytic constant-current potentiometric titration	C(+)/C(−)	32.2	0.8	32.3	0.4
	C(+)/SCE(−)	32.1	0.7	—	—
	C(−)/SCE(+)	32.4	0.6	32.1	1.0
Potentiometric titration*	I^-/SCE	32.3	1.0	32.3	1.0
Conductometric titration*		32.1	0.7	32.1	0.7
Catalytic potentiometric titration ($i = 0$)	Pt/MSE	33.1	0.8	33.1	0.8

a, Quantity found (μg); *b*, relative standard deviation (%).
* In the absence of components of the indicator reaction.

end-points by reaction with the ion to be titrated. Chlorides and bromides give a negative error in silver determination, by precipitation, and in catalytic titrations of palladium and mercury change the shape of the titration curves by making the indicator reaction much faster even before the equivalence point, and cause trouble in end-point location.

Thiocyanate causes negative error by complex or precipitate formation, but in determination of mercury, the titration curve shape may be changed considerably by oxidation of thiocyanate by Ce(IV). This effect is not pronounced in determination of palladium and silver, since the tolerance limit is relatively small anyway.

Iodate causes premature end-points and changes the shape of the titration curves, probably[15] because reduction with As(III) produces iodide, which catalyses the indicator reaction even before the equivalence point.

Lead does not interfere with the palladium and mercury determinations, but causes a negative error in the silver titration, though the sulphuric acid necessary for the indicator reaction will precipitate most of it.

Catalytic titration of mercury in Unguentum Hydrargyri

The constant-current potentiometric [C(+)/SCE(−)] titration [Ce(IV)–As(III) system] gave a mean mercury content of 6.19% (0.7% relative standard deviation), in good agreement with the comparative method, which gave 6.18% (1.0% relative stan-

Table 5. Determination of palladium chloride by various methods (6 titrations in each case)

Method of end-point determination		Indicator reaction: Ce(IV)–As(III)				Ce(IV)–Sb(III)			
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Catalytic amperometric titration	C(+)/C(−)	857.0	0.4	90.3	0.2	854.7	0.4	89.9	0.4
	C(+)/SCE(−)	847.7	0.3	87.5	0.4	847.9	0.6	90.1	0.4
	C(−)/SCE(+)	850.4	0.4	90.1	0.3	836.9	0.3	—	—
Catalytic constant-current potentiometric titration	C(+)/C(−)	847.7	0.6	89.8	0.6	852.2	0.9	89.9	0.5
	C(+)/SCE(−)	847.7	0.5	91.7	0.1	842.4	0.1	90.0	0.4
	C(−)/SCE(+)	856.8	0.3	89.9	0.5	835.2	0.6	89.8	0.3
Potentiometric titration*	I^-/SCE	850.4	0.6	89.9	0.4	850.4	0.6	89.9	0.4
Catalytic potentiometric titration ($i = 0$)	Pt/MSE	818.9	0.3	91.7	0.7	818.9	0.3	91.7	0.7

a, Quantity found (μg); *b*, relative standard deviation (%).
* In the absence of components of the indicator reaction.

Table 6. Determination of mercury(II) nitrate by application of the indicator reaction Ce(IV)–As(III) and mercury(II) chloride by application of the indicator reaction Ce(IV)–Sb(III), using various methods (6 titrations in each case)

Method of end-point determination		Indicator reaction: Ce(IV)–As(III)				Ce(IV)–Sb(III)			
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Catalytic amperometric titration	C(+)/C(−)	1560	0.8	152.4	0.6	1284	0.4	128.1	1.0
	C(+)/SCE(−)	1550	0.4	152.3	0.2	1293	0.3	129.4	0.8
	C(−)/SCE(+)	1546	0.5	—	—	1286	0.2	—	—
Catalytic constant-current potentiometric titration	C(+)/C(−)	1561	0.4	154.2	0.6	1298	0.7	130.2	0.6
	C(+)/SCE(−)	1555	0.3	153.5	0.7	1317	0.3	131.0	0.7
	C(−)/SCE(+)	1558	0.3	150.7	0.0	1294	0.6	130.1	0.4
Potentiometric titration*	I^-/SCE	1566	0.6	153.0	1.2	1310	0.6	128.0	1.2
Conductometric titration*		1529	0.6	—	—	—	—	—	—
Catalytic potentiometric titration ($i = 0$)	Pt/MSE	1540	0.3	158.0	0.3	1288	0.3	132.2	0.3

a, Quantity found (μg); *b*, relative standard deviation (%).
* In the absence of components of the indicator reaction.

Table 7. Maximal tolerance ratios of non-interfering concentration of foreign and titrated ions in catalytic titrations of $6.3 \times 10^{-4}M$ $AgNO_3$, $1.6 \times 10^{-4}M$ $PdCl_2$, and $1.6 \times 10^{-4}M$ $Hg(NO_3)_2$

Foreign ion (F)	Maximal concentration ratio		
	F:$AgNO_3$	F:$PdCl_2$	F:$Hg(NO_3)_2$
Cl^-	1:25	>1000:1	1000:1
Br^-	1:150	10:1	2:1
SCN^-	1:23	1:20	2:1
S^{2-}	1:16	1:40	1:81
IO_3^-	1:14	1:20	1:4
IO_4^-	1:25	10:1	10:1
$C_2O_4^{2-}$	1:3	12:1	5:1
Pd(II)			1:50
Hg(II)	1:127	1:17	
Cd(II)	>1000:1	1000:1	1000:1
Pb(II)	4:5	>1000:1	1000:1
Ag(I)			1:57

dard deviation), and can be used for quality control of *Unguentum Hydrargyri.*

CONCLUSIONS

All the electrodes, both indicator-reaction systems and both the amperometric and constant-current potentiometric methods are satisfactory for the purpose. The Ce(IV)–As(III) reaction and the graphite electrode are somewhat advantageous, the latter owing to the relatively large signal change at the end-point and the simple electrode pretreatment procedure.

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ANALYTICAL APPLICATIONS OF ESCA TO SAMPLES IN POWDER FORM

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Summary—Analytical applications of ESCA to samples in powder form are described. These include the estimation of atomic ratios of metallic elements to oxygen in some typical oxides, the construction of calibration curves and the investigation of precipitation phenomena. The calibration curve method is useful in combination with the calculation method when the appropriate sample preparation has been carried out.

When samples in powder form are analysed by means of ESCA (XPS and XAES), the particle size, crystal structure, mixing states and adsorption of gases on surfaces have presented problems.[1–5] In this paper the analytical applications of ESCA to samples in powder form are investigated. These include the estimation of atomic ratios of metallic elements to oxygen in some typical oxides, the preparation of calibration curves for quantitative use and the observation of precipitation phenomena.

In order to obtain the atomic ratios of the elements in oxides semi-quantitatively, equation (1)[6] has been used.

$$\frac{N_a^l}{N_b^m} = \frac{n_a \sigma_a^l \lambda_a^l S_a^l}{n_b \sigma_b^m \lambda_b^m S_b^m},$$

$$\frac{N_a^{lA}}{N_b^{mA}} = \frac{n_a \sigma_a^l \lambda_a^{lA} S_a^{lA} \gamma_a^{lA}}{n_b \sigma_b^m \lambda_b^{mA} S_b^{mA} \gamma_b^{mA}}$$

or

$$\frac{N_a^{lA}}{N_b^m} = \frac{n_a \sigma_a^l \lambda_a^{lA} S_a^{lA} \gamma_a^{lA}}{n_b \sigma_b^m \lambda_b^m S_b^m} \qquad (1)$$

where n is the concentration of the element in terms of atoms per unit volume, N is the intensity of the ejected photoelectrons or X-ray excited Auger electrons (the latter indicated by superscript A) from levels l and m, σ is the photoionization cross-section, λ is the mean free path for electrons in the samples, S is the spectrometer sensitivity factor (including the efficiency of the detector), and γ is the X-ray-excited Auger intensity factor for XAES.[6] These equations are also used in the discussion of the calibration curve method, and the original form of the equation is employed in the consideration of precipitation phenomena.

EXPERIMENTAL

Experiments were performed with an AEI-ES 200 electron spectrometer with Al $K_{\alpha_{1,2}}$ (1486.6 eV) or Mg $K_{\alpha_{1,2}}$ (1253.6 eV) radiation. The pressure in the sample chamber was maintained between 10^{-8} and 10^{-9} mmHg during experiments. The instrument was calibrated so that the difference between the photoelectron peak of Au $4f_{7/2}$ and the Fermi level of palladium was 84.0 eV. The binding energy of the C 1*s* line, due to carbon contamination from the vacuum system, was 285.0 ± 0.2 eV with respect to the Au $4f_{7/2}$ peak.

The spectrometer was operated in the computer-controlled scanning mode. Each spectrum was scanned for a preselected period of time during each cycle (with repetition of the cycle). The relative areas of spectral peaks taken on an analogue scan were measured (including satellite and plasmon peaks).[7]

The powder samples used in this experiment were prepared by the usual inorganic synthetic procedures and the composition and structure were determined by chemical analysis and X-ray diffraction. The samples were tightly pressed onto a copper or platinum grid (100–200 mesh), or onto an annealed aluminium plate under a pressure of 1–2 ton/cm^2.

RESULTS AND DISCUSSION

Calculation of atomic ratios

The atomic ratios of oxygen to metallic elements in some oxides have been obtained (Table 1). There is a problem in the measurement of intensities with some oxides having spectra which exhibit satellite peaks, *e.g.*, TiO_2, Cr_2O_3, Fe_2O_3, CoO, NiO and CuO. Examples of the spectra in the 2*p* region for TiO_2, Cr_2O_3 and Fe_2O_3 are shown in Fig. 1. The background is represented by a straight line and the shaded areas are measured to calculate intensities. There is sufficient energy resolution to permit the measurement of the background in the region between the main and satellite peaks for the 2*p* regions of the metallic elements in TiO_2, CoO, NiO and CuO. For the 2*p* regions of Cr_2O_3 and Fe_2O_3, the intensity ratios of the $2p_{3/2}$ peak to the $2p_{1/2}$ peak were calculated by using equation (1). The approximate compositions of the oxides can be estimated from the results in Table 1, provided that the oxides are not unstable, easily oxidized in air or reduced in the spectrometer, *e.g.*, Cu_2O.

Table 1. Results of the application of equation (1) to oxides

Oxide	Spectral lines measured	Atomic ratio (O/M) Theoretical	Experimental	Sample
MgO	O 1s, Mg 2p	1.0	1.30	Powder
Al_2O_3	O 1s, Al 2s	1.5	1.46	Powder, α-Al_2O_3
SiO_2	O 1s, Si 2p	2.0	1.98	Plate, commercial
TiO_2	O 1s, Ti $2p_{3/2}$	2.0	2.06	Powder
Cr_2O_3	O 1s, Cr $2p_{3/2}$	1.5	1.56	Powder
Fe_2O_3	O 1s, Fe $2p_{3/2}$	1.5	1.47	Powder, α-Fe_2O_3
CoO	O 1s, Co $2p_{3/2}$	1.0	1.28	Powder, commercial
Co_3O_4	O 1s, Co $2p_{3/2}$	1.33	1.50	Powder
NiO	O 1s, Ni $2p_{3/2}$	1.0	0.97	Powder
Cu_2O	O 1s, Cu $2p_{3/2}$	0.5	0.74	Powder
CuO	O 1s, Cu $2p_{3/2}$	1.0	0.98	Powder from $Cu(NO_3)_2$
CuO	O 1s, Cu $2p_{3/2}$	1.0	0.86	Powder from $CuCO_3$
CuO	O 1s, Cu $2p_{3/2}$	1.0	0.87	Powder from Cu_2O

Calibration curves for quantitative use

The preparation of standard samples for quantitative use (for ESCA) is difficult, but when powdered samples are used calibration curves are necessary and may be constructed in some cases. Satisfactory calibration curves have been obtained for samples fused with some flux materials, because of their homogeneity and the matrix dilution.[3,4] Calibration curves can be constructed after appropriate sample preparation with crystallographically homogeneous samples. Calibration curves cannot be constructed from ESCA data for alloy samples in which one element tends to cover the surface after polishing, as in the alloys of soft metals such as Al, Pb and Ag. The calibration curve is defined as the correlation between the intensity ratio to the reference in the same sample and the atomic ratio of the element to be analysed. In Figs. 2a and 2b, the calibration curves for some binary oxide systems, prepared by the co-precipitation method, are shown. The structures of these oxides are confirmed by X-ray diffraction, and they are solid solutions, stable in air and in high vacuum. For the combinations Cr $2p_{3/2}$/Fe $2p_{3/2}$ for Cr_2O_3–Fe_2O_3, Al 2s/Cr $2p_{3/2}$ for Al_2O_3–Cr_2O_3, Co $2p_{3/2}$/Mg 2p for CoO–MgO and Ni $2p_{3/2}$/Mg 2p for the NiO–MgO systems, linear calibration curves could be obtained. The atomic ratios calculated by using equation (1) and the calibration curves for Cr 3p/Fe 3p for Cr_2O_3–Fe_2O_3, for Co 2p/Mg 2p for CoO–MgO, and for Ni 2p/Mg 2p for NiO–MgO, were different, however, from those obtained by chemical analysis. There are two main reasons for this inconsistency. One may be the difference in photoionization cross-section between Schofield's values[8,12] and the values for the outer subshell levels of these oxides. The photon–electron and electron–electron interactions in the outer shells of these oxides (*e.g.*, Fe 3p, Cr 3p and Mg 2p) are not sufficiently simple to permit the use of photoionization cross-sections calculated by the relative HFS method. The second is the presence of satellite and overlapping peaks, making the background correction inaccurate. In the Cr 3p/Fe 3p combination for the Cr_2O_3–Fe_2O_3 system, the satellite for Cr 3p overlaps the foot of the main peak for Fe 3p and its satellite for Al $K_{3,4}$. The Fe 3p satellite overlaps the Cr 3s satellite for Al $K_{3,4}$. The employment of spectral lines from inner subshell levels gives good agreement between calculated and analytical results. A third possibility would be the attenuation caused by a contaminant over-layer, but this was regarded as negligible in this work.

The calibration curve method is useful when for some oxide samples the spectral intensities from outer levels are employed, for which the application of calculated photoionization cross-sections cannot be recommended. Further, the combination of calibration curves and equation (1) is a useful tool for semi-quantitative analysis, when the chemical shift can be measured.

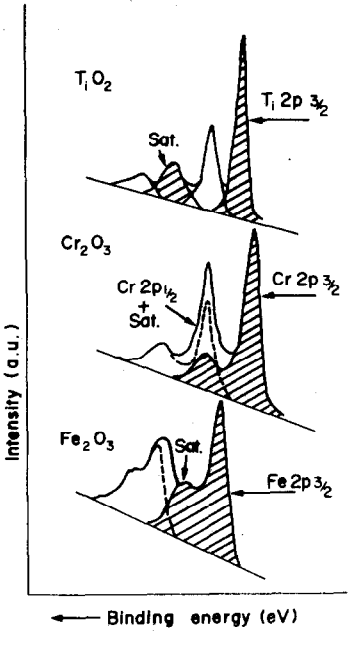


Fig. 1. Spectra of the 2p regions for TiO_2, Cr_2O_3 and Fe_2O_3 (a.u. = arbitrary units).

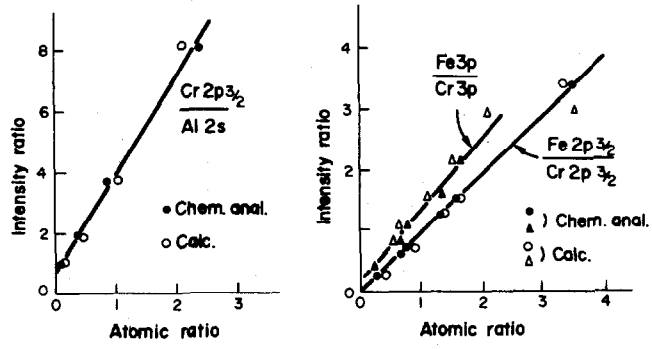


Fig. 2(a). Calibration curves and atomic ratios calculated for the Al_2O_3–Cr_2O_3 and for the Fe_2O_3–Cr_2O_3 binary systems.

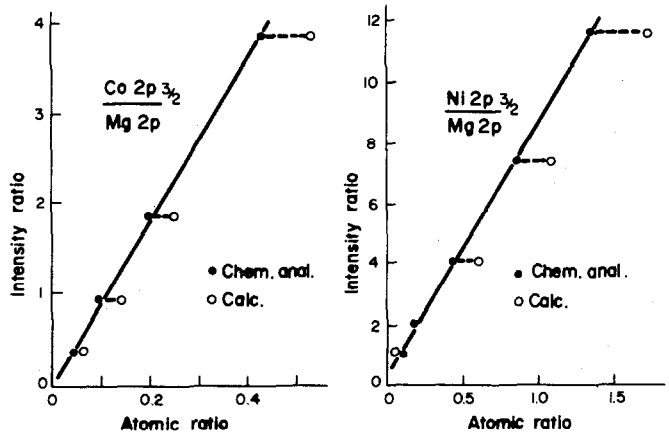
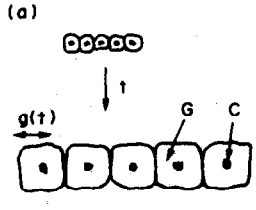


Fig. 2(b). Calibration curves and atomic ratios calculated for the CoO–MgO and for the NiO–MgO binary systems.



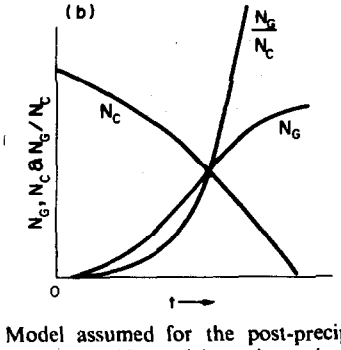


Fig. 3(a). Model assumed for the post-precipitation. (b) ESCA intensity (N_G, N_C) and intensity ratio (N_G/N_C) *vs.* time of aging of precipitate (t).

Observation of precipitation phenomena

In the separation procedures used for chemical analysis, the precipitation method is widely used. ESCA has been applied to investigate the mechanisms of co-precipitation (contamination) and deposition. There are some accounts of the origins of the contamination of precipitates such as adsorption, co-precipitation and post-precipitation.[9] For adsorption and post-precipitation, the model given in Fig. 3(a)

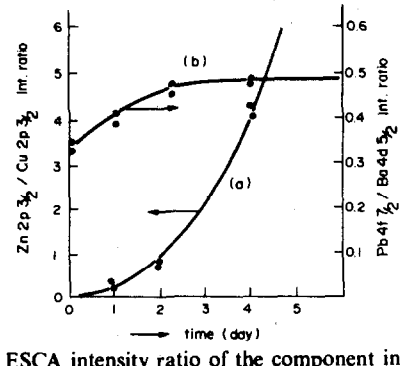


Fig. 4. ESCA intensity ratio of the component in precipitate *vs.* aging time of the precipitate. (a) CuS–ZnS, (b) $PbSO_4$–$BaSO_4$.

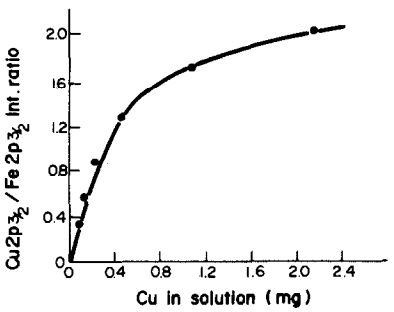


Fig. 5. Cu deposition on Fe powder, from solution, showing change of ESCA intensity.

was assumed. In this model three assumptions are made. A component G is adsorbed on a second component C and grows at a rate $g(t)$ in time t. The growth occurs at the same rate in all directions. The effect of the surface roughness of the precipitate is negligible. Now the electron intensities from level 1 of the component G, N_G, may be expressed by equation (2), if $g(t)$ is less than λ_G^1:

$$N_G \frac{N_0 n_G \sigma_G^1 S_G^1}{\sin\theta} \times \left\{ \int_0^\infty \exp\left(\frac{-Z}{\lambda_G^1 \sin\theta}\right) dZ \exp\left(\frac{-g(t)}{\lambda_G^1 \sin\theta}\right) + \int_0^{g(t)} \exp\left(\frac{-Z}{\lambda_G^1 \sin\theta}\right) dZ \right\} (\sim 2)g(t)$$

$$N_G \sim N_0 n_G \sigma_G S_G^1 \lambda_G^1 (\sim 2)g(t) \quad (2)$$

On the other hand, the electron intensities from level m of the component C, which is assumed to have infinite thickness, are given by equation (3), so relationship (4) is obtained.

$$N_C \sim \frac{N_0 n_C \sigma_C^m S_C^m}{\sin\theta(\sim 2)g(t)} \int_0^\infty \exp\left(\frac{-Z}{\lambda_C^m \sin\theta}\right) dZ \times \exp\left(\frac{-g(t)}{\lambda_G^1 \sin\theta}\right) \sim \frac{N_0 n_C \sigma_C^m S_C^m}{\sin\theta(\sim 2)g(t)} \exp\left(\frac{-g(t)}{\lambda_G^1 \sin\theta}\right) \quad (3)$$

$$\frac{N_G}{N_C} \sim \frac{n_G \sigma_G^1 S_G^1 \lambda_G^1}{n_C \sigma_C^m S_C^m \lambda_C^m} \times \frac{[(\sim 2)g(t)]^2}{\exp\left(\frac{-g(t)}{\lambda_G^1 \sin\theta}\right)} \times \lim_{t\to\infty} \frac{N_G}{N_C} = \infty \quad (4)$$

The expected shapes of the curves for these equations are as shown in Fig. 3(b). The X-ray-excited Auger electron spectra may be used in these equations. When the mechanism of co-precipitation is solid solution, the value of N_G/N_C is nearly constant. Two typical cases of co-precipitation were tested, one being post-precipitation and the other solid solution.[9]

A mixture of 2.17 mg of copper and 2.32 mg of zinc in 0.3*M* hydrochloric acid solution was precipitated with hydrogen sulphide. The precipitate was filtered off immediately, or after one day, two days and four days standing. The precipitates were dried and the peak intensities of Cu $2p_{3/2}$ and Zn $2p_{3/2}$ measured. The results are shown in Fig. 4(a). It is clear that post-precipitation of zinc sulphide on copper sulphide occurs according to the model of Fig. 3.

A mixture of 50.0 mg of lead and 50.0 mg of barium was precipitated as sulphate with an excess of sulphuric acid. The precipitate was filtered off and dried as for the copper/zinc mixture and ESCA measurements were carried out. The results for Ba $4d_{5/2}$ and Pb $4f_{7/2}$ are shown in Fig. 4(b). It can be seen that a solid solution is formed, and there is no post-precipitation.

The precipitation of copper on iron metal in 0.05*M* hydrochloric acid was investigated. In Fig. 5, the results of deposition of copper on iron powder (*ca.* 200-mesh particle size) are shown. The shape of the curve is similar to that obtained for co-precipitation and can be used as a calibration curve for the determination of small amounts of copper in solution after separation by deposition. It was confirmed that ESCA can be applied in the study of precipitation, deposition and other surface phenomena.[10,11]

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INVESTIGATION OF THE USEFULNESS OF NTA, EDTA AND DTPA IN SEPARATION OF SOME PLATINUM METALS ON CELLULOSE EXCHANGERS

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Summary—The possibility of using NTA, EDTA and DTPA as complexing agents for separation of some platinum group ions on cellulose ion-exchangers has been investigated. The greatest differences in the affinities of Pd(II) and Pt(IV) toward the cellulose ion-exchangers are obtained in the presence of DPTA, and Cellex D (as ion-exchanger) in hydroxide form. The column separation of Pd(II) from Pt(IV), Rh(III) from Pd(II) and of a Rh(III)–Pd(II)–Pt(IV) mixture can be achieved with DPTA and chloride solutions. The method can be for determination of the components of Rh–Pd–Pt alloys.

A variety of ion-exchangers can be used for separation of platinum metals. Among others, cellulose exchangers seem to be worthy of special notice. In previous papers[1,2] it was shown that they exhibit lower selectivity than several other exchangers for platinum-metal chloro-complexes. It appeared, however, that from the point of view of column separation, the lower selectivity of cellulose exchangers should be regarded as an advantage. There is no difficulty with quantitive elution of those platinum metals having chloro-complexes which exhibit strong affinity for ion-exchangers [Pt(IV), Pd(II)], though such problems are typical when other resins are used.[3,4] Unfortunately the selectivity differences of cellulose exchangers are insufficient for separation of all pairs of platinum metals in the presence of sodium chloride (or hydrochloric acid). Preliminary investigations have revealed that several separations (*e.g.*, Pd–Pt) are possible with the help of organic complexing agents. Except for thiourea[5] organic agents have not been used so far for platinum-metal separation on cellulose exchangers.

The aim of the work described here was to evaluate the possibility of Rh–Pd, Pd–Pt and Rh–Pd–Pt separation on cellulose exchangers by use of chloride and organic complexes. NTA, EDTA and DTPA were the complexants examined.

EXPERIMENTAL

Exchangers

The cellulose ion-exchangers DEAE (diethylaminoethylcellulose) and TEAE (triethylaminocellulose), supplied by Bio-Rad Laboratories as Cellex D and Cellex T, were used. The specified ion-exchanger capacities were 0.88 meq/g for Cellex D and 0.74 meq/g for Cellex T (we found 0.80 and 0.69 meq/g respectively). The ion-exchangers were used in chloride or hydroxide form. The chloride form was obtained by washing 2 g of ion-exchanger with 50 ml of 0.5*M* hydrochloric acid,[6] followed by doubly distilled water until the pH was 7. The hydroxide form was prepared by treating 2 g with 50 ml of 0.5*M* sodium hydroxide[6] and washing with water until the pH was 7.

Solutions

$RhCl_3.3H_2O$ was dissolved in 10 ml of concentrated hydrochloric acid, then the solution was evaporated to low bulk, diluted to 500 ml with water and standardized gravimetrically with thionalide.[7] An H_2PtCl_6 solution containing 14.14% of platinum was diluted to obtain the Pt(IV) solution, which was standardized by precipitation of the ammonium salt.[8] $PdCl_2$ was dissolved in 10 ml of concentrated hydrochloric acid, then the solution was evaporated to low bulk, diluted to 500 ml, and standardized gravimetrically with dimethylglyoxime.[9]

Columns

Conventional ion-exchange columns (0.98 cm bore, 25 cm long) fitted with Rotaflo TF 12/Cl/13 stopcocks were used.

Determination of metal ion retention, by the static method

The ion-exchanger (200 mg) was mixed with 20 ml of metal ion solution (Pt $2.05 \times 10^{-4}M$, Pd $2.25 \times 10^{-4}M$, Rh $2.45 \times 10^{-4}M$), at a level corresponding to 3–5% of the ion-exchanger capacity, the test solution containing the complexing agent and being adjusted to the desired pH after addition of the exchanger. The mixture was shaken for 24 hr and then the metal-ion concentration in the solution was determined by graphite-furnace AAS under the conditions given in Table 1. Retention of the metal ion was calculated as a fraction of the initial quantity of metal ion in the solution.

Table 1. Conditions for determination of Rh, Pt and Pd by AAS

Element	Wavelength, *nm*	Band-width, *Å*	Sample, *μg*
Rh	340.3	2	5, 10, 20
	350.4		
Pt	266.0	7	20
	273.4	2	10, 20
Pd	244.7	2	10, 20
	340.4		20

Column separation procedure

A column containing 1.4 g of Cellex D (hydroxide form, bed-height 8.5 cm) was loaded with 5 ml of metal chlorocomplex solution at pH 1–2, and eluted at a flow-rate of 0.7 ml/min with the following eluents.

1. For Rh–Pd separation:

   Rh—50 ml of 0.01*M* HCl in 0.1*M* NaCl;
   Pd—100 ml of 1*M* HCl.

2. For Pd–Pt separation:

   Pd—70 ml of 0.01*M* DTPA (pH 8);
   Pt—100 ml of 0.1*M* NaOH.

3. For Rh–Pd–Pt separation:

   Rh—50 ml of 0.01*M* HCl in 0.1*M* NaCl;
   Pd—70 ml of 0.01*M* DTPA (pH 8);
   Pt—100 ml of 0.1*M* NaOH.

RESULTS AND DISCUSSION

The static studies were evaluated by plotting (1) % retention *vs.* pH and (2) % retention *vs.* chloride concentration. A constant molar ratio of metal to ligand (1:100) was used throughout. This method of interpretation gives a more practical measure of the ion-exchanger affinity than conventional analysis of distribution coefficients.

The results of static studies on retention of Pd(II) on the chloride and hydroxide forms of both exchangers are shown in Fig. 1 as a function of sodium chloride and hydrochloric acid concentrations. Results obtained previously[1] for Pt(IV) and Rh(III) are given for comparison, and were selected to correspond to maximum selectivity for the Pd–Pt and Pd–Rh pairs. It can easily be seen that there is no possibility for separation of Pd and Pt, but Rh and Pd should be separable.

The results of static studies on Pd and Pt retention in the presence of NTA, EDTA and DTPA are shown in Figs. 2–4. The Pd retention *vs.* pH curves are similar for EDTA and DTPA. Retention in the presence of EDTA and DTPA is very low at pH > 6, but in presence of NTA is always higher than 30%.

Special attention should be paid to the Pd/NTA plot in Fig. 2. The curve is very characteristic and similar in shape to that for the pH-dependence of the absorbance at λ_{max} for the 1:1 complex.[10] This can be taken as evidence of equilibrium between the exchanger and the Pd–NTA complex (not the chlorocomplex). Low retention of Pd in acid media suggests that protonated (neutral) complexes must be present in the solution. Lower acidity leads to deprotonation of the complexes, and negatively charged complexes appear, which can be of the 1:1, 1:2 or mixed (with OH^-) types.[10,11]

Retention plots for Pd(II) in the presence of NTA resemble in shape the general dependence of retention on ligand concentration suggested by Fronaeus.[12]

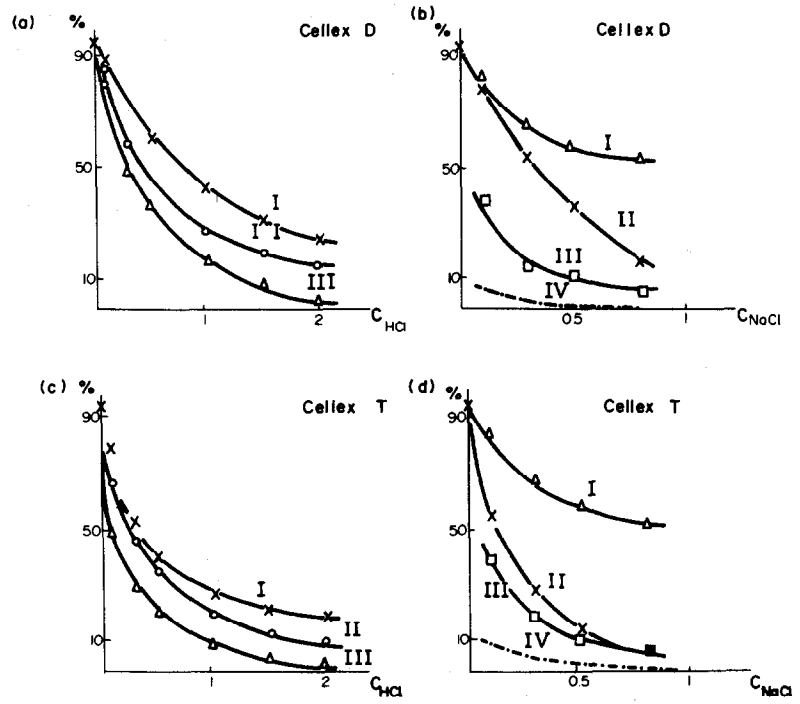

Fig. 1. (a,c) Retention of Pd(II) and Pt(IV) on Cellex D and Cellex T as a function of concentration (mole/l.) of HCl: I—Pd(II) on the exchanger in Cl^- form; II—Pt(IV) on the exchanger in Cl^- form; III—Pd(II) on the exchanger in OH^- form. (b,d) Retention of Pd(II) and Rh(III) on Cellex D and Cellex T as a function of concentration (mole/l.) of NaCl: I—Pd(II) on the exchanger in OH^- form; II—Pd(II) on the exchanger in Cl^- form; III—Rh(III) on the exchanger in OH^- form; IV—Rh(III) on the exchanger in Cl^- form.

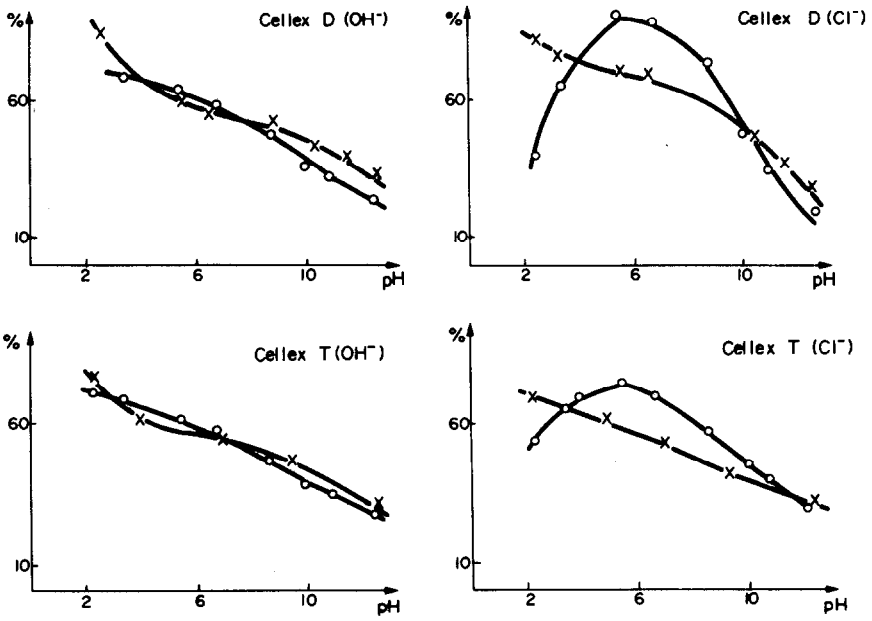

Fig. 2. Retention of Pd and Pt on Cellex D and Cellex T in presence of NTA, as a function of pH. O—Pd(II); × —Pt(IV).

The presence of EDTA and DTPA (Figs. 3 and 4) causes the greater decrease in retention, presumably because the Pd–EDTA and Pd–DTPA complexes are much more stable than the NTA complex.[11]

For Pt(IV) the retention *vs.* pH plots are very similar for all three organic ligands, the retention being greatly decreased from the 100% retention of the chloro-complexes. Pt retention decreases with increase in pH in presence of EDTA or DTPA, but the retention is greater than for Pd(II), because of complex formation between Pt(IV) and EDTA or DTPA. Platinum forms fully protonated complexes at lower pH values,[11,13] which explain the retention of Pt(IV) being higher than that of Pd(II) at low pH values.

There is no consensus about the oxidation number

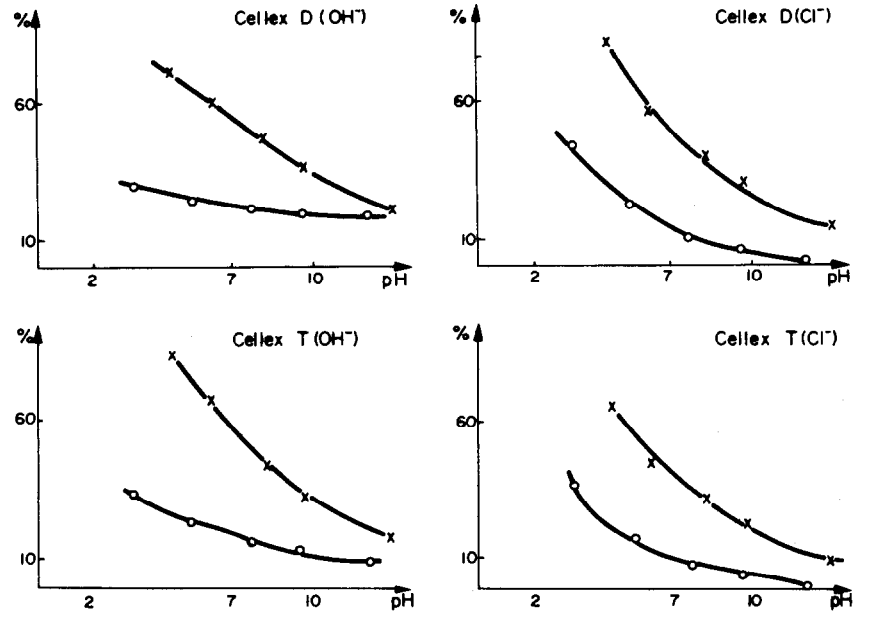

Fig. 3. Retention of Pd and Pt on Cellex D and Cellex T in presence of EDTA, as a function of pH. O Pd(II); × —Pt(IV).

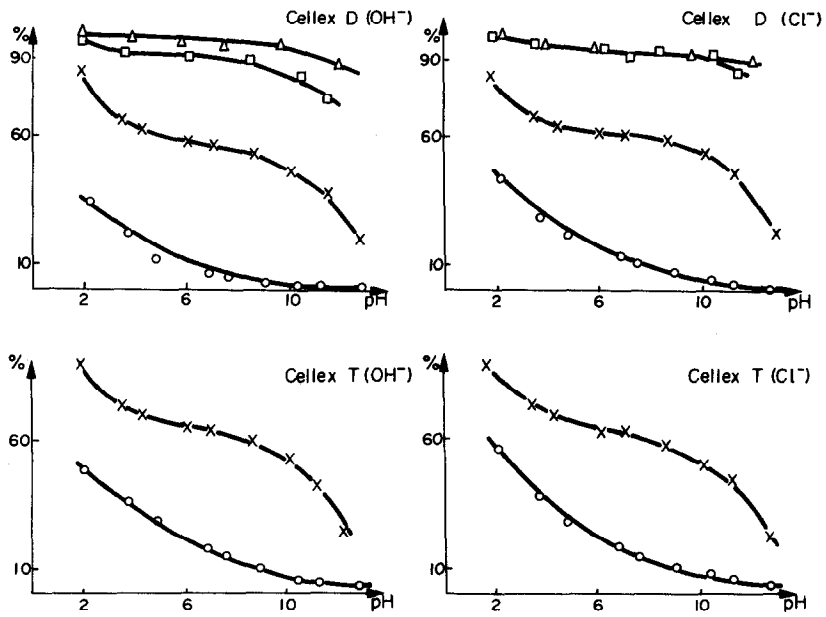

Fig. 4. Retention of Pd and Pt on Cellex D and Cellex T in presence and absence of DTPA, as a function of pH. × —Pt(IV) in presence of DPTA; ○—Pd(II) in presence of DTPA; △—Pd(II) in absence of DTPA; □—Pt(IV) in absence of DTPA.

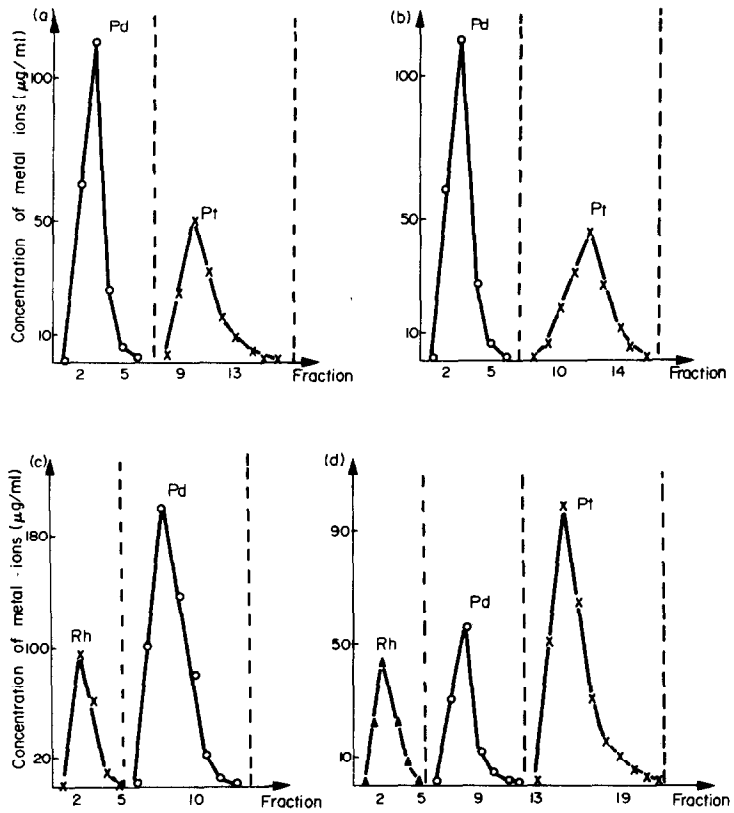

Fig. 5. Elution curves showing the separation of metal ions on Cellex D in OH^- form. Eluents: (a) 70 ml of 0.01*M* DTPA (pH 8) for Pd, 100 ml of 0.1*M* NaOH for Pt; (b) 70 ml of 0.01*M* DTPA (pH 8) for Pd, 100 ml of 0.01*M* DTPA (pH 12.9) for Pt; (c) 50 ml of 0.01*M* HCl–0.1*M* NaCl for Rh, 100 ml of 1*M* HCl for Pd; (d) 50 ml of 0.01*M* HCl–0.1*M* NaCl for Rh, 70 ml of 0.01*M* DTPA (pH 8) for Pd, 100 ml of 0.1*M* NaOH for Pt.

Table 2. Results of separation of Rh(III)–Pd(II) on Cellex D (OH^-)

Taken, *mg*		Eluted, *mg*	
Rh	Pd	Rh	Pd
1.46	2.39	1.46	2.42
		1.44	2.39
		1.43	2.35
		1.47	2.36
1.46	3.59	1.44	3.54
		1.46	3.62
		1.47	3.57
		1.43	3.51
1.46	5.99	1.44	5.92
		1.46	6.03
		1.48	5.97
		1.46	5.92
1.46	9.58	1.42	9.54
		1.46	9.47
		1.41	9.42
		1.47	9.57

Table 3. Results of separation of Pd(II)–Pt(IV) on Cellex D (OH^-)

Taken, *mg*		Eluted, *mg*	
Pd	Pt	Pd	Pt
1.20	5.62	1.21	5.58
		1.20	5.47
		1.18	5.59
		1.17	5.48
5.99	1.87	5.97	1.83
		5.95	1.86
		5.91	1.82
		5.89	1.85
5.99	0.94	5.99	0.95
		5.96	0.92
		5.92	0.91
		5.89	0.94
2.39	1.87	2.38	1.83
		2.39	1.84
		2.33	1.87
		2.34	1.86
1.20	3.74	1.23	3.73
		1.17	3.72
		1.21	3.66
		1.15	3.65

Table 4. Results of separation of Rh(III)–Pd(II)–Pt(IV) on Cellex D (OH^-)

Taken, *mg*			Eluted, *mg*		
Rh	Pd	Pt	Rh	Pd	Pt
0.73	1.20	3.74	0.70	1.16	3.74
			0.69	1.20	3.66
			0.74	1.18	3.68
			0.75	1.22	3.72
0.73	2.39	3.74	0.75	2.36	3.73
			0.70	2.35	3.69
			0.69	2.39	3.67
			0.74	2.38	3.71
0.73	2.39	5.62	0.71	2.35	5.60
			0.75	2.39	5.48
			0.73	2.36	5.59
			0.69	2.38	5.49

Table 5. Determination of composition of Rh–Pd–Pt alloy (Rh–5%, Pd–15%, Pt–80%)

Found, %		
Rh	Pd	Pt
4.9	15.0	81.7
4.8	14.7	81.3
5.0	14.4	79.9
4.9	14.6	80.5
4.8	14.9	79.4
4.9	14.4	80.7
4.9 ± 0.1*	14.7 ± 0.3*	80.6 ± 1.0*

* Mean and range (95% confidence limits).

of platinum in its EDTA and DTPA complexes. Jezierskaya and Kisieleva[14] suggest reduction to Pt(II) occurs in solutions containing EDTA. Static investigations have revealed that the greatest difference between Pd(II) and Pt(IV) retentions is observed in the presence of DTPA when the hydroxide form of Cellex D is used.

The column separation results are in good agreement with the static studies. Rh–Pd separation is possible with the chloro-complexes (Table 2).

Analysis of the static studies shows the impossibility of separation of the Pd(II) and Pt(IV) chloro-complexes. However, this pair can be separated in the presence of DTPA (Table 3). Pt(IV) is easily eluted with 0.1*M* sodium hydroxide.

It is possible that during elution of Pd with DTPA, platinum forms a complex with the organic ligand, because of the catalytic effect of hydroxide ions (the pH is 8) on the substitution process. Such catalysis has been described for the Rh(III) chloro-complex, which is more inert than the Pt(IV) complex.[15] Retention of Pt(IV) chloro-complexes on cellulose anion-exchanger (see Fig. 4) in basic media is quantitative. Dynamic data analysis also established that for elution of Pt(IV) in the presence of DTPA a higher OH^- concentration is needed. As mentioned above, there is a possibility of Pt(IV) reduction during transition into the DTPA-complex.

The static and dynamic results suggest the possibility of separation of the Rh(III)–Pd(II)–Pt(IV) system, and Tables 4 and 5 show the separation to be quantitative. Iridium must be absent.

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SHORT COMMUNICATIONS

IODAMINE-T AS AN OXIDIMETRIC TITRANT IN AQUEOUS MEDIUM

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Summary—Iodamine-T, the iodine analogue of chloramine-T, can be used for the direct oxidimetric titration in aqueous medium of As(III), Sb(III), Tl(I), ascorbic acid, hydroquinone, hydrazine, semicarbazide, thiourea, thiocyanate and sulphite. Back-titration can be used for the determination of sulphide, thiosulphate, dithiocarbamate, xanthate, thiosemicarbazide, Reinecke's salt, mercury(II) tetrathiocyanatocobaltate(II) and mercury(II) tetrathiocyanatozincate (II).

The use of chloramine-T as a titrimetric reagent has been well established by Bishop and Jennings.[1] Dichloramine-T, dibromamine-T and bromamine-T have been developed as oxidimetric titrants by Nair and co-workers.[2–5] No systematic analytical work seems to have been done on iodamine-T, the iodine analogue of chloramine-T, although it was used earlier for the determination of certain phenols by iodination.[6] In this paper, we report on its use for titrations of the types for which chloramine-T and bromamine-T have been used.

EXPERIMENTAL

Reagents

Preparation of iodamine-T.[7] Iodamine-T is the potassium salt of *N*-iodo-*p*-toluenesulphonamide, $CH_3C_6H_4SO_2NIK$. A solution of *p*-toluenesulphonamide (4.5 g) in the minimum quantity of 10% aqueous potassium hydroxide solution is slowly added to a small excess of iodine solution (9 g of iodine and 18 g of potassium iodide in 20 ml of water) and the tri-iodide crystallizes out; 50% potassium hydroxide solution is added dropwise until the solid is taken up, and iodamine-T then separates as a yellow crystalline powder. It is collected, quickly washed with cold saturated potassium chloride solution, pressed between filter papers and dried over phosphorus pentoxide. It is recrystallized from hot water. Analysis of a typical sample gave I 38.6%, N 4.2%, S 9.2%; $CH_3C_6H_4SO_2NIK$ requires I 38.9%, N 4.18%, S 9.55%.

Stock solutions of iodamine-T. Solutions of iodamine-T in water are unstable (precipitation occurs after one day) but can be made fairly stable (no precipitation in 3 months) by addition of alkali. An approximately 0.1*N* (0.05*M*) solution was made by dissolving 17 g of dry iodamine-T in 1 litre of 0.1*M* potassium hydroxide and kept in an amber coloured bottle. A solution kept in a colourless bottle was found to decrease in strength by about 3.3% over a period of 3 months, whereas a solution kept in an amber coloured bottle showed no change. The solution was standardized by the iodometric method suggested for chloramine-T.[1]

Reductants. Standard solutions of antimony(III), ascorbic acid, hydroquinone, hydrazine, semicarbazide, thiourea, thiocyanate, sulphite, sulphide, thiosulphate, dithiocarbamate, xanthate, thiosemicarbazide and Reinecke's salt were prepared by dissolving known amounts in water and making up to standard volumes. Arsenic(III) solutions were prepared by dissolving known amounts of arsenious oxide in 1*M* sodium hydroxide, neutralizing with 1*N* sulphuric acid and making up to known volume with water. Solutions of thallium(I) were prepared by dissolving known weights of thallous carbonate in the minimum of acetic acid and making up to volume with water. The strengths of these solutions were checked by standard methods.[8,9] Mercury (II) tetrathiocyanatocobaltate(II) and mercury(II) tetrathiocyanatozincate(II) were prepared by the literature method.[9] Their purity was determined by the total sulphur estimation employing wet oxidation in acid medium.[9]

Procedures

Direct titration with starch as indicator. To a measured volume (5–20 ml) of reductant solution (except sulphite) an equal volume of saturated mercuric chloride solution (if needed), 10 ml of concentrated hydrochloric acid and 1 ml of 1% starch solution were added. The solution was diluted to 100 ml and titrated with iodamine-T solution until blue. For sulphite, a reverse titration was used, a known volume (10–20 ml) of iodamine-T solution being mixed with 10 ml of concentrated hydrochloric acid and 1 ml of 1% starch solution, diluted to 100 ml and titrated with sulphite solution till colourless.

Two-phase indication titration. To a measured volume (10–25 ml) of the reductant solution (except sulphite) an equal volume of saturated mercuric chloride solution (if needed), 10 ml of concentrated hydrochloric acid and 5 ml of carbon tetrachloride were added and the reaction mixture was diluted to 100 ml. The mixture was titrated with iodamine-T solution with vigorous shaking after each addition till the permanent faint pink appeared in the organic layer. For sulphite a reverse titration was used.

Back-titration. To a measured volume (40 ml) of iodamine-T solution a known volume (5–20 ml) of reductant solution or known weight (10–30 mg) of insoluble reductant, 10 ml of 2*M* potassium hydroxide and 10 ml of saturated mercuric chloride solution (if needed) were added, and the mixture was heated at 60° on a water-bath, or kept at room temperature, for 10–15 min, then acidified with 10 ml of 5*M* hydrochloric acid. Finally 20 ml of 10% potassium iodide solution were added and the iodine liberated was titrated with thiosulphate solution, starch being added as indicator near the end point.

Table 1. Titrimetric determinations with iodamine-T

Reductant	Titrimetric method*	Range of reductant taken, *mmole*	No. of experiments	Standard deviation, *μmole*	Maximum error, %
As(III)	(a)	0.27–0.84	10	2.54	0.4
	(b)	0.24–0.79	10	2.30	0.4
Sb(III)	(a)	0.28–0.78	10	1.85	0.5
	(b)	0.27–0.84	10	2.06	0.3
Tl(I)	(a)	0.41–0.79	10	1.15	0.2
	(b)	0.20–0.79	10	2.58	0.4
Ascorbic acid	(a)	0.27–0.98	10	2.65	0.5
	(b)	0.23–0.94	10	1.92	0.3
Hydroquinone	(a)	0.48–0.97	10	2.41	0.4
	(b)	0.28–0.82	10	2.72	0.4
Hydrazine	(a)	0.24–0.55	10	1.43	0.3
	(b)	0.24–0.57	10	1.74	0.4
Semicarbazide	(a)	0.23–0.57	10	2.07	0.4
	(b)	0.21–0.46	10	1.44	0.3
Thiourea	(a)	0.12–0.26	10	2.47	0.4
	(b)	0.13–0.29	10	1.69	0.4
Sulphite	(a)	0.41–0.79	10	2.13	0.5
	(b)	0.49–1.05	10	1.05	0.3
Thiocyanate	(b)	0.20–0.47	10	1.59	0.4
Sulphide	(c)	0.04–0.15	8	2.52	0.5
Thiosulphate	(c)	0.01–0.13	8	6.72	0.3
Dithiocarbamate	(c)	0.01–0.10	8	1.81	0.6
Xanthate	(c)	0.02–0.08	8	3.26	0.5
Thiosemicarbazide	(c)	0.01–0.12	8	6.52	0.6
Reinecke's salt	(c)	0.03–0.09	8	2.86	0.4
$Hg[Co(SCN)_4]$	(c)	0.03–0.09	6	2.80	0.3
$Hg[Zn(SCN)_4]$	(c)	0.02–0.07	6	4.24	0.5

* (a) Direct, starch indicator; (b) direct, two-phase indication; (c) back-titration.

In all cases, blanks were done concurrently under identical conditions, but blank corrections were found to be negligible.

RESULTS AND DISCUSSION

Statistical data for all three methods are given in Table 1. Direct titration with starch as indicator was successful only for arsenic(III), antimony(III), thallium(I), ascorbic acid, hydroquinone, hydrazine, semicarbazide, thiourea and sulphite, and the two-phase indication titration was successful for the same reductants and also for thiocyanate. Only the back-titration method was suitable for the other reductants tested.

The reduction half-reactions of iodamine-T in acid and alkaline media are:

$$RNI^- + 2H^+ + 2e^- \rightarrow RNH_2 + I^- \text{ (acid medium)} \quad (1)$$

$$RNI^- + 2H_2O + 2e^- \rightarrow RNH_2 + I^- + 2OH^- \text{ (alkaline medium)} \quad (2)$$

where $R = CH_3C_6H_4SO_2-$

In the direct titrations mercuric chloride must be added except in the case of ascorbic acid and sulphite, and for sulphite a reverse titration method is necessary. In the back-titration method heating at 60° for 10–15 min is needed for sulphide, thiosulphate, dithiocarbamate, xanthate and thiosemicarbazide; there is no need to add mercuric chloride. The thiocyanate complexes react at room temperature but require the addition of mercuric chloride. All the direct titrations are done in acid medium, and the back-titrations in alkaline medium.

As expected from the redox half-reactions, 2 equivalents of oxidant are consumed per mole of arsenic(III), antimony(III), thallium(I), ascorbic acid, hydroquinone and sulphite; 4 equivalents for hydrazine and semicarbazide; 6 equivalents for thiocyanate; 8 equivalents for thiourea, sulphide and thiosulphate; 12 equivalents for thiosemicarbazide; 14 equivalents for dithiocarbamate and xanthate; and 24 equivalents for Reinecke's salt, and mercury(II) tetrathiocyanatocobaltate(II) and mercury(II) tetrathiocyanatozincate(II) (each of these three complex thiocyanates contains 4 moles of thiocyanate per mole).

It may be noted that hydrazine nitrogen is oxidized to elemental nitrogen, whereas amino-group nitrogen is unaffected. Sulphur in the sulphur compounds is oxidized to sulphate, while thiocyanate is oxidized to sulphate and cyanide as with bromamine-T.[5] The function of the mercuric chloride added in these titrations is probably to remove the iodide formed during the reactions and hence to shift the reaction in the

forward direction. The formal redox potential of the iodamine-T/*p*-toluenesulphonamide couple at 30° was found to be +1.059 V at pH 3 and +0.506 V at pH 10.

Attempts to use di-iodamine-T failed because of instability of the compound.

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A RAPID AND SELECTIVE METHOD FOR DIRECT GRAVIMETRIC DETERMINATION OF VANADIUM(V) AND ITS APPLICATION TO ALLOY STEEL AND SOME ROCKS

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Summary—*N-o*-Iodobenzoyl-*o*-tolylhydroxylamine has been synthesized and used as gravimetric reagent for direct determination of vanadium(V). The complex dried at 105–120° has the definite composition $VO(C_{14}H_{11}O_2NI)_2Cl$. The method is simple, rapid, sensitive and selective and gives results reproducible within 0.1%. Precipitation is quantitative at room temperature over a wide range of acidity. The method is satisfactory for alloy steels and some rocks. The very low conversion factor (0.0632) is favourable for determining very small amounts of vanadium.

Few inorganic reagents have been recommended for direct gravimetric determination of vanadium(V). The silver vanadate method[1,2] is not at all selective and suffers from many disadvantages. The mercurous vanadate method[3] calls for ignition to V_2O_5 before weighing, which requires precautions to be taken against the poisonous mercury vapour produced during the ignition; this method is very unselective and time-consuming. The volatility of V_2O_5 at certain temperatures, yielding non-reproducible results, is a serious drawback for all the ignition methods.

In recent years various organic reagents, particularly BPHA (*N*-benzoylphenylhydroxylamine) and its analogues, have been extensively employed for the determination of metal ions. BPHA and its analogues have not been used for gravimetric determination of vanadium by direct weighing of the complex even though most of the disubstituted hydroxylamine derivatives form water-insoluble metal chelates with vanadium(V). This is mainly due to their thermal instability even at 100°, appreciable solubility of the chelate in most organic solvents, and insolubility of the reagent at the working pH.

The present paper describes a simple, rapid and highly selective method for vanadium(V) over range of acidity at room temperature, by means of a newly synthesized reagent, *N-o*-iodobenzoyl-*o*-tolylhydroxylamine. The method is sensitive, vanadium(V) being determined with reasonable accuracy at concentrations as low as 20 mg/l. The method works satisfactorily for vanadium in alloy steels and rocks.

EXPERIMENTAL

Preparation and properties of the chelating agent

First *o*-iodobenzoyl chloride was prepared by treatment of 25 g of *o*-iodobenzoic acid with a slight excess of thionyl chloride under reflux on a water-bath for 60–90 min. When the reaction was over, 20–30 ml of benzene were added and the solvent and excess of thionyl chloride were distilled under reduced pressure. The residue in the flask was *o*-iodobenzoyl chloride. An ethereal solution of *N-o*-tolylhydroxylamine, prepared according to Pal and Majumdar,[4] was taken in a 600-ml beaker and 200 ml of water were added to it. The mixture was made slightly alkaline with sodium bicarbonate and kept in an ice-bath. An excess of *o*-iodobenzoyl chloride was then added very slowly from a dropping funnel, with thorough mechanical stirring. Sodium bicarbonate was added from time to time to keep the mixture alkaline. When the reaction was over (*i.e.*, the mixture failed to blacken Tollen's reagent), addition of the acid chloride was stopped and stirring was continued for another 30 min. The ethereal layer was separated and the ether evaporated. The liquid was decanted and the residue triturated with 10% sodium bicarbonate solution to remove entrapped and adhering acid chloride. The aqueous layer was decanted and the residue was washed several times with water and then extracted with concentrated ammonia solution 3 or 4 times. The extract was added slowly, dropwise, to ice-cold 4*M* hydrochloric acid with stirring, and *N-o*-iodobenzoyl-*o*-tolylhydroxylamine (IOBOTHA) separated out as snow-white crystals which were filtered off and washed with petroleum ether (b.p. 40–60°). The hard lump thus obtained was ground, then dissolved in glacial acetic acid; next 2 ml of petroleum ether were added dropwise, followed by water until a turbidity appeared. The product was allowed to settle and was filtered off (m.p. 119–120°). Found: C 47.7%, H 3.3%, N 4.0%, I 35.9%; $C_{14}H_{12}O_2NI$ requires: C 47.74%, H 3.34%, N 3.98%, I 35.9%.

The structural formula is given (Fig. 1)

The reagent is insoluble in water but highly soluble in acetic acid and most organic solvents. A 1% solution in glacial acetic acid was used as reagent.

Standard solutions

A standard solution of vanadium(V) was prepared by dissolving 2.699 g of ammonium metavanadate in dilute ammonia, diluting to 500 ml and standardizing by the Mohr[5] and EDTA[6] methods. Standard solutions of niobium, tantalum, titanium, zirconium and hafnium were prepared by fusing about 150 mg of the "Specpure" oxide (Johnson Matthey) with 1.5 g of potassium bisulphate and dissolving the cooled clear melt in 100 ml of 5% tartaric acid solution, and standardized gravimetrically.[4,7]

For solutions of other cations (metal concentration ~ 10 mg/ml), the nitrates, chlorides and sulphates were used:

tartaric acid or oxalic acid was added to the solutions of those metal ions (*e.g.*, As, Sb, Bi) which hydrolyse.

Procedure

A known volume of the standard solution containing 2–25 mg of vanadium was diluted to 100 ml with enough concentrated hydrochloric acid to make the acid concentration between 4 and 40% v/v. About 10 ml of glacial acetic acid were added and the 1% reagent solution was added dropwise with stirring until no further precipitation was observed, and then 1–2 ml in excess. The mixture was stirred for 15–20 min to complete the coagulation. The precipitate was filtered off on a previously weighed sintered-glass crucible (porosity 3), washed with 100–150 ml of 1–3% acetic acid and finally thrice with 15–20 ml portions of distilled water to remove any free reagent and acetic acid. The precipitate was dried at 105–110° to constant weight. The conversion factor is 0.0632.

Separation of vanadium from molybdenum

Aliquots of standard solutions of vanadium(V) and molybdenum(VI) were mixed and diluted to 100–150 ml with water and hydrochloric acid was added to give an acidity of 1–3*M*, followed by sodium metabisulphite (1 g) to reduce molybdenum and vanadium quantitatively. The mixture was then treated with a slight excess of the 1% reagent solution in acetic acid. Molybdenum was quantitatively precipitated. Vanadium remained in solution [V(IV) does not give any precipitation with the reagent]. The molybdenum complex was filtered off, then the filtrate boiled to remove SO_2, and after cooling was again filtered. In the filtrate V(IV) was oxidized to V(V) with permanganate solution in 3*N* sulphuric acid. The acidity of the solution was then adjusted to 10–20% v/v hydrochloric acid, and 10 ml of acetic acid were added followed by 1% acetic acid solution of the reagent. The mixture was stirred for 10–20 min and the violet precipitate was filtered off on a porosity-3 sintered-glass crucible and washed with 1% acetic acid. The complex was dried at 110° to constant weight and weighed as $VO(C_{14}H_{11}O_2NI)_2Cl$.

Separation of tungsten from vanadium

The mixture containing vanadium(V) and tungsten(VI) was treated with concentrated hydrochloric acid to precipitate tungsten as tungstic acid which was filtered off and washed. When the W(VI) is in large excess, double precipitation is necessary. The filtrate was reduced to minimum volume and then 200 mg of sodium fluoride dissolved in 10–25 ml water were added. The acidity was adjusted to 10–20% v/v hydrochloric acid and vanadium(V) was determined as described in the general procedure.

Determination of vanadium in steel

Weigh about 0.5 g of steel accurately into a 250-ml Erlenmeyer flask and heat carefully with 50 ml of 5*N* sulphuric acid until the sample dissolves. Add conc. nitric acid (5 ml) dropwise to the hot solution to dissolve any black particles. Place a small funnel in the neck of the flask, heat to fuming and fume for half an hour on a hot asbestos board, cool, add 25 ml of water and boil for few min. Cool, then filter off the WO_3 and MoO_3 and wash with water. Precipitate any remaining molybdenum after reduction with sodium metabisulphite as already described. Filter off the precipitate and wash with water. Continue as described for separation of molybdenum from vanadium, adding sodium fluoride to mask any tungsten that has escaped precipitation.

Determination of vanadium in rock samples

Transfer about 1–4 g of finely ground (100-mesh) rock sample into a nickel crucible containing a bed prepared by melting of 6 g of sodium hydroxide and 2 g of sodium carbonate. Add a pinch of potassium nitrate, cover the mixture with sodium carbonate and heat to low redness until fusion begins, then increase the heat until a clear melt is obtained. Leach the cooled melt with boiling water and quantitatively transfer to a 250-ml beaker. Filter, neutralize with hydrochloric acid and precipitate titanium and iron as hydroxides. Use a double precipitation for large amounts of titanium and iron. Oxidize the filtrate and washings with permanganate, add sodium fluoride as masking agent for residual titanium, adjust the hydrochloric acid concentration to 10–30% v/v, and determine the vanadium as already described.

RESULTS AND DISCUSSION

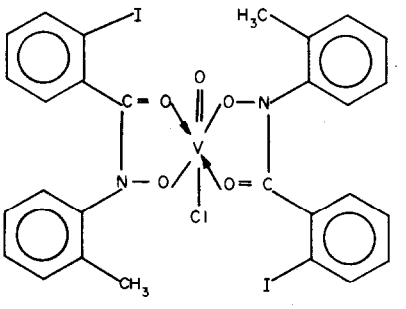
Effect of acidity

Of the mineral acids, hydrochloric acid is found to be best because the chelate is easily coagulated, there is no oxidative decomposition of the reagent at higher acid concentrations, and precipitation is quantitative over a wide range of acidity (3–25% v/v hydrochloric acid), though the reagent may precipitate at still higher acid concentration. The precipitation can also be done in 1–4*N* sulphuric acid, and is accelerated if chloride is added. If the 1% reagent solution in glacial acetic is diluted 20-fold with 3–30% v/v hydrochloric acid (*i.e.*, 0.4–4*M*) there is no precipitation of reagent even on prolonged standing at room temperature. Hence a glacial acetic acid solution of the reagent is used, to prevent precipitation of the free reagent.

Composition of the complex

The dried blue–violet complex was analysed for vanadium,[5] iodine, chlorine and nitrogen. Found: V 6.26%, I 32.3%, N 3.4%, Cl 4.6%; $VO(C_{14}H_{11}O_2NI)_2Cl$ requires V 6.32%, I 31.5%, N 3.47%, Cl 4.4%.

The structure of the complex is suggested to be



Thermal analysis showed that decomposition begins at 166° and is complete at 490° with a weight loss of 88.8%, in agreement with the formula proposed, if V_2O_5 is the ignition product (Fig. 1).

Interferences

In 10–20% hydrochloric acid medium the following cations and anions were found to cause less than 0.2% error in determination of 9.4 mg of vanadium when present in the amounts given in parentheses: Fe(III), Zn, Hg(II), Pb, Ca, Ba, Sr, Cr(III), Al, Th, Cl^-, $S_2O_8^{2-}$ (200 mg each); acetate (160 mg); Ni, tartrate (150 mg); Cd, oxalate (120 mg); Cu(II), Sb(V), As(III), SCN^-,

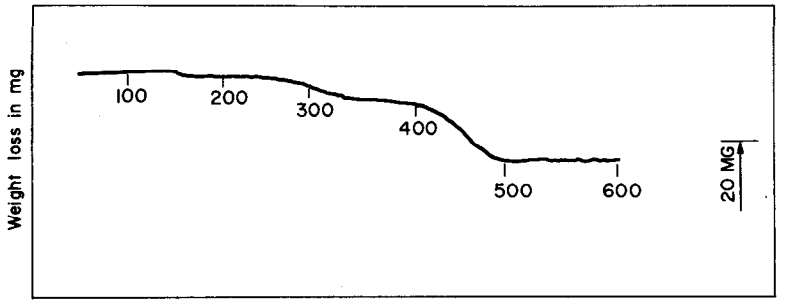


Fig. 1. Thermogravimetric curve for *N*-*o*-iodobenzoyl-*N*-*o*-tolylhydroxyl amine-vanadium(V) compound (blue).

Table 1. Analysis of alloy steel and rock samples

Sample	Composition	Vanadium found, %		
		Present method	Spectrophoto-metric method	Spectrographic method
British Chemical Standard High Speed Alloy Steel BAS 64b.	0.9% C, 4.55% Cr, 4.95% Mo, 7.05% W, 1.99% V,	1.98 2.01 1.98	1.99	—
Hornblendite	Ti, Fe, Sr, V, Ca, Mg, SiO_2 *etc.*	0.090 0.088 0.088	—	0.10
Eastern Ghat rocks	(1) Fe, Al, Ca, Mg, Zn, Cu, V	1.27 1.28	1.30	1.28
	(2) Cu, Pb, Zn, Ni, Co, Mn, Cr, Ba, Ti, Zr, V, Al, Fe	0.030 0.030 0.029	0.035	0.032
	(3) Cu, Pb, Zn, Ni, Co, V, Mn, Cr, Ba, Ti, Zr, Al, Fe	0.029 0.030 0.029	0.032	0.030

PO_4^{3-}, F^-, citrate, EDTA (100 mg); ascorbic acid (60 mg); Pd(II), NO_3^-, ClO_4^- (50 mg). If 200 mg of sodium fluoride are added, 50 mg of Zr and Ti and 15 mg of W(VI) can be tolerated; higher amounts of tungsten require prior separation as tungstic acid, and fluoride should also be added. Mo(VI) (50 mg) requires prior separation, Nb (50 mg) needs prior precipitation as hydroxide, and the error is slightly larger (−0.4%) for 50 mg of Ta (after precipitation as hydroxide). The figure given are those tested and not the maximum amount tolerable.

Applications

Typical results are shown in Table 1.

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COLORIMETRIC DETERMINATION OF DIMETHOATE OR OMETHOATE

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Summary—A simple, sensitive and rapid colorimetric method is described for determining dimethoate [*O,O*-dimethyl-*S*-(*N*-methylcarbanoylmethyl)phosphorodithioate] and omethoate by an enzymatic method using pig liver acetone powder as enzyme source and *p*-nitrobenzenediazonium fluoroborate as the chromogenic reagent. This colorimetric method is more sensitive than non-enzymatic methods. Inhibition can be detected at ng levels and amounts ranging from 50 to 1000 ng of omethoate and from 1 to 10 μg of dimethoate can be estimated.

The enzymatic methods involving cholinesterase (ChE) inhibition for the detection of organophosphorus pesticides on thin-layer chromatograms[1–5] and estimation by fluorimetry,[6] potentiometry[7] and gas-liquid chromatography[8,9] are more sensitive than the non-enzymatic methods.[10–14] Attempts have been made by one of us to simplify further the procedures for measurement of ChE inhibition of organophosphorus pesticides, employing a simple colorimetric method for methyl parathion and phosphamidon which is rapid and sensitive at nanogram levels.[15,16] The same approach is used here for determination of dimethoate or omethoate. "Pig liver acetone powder" is used as the enzyme source, and *p*-nitrobenzene diazonium fluoroborate is used as the chromogenic agent (it gives greater sensitivity than other chromogenic salts[1]). The method takes 30 min to perform, including the preincubation time. The method is sensitive and suitable for dimethoate or omethoate residue estimation in wheat and may find application in clinical, forensic and residue analysis.

EXPERIMENTAL

Reagents

All chemicals were analytical grade. Dimethoate (95%) (Rallis India Ltd., Bombay) was used for preparing acetone solutions of various concentrations. Omethoate was prepared by oxidizing dimethoate on a TLC plate by exposure to bromine vapours[15–17] and extracted repeatedly with acetone. Pig liver acetone powder (Sigma Chemical Co., USA) was homogenized in ice-cold distilled water to give a 0.2% suspension and used immediately as the enzyme source. 1-Naphthyl acetate solution in acetone, 0.1*M*. *p*-Nitrobenzenediazonium fluoroborate solution, 0.4% in acetone. Barbital–hydrochloric acid buffer (0.2*M*, pH 7.4) was prepared with doubly distilled water.

ChE assay

The reaction mixture (1.0 ml) contained 200 μg of enzyme, 0.1 ml of barbital (pH 7.4) and 1–10 μg of dimethoate or 50–1000 ng of omethoate (in acetone). The controls contained distilled water and acetone instead of the pesticides. The reaction mixture was incubated for 10 min at 28°. Then 1 μmole of 1-naphthyl acetate (0.01 ml of its 0.1*M* solution in acetone) was added to each and the reaction mixtures were incubated for exactly 1 min more. The enzymatic reaction was stopped by the addition of 4.0 ml of glacial acetic acid. Immediately, 0.1 ml of 0.4% *p*-nitrobenzenediazonium fluoroborate in acetone was added, the samples were allowed to stand for 10 min and the colour was measured at 500 nm in 1-cm cells. The amount of 1-naphthyl acetate metabolized was read from a standard graph relating absorbance to amount metabolized. The ChE activity of the test sample (with pesticide) and control (without pesticide) were compared and the per cent ChE inhibition was calculated by reference to the control ChE activity as 100%.

Residue estimation

A 50-g sample of wheat free from organophosphorus pesticides was uniformly mixed with different concentrations (Table 2) of dimethoate in 10 ml of acetone. After 48 hr the dimethoate residues were extracted according to the procedure of Mendoza and Shields.[18] The residue extracted was appropriately diluted and dimethoate determined as described above.

RESULTS AND DISCUSSION

The initial rate of the enzyme reaction was studied with respect to time, and substrate and enzyme concentrations, and these factors were appropriately chosen for assaying ChE activity in pig liver acetone powder.

The amount of acetone in the reaction mixture until incubation is complete must not exceed 0.1 ml. Further addition of acetone after the incubation is without effect.

The ChE activity was determined when various amounts of dimethoate and omethoate had been added (Table 1). A least-squares calculation gave a linear calibration with a relative standard deviation of about 2% for the slope; the r.s.d. values for the intercept were 20% for the dimethoate calibration and 2% for the omethoate. The results showed that omethoate is a more powerful inhibitor than its parent compound. This suggests that the method could be made more sensitive for dimethoate by converting the residues into their oxidation products. Thus dimethoate can be estimated as omethoate at the nanogram level by this method. The method developed is much more

Table 1. ChE inhibition* by dimethoate and omethoate

Dimethoate, μg	ChE inhibition,† %	Omethoate, ng	ChE inhibition,† %
1	8.2 ± 1.4	50	16.3 ± 2.2†
2	14.1 ± 1.7	100	19.3 ± 2.0
3	19.9 ± 1.6	200	25.4 ± 2.7
4	27.3 ± 1.0	300	31.4 ± 1.5
5	32.0 ± 1.2	400	37.2 ± 1.3
6	38.2 ± 1.9	500	42.5 ± 0.9
7	44.1 ± 0.8	600	48.4 ± 1.2
8	49.5 ± 1.0	700	51.3 ± 0.5
9	53.0 ± 0.9	800	56.0 ± 1.4
10	58.3 ± 0.9	900	60.5 ± 1.7
		1000	65.3 ± 2.6

* 100% Enzyme activity = 30 μmole of 1-naphthyl acetate metabolized per mg of enzyme per hour.

† Values are means of 4 observations ± range.

Least-squares fitting of the best straight lines gave the equations:

ChE inhibition (%) = 5.6 × dimethoate (μg) + 3.5.
ChE inhibition (%) = 0.051 × omethoate (ng) + 15.4.

For the general equation $y = ax + b$, where a and b are the slope and intercept, the standard deviations for dimethoate were 0.12 for a and 0.8 for b, and for omethoate were 0.0013 for a and 0.8 for b.

Table 2. Estimation of dimethoate residues in wheat

Dimethoate added, μg	Dimethoate recovered,* μg
5	4.9 ± 0.2
8	7.9 ± 0.1
10	10.0 ± 0

* Values are mean of 4 observations ± range.

sensitive than the non-enzymatic colorimetric methods reported,[10–14] which have determination limits ranging from 50 μg to 1 mg. The method is as sensitive as the earlier combination of ChE inhibition with spectrofluorometry,[19,20] but is simpler. The use of a semipurified system such as the liver acetone powder as a source of ChE is more advantageous than use of live enzyme sources because the acetone powder can be instantly used and preserved indefinitely. The need for expensive GLC instruments or spectrofluorimeters is also avoided. The method is not applicable to mixtures of ChE-inhibitors of course, and a preliminary separation is needed for mixed residues.

Dimethoate residues in fortified samples have been estimated by adopting the extraction technique of Mendoza and Shields.[18] Appropriate volumes of extracts of dimethoate and omethoate were analysed (Table 2) and the method was found satisfactory.

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APPLICATION OF SIMPLIFIED COMPLEMENTARY TRISTIMULUS COLORIMETRY TO CHEMICAL KINETICS IN SOLUTION—II

DETERMINATION OF RATE CONSTANTS OF CONSECUTIVE IRREVERSIBLE FIRST-ORDER REACTIONS

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Summary—A simple but highly reproducible method based on simplified complementary tristimulus colorimetry is proposed for determination of the rate constants of two consecutive irreversible first-order reactions. For the binary system the complementary colour points can be calculated in the same manner as in conventional tristimulus colorimetry. Hence the mole fractions of the three reacting species can be calculated as a function of time. Application of the method is illustrated by the assay of xanthine oxidase with hypoxanthine.

Much work has been done on the kinetics of consecutive reactions catalysed by enzymes.[1,2] The main problem in analysis of such systems is determination of the individual rate constants. Several methods have been proposed for resolving the rate constants for consecutive first- or pseudo first-order reactions.[3,4] However, the procedures used are somewhat complicated. In the previous paper,[5] simplified complementary tristimulus colorimetry (SCTS method) was used for determining first- or pseudo first-order rate constants. In the present work the SCTS method is modified in order to determine the individual rate constants of consecutive first- or pseudo first-order reactions. The procedure is simple and gives highly reproducible results. It allows direct analysis of kinetic data and estimation of the rate constants without placing any restriction on the initial concentrations of reactants.

In the SCTS method, the absorption is measured at three wavelengths (u, v and w) suitably chosen from the absorption spectra of the species present. The absorbances at time t are denoted by A_{tu}, A_{tv} and A_{tw}, respectively. The complementary colour point, Q_{tu}, corresponding to wavelength u at time t, is given by

$$Q_{tu} = \frac{A_{tu}}{J} \tag{1}$$

where J is the sum of A_{tu}, A_{tv} and A_{tw}. By Beer's law

$$J = ECl \tag{2}$$

where E, C and l are the overall absorptivity, the analytical concentration and the path-length, respectively. The complementary colour points corresponding to wavelengths v and w are defined similarly.

The colour point of a reaction mixture containing all three reacting species at a given time can be located within the triangle formed by the colour points for the individual species (Fig. 1). It has been shown[5,6] that the colour point Q_{tu}, Q_{tv} can be resolved as described by Flaschka[7] into the colour points lying on the straight lines forming the sides of the triangle. The mole fraction of a species, *e.g.*, R in a mixture of R and P, can be calculated from

$$q = \frac{E_P(Q_{Pu} - Q_{tu})}{E_R(Q_{tu} - Q_{Ru}) + E_P(Q_{Pu} - Q_{tu})} \tag{3}$$

where E_R and E_P are the overall absorptivities, Q_{Ru} and Q_{Pu} the colour point co-ordinates for the species R and P, and Q_{tu} is the colour point at time t for the same wavelength on the side Q_{Ru}, Q_{Pu}. Analogous

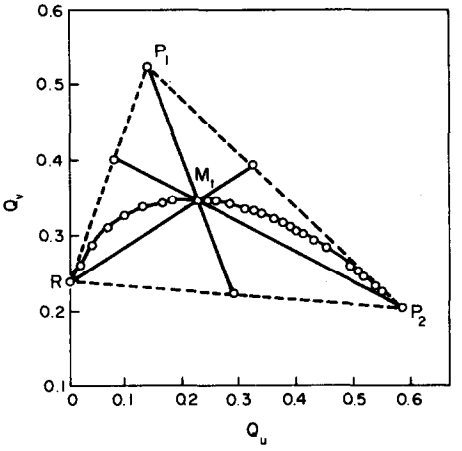

Fig. 1. Q_u–Q_v plot for oxidation of hypoxanthine to uric acid by xanthine oxidase. Complementary colour point: R: hypoxanthine; P_1: xanthine; P_2: uric acid; M_t: reaction mixture of R, P_1 and P_2 at a given time. Reaction solution [$0.75 \times 10^{-4}M$ hypoxanthine, $10^{-4}M$ EDTA, $0.02M$ phosphate buffer (pH 7.5)] contained 0.02 unit of xanthine oxidase in a final volume of 6.0 ml; temperature: 37 ± 0.1°. The following wavelengths were chosen: u: 290 nm; v: 270 nm; w: 250 nm.

equations for the mole fraction can be obtained for wavelengths v and w.

Consider now the consecutive irreversible first-order reactions

$$R \xrightarrow{k_1} P_1 \xrightarrow{k_2} P_2$$

The rate constants k_1 and k_2 are determined by use of the concentrations of the reactant R, the intermediate product P_1 and the final product P_2 and the equations[3]

$$[R]_t = [R]_0 e^{-k_1 t} \tag{4}$$

$$[P_1]_t = \frac{[R]_0 k_1 (e^{-k_2 t} - e^{-k_1 t})}{k_1 - k_2} \tag{5}$$

$$[P_2]_t = [R]_0 + [R]_0 \left(\frac{k_2}{k_1 - k_2} e^{-k_1 t} - \frac{k_1}{k_1 - k_2} e^{-k_2 t} \right) \tag{6}$$

where $[R]_t$, $[P_1]_t$, $[P_2]_t$ and $[R]_0$ are the concentrations corresponding to the reacting species at time t and at the start of the reaction, respectively.

In the triangle formed by the complementary colour points of R, P_1 and P_2, the mole fraction of R at time t is found on the system line corresponding to the R–P_1 mixture and is defined by

$$q_{Rt} = \frac{[R]_t}{[R]_t + [P_1]_t} \tag{7}$$

The colour point of a mixture of R, P_1 and P_2 can be resolved into a new colour point of a mixture of R and P_1 on the corresponding system line (see Fig. 1) and the q_{Rt} value can be calculated by equation (3) from this colour point. The mole fractions $q_{P_1 t}$ and $q_{P_2 t}$ of P_1 and P_2 for this binary system at time t can be similarly estimated with the aid of the colour points on the P_1–P_2 and P_2–R system lines. Then the mole fractions of R, P_1 and P_2 in the ternary system R–P_1–P_2 can be obtained from the values of q_{Rt}, $q_{P_1 t}$ and $q_{P_2 t}$ for the binary systems. Since $[R]_0 = [R]_t + [P_1]_t + [P_2]_t$, the rate constants k_1 and k_2 can be determined by means of equations (4), (5) and (6) with the use of these values.

EXPERIMENTAL

Reagents

Reagents of analytical grade and distilled water were used throughout. Xanthine oxidase (milk EC 1.2.3.2) was purchased from the Boehringer–Mannheim–Yamanouchi Co.

Apparatus

Spectrophotometric measurements and calculations of complementary colour points were made in the manner reported previously.[5]

Procedure

The conversion of hypoxanthine or xanthine into uric acid by xanthine oxidase was followed by the approach described in the literature.[8,9] All the experiments were performed in 0.02M phosphate buffer (pH 7.5) at 37 ± 0.1°.

RESULTS AND DISCUSSION

Xanthine oxidase catalyses the oxidation of hypoxanthine to uric acid, utilizing oxygen as the electron acceptor:[10]

$$\text{Hypoxanthine} + 2H_2O + 2O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Uric acid} + 2H_2O_2$$

In general the enzyme has been assayed by measuring the rate of formation of uric acid. However, it is known that the spectrophotometric measurement at 290 nm of the rate of formation of uric acid is complicated, because the xanthine present as an intermediate absorbs markedly in the region from 230 to 310 nm. If three suitable wavelengths are chosen for the SCTS method, the complementary colour points for the ternary system of hypoxanthine, xanthine and uric acid can be found in the Q_u–Q_v plot, within the triangle formed by the colour points for the individual reac-

Table 1. Summary of results obtained for the apparent pseudo first-order rate constant, $10^2 k_1$ (min^{-1}),* in conversion of hypoxanthine into xanthine by xanthine oxidase†

u \ v§	260 nm	265 nm	270 nm	275 nm	280 nm
280 nm	4.23 ± 0.02	4.23 ± 0.03	4.35 ± 0.03	4.25 ± 0.01	4.25 ± 0.07
285 nm	4.23 ± 0.05	4.24 ± 0.04	4.23 ± 0.02	4.25 ± 0.02	4.23 ± 0.04
290 nm	4.37 ± 0.04	4.25 ± 0.03	4.23 ± 0.07	4.22 ± 0.04	4.33 ± 0.04
295 nm	4.31 ± 0.01	4.25 ± 0.02	4.21 ± 0.04	4.22 ± 0.05	4.25 ± 0.03
300 nm	4.22 ± 0.03	4.23 ± 0.02	4.22 ± 0.04	4.22 ± 0.01	4.80 ± 0.03

* The values of the means and standard deviations are for three determinations. Analysis of variance for crossed two-way classification (A rows, B columns) gave the following results. Variances (degrees of freedom): $s_A^2 = 0.82 \times 10^{-6}$ (4), $s_B^2 = 2.5 \times 10^{-6}$ (4) within groups $s_w^2 = 1.25 \times 10^{-6}$ (16). The F-test performed on the quotients,

$$F^{(A)} = s_A^2/s_w^2 = 0.656, \text{ and } F^{(B)} = s_B^2/s_w^2 = 2.00$$

revealed that the differences are not significant at the 99% level of probability.

† All experiments were carried out at pH 7.5 (0.02M phosphate buffer) and temperature 38 ± 0.1 : $0.75 \times 10^{-4} M$ hypoxanthine; $10^{-4} M$ EDTA; 0.02 units of xanthine oxidase.

§ w: 250 nm.

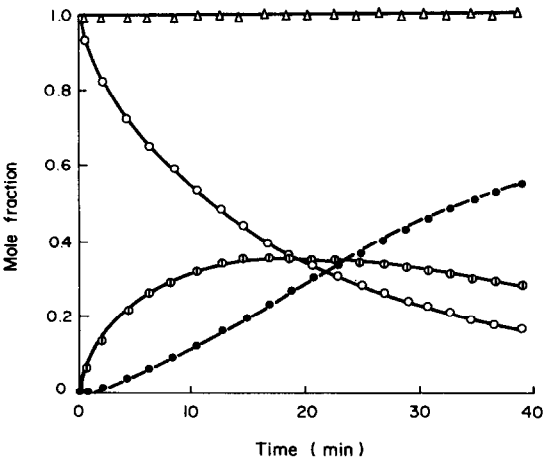

Fig. 2. Mole fraction *vs.* time curves of hypoxanthine, xanthine and uric acid for the hypoxanthine oxidation. The procedures are described in the legend to Fig. 1. The mole fractions were calculated from the complementary colour points by equation (3) as described in the text: —○— hypoxanthine; —◐— xanthine; —●— uric acid; —△— sum.

tion species. In the study, the wavelengths u, v and w chosen were those for the absorption maxima in the spectra of uric acid (290 nm), xanthine (270 nm) and hypoxanthine (250 nm) under the conditions used. Other wavelength combinations could also be chosen.[11] For example, several wavelengths were used for u and v, and the measurements made at them were examined by analysis of variance, which showed systematic errors to be absent (Table 1). A typical Q_u–Q_v plot is shown in Fig. 1. The complementary colour points for a reaction mixture of hypoxanthine, xanthine and uric acid as a function of time were resolved into the colour points on the hypoxanthine–xanthine, xanthine–uric acid and uric acid–hypoxanthine system lines by the procedure above, and the mole fractions of the three species as a function of time were calculated from their colour points by equation (3). The results are shown in Fig. 2. The apparent first-order rate constants of the two steps in the reaction were determined by equations (4), (5) and (6) from the mole fractions of the three species. The initial velocity for the oxidation of hypoxanthine to xanthine by xanthine oxidase under the given conditions could also be calculated (Table 2). The enzyme calibration curve, which was prepared by applying the method to a series of standard xanthine oxidase solutions covering the range 0.005–0.01 units, was linear and passed through the origin. For a check on the accuracy, the conversion of xanthine into uric acid by the same enzyme was examined under the same conditions and the initial velocity for the oxidation of xanthine was determined. Table 3 compares the present method for assay of xanthine oxidase by xanthine with the other method.[12] The coefficient of correlation for the two methods was 0.989, and the results showed close agreement between the two methods.

Table 2. Apparent pseudo first-order rate constant and initial oxidation rate for xanthine in conversion of hypoxanthine into uric acid*

Wavelength	Apparent pseudo first-order†§ rate constant, 10^2k_1, min^{-1}	Initial oxidation rate†, $\mu mole.l^{-1}.min^{-1}$
u (290 nm)	5.01 ± 0.04	3.76 ± 0.03
v (270 nm)	5.01 ± 0.02	3.75 ± 0.02
w (250 nm)	4.99 ± 0.06	3.74 ± 0.01

* Reaction solutions consisted of $0.75 \times 10^{-4}M$ hypoxanthine, $10^{-4}M$ EDTA and 0.02 unit of xanthine oxidase in a final volume of 6.0 ml. All experiments were carried out at pH 7.5 (0.02M phosphate buffer) and temperature 37 ± 0.1°.

† Values are the mean ± SD of five determinations.

§ Xanthine: E: 1.509×10^4 l.mole^{-1}.cm^{-1}; Q_u: 0.131; Q_v: 0.526; Q_w: 0.344. Uric acid: E: 1.953×10^4 l.mole^{-1}.cm^{-1}; Q_u: 0.577; Q_v: 0.205; Q_w: 0.217.

Table 3. Comparison of the present assay method with another method

Method*	Initial oxidation rate†, $\mu mole.l^{-1}.min^{-1}$		Reference
A	u (290 nm)	3.71 ± 0.13	This paper
	v (270 nm)	3.72 ± 0.11	
	w (250 nm)	3.66 ± 0.14	
B		3.70 ± 0.20	(12)

* (A) Reaction solutions consisted of $0.73 \times 10^{-4}M$ xanthine, $10^{-4}M$ EDTA and 0.02 unit of xanthine oxidase in a final volume of 6.0 ml. (B) Reaction solutions consisted of 0.5×10^{-6}M xanthine, $10^{-7}M$ EDTA, 1.0 ml of 0.1% gelatin solution, 1 mg of nitro blue tetrazolium, 1.0 ml of phenazine methosulphate and 0.02 unit of the enzyme in a final volume of 3.5 ml. The rate was followed by an increase in absorbance at 545 nm. All the experiments were carried out as described in the legend to Table 2.

† Values are the mean ± SD of five determinations.

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ANALYTICAL DATA

STABILITY CONSTANTS AND THERMODYNAMIC FUNCTIONS OF MOLYBDENUM AND URANIUM CHELATES FORMED WITH DL-α-AMINOBUTYRIC ACID

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Summary—The stability constants and thermodynamic functions involved in the formation of Mo(VI) and U(VI) chelates with DL-α-aminobutyric acid have been determined potentiometrically. It is observed that in the case of Mo(VI) system three chelates ML, ML_2 and ML_3 are formed stepwise, whereas in the U(VI) system only two chelates ML and ML_2 are formed before precipitation occurs, and both steps occur almost simultaneously. Results show that entropy makes a predominant contribution to the stability of both Mo(VI) and U(VI) chelates.

The metal chelates of α-aminobutyric acid with some transition metal ions were studied earlier by various workers[1–10] and their stability constants and thermodynamic data are available. In previous publications[11–13] from this laboratory the stability constants of some amino-acid chelates were reported. Their formation was studied potentiometrically by the Irving and Rossotti method.[14] This paper gives the results of a similar study of molybdenum(VI) and uranium(VI) chelates formed with DL-α-aminobutyric acid. Their stability constants, and thermodynamic parameters have been determined.

RESULTS AND DISCUSSION

The protonation constants at 25° and 0.1*M* ionic strength (KNO_3) are log $K_1 = 9.50$ and log $K_2 = 2.30$.

The pH-titrations show that in the U(VI) chelate systems, precipitation starts from pH ~5.5, whereas for Mo(VI) the solutions remain clear throughout the titration.

The formation curves ($\bar{n}$ *vs.* pL) show that for Mo(VI) $\bar{n}$ approaches a maximum value of 3 but for U(VI) $\bar{n}$ approaches a maximum value of 2 (before precipitation occurs).

Analysis of the formation curve shows that for the Mo(VI) chelates the K_1 and K_2 values are rather close ($K_1/K_2 \sim 10$) whereas K_3 may be taken to be almost independent of K_1 and K_2 ($K_2/K_3 \sim 10^{3.7}$). Hence, the whole formation curve can be resolved into two regions ($0 < \bar{n} < 2$ and $2 < \bar{n} < 3$) which can be treated separately. The value of K_3 can be read directly from the curve [log K_3 equals $pL_{(\bar{n}=2.5)}$]. It can also be computed by the average-value method in the second region. For calculation of K_1 and K_2 the first region can be taken as a system for which $N = 2$, and the values of K_1 and K_2 computed by the correction-term method. Alternatively the approximate constants obtained by the half-$\bar{n}$ method can be further refined by successive approximation.

For the U(VI) chelates the K_1 and K_2 values are very close, log K_1 − log K_2 being <0.5. Albert[15] has pointed out that under such conditions K_1 and K_2 cannot satisfactorily be computed separately; instead, log β_2 values may be obtained from the relation:

$$\log \beta_2 = \log \bar{n} - \log(2 - \bar{n}) - 2 \log[L]$$

Hence in this case log β_2 values were calculated in this way. However, attempts were also made to compute K_1 and K_2 separately by the use of the least-squares method, which gave quite satisfactory results, the value of log K_1 + log K_2 thus obtained being in good agreement with log β_2 calculated from Albert's equation.

In Tables 1–3, the protonation and stability constant data at different temperatures and ionic strengths are reported. Stability constants at zero ionic strength were obtained by extrapolation (log K *vs.* $\sqrt{\mu}$). The results show that the stability constants decrease with increasing temperature and ionic strength.

Thermodynamic parameters for the chelate systems are reported in Table 4. ΔH values were obtained by the temperature-coefficient method and the values of ΔG and ΔS were calculated in the usual way.

The results show that the entropy term makes

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Table 1. Stability constants of Mo(VI) and U(VI) chelates with DL-α-aminobutyric acid ($\mu = 0.1M$ KNO_3, temp. 25°)

Cation	pH-range for $\bar{n}$-calculations	Computational method	log K_1	log K_2	log K_3	log β_n
Mo(VI)	3.2–9.5	Half-$\bar{n}$ values	8.31	7.30	3.62	—
		Average-value method for K_3 only ($2 < \bar{n} < 3$)	—	—	3.62	—
		Correction-term method ($0 < \bar{n} < 2$)	8.15	7.46	—	—
		Successive approximation method	8.16	7.45	3.62	19.23 ± 0.01
U(VI)	3.7–5.3	Half-$\bar{n}$ values	7.78	7.27	—	—
		Albert's equation	—	—	—	15.09
		Least-squares method	6.48	8.59	—	15.07 ± 0.02

Table 2. Stability constants of Mo(VI) and U(VI) DL-α-aminobutyrate chelates at different temperatures ($\mu = 0.1M$ KNO_3)

Cation		20°	25°	30°	35°	40°
H^+	log K_1	9.63	9.50	9.34	9.19	9.05
	log K_2	2.34	2.30	2.25	2.22	2.20
Mo(VI)	log K_1	8.21	8.16	8.11	8.05	8.00
	log K_2	7.48	7.45	7.42	7.40	7.37
	log K_3	3.64	3.62	3.59	3.56	3.54
U(VI)	log K_1	6.54	6.48	6.42	6.36	6.31
	log K_2	8.62	8.59	8.55	8.52	8.48

Table 3. Stability constants of Mo(VI) and U(VI) DL-α-aminobutyrate chelates at different ionic strengths (KNO_3; 25°)

Cation		$\mu = 0.2$	$\mu = 0.1$	$\mu = 0.05$	$\mu = 0.02$	$\mu = 0.00$*
H^+	log K_1	9.52	9.50	9.45	9.36	—
	log K_2	2.30	2.30	2.28	2.25	
Mo(VI)	log K_1	8.15	8.16	8.18	8.21	8.27
	log K_2	7.44	7.45	7.46	7.49	7.55
	log K_3	3.62	3.62	3.63	3.65	3.70
U(VI)	log K_1	6.46	6.48	6.51	6.55	6.64
	log K_2	8.58	8.59	8.61	8.65	8.73

* Extrapolated values at zero ionic strength (log K *vs.* $\sqrt{\mu}$).

Table 4. Thermodynamic parameters for Mo(VI) and U(VI) DL-α-aminobutyrate chelate systems (temp. 25°, $\mu = 0.1M$ KNO_3)

Cation	$-\Delta H_{\beta_1}$	$-\Delta H_{\beta_2}$	$-\Delta H_{\beta_3}$	$-\Delta G_{\beta_1}$	$-\Delta G_{\beta_2}$	$-\Delta G_{\beta_3}$	ΔS_{β_1}	ΔS_{β_2}	ΔS_{β_3}
Mo(VI)	4.5	6.7	8.9	11.14	21.30	26.24	25	53	64
U(VI)	4.9	7.8	—	8.84	20.56	—	13	43	—

ΔH and ΔG in kcal/mole; ΔS in cal.mole^{-1}.deg^{-1}.

dominant contribution to the stability of both chelates.

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EQUILIBRIUM STUDIES OF β-DIKETOARYL AZO COMPOUNDS WITH COBALT(II), NICKEL(II) AND COPPER(II)

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Summary—The interactions of some β-diketoaryl azo substituted compounds with Co(II), Ni(II) and Cu(II) are discussed. The non-protonated form of the ligand is the main complexing species. The stability constants have been evaluated. The effect of substituent groups on the complexing ability of the ligands is discussed.

Although there has been interest in the synthesis and dye properties of β-diketoaryl azo compounds,[1–7] nothing has been published concerning their structure and the stability of their metal chelates. This paper reports the results of potentiometric and spectrophotometric studies of the compounds of cobalt(II), nickel(II) and copper(II) with a series of β-diketoaryl azo compounds.

EXPERIMENTAL

Reagents

Ligands. Benzoylacetone was coupled with the required diazonium salts in cold sodium hydroxide solution.[4] The crude products were recrystallized from ethanol. The physical constants of the compounds prepared agreed with published values.[7] Stock reagent solutions (0.1–0.15*M*) were prepared by dissolving a known weight of each ligand in ethanol.

Metal ion stock solutions. Prepared from the chlorides and standardized complexometrically.[8]

Apparatus

The Radiometer 28 pH-meter used was calibrated with two standard buffers at pH 4.01 and 7.00. The pH-meter readings (*B*) recorded in 60% v/v ethanol medium were converted into hydrogen-ion concentrations by the relation

$$-\log[H^+] = B + \log U_H$$

where U_H is the correction factor for the solvent composition and ionic strength. The ionic strength was kept at 0.10 with hydrochloric acid and sodium chloride. A value of 0.25 ± 0.01 was obtained for log U_H in 60% ethanol medium ($\mu = 0.10$) at 25°.

Procedure

The stability constants were determined spectrophotometrically by mixing solutions containing the ligand and the metal salt and keeping the ionic strength at 0.1*M* by addition of sodium chloride. The pH was measured and the spectrum was recorded against either the ionic medium or 60% aqueous ethanol (both gave the same absorbances). The pH was increased by dropwise addition of concentrated carbonate-free sodium hydroxide solution made up in the same solvent. The total change in volume did not exceed 1%, so no correction was made to the total concentrations of ligand and metal ion.

The ligands were titrated with sodium hydroxide solution at controlled ionic strength in presence and absence of the metal salt.

RESULTS AND DISCUSSION

The acid–base equilibria of the ligands in aqueous ethanol solution may be represented by the following scheme:

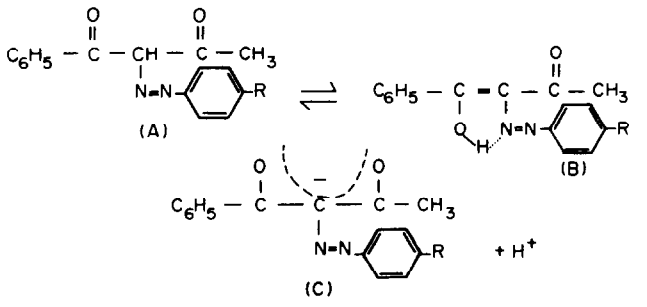

Table 1. Acid dissociation constants (pK^*) of β-diketoarylazo compounds and stability constants ($\log \beta_n$) of their constants in 60% ethanol–water at 25 ± 0.1°C and $\mu = 0.10$

Substituent	pK^*	Co(II)			Ni(II)			Cu(II)		
		pK_1	pK_2	$\log \beta_2$	pK_1	pK_2	$\log \beta_2$	pK_1	pK_2	$\log \beta_2$
H	10.50	5.65	6.27	11.92	5.30	6.0	11.3	5.5	7.75	13.25
p-NO_2	9.88	5.00	5.60	10.60	4.70	5.25	9.95	4.75	7.4	12.15
p-OCH_3	10.02	5.15	5.3	10.35	4.9	5.4	10.3	5.1	7.5	12.45
p-Br	10.25	5.4	5.85	11.25	5.1	5.6	10.70	5.4	7.45	12.85
p-CH_3	10.60	5.73	6.42	12.15	5.43	5.97	11.40	5.6	7.8	13.40

The substituent R was varied to show any inductive and conjugative effects on the electronic character of the ligand.

The overall acid dissociation constant, K^*, is defined by the equations:

$$HL \rightleftharpoons H^+ + L^-$$

$$K^* = [H^+][L^-]/[HL]$$

where HL and L^- stand for the undissociated ligand and its carbanion-enolate respectively.

The electronic spectrum of a $2 \times 10^{-5} M$ solution of the p-NO_2 substituted compound, as a representative example, in the pH range 3.06–12.85 gives two characteristic bands, one with λ_{max} at 380 nm and the other at 505 nm. The absorbance of the first band decreases with increasing pH and vanishes at pH 12.85. The reverse occurs with the second band. The two bands coexist at pH 10.85. A clear isosbestic point is apparent at 416 nm, indicating the presence of more than one absorbing species. The forms A and B are considered to absorb in the shorter wavelength region of the spectrum and C in the longer wavelength region. The equation used in calculating K^* was:[9]

$$K^* = [H^+]_x - M[H^+]_{x+y}/(M - 1)$$

where

$$M = (A_{x+y} - A_{x-1})([H^+]_{x-1} - [H^+]_x)/$$
$$(A_x - A_{x-1})([H^+]_{x-1} - [H^+]_{x+y}).$$

The results of the computations are given in Table 1, showing that the acidity of the ligands depends on the nature of the substituent (Hammett function σ).

The curve of the average molar absorptivity $\bar{\epsilon}$ *vs.* $\log[L^-]$ was smooth and independent of the initial concentration of both the metal ion and the ligand used, indicating that a non-protonated species $[L^-]$ is the complexing species. The concentration $[L^-]$ was calculated from

$$[L^-] = T_{HL}/\{1 + ([H^+]/K^*)\}$$

where T_{HL} was the total ligand concentration and was much higher than the metal ion concentration (by a factor of about 30).

The molar absorptivity ϵ_n of the metal chelate species is given by the equation:

$$\epsilon_n = \epsilon_{n-1} + \Delta A\{(1/K_n) + [L]\}/[L]T_M$$

where T_M is the total metal ion concentration. The K and ϵ values were applied to calculate $\bar{\epsilon}_n$ and β_n. The value of $\bar{\epsilon}$ was calculated as follows:

$$\bar{\epsilon} = \Sigma \epsilon_n \beta_n [L]^n / \Sigma \beta_n [L]^n$$

The results show that the stability of a given chelate species increases with the basic strength of the carbanion-enolate ion. Thus the stability constant is highest for the p-CH_3 chelate and lowest for the p-NO_2. This suggests that the ligand is coplanar with the chelate ring, the electron-donating groups increasing the electron density on the nitrogen and oxygen atoms, leading to more covalent M–O and/or M–N bonds. This conclusion accords with the observation that the conjugate bases of strong acids form weak complexes with metal ions and *vice versa*.[10] An attempt was made to relate the acid dissociation of the ligands and the stability constant of their chelates. The linearity between $\log \beta_n$ and pK^* could be

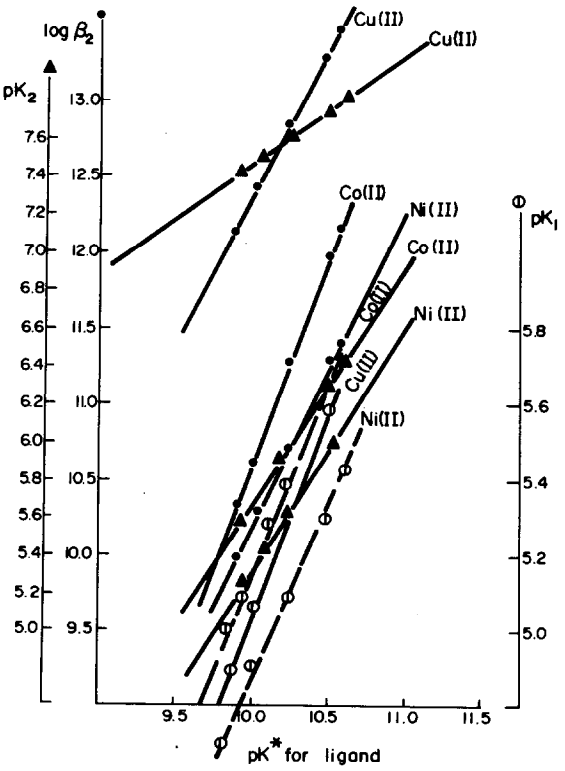

Fig. 1. ● $\log \beta_2$ *vs.* pK^*; ▲ pK_2 *vs.* pK^*; ⊙ pK_1 *vs.* pK^*.

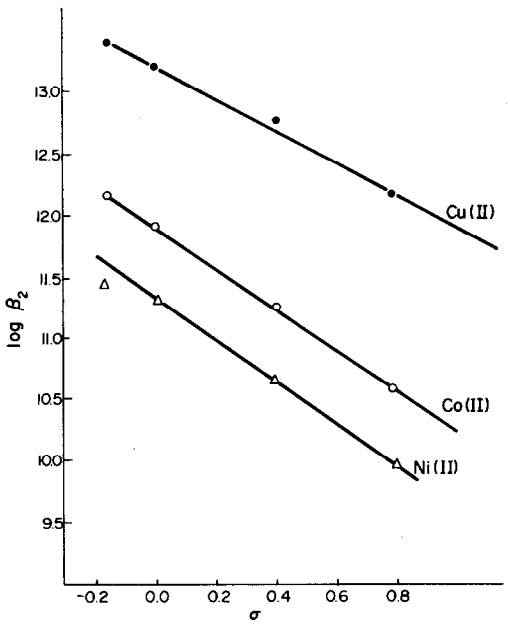

Fig. 2. log β_2 *vs.* σ.

checked from the relation $\log \beta_n = apK^* + b$ where a and b are constants. Similar linear relations have been reported for a series of 1:1 complex ions with one metal and a set of similar ligands.[11–13] The slope, a, of the pK^* *vs.* $\log \beta_n$ plot would be unity if the bonding were similar in both the ligand HL and the complex ML, and deviations from unity result from π-bonding in metal complexes.[14] The latter is apparent for cations that function as π-electron donors, and a will exceed unity for a cation which is a π-electron acceptor.[14] The results (Fig. 1) indicate that the bonding between the metal ion and the ligand is similar in nature and that the metals behave as π-electron acceptors. The slopes, a, for the copper, cobalt and nickel systems were found to be 1.8, 2.8 and 2.0 respectively. Plots of $\log \beta_n$ or pK_1 or pK_2 values against the substituent function, σ, were linear (Fig. 2) with slopes of -1.27, -1.70 and -1.80 for the copper, cobalt and nickel systems respectively. This indicates that the complex-formation is favoured by electron-donating substituents which increase the electron density on the co-ordination sites of the ligand (N, O), and suggests that the metals can act as π-electron acceptors. However, if the metal ions are π-electron donors the electron-donating substituents on the ligand would decrease. Electron-attracting groups increase the complex stability. The linear plots of $\log \beta$ *vs.* σ indicate that the bonding is similar in all the systems under investigation, irrespective of the substituent and the metal. There is a great similarity between these systems and those of acetoacetanilide.[13]

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ANNOTATION

METAL CHELATES OF PHOSPHONATE-CONTAINING LIGANDS–VI

COMPLEXES OF ETHYLENEDIAMINETETRA(METHYLENEPHOSPHONIC) ACID WITH Cd, Mg, Ca AND Ba

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Summary—A correction is made to results previously obtained, as a result of further calculations.

In the first paper of this series,[1] the stability constants of the title complexes were calculated on the assumption that only the deprotonated complex (ML) is present in significant concentrations in highly alkaline solutions and that the concentration of protonated species could be neglected. Back-calculations from these constants have revealed, however, that the method proposed for evaluation of the constants is not completely satisfactory. Consequently, it was deemed wise to consider alternative calculation methods to allow for the co-existence of different protonated species in the same buffer region.

CALCULATIONS

A FORTRAN IV program run on an ECLIPSE S/230 tea computer and based on the set of equations given by Yoshino *et al.*[2] was used to calculate the values of the chelate formation constants, K^{M}_{ML}. In each case, the average number of protons bound to the complexes, $\bar{n}_H$ was calculated from the relationship:

$$\bar{n}_H = \sum_{i=1}^{N} i[MH_iL] \Big/ \sum_{i=0}^{N} [MH_iL] = \frac{(8-a)T_L - [H] + [OH]}{T_L} \quad (1)$$

where T_L is the total ligand concentration and a is the number of moles of base added per mole of ligand.

Table 1. Values of $\bar{n}_H$ calculated from equation (1) for the different complex species

	$\bar{n}_H$			
a	Mg	Ca	Ba	Cd
4.00	3.997	3.994	3.997	3.897
5.00	3.000	3.000	3.001	2.993
6.00	2.007	2.006	2.001	2.000
7.00	1.146	1.126	1.013	1.023
8.00	0.650	0.471	0.057	0.153

Substituting the overall chelate protonation constants, $\beta^{H}_i = [MH_iL]/[H]^i[ML]$, in equation (1) produced the general relationship,

$$\bar{n}_H + [H][\bar{n}_H - 1]\beta^{H}_1 + [H]^2(\bar{n}_H - 2)\beta^{H}_2 + \ldots + [H]^N(\bar{n}_H - N)\beta^{H}_N = 0 \quad (2)$$

This equation was then subjected to numerical analysis for the N protonation constants. As a first approximation, this set of constants was fed into the program and the corresponding value of K^{M}_{ML} calculated from different points along the titration curve. Iterative calculations were continued to get the set of β^{H}_i values which gave the best agreement of the K^{M}_{ML} values.

RESULTS AND DISCUSSION

The values of $\bar{n}_H$ listed in Table 1 indicate the presence of a tetraprotonated complex species $[MH_4L]$, at the first inflection on the titration curves ($a = 4$). Further addition of base is accompanied by deprotonation of this species to give tri-, di-, mono- and finally the completely deprotonated species. Table 2 lists the values obtained for the formation constants K^{M}_{ML} and the complex protonation constants, $K^{H}_i = [MH_iL]/[H][MH_{i-1}L]$, for each metal ion. The order of stability is shown to be Cd > Ba > Ca > Mg, as indicated by the titration curves. It is of

Table 2. Formation constants of ENTMP with magnesium calcium, barium and cadmium ions at 25° (0.1M KNO_3)

	log K			
	Mg	Ca	Ba	Cd
$M^{2+} + L^{8-} \rightleftharpoons ML^{6-}$	5.69	6.93	11.14	16.53
$ML^{6-} + H^{+} \rightleftharpoons MHL^{5-}$	10.60	10.33	9.22	10.01
$MHL^{5-} + H^{+} \rightleftharpoons MH_2L^{4-}$	9.10	9.59	8.41	8.04
$MH_2L^{4-} + H^{+} \rightleftharpoons MH_3L^{3-}$	8.23	7.50	7.14	5.99
$MH_3L^{3-} + H^{+} \rightleftharpoons MH_4L^{2-}$	7.07	6.78	6.25	4.57

interest to note that the value of K_1^H for the magnesium chelate is about the same as that reported for the free ligand itself.[1] Comparison between the K_1^H values for the different species shows that the constants decrease from Mg to Ba. As the effective ionic radii for the hexaco-ordinated ions[3] are 0.72, 0.95, 1.00 and 1.34 Å for Mg, Cd, Ca and Ba respectively, it can be concluded that the repulsive forces produced by loss of the first proton increase markedly with decreasing ionic radius and reach a limiting value in the case of magnesium.

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SOLVENT EXTRACTION OF CHROMIUM: A REVIEW

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Summary—The separation of chromium by solvent extraction is reviewed.

Chromium exists mainly in two oxidation states in solution, Cr(III) and Cr(VI). The first is the more stable and exhibits a tendency to form inert complexes. In the presence of non-complexing anions (*e.g.* ClO_4^-), the ion exists as $Cr(H_2O)_6^{3+}$ and its hydrolysis products, the predominant species at various pH values being $Cr(H_2O)_5OH^{2+}$ (pH 4.6) and $Cr(H_2O)_4(OH)_2^+$ (pH > 4.6).[1] The formation of polynuclear olated species ($[(H_2O)_5Cr{-}O{-}Cr(H_2O)_5]^{5+}$) by hydrolytic processes is also suggested,[2,3] the degree of hydrolysis depending upon the pH of the solution, temperature and the nature of the anion.

In aqueous systems chromium(VI) exhibits the equilibria,[4–6]

$$2CrO_3 + H_2O \rightleftharpoons H_2Cr_2O_7$$

$$CrO_3 + H_2O \rightleftharpoons H_2CrO_4$$

$$H_2Cr_2O_7 \rightleftharpoons H^+ + HCr_2O_7^-, K = 10^6$$

$$HCr_2O_7^- \rightleftharpoons H^+ + Cr_2O_7^{2-}, K = 0.85$$

$$H_2CrO_4 \rightleftharpoons H^+ + HCrO_4^-, K = 1.21$$

$$HCrO_4^- \rightleftharpoons H^+ + CrO_4^{2-}, K = 3.21 \times 10^{-7}$$

The relative proportions of the different species will depend upon both pH and the total concentration of chromium(VI). Table 1 indicates which species predominate at various pH values and total chromium concentrations.[7]

The predominant monomeric species $HCrO_4^-$ in the pH range 1–5 forms esters with other species containing $-OH$ groups. The formation of dichromate is the archetype of this reaction.

$$HCrO_4^- + HCrO_4^- \rightleftharpoons Cr_2O_7^{2-} + H_2O$$

Changes in the electronic absorption spectra of $HCrO_4^-$ in the presence of oxyacids such as HSO_4^-, $H_2PO_4^-$, $H_2PO_3^-$ and $HS_2O_3^-$ are interpreted as indicating the formation of the 1:1 esters, $CrSO_7^{2-}$,[8] $HCrPO_7^-$,[9] $HCrPO_6^{2-}$,[9] and $CrS_2O_6^{2-}$,[8] respectively. In addition, qualitative evidence for complex formation has been reported for acetic acid.[10] Similarly, substitution of $-OH^-$ in $HCrO_4^-$ has also been observed in the reactions,[9]

$$H^+ + Cl^- + HCrO_4^- \rightleftharpoons CrO_3Cl^- + H_2O$$

$$H^+ + SCN^- + HCrO_4^- \rightleftharpoons CrO_3SCN^- + H_2O$$

Chromium, which is highly toxic, is used as a corrosion inhibitor and finds its way into waste water from cooling towers. Industrial effluents contain significant quantities of chromium. Stainless steel is a commonly used material of construction in reactor technology and the reactor cooling waters must be routinely analysed for corrosion products, including chromium. Further, an understanding of the chemical separation and oxidation states of trace elements in sea water is important both environmentally and geochemically. The presence of chromium in trace quantities in sea-water has an influence on aquatic life. The maximum concentration of chromium(VI) presently permitted in potable water supplies is 0.05 mg/l.[11] Very little is known about the ultimate fate of chromium(VI) in natural waters. Chromium(III), which is less toxic, can exist in a variety of complexed or hydrolysed forms and may be associated with particulate matter. The environmental consequences of long-term exposure to high concentrations of such substances are uncertain, but the chromium seems to interfere with the enzymatic sulphur uptake of cells[12] and affect the lungs, liver and kidneys.[13,14] While there have been no reports of the oral toxicity of chromium(III), it is considered necessary for the maintainance of a normal glucose tolerance factor.[13,15]

In view of these facts, the separation and determination of chromium at trace levels has received considerable attention. For ease of manipulation and routine operation, solvent extraction procedures are preferred,[16,17] and these can be classified in two groups, concerned with chromium(VI) and chromium (III).

EXTRACTION OF CHROMIUM(VI)

Bock[18] used diethyl ether to extract chromium(VI) and methyl isobutyl ketone has also been used to extract chromium(VI) from hydrochloric acid.[19–25] Katz *et al.*[25] showed that the extraction efficiency decreases progressively with type of mineral acid in the order, hydrochloric, nitric, perchloric and sulphuric. They suggested a mechanism involving the formation of an ion-pair in the organic phase ($R_1R_2COH^+X^-$), the anion of which is exchanged with the anionic

Table 1. Variation in chromium species present, with pH and concentration*

pH	Total concentration of chromium, M				
	10^{-3}	10^{-2}	10^{-1}	1	10
−1	H_2CrO_4	H_2CrO_4	H_2CrO_4	$HCr_2O_7^-$	$HCr_2O_7^-$
0	$HCrO_4^-$	$HCrO_4^-$	$HCr_2O_7^-$	$HCr_2O_7^-$	$HCr_2O_7^-$
1–6	$HCrO_4^-$	$HCrO_4^-$	$Cr_2O_7^{2-}$	$Cr_2O_7^{2-}$	$Cr_2O_7^{2-}$
8–9	CrO_4^{2-}	CrO_4^{2-}	CrO_4^{2-}	CrO_4^{2-}	CrO_4^{2-}

* In $\sim 10^{-4}M$ solutions of chromium(VI) only $HCrO_4^-$ need be considered in the pH range 1–5.

chromium(VI) species. The variation in the abilities of ketones to extract chromium(VI) from hydrochloric acid and in the extraction of chromium(VI) from various acids by methyl isobutyl ketone is attributed to differences in the properties of the ion-pair.

White and Ross[26] employed tri-n-octylphosphine oxide (TOPO) to extract macro-concentrations of chromium(VI). Recently, Niitsu and Sekine[27] have described the extraction of chromic acid from various acids with TOPO in hexane. They determined the equilibrium constants for the first dissociation of chromic acid and for the formation of chlorochromate in the aqueous phase. Specker and Arend,[28, 29] Ishimori[30] and Shevchenko *et al.*[31] studied various aspects of the extraction of chromium(VI) with tributyl phosphate (TBP). Tuck and Walters[32] studied the TBP extraction of chromium(VI) from perchloric, hydrochloric, sulphuric, nitric and hydrobromic acid media at both trace and macro-concentrations and suggested that the species extracted from hydrochloric and hydrobromic acid is $HCrO_3X$ (X = Cl or Br) while from other acids it is $H_2Cr_2O_7$.

The extraction of chromium(VI) as the blue peroxychromic acid into various solvents such as ether,[33] ethyl acetate,[34–36] hexane,[20] TBP[37] and ethyleneglycol monoethyl ether[38] has been reported. All these solvents readily extract the unstable complex but the extracts rapidly decompose. Sastri and Sundar[33,39] extracted peroxychromic acid with TBP in benzene for a spectrophotometric determination of chromium. According to them the colour remains stable for up to 30 hr. The chromium can also be stripped and titrated. The detection limit for chromium is $5 \times 10^{-5}M$ (2.6 μg/ml).

Several ion-association systems have been used, including the triphenylsulphonium,[40] tetraphenylphosphonium,[41] tetraphenylstibonium,[42] and the triphenylselenium[43] systems. Methyl Violet[44] and diantipyrylmethane[45] are also said to extract chromium(VI) quantitatively. Maeck *et al.*[46] used the tetrabutylammonium cation to extract chromium as $HCrO_4^-$ or $HCr_2O_7^-$ into methyl isobutyl ketone. Winkhaus and Uhrig[47] reported the extraction of CrO_4^{2-} by the di-π-cyclopentadienyltitanium cation. Schweitzer and McCarty[48] reported the extraction of $HCrO_4^-$ by the triphenyltin cation.

The extraction of chromium(VI) by various amines from acid solution has also been reported.[49–51] Fasalo *et al.*[52] reported that extraction is quantitative from hydrochloric and sulphuric acid (down to 0.1N) with tribenzylamine in chloroform, but only partial from nitric, acetic and citric acid media, and negligible from phosphoric acid. Shevtshuk and Simonova[52] employed tribenzylamine for the separation of chromium(VI) from an Mg–Cr ferrite.

Sastri and co-workers[54–57] showed that peroxychromic acid is extracted quantitatively into tri-n-octylamine (TOA) or Aliquat-336 with benzene as diluent. They reported that the complex is stable for 10 hr in Aliquat-336 and 2½ hr in TOA and suggested methods for an extractive spectrophotometric determination. They showed that the chromium(VI) was extracted as the haloperoxychromate $[CrO(O_2)_2X^-$, X = Cl or Br$]$[55,57] from hydrochloric and hydrobromic acid solution.

Hála *et al.*[58] studied the extraction of chromium(VI) by 2,3,5-propyltriphenylphosphonium cations into chloroform by using chromium-51 as tracer. It was found that under the conditions used the chromium(VI) is extracted as the ion-pair, $B^+HCrO_4^-$ (B^+ = cation). By using various buffers these workers concluded that the function of the acid in the extraction process is limited to its effect on the equilibrium

$$2HCrO_4^- \rightleftharpoons Cr_2O_7^{2-} + H_2O$$

The possible formation of CrO_3A^- (A^- = anion of the acid) in aqueous mineral acid solutions and its role in the extraction process does not appear to have been considered by these workers.

Deptula[59–62] studied the extraction of chromium(VI) by TOA from various mineral acid solutions. According to him the extracted species is CrO_3Cl^- from concentrated hydrochloric acid and $Cr_2O_7^{2-}$ from other mineral acids. He reported that the extraction from phosphoric acid involves the reaction

$$HCr_2O_7^- + H^+ + R_3N \rightleftharpoons R_3NH_2Cr_2O_7$$

where R_3N is the tertiary amine. He further observed that the distribution of chromium(VI) decreased gradually with increasing acid concentration for all mineral acid systems except hydrochloric acid, for which there is a minimum at about 0.1M concentration, and he attributes this to a change in the composition of the extracted species. He also studied[62] the synergistic and antagonistic effects of mixtures of TOA and alkylphosphoric acid solutions in the extraction of chromium(VI) from various mineral acids. The compositions of the extraced species were determined and mechanisms suggested for the synergistic and antagonistic effects. Federov and Zhdanov[63] studied the extraction of chronium(VI) by TOA from sulphuric acid and reported the extracted species as CrO_4^{2-}. Iqbal and Ijaz[64] used diphenyl-2-pyridylmethane in chloroform as a liquid anion-exchanger to extract chromium(VI) quantitatively from hydrochloric acid.

In view of this divergence of opinion on the nature of the extracted species, Rao and Sastri[65–67] made a

systematic study of the extraction of chromium(VI) from mineral acids by trilaurylamine in chloroform, using the radiotracers ^{36}Cl, ^{35}S and ^{32}P. They found that from hydrochloric acid a mixture of CrO_3Cl^- and $Cr_2O_7^{2-}$ or $HCrO_4^-$ is extracted but only $Cr_2O_7^{2-}$ from sulphuric or phosphoric acid.

EXTRACTION OF CHROMIUM(III)

Fewer studies have been reported on the liquid–liquid extraction of chromium(III), because of the inertness of its complexes. The half-life of the exchange of co-ordinated water has been reported[68] as 40 hr with rate constants ranging from[69] 2×10^{-5} to[68] 4.8×10^{-6} sec^{-1}.

McKaveney and Freiser[70] reported no extraction of chromium(III) from sulphate medium into a 1:1 mixture of chloroform and acetylacetone in the pH range 0–6.0 in 30 min of shaking. This was attributed to the extremely slow rate of formation of the metal chelate. This property has been exploited to separate chromium(III) from Al, Fe, V, Mo and Ti. Morrison and Freiser[17] recommended refluxing the aqueous chromium(III) solution with acetylacetone at pH 6 for 1 hr to bring about its complete extraction. Later McKaveney and Freiser[71] reported quantitative extraction of Cr(III) acetylacetonate into 1:1 mixtures of chloroform and acetylacetone over a wide pH range, and used the colour of the extract for determination of chromium in a wide variety of alloys, claiming it to be superior to the diphenylcarbazide method. Hellwege and Schweitzer[72] made a detailed kinetic study of this extraction from perchlorate and chloride media. They found that the rate of formation of the metal acetylacetonate is slower than the rate of transfer into the organic phase and this determines the overall rate of extraction. Zolotov *et al.*[73] have studied the catalytic acceleration of the extraction of Cr(III) acetylacetonate and reported that the extraction rate is increased by addition of ethanol or acetone to the aqueous phase and decreased by the addition of salts, in the order $NaNO_2 > NH_4F > NaI > KI > KBr$. Freger *et al.*[74] have also used acetylacetone for the extraction of chromium(III) at elevated temperatures.

Petrukin *et al.*[75] studied the extraction of chromium(III) with thenoyltrifluoroacetone (TTA) in benzene. They found that chromium(III) is not extracted in the absence of fluoride and that the extraction rate increases with fluoride concentration and pH. They reported that while chromium(III) is extracted rather quickly immediately after the addition of fluoride no extraction occurs if the extraction is done several days after the addition of the fluoride. They explained this behaviour by assuming that the fluoride forms intermediate complexes which destroy the primary hydration shell of chromium(III), but do not prevent formation of extractable Cr(III)–TTA complexes. However, it is necessary to carry out the complex formation before the inert CrF_6^{3-} is formed, a process which requires several days. These conclusions were confirmed by EPR studies. De and Majumdar[76] found that a Cr(III)–TTA species was extracted, and later De and Sahu[77] showed that the extracted species are $Cr(TTA)_3$ and $Cr(TTA)_2OH.H_2O$ from 1*M* chloride medium (pH 3–5.5) and $Cr(TTA)(SO_4)_2^{2-}$ and $Cr(TTA)_3$ from 0.5*M* sulphate medium (pH > 5.7).

Pietsch and Pichler[78] studied the extraction of the chromium(III) complex with dibutylarsenic acid into various organic solvents. The reaction between chromium(III) and sodium diethyldithiocarbamate has also been studied,[79,80] Starý reported[81] that chromium(III) is not extracted by chloroform solutions of oxine at room temperature at any pH, but on heating an extractable Cr(III) oxinate is formed. Bodnya *et al.*[82] studied the kinetics of extraction of chromium(III) by oxine solutions into various organic solvents from acetate media. They observed that the rate of extraction increases with the concentration of sodium acetate and with temperature.

The extraction of the chromium(III) complex with *m*-nitrobenzamidoxime into isoamyl alcohol was studied by Manolov *et al.*[83]. Freger *et al.*[84] studied the extraction of trace amounts of chromium(III) complexed with 1-phenyl-3-methylpyrazol-5-one. The extraction at elevated temperatures of chromium(III) with TTA, oxine, diethyldithiocarbamate and 1-phenyl-3-methyl-4-benzoylpyrazoline has been reported by various workers.[74, 85–87] Recently Sebastian and Hilderbrand[88] investigated the use of salicylic, thiosalicylic and phthalic acids as complexing agents for the solvent extraction of chromium(III) from aqueous solution into n-butanol. The extraction conditions were optimized with respect to pH, heating time, choice of buffer and concentration of salting-out agent. An extraction efficiency exceeding 97% was reported for the mixed phthalate–thiosalicylate complex.

John and Udy[89] used TBP for the extraction of chromium(III) from perchloric acid medium and reported that hydrolysed species are extracted in this system. According to them $CrClO_4(H_2O)_5^{2+}$ is the major constituent of the system at high acidities. Halpern *et al.*[90] studied the extraction of chromium(III) from hydrochloric acid solution into tributoxyethyl phosphate, using ^{51}Cr as radiotracer. They determined the degree of solvation of the extracted species. Smelov *et al.*[91,92] have studied various aspects of the extraction of chromium(III) with di-(ethylhexyl) hydrogen phosphate from acetate and nitric acid media. They reported the effect of various diluents on the extraction. The extraction of chromium(III) with di-(ethylhexyl)phosphoric acid (HDEHP, H_2A_2) in benzene from sulphuric acid has been reported[93] and the extracted species suggested to be CrA_3. The corresponding extraction from hydrochloric acid has also been investigated.[94] Imura *et al.*[95] studied the extraction of chromium(III) by TOPO in various inert solvents from trichloroacetate buffer at pH 3.5–6.0.

They reported the extracted species as $Cr(H_2O)_6^{3+}.(TCA^-)_3.3TOPO$ (TCA^- = trichloroacetate).

The use of several ion-association systems for extraction of chromium(III) has also been reported. Florence *et al.*[96] studied the extraction from concentrated halide solutions by tricaprylamine. They found that chromium(III) is readily extracted from aqueous concentrated lithium chloride media and that the extraction is more pronounced from methanolic than from aqueous solutions. The halides affect the extraction efficiency in the order LiCl > LiBr > LiI and the extracted species from chloride medium is $CrCl_5^{2-}$. Douglas and William[97] studied the extraction of chromium(III) from sulphate solutions into Primene JMT at different temperatures and found that heating increased the rate. The amount of chromium(III) extracted decreased with increasing acidity

Table 2. Extraction systems

Aqueous phase	Organic phase	Sample	References
HCl (1*M*)	4-Methyl-2-pentanone (MIBK)	NBS aluminium-base alloys, steel, cast iron, Monel metals and clay	113, 114
HCl (<3*M*)	MIBK	Chromite or chromium-bearing ores and steel	21, 115
HCl (1*M*)	MIBK	Stainless steel subjected to corrosion by thoria slurries	20
HCl (1*M*)	Tribenzylamine (TBA) in MIBK	Fission products, stainless steel	20, 46
HCl	Triphenylselenium in chloroform	Cast iron and steel samples	43
HCl, H_2SO_4 (>0.1*N*)	TBA in chloroform	Refined aluminium, crude oil, asphalt and polyphenyl	52
H_2SO_4 (0.2*N*)	TBA in chloroform	Mg–Cr ferrite	53
H_2SO_4	Tributylphosphate (TBP) in benzene or ethyl acetate	Separation of chromium from other metals	33, 34, 37, 39, 54, 116, 117
HCl (1*M*), NH_4Cl (1*M*)	TBP in xylene	Analysis of alloys	118
HCl (1–7*M*), H_2SO_4 (1–4*M*)	TOPO in cyclohexane	Concentrated alkali metal chloride or sulphate solutions	26, 119
$Fe(phen)_3^{3+}$ (50-fold ratio to Cr) pH 3–4 (phen = 1.10-phenanthroline)	Nitrobenzene	Steels	120
HCl	Tri-n-octylamine (TOA) in benzene	Spectrophotometric determination of chromium	121
H_2SO_4	Trioctylmethylammonium chloride ($TOMA^+Cl^-$) in chloroform, TOA-zephiramine-MIBK	Determination of chromium(VI)	122–124
Acid medium	TOA	Determination of chromium(VI)	125
H_2SO_4	Alamine-336 in xylene	Ilmenite, titanium dioxide pigments	126
	Mesityl oxide MIBK	Aluminium salts	127, 128
Cupferron and H_2SO_4 (0.03–0.04*N*)	Chloroform	Chromium in molybdenum matrix	129, 130
Diethyldithiocarbamate, pH 4	MIBK	Sea-water	131
Tartaric acid and oxalic acid pH 2–2.5	α-Naphthylamine–isoamyl alcohol	Steels and magnesite bricks	132
HCl (1*M*)	2-Hexylpyridine in chloroform	Separation from U, Th, fission products and first row transition elements	133
HCl (pH 2)	Aliquat-336 in toluene	Sea-water	134, 135
KSCN (1*M*)	Ammonium pyrrolidinedithiocarbamate (APDC)–MIBK	Sea-water	136
	Potassium benzylxanthate–MIBK	Estimation of chromium(VI)	137
HCl	MIBK, TOA in benzene	Separation of Cr(III)/Cr(VI) in the hot atom chemistry of the element	138
H_2SO_4	Primine JMT	Separation of Fe(III) from Cr(III) and Cr(III) from Cu(II)	97
Pyridylazoresorcinol (PAR)	Tetradecylbenzylammonium chloride in chloroform	Estimation of chromium(III)	139
Weakly acidic solution	$TOMA^+Cl^-$ in chloroform	Estimation of Cr(III) as Cr(III)–DCTA complex	140, 141
1-Pyridylazonaphthol (PAN)	MIBK	Estimation of Cr(III) as Cr(III)–PAN complex	142
	m-Nitrobenzamidoxine in amyl alcohol	Estimation of Cr(III) at μg level	83
	Aminopyridinedithiocarbamate	Chromium in ashed serum	143
		Chromium in 25% brine solution	86
	Sodium diethyldithiocarbamate in chloroform	Estimation of trace Cr(III)	144
CH_3COOH	Oxine in chloroform	Estimation of Cr(III)	145

of the aqueous phase, indicating a connection between the slow rates of extraction and hydrolysis of the chromium(III) species. The separation of Fe(III) from Cr(III) and Cr(III) from Cu(II) in sulphate media by extraction with Primene JMT is reported to be feasible.

Though the well-defined anionic thiocyanate complexes of chromium(III) are repeatedly reported to be non-extractable by liquid anion-exchangers,[98,99] McClellan *et al.*[100] have demonstrated that under suitable conditions they can be quantitatively extracted with TOA in carbon tetrachloride. Subsequently DeJong and Brinkman[101] identified the extracted species as $Cr(SNC)_6^{3-}$ at high chromium(III) concentrations and $Cr(SNC)_4(H_2O)_2^-$ at low. They suggested a method for the separation of chromium(III) from bivalent metal ions such as cobalt and nickel.

Rao[102] studied the extraction of the chromium(III)–EDTA complex with Aliquat-336 in the pH range 2–6. Maximum extraction was observed at pH 3.8 and the extracted species was identified as $CrY(H_2O)^-$ (H_4Y = EDTA). At higher pH (>7.0), the blue complex $Cr(OH)Y^{2-}$ formed is not extractable. The diluent has a marked effect on the extraction, with nitrobenzene giving maximum extraction (80%). Irving and Al Jarrah[103,104] also studied the extraction of the chromium(III)–EDTA complex with tetra-n-hexylammonium chloride in dichloromethane and reported the extracted species as $CrY(H_2O)^-$ in the form of $R_4N^+[CrY(H_2O)]^-$ (R = n-hexyl, Y = EDTA anion) over the pH range 3.6–6.0 with maximum extraction at pH 4.7. According to them the neutral $CrHY(H_2O)$ and the anionic $CrY(OH)^{2-}$ and $CrY(OH)_2^{3-}$ species are not extractable. The extraction of the chromium(III)–NTA complex with Aliquat-336 in dichloromethane has also been reported,[105] the extracted species being $CrZ(H_2O)(OH)^-$ and $CrZH_2O(OH)_2^{2-}$ (Z = NTA anion) at pH 9.0. At a still higher pH, the olive green $CrZ(OH)_3^{3-}$ is formed, but is extracted only to a negligible extent. Sastri and co-workers[67,106–108] studied the extraction of the anionic dicarboxylate complexes by trilaurylamine and Aliquat-336. Rao and Sastri[106] reported quantitative extraction of tris(oxalato)chromate(III) by trilaurylamine in chloroform from various mineral acid solutions. They studied the effects of mineral acids and diluents on the extraction.

The formation of intermediate oxidation states of chromium when chromium(VI) is reduced in the presence of complexing agents has been postulated by several workers.[109–111] Rao and Sastri[112] have observed that a red complex of intermediate chromium oxidation state is formed with EDTA when chromium(VI) is reduced with hydrogen peroxide in the prescence of EDTA. The red complex may be extracted into Aliquat-336 but slowly changes to an inextractable blue species. They studied the effect of diluents on the extraction and reported a maximum extraction of 78% with nitrobenzene.

APPLICATIONS

The separation and determination of chromium by solvent extraction coupled with various other techniques such as spectrophotometric and radiochemical methods is summarized in Table 2.

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DETERMINATION OF CHROMIUM IN ORES, ROCKS AND RELATED MATERIALS, IRON, STEEL AND NON-FERROUS ALLOYS BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY AFTER SEPARATION BY TRIBENZYLAMINE–CHLOROFORM EXTRACTION

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Summary—A method for determining trace and moderate amounts of chromium in ores, concentrates, rocks, soils and clays is described. After fusion of the sample with sodium peroxide, the melt is dissolved in dilute sulphuric acid. The chromium(III) produced by the hydrogen peroxide formed is co-precipitated with hydrous ferric oxide. The precipitate is dissolved in 0.7*M* sulphuric acid and chromium oxidized to chromium(VI) with ceric ammonium sulphate. The chromium(VI) is extracted as an ion-association complex into chloroform containing tribenzylamine and stripped with ammoniacal hydrogen peroxide. This solution is acidified with perchloric acid and chromium determined by atomic-absorption spectrophotometry in an air–acetylene flame, at 357.9 nm. Barium and strontium do not interfere. The procedure is also applicable to iron and steel, and nickel–copper, aluminium and zirconium alloys. Up to 5 mg of manganese and 10 mg each of molybdenum and vanadium will not interfere. In the absence of vanadium, up to 10 mg of tungsten will not interfere. In the presence of 1 mg of vanadium, up to 1 mg of tungsten will not interfere.

A reasonably simple and reliable atomic-absorption (AAS) method for the determination of trace and moderate amounts of chromium in diverse ores and mill products was required for use in routine work in the CANMET chemical laboratory and for possible future use in the Canadian Certified Reference Materials Project (CCRMP). From the literature it is apparent that the determination of chromium by AAS is subject to many interference effects which depend on the oxidation state of the chromium, matrix composition, acid medium, flame type and stoichiometry, and region of observation in the flame.[1–5] Recent work also suggests that the use of various compounds (aluminium, calcium, ammonium and strontium chlorides, potassium and sodium sulphates, potassium pyrosulphate and ammonium hydrogen fluoride) that have been recommended as masking or releasing agents to minimize or suppress interferences is not always reliable because their efficiency also depends on the sample matrix and on the acid medium employed.[1] Moreover, few if any of these reagents are universally applicable. Consequently, it was considered that a suitable method would involve a relatively selective solvent extraction and preconcentration step.

Numerous AAS methods based on the separation and preconcentration of chromium by extraction of its ammonium pyrrolidinedithiocarbamate, sodium diethyldithiocarbamate, oxine, potassium benzylxanthate and thenoyltrifluoroacetone complexes have been reported,[6–8] but none of these extraction procedures is very selective, because many other elements also form similar extractable complexes. Several recent methods involve the extraction of chromic acid into methyl isobutyl ketone (MIBK) from hydrochloric[9,10] and potassium bromide–sulphuric acid media.[11] Other methods involve its extraction as an ion-association complex from hydrochloric acid media into xylene containing the high molecular-weight amine, Amberlite LA-1 [*N*-dodecyl(trialkylmethyl)amine],[12] or into MIBK containing the quaternary ammonium compound, Zephiramine (tetradecyldimethylammonium chloride).[13] In these methods, chromium is determined by direct aspiration of the organic solution into the flame. However, these extraction procedures are also not very selective because of the co-extraction of similar anionic chloro- or bromo-complexes.[14,15] Because the literature indicates that the extraction of chromium(VI) with solutions of high molecular-weight amines, particularly tertiary amines such as trioctylamine and tribenzylamine, is considerably more selective from sulphuric than from hydrochloric acid media,[14–19] the applicability of this type of separation procedure to the determination of small amounts of chromium in ores was investigated. Because of the greater stability and ease of preparation of aqueous calibration solutions, particularly for routine work, the AAS determination of chromium in aqueous medium was also investigated.

This paper describes the determination of chromium in ores, concentrates, rocks, soils and clays after its preliminary separation, by co-precipitation with hydrous ferric oxide, from the sodium salts resulting from sample decomposition by fusion with sodium peroxide. Chromium is subsequently separated from iron, which interferes strongly in its determination by AAS in an air–acetylene flame, [2,5,20] and from other co-precipitated elements by the extraction of chromium(VI), from a 0.7*M* sulphuric acid medium, into chloroform containing tribenzylamine. Chromium is then stripped into 1% ammonia solution containing hydrogen peroxide. The applicability of this extraction method to the direct determination of chromium in iron and steel and in nickel–copper, aluminium and zirconium alloys is shown.

EXPERIMENTAL

Apparatus

A Varian Techtron Model AA6 spectrophotometer, equipped with a 10-cm laminar-flow air–acetylene burner and a chromium hollow-cathode lamp, was used under the following conditions (Note 1).

Wavelength: 357.9 nm
Lamp current: 5 mA
Spectral band-pass: 0.20 nm
Height of light-path above burner slot: 6 mm
Acetylene flowmeter reading: 3.0 (~2.5 l./min)
Air flowmeter reading: 6.0 (~12.5 l./min)
Flame: luminous, fuel-rich
Aspiration rate: 1 ml/min

Reagents

Standard chromium solution, 1000 μg/ml. Dissolve 1.415 g of pure potassium dichromate (dried at 105° for 1 hr) in water and dilute to 500 ml with water. Prepare a 100-μg/ml solution by diluting 20 ml of this stock solution to 200 ml with water.

Iron(III) sulphate solution (1 ml ≡ 10 mg of iron). Dissolve 25 g of ferric sulphate monohydrate in hot water containing 5 ml of concentrated sulphuric acid, cool and dilute to 500 ml with water.

Sulphuric acid, 0.7M. Dilute 80 ml of 50% v/v sulphuric acid to 1 litre with water.

Sulphuric acid, 1.4M. Dilute 160 ml of 50% v/v sulphuric acid to 1 litre with water.

Tribenzylamine. A 3% solution in chloroform.

Perchloric acid, 50% v/v. Mix equal volumes of perchloric acid and water.

Procedures

Calibration solutions. Add 1 ml of 50% v/v perchloric acid and ~30 ml of water to each of eight 100-ml beakers; then, from a burette, add to the first seven beakers 0.5, 1, 2, 3, 4, 5 and 6 ml respectively, of the 100-μg/ml standard chromium solution. The last beaker contains the zero calibration solution. Add 4 drops of 30% w/v hydrogen peroxide to each beaker, mix and evaporate the solutions to ~10 ml to destroy most of the excess of hydrogen peroxide. Cool and transfer the resulting solutions to 100-ml standard flasks containing 9 ml of 50% v/v perchloric acid. Dilute each solution to volume with water and mix (Note 2).

Ores, concentrates, rocks, soils and clays. Depending on the expected chromium, iron and aluminium contents, transfer 0.4–1 g of powdered sample, containing not more than ~150 mg of iron and ~100 mg of aluminium (Notes 3 and 4), to a 30-ml zirconium crucible. Add 4 g of sodium peroxide and mix thoroughly. Cautiously fuse the mixture over an open flame and keep it in the molten state for ~30 sec to ensure complete decomposition. Allow the melt to cool, then transfer the crucible to a covered 400-ml Teflon beaker containing ~50 ml of water and 25 ml of 50% v/v sulphuric acid. When the melt has dissolved, remove the crucible after washing it thoroughly with water, then cover the beaker (Note 5) and evaporate the solution to ~50 ml. Remove the cover and wash down the sides of the beaker with water. Add 5 ml of concentrated hydrofluoric acid and 10 ml of concentrated hydrobromic acid and evaporate the solution until ~5 ml of sulphuric acid remain. Cool, add ~100 ml of water and, if necessary, add sufficient iron(III) sulphate solution for at least 100 mg of iron to be present. Cover and heat to dissolve the soluble salts, then cool the solution to room temperature. Carry a blank, with ~100 mg of iron(III) added, through the whole procedure.

Add sufficient concentrated ammonia solution to each solution to precipitate iron as the hydrous oxide, then add 5 ml in excess and boil the solution to coagulate the precipitate. Filter off (Whatman No. 40 paper) and transfer the bulk of the insoluble material to the filter paper with 5% ammonia solution. Wash the beaker twice and the paper and precipitate three times with 5% ammonia solution. Discard the filtrate. Place the original beaker under the funnel and add 25 ml of hot 0.7*M* sulphuric acid to the filter. Carefully, to avoid tearing the paper, break up the gelatinous precipitate with a glass rod to aid in dissolution. Wash the paper twice more with 25-ml portions of hot 0.7*M* sulphuric acid, then once with 15 ml of the hot acid solution.

If the sample contains ~1 mg or less of chromium, wash the paper twice with water. Discard the paper, wash down the sides of the beaker with 10 ml of the hot acid solution and evaporate the solution to ~50 ml.

If the sample contains more than 1 mg of chromium, wash the paper twice with 0.7*M* sulphuric acid, then transfer the solution to a 200-ml standard flask, using 0.7*M* sulphuric acid to wash the beaker. Dilute to volume with the acid and mix. Transfer a suitable fraction (10–100 ml) of the solution, containing up to ~1 mg of chromium, to a 250-ml beaker. If necessary, add sufficient 0.7*M* sulphuric acid to give a total of 100 ml, and evaporate the solution to ~50 ml.

Add 300 mg of ceric ammonium sulphate (Note 6) to the solution, cover the beaker and, to ensure the complete oxidation of chromium, boil until salts start to form or fumes of sulphur trioxide start to appear. Add 50 ml of water and heat gently to dissolve the salts. Cool and transfer the solution to a 250-ml separatory funnel marked at 100 ml, filtering it if necessary (Whatman No. 40 paper) and washing the beaker *etc.* thoroughly with water. Dilute the solution to the 100-ml mark with water. Add 10 ml of 3% tribenzylamine solution, stopper and shake for 1 min. Allow several min for the layers to separate, then drain the chloroform phase into a 125-ml separatory funnel containing 10 ml of methanol. Extract the aqueous phase in a similar manner with three 5-ml portions of tribenzylamine solution (Note 7). Add 25 ml of 1% ammonia solution and 4 drops of 30% hydrogen peroxide to the combined extracts, stopper and shake for 1 min. After the layers have separated, discard the chloroform layer. Add 1 ml of 50% v/v perchloric acid to the aqueous phase (Note 8), mix thoroughly, then add 5 ml of chloroform and shake for ~30 sec to extract tribenzylamine; discard the chloroform layer. Wash the aqueous layer with another 5 ml of chloroform, then transfer it to a 100-ml beaker and heat gently in a hot water-bath to remove the residual chloroform. Evaporate the blank solution to ~5 ml (Note 9) to destroy most of the excess of hydrogen peroxide, then transfer it to a 10-ml standard flask and dilute to volume with water. Depending on the expected chromium content, evaporate

the sample solution to 5–10 ml, then, if necessary, add sufficient 50% v/v perchloric acid for 1 ml to be present for each 10 ml of final solution in excess of 10 ml (Note 10). Transfer the solution to a standard flask of appropriate size (10–200 ml), dilute to volume with water and mix.

Measure the absorbances at 357.9 nm when the resulting solutions are aspirated into a reducing air–acetylene flame (Note 11). Calculate the chromium contents (in mg) from the sample and blank absorbances and those obtained concurrently for standard solutions that bracket the sample and blank concentrations. Correct the results by subtracting the blank.

Iron and steel. Transfer 0.1–0.5 g of sample, containing up to 5 mg of chromium and not more than ~25 mg of manganese, 50 mg of molybdenum and 5 mg each of tungsten and vanadium (Note 12), to a 250-ml Teflon beaker. Cover the beaker and add 40 ml of 50% v/v sulphuric acid and 10 ml of concentrated nitric acid. Heat gently until the sample is decomposed, then remove the cover and wash down the sides of the beaker with water. Add 2 ml of concentrated hydrofluoric acid (Note 13) and evaporate the solution until copious fumes of sulphur trioxide are evolved. Cool, add 50 ml of water and heat to dissolve the soluble salts. Filter (Whatman No. 40 paper) into a 250-ml standard flask, transfer any insoluble material quantitatively to the filter and wash three times with water. Reserve the beaker.

Transfer the paper and residue to a 30-ml zirconium crucible, burn off the paper at low temperature and ignite at ~600°. Cool the crucible, add 0.5 g of sodium peroxide (Note 14) and fuse the mixture over an open flame. After cooling, place the crucible upright in the original beaker. Cover the beaker, add ~15 ml of water to the crucible, then carefully add 6 ml of 50% v/v sulphuric acid. When the melt has dissolved, remove the crucible after washing it with water. Add 3 drops of concentrated hydrofluoric acid to the beaker and, to remove hydrogen peroxide, evaporate the solution until copious fumes of sulphur trioxide are evolved. Cool, add ~30 ml of water and heat to dissolve the salts. If necessary, filter (Whatman No. 40 paper) into the standard flask containing the initial filtrate and wash the beaker and paper three times with water. Discard the paper and dilute the combined filtrates to volume with water. Carry a blank through the whole procedure.

Transfer a suitable fraction (up to 50 ml) of the sample solution, containing up to ~1 mg of chromium, to a 250-ml beaker and, if necessary, add sufficient 1.4*M* sulphuric acid for the total volume of the resulting solution to be 50 ml. Add 300 mg of ceric ammonium sulphate, cover the beaker and proceed with the oxidation, extraction (Note 15) and determination of chromium as described.

Aluminium-base, zirconium-base and nickel-copper alloys. Transfer up to 0.2 g of sample, containing up to ~1 mg of chromium and not more than ~5 mg of manganese, to a 250-ml Teflon beaker. Cover the beaker, add 10 ml each of 50% v/v sulphuric acid and 50% v/v nitric acid and heat gently until the sample is decomposed. Remove the cover, wash down the sides of the beaker with water, then add 2 ml of concentrated hydrofluoric acid (Note 13) and evaporate the solution until copious fumes of sulphur trioxide are evolved (Note 16). Cool, add 50 ml of water, heat to dissolve the salts, then proceed with the oxidation, extraction and determination of chromium as described above.

Notes

1. A luminous, moderately reducing air–acetylene flame is required to obtain the highest sensitivity for chromium. The height at which the beam from the hollow-cathode lamp passes through the flame is also very important.[20] Therefore, after all other instrumental parameters have been set, the acetylene flow-rate and the height of the light-path above the burner should be adjusted to give maximum absorbance when a solution containing chromium is aspirated into the flame.

2. The calibration solutions are stable for at least 2 weeks.

3. The use of samples containing more iron and aluminium is not recommended because the hydrous oxide filtration step becomes unduly slow.

4. The sample should not contain more than ~10 mg of tungsten.

5. The solution should be kept almost completely covered during the initial evaporation to avoid loss by spray.

6. Ceric compounds other than the sulphate are not recommended. Nitrate compounds can result in the formation of nitric acid which is partly co-extracted into tribenzylamine–chloroform solution.[21]

7. The fourth extraction is not necessary if the second extract is colourless.

8. If much chromium is present, the solution will become blue because of the formation of perchromic acid. However, this compound is unstable and the solution will soon become colourless.

9. Chromium is lost by volatilization if the blank or sample solution is evaporated to fumes of perchloric acid or to dryness.

10. Additional 50% perchloric acid is not required if the final volume of the solution is to be 10 ml, because 1 ml is added to acidify the dilute ammonia solution used for the stripping of chromium(VI). For final sample solution volumes of 25, 50, 100 or 200 ml, add 1.5, 4, 9 or 10 ml, respectively.

11. Scale expansion (~2–5-fold) is recommended for the determination of ≤2 μg/ml of chromium. For stability, the burner should be allowed to warm up for at least 5 min before measurements are made.[4]

12. If tungsten is absent, up to 50 mg of vanadium can be present. Conversely, if vanadium is absent, up to 10 mg of tungsten can be present.

13. If tin or antimony is present, add 10 ml of concentrated hydrobromic acid at this stage.

14. Because large amounts of sodium sulphate inhibit the extraction of small amounts of chromium, more sodium peroxide should not be added unless a small fraction of the final solution is used for the subsequent extraction of chromium.

15. If tungsten trioxide is present, use Whatman No. 42 paper to filter the solution before the extraction step.

16. Not too much sulphuric acid should be removed by evaporation, because a concentration of ~0.7*M* is required for the extraction step.

RESULTS

Separation of chromium by tribenzylamine–chloroform extraction

Dichromate[22] can be extracted as an ion-association complex from dilute hydrochloric or sulphuric acid media into various organic solvents containing long-chain, high molecular-weight aliphatic amines such as tribenzylamine (TBA), methyl di-n-octylamine,[21] tri-n-octylamine (TOA)[22] and Amberlite LA-1.[12] Tertiary amines are the most efficient extractants.[23] From the literature[14–19] it is apparent that the extraction of chromium is more selective from sulphuric acid because anionic chloro-complexes are co-extracted from hydrochloric acid media. Furthermore, sulphuric acid, unlike hydrochloric, is not co-extracted into chloroform containing TBA[21] or benzene containing TOA.[22] For these reasons, and

because of the danger of reduction of chromium(VI) if hydrochloric acid is used, the use of sulphuric acid media was investigated. TBA was chosen as the extractant because it is readily available and chloroform was used as the diluent because of its high density. It is more convenient than benzene when multiple extractions are required.

Preliminary tests showed that up to at least 1 mg of chromium(VI) can be quantitatively extracted from 100 ml of 0.5–1*M* sulphuric acid in successive extractions with one 10-ml and three 5-ml portions of 3% TBA in chloroform. An intermediate concentration of 0.7*M* sulphuric acid was chosen for subsequent work.

Stripping and atomic-absorption determination of chromium

Extremely low results were obtained for chromium by AAS in an air–acetylene flame in initial tests in which the TBA extracts were treated with nitric and perchloric acids to destroy TBA, followed by evaporation of the solution to strong fumes of perchloric acid or to dryness. This loss of chromium was considered to be due to the formation of volatile chromyl chloride with the chlorine produced during the decomposition of perchloric acid.[24] Subsequent tests showed that chromium(VI) cannot be completely stripped from the chloroform phase with either 50% v/v nitric acid or ~3% v/v perchloric acid containing hydrogen peroxide to reduce chromium (VI) to chromium(III), but shaking the extract with 1% v/v ammonia solution is effective for low levels of chromium. However, at the 1-mg level, a small amount of chromium (up to ~50 μg) often, but not always, remained in the chloroform phase, particularly if it was slightly cloudy after the back-extraction step. Although this residual chromium could be recovered by a second back-extraction, this was inconvenient because it required transfer of the organic layer to a second separatory funnel. It was thought that the difficulty might be due to formation of colloidal aggregates[22,25] in the TBA-chloroform phase, and subsequent tests showed that chromium can be completely recovered if a solvent of high dielectric constant (methanol or acetone) is added to the extract before the stripping step. It was found that washing the ammoniacal chromium(VI) layer with chloroform to remove any excess of TBA can result in formation of an emulsion. Consequently, the excess of TBA is best removed by washing with chloroform after the ammoniacal solution has been treated with hydrogen peroxide and acidified with perchloric acid, to reduce chromium(VI) to chromium(III).

Because the atomic absorbance of chromium depends on its oxidation state[4,5,26] and on the ionic species[27] present in solution, the chromium in the calibration solutions must be in the same form as in the sample solutions. This is conveniently done by treating potassium dichromate solutions in the same way as the sample solutions obtained after the back-extraction of chromium.

Separation of chromium by co-precipitation with hydrous ferric oxide

Fusion with sodium peroxide is usually used for the decomposition of ores and related materials containing chromium in the form of chromite. However, tests showed that the large amounts of sodium sulphate produced when the melt is acidified with sulphuric acid inhibit the extraction of small amounts of chromium with TBA, especially with smaller amounts of chromium and higher sulphuric acid concentrations. No chromium was extracted from 0.5*M* sulphuric acid containing 5 μg of chromium(VI) and 5 g of sodium sulphate, whereas ~35 and 90% were extracted at the 20- and 50-μg levels, respectively. Approximately 30 and 70% were extracted at the 20- and 50-μg levels, respectively, from 1*M* sulphuric acid. Up to ~0.3 g of sodium sulphate can be present in 0.5–1*M* sulphuric acid medium without producing significant error at the 1–2 μg chromium level. Similar interference from large amounts of sulphates has been reported for the TOA–benzene extraction system.[22]

Co-precipitation of chromium(III) with hydrous ferric oxide from ammoniacal medium is often used for preliminary separation of chromium[20,28] and tests showed that this was an effective means of separating it from sodium sulphate before the extraction step. The chromium(VI) produced in the fusion is reduced to the tervalent state (required for the co-precipitation) by the hydrogen peroxide produced when the melt is dissolved in dilute sulphuric acid. However, because peroxide oxidizes chromium(III) to chromium(VI) in alkaline media,[29] the excess of hydrogen peroxide must be completely removed, before the co-precipitation step, by fuming with sulphuric acid. The hydrous oxide precipitate can readily be dissolved in 0.7*M* sulphuric acid before the extraction, but the chromium(III) must be re-oxidized to chromium(VI).

Oxidation of chromium

Ammonium persulphate with silver nitrate as catalyst is usually used to oxidize chromium(III) in acid media, but ceric ammonium sulphate[10] was used in this work because it introduces less sulphate into the solution and because silver nitrate would lead to co-extraction of nitric acid.[21] Tests showed that ceric ammonium sulphate is an effective oxidant, but that complete oxidation requires a relatively concentrated sulphuric acid medium. Consequently, during the oxidation step, the solution must be evaporated until salts start to form or fumes of sulphur trioxide just start to appear.

Effect of diverse ions

Recent work has shown that uranium(VI), molybdenum(VI), vanadium(V) and platinum(IV) are co-extracted from ~0.1*M* sulphuric acid into chloroform[18] or benzene[19] solutions of TOA. Earlier work showed that, depending on the acid concentration

and the diluent used, rhenium(VII), niobium, tantalum, mercury(II) and zirconium can also be partly extracted from sulphuric acid media into various organic solvents containing tertiary amines.[14–16] The effects of rhenium, uranium, platinum and mercury were not considered, because only minor or trace amounts of these elements are usually present in most ores and related materials. Furthermore, rhenium and mercury would be removed by volatilization during the initial fuming with sulphuric acid in the presence of hydrobromic acid, and platinum would be removed during the co-precipitation step. Vanadium and zirconium are not extracted into TBA solution under the proposed conditions, and niobium and tantalum (also titanium) form insoluble hydrolysis products during the initial sample preparation step. These compounds, and any lead sulphate present, would be left on the filter when the hydrous oxides from the co-precipitation are dissolved. Molybdenum(VI) is only slightly co-extracted and, in the absence of vanadium, and if chromium is separated from most of the molybdenum by the co-precipitation step, up to ~100 mg can be present in the sample without causing error in the final determination of chromium in an air–acetylene flame. Up to ~20 mg each of molybdenum and vanadium can be present if chromium is first separated by co-precipitation, and up to ~10 mg of each will not interfere if they are present during the extraction step.

Large amounts of tungsten cause low results for chromium because of the formation of tungsten trioxide which occludes chromium. Up to ~10 mg each of tungsten and vanadium will not interfere if chromium is first separated from the bulk of these elements by the co-precipitation, and if the residual tungsten trioxide is removed by filtration before the extraction. In the absence of vanadium, up to ~10 mg of tungsten can be present during the oxidation step without causing error in the result. However, more than ~1 mg of each will interfere during this step if present simultaneously. The mechanism by which vanadium interferes in the presence of molybdenum and tungsten is not known, but it probably involves formation of heteropoly vanadium–molybdenum and vanadium–tungsten complexes. In the absence of molybdenum and tungsten, up to 100 mg of vanadium(V) will not interfere in the extraction of chromium.

Up to at least 30 mg of phosphate, 50 mg of arsenic(V), bismuth and titanium(IV), 100 mg of zirconium, copper(II), zinc, nickel and aluminium will not interfere either in the co-precipitation or in the extraction and subsequent determination of up to ~1 mg of chromium. Barium, strontium and lead will not interfere because they do not form insoluble chromates under the proposed conditions. Cerium(IV) oxidizes manganese(II) to manganese dioxide which can be filtered off before the extraction. Up to ~5 mg of manganese(II) will not interfere in the oxidation of up to 1 mg of chromium(III) when the recommended amount of ceric ammonium sulphate is used. Manganese in larger amounts causes low results because it is preferentially oxidized and uses up the cerium(IV) required for the oxidation of chromium. However, up to ~100 mg of manganese can be present if chromium is separated from most of it by the co-precipitation with iron(III). Possible interference from antimony and tin, which, depending on the amount present, may form insoluble hydrolysis products during the oxidation step, is avoided by removing them by volatilization as the bromides during the sample preparation step. Arsenic, germanium and selenium are also removed under these conditions.

Applications

To test the reliability of the proposed method, it was applied to the analysis of a CCRMP ore and two rock samples for which values are given for information purposes,[30] to six diverse CCRMP ores and concentrates to which a known amount of chromium was added, and to a CCRMP rock sample[30] and four soil samples[31] that have been certified for chromium. It was also applied to two National Bureau of Standards (NBS) certified reference clay samples. Various NBS and British Chemical Standards (BCS) certified reference iron and steel samples and nickel-copper, aluminium and zirconium alloys were also analysed after the separation of chromium by TBA extraction. The results of these analyses, which are the means of three or four AAS measurements, are given in Tables 1 and 2.

DISCUSSION

Table 1 shows that the results obtained for the CCRMP reference ores and rocks, UM-1, UM-2 and UM-4, are in reasonably good agreement with the CCRMP values given for information purposes, which are the results of a single analysis by AAS.[32] The results obtained for the gabbro sample, MRG-1, the soil samples, SO-1 to SO-4, and the clay samples are also in good agreement with the certified values. Those obtained for the CCRMP ores and concentrates, to which a known amount of chromium was added, agree with the total calculated amount present. Table 2 shows that the results obtained for the NBS and BCS iron, steel and non-ferrous alloys are in excellent agreement with the certified values.

An advantage of the proposed extraction method over those involving extraction with TOA solutions from ~0.1–0.2*M* sulphuric acid media[18,19] is the higher sulphuric acid concentration required, because this is also more practical for the preliminary sample preparation. The method for iron and steel also has some advantages over a recent AAS method,[10] which is based on the extraction of chromium(VI) into MIBK from a hydrochloric acid medium containing sodium fluoride to complex iron, and in which chromium is determined in the organic phase. This extrac-

Table 1. Determination of chromium in CCRMP reference ores, concentrates, rocks and soils and in NBS clays

Sample	Nominal composition, %	Certified value and 95% confidence limits, % Cr	Cr found, %
UM-1 Nickel-copper-cobalt ore	36.7 SiO_2, 1.0 Al_2O_3, 17.2 FeO, 36.1 MgO, 2.3 CaO, 3.5 S	0.31*	0.31_2
PR-1 Molybdenum ore	39.2 Si, 2.4 Al, 1.2 Fe, 1.4 Ca, 0.8 S, 0.6 Mo (0.0017 Cr)†	0.011_7‡	0.012_1
CD-1 Antimony ore	32.9 Si, 5.5 Al, 2.8 Fe, 0.6 Mg, 1.4 Ca, 3.1 S, 3.6 Sb, 0.7 As (0.0091 Cr)†	0.019_1‡	0.020_1
CT-1 Tungsten ore	17.2 Si, 2.9 Al, 8.6 Fe, 2.0 Mg, 12.2 Ca, 8.1 S, 0.7 Mn, 1.0 W (0.0028 Cr)†	0.012_8‡	0.012_5
CZN-1 Zinc concentrate	1.0 SiO_2, 10.9 Fe, 7.5 Pb, 44.7 Zn, 30.2 S (N.D. Cr)†	0.010_0‡	0.0098
CPB-1 Lead concentrate	0.7 SiO_2, 8.4 Fe, 64.7 Pb, 4.4 Zn, 17.8 S, 0.4 Sb (N.D. Cr)†	0.010_0‡	0.0095
CCU-1 Copper concentrate	2.6 SiO_2, 30.8 Fe, 3.2 Zn, 24.7 Cu, 35.6 S (N.D. Cr)†	0.010_0§	0.0099
UM-2 Ultramafic rock	39.2 SiO_2, 7.2 Al_2O_3, 13.0 FeO, 25.5 MgO, 4.7 CaO, 0.9 S, 0.2 TiO_2	1.03*	0.99_8, 0.98_5**
UM-4 Ultramafic rock	39.4 SiO_2, 9.0 Al_2O_3, 12.8 FeO, 22.5 MgO, 6.3 CaO, 0.4 S, 0.4 TiO_2	1.77*	1.80, 1.82**
MRG-1 Gabbro	39.2 SiO_2, 8.5 Al_2O_3, 17.8 Fe_2O_3, 13.5 MgO, 14.7 CaO, 3.7 TiO_2, 0.01, BaO, 0.03 SrO	$0.04_8\mu$††	0.048_5, 0.049_9**
SO-1 Regosolic clay soil	25.7 Si, 9.4 Al, 6.0 Fe, 2.3 Mg, 1.8 Ca, 0.5 Ti, 0.1 Ba, 0.03 Sr	0.0160 (0.0145–0.0175)¶	0.017_0, 0.017_6**
SO-2 Podzolic B horizon soil	25.0 Si, 8.1 Al, 5.5 Fe, 0.5 Mg, 2.0 Ca, 0.9 Ti, 0.1 Ba, 0.03 Sr	0.0016 (0.0014–0.0018)¶	0.0007, 0.0010**
SO-3 Calcareous C horizon soil	15.9 Si, 3.1 Al, 1.5 Fe, 5.1 Mg, 14.8 Ca, 0.2 Ti, 0.03 Ba, 0.02 Cr	0.0026 (0.0023–0.0029)¶	0.0024, 0.0028**
SO-4 Chernozemic A horizon soil	32.0 Si, 5.5 Al, 2.4 Fe, 0.5 Mg, 1.1 Ca, 0.3 Ti, 0.08 Ba, 0.02 Sr	0.0061 (0.0055–0.0066)¶	0.0063, 0.0065**
NBS-97 Flint clay	42.9 SiO_2, 38.8 Al_2O_3, 1.0 Fe_2O_3, 2.4 TiO_2, 0.3 ZrO_2, 0.02 BaO	0.054_1††	0.065_7, 0.062_1**
NBS-98 Plastic clay	59.1 SiO_2, 25.5 Al_2O_3, 2.1 Fe_2O_3, 0.7 MgO, 1.4 TiO_2, 0.06 BaO	0.014_4††	0.014_3

* CCRMP value given for information only (not certified).
† Chromium found by the proposed method—N.D. means none detected.
‡ Value includes chromium present† and 100 μg added to a 1-g sample.
§ Value includes chromium present† and 50 μg added to a 0.5-g sample.
¶ CCRMP value reported in μg/g.
** Results for two individual samples.
†† Calculated from Cr_2O_3.

Table 2. Determination of chromium in NBS and BCS iron, steel and non-ferrous alloys

Sample	Nominal composition, %	Certified value and range, % Cr	Cr found, %
NBS-3 White iron	2.3 C, 0.4 Mn, 1.0 Si, 0.01 Mo, 0.01 V	0.051 (0.049–0.052)	0.052
NBS-5L Cast iron	2.6 C, 0.7 Mn, 0.3 P, 1.8 Si, 1.0 Cu, 0.02 Mo, 0.03 V	0.148 (0.144–0.150)	0.152
NBS-6E Cast iron	2.6 C, 1.4 Mn, 0.4 P, 2.3 Si, 0.3 Cu, 0.02 Mo, 0.02 V	0.074 (0.064–0.082)	0.073
NBS-7f Cast iron	2.8 C, 0.4 Mn, 0.9 P, 1.9 Si, 0.05 V	0.015*	0.013
NBS-10g Bessemer steel	0.2 C, 0.9 Mn	0.008 (0.006–0.01)	0.0076
NBS-19g Acid open-hearth steel	0.2 C, 0.6 Mn, 0.2 Si, 0.01 Mo, 0.01 V	0.374 (0.369–0.380)	0.383, 0.376
NBS-30e Chromium–vanadium steel	0.5 C, 0.8 Mn, 0.3 Si, 0.15 V, 0.01 Mo	0.934 (0.929–0.939)	0.951, 0.949
NBS-36a Chromium–molybdenum steel	0.1 C, 0.4 Mn, 0.4 Si, 0.2 Ni, 0.9 Mo	2.41 (2.39–2.43)	2.42, 2.36†
NBS-55c Open-hearth iron		0.003*	0.0026, 0.0027
NBS-107a Nickel–chromium–molybdenum cast iron	2.7 C, 0.6 Mn, 0.3 P, 1.4 Si, 1.0 Ni, 0.8 Mo, 0.03 V	0.479 (0.469–0.491)	0.494, 0.491
NBS-132 Molybdenum–tungsten–chromium–vanadium steel	0.8 C, 0.3 Mn, 0.2 Si, 1.6 V, 7.1 Mo, 6.3 W	4.11 (4.06–4.14)	4.11, 4.15†
NBS-134a High speed steel	0.8 C, 0.2 Mn, 0.3 Si, 1.3 V, 8.4 Mo, 2.0 W	3.67 (3.64–3.69)	3.71, 3.69†
NBS-153 Molybdenum–cobalt steel	0.5 C, 0.2 Mn, 0.2 Si, 2.0 V, 8.4 Mo, 1.6 W, 8.5 Co	4.14 (4.12–4.17)	4.12, 4.08†
NBS-159 Chromium–molybdenum (silver bearing) steel	0.5 C, 0.8 Mn, 0.3 Si, 0.05 V, 0.4 Mo, 0.1 Ag	1.00 (0.99–1.03)	1.01, 1.00†
NBS-363 Low-alloy steel	0.6 C, 1.5 Mn, 0.7 Si, 0.3 Ni, 0.3 V, 0.03 Mo, 0.05 W	1.31*	1.32, 1.32†
BCS-273 Mild steel	0.05 Mo, 0.3 W	0.075	0.076
NBS-85a Aluminium alloy	2.5 Cu, 1.6 Mg, 0.7 Mn, 0.4 Ni, 0.2 Fe, 0.1 Si	0.231 (0.226–0.24)	0.233
NBS-86c Aluminium alloy	7.9 Cu, 1.5 Zn, 0.9 Fe, 0.7 Si	0.029 (0.022–0.032)	0.030
NBS-87a Silicon–aluminium alloy	6.2 Si, 0.6 Fe, 0.6 Ni, 0.4 Mg, 0.3 Mn, 0.2 Ti	0.11*	0.121
NBS-162a Nickel–copper alloy	64.0 Ni, 30.6 Cu, 2.2 Fe, 1.6 Mn, 0.9 Si, 0.5 Al	0.042 (0.036–0.048)	0.041
NBS-360 Zircalloy-2	1.4 Sn, 0.2 Fe	0.114*	0.107

* NBS provisional result.

† Results obtained by taking duplicate fractions of the same sample solution through the extraction step.

tion procedure was investigated in initial work but was ultimately abandoned because two extraction steps, requiring the use of two separatory funnels, are needed for the complete extraction of 1 mg of chromium. The solution must also be cooled to $\leq 10°$ to prevent the reduction of chromium(VI) when hydrochloric acid is added before the extraction, and also to increase the distribution coefficient.[33] Furthermore, the MIBK extract is not always stable.[10] In the proposed method, cooling is not required before extraction and the final sample solution is stable.

In the method for iron and steel, it is recommended that the fraction taken for extraction should not contain more than ~1 mg each of tungsten and vanadium. However, up to ~10 mg of each can be present if chromium is first separated from the bulk of these elements by co-precipitation with iron(III) as in the method for ores and related materials. This requires the addition of 4 or 5 drops of 30% hydrogen peroxide to ensure that chromium is in the tervalent state, followed by evaporation of the solution to fumes of sulphur trioxide to remove the excess of hydrogen peroxide before the co-precipitation step.

A perchloric acid medium was chosen for the determination of chromium because it has very little effect on the determination in an air–acetylene flame when a single-slot burner is used.[3,34] A 5% v/v concentration was used for convenience because ~0.5 ml of the concentrated acid is required to neutralize and slightly acidify the ammonia solution used for the back-extraction of chromium. Therefore, this amount is present, as ammonium perchlorate and perchloric acid, when a 10-ml final sample solution volume is used. The concentration of the excess of perchloric acid under these conditions is ~2% by volume, and this concentration can also be used in the proposed methods, but that in the calibration solutions should then be adjusted accordingly.

The proposed methods are suitable for ores and related materials containing as little as ~0.0002% of chromium and for iron, steel and non-ferrous alloys containing $\geq 0.001\%$. However, the accuracy that can be obtained at these levels depends on the magnitude of the reagent blank. In this work, the blank varied from ~4 to 7 μg of chromium after sample decomposition by fusion, and from 1 to 2 μg after decomposition with acids. There is also a slight positive error (~7% at the 10–20-μg level) associated with the determination of small amounts of chromium when the final sample volume is 10 ml. This is due to the enhancing effect of the ammonium perchlorate in the solution. This effect is compensated for by the blank correction when the blank and sample solutions contain comparable amounts of chromium. No significant error occurs when the final sample solution is diluted to ≥ 25 ml. Consequently, greater accuracy will be obtained at low levels of chromium if the final solution can be diluted to 25 ml. The mean standard deviations at the 20- and 1000-μg levels of chromium were 1.5 and 10 μg respectively (21 and 15 variates).

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POLAG—A GENERAL COMPUTER PROGRAM TO CALCULATE STABILITY CONSTANTS FROM POLAROGRAPHIC DATA

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Summary—A general computer program, POLAG, that will calculate stability constants from polarographic data, has been written. The program requires no pretreatment of the experimental data. Any equilibrium model, consisting of species having the general formula $M_mH_j(OH)_kL_nL'_p$ may be fitted to the data. The performance and versatility of POLAG has been demonstrated by reprocessing polarographic data previously published by four different research groups.

A recently published review[1] on stability-constant determination aided by computer programs of various levels of sophistication covered the literature up to about 1976, and noted that about 100 programs, or modifications of existing programs, have been published. It is therefore surprising that only two references, of the 163 listed in the review, report the use of a computer program to calculate stability constants from polarographic data.[2,3] Both publications adopt essentially the DeFord and Hume[4,5] method, thereby incorporating certain approximations. Meites[6] has also described a program based on the DeFord and Hume approach.

Although experimental uncertainties, seemingly inherent in polarography, make the technique potentially less attractive as a method of studying complex equilibria, it is nonetheless a useful approach. Meites[7] and Crow[8] provide valuable practical details which, if observed, can lead to data from which reliable constants may be determined.

It is the objective of this publication to provide a rigorous and approximation-free method of processing reversible polarographic data so as to obtain reliable estimates of the stability constants for complex formation. It will be demonstrated that the program is applicable to equilibria of the type:

$$M + nL \rightleftharpoons ML_n; \quad n = 1,2 \ldots N$$

as well as:

$$M + nL + pL' \rightleftharpoons ML_nL'_p; \quad n,p = 0,1 \ldots N(P)$$

whether or not the data are dependent on pH.

METHOD

The program, POLAG, is derived in large part from SQUAD,[9] which in turn was based on the algorithms used in SCOGS.[10] In other words, POLAG employs well tried and tested numerical methods. It has been noted earlier that the most common method of extracting stability constants from polarographic data employs the approach detailed by DeFord and Hume.[4,5] The starting equation for this method is:

$$F = \exp\left[\frac{nF}{RT}(E_{1/2a} - E_{1/2c})\right] + \ln\left(\frac{I_{da}}{I_{dc}}\right) \quad (1)$$

$$F = 1 + C_L\beta_1 + C_L^2\beta_2 \ldots C_L^n\beta_N \quad (2)$$

where $E_{1/2a}$ and $E_{1/2c}$ are the half-wave potentials for the uncomplexed and complexed metal ion respectively; I_{da} and I_{dc} are the diffusion currents for uncomplexed and complexed metal ion; C_L is the analytical concentration of the ligand and β_N is the overall stability constant of the Nth complex. Equation (2) is based on the assumption that since $C_L \gg C_M$, then $[L] = C_L$. The graphical analysis of equation (2) is discussed in detail by Crow.[8] However, the method is, of necessity, tedious and the patience of the researcher may be exhausted before all reasonable models have been tested.

It is often advantageous to use a second ligand to provide competition for the metal ion. In this situation the method of Schaap and McMasters[3,11] is used as originally published or with modification.[12] However, since the application of the method is, in essence, based on the graphical approach used by DeFord and Hume, the ease of data-processing is questionable. The technique is also based on the assumption that C_L and $C_{L'} \gg C_M$, where $C_{L'}$ is the analytical concentration of the second ligand.

The exact form of equation (2) is:

$$F = 1 + [L]\beta_1 + [L]^2\beta_2 \ldots [L]^n\beta_N \quad (3)$$

which is derived from:

$$F = \frac{C_M}{[M]}. \quad (4)$$

POLAG is a non-linear least-squares iterative program that seeks to minimize U, the sum of squares of the residuals, *i.e.*,

$$U = \sum_1^N (F_{CALC} - F_{OBS})^2 \quad (5)$$

where N is the number of data points; F_{OBS} is given by equation (1) and represents the experimental data; F_{CALC} is obtained from equation (4).

Consider the formation of the general complex $M_mH_j(OH)_kL_nL'_p$. For the present, let $j = k = 0$. The mass-

balance equations for the metal and the two ligands are:

$$C_M = [M] + \sum m[M_mL_nL'_p] \quad (6)$$

$$C_L = [L] + \sum n[M_mL_nL'_p] \quad (7)$$

$$C'_L = [L'] + \sum p[M_mL_nL'_p] \quad (8)$$

$$C_M = \sum_0^m \sum_0^n \sum_0^p m\beta_{mnp}[M]^m[L]^n[L']^p \quad (9)$$

$$C_L = \sum_0^m \sum_0^n \sum_0^p n\beta_{mnp}[M]^m[L]^n[L']^p \quad (10)$$

$$C'_L = \sum_0^m \sum_0^n \sum_0^p p\beta_{mnp}[M]^m[L]^n[L']^p \quad (11)$$

where

$$\beta_{mnp} = \frac{[M_nL_nL'_p]}{[M]^m[L]^n[L']^p}. \quad (12)$$

Thus for given values of β_{mnp}, C_M, C_L and C'_L, equations (9)–(11) may be solved to give [M]. Hence F_{CALC} depends on the particular combination of m, n and p and the values of β_{mnp}. POLAG employs the same algorithm that is used in SPECON II.[3] Therefore, various equilibrium models may be fitted to the polarographic data simply by changing the input data values of m, n and p together with appropriate initial values for β_{mnp}. An additional mass-balance equation is used when j or $k \neq 0$.

Traditionally $E_{1/2s}$ is obtained from solutions of the metal ion without added ligand(s), or plotting $E_{1/2}$ *vs.* C_L and extrapolating to $C_L = 0$ where $E_{1/2s} = E_{1/2}$ ($C_L = 0$). POLAG allows the user to include $E_{1/2s}$ as an adjustable parameter. Alternatively, the initial value may be obtained from the more traditional approaches.

POLAG is written in ANSI standard FORTRAN. A documented version, together with sample data, is available from the author upon request.

RESULTS AND DISCUSSION

A number of published polarographic studies, where the basic data were given, have been re-examined. The results of reprocessing these data are presented below.

Cadmium–chloride[14]

This study demonstrated the use of differential pulse polarography (DPP) for the determination of stability constants. The cadmium–chloride system was studied and stability constants were obtained graphically by the DeFord and Hume[4,5] method. These data have been analysed by means of POLAG, and the results are presented in Table 1, entry II. It is clear that the data are of exceptionally high quality. When $E_{1/2s}$ is refined simultaneously with the stability constants, entry III, the overall fit of the data, given by:

$$\sigma_{DATA} = \sqrt{\frac{\Sigma(E_{obs} - E_{calc})^2}{\text{degrees of freedom}}} \quad (13)$$

is only 2.32×10^{-5}. $E_{1/2s}$ (refined) is within 0.4 mV of the experimentally determined value. This is the only one of the studies re-examined here that employed DPP, and it will be seen that σ_{DATA} is lower by a factor of at least 20 for this system than that for any other.

The effect of using equation (4) rather than the approximate equivalent, equation (2), in the Nernst equation is small but not insignificant.

Cadmium–thiocyanate[5]

This study was the experimental verification of the adaptation by DeFord and Hume[4,5] of Leden's[15] method. $E_{1/2}$ was measured to within 0.1 mV and the results of each experiment could be duplicated to better than 1 mV.

The reported data were processed by POLAG for several different combinations from ML to ML_6, inclusive, with and without simultaneous refinement of $E_{1/2s}$. The results for those models that converged are presented in Table 2.

These data are about 100 times less precise than the data of Heath and Hefter.[9] The original model, for complexes ML–ML_4, inclusive, would refine only when $E_{1/2s}$ was fixed at the published value. This model is in doubt, because of the high standard deviation of the constant for ML_3. More realistic standard deviations for the constants were obtained for the model ML_1, ML_2 and ML_4, trial III, Table 2. When $E_{1/2s}$ was refined for this model, trial IV, all refined parameters, including $E_{1/2s}$, were nearly identical. Non-convergence was noted for the model ML_1, ML_2 and ML_3, $E_{1/2s}$ fixed or refined. It is reasonably

Table 1. Cadmium–chloride system, I = 1.0*M*, *T* = 25°C

Model	log β			$E_{1/2s}$, *V*	σ_{DATA}, *V*
	ML	ML_2	ML_3		
I[a]	1.352_2	1.748_1	1.544_0	−0.5885[b]	—
II	1.329_7 0.002_5	1.736_3 0.006_4	1.514_7 0.011_8	−0.5885[b]	3.84×10^{-5}
III	1.304_8 0.009_0	1.738_6 0.003_8	1.480_0 0.014_5	−0.5889[d] 0.0001	2.32×10^{-5}

[a] Published values for constants.
[b] $E_{1/2s}$ used in original study.
[c] Standard deviation of constant.
[d] $E_{1/2s}$ varied simultaneously with stability constants.

clear that ML_3 either does not contribute significantly to the data or does not exist.

This example demonstrates the inherent difficulty in using the method of DeFord and Hume. The method requires subjective judgements to be made when selecting the next $F_{(i)}$ function to be plotted, and the data to be included in the linear sections of the plot. The reader is referred to the paper of Hume *et al.*,[4] which discusses the various rationales for accepting or rejecting data before plotting. These authors have also plotted "percentage of cadmium in various forms as a function of free thiocyanate ion concentration" (Ref. 5, Fig. 3). ML_3 is responsible for less than 5% of the cadmium and then only when C_{SCN} is greater than 0.5*M*. In other words, the contribution of the proposed complex, ML_3, to the observed polarographic data is very small, so the existence of ML_3 is questionable.

Momoki, Ogawa and Sato[12] re-investigated this system as part of a study on improved measurement techniques for polarography. Their results are in agreement with those presented here.

Meites[6] has also reprocessed the data of Hume *et al.*, using a non-linear regression algorithm to evaluate the coefficients (stability constants) of the polynomial equation (2). The results are presented in Table 2, entry VI, and are seen to be in close agreement with those of Hume *et al.* Meites also found that the models involving complexes ML–ML_5, inclusive, and ML–ML_6, inclusive, also fitted the data equally well. The values of the constants for these models indicated that ML_3 was the predominant species and ML_4 was the least significant. These models, when fitted to the original data with the aid of POLAG, caused the refinement process to diverge. POLAG will terminate if the standard deviation of any constant rises above 100. This situation is always encountered when an erroneous model is selected. For example, the model ML–ML_5, inclusive, gave $\log \beta_1 = 0.39 \pm 12.2$; $\log \beta_2 = 2.27 \pm 2.2$; $\log \beta_3 = 0.26 \pm 203.3$; $\log \beta_4 = 1.90 \pm 44.1$; $\log \beta_5 = -0.24 \pm 174.5$; $\sigma_{DATA} = 1.1 \times 10^{-2}$. The starting values for β_1–β_5 were those quoted by Meites.[6] Thus POLAG gives clear indications of whether a model is an appropriate fit to the data. The differences between employing polynomial fitting and conventional non-linear least squares have been discussed by Gans[16] and in detail by Bond.[17]

Cadmium–thiocyanate–nitrate system[17]

To demonstrate the applicability of POLAG to ternary systems, the original data reported by Momoki *et al.*[12] have been reprocessed. The results of testing several different models are shown in Tables 3 and 4. In each model the value of $E_{1/2s}$ has been fixed at the reported value of −0.5594 V. It is clear from Momoki's data (Ref. 7, Table V) that both $Cd(SCN)_n$ and $Cd(SCN)_n(NO_3)_m$ are present in solution. The first entry in Table 3 presents the results of the refinement process when it is assumed that only one complex exists in solution. The remaining models, II–VII, are the refined constants for various combinations of ML–ML_4. In fact, all the mathematical combinations of ML_1, ML_2, ML_3 and ML_4 were tried, but only those models that converged are reported. There is little to choose between models III, V, VI and VII according to the values of σ_{DATA}. However, it appears that the most likely model is VI followed by V and VII, when the values of $\sigma(\log \beta)$ are considered.

Table 4 reports the results of including ternary complexes in the model. Many other models were tried but did not converge and have not been

Table 2. Cadmium–thiocyanate system,[5] $I = 2.0M$, $T = 25°C$

Model	log β				$E_{1/2s}$, V	σ_{DATA}, V
	ML	ML_2	ML_3	ML_4		
I[a]	1.04	1.75	0.78	1.78	−0.5724[b]	—
II	1.119_3 0.308_4	1.572_3 0.803_6	1.476_6 1.897_3	1.883_9 0.344_4	−0.5724	2.32×10^{-3}
III	1.059_7 0.189_8	1.718_5 0.153_2	—	1.955_0 0.071_6	−0.5724	2.31×10^{-5}
IV	1.059_4 0.317_1	1.718_4 0.397_2	—	1.951_2 0.314_3	−0.5725[c] 0.0022	2.11×10^{-3}
V[d]	1.04	1.84	—	1.87	−0.5724	—
VI[e]	1.03	1.73	0.69	1.77	−0.5724	—

[a] Published values for constants.[5]
[b] $E_{1/2s}$ used in original study.
[c] $E_{1/2s}$ varied simultaneously with stability constants.
[d] Published values of Momoki, Ogawa and Sato.[12]
[e] Published values of Meites.[6]

Table 3. Cadmium–thiocyanate–nitrate system, $I = 2.0$, $T = 25°C$, binary models only

Model		ML	ML_2	ML_3	ML_4	
I	$\log \beta$	2.262_1	2.560_4	2.758_5	2.880_4	
	$\sigma(\log \beta)$	0.066_9	0.021_4	0.051_5	0.092_3	
	σ_{DATA}, V	1.18×10^{-2}	3.65×10^{-3}	8.37×10^{-3}	1.43×10^{-2}	
		$\log \beta$				
		ML	ML_2	ML_3	ML_4	σ_{DATA}, V
II		0.942_7	2.533_1	—	—	3.58×10^{-3}
		0.319_6	0.029_7			
III		1.698_2	—	2.535_5	—	1.45×10^{-3}
		0.019_7		0.013_8		
IV		—	2.463_7	2.016_9	—	3.29×10^{-3}
			0.042_0	0.158_1		
V		1.633_2	1.656_6	2.479_2	—	1.41×10^{-3}
		0.0415	0.227_3	0.0349		
VI		1.501	2.243_1	—	2.213_6	1.34×10^{-3}
		0.044_8	0.034_9		0.034_3	
VII		1.553_6	2.109_8	2.017_0	2.031_6	1.34×10^{-3}
		0.060_9	0.157_2	0.400_1	0.207_4	

reported. It is significant to note that, of the binary models tested, only V and VI, Table 3, permitted the successful inclusion of ternary complexes. It can be seen from Table 4 that, apart from models IV and VI, each model led to low and comparable values for σ_{DATA}, but I–IV resulted in several high values for $\sigma(\log \beta)$. On the other hand, the basic model ML, ML_2 and ML_4 generally resulted in much better values for $\sigma(\log \beta)$.

It is generally true that σ_{DATA}, giving the overall fit to the data, is a useful tool for discriminating between two models when the σ values are 6×10^{-4} and 1.5×10^{-3} V. When the values are as close as 5×10^{-4} and 6×10^{-4} V, σ_{DATA} ceases to be the indicator of choice. In these situations an attempt must be made to base judgements on the individual values of $\sigma(\log \beta)$ for each constant of the various models. In either situation the most important criterion for the

Table 4. Cadmium–thiocyanate–nitrate system, $I = 2.0$, $T = 25°C$, ternary models only

	$\log \beta$							
Model	ML	ML_2	ML_3	ML_4	MLL′	ML_2L'	ML_3L'	σ_{DATA} V
I	1.445_2	1.196_5	2.593_9	—	1.387_7	—	—	5.74×10^{-4}
	0.030_1	0.295_8	0.012_8		0.319_1			
II	1.463_3	1.100_0	2.594_9	—	1.374_3	0.562_2	—	5.82×10^{-4}
	0.039_1	0.507_3	0.013_1		0.054_4	1.382		
III	1.442_7	1.280_9	2.515_4	—	1.321_9	—	0.526_9	5.40×10^{-4}
	0.030_5	0.221_5	0.013_3		0.035_5		1.566	
IV	1.690_2	1.062_1	2.543_5				1.903_0	1.41×10^{-3}
	0.040_7	1.109_6	0.034_4				0.180_4	
V	1.332_5	2.152_7	—	2.229_5	1.190_1	—	1.688_8	6.34×10^{-4}
	0.044_2	0.027_0		0.016_1	0.053_8		0.121_2	
VI	1.577_1	2.157_1	—	2.284_9	—	—	2.030_5	1.16×10^{-3}
	0.037_3	0.056_0		0.028_8			0.180_5	
VII	1.247_1	2.262_5	—	2.328_2.	1.317_0	—	—	7.17×10^{-4}
	0.055_3	0.019_6		0.016_5	0.046_2			
VIII	1.317_1	2.216_2	—	2.336_1	1.210_4	1.327_5	—	6.95×10^{-4}
	0.055_9	0.035_1		0.016_4	0.091_0	0.252_4		

right model remains the chemical validity of the proposed complexes.

For this system there is no clear-cut decision to be made between models V, VII and VIII, but the data analysis does indicate that ternary complexes are formed. Models I, II and III are less favourable choices because of the high values for $\sigma(\log \beta)$. Momoki *et al.*, from their analysis of the same data, concluded that the following species were present; $Cd(SCN)^+$ (18.3 ± 1.4), $Cd(SCN)_2$ (137 ± 13), $Cd(SCN)_3^-$ (87.6 ± 26.2), $Cd(SCN)_4^{2-}$ (178 ± 14), $Cd(NO_3)^+$ (1.28 ± 0.60), $Cd(SCN)(NO_3)$ (8.6 ± 6.9) and $Cd(SCN)_2NO_3^-$ (35.6 ± 16.2). Values in parentheses are for β and $\sigma(\beta)$. In the present study no evidence was found for $Cd(NO_3)^+$, and it is doubtful whether $Cd(SCN)_3^-$ exists at detectable concentrations. Models V, VII and VIII, Table 4, give average log β values of about 1.3, 2.2 and 2.3 for ML, ML_2 and ML_4, respectively. The values for the same species, reported in Table 2, model IV, are 1.1, 1.7 and 2.0. Agreement between these two sets is not good, and this may be due to differences in experimental procedures and the quality of the instrumentation.

Cadmium (copper)–ethylenediamine–oxalate systems[11]

Almost all mixed-ligand investigations have followed the procedures developed by Schaap and McMasters.[11] These authors examined the mixed-ligand complexes of ethylenediamine and oxalate with cadmium or copper. The published polarographic data have been reprocessed by using POLAG.

Before the results obtained from POLAG are presented, certain problems with the reported data of Schaap and McMasters need to be discussed. The equation developed by these authors is:

$$F_{00}(X,Y) = A + B[X] + C[X]^2 + D[X]^3 \quad (14)$$

where A, B, C and D are functions of the various stability constants for species present in solution, and of [Y]. X and Y are the two ligands. F_{00} (X,Y) is also defined in terms of the experimentally measurable quantities $E_{1/2a}$, $E_{1/2c}$, *etc.* The difficulty in the use of equation (14) arises from the calculation of [X] and [Y]. Given the pK_a values of oxalic acid (Y) and the experimental pH range, not lower than 5.04, it is perfectly reasonable to assume that *before complexation* $[Y] = C_y$. However, the same is not true for ethylenediamine. Further, since the complexes of both cadmium and copper with ethylenediamine are moderately stable, it is not reasonable to say, as the authors have done, that "... concentration of "free" ethylenediamine, [en], was calculated from the pH and total amount present, using the appropriate pK values...". There is a complicated set of equilibria established, with competition between the two ligands for the metal ion, and between protons and the metal ion for ethylenediamine. Therefore, the assumptions embedded in their statement are not necessarily true, nor is there a simple way in which these assumptions may be checked.

The second problem is that for the cadmium study no total concentration of ethylenediamine taken was reported. Back-calculations were done by using the quoted values of [en] and the pK_a values in an attempt to obtain values for C_{en}. However, some doubt still exists as to the true values of C_{en} used since the authors have reported three sets of values for the pK_a values, that are concentration-dependent.

The results obtained from POLAG are shown in Table 5 for cadmium, and in Table 6 for copper. Given the pH range and ligand concentrations studied, it would, in principle at least, be possible to refine constants for all complexes between cadmium, ethylenediamine and oxalate. Before processing the data with POLAG, some SPECON II calculations were done. The results showed that 97% of the oxalate was either part of the ternary complexes or present as Ox^{2-}. Subsequent attempts to refine the stability con-

Table 5. Cadmium–ethylenediamine–oxalate system,[11] I = 1.0M, T = 25.0°C

Stoichiometry of complex				Ref. 11	This study	
Cd	H	En	Ox	log β	log β	$\sigma(\log \beta)$
1	0	1	0	5.60	5.62_8	0.07_1
1	0	2	0	10.63	10.73_4	0.05_3
1	0	3	0	12.10	12.58_6	0.17_2
1	0	0	1	2.61	2.61[a]	—
1	0	0	2	4.14	4.14[a]	—
1	0	0	3	5.04	5.04[a]	—
1	0	1	1	7.87	7.90_2	0.00_1
1	0	2	1	11.44	11.29_5	0.13_5
1	0	1	2	8.81	8.38_7	0.23_7
0	1	1	0	10.06 or 9.85[b]	10.11_6	0.01_3
0	2	1	0	17.44 or 17.16[b]	17.40_4	0.00_8
0	1	0	1	3.60 or 3.43[b]	3.43[a]	—
0	2	0	1	6.37 or 5.33[b]	5.33[a]	—

$\sigma_{DATA} = 1.34 \times 10^{-3}$ V

[a] Constants not refined by POLAG.
[b] Concentration-dependent pK_a values from Ref. 11.

Table 6. Copper–ethylenediamine–oxalate system,[11] $I = 1.0M$, $T = 25.0°C$

Stoichiometry of complex				Ref. 11	This study	
Cu	H	En	Ox	log β	log β	σ(log β)
1	0	1	0	10.75[a]	11.95_4	0.16_9
1	0	2	0	10.49	20.19_4	0.06_5
1	0	1	0	5.70	5.70[b]	—
1	0	2	0	9.22	9.53_7	0.17_7
1	0	1	1	15.40	15.43_7	0.06_8
0	1	1	0	10.06 or 9.85[c]	10.40_4	0.02_3
0	2	1	0	17.44 or 17.16[c]	17.35_5	0.03_1
0	1	0	1	3.60 or 3.43[c]	3.43[b]	—
0	2	0	1	6.37 or 5.33[c]	5.33[b]	—

$\sigma_{DATA} = 6.97 \times 10^{-4}$ V

[a] Mean value taken from literature earlier than 1961.
[b] Constants not refined by POLAG.
[c] Concentration-dependent pK_a values from Ref. 11.

stants for Cd(Ox), $Cd(Ox)_2^{2-}$ and $Cd(Ox)_3^{4-}$ were unsuccessful owing to the low concentration (<2% of C_{Cd}) of these complexes. The results of the SPECON II calculations showed that, in this situation, $[HOx^-]$ and $[H_2Ox]$ were also very low. Thus, although the pK_a values for oxalic acid are in doubt, they have little or no influence on the final outcome of the distribution of cadmium among the various complexes. Of thirteen possible complexes, refined constants have been obtained for eight.

This approach to the data-processing deserves comment. As stated earlier, the pK_a values and total concentrations of ethylenediamine taken are not known with certainty. Schaap and McMasters' data were processed with POLAG, using values for C_{en} obtained from the back-calculations, giving constants for the ternary complexes and binary complexes of ethylenediamine and cadmium. These values were then fixed and the pK_a values of ethylenediamine refined. Then the new pK_a values were fixed and the constants for the binary and ternary complexes refined. This process was repeated until there were no further changes in all eight constants. New values for C_{en} were chosen and the stability constant refinement process repeated. The results, shown in Table 4, are for C_{en} equal to $0.207M$ and $0.0208M$. Initial back-calculation had given values of $0.209M$ and $0.026M$.

Thus, despite the difficulties with the reported data, it can be seen that most of the stability constants obtained by POLAG are in good agreement with the original published values. The value obtained by POLAG for $Cd(en)_3^{2+}$ may be in doubt. According to the constants in Table 4 this complex is only significant at pH 9.5 and above. $Cd(en)(Ox)_2^{2-}$ is only significant at pH 7 and below.

Generally, it is not good practice to attempt to seek stability constants of binary complexes and ternary complexes simultaneously. The preferred approach is to build up a base of information from sets of experiments for each binary system. However, the point has been made that POLAG can refine any set of selected constants dictated by the input data. Also the use of POLAG does not involve the user in any doubtful or prejudicial assumptions.

The copper–ethylenediamine–oxalate system was treated in much the same manner. The set of original data included the total concentrations of ethylenediamine taken.

After the first few attempts at refining these data it appeared that the data subset, obtained by Schaap and McMasters for $C_{en} = 0.205M$ and $C_{ox} = 0.247M$, Series II, possessed some type of systematic error. This was evident from the values of the residuals, $(E_{OBS}-E_{CALC})$, for each pH. When this subset of data was removed, refinement of the stability constants gave the values shown in Table 5, with σ_{DATA} found to be 6.97×10^{-4} V. No evidence was found in this system, or in the previous one, for protonated or hydroxy complexes. It was possible to refine the stability constant for $Cu(Ox)_2^{2-}$ but not for Cu(Ox). Indeed, when the latter complex was omitted from the model the refined constants were identical to within ± 0.001.

Again it should be noted that the stability constants for the binary complexes do not agree too well with those found by Schaap and McMasters from separate investigations of each binary system.

CONCLUSIONS

This study has shown that POLAG can successfully handle reversible polarographic data from any chemical system consisting of up to two ligands with one metal, with or without competition from protons. No approximations are necessary. Since model changes are made simply by changing one or more data cards, many models may be tested. In situations where many models may be chemically feasible POLAG gives very clear evidence of an incorrect model. It must be remembered, however, that no non-linear least-squares program will always provide an unequivocably "correct" model. This has been demonstrated with the cadmium–thiocyanate–nitrate system. The number of "most likely" models can be reduced, however, by exercising utmost care in obtaining the data.

Often, by repeating the investigations for different metal ion and/or ligand concentrations and comparing the selection of "most likely" models obtained from each set of data, the nature of the minor species can be determined with greater confidence.

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COLLECTION OF MERCURY FROM ARTIFICIAL SEA-WATER WITH ACTIVATED CARBON*

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Summary—Nanogram amounts of mercury(II) and methylmercury in artificial sea-water containing mineral acids as preserving reagents were shown to be collected quantitatively with activated carbon. Mercury concentrated on activated carbon was determined directly by combustion, trapping on gold and electrothermal atomic-absorption spectrophotometry. The activated carbon was purified by heating at 350° for 2 hr. Sulphuric acid and hydrochloric acid were purified by treatment with activated carbon. Interference from iodide was eliminated by using a carbonate buffer wash before the atomic-absorption measurement. Less than 4 ng of mercury in 200–300 ml of artificial sea-water, whether acidified or not (with sulphuric, hydrochloric or nitric acid), was satisfactorily collected with 100 mg of activated carbon. Mercury was also collected quantitatively after oxidative treatment of artificial sea-water.

Previous work[1] has shown that traces of mercury can be collected with activated carbon from dilute aqueous solutions containing preserving reagents. The purpose of the present work was to test the applicability of the collection method to salt solutions such as artificial sea-water. Das and van der Sloot[2] determined mercury in sea-water by neutron activation after preconcentration with activated carbon. However, the present work is different from their work in many respects, including the mercury level. The present work describes purification of the activated carbon, effects of mineral acids that have been used as preserving reagents in mercury collection,[3–6] and determination of mercury by combustion followed by trapping on gold, and electrothermal atomic-absorption spectrophotometry. Less than 4 ng of mercury can be collected from 200–300 ml of acidified artificial sea-water with 100 mg of activated carbon. Recovery of mercury from artificial sea-water after oxidative treatment is also satisfactory.

EXPERIMENTAL

Reagents and apparatus

Activated carbon powders (Merck, GR grade, No. 2186 and Wako Pure Chemical Industries, Ltd., reagent grade) were used after purification described below.

Standard mercury(II) solution (Hg 100 mg/l.) was prepared by dissolving 0.1361 g of $HgCl_2$ (99.5% pure) in 0.1 *M* nitric acid containing 10 mg of L-cysteine per litre, and diluting to exactly 1 litre with the same solvent. Working standard solutions were prepared by diluting the stock solution with the solvent used.

Standard methylmercury chloride solution (Hg 101 mg/l.) was prepared by dissolving 0.1286 g of CH_3HgCl (98% pure) in water and diluting to exactly 1 litre with water. Working standard solutions were prepared by diluting the stock solution with water before use.

Artificial sea-water[7] was prepared by dissolving 23.48 g of NaCl, 10.64 g of $MgCl_2.6H_2O$, 0.040 g of $SrCl_2.6H_2O$, 0.026 g of H_3BO_3, 3.917 g of Na_2SO_4, 1.102 g of $CaCl_2$, 0.664 g of KCl, 0.096 g of KBr, 0.192 g of $NaHCO_3$, and 65 μg of KI[8] in about 800 ml of water and making up to 1000 ml with water. This artificial sea-water contained about 1 ng of mercury per litre.

Water was purified by distilling demineralized water in a glass still and treating the distillate with activated carbon. Purification of the mineral acids is described below.

Filters (Millipore, glass fibre prefilter, AP40) were used after heating in a muffle furnace at 350° for 2 hr.

An atomic-absorption instrument (Nippon Instruments Co. Ltd., Rigaku Mercury SP) was used to determine mercury. In this instrument, the sample is decomposed by heating in a furnace, then the vapour containing mercury is washed with water or buffer solution and the mercury collected on gold. The mercury is volatilized by heating the gold amalgam at 600° and collected again in a second trap containing gold; it is then volatilized from the second trap by heating to 640° and finally determined by atomic-absorption.

Procedure

Transfer 200 or 300 ml of artificial sea-water (acidified or not), containing 0–4 ng of mercury, to an Erlenmeyer flask with a ground-glass stopper. Add 100 mg of activated carbon, stopper the flask, and swirl the solution occasionally. After 1 hr, filter off the carbon at the pump. Transfer the carbon from the flask to the filter with a small amount of water. Roll the filter plus carbon and transfer it to a porcelain boat containing activated alumina. Cover the filter and carbon with activated alumina, sodium carbonate, and calcium oxide, in that order. Place the boat in the furnace of the atomic-absorption instrument and determine the mercury. Before use, heat the activated alumina, sodium carbonate and calcium oxide at about 700° to remove mercury.

Construct the calibration curve by adding 0–4 ng of mercury to the filter plus carbon obtained by applying the procedure in the absence of added mercury, and measuring the peak heights. It should be linear.

Determine a blank by carrying water (acidified or not) through the entire procedure.

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† Reprint requests.

Table 1. Purification of activated carbon* by treating with nitric acid

$[HNO_3]$, *M*	Treatment	Purified activated carbon: Hg content, *ng/g*	Purified activated carbon: Hg collection, %
6	10 min contact	2.2	80
8	10 min contact	1.8	79
8	60 min contact including 15 min warming	1.1	78
16	60 min contact including 15 min warming	1.3	76

* Merck activated carbon; initial Hg content, 3.5 ng/g.

RESULTS AND DISCUSSION

Purification of activated carbon

Nitric acid treatment. Two g of Merck activated carbon were stirred in 50 ml of 6–16*M* nitric acid, filtered off, washed with water, and dried at 105°. The results are shown in Table 1. Because of the incomplete removal of mercury from the activated carbon and incomplete collection of mercury with the purified carbon, this method was abandoned.

Heat treatment. Merck activated carbon was heated in an ordinary muffle furnace. As shown in Table 2, mercury was effectively removed from carbon by heating at 350° for 2 hr. Collection of mercury with the purified carbon was satisfactory. Activated carbon heated at 500° for 1 hr also showed good adsorption of mercury, but the weight loss during the heating was larger. Heating at 350° for 2 hr was the method chosen.

Table 2. Purification of activated carbon by heating in a muffle furnace

Temperature, °C	Time, *hr*	Purified activated carbon: Hg content, *ng/g*	Purified activated carbon: Hg collection, %	Weight loss during heating, %
Merck (initial)		3.5	—	
105	1	4.5	—	10.8
200	1	4.0	—	10.4
300	1	1.9	100	11.0
	2	1.8	—	11.1
	4	1.1	—	11.5
	8	0.5	—	12.2
350	1	0.4	—	12.5
	2	0.1	101	12.8
	3	<0.1	100	14.2
400	1	<0.1	98	16.8
500	1	<0.1	100	25.0
Wako (initial)		30	—	
105	1	—	—	9.0
350	1	0.8	—	16.7
	2	0.8	101	23.9
	3	0.3	—	31.5

— = Not examined.

Purification of mineral acids

Uchino *et al.*[9] have studied removal of mercury from mineral acids and other reagents by means of a chelating resin and activated carbon. Figure 1 shows that sulphuric acid (<9.8*M*) and hydrochloric acid (<5.8*M*) can be purified by treating them with activated carbon. This purification method was therefore adopted. Nitric acid cannot be purified in this way, but the mercury content of the concentrated nitric acid used in this work was so low (about 0.01 ng/ml) that further purification was not necessary.

Collection of mercury from artificial sea-water

Recovery of mercury was low when the procedure was applied in the presence of iodide and water was used as the wash liquid before the mercury was trapped on the gold. The interference decreased with increasing pH of wash liquid and was eliminated by using carbonate buffer solution (0.025*M* $NaHCO_3$–0.025*M* Na_2CO_3, pH 10). This finding supports the idea[1] that the low recoveries were caused during the determination and not during the collection. The carbonate buffer was adopted.

Conditions were studied for the collection of mercury(II) from artificial sea-water not acidified (pH 8.0) or acidified with sulphuric acid, hydrochloric acid, or nitric acid.

The recovery of mercury was practically independent of the amount of activated carbon used, in the range 20–250 mg, Fig. 2. The amount selected for use was 100 mg.

Recoveries of mercury were almost quantitative when the solution and activated carbon were stirred occasionally during a period of 5–60 min.

The effect of the mineral acid concentration on the collection of mercury is shown in Fig. 3. Good recoveries were obtained from artificial sea-water whether

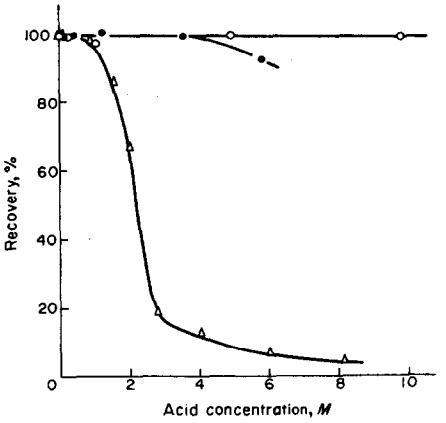

Fig. 1. Collection of mercury(II) from different acid solutions. Activated carbon, 100 mg; contact time, 1 hr. ○, H_2SO_4, 50 ml, 20 ng of Hg(II); ●, HCl, 25 ml, 40 ng of Hg(II); △, HNO_3, 100 ml, 2 ng of Hg(II).

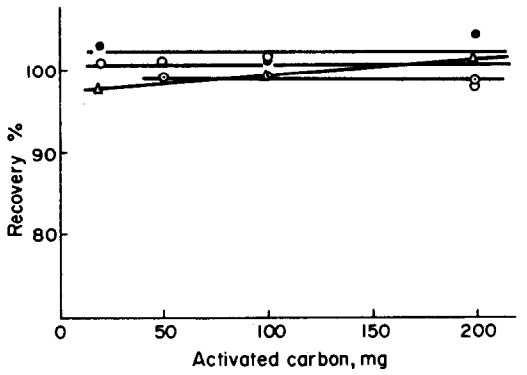

Fig. 2. Effect of amount of activated carbon on the collection of mercury(II) from artificial sea-water. Solution, 200 ml; Hg(II), 3 ng; contact time, 1 hr. ⊙, Unacidified artificial sea-water; ○, artificial sea-water, 0.2*M* in H_2SO_4; ●, artificial sea-water, pH 1.6 (0.03*M* in HCl); △, artificial sea-water, 0.1*M* in HNO_3.

unacidified or made up to 0.7*M* in sulphuric acid, 1*M* in hydrochloric acid, or 0.3*M* in nitric acid.

Calibration curves were constructed by treating water (not acidified and acidified) and artificial sea-water (not acidified and acidified) with activated carbon, filtering, adding 0–4 ng of mercury to the filter plus carbon, and measuring the peak height. The results are shown in Table 3. All the curves were the same, within experimental error. It is therefore more economical to prepare the standards with water.

A preliminary investigation indicated that the mercury content of sea-water was lower than the literature values.[2,3] It became necessary to decrease the blank value by treating the sulphuric acid and water with activated carbon twice, to increase the sample volume to 300 ml, and to use the 2-mV range of the recorder. The results obtained under these conditions are included in Table 3. Recoveries of mercury from purified artificial sea-water were also satisfactory.

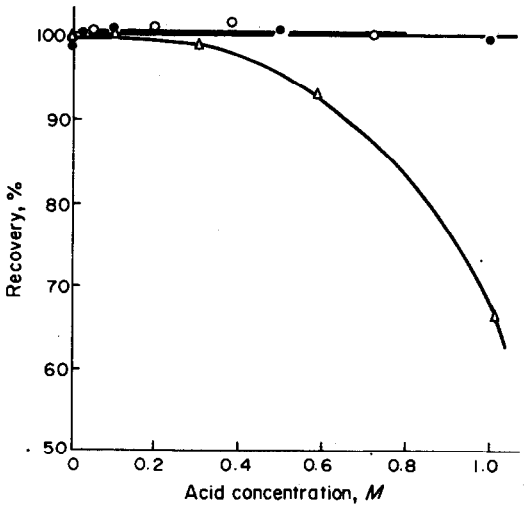

Fig. 3. Effect of acid concentration on the collection of mercury(II) from artificial sea-water. Solution, 200 ml; Hg(II), 3 ng; activated carbon, 100 mg; contact time, 1 hr. ○, Artificial sea-water plus H_2SO_4; ●, artificial sea-water plus HCl; △, artificial sea-water plus HNO_3.

Table 3. Collection of mercury(II) from artificial sea-water

Artificial sea-water	Average recovery, % calibration curve: Water	Average recovery, % calibration curve: Artificial sea-water	Blank, Hg, *ng*	R.S.D., % *n* = 6 (added Hg, *ng*)
Without oxidative treatment				
Not acidified	98[a]	97	0.14	2.3 (2.0)
0.2*M* H_2SO_4	98[a]	98	0.30	1.5 (2.0)
0.2*M* H_2SO_4	97[b]	—	0.03	2.4 (0.00)[c] 3.2 (0.10) 3.1 (0.50)
0.2*M* H_2SO_4	100[b,d]	—	0.04	2.2 (0.10) 2.5 (0.50)
pH 1.6 (0.03*M* HCl)	100[a]	99	0.15	1.8 (2.0)
0.1*M* HNO_3	99[a]	99	0.15	2.0 (2.0)
With oxidative treatment				
0.2*M* H_2SO_4	—	104[b]	0.30	4.2 (0.00)[c,f] 8.5 (0.30)[c]

Activated carbon, 100 mg.
— = Not examined.
a = Solution, 200 ml. Recovery was determined by adding 0–4 ng Hg.
b = Solution, 300 ml. Recovery was determined by adding 0–4 ng Hg.
c = When no Hg(II) was added, 0.41 ng of Hg was found.
d = Artificial sea-water purified by treating with activated carbon.
e = *n* = 5.
f = When no Hg(II) was added, 0.33 ng of Hg was found.

With purified Wako activated carbon, an average recovery of 97% was obtained from artificial sea-water made 0.2*M* in sulphuric acid.

Results for the collection of methylmercury are shown in Table 4. The recoveries were a little lower than those for mercury(II). Merck activated carbon gave better recoveries than did Wako activated carbon; 87% of methylmercury was collected with the

Table 4. Collection of methylmercury chloride from artificial sea-water

Artificial sea-water	Average recovery, % calibration curve: Water	Average recovery, % calibration curve: Artificial sea-water	Blank, Hg, *ng*	R.S.D., % *n* = 6 (added Hg, 2.0 ng)
Not acidified	95	95	0.23	2.2
0.2*M* H_2SO_4	96	96	0.37	2.9
pH 1.6 (0.03*M* HCl)	97	96	0.20	1.5
0.1*M* HNO_3	97	96	0.22	2.0

Solution, 200 ml; activated carbon, 100 mg.
Recovery was determined by adding 0–4 ng Hg.

Table 5. Collection of mercury(II) from various solutions after treatment with $KMnO_4$ and $K_2S_2O_8$

Solution composition	Average recovery, %
Artificial sea-water	104
Artificial sea-water*	101
Artificial sea-water and water (1:1)	96
NaCl–KBr–KI†	100
NaCl–KI†	93
KI†	98
NaCl–KBr†	33
NaCl§	33
Water	28

Each solution was made 0.2*M* in sulphuric acid. Volume of solution, 300 ml; Hg(II), 0.5 ng; activated carbon, 100 mg.

* Artificial sea-water was purified by treating with activated carbon.

† The concentrations were the same as those in artificial sea-water.

§ 0.5–9 g in 300 ml.

latter from artificial sea-water made 0.2*M* in sulphuric acid.

The effect of sulphide and a few organic substances on the collection of mercury was examined, and 0.2 mmole of sodium sulphide, tartaric acid or citric acid, 2 ml of ethanol or 0.2 ml of benzene was found to have no effect (error < 3%) on recovery of 3 ng of mercury from 200 ml of artificial sea-water made 0.2*M* in sulphuric acid.

Collection of mercury from artificial sea-water after oxidative treatment

Sea-water samples are often treated with oxidizing agents before determination of total mercury. Therefore, collection of mercury from artificial sea-water after oxidative treatment was investigated. The treatment described by the Mercury Analysis Working Party of BITC[10] was first examined. Because large amounts of oxidizing agents were used, a high blank value (about 0.8 ng of Hg) was obtained. The treatment described by Robertson[5] was next examined and a lower blank value (0.3 ng of Hg) was obtained. The following procedure was worked out.

Transfer 300 ml of artificial sea-water (0.2*M* in sulphuric acid) containing 0–4 ng of mercury to an Erlenmeyer flask with a ground-glass stopper. Add 7.5 ml of 16*M* nitric acid, 6 ml of 50-g/l. potassium permanganate solution and 6 ml of 50-g/l. potassium peroxodisulphate solution. Stopper the flask lightly and heat in a water-bath at 80° for 2 hr. Cool the flask to room temperature and add 3 ml of 120-g/l. hydroxylammonium chloride solution. Then continue as described in the procedure.

In order to determine blank values and calibration methods, collection of mercury(II) from various solutions was investigated after the treatment described above. As shown in Table 5, recoveries of mercury from solutions containing iodide were acceptable (93–104%), but those from solutions not containing iodide were low (about 30%). Iodide shows a beneficial effect on the collection of mercury, possibly by formation of the HgI_4^{2-} complex.[11] It is probably best to add some iodide to the sample as a precaution. When no heating was used in the oxidative treatment, the recovery of mercury was about 44%, probably because of the presence of residual peroxodisulphate. Heating the solution as described above eliminated this difficulty. On the basis of the results, artificial sea-water purified with activated carbon was used for blanks and calibration curves.

The results obtained by applying the oxidative treatment are included in Table 3.

Application to sea-water

Two samples of surface coastal sea-water were taken at Oarai, Ibaraki-ken. Immediately after sampling in glass bottles, the samples were made 0.2*M* in sulphuric acid. The results obtained with and without the oxidative treatment are shown in Table 6; they were the same. This suggests that the oxidative treatment is probably unnecessary for the determination of total mercury in sea-water. The same results were

Table 6. Mercury content of sea-water at Oarai, Ibaraki-ken

Sample	Blank, Hg, *ng*	Hg content, *ng/l.* Calibration curve method	Hg content, *ng/l.* Standard addition method	Salinity, ‰
No. 1				
Without oxidative treatment	0.06	2.2 (3.7; $n = 4$)*	2.2	33.16
With oxidative treatment	0.26	2.2 (14; $n = 4$)*	2.3	
		Average 2.2	Average 2.3	
No. 2				
Without oxidative treatment	0.06	1.8 (8.3; $n = 5$)*	1.8	33.26
With oxidative treatment	0.26	1.5 (9.4; $n = 4$)*	1.5	
		Average 1.7	Average 1.7	

Sample, 300 ml; activated carbon, 100 mg.

* R.S.D., %.

obtained by the calibration curve method and the standard addition method. The average mercury content was 2.0 ng/l., in good agreement with the recent value of 2–3 ng/l. for coastal sea-water.[12]

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PROPERTIES OF *cis*- AND *trans*-BIS(CROWN ETHER)S FOR COMPLEXATION AND EXTRACTION OF ALKALI METAL PICRATES

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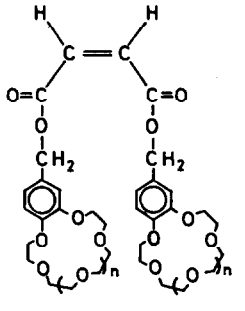
Summary—The properties of *cis*- and *trans*-bis(crown ether)s containing benzo-15-crown-5 or benzo-18-crown-6 units as complexants and extractants for alkali metal picrates have been studied. The optical spectra suggest that the *cis*-bis(crown ether)s can form intramolecular 2:1 crown ether unit/cation complexes with particular metal cations easily, while the *trans*-bis(crown ether)s can form only 1:1 crown ether unit/cation complexes because of the unfavourable *trans* configuration for the formation of the 2:1 complexes. It was found that the *cis* isomer possesses much higher extractive power than the *trans* isomer for the metal cations, which also reflects their complexing properties. The extraction equilibrium constants and thermodynamic quantities have been also evaluated, and the effect of the stereochemical structure of the bis(crown ether) on the complexing and extractive properties is discussed.

Macrocyclic polyethers (crown ethers) all have a cavity, the size of which affects their cation-complexing selectivity considerably. The selectivity of the monocyclic crown ether is, however, not so high.

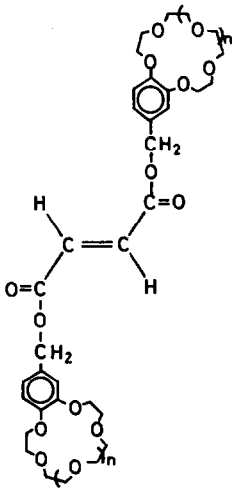
Bis(crown ether)s containing crown ether units at the end of a short aliphatic chain generally show different cation-complexing properties from the corresponding monocyclic crown ethers.[1–7] The complexing property of bis(crown ether) derivatives is closely related to the fact that many monocyclic crown ethers form sandwich-type complexes with two crown ethers per cation if the cation is too large to fit into the crown ether ring. That is to say, it seems likely that the bis(crown ether)s form the 2:1 crown ether unit/cation complexes with particular metal cations intramolecularly and more easily than the corresponding monocyclic crown ethers, by co-operative action of the two adjacent crown ether units.[1,2] Thus, the bis(crown ether)s often exhibit excellent selectivity in complexing and extracting cations which tend to form the 2:1 complexes with the corresponding monocyclic crown ethers.[2–4,7]

This motivated us to synthesize various stereoisomers of bis(crown ether)s and elucidate the effect of stereochemical structure on their complexing properties. In preliminary experiments on solvent extraction of alkali metal picrates with some stereoisomers of bis(crown ether)s, pronounced difference in extractive power was observed between *cis*- and *trans*-bis(crown ether)s, **I** ($n = 1$) and **II** ($n = 1$), which are derived from maleic and fumaric acids, respectively.[8]

In this paper, we are concerned in detail with the complexing and extractive properties of bis(crown ether)s **I** ($n = 1,2$) and **II** ($n = 1,2$) for alkali metal picrates. Bis(crown ether)s **III** ($n = 1,2$) derived from succinic acid were also employed for comparison.



I (n=1,2)



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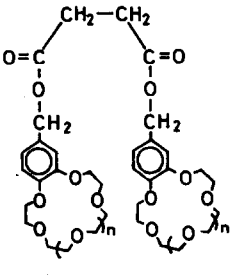

III (n=1,2)

* Reprint requests.

Attempts were made to estimate extraction equilibrium constants and thermodynamic quantities of extraction, and discuss the correlation between the complexing or extractive properties and the configuration of the bis(crown ether).

EXPERIMENTAL

Materials

The synthesis of bis(crown ether)s, **I** (n = 1), **II** (n = 1) and **III** (n = 1,2) has been described elsewhere.[5,6,8] Bis(crown ether)s **I** (n = 2) and **II** (n = 2) were obtained in an analogous way, and their properties and analytical data are as follows. **I** (n = 2): colourless oil; ^{1}H-NMR (δ, $CDCl_3$): 5.04 (CH_2Ar, 4H, s), 6.24 (CH, 2H, s); M^+: 764; $C_{38}H_{52}O_{16}$ requires C 59.67% H 6.85%; analysis gave C 59.2%, H 7.0%. **II** (n = 2): m.p. 105–106°; ^{1}H-NMR (δ, $CDCl_3$); 5.12 (CH_2Ar, 4H, s), 6.83 (CH, 2H, s); M^+ 764; $C_{38}H_{52}O_{16}$ requires C 59.67%, H 6.85%; analysis gave C 59.2%, H 6.9%.

Sodium, potassium, rubidium, and caesium picrates were prepared according to Fuoss's method.[9] Tetrahydrofuran (THF) and chloroform were purified in the usual manner, and demineralized water was used.

Optical spectra

Optical spectra of bis(crown ether)–cation complexes were measured in THF, with a Hitachi 340 recording spectrophotometer. The measurement was also done in the chloroform phase immediately after extraction of the alkali metal picrates, in order to elucidate the types of complexes.

Extraction

Chloroform and water were saturated with each other before use, to prevent volume changes in the phases during extraction. Equal volumes (10 ml) of bis(crown ether) solution in chloroform and alkali metal picrate aqueous solution were introduced into a stoppered flask, and then shaken for 40 min at 25°. For determination of thermodynamic quantities, extractions were also carried out at 15, 20, and 30°. After phase separation, 2 ml of acetonitrile were added to 2 ml of the chloroform phase, and the picrate concentration in the mixture was determined spectrophotometrically (λ_{max} 374 nm; ϵ (10^4 l.mole^{-1}.cm^{-1}): Na^+ 1.86, K^+ 1.88, Rb^+ 1.88, Cs^+ 1.86).

Calculation of extraction equilibrium constant

The extraction equilibrium constants (K_e) were determined graphically.[4] The extraction equilibrium between an aqueous solution of metal cation (M^+) and picrate anion (Pi^-), and a chloroform solution of crown ether (CE) can be defined for 1:1 crown ether unit/cation complexes by the following equations.

$$M^+_{aq} + Pi^-_{aq} + CE_{org} \rightleftharpoons M(CE)Pi_{org}$$

$$K_e = [M(CE)Pi]_{org}/[M^+]_{aq}[Pi^-]_{aq}[CE]_{org}. \quad (1)$$

Some approximations can be introduced, as explained earlier,[4] because of the negligible dissociation of $M(CE)Pi_{org}$ in chloroform,[10] and equation (1) is rewritten as equation (5):

$$D = [M(CE)Pi]_{org}/[M^+]_{aq} \quad (2)$$

$$[M^+]_{aq} = [Pi^-]_{aq} = M^0 - A \quad (3)$$

$$[CE]_{org} = (CE)^0 - A \quad (4)$$

$$D = K_e(M^0 - A)[(CE)^0 - A] \quad (5)$$

where M^0 and $(CE)^0$ denote the initial concentrations of alkali metal cation and crown ether, respectively, and D is the distribution coefficient of metal cation between the organic and aqueous phases. In this study, all concentrations of crown ether are represented for convenience by those of the crown ether units of the bis(crown ether)s, *i.e.*, the benzo-15-crown-5 or benzo-18-crown-6 unit, not by those of the bis(crown ether) molecules. A refers to the concentration of picrate transferred to the organic phase, which can be determined spectrophotometrically. If the bis(crown ether) forms a 2:1 crown ether unit/cation complex intramolecularly, equation (6) can be applied.

$$D = K_e(M^0 - A)[(CE)^0 - 2A]. \quad (6)$$

The plots of $-\log D$ *vs.* $-\log(M^0 - A)[(CE)^0 - xA]$ (where x = 1 or 2) should give a straight line with a slope of 1 if the approximations above are reasonable, and then $\log K_e$ can be obtained from the intercept of the straight line.

RESULTS AND DISCUSSION

Optical spectra of bis(crown ether)–alkali metal complexes

In order to get some information about the complexing properties of the *cis*- and *trans*-bis(crown ether)s, **I** (n = 1,2) and **II** (n = 1,2), for alkali metal picrates, the spectral change of their complexes in THF was followed at constant picrate concentration and varying crown ether unit concentration. Bis(crown ether)s **III** (n = 1,2), which are more flexible

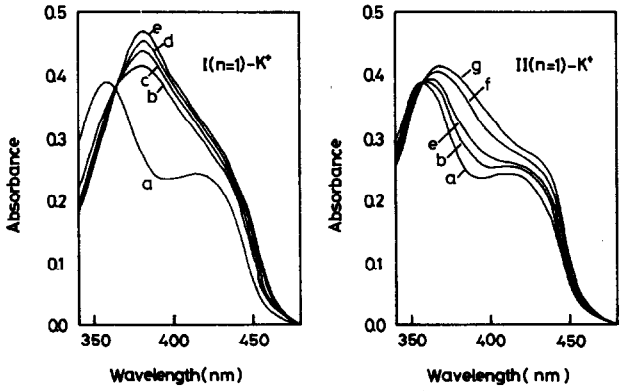

Fig. 1. Optical spectra of potassium picrate solution in THF in the presence of various amount of *cis*- and *trans*-bis(crown ether)s **I** and **II** (n = 1). [potassium picrate]: 2.5 × 10^{-5}*M*, [crown ether unit]/[potassium picrate]: *a*, 0; *b*, 2; *c*, 3; *d*, 5; *e*, 10; *f*, 30; *g*, 50.

than the *cis*- and *trans*-bis(crown ether)s, were also employed for comparison.

Spectral changes for the *cis*-bis(crown ether) **I** ($n = 1$) and *trans*-bis(crown ether) **II** ($n = 1$) systems with potassium are shown in Fig. 1. The optical spectrum of potassium picrate has a main absorption band at 357 nm in THF.[2,10] Addition of only an equimolar amount of bis(crown ether) **I** ($n = 1$) to the picrate solution in THF causes a pronounced red shift of the main absorption band of the picrate anion from 357 to 381 nm, whereas only a slight red shift from 357 to 365 nm was observed even on addition of a large excess of bis(crown ether) **II** ($n = 1$).

Bourgoin *et al.*[2] have reported that the absorption band of the picrate near 380 nm is indicative of the formation of loose ion-pairs, which, in turn, suggests that of 2:1 crown ether unit/cation complexes, where a cation is sandwiched by two crown ether units. On the other hand, a slight red shift of the absorption band on addition of crown ether suggests the formation of tight ion-pairs and 1:1 crown ether unit/cation complexes. That is to say, *cis*-bis(crown ether) **I** ($n = 1$) can form intramolecularly sandwiched 2:1 complexes with potassium easily, whereas, owing to the unfavourable *trans* configuration, *trans*-bis(crown ether) **II** ($n = 1$) can form only 1:1 complexes, where a crown ether unit in the bis(crown ether) molecule interacts with a potassium cation. It was similarly suggested that bis(crown ether) **I** ($n = 1$) forms 2:1 crown ether unit/cation complexes with rubidium and caesium as well as with potassium, and that bis(crown ether) **II** ($n = 1$) forms 1:1 crown ether unit/cation complexes with all the alkali metal cations employed here. However, in spite of the favourable structure of bis(crown ether) **I** ($n = 1$), it does not seem to form a 2:1 crown ether unit/cation complex with sodium, probably because a sodium cation can fit into the cavity of the crown ether unit, as observed in the sodium complex of the corresponding monocyclic crown ether, benzo-15-crown-5. In the bis(crown ether) **III** ($n = 1$) systems, spectral changes similar to those of the bis(crown ether) **I** ($n = 1$) systems were observed, suggesting that their complexing properties are almost the same.

The bis(benzo-18-crown-6) systems differ significantly from the bis(benzo-15-crown-5) systems in complexing properties, as also described previously.[4–7] Only in the bis(crown ether) **I** ($n = 2$) and **III** ($n = 2$) systems with caesium was a pronounced red shift of the optical spectrum similar to that for the bis(crown ether) **I** ($n = 1$) and **II** ($n = 1$) systems with potassium observed, suggesting the formation of the intramolecularly sandwiched 2:1 complex. In all other systems of bis(benzo-18-crown-6), **I** ($n = 2$)–**III** ($n = 2$), the formation of the 1:1 crown ether unit/cation complex seems to be preferred to that of the 2:1 complex.

* The concentration refers to moles of crown ether unit, *i.e.*, benzo-15-crown-5 or benzo-18-crown-8, per litre.

Table 1. Stoichiometry of alkali metal complexes of bis(crown ether)s **I**, **II**, and **III** ($n = 1,2$)

Bis(crown ether)	Na^+	K^+	Rb^+	Cs^+
I ($n = 1$)	1:1	2:1*	2:1*	2:1*
II ($n = 1$)	1:1	1:1	1:1	1:1
III ($n = 1$)	1:1	2:1*	2:1*	2:1*
I ($n = 2$)	1:1	1:1	1:1	2:1*
II ($n = 2$)	1:1	1:1	1:1	1:1
III ($n = 2$)	1:1	1:1	1:1	2:1*

The ratio is that of the number of crown ether units per cation.

* A cation is sandwiched intramolecularly by the two crown ether units in a bis(crown ether).

The stoichiometry of the bis(crown ether)/cation complexes supported by the spectral change is summarized in Table 1. The marked difference in complexing ability between *cis*- and *trans*-bis(crown ether)s indicates that the configuration of the bis-(crown ether)s is a very important factor in forming the intramolecular 2:1 crown ether unit/cation complex. There is, however, little difference in complexing ability between bis(crown ether)s **I** ($n = 1,2$) and **III** ($n = 1,2$), as far as is indicated by the spectral change.

Extraction of alkali metal picrates with bis(crown ether)s

It interested us to see how the complexing properties of the bis(crown ether)s **I** ($n = 1,2$)–**III** ($n = 1,2$) are reflected in their extractive power. Chloroform was chosen as the water-immiscible solvent; it is a good solvent for the bis(crown ether)s used, and the dissociation of the extracted complex in it is negligible.[10] Extractions were carried out at 25° from $1 \times 10^{-2} - 1 \times 10^{-3} M$ aqueous alkali metal picrate solution with 1×10^{-3}–$1 \times 10^{-4} M$* crown ether solution in chloroform.

The results for $1 \times 10^{-2} M$ alkali metal picrate and $5 \times 10^{-4} M$ crown ether are shown in Table 2, and are expressed as D, *i.e.*, the distribution ratio of metal cation between the chloroform and aqueous phases.

In the bis(benzo-15-crown-5) extraction systems, the extractive power of bis(crown ether)s **I** ($n = 1$) and **III** ($n = 1$) for potassium, rubidium and caesium is much larger than that of bis(crown ether) **II** ($n = 1$),

Table 2. Distribution ratio on extraction of alkali metal picrates with bis(crown ether)s **I**, **II**, and **III** ($n = 1,2$)

	log D			
Bis(crown ether)	Na^+	K^+	Rb^+	Cs^+
I ($n = 1$)	−2.22	−1.71	−1.97	−2.72
II ($n = 1$)	−2.25	−2.76	−3.20	−3.46†
III ($n = 1$)	−2.22	−1.71	−2.00	−2.71
I ($n = 2$)	−2.23	−1.38	−1.46	−1.66
II ($n = 2$)	−2.29	−1.33	−1.45	−1.77
III ($n = 2$)	−2.21	−1.33	−1.45	−1.66

[crown ether unit]: $5 \times 10^{-4} M$ (†, $1 \times 10^{-3} M$) in $CHCl_3$, [picrate salt]: $1 \times 10^{-2} M$ in H_2O, at 25°.

but little difference in extractive power for sodium was observed among these bis(crown ether)s.

On the other hand, in the bis(benzo-18-crown-6) extraction systems, the extractive power of bis(crown ether)s **I** (n = 2) and **III** (n = 2) for caesium is larger than that of bis(crown ether) **II** (n = 2), whereas that of all three bis(crown ether)s **I** (n = 2)–**III** (n = 2) for sodium, potassium and rubidium is almost the same.

The optical spectrum of the chloroform phase was measured immediately after the extraction, in order to see which type of bis(crown ether)–alkali metal picrate species was extracted. The λ_{max} values for the main absorption band of the picrate anion suggest that the bis(crown ether)–cation complexes exist in the chloroform phase with the stoichiometry shown in Table 1. Comparison of the extraction data and the stoichiometry of the complexes indicates that the extraction is increased if the bis(crown ether) and alkali metal cation are capable of forming easily the intramolecular 2:1 crown ether unit/cation complex. This may be attributed to the 2:1 complex being generally more lipophilic than the 1:1 complex, the metal cation being transferred more effectively from the aqueous to the organic phase.

It should, in particular, be noted that the extractive power of the *cis*-bis(crown ether)s **I** (n = 1,2) is very different from that of the corresponding *trans*-bis(crown ether)s, although the chemical formulae are the same, and the difference must be due to the geometry of the molecules. That is to say, the *trans*-bis(crown ether) cannot exert the co-operative effect of its two crown ether units to form the intramolecular 2:1 complex. Therefore, they show only low extractive power, resembling that of the corresponding monocyclic crown ethers.[3,4]

The *cis*-bis(crown ether)s might be expected to exhibit higher extractive power than bis(crown ether)s **III** (n = 1,2) for cations which tend to be sandwiched by two crown ether units, because they possess the *cis* configuration, the partly preorganized structure for forming the lipophilic 2:1 crown ether unit/cation complex intramolecularly. However, no remarkable difference in this respect could be observed between bis(crown ether)s **I** (n = 1,2) and **III** (n = 1,2). Their similarity will be discussed later.

Extraction equilibrium constant and thermodynamic quantities

Attempts were made to estimate the extraction equilibrium constant, K_e, for the bis(crown ether) extraction systems. Equations (5) and (6) can be applied to the 1:1 crown ether unit/cation complex and the intramolecular 2:1 complex, respectively. Plots of $-\log D$ *vs.* $-\log(M^0 - A)[(CE)^0 - xA]$, where x = 1 or 2, in the extraction systems of bis(crown ether)s **I** (n = 1,2) gave straight lines with a slope of 1, indicating that the approximations such as equations (2)–(4) and the stoichiometry assumed for the complexes in the chloroform phase are reasonable; the intercept of the straight line gives log K_e. The K_e values for the other extraction systems were obtained in a similar way and the values at 25° are summarized in Table 3.

In order to determine the thermodynamic quantities for extraction of some metal cations, extractions were done at various temperatures. The log K_e value was a linear function of the inverse of the absolute temperature, and the enthalpy change, $\Delta H°$, was calculated from the slope. The entropy and free energy changes, $\Delta S°$ and $\Delta G°$, were calculated from the log K_e values at 25° and the $\Delta H°$ values, by using the equations $\Delta S° = (\Delta H° - \Delta G°)/T$ and $\Delta G° = -RT \ln K_e$. The results are listed in Table 4.

It can be seen that there is little difference in the thermodynamic values for all the bis(benzo-15-crown-5)/sodium systems and bis(benzo-18-crown-6)/potassium systems, where the alkali metal cations are extracted into the chloroform phase as the 1:1 crown ether unit/cation complexes. This finding implies that intramolecular 2:2 crown ether unit/cation complexes, containing two cations within each bis(crown ether) unit, do not exist in the chloroform phase. The formation of a 2:2 crown ether unit/cation complex would require a relationship to exist between the thermodynamic quantities and the stereochemical structure of the bis(crown ether)s, because of the effect of repulsion between two cations complexed by a bis(crown ether) units.

It is of interest that the $\Delta H°$ and $\Delta S°$ values for the bis(crown ether) **I** (n = 1)–potassium system differ considerably from those for the bis(crown ether) **III** (n = 1)–potassium system, though the $\Delta G°$ values are almost the same. This is also the case with the bis(crown ether)s **I** (n = 2) and **III** (n = 2)–caesium systems, although the difference is not so marked. The contribution of $\Delta S°$ to $\Delta G°$ in the bis(crown ether) **I** (n = 1)–potassium and bis(crown ether) **I** (n = 2)–caesium systems is larger than that in the corresponding bis(crown ether) **III** (n = 1) and **III** (n = 2) systems, respectively. It is thought that the larger entropy contribution of the bis(crown ether) **I** (n = 1,2) systems is derived to a large extent from the advantage of the partly preorganized structure, the *cis* configuration, for forming the lipophilic 2:1 crown ether unit/cation complex intramolecularly. However, the difference in entropy contribution is not sufficiently large to overwhelm that in the enthalpy contribution, and is con.

Table 3. Extraction equilibrium constants at 25°

Bis(crown ether)	log K_e			
	Na^+	K^+	Rb^+	Cs^+
I (n = 1)	3.13	4.23	3.62	2.40
II (n = 1)	3.08	2.56	2.15	1.54
III (n = 1)	3.09	4.20	3.56	2.35
I (n = 2)	3.10	5.28	4.70	4.82
II (n = 2)	3.07	5.20	4.54	3.77
II (n = 2)	3.14	5.27	4.69	4.74

The unit of K_e is $l.^2/mole^2$.

Table 4. Thermodynamic quantities (at 25°)

Bis(benzo-15-crown-5)	Na^+			K^+		
	$\Delta G°$	$\Delta H°$	$\Delta S°$	$\Delta G°$	$\Delta H°$	$\Delta S°$
I	−4.27	−7.74	−11.6	−5.77	−6.18	−1.38
II	−4.20	−7.80	−12.1	−3.49	−9.02	−18.6
III	−4.21	−7.76	−11.9	−5.73	−8.88	−10.6
Bis(benzo-18-crown-6)	K^+			Cs^+		
	$\Delta G°$	$\Delta H°$	$\Delta S°$	$\Delta G°$	$\Delta H°$	$\Delta S°$
I	−7.20	−16.0	−29.5	−6.57	−11.0	−14.9
II	−7.09	−16.1	−30.2	−5.14	−15.3	−34.1
III	−7.19	−16.3	−30.6	−6.47	−12.7	−20.9

$\Delta G°$ and $\Delta H°$ in kcal/mole, and $\Delta S°$ in cal.mole^{-1}.deg^{-1}.

pensated for by the latter, resulting in the extractive power of the bis(crown ether)s **I** (n = 1,2) being close to that of bis(crown ether) **III** (n = 1,2).

All bis(crown ether)s synthesized so far have the ability to form the 2:1 crown ether unit/cation complex intramolecularly, although the stability of the complex is affected more or less by the length and flexibility of the carbon chain linking the two crown ether units in the bis(crown ether).[2] However, bis(crown ether)s do not necessarily form a 2:1 complex, and *trans*-bis(crown ether)s **II** (n = 1,2) cannot do it. Conversely, readily extractable bis(crown ether)complexes can probably be obtained by proper design of a stereochemical structure that can form stable 2:1 intramolecular crown ether unit/cation complexes, though, unfortunately, the *cis*-bis(crown ether)s **I** (n = 1,2) do not give better performance than the bis(crown ether)s **III** (n = 1,2) in this respect.

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CHELATE-FORMING RESINS PREPARED BY MODIFICATION OF ANION-EXCHANGE RESINS

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Summary—The properties of some chelate-forming resins prepared from common anion-exchange resins by treatment with reagents bearing chelate-forming and ion-exchange groups have been studied. A resin prepared from the sulphonic acid derivative of dithizone (DzS) was found to be superior to other chelate-forming resins. Resins loaded with DzS, tetraphenylporphinetrisulphonic acid or zincon were stable in 1*M* sodium chloride. Resins prepared from sulphonazo III, arsenazo III, thiosalicylic acid or *p*-mercaptobenzenesulphonic acid were found to be unstable when exposed to sodium chloride solution.

We have reported the preparation of an effective chelate-forming resin by modification of common anion-exchange resins with the sodium salt of the sulphonic acid derivative of dithizone (NaO_3S–C_6H_4–N = N–CS–NH–NH–C_6H_4–SO_3Na, disodium (4-sulphophenyl)-1-[2-(4-sulphophenyl)hydrazide]diazenecarbothioate, abbreviated as DzS hereafter. This resin, which bears dithizone functional groups, (called DzS resin hereafter) is effective in the sorption of mercury(II).[1] In addition, a new chelate-forming resin bearing mercapto and azo groups has been prepared.[2] The success of these resins has prompted a search for new chelate-forming resins based on the same idea. The properties of DzS resin have been compared with those of other chelate-forming resins obtained from other chelating agents.

The complexing agents used are listed in Table 1. *p*-Mercaptobenzenesulphonic acid and thiosalicyclic acid were chosen as reagents which might react selectively with heavy metal ions. These chelating agents are bound to the resin by ion-exchange rather than by physical adsorption. Dithizone, which does not have ion-exchange functional groups, was chosen as an example of a chelating agent which may be attached to the anion-exchange resin by physical adsorption. In addition, zincon, sulphonazo III, arsenazo III and TPPS (tetraphenylporphinetrisulphonic acid) were chosen from commercially available chelating agents, as species which may be loaded on the anion-exchange resin by both ion-exchange and physical adsorption.

EXPERIMENTAL

Materials

The chelating agent, DzS, was prepared as reported previously.[1] *p*-Mercaptobenzenesulphonic acid, arsenazo III, sulphonazo III, zincon and TPPS were obtained commercially. A solution of $^{203}Hg(NO_3)_2$ in 0.5*M* nitric acid was purchased from New England Nuclear, Boston, Mass., U.S.A. Amberlite IRA 400 (8% divinylbenzene) in the chloride form, 100–200 mesh, was used as the anion-exchange resin. The exchange capacity of the resin was found to be 3.2 meq per g of air-dried resin (chloride form). All other reagents were of analytical-grade quality. Estuarine water was taken from Nagoya Bay, Japan and stored for about one month. The estuarine water used contains chloride (1.5×10^4 μg/ml), iron (10 μg/ml), manganese (10 μg/ml), zinc (10 μg/ml), copper (1 μg/ml) and lead (less than 1 ng/ml).

Determination of amount of chloride ion released in ion-exchange reaction

The anion-exchange resin (500 mg) was stirred for 1 hr with 50 ml of solution containing various amounts of the complexing agent. The amount of the complexing agent taken up was determined by measurement of the absorbance at the wavelength of maximum absorption (Table 1). Twenty ml of the solution were used for the determination of chloride by titration with standard silver nitrate solution. Because of its limited solubility, zincon was dissolved in 2 ml of 1*M* sodium hydroxide and diluted with phosphate buffer. In the case of dithizone, the resin was stirred for 7 hr with a solution of dithizone in acetone–water mixture (1:3) and filtered off. After the volume of the filtrate had been reduced by the use of a rotary-evaporator, the amount of chloride in it was determined.

Determination of complexing agent eluted with sodium chloride solution of hydrochloric acid

The resin, 250 mg loaded with the complexing agent (1 mmole per g of resin), was packed in a column and 100 ml of sodium chloride solution or hydrochloric acid of known concentration were passed through it at a flow-rate of 2 ml/min. The amount of the complexing agent in the effluent was determined spectrophotometrically.

Determination of exchange capacity for complexing agents

The anion-exchange resin (200 mg) was stirred with a

small excess of the complexing agent. The amount of the complexing agent in the solution was determined at regular intervals by spectrophotometry.

Determination of chelating agent adsorbed on resin in estuarine water

One g of the anion-exchange resin was stirred with 100 ml of the estuarine water containing 1 ml of 0.1*M* DzS or 8 ml of 0.1*M* 8-hydroxyquinoline-5-sulphonic acid. The pH was adjusted with hydrochloric acid and sodium hydroxide. The amount of the chelating agent present in the solution was determined periodically by spectrophotometry.

Determination of distribution coefficients

Mercury(II). A mixture of the anion-exchange resin (100 mg) and an aqueous solution containing 0.1 mmole of the complexing agent was shaken in a stoppered glass tube for 2 hr and then centrifuged. The resulting resin, loaded with the complexing agent, was washed three times with distilled water. The resin was mixed with 0.5 ml of 10^{-5}–0.1*M* mercury(II) nitrate solution in 0.1*M* nitric acid, that had been diluted to 5 ml with distilled water and contained enough ^{203}Hg as tracer to give an activity of 10^5 cpm in the 5 ml. In the case of DzS resin, 0.5 ml of 1.0*M* potassium nitrate was added before the dilution. The mixture was shaken for about 60 min and centrifuged and 1-ml aliquots were used for measurement of the radioactivity.

Copper(II). To 100 mg of the resin loaded with chelating agent (0.2 mmole/g) in a stoppered flask, 3 ml of $1.4 \times 10^{-3}M$ copper(II) and 97 ml of 0.1*M* acetate buffer were added. The pH was adjusted to below 4 by the dropwise addition of 0.1*M* nitric acid. The mixture was shaken for about 2 hr and the resin was then filtered off on a fritted-glass funnel. The appropriate amount of the filtrate was used for the spectrophotometric determination of copper(II) with diethyldithiocarbamate.[3]

RESULTS AND DISCUSSION

The reaction of DzS with the anion-exchange resin in the chloride form was found to be quantitative. Figure 1 shows the amounts of chloride released in

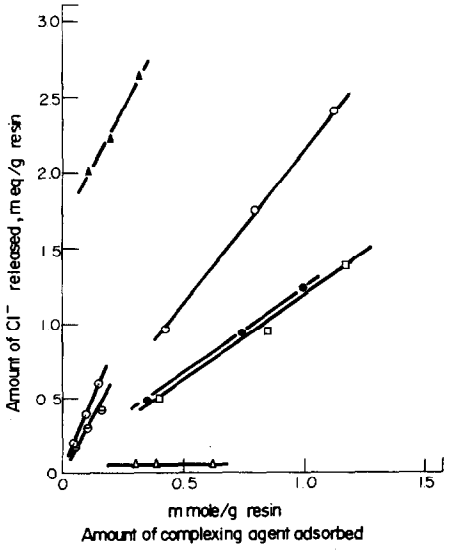

Fig. 1. Release of chloride ion from the anion-exchange resin by complexing agents. ○ DzS; ● *p*-mercaptobenzenesulphonic acid; □ sodium thiosalicylate; △ dithizone; ▲ zincon; ⊙ sulphonazo III; ⊖ arsenazo III.

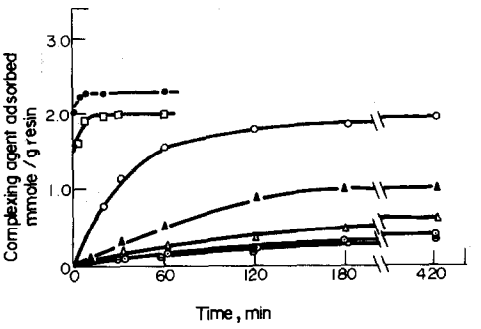

Fig. 2. Effect of time on the uptake of complexing agents. ○ DzS; ● *p*-mercaptobenzenesulphonic acid; □ sodium thiosalicylate; △ dithizone; ▲ zincon; ⊙ sulphonazo III; ⊖ arsenazo III.

the reactions with several complexing agents. The slope is related to the number of ion-exchange groups present (*e.g.*, sulphonic and carboxylic acid groups). In the case of dithizone, chloride was not released, because of the absence of an ion-exchange group in the molecule. Dithizone was found to be loaded on the anion-exchange resin by physical adsorption. Although the amount of dithizone loaded on the resin was less than that of DzS, the dithizone-loaded resin is of some value[4] as a chelating resin. It can be compared with materials in which dithizone is adsorbed on substances[5–11] of high molecular weight. Figure 2 shows the rate of uptake of some complexing agents on the anion-exchange resin. The time required for uptake was short for *p*-mercaptobenzenesulphonic and thiosalicylic acids, which may be bound to the resin purely by ion-exchange. The rate of uptake of DzS is greater than that of dithizone. The time required for loading with a third of the saturation amount of DzS (sufficient for practical use), was about 20 min, whereas that for dithizone was about an hour. DzS is thus superior to dithizone for the preparation of chelating resins, and may be held by both ion-exchange and physical adsorption. The exchange capacity, stability in sodium chloride solution and in

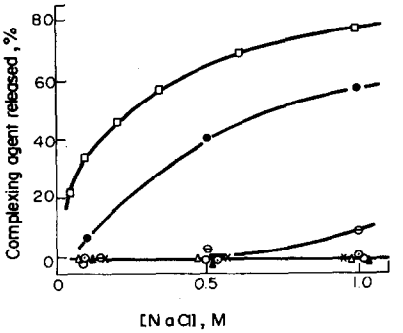

Fig. 3. Release of complexing agents from resin. ○ DzS; ● *p*-mercaptobenzenesulphonic acid; □ sodium thiosalicylate; △ dithizone; ▲ zincon; ⊙ sulphonazo III; ⊖ arsenazo III; × TPPS.

hydrochloric acid, and the time required for loading of the complexing agent, are summarized in Table 1, together with the spectral data and molecular weights. A nearly quantitative amount of DzS (based on exchange capacity) can be loaded on the anion-exchange resin. Figure 3 illustrates the stability of the resin in sodium chloride solution. Resins loaded with DzS, dithizone, zincon or TPPS are stable even in 1*M* sodium chloride. Sulphonazo III and arsenazo III remain on the resin in 0.1*M* sodium chloride, but are partially eluted with 1*M* sodium chloride. *p*-Mercaptobenzenesulphonic acid and thiosalicylic acid are released on exposure to 0.1*M* sodium chloride. In the case of DzS, dithizone, zincon, sulphonazo III, arsenazo III and TPPS, physical adsorption is considered to be responsible to some extent for their binding to the anion-exchange resin. DzS-loaded resin was found to be stable even in 5*M* hydrochloric acid and in 2*M* sodium hydroxide.[1] The high stability of resins loaded DzS, dithizone, zincon or TPPS indicates that the molecular structure of these chelating agents favours physical adsorption on the anion-exchange resin. With sulphonazo III, arsenazo III and TPPS, the bulkiness of the molecule decreases the amount loaded on the anion-exchange resin. The exchange capacity for TPPS was greater in the case of a macroreticular type of resin, which has larger cavities in its structure. DzS resin is superior to the other resins tested, although the relation between its structure and adsorption behaviour cannot be defined. Among the chelating agents tested, TPPS[12,13] and zincon[3] may have some value because of their stability on adsorption and their sensitivity in reaction with some metal ions, although large amounts of chelating agent are not loaded on the resin.

Some chelate-forming resins prepared with Bromopyrogallol Red, chromotropic acid, or Alizarin Red S have been reported.[14] Akaiwa *et al.* reported that a resin loaded with 8-hydroxyquinoline-5-sulphonic acid[15] or zincon[16] was useful for the preconcentration of some metal ions in activation analysis. In addition, Lee *et al.*[17] described similar resins, including one loaded with 7-iodo-8-hydroxyquinoline-5-sulphonic acid, but the stability in the presence of chloride was not mentioned. The advantages of DzS resin are indi-

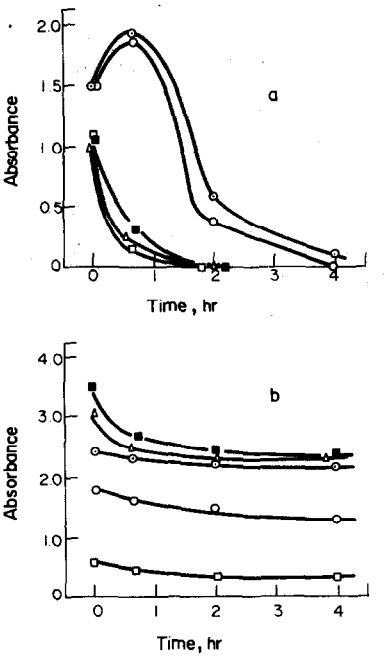

Fig. 4. Uptake of chelating agents on the anion-exchange resin in estuarine water at different pH values. ○ pH 1; ⊙ pH 3; □ pH 5; △ pH 7; ■ pH 9. (*a*) DzS at 420 nm; (*b*) 8-hydroxyquinoline-5-sulphonic acid at 390 nm.

Table 1. Properties of complexing agents

Complexing agent	Molecular weight*	Reaction time†, *min*		Complexing agent released, %‡			λ_{max} (pH), *nm*	Exchange capacity *mmole/g*
				NaCl		HCl		
		$t_{1/2}$	t	0.1*M*	1*M*	0.1*M*		
DzS	416	20	180	0	0	0	476 (7.0)	1.9
Dithizone§	256	100	420	0	0	0	450 (4.0)	0.7
Zincon	440	60	180	0	0	0	470 (8.10)	1.0
Sulphonazo III	689	90	420	0	0.4	0	565 (4.96)	0.4
Arsenazo III	776	90	420	0	10	<1	535 (4.65)	0.3
p-Mercaptobenzenesulphonic acid	190	<5	10	8	60	50	253 (1.0)	2.3
Thiosalicylic acid	154	<5	10	30	>80	50	308 (1.0)	2.0
TPPS	855	120	540	0	0	0	434 (2.0)	0.05

* For free acid form.

† The values of t and $t_{1/2}$ are the times required for the uptake of the saturation amount of the complexing agent and half of that amount, respectively.

‡ The values indicate the ratio of complexing agent released to the total amount loaded on the resin, in the medium shown.

§ In 25% acetone solution.

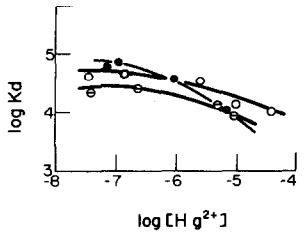

Fig. 5. Distribution coefficients of mercury(II). DzS (pH 2.3, 0.1*M* potassium nitrate, ○ 0.2 mmole of Hg per g of resin, ⊖ 0.1 mmole of Hg per g of resin), *p*-mercaptobenzenesulphonic acid (pH 2.4, ● 0.1 mmole per g of resin).

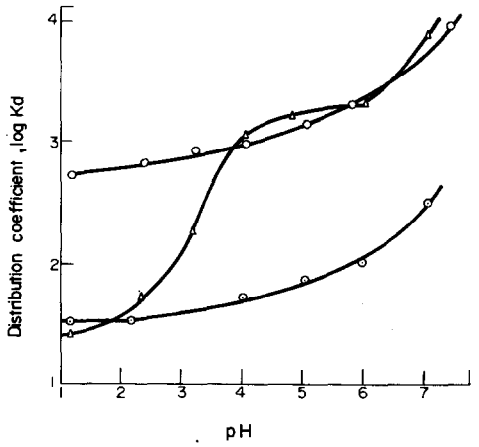

Fig. 6. Distribution coefficients of copper(II). ○ DzS, ⊙ zincon; △ sulphonazo III; copper(II), $4.2 \times 10^{-5}M$; chelating agent, 0.2 mmole per g of resin.

cated by its behaviour in estuarine water. As shown in Fig. 4, DzS is completely taken up, whereas 8-hydroxyquinoline-5-sulphonic acid is not.

Some results on the sorption of metal ions on DzS resin and on some other resins are shown in Figs. 5 and 6. The distribution coefficients (K_d) of mercury(II) on DzS resin and *p*-mercaptobenzenesulphonic acid resin in the presence of various amounts of mercury(II) are shown in Fig. 5. The value of log K_d for mercury(II) on DzS resin is very high in the presence of 0.1*M* potassium nitrate, and that on *p*-mercaptobenzenesulphonic acid resin is comparatively high. However, as expected from Table 1, *p*-mercaptobenzenesulphonic acid is released in the presence of 0.1*M* potassium nitrate. The value of log K_d for copper(II) at different pH values is shown in Fig. 6. At low pH, only DzS resin gives fairly high log K_d values. In the weakly acid and neutral region, DzS resin and zincon resin give satisfactorily high log K_d values.

In conclusion, DzS resin has been shown to be a satisfactory chelate-forming resin, superior to other similar resins described previously.

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DETERMINATION OF TOTAL IRON IN STANDARD ROCKS BY SPECTROPHOTOMETRIC TITRATION WITH EDTA

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Summary—A method for the determination of total iron in silicate rocks is described. It is based on spectrophotometric titration of iron(III) with EDTA after decomposition in a PTFE bomb. No prior separation of interfering elements is needed. The method was tested by analysis of the U.S. Geological Survey reference rocks G-2, AGV-1, GSP-1, BCR-1 and PCC-1. The same sample solutions were also analysed by atomic-absorption spectrophotometry. The agreement with published and recommended values was good.

Iron is one of the main constituents of our environment and also of many industrial products, so accurate methods for its determination are of great importance. This work deals with the determination of iron in U.S. Geological Survey standard rocks by spectrophotometric titration. These standard rocks are very suitable for testing methods of analysis, because they have been very thoroughly investigated by laboratories around the world.

The reagent, EDTA, used in the spectrophotometric titrations was standardized against metallic copper.[1] Results obtained by the spectrophotometric titration method were compared with atomic-absorption spectrophotometric measurements on the same samples. The rock samples were decomposed by hydrofluoric acid in a PTFE bomb.[2,3]

For the determination of iron in different materials the Analytical Methods Committee recommends use of atomic-absorption spectrophotometry,[4] and two spectrophotometric methods based on 1,10-phenanthroline[5] and 4,7-diphenyl-1,10-phenanthroline.[6] According to the compilation of data on USGS standards by Flanagan,[7,8] the methods most used for determination of total iron are atomic-absorption spectrophotometry and X-ray fluorescence.

EXPERIMENTAL

Equipment

For the spectrophotometric titrations, a Pye Unicam SP 6 spectrophotometer equipped with a rectangular glass titration cell with an optical path-length of 50 mm was used. Titrant was added from a Metrohm E 457 microburette. The sample solution was stirred with a Metrohm E 381 stirrer.

For atomic-absorption spectrophotometric analysis a Perkin–Elmer model 303 atomic-absorption spectrophotometer equipped with a standard three-slot air–acetylene burner head was used. The light-source was an Intensitron hollow-cathode lamp. Instrument parameters were set to the values recommended in the instrument manual.

The glassware and burettes used for volumetric measurements were calibrated.

Reagents

The disodium salt of EDTA (Merck, *pro analysi*) was used to prepare the 0.002*M* titrant. The acids used for decomposition and for adjusting pH were of analytical-reagent grade.

Procedure

Weigh 250 mg of powdered air-dried sample into a PTFE bomb containing a PTFE-coated magnetic stirrer bar. The moisture content of the material should be determined simultaneously on a separate sample. Add 0.5 ml of water as wetting agent and 5 ml of 48% hydrofluoric acid for decomposition of the sample. For basic rocks a mixture of 4 ml of 48% hydrofluoric acid, 1 ml of conc. hydrochloric acid and 1 ml of conc. nitric acid is used.

Heat the bomb at 130° for 20 minutes on an electrical hot-plate equipped with a magnetic stirrer.

Transfer the decomposed sample into a plastic beaker and add a few drops of hydrogen peroxide to oxidize any iron(II) to iron(III). To dissolve precipitated metal fluorides and to complex excess of fluoride add 50 ml of saturated boric acid solution and stir thoroughly. Finally dilute the sample solution to 250 ml in a standard flask with doubly distilled water. Store the sample solutions in polyethylene bottles.

For the spectrophotometric titrations, transfer a suitable volume (5–25 ml depending on the original iron concentration) of sample into the titration cell. Dilute the sample to 100 ml and adjust the pH simultaneously to 2–2.3 with chloroacetate buffer solution. Follow the titration at 380 nm. Determine the equivalence volume as described later in this paper.

THEORY

A thorough treatment of spectrophotometric titrations without indicators is given by Ringbom.[9] For a selective determination of a metal ion in the presence of interfering metal ions, the difference between the logarithms of the conditional constants should be at least 2, if the total concentrations of the metal ions in

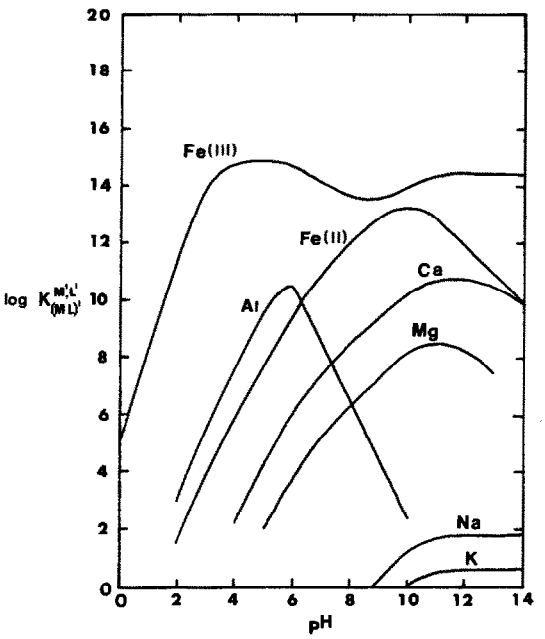

Fig. 1. Conditional stability constants of some metal–EDTA complexes as functions of pH.

question are assumed to be of the same order of magnitude.

Iron(III) forms a stable 1:1 complex with EDTA: the following equilibrium constants are given in the critical selection by Anderegg[10] for the Fe(III)-EDTA system at 20° and ionic strength 0.1: $\log K_{FeL}^{Fe;L} = 25.1$, $\log K_{FeHL}^{FeL;H} = 1.2$, $\log K_{Fe(OH)L}^{FeL;OH} = 6.42$. Thus at pH less than 1.2 FeHL dominates and at pH greater than 7.6 Fe(OH)L is formed.

Figure 1 gives the conditional constants of the metal–EDTA complexes of the main components of silicate rocks. The curves represent the maximum values of the conditional constants. Usually the presence of some buffer or interfering ions will decrease the conditional constants. Most of the values plotted are taken from Table A.6 in the book by Ringbom.[9] The conditional constants for the alkali metals were calculated from stability constants selected by Anderegg.[10]

With the help of Fig. 1, suitable conditions for the titration of iron(III) in presence of other metals can be chosen. Of the main constituents of silicate rocks, potassium, sodium, magnesium and calcium form much weaker complexes with EDTA than iron(III), so do not interfere in the determination. At pH 6, the conditional constant for Fe(III)–EDTA has a higher value than those of the other metal ions considered in Fig. 1.

Aluminium, which occurs in large amounts in rocks and minerals, often represents the most serious interference in the determination of iron. From the conditional constants it can be seen that aluminium should have no influence on the spectrophotometric titration without indicators in acid solutions.

Among the trace constituents of silicate rocks, zirconium, hafnium and indium form very stable complexes with EDTA and could possibly interfere in the determination. However, these elements usually occur in very low concentrations, so this has no practical importance.

Iron can thus be titrated selectively with EDTA in solutions of pH 0–6. In this work the pH is adjusted to about 2. At this pH, the formation of hydroxo complexes, $Fe_n(OH)_m$, does not appreciably affect the rate of the titration reaction, because the total iron concentration in the sample solution is less than $10^{-2}M$, so only a small fraction of the iron ions is present as hydroxo complexes (ref. 11, p. 47). At pH 2, the conditional constant of the iron(III)–EDTA chelate is still sufficiently high for a successful titration.

SPECTROPHOTOMETRIC TITRATIONS

The absorption spectra of solutions of Fe(III) and Fe(III)–EDTA are shown in Fig. 2. The Fe(III)–EDTA complex absorbs in the ultraviolet and near-ultraviolet regions. The greatest difference between the absorbance curves is at about 270 nm. However, the titrations in this work were performed at the more convenient wavelength 380 nm, since the absorbance is still high enough for good results.

The spectrophotometric titration of iron(III) with EDTA was reported by Uzumasa and Nishimura.[12] Figure 3 shows a typical titration curve obtained for the determination of total iron in silicate rocks. Corrections are made to the absorbance readings to compensate for dilution of the sample. The equivalence volume of the titration is found as described previously.[1] The equations for the linear parts of the titration curve are calculated by least-squares. Owing to incomplete reaction, a slight rounding of the curve is observed near the end-point of the titration: data points from this region are omitted in the calculations. The starting point of the titration can be

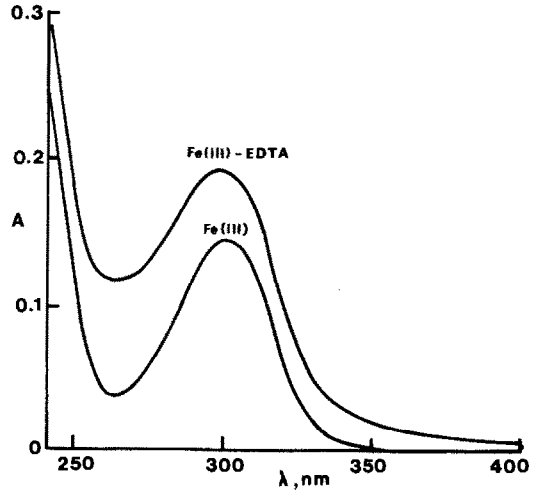

Fig. 2. The absorption spectra of $10^{-5}M$ Fe(III) and Fe(III)–EDTA solutions at pH 2.0.

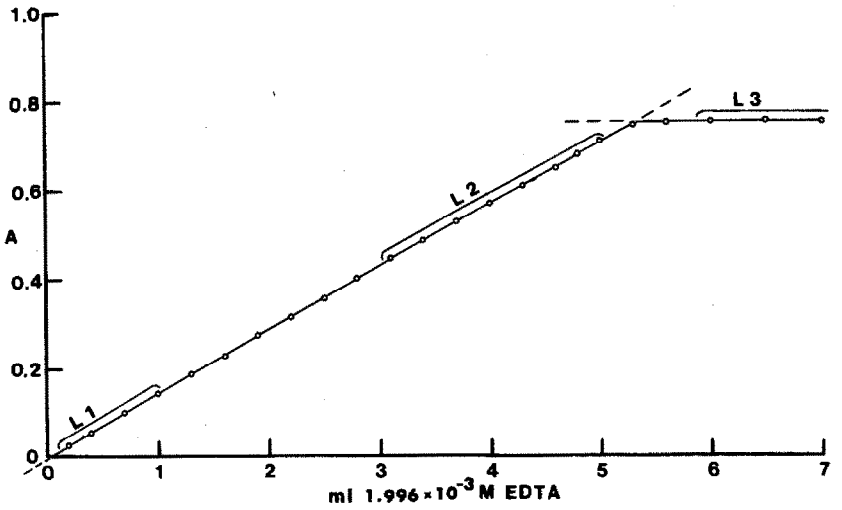

Fig. 3. Spectrophotometric titration of total iron in andesite with EDTA without an indicator at $\lambda = 380$ nm and pH = 2.3.

obtained very exactly from the equation for the line L1 in the figure. The intersection of the lines L2 and L3 gives the end-point of the titration.

A difficulty in the complexometric determination of iron arises from the slowness of the reaction between iron(III) and EDTA, especially at pH values above 2. Usually it is necessary to wait about 2 min after each addition of titrant to obtain a constant absorbance reading. During the later part of the titration a somewhat longer time between each increment of titrant is needed. The time necessary for a complete titration is about 30 min. When pure iron(III) solutions are titrated the reaction is faster.

ATOMIC-ABSORPTION ANALYSIS

In order to test the spectrophotometric titration method, the standard samples were also analysed by atomic-absorption spectrophotometry. The concentration of iron in the sample solutions was found by the method of standard addition. Normal calibration curves were not used, because of matrix effects.

The standard iron solution (0.8372 g/l.) was prepared from iron drillings, dissolved in 50 ml of 1:1 nitric acid and diluted to 1 litre. This stock solution was standardized by the titration method described in the present paper. Working standards were prepared by appropriate dilution of the stock solution.

Five determinations were made on each rock sample solution. For instance, for the granite G2 the following values were obtained: 2.58, 2.71, 2.67, 2.64, 2.70%. These give a mean value of 2.66% and a relative standard deviation of 2.0%. In general the relative standard deviations were between 1 and 2%.

DISCUSSION

The results for the determination of total iron in U.S. Geological Survey standard rocks are summarized in Table 1. The values obtained by spectrophotometric titrations and by atomic-absorption spectrophotometry are in good agreement. Only for granodiorite, GSP-1, was a deviation obtained. Table 1 also includes literature data from the compilations by Flanagan;[7,8] our values are in good agreement with these data, and are only slightly below the averages given by Flanagan.

In order to test the precision of the spectrophotometric titration method, five separate determinations of the total iron content in andesite (AGV-1) were made. The results were 6.714, 6.756, 6.742, 6.714 and 6.715%. The average is 6.728% and the standard

Table 1. Comparison of spectrophotometric-titration and atomic-absorption data with published values for total iron as % Fe_2O_3 in USGS standard rocks

USGS standard split/position	G-2 83/27	GSP-1 44/25	AGV-1 50/8	PCC-1 52/6	BCR-1 17/17
Spectrophotometric titration (this work)	2.69	4.24	6.73	8.18	13.36
Atomic-absorption spectrophotometry (this work)	2.66	4.39	6.72	8.16	13.25
Published averages[7,8] (1969)	2.77	4.33	6.80	8.54	13.51
Published averages[7,8] (1972)	2.65	4.33	6.76	8.35	13.40

deviation 0.020%. This corresponds to a relative standard deviation of 0.3‰ which is acceptable.

The results show that the spectrophotometric titration method can be used with good accuracy for determination of total iron in rock materials, and the precision is far better than that of atomic-absorption spectrophotometry. The method is selective and no prior separation of interfering elements is necessary. The method is particularly suitable for use by small laboratories because the equipment needed is inexpensive. A drawback of the method is the rather slow reaction between iron(III) and EDTA.

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SHORT COMMUNICATIONS

STANDARDIZATION OF EDTA BY SPECTROPHOTOMETRIC TITRATION, WITH METALLIC COPPER AS STANDARD

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Summary—The standardization of EDTA against electrolytically pure copper is described. The copper is dissolved and titrated spectrophotometrically with EDTA at pH 5.0 without an indicator. A relative standard deviation of 0.009% was achieved.

EDTA, ethylenediaminetetra-acetic acid, has found widespread use in titrimetry. It is used not only as a reagent in complexometric analysis, but also for standardization of many metal salt solutions used, for instance, as standards in atomic-absorption spectrophotometry. For accurate analytical work the reagent has to be standardized against some primary standard.

We have tried calcium carbonate, lead nitrate and zinc nitrate as standards for this purpose, but we have found metallic copper more successful and will describe the method in this paper. Metallic copper is weighed, dissolved and spectrophotometrically titrated with EDTA without indicator. A titration without indicator was first reported by Sweetser and Bricker.[1]

Many methods for standardization of EDTA are reported in the literature. Underwood[2] has used a similar method to ours with copper as primary standard, but his standardization procedure was only very briefly described.

Calcium carbonate is perhaps the most widely used standard and the material is recommended in several books on analytical chemistry. Vřeštál *et al.*[3] have published a comprehensive study, where the utility of several standards is investigated.

Recently the Complexiметric Standards Panel of the Analytical Methods Committee[4] has recommended pure bismuth metal as primary standard for precise standardization of EDTA. Metallic bismuth is dissolved in nitric acid and titrated spectrophotometrically with EDTA at pH 2.4 with Catechol Violet as indicator. Collaborative assays gave very small standard deviations.

EXPERIMENTAL

Apparatus and reagents

A Pye Unicam SP 6 spectrophotometer was used for the titrations. A very sensitive voltmeter built in our laboratory was used to achieve scale expansion. A rectangular titration cell of glass with an optical path-length of 50 mm was made for the spectrophotometer. For stirring, a Metrohm E 381 stirrer equipped with a glass spiral was used.

EDTA (disodium salt, Merck, *pro analysi*) was used in the experiments. Electrolytic 99.999% pure metallic copper (from Outokumpu Oy) was used as primary standard.

All burettes and glassware used for volumetric measurements were calibrated.

Procedure

Clean the copper wire with sand-paper, then immediately weigh out about 2 g of it. Dissolve it in 10 ml of nitric acid and dilute to 1 litre.

Transfer a suitable volume (5–10 ml) of this copper solution to the titration cell from a sufficiently accurate burette such as the Metrohm E 457 microburette, or, preferably, a weight-burette (in which case the density of the copper solution must be determined). Add 10 ml of acetate buffer (1*M*, pH = 5.0) and dilute the sample solution to the desired volume.

Set the analytical wavelength at 700 nm and perform the titration by adding titrant from the kind of burette used for the copper solution. Evaluate the results as described below.

RESULTS

A spectrophotometric titration of Cu(II) with EDTA at pH 5.0 is shown in Fig. 1. The values have been corrected for dilution, by the factor (initial volume + volume of EDTA added)/initial volume.

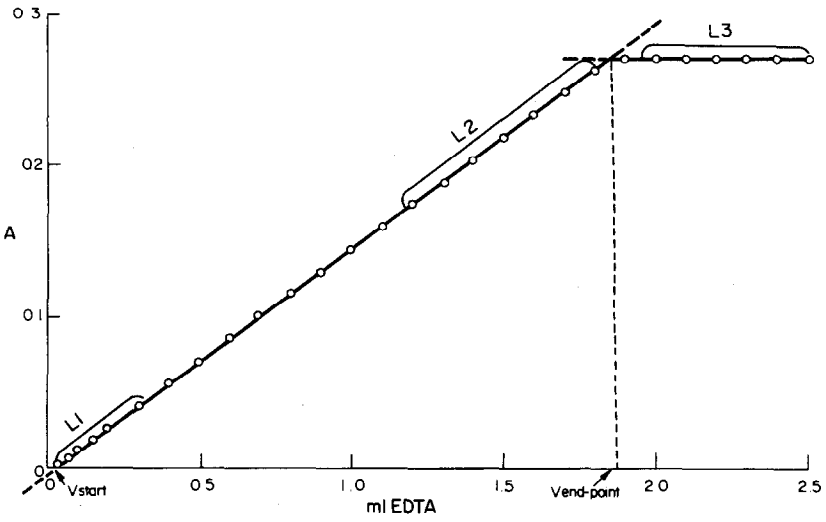

Fig. 1. Spectrophotometric titration of Cu(II) (5.8171 mg) with EDTA without indicator: λ = 700 nm; initial volume = 100 ml; pH = 5.0 [([HAc] + [Ac]) = 0.1M].

With a piston burette it is often difficult to define exactly the start of delivery of the titrant. In order to define the start more exactly, a little air is sucked into the capillary tip of the burette. As long as air is expelled from the burette tip, the titration curve is horizontal (A = 0.000). When delivery of EDTA begins, the curve begins to rise. The best straight line through the first points on the rising part of the curve is calculated. From the equation of this line, L1, the starting point of the titration can be obtained very exactly.

The end-point of the titration can be obtained from the second break of the titration curve. The best straight lines are calculated before and after the second break. The equation for L2 is calculated from data points between the half-titration point and the end-point. From the equations of the two lines L2 and L3, the end-point of the titration is calculated.

For the case illustrated in Fig. 1, the following equations were obtained by linear regression for the three lines:

L1 $Y = 0.1468X - 0.0031$ ($r = 0.9999$)
L2 $Y = 0.1465X - 0.0027$ ($r = 0.9999$)
L3 $Y = -0.0013X + 0.2709$ ($r = -0.63$)

Since line 3 is almost horizontal, the correlation coefficient obtained does not have its usual meaning. The initial volume is found to be 0.0211 ml and the end-point volume 1.8512 ml. The difference gives the consumption of EDTA.

Table 1 presents the results of a series of titrations for which a Metrohm microburette was used. For 7 titrations the mean value of the EDTA concentration was 0.04991M. The precision of the titrations is satisfactory, as can be seen from the relative standard deviation.

Since the absorbance measurements seemed to be the limiting factor in the accuracy of the standardization, a sensitive voltmeter was connected to the spectrophotometer. This allowed absorbance readings to be obtained to five significant figures, although the fifth figure was unstable. To improve the accuracy of delivery of EDTA solution, a weight-burette was used.

Five titrations performed with this equipment gave the following concentrations (M) of EDTA: 0.049866, 0.049875, 0.049870, 0.049876, 0.049867, with a mean value of 0.049871M and a relative standard deviation of 0.009%. This mean is 0.08% below that given in Table 1. The difference is probably due to decomposition of the EDTA, because the experiments with scale expansion and the weight-burette were done with the same EDTA solution about a year later than the titrations presented in Table 1. Another possible source of the deviation is the greater statistical error obtained in the titrations given in Table 1.

Table 1. Standardization of EDTA by spectrophotometric titration of copper(II) solution without indicator at λ = 700 nm and pH 5.0, titrant delivered with a Metrohm E 457 microburette

Cu taken, *mg*	Consumption of EDTA, *ml*	Concentration of EDTA, *M*
5.8171	1.8338	0.04992
14.5787	4.5920	0.04996
14.3511	4.5259	0.04990
12.7611	4.0203	0.04995
10.9541	3.4586	0.04984
11.1117	3.5025	0.04992
15.3623	4.8459	0.04989

Mean = 0.04991M; standard deviation = 0.000040M; relative standard deviation = 0.08%.

DISCUSSION

A good primary standard material should be very pure (100.00 ± 0.02%) and have a known and well defined composition. It should be stable and have a high relative molecular or atomic mass. The titration reaction should be stoichiometric and rapid and the conditional constant of the reaction should be sufficiently high.

Electrolytically manufactured copper is usually very pure. Copper is a relatively noble metal and is not oxidized as easily as, for example, zinc, nickel and bismuth. Just before the weighing, the layer of copper oxides was removed by mechanical cleaning of the wire with sand-paper. The atomic weight of copper, 63.546 is relatively low, but this drawback can be avoided by weighing a greater amount of copper and diluting.

Copper(II) forms a very stable 1:1 chelate with EDTA and the standardization can be done over a wide pH range. In the medium used in this work (pH = 5.0, $C_{Ac} = 0.1M$) the conditional constant is $10^{11.3}$. For spectrophotometric titrations without an indicator a constant of 10^5 is sufficient if the total analytical concentration of the chelate is at least $10^{-3}M$.[5] The spectrophotometric titrations can be done at other analytical wavelengths. For more dilute EDTA solutions the ultraviolet region can be used. A titration procedure without an indicator is to be preferred, because then no indicator correction is needed which could be rather complicated.[6]

The accuracy of the standardization naturally cannot be determined. Even an estimate of the accuracy is difficult. However, it is important that the method is based on sound theoretical principles. It may be mentioned that the concentration calculated directly from the weight of EDTA taken and volume of solution made was 0.05003M.

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SILANED GLASS BEADS FOR THE PRECONCENTRATION AND SPECTROPHOTOMETRIC DETERMINATION OF COBALT WITH 2-(2-PYRIDYLAZO)-5-DIETHYLAMINOPHENOL

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Summary—Silaned glass beads are applied for the preconcentration and spectrophotometric determination of cobalt with 2-(2-pyridylazo)-5-diethylaminophenol (PADAP). Traces of cobalt are collected as the coloured PADAP complex on a column of the beads, and the complex is then eluted with a small volume of ethanol–hydrochloric acid mixture and the absorbance of the eluate is measured at 575 nm. The cobalt can easily be concentrated by a factor of 50–500 in this way, and 0.1–2 μg of cobalt in 100 ml of sample solution can be determined reproducibly. High concentrations of Fe(III), Cr(III), Pb, Zn, Cu(II), Mn(II), Cd, Al, Ca and Mg can be tolerated but Pd(II) interferes.

Recently, an adsorption–elution and spectrophotometric method using Amberlite XAD-2 was proposed for the preconcentration and determination of traces of iron.[1] In this method, the tris(1,10-phenanthroline)-iron(II) complex was collected on a column of the resin, eluted with methanol and measured spectrophotometrically. The adsorption–elution method is very promising for the preconcentration of traces of toxic elements in water, but so far very little work has been done on the method. Preliminary experiments showed that the bis[2-(2-pyridylazo)-5-diethylaminophenolato]cobalt(III) ion (Co–PADAP complex) may be collected on a column of silaned glass beads and then eluted with a small volume of ethanol.

PADAP reacts with many heavy metal ions to form red to violet complexes.[2–7] Most of them are decomposed in acid medium, but the cobalt complex is stable in 0.8*M* hydrochloric acid.[5,6] The difference between the stabilities of cobalt and other metal complexes has been utilized in developing a selective method for the determination of cobalt.

The Co–PADAP complex, $Co(PADAP)_2^+$, can be extracted into organic solvents in the presence of suitable counter-ions,[8] and this has been exploited for the extraction–spectrophotometric determination of cobalt[5] and anionic surfactants.[8] However, the solvent extraction will give at best only a 50-fold concentration because of the rather high solubility of the organic solvents in water. Consequently it was decided to investigate the spectrophotometric determination of traces of cobalt by use of silaned glass beads and PADAP. The results are described below.

EXPERIMENTAL

Reagents

Adsorbent. Prepared[9,10] by treating 100–120 mesh nonporous glass beads, first with dimethyldichlorosilane and then with octadecyltrichlorosilane.

Column preparation. The column, in a tube 6 mm in diameter and 300 mm in length, was prepared by adding a slurry of the beads in ethanol until the height of the bed was 250 mm. It was washed with 0.1*M* hydrochloric acid to remove the ethanol.

PADAP. PADAP was synthesized according to Florence.[7] It was dissolved in 0.1*M* hydrochloric acid to give a 0.01% solution.

Standard cobalt solution. Prepared by dissolving cobalt chloride in 0.1*M* hydrochloric acid and standardized complexometrically.

Phosphate buffer solution, pH approx. 7. Prepared by mixing equal volumes of 0.05*M* disodium hydrogen phosphate and 0.05*M* potassium dihydrogen phosphate.

Eluent. A 1:3 v/v mixture of ethanol and 1*M* hydrochloric acid.

Procedure

To a sample solution containing less than 2 μg of cobalt, add 2 ml of PADAP solution and adjust the pH to 7 with 5 ml of the phosphate buffer solution. After 10 min, adjust the pH to 1 with hydrochloric acid, and pump the solution through the column. Rinse the column with 0.1*M* hydrochloric acid to remove the excess of PADAP. Pump the eluent through slowly and collect the 2 ml of eluate containing the reddish-violet complex, and measure its absorbance at 575 nm.

RESULTS AND DISCUSSION

Optimum conditions

Eluent. The complex collected on the column can be eluted easily with ethanol, a medium in which the

Table 1. Determination of various amounts of cobalt in various sample volume

Co taken, μg	Volume of sample soln., ml	Concn., μg/l.	Co found			Coefficient of variation, %
			No. of detns.	Mean, μg/l.	Recovery, %	
0.12	1000	0.12	5	0.13	108	7
0.12	500	0.24	3	0.23	96	—
0.29	500	0.58	3	0.56	97	—
0.116	100	1.16	9	1.16	100	1
0.579	100	5.79	5	5.83	101	1
1.16	100	11.6	3	11.8	102	—
2.32	100	23.2	3	23.3	100	—

complex has a single absorption maximum at 575 nm. In aqueous solution, the complex shows two maxima, at 575 and 530 nm, and the molar absorptivity is a little greater. In 1:3 v/v ethanol-water solution, the spectrum is the same as that in water. The eluent was acidified to be 0.75*M* in hydrochloric acid so that any PADAP which might be present in small amounts in the eluate, would have practically zero absorbance at the absorption maximum of the Co–PADAP complex.

Effect of pH. The formation of the complex is incomplete at pH values lower than 4, but, once formed, the complex is not decomposed even in 0.8*M* hydrochloric acid. Most other metal–PADAP complexes are decomposed in the strongly acidic medium.

Effect of PADAP. An addition of 2 ml of 0.01% PADAP solution is sufficient for almost complete reaction with 2 μg of cobalt. The excess of PADAP must be removed because PADAP absorbs appreciably at 575 nm. Rinsing the column with 0.1*M* hydrochloric acid is sufficient to remove the PADAP, because PADAP is protonated to give a cationic form in acid medium (the dissociation constants of PADAP in aqueous solution were determined spectrophotometrically as $10^{-1.5}$, $10^{-4.1}$ and $10^{-11.3}$).

Flow-rate. Reproducible results were obtained by passing the sample through the column at any flow-rate between 1 and 10 ml/min. The eluent was pumped through the column at a slow rate, about 1 ml/min, and the coloured fraction of the eluate was collected.

Adsorbent. Non-porous glass beads are a suitable support because the adsorbed complex is eluted completely within a 2-ml fraction of the eluate. The complex may be adsorbed only on the surface of the beads. Other adsorbents, such as Amberlite XAD-2, XAD-7 and silica gel that has been treated with octadecyltrichlorosilane retain a considerable amount of the complex, but the adsorbed complex is not easily eluted.

Reaction time. Ten minutes will suffice for almost complete reaction of 0.1 μg of cobalt with PADAP at pH 7.

Sensitivity and precision

Table 1 shows the results of recovery tests on various concentrations and volumes of cobalt solution. The calibration was done by measurement of solutions of $Co(PADAP)_2Cl$ synthesized by the reported method.[8] A 500-fold concentration can be achieved for 0.1 μg of cobalt per litre. Reproducible results are obtained for cobalt levels above 1 μg/l.

Interferences

Table 2 shows the effect of various cations on the determination of cobalt. High concentrations of almost all other ions except palladium do not interfere.

CONCLUSIONS

Octadecylsilane groups on treated silica gel behave like an organic extractant in the adsorption process,[11] and the Co–PADAP complex is presumably adsorbed as the $Co(PADAP)_2^+ Cl^-$ ion-pair by the same mechanism on the glass beads treated with octadecyltrichlorosilane. As the complex is bound only on the surface of the bead, it can be eluted easily with a small volume of eluent, so large concentration factors can be achieved. With the modified silica gel, or Amberlite XAD-2 and XAD-7, the complex may be held in the interior surfaces of the adsorbent and the adsorbed complex is not eluted easily. The modified glass beads may find many other applicatons for preconcentration of trace impurities in water.

Acknowledgement—This work was partially supported by a grant from the Ministry of Education of Japan.

Table 2. Effect of diverse ions on the determination of cobalt (5.79 μg/l.)

Ion	Added, μg/l.	Co found, μg/l.	Recovery, %
Fe(III)	100	5.71	99
Cr(III)	100	5.84	101
Pb(II)	100	5.75	99
Zn(II)	100	5.86	101
Cu(II)	100	5.93	102
Cd(II)	100	5.78	100
Mn(II)	100	5.89	102
Al(III)	100	5.83	101
Ca(II)	4×10^5	5.86	101
Mg(II)	1.2×10^6	5.76	100
Pd(II)	1.23	6.68	115

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ANALYTICAL DATA

MIXED-LIGAND COMPLEXES OF COPPER WITH SOME AMINO-ACIDS AND MALONATE

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Summary—The mixed-ligand complexes formed by copper(II) with an amino-acid (valine, threonine, isoleucine, aspartic acid, glutamic acid, lysine) and malonic acid have been investigated polarographically and their stability constants determined. The complexes are less stable than the corresponding complexes with oxalic acid instead of malonic, but also exhibit less disproportionation into the simple complexes, because the simple oxalate complexes are more stable than the malonate complexes.

We have continued our work on mixed-ligand complexes of copper(II) by studying the complexes formed by amino-acids and malonic acid. The same experimental methods as before[1] were used, except that malonic acid was used instead of oxalic, the pH was kept above 6.9 instead of 5.6, and three additional amino-acids were examined. The values obtained for the various complexes are shown in Fig. 1 (shown overleaf).

DISCUSSION

Comparison of the formation constants of the malonate complexes of copper and aspartic acid, glutamic acid or lysine with the constants for the corresponding oxalate complexes[1] shows that there is a greater tendency for an oxalate ion to be added to the 1:1 copper–amino-acid complex than for addition of a malonate ion, but practically no difference in the tendency for addition of amino-acid to either of the 1:1 copper–dicarboxylic acid complexes. The addition of the amino-acid to the dicarboxylate 1:1 complex is favoured by an amount corresponding to the statistical factor for mixed-ligand complex formation. The greater stability of the mixed-ligand complexes relative to that of the bis(dicarboxylic acid) complexes is due to the entropy effect arising from the lower charge on the mixed-ligand complex. The disproportionation constant for the reaction

$$2Cu(XY) \rightleftharpoons Cu(X)_2 + Cu(Y)_2$$

is given by

$$\log K_{dis} = \log K_{Cu(X)_2} + \log K_{Cu(Y)_2} - 2 \log K_{Cu(XY)}$$

If the disproportionation is purely statistical in nature, log K_{dis} should be -0.60. It ranges from -0.77 to -1.30 for the oxalate complexes, but from -1.50 to -1.95 for the malonate complexes, showing that the latter are less prone to disproportionation, because the bis(malonate) complex is less stable than the bis(oxalate) complex.

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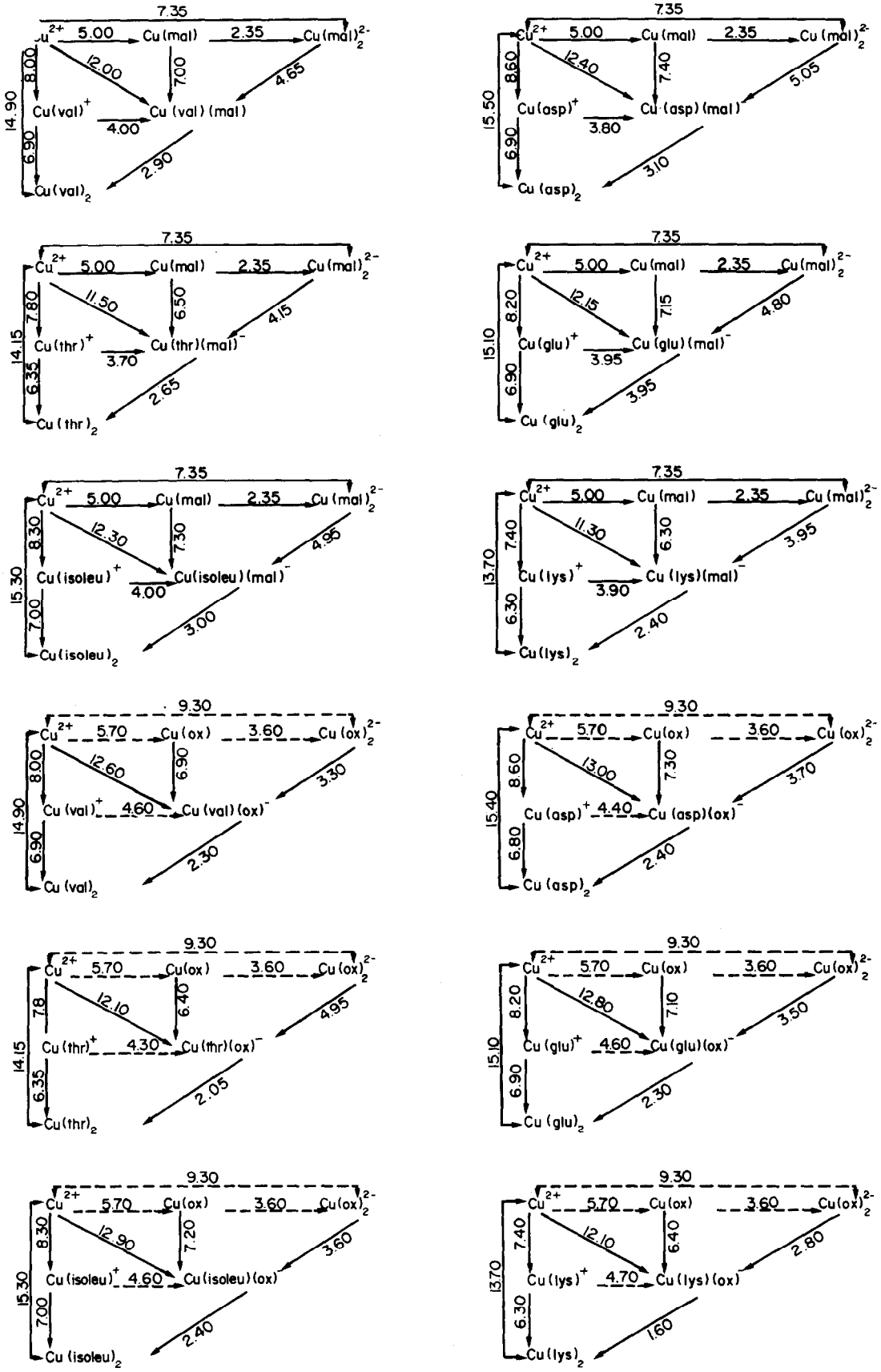

Fig. 1. The log K values for the various complexes.

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FORMATION CONSTANTS OF MERCURY(II) COMPLEXES WITH ETHYLENEDITHIODIACETIC AND DIETHYLENETRITHIODIACETIC ACIDS

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Summary—The logarithmic values of the stability constants of the mercury(II) complexes with ethylenedithiodiacetic and diethylenetrithiodiacetic acids were found to be 18.99 ± 0.07 and 19.05 ± 0.10 respectively.

Complexes of Hg(II) with ethylenedithiodiacetic acid (ethanedithiodiacetic acid, HOOC–CH_2–S–CH_2CH_2–S–CH_2COOH) and with diethylenetrithiodiacetic acid [thiobis(ethaneacetic acid), HOOCCH_2–S–CH_2CH_2–S–CH_2CH_2–S–CH_2COOH] have been investigated by spectrophotometry.[1,2] The complex species were found to have a metal to ligand ratio of 1:2 in acidic medium, and to be successively protonated as the acidity increased. Owing to the high stability of the complexes, the stability constants could not be determined: only the successive protonation constants of the various species could be derived.

Ethylenedithiodiacetic acid has been used for the spectrophotometric determination[1] and spectrophotometric titration[3] of mercuric ions at 260 nm, with high selectivity and good sensitivity.

To determine the formation constants of the two complexes, free mercury-ion concentrations at various pH values, at 25° and $I = 0.5M$ have now been measured potentiometrically with the aid of a mercury electrode.

EXPERIMENTAL

Ethylenedithiodiacetic acid (K & K) was used without further purification. Diethylenetrithiodiacetic acid was prepared as described by Podlaha.[4] Mercuric perchlorate was prepared by dissolving mercuric oxide (Merck) in perchloric acid. The ionic strength was adjusted to $0.5M$ with sodium perchlorate.

Potentiometric measurements were made at 25.0 ± 0.1° in the following cells:

Reference electrode | test solution | Hg. (A)

Reference electrode | test solution | glass electrode. (B)

The reference electrode was an Ag/AgCl electrode with a liquid bridge of $0.50M$ sodium perchlorate, as described by Forsling *et al.*[5] and the mercury electrode was similar to that of Reilley and Schmid.[6]

In a constant ionic medium the activities can be replaced by concentrations,[7] so that the emf (mV) of cells (A) and (B) can be expressed as:

$$E_A = E_A^0 + 29.58 \log[Hg^{2+}] + E_j$$

$$E_B = E_B^0 + 59.16 \log[H^+] + E_j$$

where E_j is the junction potential ($-100[H^+]$ mV under our experimental conditions) and E_A^0 and E_B^0 are constants determined in the absence of ligand before each set of measurements. The formation of Hg(I) at the mercury electrode was taken into account, the value 129.2 being used for $K = [Hg_2^{2+}]/[Hg^{2+}]$.[8] Complexation of Hg(I) by the ligands was negligible.

RESULTS AND DISCUSSION

Free mercury(II) concentrations were determined in solutions with $C_L > 2C_M$ and $-\log[H^+] = 2.5$–5.

For each value of $-\log[H^+]$ the formation constant for the 1:2 metal complex was calculated from the equations

$$[HgL_2'] = C_M - [Hg^{2+}]$$

$$[L^{2-}] = (C_L - 2[HgL_2'])/\alpha_L$$

$$\beta_2' = \frac{[HgL_2']}{[Hg^{2+}][L^{2-}]^2}$$

$$\alpha_L = 1 + \beta_{011}[H^+] + \beta_{021}[H^+]^2$$

where

$$[HgL_2'] = [HgL_2^{2-}] + [HgHL_2^-] + [HgH_2L_2].$$

β_{pqr} represents the formation constant of the species $Hg_pH_qL_r$, so β_{011} and β_{021} are the overall protonation constants of the ligands with the values:

$\log\beta_{011} = 3.90$; $\log\beta_{021} = 7.13$ for ethylenedithiodiacetic acid,

$\log\beta_{011} = 3.97$; $\log\beta_{021} = 7.18$ for diethylenetrithiodiacetic acid.

Table 1 gives experimental values for the system mercury(II)–ethylenedithiodiacetic acid. From the known values of the protonation constants of the

Table 1. Calculation of the stability constant of the mercury(II) complex of ethylenedithiodiacetic acid at various $-\log[H^+]$ values ($C_M = 1 \times 10^{-4}M$, $C_L = 3 \times 10^{-4}M$)

$-\log[H^+]$	$-\log[Hg^{2+}]$	$2\log\alpha_L$	$\log\beta_{102}$
3.21	12.91	2.08	18.99
3.47	13.61	1.44	19.05
3.68	13.96	1.02	18.98
3.87	14.26	0.73	18.99
4.12	14.50	0.45	18.95
4.57	14.86	0.18	19.04
5.24	14.87	0.04	18.91
			Mean 18.99 ± 0.07

Table 2. Logarithms of the formation constants of the mercury(II) complexes

Equilibrium	Ethylenedithiodiacetic acid	Diethylenetrithiodiacetic acid
$Hg^{2+} + 2L^{2-} \rightleftharpoons HgL_2^{2-}$	18.99 ± 0.07	19.05 ± 0.10
$HgL_2^{2-} + H^+ \rightleftharpoons HgHL_2^-$	2.10[1]	3.76[2]
$HgHL_2^- + H^+ \rightleftharpoons HgH_2L_2$	0.41[1]	1.58[2]

complex species, it is expected that the contribution of these complexes is negligible at the values of $-\log[H^+]$ considered, so the constant evaluated is β_{102}. This is confirmed by the calculated values of the formation constant being independent of $[H^+]$.

Table 2 summarizes the values found for the equilibrium constants of the mercuric complexes.

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ANNOTATION

ANALYSIS OF AUTOMOBILE-EXHAUST EMISSION-CONTROL CATALYSTS

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Summary—A review is given of methods used and problems arising in determination of platinum metals in catalysts used for treatment of automobile exhausts, and the results of laboratory and interlaboratory tests are discussed.

The enforcement of the 1970 Clean Air Act by the Environmental Protection Agency (EPA) led to the introduction of catalytic converters for automobile exhaust gases, starting with the 1975 model cars. In the U.S.A., General Motors through its AC Spark Plug Division initially chose the pellet form while Ford Motor Co. and Chrysler Corp. preferred monolithic type catalysts. During the last year General Motors has also been phasing in monoliths.

The pelleted catalysts, referred to as spheres, beads, pills or extrudates, depending on the shape or size, consist of a thin layer of platinum and palladium in approximate ratio 5:2 deposited on a substrate of γ-alumina. Typical catalysts, in use from 1975 to 1979, contain in the case of the so-called "low-load" form about 370 ppm of platinum and about 160 ppm of palladium and for the "high-load" variety approximately 60% more of both elements.

During the last few months, a number of new platinum–palladium combinations have been introduced to satisfy the stricter air-quality requirements of the EPA, starting with 1980 cars, and also to accommodate a greater variety of car and/or engine sizes. At the present time, the platinum content of pellets may range from 350 to 1000 ppm, that of palladium from 150 to 850 ppm; the platinum–palladium ratio may range from 1:0.4 to 1:2.

California's stricter air-quality standards led to the early introduction of pelleted platinum–rhodium catalysts. Rhodium is now widely used, in combination with platinum, in addition to or instead of palladium. The platinum–rhodium ratio was initially about 7:3. At the present time it is about 11:1. The rhodium-bearing catalysts are called "three-way" catalysts, since they not only oxidize carbon monoxide and hydrocarbons but also remove various nitrous oxides (usually referred to as "NOX").

The monolithic catalysts are basically cordierite-type honeycombs with the precious metals (usually platinum and rhodium in the ratio of 10:1 or 11:1) deposited on large surface area γ-alumina which in turn is dispersed on a honeycomb consisting of aluminium magnesium silicates and/or calcium aluminium silicates. General Motors also uses platinum–palladium monoliths containing approximately 800 ppm of platinum and 400 ppm of palladium.

EVALUATION OF CATALYSTS

The preliminary evaluation of exhaust-control catalysts includes performance tests carried out at the plants of the car manufacturers, involving sophisticated dynamometer equipment which measures, among other things, the characteristics and efficiency of catalysts under conditions involving various parameters of air–fuel ratios, equivalent speeds, and variations in driving conditions.

One of the production acceptance criteria used by the AC Spark Plug Division is the amount of precious metal contained in a cubic foot of catalyst. Since the bulk density varies between 37 and 43 lb/ft^3, the required amount of platinum and palladium varies accordingly. Typically, for the low-load catalyst, the specification asks for 0.237 troy oz of platinum and 0.095 oz of palladium per ft^3 of pellets. This corresponds to about 380 ppm of platinum and 152 ppm of palladium.

XRF control methods

AC Spark Plug Division and its suppliers extensively use X-ray fluorescence for internal checking purposes. Sample preparation for XRF is simple. The pellets or monoliths are ground to 100-mesh size. While some suppliers prefer to calcine the sample at about 1200° before compacting, others have observed that variations in the Al_2O_3 morphology, that is changes from γ-alumina to α-alumina or θ-alumina, did not cause any measurable effects on the XRF intensity, nor did they notice any effect caused by large variations in the moisture content.

AC Spark Plug Division uses a flat wafer obtained by compression of 10 g of catalyst with a 30,000 psi press. This wafer is about $\frac{3}{8}$ in. thick. It was found that an acceptable precision can be obtained with 2-g wafers, so it does not matter much if the sample available is small. In fact, by diluting the available sample with γ-alumina, it is possible to analyse accurately as little as 0.05 g of sample.

Most laboratories carry out their tests with wavelength-dispersive X-ray spectrometers using chromium radiation; others use molybdenum targets. Most laboratories use a 2.84-Å *d*-spacing (110) lithium fluoride analyser crystal and count for about 100 sec. A precision of 3% (2 standard deviations) is claimed by most organizations.

As mentioned before, the "three-way" catalysts frequently contain palladium and rhodium together. This causes a problem with XRF methods since the stronger and broader palladium line interferes with the best rhodium line. To overcome this problem, some use very narrow (0.15 mm) collimator slits, together with a 2.84-Å *d*-spacing (110) LiF analyser crystal to achieve sufficient dispersion and resolution to separate the two lines. Thus, as little as 20 ppm of rhodium can be measured in the presence of up to 200 ppm of palladium.

Energy-dispersive XRF analysers employing radioisotopes as a source are also widely used. In this technique, secondary-emitting targets can be selected to excite extremely low loadings of precious metals. For instance, americium-241 is being used to excite a tin target. The emitted tin *K*-radiation very efficiently excites rhodium, permitting the detection of as little as 5 ppm in some catalysts. However, when palladium is present in significant concentrations, the proximity of its *K*-radiation can interfere with the measurement of rhodium unless instrumentation having very good energy resolution is available. Similarly, in some situations, measurement of palladium can be interfered with by the proximity of the excitation tin *K*-radiation. In this case, an antimony target can be used instead for determining both palladium and rhodium. Another isotope used to excite secondary-emitting targets is cadmium-108. It is being used to some extent for the determination of platinum.

Two major difficulties have been encountered with all XRF methods. The first is caused by the inhomogeneity of distribution of the precious-metal powder on the supports. It is obvious that the dispersion of the precious metals in the calibration standards should match that found in actual samples. In the opinion of some, it is questionable whether this aim can be achieved. Some organizations attempt to avoid this problem by calcining their samples to coalesce the precious metals to "equilibrium" crystalline size. However, others express doubts as to whether all samples will reach the same equilibrium crystal size, and in their opinion this technique means just trading one uncertainty for another.

The second major difficulty consists in the unlimited variety of mixtures of precious metals together with various stabilizers found in catalysts. To develop accurate XRF procedures, it is necessary to have available an adequate supply of reliable standards covering the exact range of promoters and stabilizers found in the sample matrix.

It is, therefore, nor surprising that all the suppliers of catalysts to AC Spark Plug Division are using XRF only for quality control. Some of the suppliers have chemical facilities too; others rely on companies like Ledoux & Company to monitor their products by chemical methods.

Chemical analysis of automotive catalysts

Because of the limitations of the XRF methods, the AC Spark Plug Division bases acceptance of each lot of catalyst on the results of chemical analysis. Chemical methods have been shown to meet their requirement of an error not exceeding about $\pm 1\%$ relative, even though the content of platinum may be as low as 350 ppm, of palladium as low as 140 ppm and of rhodium as low as 50 ppm.

Ledoux & Company have determined the precious metal content of catalysts for General Motors on an exclusive basis by chemical procedures since 1975 and the final results of the work have been used as an acceptance or rejection criterion for more than 2500 lots of catalysts.

Our analytical programme covering these catalysts includes the spectrographic examination of the platinum and palladium used by some of the suppliers. The sponge used must meet certain specifications for purity and precious metal content. Next, several suppliers submit to us samples of the precious metal solutions, which eventually will be coated onto the catalyst, for a check on their own findings. Obviously, any error at this stage could lead to disastrous financial consequences. These solutions are always analysed by gravimetric methods on comparatively large sample weights to ensure a high degree of accuracy. Then follows the analysis of each lot of catalyst shipped to AC Spark Plug Division. Primary scrap sold to Gemini Industries is also analysed before reprocessing. Finally, the platinum and palladium recovered by Gemini is spectrographically examined for purity for AC Spark Plug Division. The evaluation of the precious metal catalysts, however, occupies the central part of our analytical activities.

Neutron-activation analyses by General Motors have indicated that the homogeneity of the pellets is so poor that the analytical sample must weigh at least 300 g and 10–15-g portions of the pulverized sample must be used for the analytical tests. Monolithic samples are usually even less homogeneous. It is, therefore, common practice to determine the precious metal content of the top, middle and bottom sections of each monolith.

To ensure accuracy, we carry out all tests in triplicate, using three different sample weights ranging between 10 and 15 g.

Our analytical procedures have gone through several phases of development.

First method

Our first technique was based on the conversion of the γ-alumina into α-alumina by heating the powdered sample for several hours at about 1200°. Loss of platinum and palladium as chlorides was avoided by mixing the sample with ammonium carbonate before calcining. The calcined powder was treated with *aqua regia* to extract the precious metals, then was re-ignited under the same conditions and retreated with *aqua regia*. More than 90% of the platinum and palladium were extracted, together with small quantities of alumina. The remaining platinum and palladium were recovered from the residue by fire-assay, with silver as a collector.

We used this procedure for about half a year, correcting for the incomplete extraction of platinum and palladium by running synthetic standards through all steps of the precedure. In connection with this procedure, it should be emphasized that powdered α- and γ-alumina are strong absorbers of precious metals and that contact time must therefore be kept to a minimum.

Second method

The method which we used[2] for the next four years was based on the opposite approach. Briefly, the method consists in dissolving the alumina by treatment with a mixture of sulphuric and phosphoric acids, precipitating the platinum and palladium with hydrogen sulphide, dissolving the ignited sulphides in *aqua regia*, separating the platinum and palladium by a dimethylglyoxime–chloroform extraction, determining the platinum by differential spectrophotometry, and palladium by atomic-absorption spectrophotometry.

Third method

Our third method, which was phased in during the last year, consists in the decomposition of 12–15-g portions of the sample by heating with 105 ml of a 1:1:1 mixture of phosphoric acid, sulphuric acid and water and dissolving platinum, palladium and/or rhodium with bromine and hydrochloric acid.

In the case of pelleted γ-alumina catalyst, the resulting solution is often clear. If this is not the case, any precipitate is filtered off, ignited, fused with a little bisulphate and cooled, then the melt is leached with dilute hydrochloric acid containing bromine, and the solution is added to the main solution.

In the case of monolithic catalysts, the residue is usually substantial and consists of α-alumina, calcium aluminium silicate, and/or magnesium aluminium silicate. It may retain substantial amounts of platinum, palladium or rhodium. For the recovery of the precious metals the residue is filtered off, ignited, and subjected to a fire-assay fusion and cupellation, 100 mg of gold being used as a collector for the residual platinum and rhodium, or 100 mg of silver as a collector for residual platinum and palladium.

The gold is dissolved in *aqua regia*, followed by an oxalate precipitation in hydrochloric acid medium. After filtration, platinum and rhodium are recovered from the filtrate by evaporation with nitric and perchloric acids, followed by dissolution of the residue in hydrochloric acid. This is added to the main solution. If silver is used as a collector (platinum–palladium monolith) the bead is dissolved in nitric acid, silver precipitated and platinum and palladium are dissolved by the addition of hydrochloric acid.

In the case of platinum–palladium catalysts or platinum–palladium–rhodium catalysts, palladium is now removed by a dimethylglyoxime or furildioxime extraction into chloroform and determined either photometrically or by atomic-absorption spectroscopy. In the absence of palladium, the extraction step is obviously omitted. After the removal of the palladium, the dimethylglyoxime or furildioxime is destroyed by continuous heating with repeated additions of 20-ml portions of bromine water. If a rhodium determination is required, the solution is transferred to a 500-ml standard flask and diluted to volume. Rhodium is determined in this solution by atomic-absorption spectroscopy, the standards containing various amounts of rhodium and approximately the same amount of alumina and phosphoric and sulphuric acids as the samples. If no rhodium determination is required, the solution is evaporated to about 300 ml and is then ready for the platinum determination.

The whole procedure is applied to a blank consisting of the same substrate as the samples being analysed. This step is very important, inasmuch as the total matrix of the sample and all the acids are present when rhodium and platinum are measured. The blank is insignificant in the case of pelleted catalysts, because usually no stabilizers or promoters are added. In the case of monolithic catalysts, the blank will also be low, as long as the sample solutions contain no coloured species which absorb at the 403 nm wavelength of the platinum–stannous chloride complex. After the determination of the rhodium, the 500-ml standard flask is refilled to volume by adding the required quantity of water from a burette so that the dilution factor can be calculated. The solution is transferred to a beaker, evaporated to about 300 ml, transferred to a 500-ml standard flask and diluted to volume. The platinum is then determined spectrophotometrically. A detailed description of the stannous chloride procedure has been published.[2]

Fourth method

Because of engineering difficulties, the distribution of the precious metals on the monoliths is quite uneven and, as already mentioned, it is customary to analyse powdered portions of the top, middle and bottom of each sample. During the last few months, we have developed a method which allows the quanti-

Table 1. Effectiveness of calcination–acid extraction procedure

Total found		Recovered from *aqua regia* solution				Recovered from Ag bead after fire assay treatment of residue			
Pt, *mg*	Pd, *mg*	Pt, *mg*	Pt, % of total	Pd, *mg*	Pd, % of total	Pt, *mg*	Pt, % of total	Pd, *mg*	Pd, % of total
3.58	1.40	3.27	91.3	1.30	92.8	0.31	8.7	0.10	7.2
4.20	1.71	4.02	95.7	1.66	97.1	0.18	4.3	0.05	2.9
5.23	2.09	5.00	95.6	2.00	95.6	0.23	4.3	0.09	4.3
3.70	1.50	3.45	93.3	1.42	94.7	0.25	6.7	0.08	5.3
4.48	1.80	4.31	96.2	1.72	95.6	0.17	3.8	0.08	4.4
5.50	2.22	5.13	93.3	2.15	96.8	0.37	6.7	0.07	3.2
5.62	2.21	5.40	96.1	2.14	94.7	0.22	3.9	0.12	5.3
6.74	2.66	6.34	94.1	2.46	92.5	0.40	5.9	0.20	7.5
8.45	3.38	7.73	91.5	3.05	90.2	0.72	8.5	0.33	9.8

tative stripping of platinum, palladium and/or rhodium from pieces of monolith without the necessity to pulverize the sample. Pieces weighing between 50 and 100 g are boiled with *aqua regia* and bromine water, and the solution is decanted; this is repeated twice more. The precious metals plus γ-alumina plus enhancers and/or stabilizers are quantitatively separated from the cordierite "skeleton". The combined solutions are transferred to a standard flask. For the determination of rhodium, portions of this solution, representing 7–15 g of sample, are evaporated with sulphuric and phosphoric acids to remove the *aqua regia*. In the resulting solution rhodium is determined by atomic-absorption spectroscopy. For the determination of platinum similar portions of the master solution are evaporated with sulphuric and phosphoric acids and the platinum is determined by differential spectroscopy. If palladium is present (up to now monolith samples contain either platinum and palladium or platinum and rhodium), platinum and palladium are separated by a dimethylglyoxime or furildioxime extraction into chloroform.

It should be emphasized that the analysis of solid pieces of monolith instead of the pulverized sample has not only cut our analysis time to less than half, but has also provided us with a more representative sample.

VERIFICATION

First method

The effectiveness of the calcination–acid extraction procedure is demonstrated in Table 1. While the extraction of platinum and palladium is 95% complete on average, the recovery of the platinum and palladium from the acid-insoluble material is difficult and requires the use of a cumbersome fire-assay procedure.

Second method

In 1975 General Motors carried out a round-robin programme. "The study was undertaken to determine the level of agreement or disagreement in results between those laboratories involved in measuring platinum and palladium loadings on pelleted catalysts used in the General Motors exhaust converter".[3]

Ledoux & Company participated in this assessment study and applied the method then in use (see "Second Method"). Laboratory X made use of a chemical procedure,[4] and Laboratory Y used a proprietary atomic-absorption method after the collection of platinum and palladium with sodium formate in sulphuric acid medium. In addition, four organizations analysed the samples by XRF methods. The study involved two different substrates. From each substrate a low-load "standard" was prepared containing nominally 350 ppm of platinum and 150 ppm of palladium. In addition, from one of the substrates a high-load "standard" was prepared containing nominally 560 ppm of platinum and 240 ppm of palladium. The "standards" were prepared by ball-milling 300-g portions of pellets with distilled water, to produce fine powder suspended in a thin slurry. The slurry was dried into a solid cake, which then was repowdered and blended. The resulting blended powder was placed in glass bottles, three of which were selected at random from each sample lot and forwarded to each laboratory. For precision data see Tables 2 and 3. Since the true platinum and palladium content of the "standards" was unknown no statement could be made about accuracy.

The nature of the catalyst substrate significantly affected the precision and accuracy of the X-ray measurements. Standards prepared from substrate no. 1 could be analysed accurately only by laboratories calibrating with substrate no. 1 and standards prepared from substrate no. 2 could be analysed accurately only by laboratories using substrate no. 2 for calibration.

A statistical *F*-test was performed to determine which of the observed differences between laboratories were statistically significant when compared with the repeatability limits of the measurements. It was found that in the case of platinum, to be considered significant the differences between two labora-

Table 2. Precision obtained by three laboratories using chemical procedures

	Sample a		Sample b		Sample c	
	Pt	Pd	Pt	Pd	Pt	Pd
Ledoux & Co.						
Mean, % w/w	0.05668	0.02258	0.03583	0.01402	0.03860	0.01530
Std. devn., % w/w	0.00032	0.00018	0.00020	0.00010	0.00020	0.00006
Relative std. devn., %	0.56	0.80	0.56	0.71	0.52	0.39
Laboratory X						
Mean, % w/w	0.05682	0.02255	0.03578	0.01385	0.03810	0.01532
Std. devn., % w/w	0.00035	0.00028	0.00045	0.00014	0.00030	0.00015
Relative std. devn., %	0.62	1.24	1.26	1.01	0.79	0.98
Laboratory Y						
Mean, % w/w	0.05780	0.02285	0.03777	0.01417	0.03870	0.01547
Std. devn., % w/w	0.00077	0.00015	0.00037	0.00016	0.00018	0.00008
Relative std. devn., %	1.33	0.66	0.98	1.13	0.47	0.52

tories must equal or exceed the following relative errors:

Sample a 1.57%
Sample b 1.88%
Sample c 1.80%

For palladium measurements the difference must equal or exceed the following relative errors:

Sample a 2.38%
Sample b 2.49%
Sample c 1.67%

No significant differences were found in the case of the palladium measurements. Table 3 presents the corresponding platinum data which indicate that Ledoux & Company and Laboratory X are in excellent agreement, while both laboratories agree with Laboratory Y only in the case of sample c.

Part II of the same study was undertaken in 1977, (*a*) to determine whether one or more laboratories could provide sufficiently accurate wet analysis to qualify as a source of wet analyses for XRF calibration standards and (*b*) to resolve the 2–3% bias observed between Laboratory Y and Ledoux & Company.[5]

In addition to Ledoux & Company, two supplier laboratories employing chemical methods participated in this study. Part II of the study was based on the analysis of individual synthetic "standards", prepared by cutting high-purity platinum and palladium wire to length, weighing the cut wire to the nearest μg and dissolving it with *aqua regia* in clean bottles equipped with ground-glass stoppers. After dissolution and two evaporations to dryness, with intermittent addition of hydrochloric acid, the salts were dissolved in water and approximately 15 g of 150-mesh substrate were introduced with stirring. The stirring rod was rinsed, then the bottles were placed in an oven at 95° and the contents were evaporated and dried.

Table 3. Statistically significant differences between laboratories (Lab A − Lab B) in measurements

	Lab B		
Lab A	Ledoux & Co.	Laboratory X	Laboratory Y
Ledoux & Co.			
Sample a	—	*	−1.98
Sample b	—	*	−5.24
Sample c	—	*	*
Laboratory X			
Sample a	*	—	−1.70
Sample b	*	—	−5.37
Sample c	*	—	*
Laboratory Y			
Sample a	+1.98	+1.70	—
Sample b	+5.24	+5.37	—
Sample c	*	*	—

* No statistically significant difference; degrees of freedom: 5 for each laboratory.

Three different "loadings" were employed.

1. Low load: ~6 mg of Pt, 2.4 mg of Pd
2. High load: ~10 mg of Pt, 4 mg of Pd
3. Pt only: ~10 mg of Pt

Six bottles of each loading type, encompassing as wide a range of weights as practical, were selected and forwarded to each of the three laboratories. Each laboratory performed its usual wet analysis for platinum and palladium, with whatever modifications were necessary to accommodate a 15-g sample size. The results of each analysis were reported as μg of platinum and palladium.

Immediately after completing the 18 analyses, each laboratory forwarded its results to the AC Spark Plug Materials Laboratory for comparison with the "known weights of added wire".

CONCLUSION

(A) Wet analyses for platinum yielded the following "apparent" average errors and precisions (2 standard deviations).

Laboratory	Error, %	Precision, %
LEDOUX		
Low load	−1.05	±2.9
High load	−0.60	±0.96
Overall	−0.83	±2.16
SUPPLIER A		
Low load	−6.17	±2.88
High load	−4.08	±4.06
Overall	−5.13	±3.53
SUPPLIER B		
Low load	5.90	±8.23
High load	5.83	±1.76
Overall	5.87	±5.95

(B) Wet analysis for palladium yielded the following corresponding results.

Laboratory	Error, %	Precision, %
LEDOUX		
Low load	−2.24	±9.72
High load	−1.05	±2.06
Overall	−1.65	±7.03
SUPPLIER A		
Low load	−5.78	±3.56
High load	−4.54	±3.68
Overall	−5.16	±3.62
SUPPLIER B		
Low load	3.22	± 4.47
High load	−2.41	±11.68
Overall	0.41	± 8.87

While in this study the error statement given is justified, the precision must be termed "apparent", because the various synthetic standards were prepared separately but are treated statistically as originating from one and the same sample.

Third method

Another study was carried out by General Motors, this time to determine the accuracy and precision associated with the chemical analysis for platinum and rhodium contents in coated 3-way monoliths produced by one of its suppliers.[1]

As in the case of Part II of the study just described, samples with "known" loadings were prepared by G.M. personnel in each of the two participating laboratories. Samples with "known" loadings were prepared by the addition of standard platinum and rhodium solution to a slurry of two different types of finely ground blank monolith which were coated with an alumina wash-coat containing a number of proprietary enhancers and stabilizers.

The entire sample preparation procedure was observed by a "neutral" representative of the Department of Chemistry of a major U.S.A. university, who removed all prior identification and relabelled each beaker with his own code.

Results

Wet analysis of platinum in 3-way monoliths yielded the following "apparent" average errors and precisions (2 standard deviations).

	Errors, %	Precision, %
Low-load monolith		
Ledoux	+4.3	±7.4
Supplier	+3.7	±7.3
High-load monolith		
Ledoux	+1.6	±2.6
Supplier	0	±5.4

Wet analysis for rhodium in 3-way monoliths yielded the following corresponding results.

	Error %	Precision, %
Low-load monolith		
Ledoux	+1.1	±3.6
Supplier	0	±4.8
High-load monolith		
Ledoux	+0.7	±2.2
Supplier	−4.0	±3.8

As in the case of the previous study, the accuracy statement is justified while the precision statement must be qualified as "apparent".

Fourth method

It was mentioned before that generally speaking the distribution of the precious metal on monoliths is uneven, necessitating the analysis of top, middle and bottom portions of the sample. In order to account for discrepancies in results and determine the degree of homogeneity, AC Spark Plug Division selected at random 3 monolith pieces from a batch of several thousand. Each piece was subdivided into 3 sections, top, middle and bottom, and each section was cut again into four subsections. Two of the subsections were pulverized and portions sent to Ledoux & Co. and to the monolith supplier. The two other subsections were left intact and sent to Ledoux & Co.

Thus, Ledoux & Co. received for each monolith 6 powdered samples and 6 "pieces" for a total of 18 powdered samples and 18 pieces. All 36 samples had been identified with a code only known to General Motors. For the powdered sample we used the third method, and the fourth method for the pieces. We also determined the platinum and rhodium content in portions of the supplier's coded sample solutions prepared from powdered samples. After reporting our results, we were informed of the following outcome for the overall averages.

	Average Pt content, %	Average Rh content, %
Ledoux pieces	0.250	0.0218
Ledoux powder	0.251	0.0215
Ledoux, solutions from supplier	0.250	0.0213

Though these results give no information about precision or homogeneity, we regard the agreement between the means as gratifyingly satisfactory.

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TALANTA REVIEW*

THE CHANGING FACE OF LABORATORY AUTOMATION: PRESENT AND FUTURE TRENDS

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Summary—A review is given of current developments in automation of laboratory methods, with emphasis on the managerial outlook demanded from the modern analytical chemist and on the underlying philosophy of automation. The influence of microprocessor technology on laboratory instrumentation is discussed, and future trends predicted.

The history of automatic or automated analysis is difficult to trace for two reasons; on the one hand there is a complete lack of an accepted definition and on the other the field of automation spans a range of disciplines. Some time ago the International Union of Pure and Applied Chemistry (IUPAC) defined automation as being "the use of combinations of mechanical and instrumental devices to replace, refine, extend, or supplement human effort and facilities in the performance of a given process, in which at least one major operation is controlled without human intervention, by a feedback mechanism" and mechanization as "the use of mechanical devices to replace, refine, extend, or supplement human effort". The distinction between the two terms is clear. IUPAC recommends that "automation" be reserved for those systems involving feedback loops. This is logically sound. However, very seldom does a feedback loop enhance the potential of a system nor does it further reduce the manpower associated with the analytical process. It has become common practice now to refer to instruments or devices as automatic where they do achieve a reduction, partial or complete, in some phases of the analysis procedure. Recently the IUPAC Commission on Automation produced a provisional document entitled "Characteristics and attributes of instruments intended for automated analysis in clinical chemistry". Whilst this document is still under discussion, there are many who suggest that in itself it represents a considerable achievement in bringing together a range of views and that there is little to be gained by continued modification to it. Further confusion abounds because very often instruments are described as "fully automatic" when they are either microprocessor-controlled or include on-line data collection and analysis modules. These instruments are not in any sense "fully automatic".

An analytical procedure is in itself a systems problem and the sampling, pretreatment, measurement, data collection and reduction, and final reporting all have to be considered in a fully automatic approach. Likewise computerization is often considered as being synonomous with automation; but, although microprocessor technology is certainly changing the face of automatic instrumentation and influences both the control aspects as well as data reduction, computerization represents only a part of the overall problem. In this sense computers can be considered as tools of the trade within the area of automation.

Automation has been applied for a number of years in process control instrumentation but the major impetus to introduce automatic devices in the laboratory stems from three sources: (*a*) the introduction of the continuous flow principles as outlined by Skeggs,[1] (*b*) the general demand for clinical chemical measurements, which presents a ready and sizable market for instrument companies and, more importantly, (*c*) the ability to handle large volumes of data and package them in a form suitable for presentation to analysts and customers, through the use of mini and micro computer systems linked to the control computer. The availability at reasonable price of computer power and the various associated peripherals that are also imperative for a viable computer system is seen as being the most important feature of future growth in this area. However, this must be balanced against the ever increasing cost of reliable and effective software, which is also required to maximize the usefulness of computer hardware. A major problem is that the jargon and computer languages present a formidable barrier for the inexperienced to overcome. Rapid progress towards more effective automation demands that a concerted effort is made to develop

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the software. This is, however, a global problem and the first priority must be suitable software, for handling intercomputer communications, in particular. The BASIC computer language is presently favoured for use with more sophisticated instruments but this offers only limited advantages and is a very real stumbling block to future progress. This problem is discussed in more detail later in this paper.

Because of the importance of the advances made in the field of clinical chemistry, instrument companies have addressed this market vigorously. There is however another and even more exciting market in industrial process control. In this area there are real problems of sample handling and sample matrix effects. In contrast, the clinical market is fortunate in that its problems mainly relate to blood and urine and the problems experienced in the industrial area are minimized. One significant problem in the clinical market, however, which has repercussions on the other market sectors, is that it takes a long time for an instrument to be designed, developed, evaluated and accepted into use. The time scale for this is of the order of ten years. This puts considerable constraints on instrument development and only the most successful instrument companies can afford the financial investment required for such developments. Indeed, many companies have attempted and failed to break into this market, often investing very large sums without producing an accepted instrument. There are signs that companies are becoming increasingly aware of the industrial market and some attempts have been made to develop a systematic approach to this problem. The recently introduced Mettler range of automatic instruments provides one example.[2]

Automatic chemistry draws on a whole range of disciplines. Therefore the student of automation or the systems designer must be willing to use technology developed for achievements in one sector and to apply it to another area, which is often only loosely related to the first. There are therefore problems of education, communication and specification, and this review would not be complete without some reference to such problems. The area on which automatic chemistry impinges is vast. It is therefore not possible to provide a comprehensive coverage in this review. An attempt will be made to highlight some of the more interesting and more recent avenues of development.

EDUCATION, SPECIFICATION AND COMMUNICATION

These three aspects present many problems in automatic analysis. Here they are considered separately but that they are highly interactive is obvious.

Education

For the laboratory with analytical needs which can be met by purchasing a commercial automatic analyser and using it strictly in accordance with the maker's instructions, methods, and servicing, the training needs for the operators can usually be met through a course provided by the manufacturer. This need is well catered for by the major manufacturers, most of whom offer training at their own premises on a regular or as-required basis. But for those laboratories having a large and varied work-load, commercial automatic analysers are likely to be viewed as facilities to be modified to meet new needs. Frequently the chemical methods needed will be developed by the laboratory staff. In these circumstances it is essential that several of the staff develop an in-depth understanding of the subject, including component design and performance, sample-transport mechanism, data processing, and control technology. For such people the education and training requirements are more demanding. At present these cannot be met in a wholly satisfactory manner. The underlying training requirement is to create an understanding not only of the relevant scientific principles, but also of the philosophies of automation, which are evolving rapidly as the range of applicability of automatic analysis continues to grow.

To educational establishments the teaching of automatic analysis poses three problems. It is an interdisciplinary subject involving engineering, electronics, and computer disciplines in addition to chemistry; equipment for demonstration purposes is expensive; and at the present time very few teaching staff have more than a rudimentary experience of the subject. Nevertheless, colleges are generally aware of the problems and have made important, though limited, attempts to overcome them. For example Betteridge *et al.*[3] have described a microprocessor-controlled automatic titrator which is of value as a teaching aid, and the relatively inexpensive and simple flow-injection technique seems ideal for the same purpose. In the USA, Malmstadt[4] has studied the teaching aspects for some years and has developed a modular experimental approach; teaching manuals and equipment for this are available for purchase.

In the UK, automatic analysis is taught, albeit to a limited extent at present, in a number of postgraduate M.Sc. and diploma courses devoted to instrumental analysis. With increasing experience and availability of equipment such courses should become more effective in presenting the principles of the subject. But the important philosophical aspects are preferably presented by experienced workers in the field. They can pass on not only the practice but the thinking that generates progress in automatic analysis. There is ample scope for specially designed short courses, with the use of equipment loaned by manufacturers to illustrate development. Encouraging progress is being made; for example, discussion and workshop sessions on automatic analysis now feature at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy and at least one region of the American Chemical Society has sponsored training schools in the subject.

Recently Betteridge, Porter and Stockwell[5] organized, under the auspices of the Chemical Society, a Summer School on Automatic Analysis at University College Swansea, which drew together world experts to present the foundation lectures/programmes. This series of lectures covered the various facets of automation, including management and economics in addition to developments in philosophy and techniques. More recently introduced techniques such as thin-film chemistry, the use of microcomputer-controlled systems and flow-injection analysis were discussed in some depth. Table 1 illustrates the scope of the lecture programme. In addition, a range of tutorial sessions catering for the participants' own particular interests was arranged. Tutors comprised the lecturing staff and local experts. Twenty practical experiments made available by a number of instrument companies and other institutions enabled the course members to gain first-hand experience of a wide range of instruments. One novel feature of the course was a tutorial session in which the course members had to develop a strategy for finding an automated solution to an important analytical problem. This problem related to the analysis of cigarette smoke to determine its tar, nicotine and carbon monoxide levels. Small groups considered the problem first and then combined into three large groups each of which prepared a joint presentation and discussed the development strategy. At the end of the course each of these three groups made a formal presentation. Copeland from the Laboratory of the Government Chemist then described the actual approach taken to solve the problem. The various approaches were then discussed and contrasted by the lecturers and course members. This problem and discussion served to illustrate the various philosophies and principles outlined by the course staff throughout the course. The response to this course was particularly enthusiastic, and whilst there are undoubtedly improvements and modifications that could usefully be made in any future venture, the success of the course proved that the basic formula was correct and above all that this type of course is needed in the UK and, no doubt, elsewhere.

Specification of the analytical problem

To derive full benefit from the introduction of auto-

Table 1. Organization of summer school

Form of teaching	Time allocated	Instructors
Lectures	16 × 45 min = 12 hr	H. Bartels, Discrete analysers D. Betteridge, Microprocessors G. K. E. Copeland, Tobacco smoke problem D. R. Deans, GLC M. B. Denton, Spectrochemical methods, Future of automation J. K. Foreman, Management E. H. Hansen, Flow injection analysis F. L. Mitchell, Introduction to automation, New techniques in clinical analysis H. L. Pardue, New electronics, Kinetics D. G. Porter, Make or buy J. Ruzicka, Flow injection analysis P. G. Sanders, Clinical P. B. Stockwell, Computerization or automation?
Tutorials	(a) Course problem 3 × 45 min (b) Specialist 4 × 45 min Total 5¼ hr	Lecturers and T. Alliston, B. Karlberg, J. Scott, J. M. Skinner, J. Telford
Practicals	(a) Fixed exercises 4 × 1½ hr (b) Free choice 2 × 1 hr + Wed. afternoon Total = (6 + 3) hr	Beckman, Bifok, Chem Lab, C.I. Electronics, E.D.T. Mettler, Phase Separations, Pye, Spectra Physics, Technicon, Varian, Vickers, Laboratory of the Government Chemist, ICI Petrochemicals

matic equipment it is essential that proper consideration is given, at the outset, to specifying the analytical requirement in sufficient detail to ensure that the characteristics of the equipment installed match the analytical needs as closely as possible. This is a major new role for the analytical chemist and one for which laboratory management, manufacturers, and educational institutions must develop, on a continuing basis, principles for guidance. Specification is of fundamental importance where automatic analysers are to be designed and constructed rather than purchased from commercial suppliers. Over-design is expensive and time-consuming; under-design can lead to the inability of the product to meet the full analytical requirement, a frustration which often cannot easily be eliminated by later modifications. Specification includes the chemical procedure to be used. Direct conversion of a manual method for automatic operation may not always prove satisfactory, especially if the manual method includes steps, such as precipitation, which do not conveniently lend themselves to automation. In such instances it is often preferable to modify the method so that the full economic and scientific benefits of automation can be incorporated. Considerable advantages can be gained if such a study is undertaken, even if the result is that new manual procedures are introduced rather than automatic ones.

If proper attention is given to calibration and standardization a well designed, constructed and maintained automatic analyser will operate reproducibly over long periods in the hands of a trained operator.

In recent years a large number of experienced practising analytical chemists have witnessed the introduction of automatic techniques into their laboratories; indeed, many have helped to initiate the changeover in the interest of the efficiency of the laboratory. As the dependence on manual methods of analysis is reduced, the analyst becomes conscious that the demand for his manipulative skill and experimental judgement is being eroded. At first sight his role appears to have been downgraded in that his accumulated skill and expertise are being replaced by a requirement merely to operate an instrument which is, unlike himself, largely incapable of responsive judgement. He may also feel less able to defend the results produced by the automatic instrument with the same personal conviction that he can apply to his manual work. Closer examination reveals that the analyst's function has not been downgraded; in a laboratory committed to automatic analysis he holds a central and indispensible role, albeit very different in character from his previous one, but the revised role may require some adaptation and change in attitude, notably in taking a much broader view of analysis than hitherto. The analytical chemist retains ultimate responsibility for the status and quality of the results produced by the machine, and it is imperative that, with the full co-operation of the manufacturer or in-house designer, he acquires an understanding of the principle and operation of the machine to utilize its strengths and be aware of its shortcomings. He will come to appreciate that, if the instrument performance has been correctly specified and achieved then the reliability of the chemistry of the method may be the limiting factor in the quality of the results. Thus the analyst has not conceded responsibility for the methods used; indeed he can exploit his analytical knowledge to modify the chemistry to make it more compatible with the instrumental facility and more resilient to minor variations in sample composition, pH *etc.* in order to offset any lack of responsiveness of the treatment to such changes.

The analyst indeed gains an additional responsibility, that of specifying the requirements for, and performance of, any new automatic equipment to be purchased or constructed, with particular reference to stages or parameters where close control is critical. This is, in general, a new and unfamiliar task for the analytical chemist, and he can only discharge it effectively with the active collaboration of colleagues experienced in other relevant scientific disciplines. The alternative, which can be fraught with pitfalls, is to accept manufacturers' literature at face value. The importance of devising a proper specification for the analytical requirement cannot be over-stressed. It is essential, however, to see clearly the introduction of automation in its widest sense and to consider the analytical calculating and reporting procedures from the outset. The analytical requirements must be clearly defined and may not be the same as those of the corresponding manual procedure.

In summary, the introduction of automation into an analytical laboratory leads the analytical chemist away from experimental individualism towards a managerial team-consciousness in which the origin of the samples and the requirements of their originator explicitly assume an importance equal to the analysis itself.

Figure 1 attempts to illustrate the various facets of designing and developing an automatic analytical system. From this it can be seen that the overall solution is most likely to be a compromise between chemistry and both computer hardware and software. It can be seen that for any particular problem all the necessary technology could be purchased directly from a commercial company. Equally all the development could be done "in house", although the most realistic solution is likely to be a hybrid of commercial modules and software and home-made devices, with some of the software modified to suit the particular needs of the laboratory. The role of the laboratory within the organization of which it is a part has an overriding influence on the specification. Whilst the specification and production of a device is a multidisciplinary task, the analyst has a vital role and he alone can properly define the constraints, be they chemical, statutory or legal. In-house construction should not be undertaken lightly and Porter and Stockwell[6] and Carlyle[7] have discussed these problems in some depth.

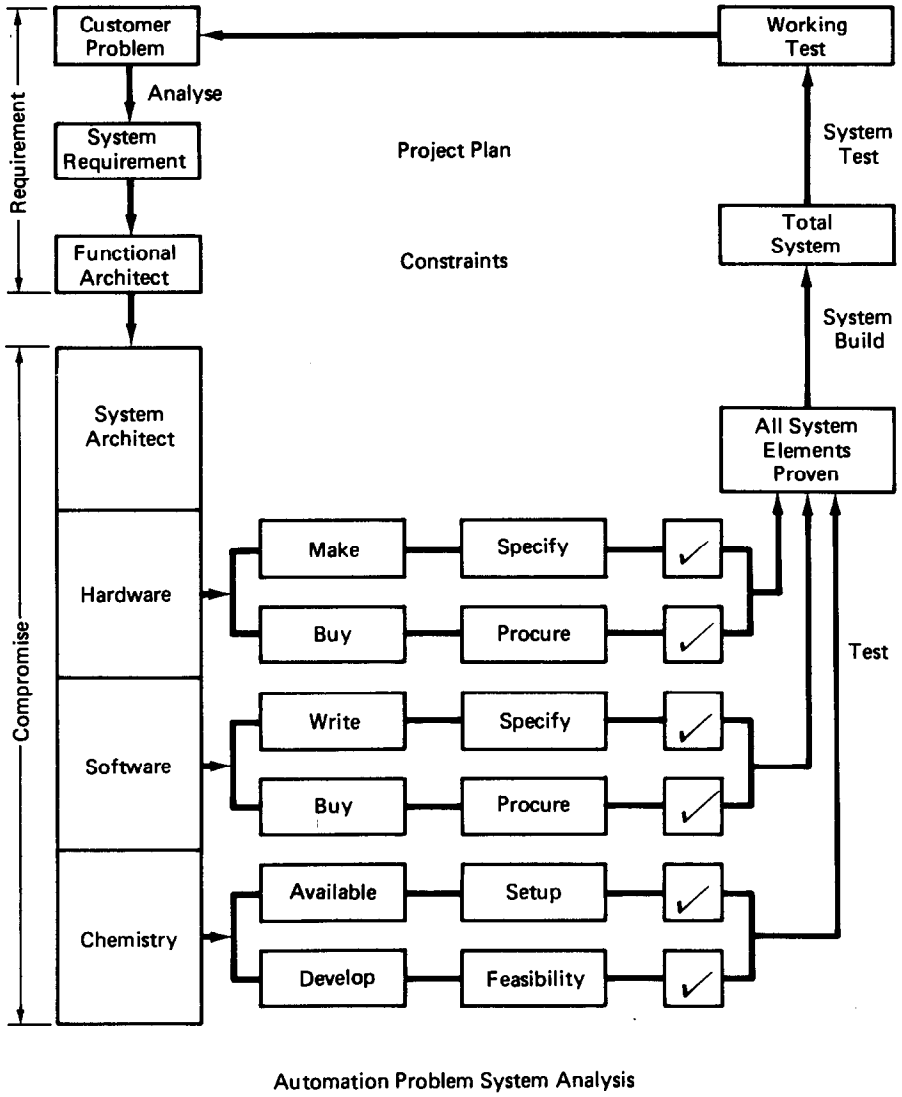

Fig. 1. A systematic approach to solving problems of laboratory automation.

Communications

From the above it is obvious that a systems designer for laboratory automation must be conversant with and able to utilize a whole range of disciplines, from chemistry to electronics and from computer software to statistics and instrument design. The user of the automation, his manager and the customer for the analytical results also have a contribution to make in deciding the specification. Very often the user with little or no experience of automation defines his requirements in terms he can understand. This is often not the best approach. The technical literature now more than ever presents the view that existing instrumentation can and does solve any problem the user might have. At the specification stage it is necessary to establish what are the needs for the instrumentation and the solution to the problem will then be a compromise between the economic and scientific requirements. The solution will also attempt to make use of the technology and resources available to the organization. Unfortunately, there is a problem of communication, because every discipline, despite the apparent belief that only computing suffers from this problem, has its own jargon behind which the specialists hide. This represents a barrier to the chemist. However, given a willingness to overcome this problem, it is quite possible to make an assessment of the abilities of each technique. As yet there are few books which attempt to cover the area of automatic chemistry, but Stockwell and Foreman have founded a series of books on automatic chemistry, published by Horwood at Chichester.[8] Technical papers which relate to automation have most often been presented in analytical journals dealing with the basic problems, and even then the details of the automation are often less well documented than the chemistry. Aspects of management, education and economics are given scant treatment despite their importance. Recently the *Jour-*

nal of Automatic Chemistry has been launched to fill this void in the literature, and initial experience has shown that there is a wealth of technical experience awaiting publication.

DEVELOPMENTS IN THE PHILOSOPHY OF AUTOMATION

The overriding benefits of automation are economic in nature. The rapid introduction of automation into clinical laboratories and the large market open to potential manufacturers have prompted many developments in instrumentation. Far less consideration has been given to the philosophical approaches to the subject. Until recently these could be considered in three categories: (*a*) the approach in which methodologies are adapted to the techniques available such as in continuous flow analysis, (*b*) a total systems approach such as that developed at the Laboratory of the Government Chemist, which attempts to define the total analytical requirement, including transmission of the result to the customer and which uses available techniques to solve the problem in the most effective manner,[9] and (*c*) the approach described by Arndt and Werder[2] in which a systems study is made of the available manual procedures, which are then broken down into a number of steps, a complete rationalization of which then generates a flexible range of equipment designs to meet the varied needs of an analytical laboratory. While the first approach typifies that followed by the majority of the instrument companies, the second is obviously the province of the systems designer working in a multidisciplinary laboratory. The third approach offers a fresh and encouraging input from one of the world's major instrument companies embarking on an automation programme in concert with an industrial chemical company already committed to the principles and benefits of automating their work pattern.

Many of the developments in automatic chemistry, including the introduction of continuous flow analysis and radio immunoassay, have evolved from the clinical environment. Another exciting development, which offers a fourth approach to automation, is that of thin-layer or solid-phase chemistry.[10] The chemistry is carried out on dry-to-touch, separate but interacting layers on a supporting membrane and has been described by Curme *et al.*[11] and Spayd *et al.*[12] The technology developed for photographic processes has been perfected to such a pitch that up to 16 different layers can be produced with a high degree of precision. Although photographic principles are not directly involved they have been transferred to the analysis of blood and serum. The details of the technique are discussed in later sections of this paper, but in principle a small section of film, termed a chip, is used to transport the sample and to perform the essential chemistry through a series of layers designed for the appropriate analysis. Photographic film of high quality has been in production for a number of years and the precision shown for the thin-layer approach to clinical applications can be attributed to the experience of first black and white and then colour photography.

The relative merits of the first three philosophies have been described by Stockwell[13] whereas the potential of the fourth can be seen from a brief consideration of the detail of the technique. In clinical chemistry a new concept requires a considerable time for complete and thorough evaluation before its acceptance into routine use. The Du Pont aca system, which first introduced prepackaged reagent analysers in the late 1960s, is finding increasing acceptance but only after a hesitant start to its use. The solid-phase approach, as developed by Eastman Kodak, is in the early stages of detailed evaluation in the USA and Europe.

AUTOMATIC INSTRUMENTATION—RECENT DEVELOPMENTS IN TECHNIQUES AND INSTRUMENTATION

Besides understanding the philosophy and concepts of automatic instrumentation it is necessary to have a clear economic assessment of the advantages to be gained from the introduction of automation. There can be no substitute for practical experience in solving such problems. The field is wide and in this paper a complete review is not practical. A range of recent developments is reviewed, however, in the hope of stimulating the reader into deeper research to find the best solution to the particular problems in hand. It is of considerable importance to evaluate the various developments that have become available through both large and small instrumentation companies.

The DACOS analyser

The concepts of centrifugal analysers were first outlined by Anderson[14] in his description of the GEM-SAEC (General Medical Sciences—Atomic Energy Commission) analyser. Samples and reagents are added near the centre of a centrifuge rotor, and on rotation all move to and are mixed in cuvettes at the periphery where the chemical reaction proceeds and the cuvettes are monitored as they cut a light beam. Mixing occurs simultaneously and quickly, and readings of absorbance are obtained during each revolution, which makes the approach highly suitable for kinetic assays. The one major disadvantage is that though several analyses are run in parallel, the system is discontinuous and the rotor has to be stopped for reloading; it is also difficult to carry out more than one type of assay in a rotor at any one time. This problem has been overcome in the DACOS approach (Discrete Analyser with Continuous Optical Scanning) described by Snook *et al.*[15] In this approach the reaction tubes are situated at the periphery of the rotor, which turns in discrete steps, a few degrees at a time, to enable tubes to be loaded with sample and subsequently washed when measurements are com-

plete. Instead of the tubes rotating rapidly through the light-beam, itself rotates on the same axis as the rotor. The signal pattern from this type of analysis is therefore identical to that from the conventional centrifugal analysis approach. Long reaction times can be accommodated either by slowing the motion of the rotor or by suppressing the washing, sample and reagent addition phases for one or more cycles; the scanning speed can also be varied within wide limits.

Figure 2 shows the general arrangement of the absorptiometer with 100 optical cuvettes radially dispersed around the vertical axis of the reaction rotor. The optical components of the dual-channel monochromator are rigidly mounted within a machined aluminium housing (which is not shown in the diagram), and the housing itself is solidly fixed to the top of the rotor.

The precision optical shaft encoder accurately relates the signals from the photomultiplier with the appropriate cuvettes. The shaft encoder tube, which contains the reflecting prism, is driven by a small synchronous motor and associated gear train, which provides the rotation of the scanning beam. The photomultiplier and the collecting lenses of the detector optics are also mounted rigidly on the rotor, so that the relative positions of all the optical components, except for the rotating reflecting prisms, are rigidly fixed with respect to each other.

The cuvette-cleaning, sample-transfer and reagent-addition mechanism remains static relative to the main-frame of the equipment. The analytical rotor is moved through 3.6° with respect to the equipment main-frame every 24 sec. Since the rotor carries within itself the continuously scanning beam of the absorptiometer, another monitoring device is required to define the scanning sector, and to relate the effect of the double movement of the beam to the main-frame. This is achieved by fitting a light-emitting diode (LED) to the end of the arm A, which is fixed to the rotating encoder tube T. A light-shield (not shown), drilled with 100 holes on its circumference, covers this rotating miniature light-source, and the passage of the light behind the 100 holes is monitored by two photodetectors fixed to the equipment main-frame. The detectors are not adjustable and always define the maximum reading sector. This low-resolution encoder provides the interrupts necessary to define the START and END of the reading sector. The computer software routines recognize these interrupts and relate the data to the appropriate cuvettes, thus avoiding any mismatching of the data recorded.

The flexibility required to handle procedures where a lag phase is needed or where reagents must be added, is incorporated into the software routines. In this manner, any data points which do not meet the specified criteria for the analysis are ignored. Stepping of the reaction rotor to receive a new sample is achieved by a simple rim drive arrangement which is activated when the scanning beam leaves the reading sector on the 12th scan.

Because of the large yield of analytical data it is necessary to integrate a computer into the overall design, chiefly for data-handling but also in part to function as a process controller. Although Snook *et al.*[15] used a Texas 960A computer, microcomputer systems would also be suitable.

The classification of kinetic methods proposed by Pardue[16] is adopted in the software philosophy. The

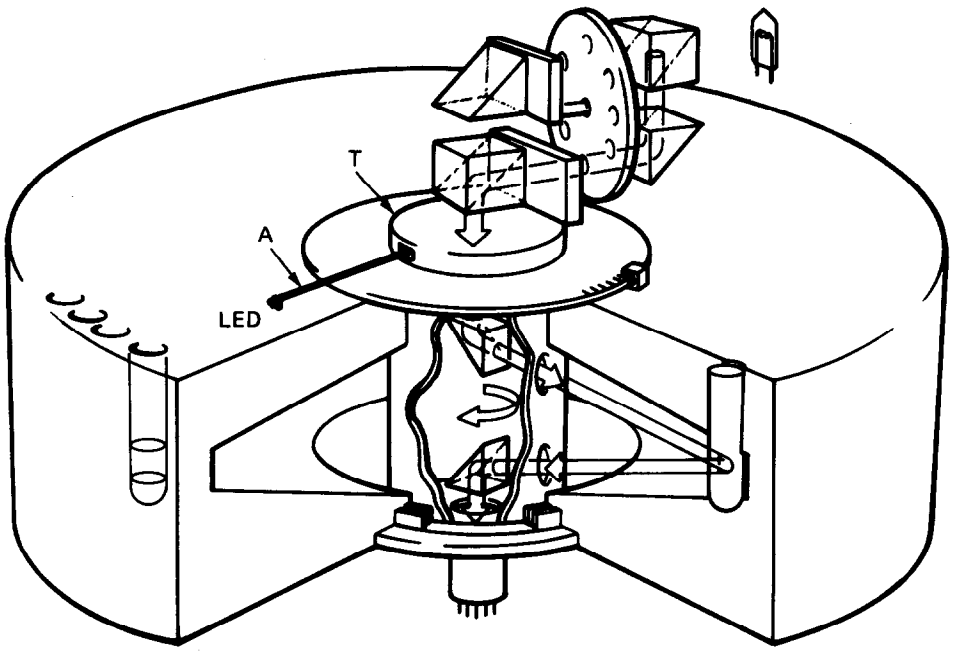

Fig. 2. Schematic diagram of discrete analyser with continuous optical scanning[15] (reprinted from *J. Automatic Chem.*, 1979, **1**, 72, by permission of the copyright holder, United Trade Press Ltd, London).

defined objective of measurement in this system is to obtain the best regression fit to a minimum of 10 data points, taken over either a fixed time (*i.e.*, the maximum time for slow reactions) or variable time (for reactions complete in less than 34 min, which is the maximum practical observation time). In an analytical system generating information at the rate of 50 datum points per second, with reactions being monitored for up to 2040 sec, effective data-reduction is of prime importance. To reduce this large quantity of analytical data to more manageable proportions an algorithm was devised which optimizes the time-base of the measurements for each individual specimen.

The calculation routines for the regression slope and its standard error are time-shared with the data-acquisition before the cuvette is cleaned ready to receive a new sample at the solution-transfer position. When the scanning beam leaves the reading sector other computer-controlled tasks begin. The printing of results is also time-shared with the mathematics packages. The system is very flexible and overcomes many of the objections to other conventional centrifugal analysers; it can monitor reactions which take place over times ranging from 18 sec to 45 min. The principles embodied in the system described by Snook *et al.*[15] will be incorporated into a new instrument currently being developed by Coulter Scientific Inc., Florida, USA.

Automated single-test analysis system

The approach to laboratory automation described by Arndt and Werder[2] involved close co-operation of a commercial instrument company with a laboratory attached to a large industrial chemical organization. The analytical work-load in industrial wet-chemistry laboratories is characterized by a multitude of procedures and methods as well as small numbers of samples in serial runs of analyses. This has been one of the reasons for resistance to automation. A new approach was needed and this has emerged as a modular subdivision of manual analytical procedures into basic operations. These are assigned execution parameters that determine the detailed operation. From this it is possible to derive a conceptual design of an automatic analysis system with which it is possible to run individual samples and small and large series of samples. Each sample is contained in its own cup, the automated units are self-cleaning, and existing analytical procedures may be used. A complete range of instruments based on these principles is available from Mettler AG, and instruments are easily tailored to suit an individual laboratory's requirements. Control is either by desk calculator or for more sophisticated requirements by use of a system of computers to control individual modules and co-ordinate overall control and reporting.

This approach represents a significant advance on the philosophy adopted by instrument manufacturers. Once the utility of the approach has been proved in industrial laboratories it will be interesting to see how far the philosophy will develop and solve problems directly rather than by simply mimicking manual procedures.

The instrumentation developed employs six types of elemental units for (*a*) automation of the basic operations, (*b*) sample transport, (*c*) central control, (*d*) the entry and weighing station, and (*e*) the output for results and logistics. These are discussed briefly here.

The heart of the system comprises the automatic units that carry out the basic operations. The individual steps of the operations are specified in a set of execution parameters which are controlled and monitored by a microprocessor which also calculates the basic results. Apart from monitoring all operations for correct action, it is necessary to avoid the generation of incorrect analytical results and to signal the malfunctioning of any subsystem. The machine operation should allow the state of any procedure or module to be readily and easily visible.

The sample-transport mechanism is the physical link between the units for the basic operations and it moves the sample cups to the entry ports. The sample identification system ensures that samples are available to the appropriate unit at the right time. The mechanism functions like a railway system; it receives a command to move a cup containing a standard volume of sample from one place to another and then waits for the next instruction, which may require transport of the next sample cup or of the same sample to a different module.

Whereas the microprocessor controls an individual basic operation, the central computer, which has all the analytical procedures held in its memory, controls the particular analytical procedure required. At the appropriate time the central computer transmits the appropriate set of parameters to the corresponding units and provides the schedule for the sample-transport operation. All units are monitored to ensure proper functioning. If any of the units signals an error, a predetermined action such as disposing of the sample is taken. The basic results from the units are transferred to the central computer, the final results are calculated, and the report is passed to the output terminal. These results can also be transmitted to other data-processing equipment for administrative or management purposes. The central control is, therefore, the leading element in a hierarchy of processors. Figure 3 shows a schematic diagram of the system. The configuration of any particular system can be tailored to individual laboratory requirements. The entry and weighing station is the most important interface between man and machine. Brief comments arising from visual inspection of samples may be entered, and these will appear with the analytical results.

A sample of specified weight is normally required in the procedure. An interactive form of weighing is used which indicates on the display or printing unit of the entry station whether or not the sample has been

accepted. Before analysis it is necessary to specify the code number of the analytical method that is to be used, and to store this in the memory of the central control. To indicate where samples are located it is necessary to identify them before weighing. Optical readers are therefore mounted on the sample-transport mechanism to register each sample. The sample is identified by a unique code placed on the outside of the sample cup.

After all the primary input data have been entered by the operator the sample is placed on the transport mechanism. Once initiated, the remaining steps are carried out completely automatically.

The basic results from the individual units are processed and then combined to form the final result which is produced on the report printer. Results that deviate from an expected value by more than a preset tolerance may be marked or commented on. Additional information such as sample identification and origin is also made available. To ensure complete control by the analyst, the basic raw results may also be recorded in analogue form. Sample identification is provided so that the data can be re-analysed.

Fully automatic systems require careful monitoring of the supply of reagents and the disposal of waste chemicals. To achieve this, fluid levels are monitored, and if they are low, an alarm signal is issued to the operator.

The whole system with its internal and external interfaces is designed so that it can be adapted to the needs of any particular analytical wet-chemistry laboratory, and so that responsibility for the analysis can be assigned to the operator. The system can be arranged in many ways and one of these, relating to the SR10 systems titrator, is shown in Fig. 4. The samples enter the unit at the entry station. Next the sample carrier moves the sample to the dispensing station where solvents or reagents are dispensed. The treated sample is then moved to one of four working stations, each of which can be fitted with a combination of a measuring and a reference electrode; it is also possible to attach a sensor for photometric titration. Up to six burette tips may be inserted at each working station.

Before the sample is moved to the desired working station the burette tips and the electrodes are rinsed, and the burette tips are primed to provide fresh solution. A washing cup that contains the conditioning solution for the electrodes while they are not in use is lowered and replaced by the sample cup.

The titration cycle comprises first a homogenizing period which allows dissolution of solid samples, flushing with an inert gas, or application of a chemical reaction. The sample may also be heated to a predetermined temperature. Next, a precise volume of a reagent or reagents is dispensed if required. While the sample is being stirred a titration can be performed, either to a relative or an absolute end-point, or incrementally with or without equilibrium detection. Several titration modes are available, including potentiometric, amperometric, voltammetric and spectrophotometric.

The titration cycle, like most of the other functions, may be repeated at will. Back-titrations are therefore possible as well as multiple titrations for multi-component analyses. At the end of the cycle the sample is returned to the sample transport. All dispensing is from a multi-burette system with up to 20 dispensing assemblies each with a total delivery volume of 10 or 20 ml.

A desk calculator, Hewlett Packard model 9815A, which uses a magnetic tape cartridge for storage of analytical procedures and programs is the control computer in this application. The procedures are entered and stored in a conversational manner. The software of the desk calculator transmits the execution parameters to the titrator, accepts raw data, and calculates the final result. The analytical procedure to be used, with any auxiliary data, is first specified on the keyboard, then the sample is weighed (if necessary) and put on the transport; the rest of the operation is automatic. The four working stations permit configuration of the system for four different types of titration requiring different electrode combinations

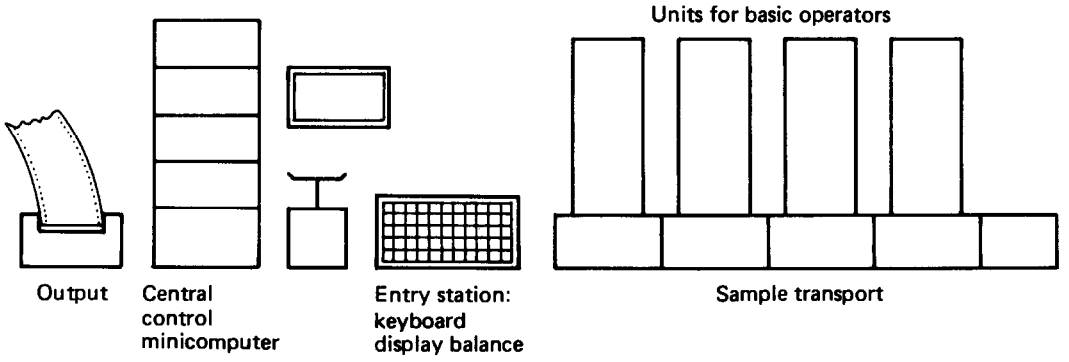

Fig. 3. Schematic representation of a system with several units for automation of basic operations, controlled by a mini-computer (reprinted from P. B. Stockwell and J. K. Foreman, *Topics in Automatic Chemical Analysis*, by permission of the copyright holder, Ellis Horwood Ltd., Chichester).

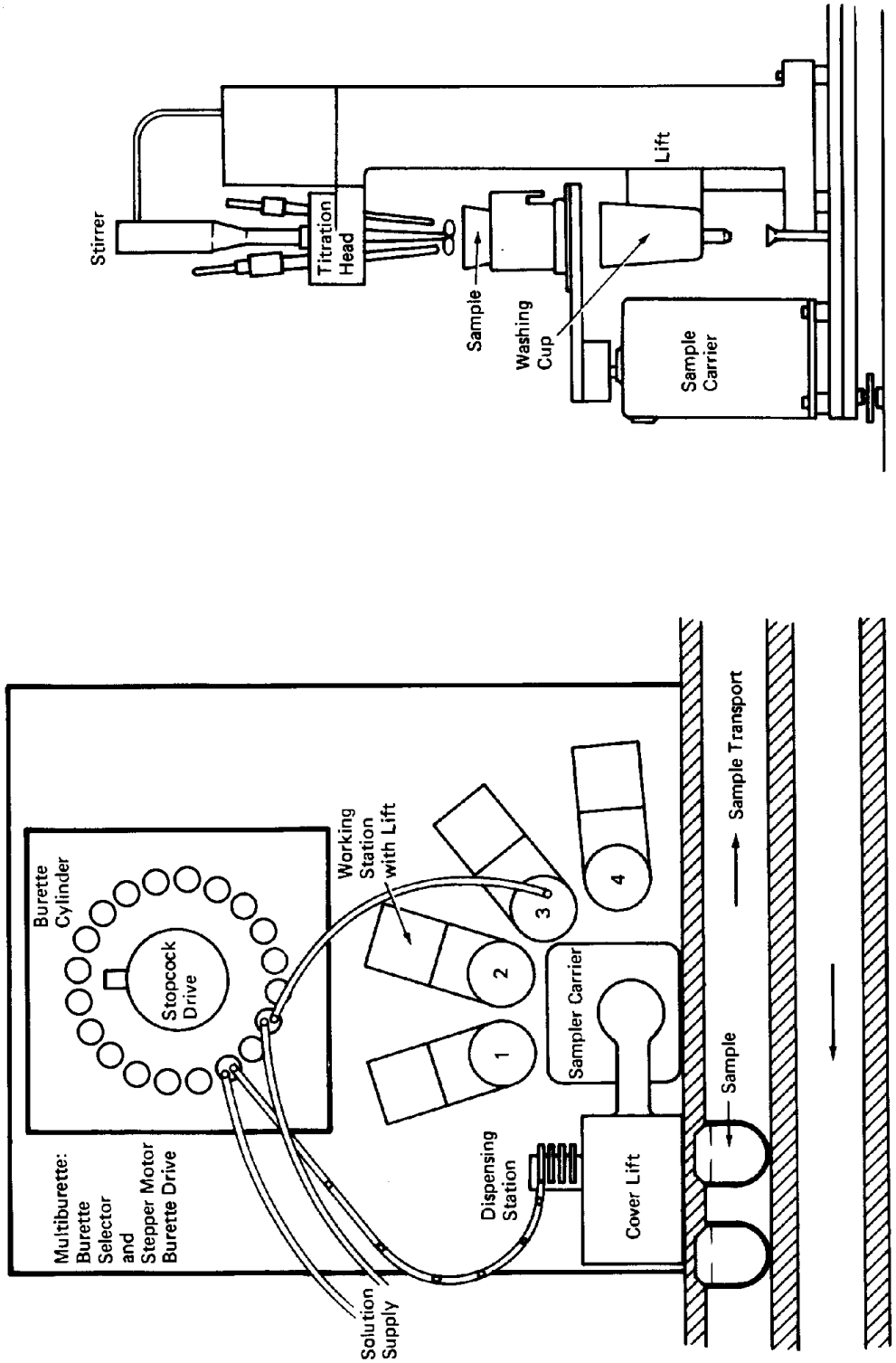

Fig. 4. (*a*) SR 10 System Titrator: arrangement of mechanical unit; (*b*) SR 10 System Titrator; working station with lift (reprinted from P. B. Stockwell and J. K. Foreman, *Topics in Automatic Chemical Analysis*, by permission of the copyright holder, Ellis Horwood Ltd., Chichester).

and reagents. The universal design permits performance of a range of functions which are specified by the user and depend on the hardware configuration (electrodes, reagents, amplifiers, options *etc.*) and the assay methods stored in the software. The accuracy of results, chemistry permitting, is better than 0.1%. Transfer of a manual process to the automatic regime takes only 1–2 hr. Whilst this approach to laboratory automation is expensive, an organization with a large number of titration tasks can easily justify the investment, *i.e.*, it can amortize the instrument over 3 or 4 years. The manufacturers claim that the device can operate at the combined speed of up to ten skilled analysts.

The DuPont "aca"

It seems at first sight foolish to include in the area of recent developments a description of an instrument first introduced in 1968. But the aca analyser is unique in design and despite a chequered beginning it has now become firmly established in the market place. Recent developments in the aca in terms of automation and indeed computerization have ensured that current technology is included in its design.

DuPont claim that the aca can be used immediately from start-up, zero time is required for changeover from one test to another, and single tests can be run. Its output rate is determined by the number of tests requested rather than by the number of specimens entered. In fact, the same time is taken for a single test on ten specimens as for ten tests on the same specimen. Any number of tests from a set of 30 rather complex wet-chemistry procedures can be chosen. Some of them are unusual and are peculiar to this instrument. Almost 100 different reagents are used in these tests, but a reagent delivery system is not required. A single pump delivers the samples and the wash-out or flushing solutions, selected from a range of six solutions by use of a simple valve sequence. Once the sample has been dispensed into the disposable reaction chamber it is sealed from atmospheric contamination up to and including the measurement stage. Subsequent reagents can be released at will within the enclosure as they are pre-pipetted into temporarily sealed pouches.

The concept utilized in the aca, *i.e.*, prepackaged chemistry, makes the instrument reasonably rugged and allows it to be used as a standby instrument for urgently required analyses. There are few moving parts and they need move only very slowly. Many of the problems commonly experienced in automatic instruments on this scale, such as pump and tube priming, leakages, reagent deterioration and contamination are largely eliminated. Also, the storage and handling of the reagents is greatly simplified. The instrument produces approximately 80 analytical results per hour, and this rate is maintained throughout the day because minimal down-time is required to ensure full operation. These features of the aca are often attractive to the clinical laboratory, and many have been installed to back up other instruments that have higher output but are less reliable. The aca has not been without problems of its own. In practice it requires an initial start-up time, and in the original designs the electronics were prone to drift and required frequent readjustment. There is no facility, in the range of determinations offered, to measure alkali-metal ions. After the deproteination and centrifugation step, serum and plasma samples must be decanted manually into the sample containers. The aca should not be handled by unskilled workers, but Du Pont operate a continuous and comprehensive training programme.

The instrument has unique qualities, but the chemical methods and principles used often differ widely from accepted manual or automatic methods. This makes it difficult to provide a proper evaluation of the processes and, of course, raises doubts in users' minds. Also, the methodology is not under the control of the working clinical chemist, who is dependent on the manufacturer not only for provision of adequate quality-control of the reagents used and the results obtained, but also for method development. The assumption that quality-control results are reasonably representative of results obtained on samples from patients is partly invalidated because synthetic samples are used for the control-tests. Such a situation would be intolerable without full co-operation between the manufacturer and clinical organizations, particularly as the philosophy adopted becomes more widely used.

The third generation of this analyser, the aca III was recently introduced into Europe at the 3rd Clinical Congress at Brighton. The instrument, along with its predecessors, provides a 24-hr availability, rapid test report, and patient identification, and it allows individual access to tests and samples. There are 35 different procedures currently available. The aca III incorporates additional features such as computer-assisted calibration, automatic instrument-standardization, and an inbuilt fault diagnosis program, and it comes complete with alpha numeric keyboard and display. The introduction of microprocessor technology has greatly increased the operator efficiency and the aca III capacity can be further expanded as methods become available to provide up to a maximum of 60 test measurements. The instrument can also communicate to any on-line laboratory data-system for further data-analysis. An additional advantage of the expandability offered is that current aca II models can be upgraded to provide the aca III capabilities. DuPont should be especially congratulated on this fact, and for keeping faith with existing customers.

Infrared reflectance spectroscopy

Applications of near infrared reflectance spectroscopy were first introduced by Norris[17] for the determination of moisture, oil and fat in cereal products. A number of instrument companies, notably Techni-

con, have developed commercial instruments. The instrument replaces a series of chemical procedures by a signal measurement in each of six infrared regions and reference to a suitable computer calibration. Such an approach offers considerable advantages and is novel. It is made further attractive by the availability of microprocessor computing power. Whilst the instrumentation has been specifically developed for the cereal market, it has much wider applications, for example to the tobacco industry. It does, however, suffer from some disadvantages. The instrument must be calibrated against a suitably accurate standard method, and unfortunately few chemical methods are available. Also, the six wavelengths were selected for the prime objective of wheat analysis, and are not the most appropriate for other types of analyses. On the other hand, one of the major advantages of the technique is that the analysis can be done on site, away from the laboratory.

Evaluation and use of the first generation of reflectance infrared instruments has indicated the necessary specification for an instrument with more flexibility. The requirements are as follows.

1. Fast, simple, flexible sample-handling.
2. Simple to operate, standardize and calibrate.
3. Capability to analyse many different products and for many constituents.
4. Ability to operate safely and accurately over wide-ranging environmental conditions.
5. High accuracy in detecting energy reflected from the sample.
6. Insensitivity to variable sample matrix effects such as particle-size variations.
7. Measurement at several specific wavelengths with narrow bandwidth to remove interferences and provide highest accuracy for the constituent measured.
8. Ability to adapt to new advances in technology in optical measurement, signal processing and data treatment.
9. Capability for rapid, easy diagnosis and correction of instrument malfunctions.

The Technicon InfraAlyser 400 has been designed to meet these requirements and is a good example of how, by the introduction of current technology, a flexible instrument results. In addition, an integrating-sphere detection system has been incorporated into the design, which, coupled with Kohler optics (as used in microscopy), allows uniform sample-illumination and good signal-to-noise characteristics. The new instrument is also more independent of sample particle-size and temperature. An integral sealed gold standard is fitted which ensures minimal drift and allows absolute readings of reflectance data to be made. Up to 20 different wavelength detectors with narrow bandwidths are available, including one for diagnostic purposes. The incorporation of microprocessor technology allows such facilities as self-calibration and the introduction of self-teaching aids. The computer system is also fully expandable to cater for a range of applications. Applications in wheat, dairy, animal feed, tobacco, and cocoa analysis and many other areas are under evaluation. Certainly some of the major obstacles to the application of the technique to a wider range of samples, especially the inflexibility, have been overcome by this new instrument.

Thin-film or solid-phase chemistry

Thin-film or solid-phase chemistry has been introduced recently by Eastman Kodak. A brief explanation of the technique was given above. The salient principles of the technique can be seen by reference to the determination of urea in serum; Fig. 5 shows a schematic diagram of the "chip" used and also illus-

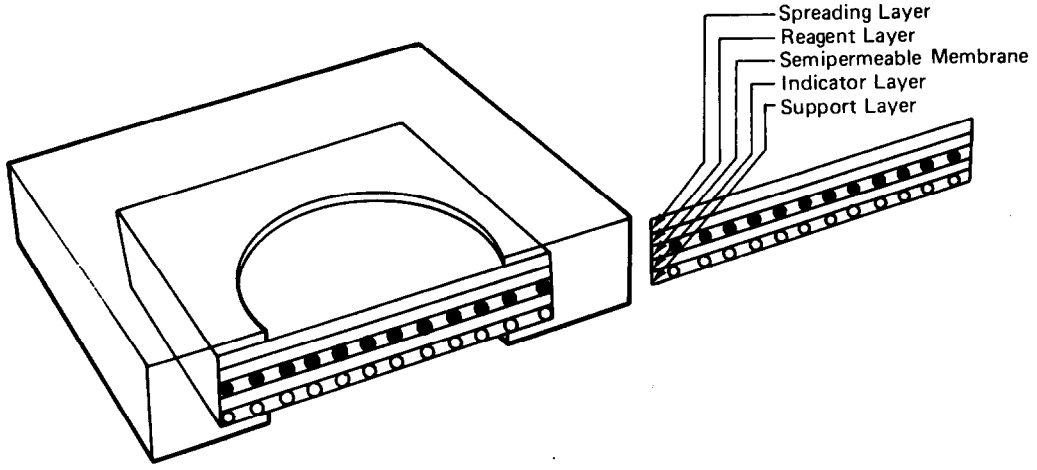

Fig. 5. Schematic diagram of the Eastman Kodak slide for solid-phase or thin-film chemistry, illustrating the chemistry integrated into the multilayers[18] (reprinted from *J. Automatic Chem.*, 1979, **1**, 273, by permission of the copyright holder, United Trade Press Ltd, London).

trates the chemistry integrated into the multilayers. The spreading layer is an isotropically porous non-fibrous layer, 80% of which is void, and has a mean pore size of 1.5 μm. The spreading and metering action, which is aided by surfactants, compensates for any differences in sample size (10 μl applied) and serum viscosity. A constant volume per unit area is then naturally applied to subsequent layers of the chip. High molecular-weight materials such as protein are removed by this layer and consequently do not interfere with the subsequent analysis. Titanium oxide is incorporated in the spreading layer to improve its reflectivity. It also acts as a white background for reflectance measurements of the colour density produced in the reagent layer. The reactant layer contains the urease which catalyses the hydrolysis of urea in the sample to produce ammonia. Water from the added serum swells the gel, allowing the urease to diffuse into the layer. The layer is buffered to pH 7.8, which maintains the ammonia at a low level and consequently extends the range of the assay. A third layer consisting of cellulose acetate butyrate with selective permeability allows non-ionic materials such as ammonia and water to pass through to the indicator layer. Ionic compounds are excluded and this provides some degree of selectivity. The indicator layer consists of a gel binder incorporating the indicator reagent, which in this example is *N*-propyl-4-(2,6-dinitro-4-chlorobenzyl)quinolinium ethane sulphonate. The free ammonia diffusing into this layer reacts with the indicator to form a dye which has a molar absorptivity of approximately 5000 $l.mole^{-1}.cm^{-1}$ and a broad absorption peak at 520 nm. The reflectance density is measured from the peak at 670 nm. The final layer is a clear polyester support upon which all the other layers are coated. It is transparent and allows measurement of the colour intensity of the compound formed in the indicator layer.

Performance trials and evaluation tests on the technique indicate that it is both reliable and accurate and in addition the specificity is sufficient to cope with most clinical requirements. A recent evaluation was made by Haeckel *et al.*[18] If this approach is successful, the use of the dispensers and tubes common in laboratories will become redundant. It may well become possible for the clinical test to be undertaken close to the patient rather than in the laboratory. Whilst the techniques have as yet only been applied to clinical applications, there are many other potential applications, for example in the water industry. However the very nature of the technique necessitates development by Eastman Kodak. Very few users will be able to influence the choice of analytical problems to be tackled by this unique approach.

Imaging detectors

One of the significant instrumental developments in recent years has been the widespread adaptation of imaging detectors for analytical spectroscopy. An imaging detector is a device that is capable of measuring different segments of an optical image simultaneously and providing electrical signals that are related to the intensity of the radiant energy emerging from these segments. Imaging detectors were designed originally for industrial applications, such as the generation of television pictures. The major advantage of imaging detectors in analytical spectroscopy is that they permit electronic selection of different wavelength elements.

In analytical applications a dispersed optical spectrum is focused on to the active surface of the imaging detector, and electronic circuits examine different segments of the detector independently to produce electrical signals which are related to the intensities at different wavelengths. Whether the interest is in single or multiple wavelength elements, the ability to select wavelengths electronically offers several potential advantages over conventional mechanical methods. These include improvements in the speed and precision of wavelength selection, in ease of automation, and in versatility of scanning. These advantages accrue because it is much easier to move electrons rapidly and reproducibly than it is to move mirrors, gratings, slits, phototubes, and other components.

Many different kinds of imaging detectors are in use for a variety of different analytical applications.[19] They include the unit gain devices such as solid-state diode arrays, either one- or two-dimensional, silicon vidicons, and the detectors with gain such as silicon intensified target tubes (SIT), intensified silicon intensified target tubes (ISIT), and image dissectors (ID). They have been applied to both molecular and atomic spectroscopy, as well as to mass spectrometry. Applications in molecular spectrometry include absorption, fluorescence, and raman spectroscopy for both equilibrium and kinetic analyses. They have also been used in a number of fundamental kinetic studies and as multiwavelength detectors in liquid chromatography. Atomic-spectrometry applications include absorption, emission and fluorescence spectroscopy with conventional optics and with two-dimensional echelle spectrometer optics. Among applications that have been reported are derivative spectroscopy and the simultaneous measurement of excitation and emission spectra in fluorescence applications. Chemical applications range from elemental determinations to basic studies of organic, inorganic, and biochemical reactions.

The silicon vidicon and the photodiode arrays are the most useful detectors for ultraviolet and visible region absorption spectrometry because of their relatively low cost. The silicon vidicon has an advantage in terms of linear dynamic range compared to currently available diodes, but the photodiodes appear to have the advantage of speed, less blooming, lower cost, and lower power consumption. For molecular luminescence spectrometry in the near ultraviolet and visible regions, SIT and/or ISIT detectors are the most suitable of the choices available, but they do not have a useful response at wavelengths shorter than

about 350 nm. For atomic spectrometry, the image dissector and secondary electron conduction tubes are the detectors of choice, although more detailed data are needed for the latter in order to differentiate objectively between them.

The advantages and limitations of imaging detectors applied to analytical spectrometry have been reviewed by Pardue.[20] They will not replace conventional approaches in the near future, but they will be used extensively as complementary alternatives to conventional detectors. It should be kept in mind that the detectors discussed here were specifically designed for television applications rather than for quantitative analytical applications. Accordingly, it is reasonable to expect that when the limitations of the particular devices have been documented, and a market has been established, improved imaging detectors specifically developed for analytical chemistry will be forthcoming.

One recent introduction, representing perhaps the most significant change in this area, is the charge injection device (CID) and its mode of read-out. This has been described in detail by Sachs and Howard;[21] in simple terms it extends the capability and scope of such devices. It allows a "pixel" to be monitored and the signal measured either destructively or non-destructively, which improves the signal-to-noise characteristics so that more meaningful data can be obtained over a spectral range where some absorption bands have greater amplitudes. The spectral range of these devices is also quite wide and Denton[22] predicts that devices with a usable signal throughout the ultraviolet (down to 200 nm) will soon be available.

Computer applications

With the rapid introduction of microprocessor technology into automated analytical instrumentation the analytical chemist might be misled into thinking that all his problems have been solved. Automatic instruments are advertised which purport to be the ultimate in design and to cope with all the potential user's needs. Often this is far from the truth. In reality, these instruments may have been designed with little if any appreciation of the requirements of the analytical chemist.

Microprocessor technology has been responsible for considerable discussion and awareness of applications. Whilst the hardware suitable for incorporation into instrumentation is cheap, the software required is expensive. The decision to include computer power in an instrument therefore poses a difficult problem. Because microcomputers are cheaper and have better reliability factors than the previous generation of minicomputers, the approaches to computer data-processing that can be taken have been modified. Most of the facilities offered by microcomputers had previously been available with minicomputers but the economic factor makes "stand alone" systems more attractive. The choice of computer facilities is a difficult decision because the technology is changing so rapidly, and with the introduction of such devices as the Commodore "PET" personal computer the need for centralization and computer support is often questioned. The strategy for computer use therefore needs careful consideration.

The problems of communication have been briefly discussed elsewhere but now more than ever the analyst cannot sit back and accept the instrumentation he is given: he must become acquainted with the scope of computers, be they mini or micro, particularly with respect to new technology. It is vital that analytical chemists enter into instrument design. There are complaints that computer systems are inadequate for the user's needs, but as often as not this can be attributed to the analytical chemist not being assertive or aware enough to become fully involved in specifying the needs.

Normally analytical chemists have little, if any, say in the decisions involved in computer purchase. This is wrong for a number of reasons, which are outlined below. Fundamentally, the choice of computer will reflect the role of the laboratory within an organization, the work pattern and also the type of staff working in the laboratory. In any computer system, the analyst should feel that he has complete control of the computer and he must not be restricted by the computer. The computer should be sympathetic to the user and communicate in a mode the user is happy with. Very often the problem arises that analysts are not aware of their own computer requirement, whilst the computer specialist or instrument company has an incomplete or erroneous appreciation of the analysts' special requirements. For any system to be suitable, the analyst must play an active part in the specification, and the choice of system must be made by those people who will implement and use the system once purchased.

Experience in the author's laboratory serves to illustrate some of the points discussed above. The choice of computer was the responsibility of the Automation and Computer Group, which is a multidisciplinary team comprising analysts working alongside specialist computer programmers and electronics experts. The role of the laboratory has been outlined in the Annual Report,[23] but for the majority of the tasks samples from a Government Department are sent to the Laboratory and analysed in one or more sections of the Laboratory and reports or test notes are issued. On the basis of these reports the Government Department concerned may take some action, such as collection of duty, prosecution of a trader or publication of a survey report for public information. A central computer system was specified in 1973/74, purchased in 1974 after many evaluations, and installed in 1975. In essence the role of the computer was fourfold: (1) data-acquisition from laboratory instruments, (2) data-reduction and reporting from instruments on-line and off-line, (3) batch processing of computer requirements previously carried out on a computer bureau facility and (4) data-base manage-

ment for pattern matching such as mass spectral data or for a normal management information system. A Rank Xerox computer system with 64 kbyte of core memory and 50 Mbyte of on-line disc store was chosen. The basic structure of this is shown in Fig. 6; the software provided offered a reliable, well-proven operating system. A Fortran compiler, along with the inherent flexibility of the hardware, allowed the Automation Group to develop the appropriate software for the applications envisaged.

The role of this computer system has been modified as the availability of first minicomputer and now microcomputer systems has been exploited. The system used has been rapidly expanded, and upgraded to provide up to 200 Mbyte of on-line disc store. For each computer linked to the central site specific software has to be developed for both the mini and micro computer and for the central site. For each application the software requirements are unique. Very often the coupling to the central site, whilst extending the capabilities of the instrument itself, has been necessitated mainly by the failure of commercial packages to meet the real needs of the users.

Currently the Automation and Computer Group is evaluating the future needs of the Laboratory. The specification for meeting future increasing needs is that the central computer will act as a central registry of programs and data and allow computer software to be transferred from one central computer to computers linked to it. Very little data-acquisition will be carried out on the central site; this function and instrument control will be the function of a distributed network of mini and micro computers. Networking, involving message passing rather than character transfer will be a fundamental role of the computer. The system will also require to have a rapid response to time-sharing needs, be equipped with all modern peripherals such as interactive graphics, and have a flexible and easy-to-use database management system. General packages should be available for linking to other computer systems to overcome problems with the present approach. Software requirements of course are paramount in the specification and the latest compilers such as PASCAL should also be included. The basic requirements in terms of computing have not changed a great deal, but the collection of information for data bases, the control of some instrument parameters and preliminary data-reduction have been shifted to the instrument site, providing more responsive control by the users. The need remains, however, for centralization to co-ordinate all the information from the distributed sites and to prepare the reports. Software is likely to be the most demanding task and the central service should be able to develop this in the most cost-effective manner. In all the discussions before final specification the needs of the analysts are in the forefront.

In instrumentation the role of microprocessors will become increasingly important and the analyst must be involved in their proper and correct implementation. Currently, many developments are simply add-on modules developed to show how up-to-date the researcher is rather than as properly integrated

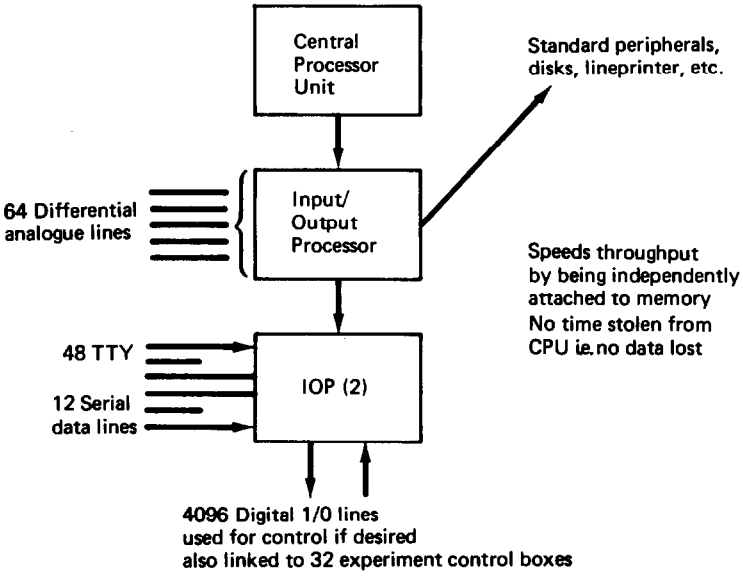

Fig. 6. Schematic diagram of the computer system required to meet the needs of the Laboratory of the Government Chemist.

systems which need microprocessor technology to exploit the scope of the instrument to greatest advantage. New technology can be profitable when it introduces increased flexibility into instrumentation, for example extending its range of sample input or the manner in which it can be used. Increased trouble-shooting and self-maintenance facilities are important, as is self-calibration and standardization. One of the great advantages of microcomputers is their inherent ability to communicate and this must be exploited to the full. This fact was not at first recognized by instrument companies. The first Hewlett Packard gas chromatographs to incorporate microprocessors were difficult to link to other computer systems. However, this situation has happily been rectified in subsequent models and the latest range is fully expandable on a modular basis. Very often instruments are described as being "intelligent" but this is a misconception. The instrument company should aim for full interaction with the analyst and to provide this by co-operation. This is particularly important with respect to the decision-making processes. It is not uncommon for analysts to require data to be presented graphically, and the use of interactive graphic terminals to develop correct analysis software packages has been invaluable in the author's own laboratory. Cheaper and more readily available peripherals of this nature should greatly extend the use of computers in analytical chemistry. One other advantage of the new technology is the possibility of more suitable detection systems. One problem with chromatographic detectors for example is the lack of linearity over a large dynamic range. If microprocessors are used, this fault can be overcome by software. In addition, detectors such as charge injection detectors have many uses in spectroscopy and these detectors not only require microcomputer power but are themselves products of the "new" technology.

One major problem remains. The rapid introduction of computers into analytical chemistry and almost all other spheres of life necessitates rapid development of computer software. But software is the most expensive facet of any instrumental development. To maintain progress future research must concentrate on the development of more flexible and simple systems which are easy to use and portable from one computer to another. Denton and Tilden[24] have developed a specific language "CONVERS" for control operations and applied this to analytical instrumentation to allow rapid transfer from a manual to automatic mode. By enabling users to overcome this stumbling block to implementation, "CONVERS" allows them to design sophisticated automated systems and to promote healthy research into automatic instrumentation. Developments such as this are encouraging but more effort will be needed over the next decade.

CONCLUSION

This review attempts to highlight some of the progress made in laboratory automation, to illustrate some current trends, and to predict some future progress. It is not a complete coverage of the field. Chromatography is mentioned only in passing; development in this particular area is reviewed elsewhere.[25] Multiple column switching has become increasingly used in gas chromatography and Deans and Peterson[26] have reviewed this area. In liquid chromatography Mills *et al.*[27] have described the advantage of microprocessor control, and Burns[28] and Frei[29] have also explained the advantages of automated pre- and post-column reactions. The use of visual display units has been especially helpful in chromatography. This approach is finding increasing use in other techniques such as atomic-absorption spectroscopy. Sample preparation, however, has received very little attention, although developments by Technicon with the evaporation-to-dryness module and by DuPont with an automated clean-up module for use before HPLC analysis, represent some limited progress.

The industrial sector still lags behind the clinical area in the introduction of automation. However, the increasing demands caused by various factors such as the increasing cost of specialized staff and economic constraints on the industrial countries will act as spurs to increasing the investment in automation. To ensure that this is an effective change with positive advantages it is important to pay attention to communication and to problems of specification. A unified approach to the philosophy of automation may well be developed.

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NOISE REDUCTION IN RELAXATION KINETIC EXPERIMENTS

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Summary—The use of high-speed digital data-acquisition devices makes it possible to obtain a large number of points (>4000) for a single experiment during a short time interval. A suitable numerical treatment capable of reducing the periodic and random noise in relaxation-curves and of providing relaxation times and amplitudes of high accuracy is presented.

Relaxation techniques can provide both kinetic and thermodynamic data for chemical reactions. Among them the temperature-jump method is the most versatile and commonly used. Its application is restricted to chemical reactions with sufficiently high normal mode absorbance changes[1] and is limited by the resolution of the instrument. Although larger temperature changes would cause correspondingly greater relaxation effects, the non-linear differential equations which must be used to describe the data are difficult to solve analytically.

Our efforts, therefore, have been concentrated on improving the performance of the instrumentation by constructing a combined stopped-flow temperature-jump apparatus of high resolution[2] coupled with a Biomation 1010 transient-recorder. In addition, the noise was considerably reduced by isolation of high-voltage and detection units as well as by heavy shielding of the phototube and the capacitance-discharge units. Further reduction of the noise by electrical means would be inordinately expensive in terms of parts and labour. Instead, we describe in this paper a numerical treatment of the noise when a large amount of data is available.

Since a major component of the noise observed on the oscilloscope has a frequency of 60 Hz or multiples of this, a fast Fourier analysis in connection with a curve-fitting subroutine was developed but found to be rather time-consuming and unsatisfactory. Several other possibilities, such as Fourier transforms or convolution techniques, have been considered, but all demand long computing times which make the use of microcomputers unsatisfactory or cause too long turn-arounds with large computers.

The approach described here is based on a simple numerical integration and the application of a multi-parametric curve-fitting program[3] to the analytically integrated relaxation functions. The program is rather fast (less than 25–50 sec on the IBM 360) and provides relaxation times and amplitudes of high accuracy. On a Radio Shack TRS 80 microcomputer using Level II Basic (total cost ~ $850) the execution time is about 60 min. This can be reduced considerably if the Fortran program for TRS 80 written by Microsoft is employed. Further improvement can be achieved by means of the readily available Z-80 assembly language programming.

EXPERIMENTAL

The Biomation Model 1010 transient-recorder reads voltage data from the photo transducer with constant sampling times which range from 0.1 μsec to 500 msec and converts the readings from analogue to digital. The 2048 (or 4096 respectively) digitized data of a typical experiment are assigned integers from 0 to 1023, which can be accessed and processed by a microcomputer. The unscaled data are then numerically integrated at a selectable interval length ΔX and fitted to a three-parameter (one relaxation) process corresponding to the analytically integrated relaxation function. The same type of analysis can be applied to a two-relaxation process by using a six-parameter equation. The relaxation times and amplitudes computed in this manner have to be multiplied by the appropriate scale factors which are read from the dial settings on the Biomation.

A polarity switch on the transient-recorder allows a change from a decreasing $[A\exp(-t/\tau)]$ to a rising relaxation function $\{A[1-\exp(-t/\tau)]\}$. The entire treatment given here is based on the latter.

THEORETICAL

Numerical integration

A rather primitive, but high-speed, numerical integration is applicable to large numbers of data, $y(x)$, at unit quantization intervals. The integration over the interval ΔX reduces to simple summation:

$$J(X) = \sum_{x=X}^{X+\Delta X} y(x) \qquad (1)$$

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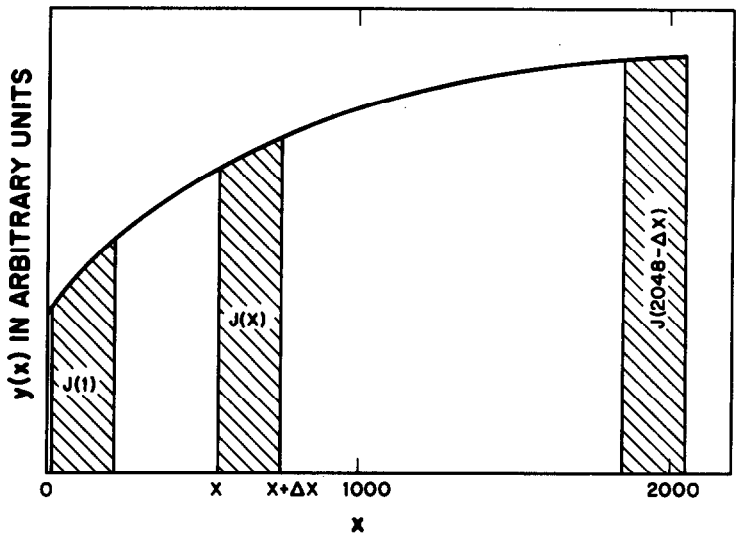

Fig. 1. Relationship between the observed data points and the fitted integrated functions.

Once again, since the data read are at unit quantization intervals, it follows that

$$J(X+1) = \sum_{x=X+1}^{X+1+\Delta X} y(x) \qquad (2)$$
$$= J(X) - y(X) + y(X+1+\Delta X)$$

Consequently, the function $J(X)$ needs to be summed only for $J(1)$, while every following value of $J(X)$ can be obtained from the preceding one by three fundamental operations, as shown in Fig. 1. For example, if $\Delta X = 500$, the value of $J(1)$ is obtained from the sum of $y(1), y(2), \ldots y(501)$ [equation (1)]. Similarly, $J(2)$ is given by the sum of $y(2), y(3) \ldots y(502)$, which is the same as $J(1) - y(1) + y(502)$ [equation (2)].

Analytical integration

The relaxation curve for one relaxation process is given by

$$y = b_1 + a_1\,[1 - \exp(-x/\tau)] \qquad (3)$$

and for two relaxation processes it is the sum of two exponential functions:

$$y = b_1 + a_1\,[1 - \exp(-x/\tau_1)] + a_2\,[1 - \exp(-x/\tau_2)]. \qquad (4)$$

Integration of equations (3) and (4) between X and $(X + \Delta X)$ results in

$$J(X) = (b_1 + a_1)\Delta X - a_1\,\tau_1[1 - \exp(-\Delta X/\tau_1)]\exp(-X/\tau_1) \qquad (5)$$

and

$$J(X) = (b_1 + a_1)\Delta X + a_2\Delta X - a_1\,\tau_1[1 - \exp(-\Delta X/\tau_1)]\exp(-X/\tau_1) - a_2\tau_2[1 - \exp(-\Delta X/\tau_2)]\exp(-X/\tau_2). \qquad (6)$$

Because in a given experiment a_1, a_2, τ_1, τ_2 and b_1 are fixed values and ΔX is an appropriately chosen constant, equations (5) and (6) can be simplified by using three (and five respectively) parameters:

$$J(X) = P_1 - [P_2 \exp(-X/P_3)] \qquad (7)$$

$$J(X) = P'_1 - [P_2 \exp(-X/P_3)] - P_4 \exp(-X/P_5). \qquad (8)$$

For computational reasons it is advantageous to have parameters P_1 and P'_1 in both equations expressed in terms of the same values of a_1 and a_2; thus

$$P'_1 = P_1 + a_2\,\Delta X, \qquad (9)$$

where a_2 has to be expressed as a function of appropriate P parameters. The relaxation times and amplitudes are then related as follows:

$$\tau_1 = P_3 \qquad (10)$$

$$\tau_2 = P_5 \qquad (11)$$

$$a_1 = \frac{P_2}{\tau_1[1 - \exp(-\Delta X/\tau_1)]} = \frac{P_2}{P_3[1 - \exp(-\Delta X/P_3)]} \qquad (12)$$

$$a_2 = \frac{P_4}{\tau_2[1 - \exp(-\Delta X/\tau_2)]} = \frac{P_4}{P_5[1 - \exp(-\Delta X/P_5)]} \qquad (13)$$

The integrated relaxation functions $J(X)$ can be fitted by using three (P_1, P_2, P_3) or five (P'_1, P_2, P_3, P_4, P_5) adjustable parameters respectively, and a multiparametric curve-fitting program[3] which uses a non-linear least-squares subroutine. In this procedure, the sum of the squares of the deviations of the calculated

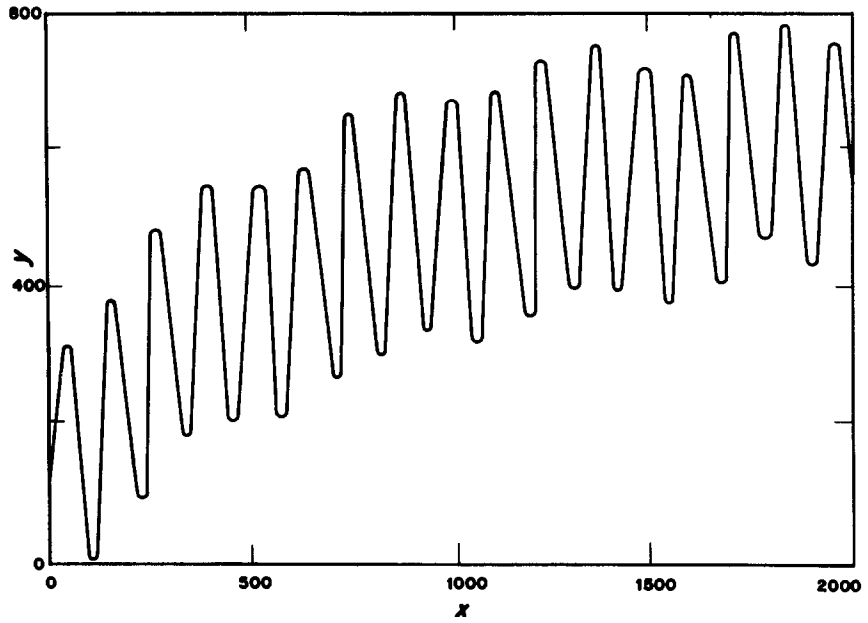

Fig. 2. Plot of the relaxation curve obtained from the standard data set.

values of $J(X)$ from the corresponding measured values is minimized by variation of the adjustable parameters.

RESULTS

As a first step the program has been tested on simulated raw data in order to obtain information on the limitations in resolution and in the accuracy of relaxation times and amplitudes that can be achieved.

The raw data were generated by use of equation (3). To simulate the experimentally observed periodical noise, values obtained from the function

$$y_{noise}(x) = c_1 \sin(x/d_1) + c_2 \sin(x/d_2) \qquad (14)$$

have been added.

The following set of parameters was used as a standard input: $\tau_1 = 700$, $a_1 = 450$, $b_1 = 100$, $c_1 = 50$, $c_2 = 150$, $d_1 = 10$, and $d_2 = 60$. The values of y were calculated for $x = 0,1,2,3 \ldots 2047$ according to equations (3) and (14). To simulate the analogue-to-digital conversion only the integer in the y-values has been considered. A plot of the curve obtained from the standard input data is shown in Fig. 2. The contribution from the noise was made higher than that usually encountered in experiments. Thus, the computation results should be indicative of the reliability of the analysis employed. For a meaningful test of the method, it is necessary to compare the relaxation times and amplitudes calculated from "experimental" curves with the exact values used in the simulated functions, in addition to use of the usual statistical criteria such as standard deviation of the fit, deviation patterns, *etc.*

Several series of computer experiments were carried out on this set of data, varying only one parameter (τ, a, b, c or d) at a time. For the numerical integration all 2048 data points were taken, but for the curve-fitting only every twentieth $J(X)$ was considered. Values at the beginning and end of the relaxation curve, corresponding to the largest deviations in real experiments, were omitted, so that less than 100 points had to be fitted. In all cases, the agreement between calculated and observed $J(x)$ values was very good as judged by the standard deviation of the fit and the random deviation pattern obtained. It should be recalled that all data used in this work represent unscaled quantities, which makes this simple treatment feasible. Appropriate scale multiplication factors (*e.g.*, sampling rate and voltage range as set on the transient-recorder) would have to be applied in order to obtain the actual relaxation times and amplitudes. The unscaled relaxation data can cover a fixed range of natural numbers, depending on the transient-recorder used. There is some limitation in the accuracy of the measured relaxation time and amplitude resulting from the fact that only integer values are obtainable from such devices (analogue-to-digital conversion). Ideally, recorders with sufficiently high resolution (10 bits or greater, corresponding to better than 0.1% full-range error) should be used to take maximum advantage of the instrument sensitivity. By comparison, a storage oscilloscope introduces an error of several per cent owing to non-linearity of the screen. The results obtained with the procedure outlined above (integration and curve-fitting with the analytically integrated function) are summarized in Figs. 3–6, where the relative errors of the relaxation time (○) and relaxation amplitude (□) are plotted

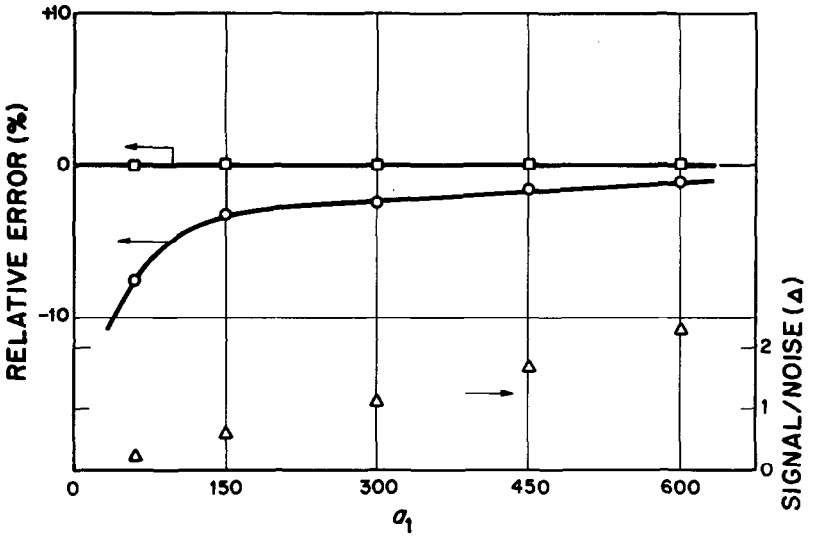

Fig. 3. Plot of the relative errors in relaxation time (○) and amplitude (□) as a function of the amplitude (a_1). The triangles (△) give the corresponding signal-to-noise ratio at mid-sweep.

against different variables of the standard set. The signal-to-noise ratio (△), given in each figure, was calculated from the noise-free value at mid-sweep.

A variation over a large range of relaxation amplitudes (Fig. 3) produced very precise relaxation amplitudes (relative error $< 0.15\%$). The relaxation times were generally less precise; however, if the relaxation amplitude was larger than 1/6 ($a_1 = 150$) of the full range (1024), the τ-values were obtained within 3% error. The large error in τ for $a_1 = 60$ is primarily due to the small number of distinct integer values available to describe the relaxation effect (giving a poor resolution) and to a fourfold noise contribution. An amplitude of 60 means that the relaxation effect (without noise) covers only 1/16 of the full range. Repeating the experiment by selecting a voltage range smaller by a factor of 2 or 5 produces a larger amplitude and minimizes the error due to the restriction on integer values. Naturally, the noise increases by the same factor, but the error due to the noise is constant because the signal-to-noise ratio remains unchanged.

Varying τ, (Fig. 4) leads to noticeable deviations of calculated relaxation times and amplitudes when τ_1 is larger than half of the sweep time. The use of a faster sampling rate causes τ_1 to fall into a favourable relaxation time range of 250–750 (3τ–8τ per sweep time). To be in this proper range the sampling rate must be changed by a factor of 3 or less, which can be accomplished with most transient-recorders. In addition, the faster sampling rate provides shorter noise

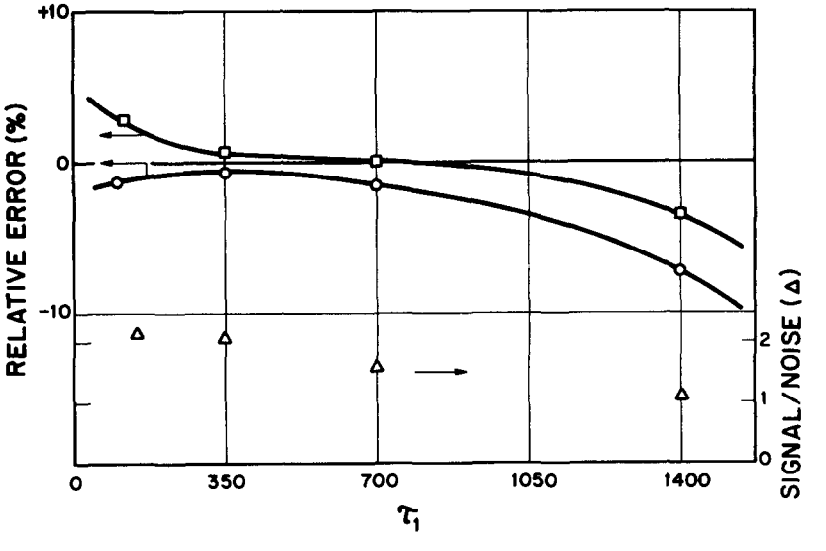

Fig. 4. The same plot as Fig. 3, as a function of the relaxation time (τ_1).

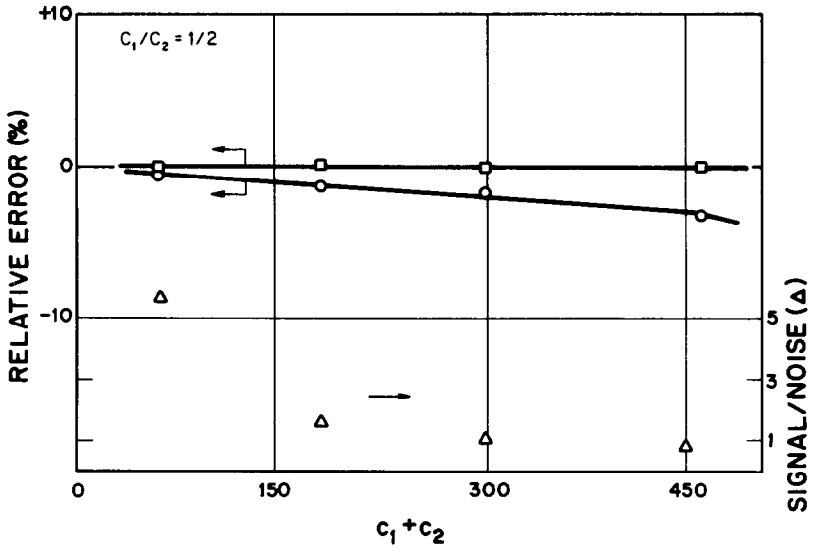

Fig. 5. The same plot as Fig. 3, as a function of the sum of the two noise frequency amplitudes, c_1 and c_2; c_1/c_2 was kept constant at 1/2.

periods and, therefore, better cancellation of the noise through integration over the same interval length, ΔX.

As expected, an increase in the noise amplitudes (c_1, c_2) exhibits essentially the same effects as a decrease in the relaxation amplitude (Fig. 5).

A variation in the noise frequency results in deviations of relaxation time of up to ~10% (Fig. 6). The larger errors are associated with the noise periods being much longer than the integration interval length (ΔX).

Table 1 gives a comparison of calculated and input values for a_1 and τ_1 for several simulated experiments for different values of ΔX. The standard data set was used except for the values of d_1 and d_2, as indicated in the table.

A substantial improvement is observed when ΔX is increased to ~1000; however, a further increase in ΔX makes the error much larger. The less accurate results for ΔX greater than half of the sweep range are due to the fact that fewer data are available to be fitted and a major fraction of the information from the last part of the relaxation curve is lost.

The computer simulations described in this work, with emphasis on a simple relaxation process having an extremely high noise contribution, demonstrate the efficiency of this numerical approach in the evaluation of the relaxation times and the amplitudes. An exten-

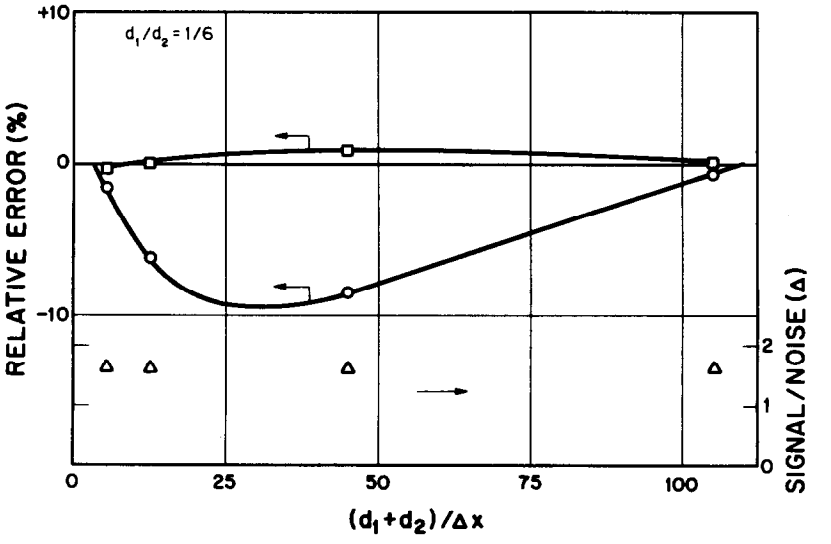

Fig. 6. The same plot as Fig. 3, as a function of the noise frequencies d_1 and d_2; d_1/d_2 was kept constant at 1/6.

Table 1. A comparison of the input and calculated values for α_1 and τ_1 at different values of ΔX

		Input values	Calculated values	Relative Error, %
$d_1 = 40$ $d_2 = 240$	$\Delta X = 200$	$\tau_1 = 700$	641	−8.4
		$a_1 = 450$	461	+2.4
	$\Delta X = 500$	$\tau_1 = 700$	692	−1.2
		$a_1 = 450$	450	0
$d_1 = 200$ $d_2 = 1200$	$\Delta X = 1000$	$\tau_1 = 700$	682	−2.5
		$a_1 = 450$	452	+0.3
	$\Delta X = 1300$	$\tau_1 = 700$	651	−7.0
		$a_1 = 450$	452	+0.5
	$\Delta X = 1600$	$\tau_1 = 700$	454	−35
		$a_1 = 450$	464	+3.0

sion of the procedure to multiple relaxation processes is straightforward.[4, 5] The program execution is very short, making it ideally suited for a dedicated microcomputer. The short turn-around allows the selection of other ranges of total voltage, or more importantly, of other sampling rates in order to optimize the accuracy of the results as judged by well-defined statistical criteria (*e.g.*, standard deviation of the fit, deviation patterns, *etc.*).

In summary, results of optimum accuracy can be obtained under the following conditions of data collection and treatment.

(1) The relaxation curve should cover as much as possible of the voltage range (Figs. 3 and 5). To achieve the optimum noise reduction by integration, all data points (including the noise) should be within the full voltage range.

(2) The sampling rate should be selected so that the resulting relaxation time is between 1/8 and 1/3 of the total sweep time (Fig. 4).

(3) The noise reduction through the integration method is obviously most effective if the integration interval length ΔX is identical with the length of the noise period. The noise period or an effective ΔX can be established with one set of experimental data and various computer runs with different ΔX values. The best ΔX can be chosen by using statistical criteria obtained from the curve-fitting program. For example, the ΔX value for a double sampling rate is just $\Delta X/2$; thus, it is actually necessary to determine the most effective ΔX only once. If ΔX is not optimized, a value between 1/10 and 1/2 of the sweep time provides results of good accuracy.

(4) Experiments with noise periods longer than twice the relaxation time are better fitted by a sine function incorporated in the integrated relaxation function to allow for one very low noise frequency.

Using the criteria described above (selecting the voltage range, the sampling rate and the integration interval length) results in an error of less than 5% for the relaxation time and 0.5% for the relaxation amplitude. These errors apply to a signal-to-noise ratio of 3:2, which was chosen to illustrate clearly the effects of the different variables.

At the highest sensitivity range together with a small amplitude, a condition usually associated with large noise, an error of 5% is considered excellent performance. Experiments with such small amplitudes (relative absorbance changes, $\Delta A/A$, smaller than 10^{-3}) are usually only performed to obtain an additional point for a limiting case.[6] In actual experiments most of the relaxation effects can be observed at a signal-to-noise ratio greater than 10:1, in which case τ_1 may be determined to within 2%.

Although the integration method described was exemplified on temperature-jump spectrophotometric experiments, its application to other techniques (*e.g.*, stopped flow, pressure jump, electric-field jump, *etc.*) using conductance, fluorescence, light-scattering, thermometry, *etc.* for detection, is equally straightforward.

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THERMOMETRIC TITRATION OF THORIUM WITH EDTA IN THE PRESENCE OF LARGE EXCESS OF NEUTRAL SODIUM SALTS

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Summary—The thermometric titration of Th(IV) in the presence of neutral sodium salts, sulphuric acid or acetic acid with EDTA has been studied. The effect of each on the observed heat values for the titration is discussed. For sodium perchlorate media, ΔH values of -9 and -21 kJ/mole have been estimated for the formation of the Th(IV)–EDTA chelate at $\mu \rightarrow 0$ and $\mu = 0.5$ ($NaClO_4$), respectively. The $-\Delta H$ values increase steadily with increase in concentration of sodium perchlorate up to at least 3*M*. For the titration of Th(IV) in the presence of a large excess of sodium nitrate the use of sodium iodide as a masking reagent has been examined: large amounts of Bi and Cu(II) are masked and a masking effect is observed for small amounts of Ni.

The presence of a large excess of neutral alkali metal salts generally interferes in the EDTA titration of a metal such as calcium or thorium. For thorium titrations with a visual end-point, it affects the conditional equilibrium constants for the Th–indicator and Th–EDTA chelates, resulting in a vague colour change near the end-point. For titrations based on use of a parameter linearly related to concentration and extrapolation of values obtained before and after the end-point, an accurate result would be obtained provided complete formation of the Th–EDTA chelate takes place during the titration. There often occur, however, such difficulties as the "loading effect" in high-frequency titration[1,2] or the "too negative half-wave potential" in amperometric titration.[3] In thermometric titrations the heat of dilution in the presence of large excess of neutral alkali metal salts is often very large, which makes it difficult to judge the end-point. Such a dilution effect can be suppressed to a considerable extent, however, by matching the salt concentration of the titrant with that of the titrand. The thermometric end-point detection method thus becomes very useful for the determination of Th(IV) in concentrated alkali metal salt solutions.

The heat of solution, dilution or mixing in non-aqueous media has been determined by thermometric titrimetry,[4,5] and the heat of dilution of the titrant has been utilized for thermometric end-point enhancement.[6] Analysis of the dilution effect for thermometric titration in aqueous electrolyte solution is very complicated, however, and the heat of reaction is usually determined in a constant ionic strength medium where the dilution effect is taken as constant. In a previous paper,[7] the effective heats of dilution of 0.159*M* Na_4EDTA for EDTA titrations in 0.1*M* ionic media were determined. In the present paper, the effective heats of dilution of Na_4EDTA are discussed in more detail, based on the data so far reported for heat of dilution of electrolyte solutions, and the effect of sodium perchlorate concentration on the observed heat values for titrations is elucidated.

EXPERIMENTAL

Apparatus

The apparatus and procedures were the same as previously described.[7] All measurements were made at 25.0°.

Reagents

Thorium nitrate was purified by repeated precipitation with hydrogen peroxide. Thorium perchlorate and chloride were prepared from the nitrate. Approximately 0.1*M* solutions of each thorium salt were prepared so that they were *ca.* 0.1*M* in the corresponding acid. The concentration of thorium and free acid was determined by the method described previously.[8] Sodium nitrate and perchlorate were recrystallized three times from water and general reagent grade sodium chloride was used without further purification. The concentration of sodium and free acid in the sodium salt stock solutions (except for the sodium chloride) was determined as before.[8] A solution of the tetrasodium salt of EDTA was prepared and standardized as described previously.[7]

For the titration of thorium in the presence of acetic acid or bisulphate, the thorium solution was prepared by adding the appropriate reagent to thorium perchlorate solution and adjusting to the desired pH with perchloric acid or sodium hydroxide, by use of a pH-meter calibrated with hydrochloric acid standards. For the titration of thorium in the presence of a neutral sodium salt the acidity of the thorium solution was adjusted with the corresponding acid. In the study of the effect of other ions, the thorium solution was prepared by adding enough sodium iodide to thorium nitrate solution (which was 1–4*M* in sodium nitrate) to give a concentration of 1.5*M* and adjusting to the desired pH with nitric acid or ammonia solution, by use of a pH-meter calibrated with nitric acid standards which were 4.0*M* in sodium nitrate.

Calculations

By an approach analogous to that outlined previously for determination of the enthalpy of the formation of the 1:1 Th–EDTA chelate,[8] it is possible to obtain ΔH^{Th}_{obs} and ΔH^{Y}_{obs}.

Thorium is titrated at pH 2.0 in the presence of acetic acid or bisulphate, HA, with Na_4EDTA. The observed effective enthalpy. ΔH^{Th}_{obs}, refers to the process:

$$(ThA_n)' + Y(titrant) \rightarrow (ThY)' + n(A)' \quad \Delta H^{Th}_{obs} \quad (1)$$

where $(ThA_n)'$ represents all thorium species present in the titrand before the titration, $(ThY)'$ all the 1:1 Th–Y species in the titrand before the end-point, $(A)'$ all the species of A liberated by formation of the Th–EDTA chelates in the titrand before the end-point. The enthalpy change, ΔH^{Th}_{obs}, is given by:

$$\Delta H^{Th}_{obs} = \Delta H_D + \Delta H^{ThY}_{eff} + \Delta H^{ThA_n(D)}_{eff} + \Delta H^{HA}_{eff} \quad (2)$$

where ΔH_D is the effective heat of dilution of Na_4EDTA; ΔH^{ThY}_{eff} the effective enthalpy of formation of the Th–EDTA chelates; $\Delta H^{ThA_n(D)}_{eff}$ the effective enthalpy of dissociation of ThA_n complexes; ΔH^{HA}_{eff} the effective enthalpy of the protolytic reaction of A.

Next, the observed effective enthalpy change ΔH^{Y}_{obs} refers to the process:

$$(A)'_b + Y(titrant) \rightarrow (Y)' + (A)'_a \quad \Delta H^{Y}_{obs} \quad (3)$$

where $(Y)'$ represents all the protonated EDTA species in the titrand after the end-point, and $(A)'_b$ and $(A)'_a$ stand for all the species of A in the titrand at and after the end-point, respectively. The enthalpy change, ΔH^{Y}_{obs}, is given by:

$$\Delta H^{Y}_{obs} = \Delta H_D + \Delta H^{H_nY}_{eff} + \Delta H^{HA}_{eff} \quad (4)$$

where $\Delta H^{H_nY}_{eff}$ is the effective enthalpy of formation of the protonated EDTA species, H_nY. The calculated values of ΔH^{Th}_{obs} and ΔH^{Y}_{obs} are obtained by calculation of each ΔH term on the right-hand side of equations (2) and (4), respectively. The data used in the calculations are mentioned below.

For titrations of thorium with Na_4EDTA in sodium perchlorate media the $\Delta H^{ThA_n(D)}_{eff}$ term is omitted in equation (2). For the 0.1*M* medium, ΔH for the formation of the Th–EDTA chelate is determined in the same manner as described previously.[8] For the 0.5*M* perchlorate medium the ΔH value cannot be determined because the value of K^{H}_{ThHY} is unknown. The value of ΔH^{ThY}_{eff} calculated from equation (2), however, approximates to the enthalpy value for the formation of the Th–EDTA chelate because that for protonation of the chelate, ΔH^{H}_{ThHY}, is low[8] (−0.3 kJ/mole at $\mu = 0.1$).

For titrations of thorium in the presence of a neutral sodium salt the ΔH^{HA}_{eff} term of equation (2) or (4) is omitted. Further, the effective heat of dilution, ΔH_D, refers to the process:

$$Y(0.154M) + MA(C_1) \rightarrow Y^{4-},MA(C_3,C_2) \quad \Delta H_D \quad (5)$$

where MA stands for the neutral alkali metal salt and the concentration of each species is shown in parentheses. The process is divided into the following steps:

$$Y(0.154M) \rightarrow Y^{4-}(C_3) \quad \Delta H^{Y}_{D} \quad (6)$$

$$MA(C_1) \rightarrow MA(C_2) \quad \Delta H^{MA}_{D} \quad (7)$$

$$Y^{4-}(C_3) + MA(C_2) \rightarrow Y^{4-},MA(C_3,C_2) \quad \Delta H^{Y,MA}_{mix} \quad (8)$$

where ΔH^{Y}_{D} and ΔH^{MA}_{D} are the effective heats of dilution of Na_4EDTA and MA, respectively; $\Delta H^{Y,MA}_{mix}$ is the effective heat of mixing at constant volume for equation (8). Such effective heat values are considered constant enough to be calculated as average values because during the titration the change in concentration of each species in the titrand is very small. It is convenient to express all the heat values in kJ per mole of EDTA. Then the heat change ΔH_D is represented by

$$\Delta H_D = \Delta H^{Y}_{D} + \Delta H^{MA}_{D} + \Delta H^{Y,MA}_{mix} \quad (9)$$

The ΔH_D value at a given ionic strength is determined in the same manner as described previously.[7,8] Further, the ΔH^{MA}_{D} value is calculated from the data so far reported for heat of dilution of electrolyte solutions,[9] in which the specific gravities of electrolyte solutions listed in International Critical Tables[10] are used. The value, d^{25}_{25}, of 1.033 for 0.154*M* Na_4EDTA solution was determined experimentally. Rearrangement of equation (9) gives

$$\Delta H_D - \Delta H^{MA}_{D} = \Delta H^{Y}_{D} + \Delta H^{Y,MA}_{mix} \quad (10)$$

The ΔH^{Y}_{D} value, which (by its definition) does not change with ionic strength, is estimated from the intercept on extrapolation of a plot of $(\Delta H_D - \Delta H^{MA}_{D})$ *vs.* μ. The ΔH_D value for 0.1*M* or 0.5*M* sodium perchlorate medium was determined in this work, the protonation constants and enthalpies of EDTA being obtained from the literature[11–15] (*cf.* reference 8). Knowledge of the protonation constants and enthalpies of EDTA over wider range of ionic strength is desirable for exact estimation of ΔH^{Y}_{D}.

The change in pH of the solution during the titration is small. The difference between ΔH^{Th}_{obs} and ΔH^{Y}_{obs}, therefore approximates to the enthalpy change of the substitution reaction, $(ThA_n)' + (Y)' \rightarrow (ThY)' + n(A)'$, which is denoted by $\delta\Delta H_{obs}$, and its value is independent of ΔH_D. Such a dilution effect as ΔH_D can be adjusted to the desired extent by the addition of neutral alkali metal salt to the titrant and thus the $\delta\Delta H_{obs}$ value is recommended as an index to the sharpness of the end-point.

RESULTS AND DISCUSSION

For titrations of thorium with Na_4EDTA in perchloric, nitric or hydrochloric acid media, the thermograms show well-defined inflection points in each acid at concentrations ranging from 0.01 to 0.1*M*. All the titration curves obtained are similar to curve (1) in Fig. 2. Moreover, the values of ΔH^{Th}_{obs} and ΔH^{Y}_{obs} at the same acid concentration are nearly equal, which suggests that the heat evolved during the titration by dissociation of thorium nitrato or chloro complexes, *i.e.*, $\Delta H^{ThA_n(D)}_{eff}$ in equation (2), is small.

The titrations at pH 2.0 in sulphuric or acetic acid media provide well-defined inflection points at conditional concentrations of acid up to 0.2*M*, and rounded inflection points at higher concentrations. The values of ΔH^{Th}_{obs} and ΔH^{Y}_{obs} are plotted against the conditional acid concentration of the thorium solution in Fig. 1. The values of ΔH^{Th}_{obs} for acetic acid media were calculated from equation (2) by using both the ΔH^{Th}_{obs} value measured for 0.01*M* perchloric acid and Portanova's data[16] for the Th–acetate system ($\mu = 1.0$), values of −15.2 and −17.4 kJ/mole being obtained for 0.05*M* and 0.1*M* acetic acid, respectively. These agree with the observed values, that is, −15.3 kJ/mole for the 0.05*M* and −17.6 kJ/mole for 0.1*M* acid, although calculated without correction for change in ionic strength. The calculated values of ΔH^{Y}_{obs} also agreed with the observed values. On the other hand, the marked increase in $-\Delta H^{Th}_{obs}$ and $-\Delta H^{Y}_{obs}$ with C_L for sulphuric acid media could not be explained by such a calculation without ionic strength taken into account. Since a ΔH_D value for the

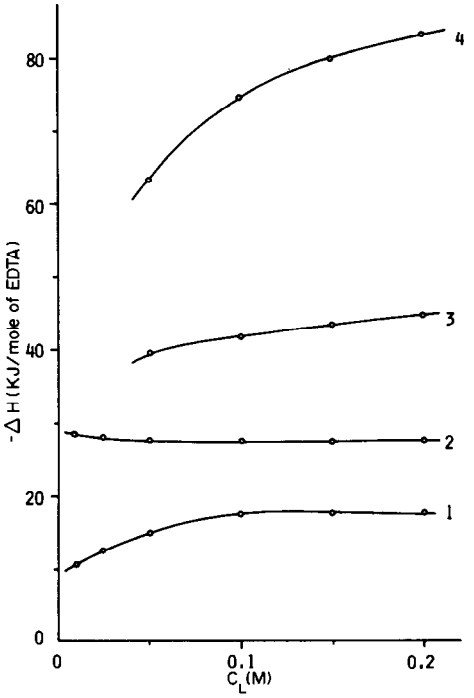

Fig. 1. ΔH^{Th}_{obs} and ΔH^{Y}_{obs} as a function of ligand concentration. Titration conditions: $C_{Th} = 7 \times 10^{-3}M$, pH 2.0, EDTA concentration of titrant = 0.154M. For acetic acid, (1) ΔH^{Th}_{obs}; (2) ΔH^{Y}_{obs}. For sulphuric acid, (3) ΔH^{Th}_{obs}; (4) ΔH^{Y}_{obs}.

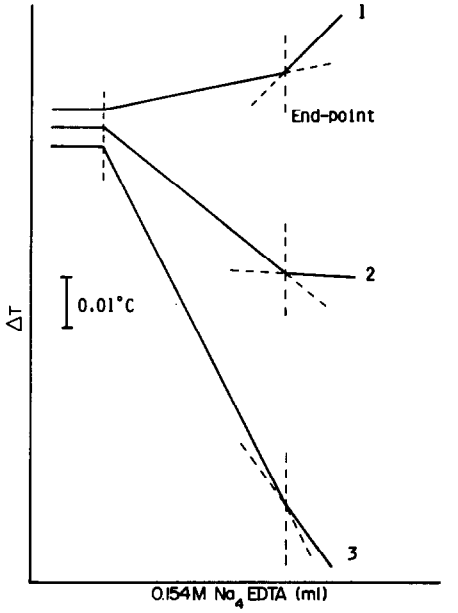

Fig. 2. Thermometric titrations of Th with Na_4EDTA in various concentrations of sodium nitrate. $C_{Th} = 7 \times 10^{-3}M$, $C_{HNO_3} = 0.01M$. (1) $C_{NaNO_3} = 0$; (2) $C_{NaNO_3} = 3.0M$; (3) $C_{NaNO_3} = 5.0M$.

medium was not simple to evaluate from known data, the value of $-(\Delta H^{Th}_{obs} - \Delta H_D)$ was calculated from equation (2) by using the relevant equilibrium constants and enthalpies[8,17,18] at $\mu = 0.1$, the value obtained for 0.05M sulphuric acid being lower than the observed value by 10 kJ/mole; in the case of $-\Delta H^{Y}_{obs}$, the calculated value was lower than the observed value by 25 kJ/mole. The disagreement between the calculated and observed values cannot be explained only by the ΔH_D term and, particularly for ΔH^{Y}_{obs}, suggests that there is an increase in $\Delta H^{H_2Y}_{eff}$ with sulphuric acid concentration.

The thermograms for titrations of thorium at pH 2.0 in the presence of sodium nitrate are shown in Fig. 2; their slopes are markedly affected by the concentration of sodium nitrate. The titration curves, nevertheless, show well-defined inflection points for sodium nitrate concentrations up to 4M. If the sodium nitrate concentration of the thorium solutions is denoted by C_{NaNO_3}, plots of C_{NaNO_3} *vs.* $-\Delta H^{Th}_{obs}$, $-\Delta H^{Y}_{obs}$ and $\delta\Delta H$ have the shapes shown in Fig. 3. The dashed curve (1) is that for $-\Delta H^{NaNO_3}_D$ [*cf.* equation (7)]. The curves for both $-\Delta H^{Th}_{obs}$ and $-\Delta H^{Y}_{obs}$ show maxima at a nitrate concentration of 0.6–0.8M and drop sharply at nitrate concentration above *ca.* 1.5M, the slopes being roughly equal to that of the dashed curve (1). Strictly speaking, the values of $-\Delta H^{Th}_{obs}$ corrected for $\Delta H^{NaNO_3}_D$, which are shown by curve (6) in Fig. 3, increase considerably with increase in C_{NaNO_3} up to 3M. The values of $(\Delta H^{Th}_{obs} - \Delta H^{NaNO_3}_D)$ are -8.8 and -21 kJ/mole for 0.1 and 1.0M sodium nitrate, respectively; the values of $(\Delta H^{Th}_{obs} - \Delta H^{NaClO_4}_D)$ are -9.0 and -22.2 kJ/mole for 0.1 and 1.0M sodium perchlorate media. This suggests that the increase in $-(\Delta H^{Th}_{obs} - \Delta H^{NaNO_3}_D)$ with C_{NaNO_3} is governed by

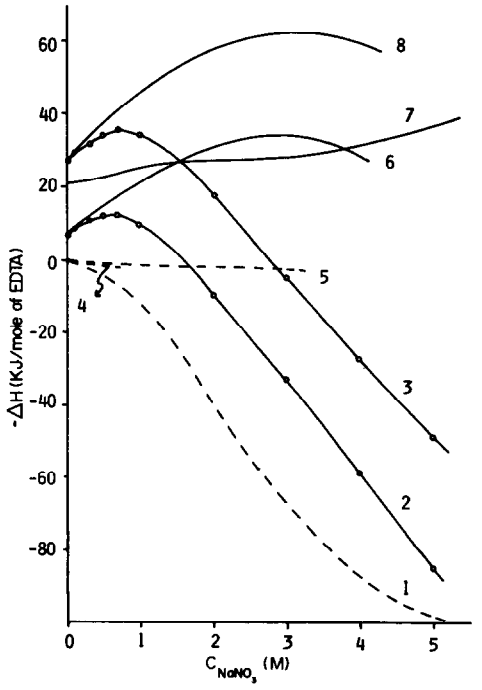

Fig. 3. ΔH^{Th}_{obs}, ΔH^{Y}_{obs} and $\Delta H^{NaNO_3}_D$ as a function of C_{NaNO_3}. Titration conditions as for Fig. 2. (1) $\Delta H^{NaNO_3}_D$; (2) ΔH^{Th}_{obs}; (3) ΔH^{Y}_{obs}; (4) $\Delta H^{Y,NaClO_4}_{mix}$; (5) $\Delta H^{NaCl,HCl}_{mix}$; (6) $\Delta H^{Th}_{obs} - \Delta H^{NaNO_3}_D$; (7) $\delta\Delta H_{obs}$; (8) $\Delta H^{Y}_{obs} - \Delta H^{NaNO_3}_D$.

ionic strength and that the heat evolved by dissociation of the nitrato complexes, *i.e.*, $\Delta H_{eff}^{ThA_n(D)}$ of equation (2), is small. The increase with C_{NaNO_3}, therefore, results from that in $-(\Delta H_{eff}^{ThY} + \Delta H_{mix}^{Y,NaNO_3})$ with C_{NaNO_3} [*cf.* equations (2) and (6)–(8)]. An effective heat of mixing at constant volume is ordinarily not used in discussing a mixing process for electrolyte solutions, but for 1:1 electrolyte solutions it can be calculated by using known data.[9,10,19,20] For example, the heats for the following theoretical processes, $\Delta H_{mix}^{NaCl,HCl}$, [*cf.* equation (8)], were calculated:

$$NaCl(0.007M) + HCl(0.093M) \rightarrow NaCl,HCl(0.007M,0.093M)$$

$$NaCl(0.007M) + HCl(0.493M) \rightarrow NaCl,HCl(0.007M,0.493M)$$

values of 0.7 and 1.1 kJ per mole of NaCl being obtained at 0.1 and 0.5*M* total salt concentration. The change in $-\Delta H_{mix}^{NaCl,HCl}$ with total salt concentration is shown by the dashed curve (5) in Fig. 3, and the change in $-\Delta H_{mix}^{Y,NaClO_4}$ with C_{NaClO_4}, which was obtained in this work, is shown by the dashed curve (4). The changes are similar to each other and thus the values of $\Delta H_{mix}^{Y,NaClO_4}$ seem to be positive and low, at least up to 1–2*M* sodium perchlorate concentration. There is no obvious reason for the value of $\Delta H_{mix}^{Y,NaClO_4}$ and $\Delta H_{mix}^{Y,NaNO_3}$ to differ very much. It is concluded that the increase in $-(\Delta H_{obs}^{Th} - \Delta H_D^{NaNO_3})$ with C_{NaNO_3} results from that in $-\Delta H_{eff}^{ThY}$, mainly from ΔH for the formation of the Th–EDTA chelate, with C_{NaNO_3}. The increase in $-(\Delta H_{obs}^{Y} - \Delta H_D^{NaNO_3})$ with C_{NaNO_3} is interpreted in the same way, and consequently is thought to result from the increase in $-\Delta H_{eff}^{HY}$ with C_{NaNO_3}. The $\delta\Delta H_{obs}$ values increase gradually with increase in C_{NaNO_3}. The titration method, therefore, is very advantageous for determining thorium in a concentrated sodium nitrate solution so long as the effect of dilution of sodium nitrate is reduced (by the addition of sodium nitrate to the titrant) to such an extent that a distinct end-point is obtained.

Titrations of thorium at pH 2.0 in the presence of sodium perchlorate give thermograms very similar to those for sodium nitrate media shown in Fig. 2. The titration curves show well-defined inflection points for perchlorate concentrations up to 4*M*. The plots of C_{NaClO_4} *vs.* $-\Delta H_{obs}^{Th}$ and $-\Delta H_{obs}^{Y}$ show maxima at $C_{NaClO_4} = 0.55M$ ($\Delta H_{obs}^{Th} = -13.2$ kJ/mole and $\Delta H_{obs}^{Y} = -36.6$ kJ/mole), and the increase in $-(\Delta H_{obs}^{Th} - \Delta H_D^{NaClO_4})$ with C_{NaClO_4} was also very similar to that for sodium nitrate media. All the effective enthalpy and heat values involved in the titrations as a function of C_{NaClO_4} are shown in Fig. 4. It is seen from the figure that the steady increase in $-\Delta H_{obs}^{Th}$ arises from that in $-\Delta H_{eff}^{ThY}$, mainly ΔH for the formation of the Th–EDTA chelate, with C_{NaClO_4}, in other words, the ionic strength. A ΔH for the formation of the Th–EDTA chelate at $\mu = 0$ was estimated by extrapolation to be -9 kJ/mole. The enthalpies of formation of the bi-, ter- or quadrivalent metal–EDTA chelates at $\mu = 0.1$ and $\mu = 0.5$ are tabulated in Table 1. It was reported by Yatsimirskii and Karacheva[21] that the $-\Delta H$ *vs.* μ plot for the formation of the calcium or nickel EDTA chelate shows a minimum at an ionic strength of 0.5–0.8. The plot for the

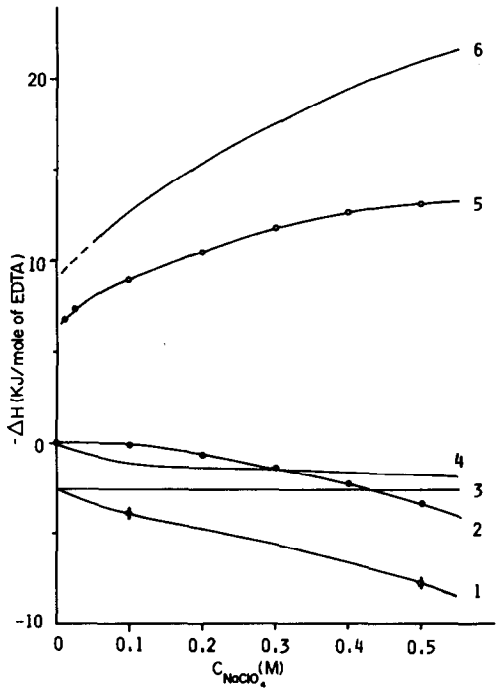

Fig. 4. Effective enthalpy and heat changes for titrations of Th with Na_4EDTA in sodium perchlorate media as a function of C_{NaClO_4}. $C_{Th} = 7 \times 10^{-3}M$, $C_{HClO_4} = 0.01M$, EDTA concentration of titrant = 0.0154*M*. (1) ΔH_D; (2) $\Delta H_D^{NaClO_4}$; (3) ΔH_D^{Y}; (4) $\Delta H_{mix}^{Y,NaClO_4}$; (5) ΔH_{obs}^{Th}; (6) ΔH_{eff}^{ThY}. (○) observed values; (●) calculated values; (ϕ) values determined by measuring ΔH_{obs}^{Y}.

Table 1. Comparison between ΔH at $\mu = 0.1$ and $\mu = 0.5$ for the formation of metal–EDTA chelates

	$\mu = 0.1$	$\mu = 0.5$	
	kJ/mole of Y		Reference
$Ca^{2+} + Y^{4-} \rightarrow CaY^{2-}$	−23.2	−19.7	21*
$La^{3+} + Y^{4-} \rightarrow LaY^{-}$	−12.3	−17.2†	22,23
$Th^{4+} + Y^{4-} \rightarrow ThY$	−12.9	−21	8

* Both values are obtained from the ΔH *vs.* μ plot.
† $\mu = 0.44$.

formation of the Th–EDTA chelate, however, shows no such minimum, but a steady increase in the $-\Delta H$ values. It is seen from Table 1 that the $-\Delta H$ values for the formation of the La–EDTA chelate increase with ionic strength similarly to those for the formation of the Th–EDTA chelate. The plot for the formation of the La–EDTA chelate also seems not to show a minimum.

Titrations of thorium at pH 2.0 in various concentrations of sodium chloride also provide well-defined inflection points at salt concentrations up to 4*M*. The plots of C_{NaCl} *vs.* $-\Delta H_{obs}^{Th}$, $-\Delta H_{obs}^{Y}$ and $\delta\Delta H_{obs}$ are shown in Fig. 5, the curves being different from those for sodium nitrate or perchlorate. The calculated ΔH_D^{NaCl} values, which are represented by the broken curve in the figure, increase gradually with increase in C_{NaCl} above 3*M*. This makes the shape of the titration curves for concentrated sodium chloride solutions different from that for concentrated sodium nitrate solutions. The $\delta\Delta H_{obs}$ values are nearly constant over the concentration range shown in the figure. The addition of sodium chloride to the titrant makes it possible to titrate thorium in more concentrated sodium chloride solution.

About 0.5 mmole of thorium in 100 ml of 0.1*M* nitric acid containing no neutral alkali metal salt was titrated with 0.154*M* Na_4EDTA, and the effect of other ions was examined. Manganese(II), lanthanum and alkaline-earth metals do not interfere even when present in 0.1*M* concentration. Less than 8 mmole of cobalt(II) or zinc and less than 4 mmole of cadmium will not interfere. The presence of the metals does not significantly affect ΔH_{obs}^{Th} in the concentration range mentioned above. For example, the ΔH_{obs}^{Th} values of -8.4, -9.4 and -10.4 kJ/mole were obtained for Co(II) concentrations of 0.01, 0.05 and 0.08*M*, respectively. On the other hand, the presence of lead affects ΔH_{obs}^{Th} even at low concentrations. ΔH_{obs}^{Th} values of -8.3, -8.9 and -11.3 kJ/mole were obtained for lead concentrations of 0, 0.005 and 0.02*M*, respectively. The values are much higher than those expected from the increase in ionic strength with lead concentration and thus indicate that the Pb–EDTA chelates partly formed in the course of the titration do not dissociate immediately. The good linearity of the thermograms obtained, however, makes it possible to determine thorium with an error of $\leqslant 1\%$ when less than 2 mmole of lead is present. Copper(II) and nickel interfere by formation of their EDTA chelates in the course of the titrations, but the use of 2,2′-bipyridyl as masking agent makes it possible to determine thorium in their presence. Up to 6 mmole of the metals can be masked at pH 2.0 by 20 mmole of the ligand. It is preferable to add dimethyl sulphoxide to the titrand when larger amounts of the metals are present.

For titrations of thorium in concentrated neutral sodium salt solutions, the addition of the salt to the titrant makes the end-points clearer. For example, to titrate thorium in $<4M$ sodium nitrate solutions, 0.07*M* Na_4EDTA (titrant) was prepared so as to be *ca.* 1*M* in sodium nitrate. In this case, the use of 2,2′-bipyridyl as masking agent was not adequate because the metal chelates were liable to precipitate under the experimental conditions. Hence, the titrand was made *ca.* 1.5*M* in sodium iodide as masking agent, whereby the precipitate of bismuth or lead iodide was redissolved. Typical thermograms and analytical results for the titrations are shown in Fig. 6 and Table 2, respectively. Lanthanum, manganese(II) and the alkaline-earth metal ions, of course, cause no interference. Bismuth, which forms stable iodo-complexes, and copper(II) which is reduced by iodide to form stable copper(I) iodide, do not interfere. Fairly large amounts of cobalt(II), zinc, cadmium, lead and uranium(IV) do not interfere. Nickel, aluminium and iron(III) do not interfere when present in several-fold

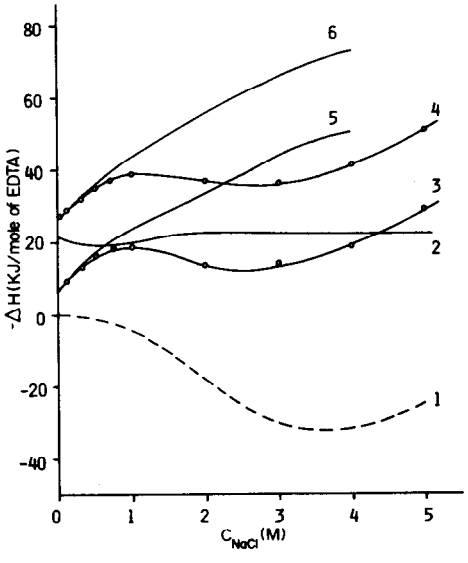

Fig. 5. ΔH_{obs}^{Th}, ΔH_{obs}^{Y} and ΔH_D^{NaCl} as a function of C_{NaCl}. Titration conditions: $C_{Th} = 7 \times 10^{-3}M$, $C_{HCl} = 0.01M$, EDTA concentration of titrant = 0.154*M*. (1) ΔH_D^{NaCl}; (2) $\delta\Delta H_{obs}$; (3) ΔH_{obs}^{Th}; (4) ΔH_{obs}^{Y}; (5) $\Delta H_{obs}^{Th} - \Delta H_D^{NaCl}$ (6) $\Delta H_{obs}^{Y} - \Delta H_D^{NaCl}$.

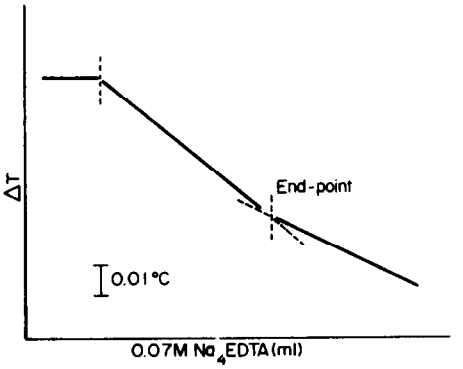

Fig. 6. Thermometric titration of Th with Na_4EDTA in the presence of a large excess of sodium nitrate. Titrand conditions as for Table 2, sodium nitrate concentration of titrant = 1.0*M*.

Table 2. Effect of diverse ions on the determination of Th in the presence of a large excess of sodium nitrate

Th taken, *mg*	Bi taken, *mg*	Other ion taken, *mg*	Th found, *mg*
11.6	418		11.7
17.4	418		17.4
17.4	627		17.5
34.8	418		35.0
34.8	627		34.7
		Pb(II)	
17.4	418	104	17.6
34.8	418	104	17.6
34.8	627	207	35.1
		U(VI)	
17.4	418	119	17.4
17.4	418	238	17.5
34.8	418	119	34.8
34.8	627	238	35.0
		Fe(III)*	
34.8	418	28	34.7
34.8	627	42	35.1
		Zr(IV)†	
17.4	418	18	17.5
34.8	418	18	34.8
34.8	627	27	34.9

Conditions: $C_{NaNO_3} = 4.0M$; $C_{NaI} = 1.5M$; pH = 1.5; total volume of titrand = 100 ml; temperature 25°C.

* With 0.1 ml of 5% ascorbic acid solution added per mg of Fe(III).

† In presence of 0.3*M* glycerine.

Table 3. Determination of Th(IV) in synthetic Bi(III)–Th(IV)–U(IV) mixtures

Th taken, *mg*	Bi present, *mg*	U present, *mg*	Average Th found,* %	Standard deviation,* %
13.2			100.3	0.5
26.4			100.2	0.4
13.2	500	200	100.4	0.5
26.4	500	200	100.3	0.4

* Ten determinations.

molar ratio to thorium. Iodide exerts a masking effect on nickel. When iron(III) is present 5% ascorbic acid is added until the iodine colour produced by the reduction of Fe(III) has disappeared. Small amounts of zirconium can be masked by glycerine.

The EDTA titrations (with visual end-point detection) of thorium in alloys or minerals often require preliminary separation of the thorium from other ions by precipitation or ion-exchange.[24–28] Such procedures are usually troublesome and time-consuming. The application of thermometric titration would offer many possibilities for the determination of thorium without such procedures. For example, thorium in monazites must be titrated immediately after addition of sodium iodide to the filtrate (3–4*N* hydrochloric acid) from removal of silica[24] and adjustment of the pH to 1.5 with 8*N* sodium hydroxide. The preliminary separation of bismuth is necessary for the titration of thorium in bismuth-based alloys.[25] However, distinct possibility for the titration of thorium in the presence of a large excess of bismuth and uranium(IV) is evident from Table 2. The application to the titration of thorium in bismuth-based alloys was therefore examined. The results are shown in Table 3. Bismuth-based alloys are dissolved in nitric acid (1 + 3),[26] so synthetic solutions were prepared by adding bismuth and uranyl nitrates to thorium solution in nitric acid (1 + 3).

Procedure

The sample solution containing 5–50 mg of thorium was diluted to about 50 ml with dilute nitric acid. Then 15 g of sodium iodide were added, followed by 18 g of sodium nitrate. The pH of the solution was adjusted to 1.5 with 8*N* sodium hydroxide (pH-meter). The solution was transferred to a Dewar vessel and titrated with 0.07*M* Na_4EDTA (1*M* in sodium nitrate) delivered from an automatic titrator.

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COMPARISON OF SAMPLE INTRODUCTION TECHNIQUES WITH A CONTINUOUSLY HEATED GRAPHITE-FURNACE ATOMIZER FOR ATOMIC-ABSORPTION SPECTROPHOTOMETRY

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Summary—A graphite furnace is described which can be operated continuously at temperatures up to 2100°C or be subjected to a temperature ramp running up to 3100°C. Pyrolytic coating of the tubes with carbon before operation was found to be necessary to ensure a satisfactory life-time. Two methods of sample introduction were investigated with a number of salt solutions: continuous nebulization combined with desolvation, with the furnace operated at constant temperature, and deposition of material from an aerosol into the furnace at 150°C for 10 sec before the conventional ramping procedure. The continuous introduction method offers ease of operation, good precision (RSD = 1–1.3%) and moderate sensitivity, but suffers from extensive interferences. The aerosol deposition method combines the merits of conventional furnace atomic-absorption with easier operation, good precision (RSD = 1.8–2.3%) and higher sensitivity.

Flames have been used widely as atomizers in atomic-emission spectrophotometry (AES), atomic-absorption spectrophotometry (AAS) and atomic-fluorescence spectrophotometry (AES). They do, however, suffer from some disadvantages. Winefordner,[1] in a study of typical graphite cells, has shown that the atom concentration can be at least 500 times higher than in a flame, giving rise to an increase in sensitivity by a factor of 10–100 for both atomic absorption and atomic emission. Woodriff and co-workers[2–7] were the first to construct and use a graphite-tube furnace with continuous sample introduction for AAS. More recently, Matousek[8] has shown that the deposition of sample (introduced as an aerosol) into the furnace elegantly combines some of the advantages of furnace AA with the convenience of this form of sampling.

EXPERIMENTAL

Measurement system

The light from an appropriate single-element hollow-cathode lamp was focused by a 50-mm diameter 75-mm focal length convex silica lens onto the blade of a mechanical chopper (Model 9479, Brookdeal Electronics Ltd., Market Street, Bracknell, Berkshire, U.K.) set to produce a modulation frequency of 285 Hz. A lens of 23 mm diameter and 50 mm focal length was used to produce a second image at the centre of the graphite-tube furnace which in turn was focused onto the entrance slit of a Varian Techtron AA4 monochromator (Techtron Pty Ltd., Melbourne, Australia). The photo-current from the photomultiplier tube was detected and amplified with a lock-in amplifier (Model 9501, Brookdeal Electronics). The resulting signals were displayed on a Servoscribe potentiometric chart recorder.

Graphite-furnace atomizer

The graphite furnace used in this study was a modification of the device described by Johnson *et al.*[9] A vertical stem at the top centre of the enclosing chamber was provided for sample introduction and another at the side for the exhaust outlet. Argon was used as the purge gas.[9] The space between the electrodes was enclosed by insulating plates made from Syndanio (Turners Asbestos Co. Ltd., Manchester). Stainless steel was used as a liner on the inner surfaces of these plates to protect them from radiant heat from the tube.

The graphite tubes, 20 mm long, 6 mm diameter and 4 mm bore, were machined from Ringsdorff high-purity graphite rods. A 3.5-mm diameter hole was drilled at the top centre to provide for introduction of the sample aerosol into the tube. Good electrical contact between the tube and the electrodes was achieved by mounting the tube in graphite end-rings. The tubes could be run continuously at temperatures up to 2100°, or up to 3100° intermittently with ramp heating.

Nebulizer

Samples were aspirated into the furnace by a concentric-flow nebulizer fabricated from stainless steel. The stainless-steel uptake capillary was 0.018 in. (0.46 mm) diameter, 0.008 in. (0.20 mm) bore, and the nebulizing orifice was 0.020 in. (0.51 mm), diameter. The uptake rate of the nebulizer varied linearly from 0.7 to 1.0 ml/min as the flow-rate of the carrier gas (argon in this case) was varied from 1.1 to 2.6 l./min. Flow-rates were measured on the low-pressure side downsteam from the nebulizing orifice. For all the elements studied the maximum attainable uptake rate yielded the highest sensitivity and was therefore used in the subsequent experiments. At the higher flow-rates the absorbance *vs.* flow-rate curve tended to level off. The reasons for this have not been established, but decreased transport efficiency through the desolvation system, decreased residence time in the furnace, and cooling of the furnace wall may have been contributory factors.

Solutions

Stock solutions were made from analytical-grade reagents dissolved in demineralized water. The nitrates were

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used for all the elements except molybdenum, for which sodium molybdate was taken, and caesium, for which the chloride was used. For the anions, the corresponding acids were taken (but ammonium chloride was also used).

Sample-introduction system

The sample-introduction system was similar to that described by Veillon and co-workers[10,11] in which the aerosol from the nebulizer was introduced into a heated chamber to evaporate the solvent, leaving tiny solid particles, and then passed through a Friedrich condenser to remove the solvent vapour from the gas stream. Dried particulate matter was then carried through about 500 mm of polyethylene tubing (6 mm bore) to a stainless-steel tube 140 mm long, 4 mm bore, sealed onto the top opening of the furnace chamber. Within the chamber an alumina injector capillary, 1 mm bore and 10 mm long, was set into the end of the stainless steel-tube with epoxy resin, and positioned so that its lower end was 3 mm above the opening in the carbon-tube furnace. Originally a stainless-steel injector was used, but this was liable to melt.

The dimensions of the desolvation chamber were quite critical, and after some experimentation a tube 250 mm long and 50 mm in diameter (150 mm long and 50 diameter was used in reference 10) was selected as providing optimum absorbance signals for samples containing the test element cadmium. For all the elements studied, increasing the temperature of the desolvation chamber resulted in greater absorbances, reaching a maximum at 345°. Further temperature increase had no significant effect on the analytical signals, so 345° was chosen as the optimal temperature. By comparing uptake and drainage volumes it was estimated that, at an uptake rate of 1.9 ml/min, approximately 0.1 ml of solvent per min reached the furnace after desolvation, the condenser removing 95% of the aspirated solvent. Analysis of the drainage solution showed the sampling-system efficiency to be quite high, *ca.* 30%.

Pyrolysis treatment of the graphite tubes

Continuous operation of a graphite tube in an environment saturated with water vapour leads to its rapid oxidation and destruction. Several authors[11–16] have described the treatment of furnace parts to produce a coating of pyrolytic graphite, which exhibits low gas-permeability, high thermal conductivity, high resistance to oxidation and improved analytical sensitivity for several elements.

Before being used for analysis, each new tube was coated by the following procedure: the nebulizer-uptake capillary was blocked to exclude air, a mixture of 97% argon and 3% methane at a flow-rate of 1.8 l./min was introduced into both the purge gas and nebulizer inlets and the tube was raised to a temperature of 2000° for 5 min. After this treatment a hard grey coating could be observed on the tube. In addition, a thin weld between the tube and the carbon contact rings was achieved which, by lowering the contact resistance between the tube and the electrodes, avoided problems of localized heating which could cause the tube to crack.

A coated tube could be operated at a temperature of 2100° for a period of 45 min. In an attempt to extend the working life of a tube a continuous flow of methane was introduced into the atomizer. Optimum flow-rates were found to be 0.2 and 0.13 l./min of methane injected into the purge gas and nebulizer flow respectively; this corresponded to a total of about 0.4% methane in the argon. When the furnace was operated continuously for 1 hr at 2000° with this flow of methane, there was no significant change in the absorbance signals from an aspirated cadmium solution. At the end of the period the surface was seen to be in good condition, with the pyrolytic coating intact. With methane present, however, the sensitivity for cadmium was reduced by 6% and the noise level increased giving a poorer limit of detection. Manning and Ediger[17] also observed a loss of sensitivity and poorer reproducibility when methane was present during the atomization step. For these reasons, the methane–argon gas mixture was not employed in the subsequent experiments.

Temperature measurements

Tube temperatures were measured with a thermocouple in the range from ambient up to 1000° and with an optical pyrometer above 1000°. Both measurements showed the temperature of the tube to vary proportionately with the applied voltage. The slopes of the two curves differed however, with a 400° discrepancy between the two values for a common voltage setting, *e.g.*, at 4 V the thermocouple indicated a temperature of 600° whereas the pyrometer indicated 1000°C. The discrepancy may have been caused by imperfect contact of the thermocouple, local cooling caused by the probe or by variations in the emissivity of the carbon surface. All temperature measurements quoted are therefore considered subject to an uncertainty of ±200°, although the thermocouple measurements are almost certainly more accurate than this at the low end of the range, and the pyrometry measurements at the high end.

RESULTS AND DISCUSSION

Continuous sample introduction

Effect of tube temperature. The effects of varying atomizer temperature for the elements Ca, Cd, Co, Mg, Pb and Zn are shown in Figs. 1 and 2. All the elements exhibited maximum absorbance below 2000° except calcium, for which the maximum probably occurs at *ca.* 2300°. The curves indicate that increasing temperature causes increased atomization efficiency but at higher temperatures rapid gas expansion probably reduces residence times and hence yields lower absorbance values. The observed optimum temperatures for the more volatile elements, *i.e.*, cadmium, lead and zinc, seem rather high but this may indicate that a significant fraction of the analyte is being atomized by the hot argon gas and not by collision with the tube wall (see below).

Effect of purge-gas flow-rate. The temperature of the furnace was nominally fixed (by using the previously determined voltage) at the optimal value for each element and the argon purge-gas flow-rate varied. The elements investigated fell into two groups; those exhibiting a maximum in the absorbance value (see Fig. 3) and those showing a steady decrease in absorbance (see Fig. 4) as the purge-gas flow was increased. The common factors between the two groups are not immediately obvious. Increasing the purge-gas flow will lower the furnace temperature below the optimum values and will also facilitate removal of free atoms from the furnace. These effects will tend to decrease the recorded absorbance. However, increased argon flow will dilute and remove more efficiently any oxidizing species from the enclosed atmosphere, which would tend to increase the absorbance. The shape of the curves probably represents the relative weights of these effects for each element.

Sensitivity, limits of detection and precision. The 2σ detection limits, concentration range (defined as the linear range above the 1% absorption level) and the relative standard deviation at 100 times the detection

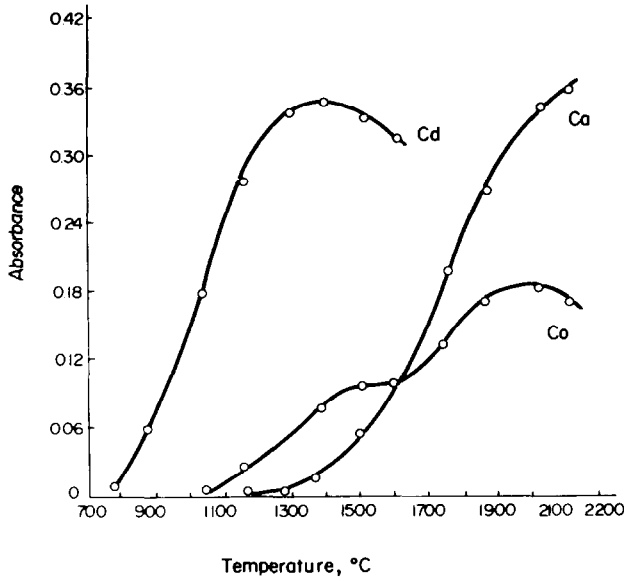

Fig. 1. Variation of absorbance with furnace temperature for Cd (0.3 ppm), Ca (1.0 ppm) and Co (1.0 ppm).

limit (measured from 10 determinations) are shown in Table 1. As expected, the detection limits do not compare favourably with those achieved with the normal injection technique, but the reproducibility is better and the working range similar.

Interferences. To investigate the effect of various additional ions on the analytical signal the concentrations of the test elements were held constant and the interference effects studied at different interferent-to-analyte concentration ratios. The results are summarized in Table 2.

Most ions produced depressions in the analytical signal, but there were some exceptions. Both calcium and magnesium showed enhancement in the presence of the easily ionizable elements sodium and caesium at low concentrations, but depression at higher concentrations. This suggests that ionization may have been suppressed but that the effect was outweighed by a depressive effect at higher interferent concentrations. Cadmium showed a consistently high enhancement (not attributable to ionization suppression) with either sodium nitrate or caesium chloride, and a similar tendency with chloride (added as ammonium chloride). The atomic absorption of zinc was depressed by low concentrations of sulphate but enhanced by high concentrations. Aluminium, silicon, vanadium, molybdenum and phosphate are all known

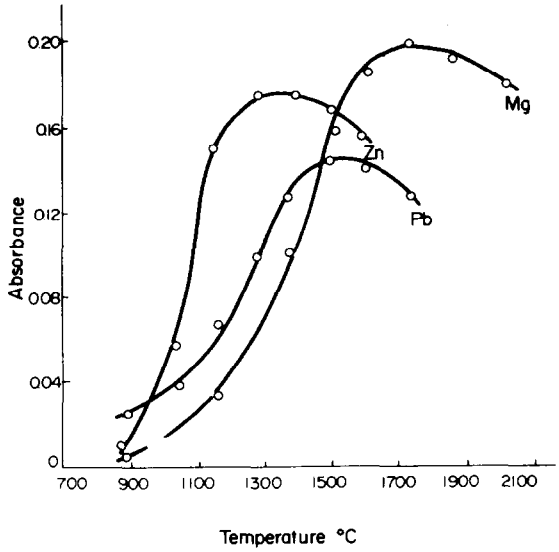

Fig. 2. Variation of absorbance with furnace temperature for Pb (5.0 ppm), Mg (0.3 ppm) and Zn (0.1 ppm).

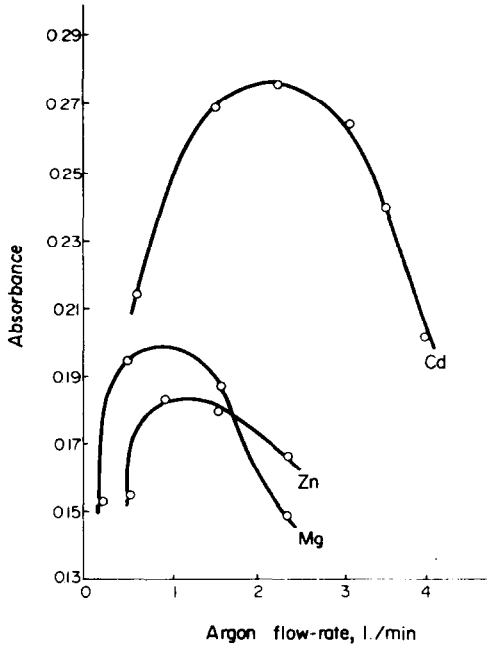

Fig. 3. Variation of absorbance with purge-gas flow-rate for Cd (0.3 ppm), Mg (0.3 ppm) and Zn (0.1 ppm).

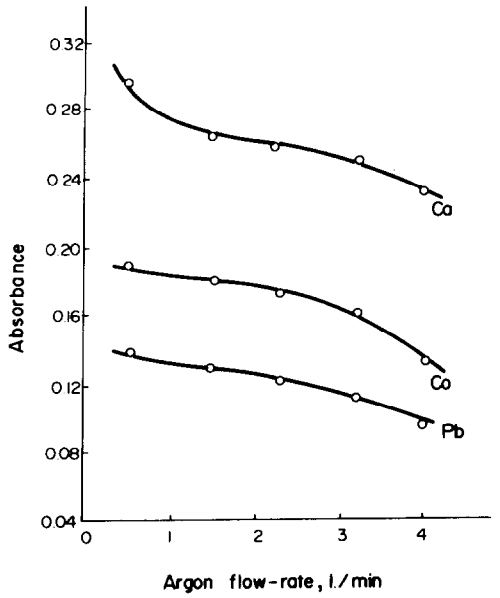

Fig. 4. Variation of absorbance with purge-gas flow-rate for Ca (1.0 ppm), Co (1.0 ppm) and Pb (5.0 ppm).

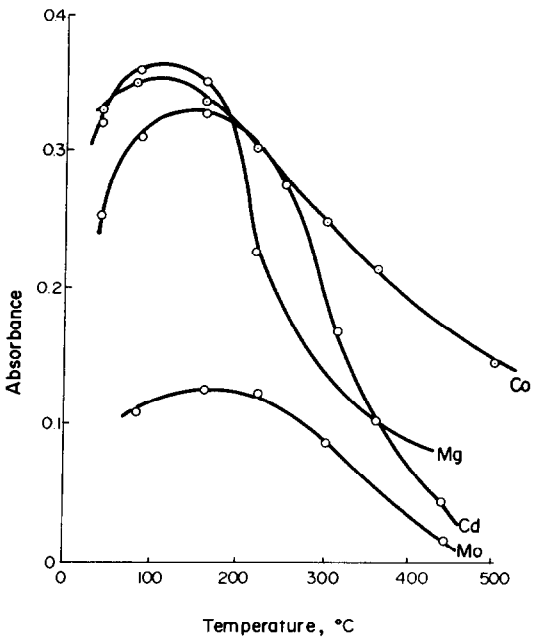

Fig. 5. Variation of absorbance with drying temperature of the furnace for Ca (5.0×10^{-3} ppm), Mo (2.5×10^{-2} ppm), Cd (1.0×10^{-3} ppm) and Mg (2.5×10^{-3} ppm), by the aerosol-deposition technique.

to form thermally stable compounds which can result in reduced atomization efficiency both for the species themselves and for other elements which may react with them or be occluded in the matrix of the nebulized particulates. All the test elements showed some depression of absorbance by this group, and cobalt was found to be the most severely affected. Conversely, lead showed remarkable freedom from interference and calcium, of all the elements tested, showed the least effect with phosphate.

The extensive interferences encountered indicate that an electrothermal furnace operated with continuous sample introduction must have a relatively low efficiency as an atomizer. This results from the rather low wall temperature of the carbon furnace, and because a significant proportion of the atomization probably takes place in the gas phase at even lower temperatures.

Aerosol-deposition method

Continuous introduction of sample aerosol into the graphite furnace gives a relatively poor absolute sensitivity because of the inefficiency of the nebulization and sample-introduction system. The discrete sample-introduction method normally used with graphite rods and tubes is limited to very small volumes, and can only give satisfactory precision in the hands of experienced operators or with automatic sampling devices. The aerosol deposition technique[7] in which sample in aerosol form is deposited onto the walls of the furnace for a pre-set period before the atomization step, can be used to overcome these difficulties.

Effect of deposition temperature. Optimal values of the operating parameters were found to be the same as for the continuous sample-introduction method. The elements studied were calcium, cadmium, magnesium and molybdenum.

The results of monitoring the absorbance as a function of the deposition temperature[8] are shown in Fig. 5. Temperatures above 200° probably result in gas expansion away from the heated surface which

Table 1. Determinations with continuous sample-introduction

Element	Wavelength, *nm*	Atomizer temperature, °C	Characteristic* concentration, *ppm*	Limit of detection, *ppm*	Linear range, *ppm*	RSD, %
Ca	422.67	2100	0.015	0.006	0.015–2	1.3
Cd	228.80	1400	0.005	0.0015	0.005–0.8	1.1
Co	240.72	2000	0.033	0.01	0.033–2.5	1.0
Mg	285.21	1750	0.0085	0.004	0.0085–1.3	1.3
Pb	283.31	1500	0.13	0.04	0.13–32	1.3
Zn	213.86	1400	0.006	0.002	0.006–0.85	1.2

* IUPAC term for that concentration which will give a 1% absorption signal under the given conditions.

Table 2. Interferences observed with continuous sample-introduction, expressed as % enhancement or depression

Interferent	Ratio: Interferent/Analyte	Cd (228.80 nm) 0.1 ppm	Ca (422.67 nm) 0.5 ppm	Co (240.72 nm) 1.0 ppm	Pb (283.31 nm) 1.0 ppm	Mg (285.21 nm) 0.2 ppm	Zn (213.86 nm) 0.1 ppm
Na	10	45	35	−20	−4	10	−8
	100	50	10	−32	−30	2	−28
	1000	50	−25	−50	−35	−11	−39
	5000	50	−35	−65	−50	−30	−55
Cs	10	28	25	−11	−30	10	−5
	100	55	40	−28	−30	20	−20
	1000	67	15	−45	−30	7	−35
	5000	67	−25	−53	−30	−15	−48
Al	10	0	−10	−30	−11	−30	−20
	100	−18	−25	−50	−21	−40	−30
	1000	−38	−38	−90	−45	−45	−52
	5000	−45	−50	−100	−45	−45	−60
Si	10	−40	−8	−40	−12	0	−12
	100	−20	−18	−80	−12	0	−20
	1000	−18	−30	−95	−12	0	−25
	5000	−18	−38	−100	−23	−15	−25
V	10	−14	−12	−25	0	−30	−10
	100	−18	−18	−55	0	−66	−16
	1000	−20	−32	−80	0	−80	−25
	5000	−23	−48	−100	0	−80	−30
Mo	10	−10	−10	−30	0	−55	−15
	100	−16	−19	−60	0	−55	−20
	1000	−16	−28	−95	0	−55	−28
	5000	−16	−35	−95	0	−70	−32
PO_4^{3-}	10	−11	0	−8	−9	−10	−25
	100	−20	−4	−30	−35	−15	−40
	1000	−42	−10	−55	−60	−18	−55
	5000	−55	−13	−55	−80	−20	−68
SO_4^{2-}	10	0	−4	−3	0	−25	−15
	100	0	−8	−6	−30	−25	−6
	1000	0	−10	−9	−40	−25	20
	5000	0	−12	−14	−40	−25	28
Cl^-	10	15	18	0	30	−3	−20
	100	30	30	0	20	−3	−31
	1000	40	42	0	0	−10	−40
	5000	55	50	−9	0	−10	−65

thus may provide an aerodynamic barrier to particle deposition. It would appear from the results that temperatures below 100° result in wetting of the furnace wall or establishment of a liquid surface film which considerably reduces the capture efficiency. From the results shown in Fig. 5, a deposition temperature of about 150° would seem to be best.

Effect of deposition time. The effect of varying the deposition time was studied by aspirating a solution containing 5×10^{-4} ppm of cadmium into the furnace with the temperature set at 150° and atomizing at a maximum temperature of 1400°. An almost linear plot of absorbance against deposition time was obtained over the range 0–20 sec; a 10-sec deposition time was used for all subsequent experiments.

Sensitivity, limits of detection and precision. These parameters are summarized in Table 3. Most important to note are the low detection limits achieved by using the relatively short 10-sec deposition period. For comparison, when the normal injection technique

Table 3. Determinations with the aerosol-deposition technique

Element	Wavelength, *nm*	Maximum atomizer temperature, °C	Characteristic concentration, *ppm*	Limit of detection, *ppm*	Linear range, *ppm*	RSD, %	Stopped-flow characteristic concentration, *ppm*
Ca	422.67	2100	7.2×10^{-5}	4×10^{-5}	7.2×10^{-5}–5.0×10^{-3}	1.8	2.4×10^{-5}
Cd	228.80	1400	2.3×10^{-5}	1×10^{-5}	2.3×10^{-5}–2.2×10^{-3}	2.3	6.5×10^{-6}
Mg	285.21	1750	3.5×10^{-5}	2×10^{-5}	3.5×10^{-5}–5.0×10^{-3}	2.2	1.7×10^{-5}
Mo	313.26	3030	1.0×10^{-3}	5×10^{-4}	1.0×10^{-3}–9.0×10^{-2}	2.0	5.5×10^{-4}

was used with the same furnace, the detection limit for molybdenum with hydrogen as purge-gas[18] was 5×10^{-3} ppm, an order of magnitude worse than the 5×10^{-4} ppm achieved here. The relative standard deviations at 100 times the detection limit are worse than those for the continuous sampling method, but are achieved at much lower concentrations. The sensitivity can be improved further, as shown in Table 3, by using the stopped-flow atomization technique. For this, the flow of the purge-gas is temporarily interrupted during the atomization step. Interferences encountered with the deposition method are probably similar to those found for the normal discrete sample-injection method.

Conclusions

The results presented show that a continuously operable graphite furnace coupled to a suitable nebulization/desolvation system has potential for routine analytical applications. For relatively volatile elements, continuous sampling can be employed with moderate detection power and good precision. The technique requires little operator skill and is readily amenable to automation and unattended operation. The principal disadvantage possessed by this technique, in common with all other electrothermal techniques, in comparison with flame atomization, is the extensive interferences that occur, but these may be minimized by appropriate choice of conditions.

However, the use of this system with the aerosol deposition technique is of greater importance than its use in the continuous sample-introduction mode. The aerosol deposition technique combines the high sensitivity of furnace techniques with the precision and convenience of operation associated with the equivalent flame method.

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CHROMATOGRAPHIE VON METALLCHELATEN—IX

ADSORPTIVE VORANREICHERUNG FÜR BESTIMMUNGEN VON KOBALT, KUPFER UND NICKEL IM MIKROGRAMM/LITER-BEREICH NACH UMKEHRPHASEN-CHROMATOGRAPHIE DER DIÄTHYLDITHIOCARBAMATE

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Zusammenfassung—Es wird ein Verfahren zur Bestimmung von Kupfer, Kobalt und Nickel als Diäthyldithiocarbamate aus wässriger Lösung im unteren µg/l.-Bereich vorgestellt. Die Trennung erfolgt mit Hilfe der Hochdruck-Flüssigkeits-Chromatographie (HPLC) auf einer RP ("reversed phase")-Phenyl-Phase, bestimmt wird photometrisch über UV/VIS-Detektion. Die notwendige Voranreicherung der derivatisierten Metallionen aus wässriger Lösung wird durch Adsorption der hydrophoben Chelate auf einer ebenfalls mit RP-Phenyl-Phase gepackten Vorsäule erreicht. Die Elution der Probe von der Vorsäule direkt auf die analytische Säule ermöglicht eine vollständige Nutzung der angereicherten Menge und schließt gleichzeitig eine Kontamination mit den zu bestimmenden Elementen aus. Das Elutionsmittel dient dabei als mobile Phase für die anschließende Trennung. Um Blindwertprobleme und Austauschphänomene zu vermeiden wurde das gesamte System ausschließlich aus Glas und Teflon als Werkstoffe aufgebaut. Quantifiziert wurde im Bereich von 0,2–10 µg/l. bei einem Anreicherungsvolumen von 50 ml. Durch Erhöhung des Anreicherungsvolumens läßt sich die Nachweis- bzw. Bestimmungsgrenze weiter erniedrigen. Begrenzende Faktoren sind dabei Blindwert- und Adsorptionserscheinungen.

Die Anwendungsmöglichkeiten der Hochdruck-Flüssigkeits-Chromatographie (HPLC) in der anorganischen Spurenanalyse wurde in mehreren Zusammenfassungen beschrieben.[1–4] Die Trennung der Metalle erfolgt nach vorangehender Derivatisierung bzw. Chelatisierung sowohl durch Adsorptions- als auch durch "reversed phase"-Chromatographie. Mit der Bildung der Metallchelate lassen sich ganz allgemein die Methoden der Chromatographie von organischen Molekülen für die Kationenanalyse einsetzen. Als geeignete Chelatbildner für die Trennung von Neutralchelaten erwiesen sich u.a. Dialkyldithiocarbamate,[3,5,6] Alkylxanthogenate,[7] Dithizon,[8] 1,2-Diketone,[9] 1,2-Diketo-bis-thiosemicarbazone,[6,10] 1,2-Diketo-bis-thiobenzhydrazone,[11] substituierte 8-Hydroxy-chinoline,[12] 8-Mercaptochinolin,[13] substituierte Formazane[14,15] und 1-Hydroxy-2-pyridinthion.[16]

Die Anwendbarkeit der HPLC auf spurenanalytische Problemstellungen macht eine Anreicherung der Elemente durch Extraktion der Chelate mit organischen Lösungsmitteln (Flüssig–Flüssig-Verteilung) vor der eigentlichen Trennung und Bestimmung notwendig. Die Flüssig–Flüssig-Verteilung hat jedoch für die Spurenanalyse im µg/l.-Bereich erhebliche Nachteile: die Extraktion gestaltet sich schwierig[3] und nur ein Teil der angereicherten Menge wird im Regelfall auch zur Bestimmung genutzt, was den Anreicherungseffekt entscheidend verringert. Einengen des Extraktes auf 20–30 µl kann dem entgegenwirken.[7]

In der vorliegenden Arbeit wird als Alternative die Anreicherung durch Adsorption der Chelate auf einer Vorsäule beschrieben, wobei die anschließende Trennung durch Umkehrphasen-Chromatographie eine vollständige Nutzung der angereicherten Probe erlaubt. Das Schema in Abb. 1 faßt die Arbeitsschritte des Verfahrens zusammen. Die Bestimmung von Kupfer, Kobalt und Nickel als Diäthyldithiocarbamate im unteren µg/l.-Bereich ist auf diese Weise mit einfachen Mitteln ohne großen Arbeitsaufwand möglich.[17] Die direkte Kopplung des Anreicher-

VIII. Mitteilung: M. Lohmüller, P. Heizmann, K. Ballschmiter, *J. Chromatog.*, 1977, **137**, 165.

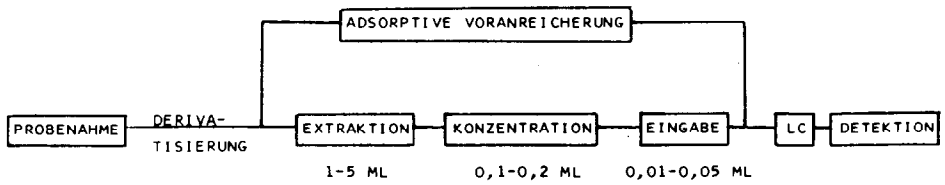


Abb. 1. Arbeitsschritte bei Anreicherung durch Extraktion und Adsorption.

ungsschrittes mit der anschließenden Trennung und Bestimmung vermindert die Gefahr der Kontamination. Schwierigkeiten ergeben sich weniger aus dem chromatographischen Verfahren als vielmehr aus Grenzflächen- und Werkstoffphänomenen beim Arbeiten mit Lösungen im µg/L-Bereich. Die Adsorption von organischen Molekülen an hydrophoben Polymeren oder alkylierten Silicagelen ist zur Spurenanreicherung aus Wasser mehrfach beschrieben worden.[18–20]

EXPERIMENTELLER TEIL

Geräte zur HPLC

Analytische Pumpe: HPLC-Pumpe M-6000 (Waters Associates Inc., Milford)

Anreicherungspumpe: Dosierpumpe Pro Minent electronic (cfg Heidelberg)

Aufgabesystem: HPLC-Probenaufgabeventil R 6031 SVK (Latek, Heidelberg)

Detektor: Spektrophotometer Typ LC-55 (Perkin-Elmer, Überlingen)

Trennsäule: Glas, Länge 10 cm, stationäre Phase Chromosorb LC-5 Phenyl, 37–44 µm

Sorptionsmaterial: Chromosorb LC-5 Phenyl, 37–44 µm (Johns-Manville, Denver, Colorado)

Eich- und Analysenvorschrift

Zur Kalibrierung werden durch Verdünnen einer schwach sauren 10 ppm-Stammlösung bezogen auf das jeweilige Metallkation wässrige Lösungen im Bereich von 0,2–10 µg/l. jeweils erneut hergestellt. Ein aliquoter Teil der Eichlösung, im Regelfall 50 ml, wird mit Hilfe eines $Na_2CO_3/NaHCO_3$-Puffers auf den pH-Wert 10,5 eingestellt. Die Derivatisierung der Metallionen erfolgt durch Zugabe eines 100–150 fachen Überschusses an Natriumdiäthyldithiocarbamat in Form einer 10^{-2}%igen wässrigen Lösung vom pH 10–11 mit anschließendem leichten Schütteln von 80–90 sec. Zur Einstellung des Adsorptions–Desorptions-Gleichgewichtes der Chelate an der Wand des 1-Liter Glaskolbens ist eine Standzeit der Reaktionslösung von 15 min erforderlich. Danach werden jeweils 50 ml direkt aus dem Reaktionsgefäß über die Vorsäule angereichert. Ein anschließendes "Spülen" der Vorsäule mit 25 ml zweifach destilliertem Wasser von pH 10,5 führt zu einer Erniedrigung des Blindwertsignals verursacht durch den Chelatbildner, ohne die Quantifizierung der angereicherten Metallchelate zu beeinflussen. Durch Umschalten des Ventils erfolgt mit Hilfe des als mobile Phase verwendeten Lösungsmittelgemisches die Elution der Chelate von der Vorsäule auf die analytische Säule.

Die zu analysierende Probelösung wird in entsprechender Weise behandelt, das zur Anreicherung verwendete Probe-Volumen beträgt ebenfalls 50 ml.

Zum Spülen bzw. Konditionieren der Gefäße siehe weiter unten. Die verwendeten Metallsalze bzw. Reagenzien besaßen sämtlich p.a. Qualität. Der Chelatbildner wurde durch Extraktion mit Chloroform von Metallverunreinigungen befreit. Zur Herstellung der Eichlösungen wurde ausschließlich zweifach über Kaliumpermanganat destilliertes Wasser verwendet.

ERGEBNISSE UND DISKUSSION

Anreicherungssystem

Es wurde gezeigt, daß sich Diäthyldithiocarbamat-Chelate auf RP*-Phasen mit Methanol/Wasser oder Acetonitril/Wasser als mobile Phase trennen lassen.[5] Benutzt man nur Wasser als mobile Phase werden die Metallchelate auf RP-Phasen vollständig retardiert, da die Probemoleküle aufgrund ihrer starken Hydrophobie aus dem Laufmittel Wasser in bzw. an die stationäre lipophile Phase gedrängt werden. Dieser Vorgang kann zur Anreicherung von Metallspuren in Form ihrer Diäthyldithiocarbamat-Chelate aus wäßriger Lösung auf einer mit RP-Phase gepackten Vorsäule ausgenützt werden. Die Chelatisierung der Metallionen erfolgt in der Analysenlösung durch Zugabe des Chelatbildners Natriumdiäthyldithiocarbamat (Na-DDTC) in 100–150fachem Überschuß in Form einer 10^{-2}%igen wäßrigen Lösung. Die Komplexierung der Metalle verläuft schnell und quantitativ. Durch die Schwerlöslichkeit der Diäthyldithiocarbamate in Wasser ist die adsorptive Anreicherung zwangsläufig auf den Spurenbereich beschränkt, da sich bei höheren Konzentrationen die Metallchelate durch Fällung der weiteren Analyse entziehen. In diesem Fall ist die Extraktion als Anreicherungsmethode anzuwenden[3] oder es sind Dithiocarbamate mit höherer Wasserlöslichkeit, z.B. die Diäthoxyäthyldithiocarbamate, einzusetzen.[6]

Die Elution der auf der Vorsäule adsorbierten Chelate erfolgt ohne weitere Zwischenschritte direkt auf die analytische Säule, wobei das Elutionsmittel gleichzeitig als mobile Phase für die Trennung dient. Bei dieser Art der Anreicherung wird die gesamte angereicherte Menge zur Bestimmung ausgenützt, was die Nachweis- und Bestimmungsgrenze erheblich erniedrigt.

Abbildung 2 zeigt den schematischen Aufbau der verwendeten Apparatur. Die Chelatlösung wird mit Hilfe einer Pumpe P2 über die Anreicherungssäule, gepackt mit einer Phenyl-Phase, 40 µm, gepumpt. Als Aufgabesystem wird dann ein Probenaufgabeventil aus Teflon benützt, bei dem Anstelle der üblichen Probeschleife die Vorsäule eingesetzt ist. Durch Umschalten des Ventils erfolgt nach Beendigung der Anreicherung die Elution der adsorbierten Chelate mit Hilfe der analytischen Pumpe P1 direkt auf die Trennsäule. Als Elutionsmittel diente ein Methanol/Wasser-Gemisch im Verhältnis 4:1 (v/v).

Da der Anreicherungsschritt keine pulsationsfreie Förderung der Probelösung erfordert, wurde hierfür eine Membranpumpe aus Teflon mit einer Förderleistung von 10 ml/min verwendet wodurch sich 50 ml der Probelösung in 5 min anreichern lassen. Das gesamte System ist mit Ausnahme der analytischen Pumpe metallfrei aufgebaut, um Blindwertprobleme

* RP = "reversed phase".

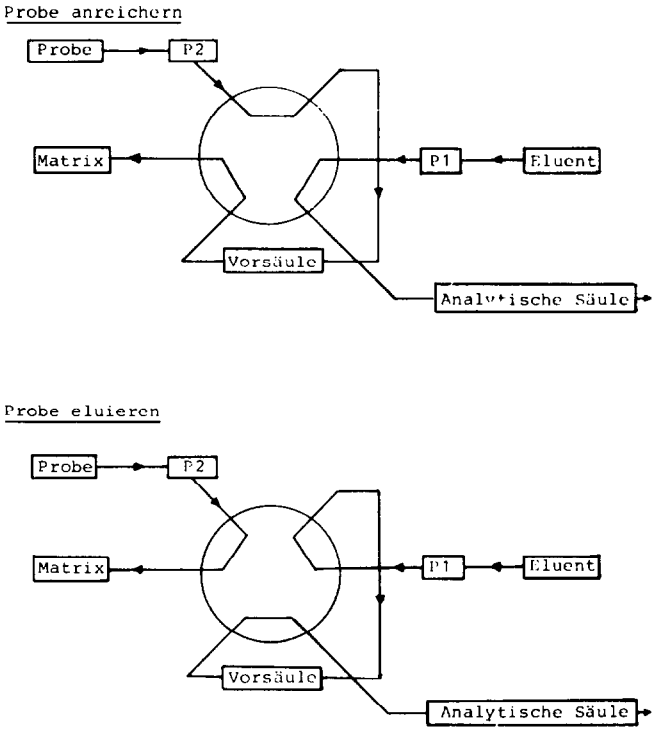


Abb. 2. Schematischer Aufbau des Systems.

und Austauschphänomene auszuschließen. Als Werkstoffe wurden Glas (analytische Säule) und Teflon (Vorsäule, Anreicherungsventil, Kapillaren) verwendet. Die Vorsäule selbst besteht aus einem Teflonrohr von 20 mm Länge und 2 mm innerem Durhmesser.

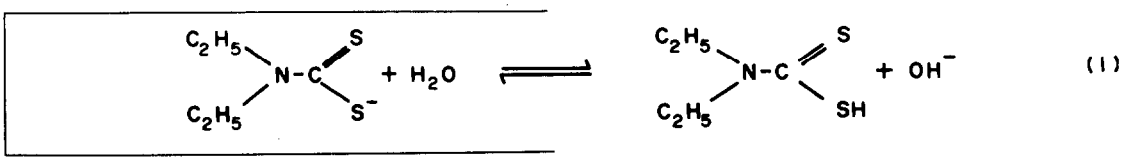
Trennleistung des Systems

Der maximal zulässige Druckabfall von 35 bar bei der Verwendung von Glas und Teflon als Werkstoffe erfordert Säuler mit ausreichender Permeabilität, was die Korngröße des verwendeten RP-Materials (>10 μm) beschränkt. Damit ist die Trennleistung zwangsläufig limitiert. Abbildung 3a zeigt die Trennung nach Anreicherung von $Cu(DDTC)_2$, $Co(DDTC)_3$ und $Ni(DDTC)_2$ auf einer trocken gepackten 15-cm Säule, stationäre Phase RP-Phenyl (40 μm), mobile Phase Methanol/Wasser 3:1 (v/v), Druckabfall 30 bar und zum Vergleich dazu Abb. 3b die optimierte Trennung derselben Chelate auf einer nach der "balanced density"-Methode[21] selbst gepackten 30-cm Säule (i.D. 3 mm), stationäre Phase Lichrosorb RP-18 (5 μm), mobile Phase Acetonitril/Wasser 3:1 (v/v) bei einem Druckabfall von 140 bar. Die Probenaufgabe der Lösung von Metalldithiocarbamaten erfolgte hier über eine Probeschleife.

Kobalt and Nickel mit Hilfe eines UV/VIS Detektors mit variabler Wellenlängeneinstellung aufgrund der unterschiedlichen Absorptionsspektren ihrer Chelate praktisch störungsfrei nebeneinander bestimmt werden, solange eine Komponente nicht in starkem Überschuß vorliegt. $Co(DDTC)_3$ und $Ni(DDTC)_2$ besitzen bei 325 nm ein Maximum, wohingegen $Cu(DDTC)_2$ bei dieser Wellenlänge nur sehr schwach absorbiert. $Co(DDTC)_3$ und $Ni(DDTC)_2$ lassen sich auf einer 15-cm Säule mit 40-μm Material trennen (vgl. Abb. 3a). Andererseits weisen im Absorptionsmaximum des $Cu(DDTC)_2$ bei 440 nm die Chelate von Kobalt und Nickel ein Minimum auf.

Blindwertprobleme

Als chelatisierendes Reagenz dient das Natrium-Salz der Diäthyldithiocarbaminsäure, welches in großem Überschuß zur Analysenlösung zugegeben wird. Diese ionogene Verbindung sollte während des Anreicherungsschrittes nicht auf der RP-Phase zurückgehalten werden und somit die Bestimmung der Metallchelate nicht stören. Messungen zeigten jedoch, daß zwar nicht das Salz, wohl aber die freie Säure, welche sich durch Hydrolyse des Salzes bildet,



(1)

Trotz dieser nicht befriedigenden Trennleistung bei Verwendung von 40-μm Material können Kupfer, adsorbiert wird und sich bein schlechter Trennleistung der Säule störend auswirkt.

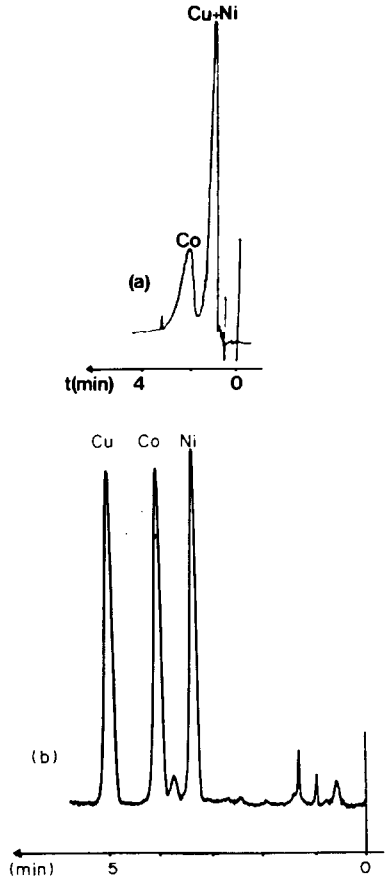


Abb. 3. Flüssigchromatographische Trennung von Co, Cu und Ni als Diäthyldithiocarbamate. (*a*) Adsorptive Anreicherung; Säule: 10 cm, LC-5 (37–44 μm); mobile Phase: Methanol/Wasser (3:1); Fluß 1 ml/min; Druckabfall 30 bar; Detektion 440 nm. (b) Probenaufgabe über Probeschleife; Säule: 30 cm, RP-18 (5 μm); mobile Phase: Acetonitril/Wasser (3:1); Fluß 1 ml/min; Druckabfall 140 bar; Detektion 250 nm.

Dieses durch den Chelatbildner verursachte Analysensignal und damit "Blindwert" läßt sich durch Erhöhung des pH-Wertes der Analysenlösung und der damit verbundenen Verschiebung des Gleichgewichtes (1) in Richtung des Salzes soweit verringern, daß eine Bestimmung von Kobalt und Nickel auch in Bereichen < 1 μg/l. nicht gestört wird. Die durch die Carbaminsäure verursachte Störung wirkt sich beim Cu-$(DDTC)_2$ von vornherein weniger stark aus, da die Carbaminsäure bei einer Wellenlänge von 440 nm nur schwach absorbiert.

Die Peaks in Abb. 3b im Bereich zwischen 0,5 und 1,5 Minuten sind nicht identifiziert worden; sie sind jedoch *organischen* Verunreinigungen zuzuordnen (Lösungsmittel-Peak, Zersetzungs-Produkt des Chelatbildners: Thiuramdisulfid, usw.). Ebenso ist der Peak zwischen dem Co- und Ni-Signal einer organischen Verunreinigung zuzuordnen.

Abbildung 4 zeigt die Abhängigkeit störender Absorption der Carbaminsäure vom pH-Wert. Dabei wurden 490 ml Wasser mit 10 ml einer 10^{-2}%igen Na-DDTC-Lösung versetzt und davon 50 ml angereichert, gemessen wurde bei einer Wellenlänge von 325 nm. Als optimal erwies sich ein pH-Wert von 10–11.

Zur Herstellung der Eichlösungen wurde zweifach über Kaliumpermanganat destilliertes Wasser verwendet (Quarz-Apparatur), um Störungen durch organische hydrophobe Spurenbestandteile, die ebenfalls mit angereichert werden, auszuschließen. Abbildung 5 zeigt den Meßwert einer 1-μg/l. Nickel-Eichlösung nach Derivatisierung, Anreicherungsvolumen 50 ml, und das entsprechende Blindwertsignal, welches sich aus dem tatsächlichen Nickel-Blindwert und dem Signal des nicht abgetrennten Chelatbildners zusammensetzt.

Quantifizierung

Zur Erstellung der Eichkurve wurden Metallionen-Lösungen im Bereich von 0,2–10 μg/l. durch Verdünnen einer 10-μg/ml. Stammlösung hergestellt. Nach Zugabe der Puffer- und der Reagenzlösung erfolgt die Derivatisierung durch leichtes Umschütteln. Aus der jeweiligen Lösung werden 50 ml über die Vorsäule angereichert und anschließend eluiert. Als Elutionsmittel dient bei Kupfer und Kobalt Methanol/

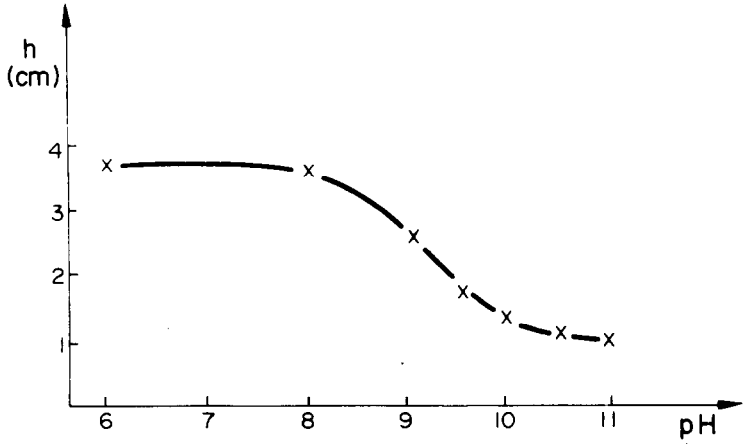


Abb. 4. Peakhöhe des Chelatbildners in Abhängigkeit vom pH-Wert.

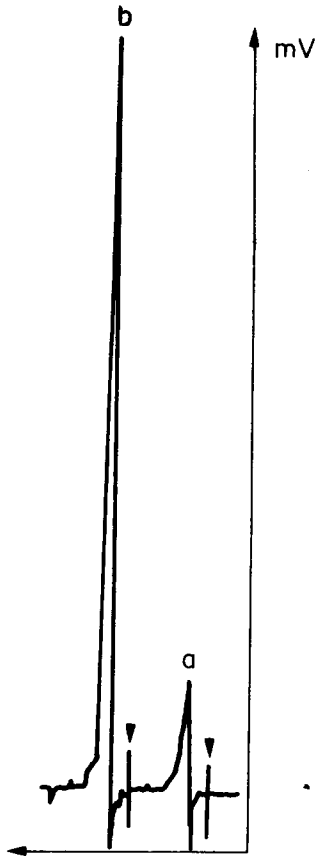


Abb. 5. Meßwert einer 1-μg/l. Ni-Eichlösung nach Derivatisierung Anreicherungsvolumen 50 ml (b), mit dem entsprechenden Blindwertsignal (a); Säule 10 cm, LC-5 (37–44 μm), mobile Phase Methanol/Wasser (3:1), UV-Detektion 325 nm.

Tabelle 1. Reproduzierbarkeit der Anreicherung für 50 ml einer 10-μg/l. Kupferlösung; Detektion 440 nm

Peakhöhe, *mm*				
141	141	139	138	138
135	139	136	141	137

$\bar{x}$ = 138,5 mm
s = 2,2 mm
V = 1,6%

Wasser 4:1 (v/v), bei Nickel Methanol/Wasser 3:1 (v/v).

Die Reproduzierbarkeit der *Anreicherung* ergab für eine 10-μg/l. Kupferlösung eine relative Standardabweichung von 1,6%. Das Anreicherungsvolumen betrug jeweils 50 ml, die Anreicherungsgeschwindigkeit 10 ml/min und der Fluß 1 ml/min. Die Anreicherung erfolgte jeweils aus der gleichen 500-ml Probelösung.

Diese Reproduzierbarkeit ist aber nur zu erreichen, wenn die Wand des Aufbewahrungsgefäßes (Duran-Glas oder Quarz) durch vorangehendes Konditionieren mit einer Chelatlösung in etwa vergleichbarer Konzentration und anschließendes vorsichtiges Spülen mit zweifach destilliertem Wasser mit den Chelatmolekülen gesättigt ist. Ist dies nicht der Fall, so macht sich in den betrachteten Konzentrationsbereichen die Adsorption an der Gefäßwand stark bemerkbar. Abbildung 6 zeigt die Verluste durch Adsorption der Chelate am Beispiel einer 10-μg/l. Kupferlösung nach der Derivatisierung, wobei das Gefäß, ein 1-liter Glaskolben *nicht* konditioniert war.

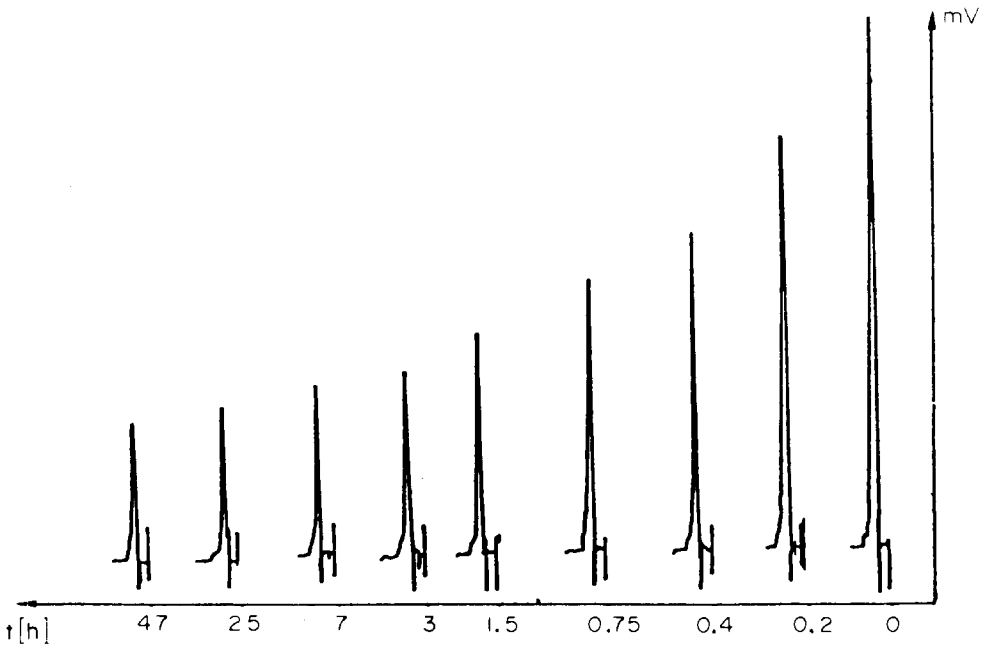


Abb. 6. Adsorptionsverluste in Abhängigkeit von der Zeit am Beispiel einer 10-μg/l. Cu-Lösung nach Derivatisierung und Anreicherung von jeweils 50 ml der Probelösung.

Tabelle 2. Reproduzierbarkeit der Anreicherung von 50 ml einer 1-µg/l. Nickellösung nach Derivatisierung; Detektion 325 nm

Ansatz	Peakhöhe, *mm*		
1	111	111	107
2	105	102	101
3	112	103	103

$\bar{x}$ = 106,1 mm
s = 4,3 mm
V = 4,0%

Die Adsorption voni onen läßt sich in wäßriger Lösung durch Verwendung von Quarz-Geräten und durch Erniedrigung des pH-Wertes unterdrücken.[22] Bei organischen Molekülen ist dies nicht möglich. In den vorliegenden Konzentrationen sind die Verluste durch Adsorption der Chelate auch bei konditionierten Glasgefäßen in den ersten Minuten nach Zugabe des Chelatbildners nicht vermeidbar. Während der Einstellung des Adsorptions–Desorptions-Gleichgewichtes an der Gefäßwand änderten sich die Meßwerte stetig. Hat sich das Gleichgewicht einmal eingestellt, bleiben die Meßwerte über einen längeren Zeitraum konstant bei einer relativen Standardabweichung von $<5\%$, in Konzentrationsbereichen <1 µg/l. von $<10\%$. Dieser unvermeidbare systematische Fehler durch die Adsorptionsverluste läßt sich jedoch durch eine Standardisierung des gesamten Verfahrens klein und konstant halten, sodaß über eine entsprechende Eichung die Richtigkeit der Analysenergebnisse gegeben ist. Tabelle 2 zeigt die Reproduzierbarkeit der Anreicherung am Beispiel einer 1-µg/l. Nickellösung nach Einstellung des Adsorptionsgleichgewichtes an der Gefäßwand. Aus drei verschiedenen Ansätzen der Eichlösung wurden jeweils dreimal 50 ml angereichert. Abbildung 7 zeigt die Eichkurven zur Bestimmung von Kupfer, Kobalt und Nickel. Bei der Kupferbestimmung wurde die Eichung aus einem Duran-Glasgefäß (Gerade I) und aus einem Quarz-Glasgefäß (Gerade II) vorgenommen. Man erkennt die unterschiedlichen Adsorptionseigenschaften der Gläser. Daraus folgt, daß sowohl Eichung als auch Probenahme und Analyse aus bzw. in demselben Gefäß erfolgen muß.

Die Wiederfindegrate wurde nicht benau bestimmt. Sie läßt sich jedoch aus der Höhe der Extinktion bei bekanntem Volumen der Mikrodurchflußküvette des UV/VIS-Detektors und bekannten molaren Extinktionskoeffizienten der Metallchelate grob abschätzen. Sie liegt über 65%.

Anwendungen

Die beschriebene Methode ist zur Untersuchung von Elementspuren in wäßrigen Lösungen geeignet. Da nach Aufschlußverfahren wäßrige Lösungen erhalten werden, ist eine Anwendung auch für feste Proben geeignet, insbesondere für die Bestimmung kleinster

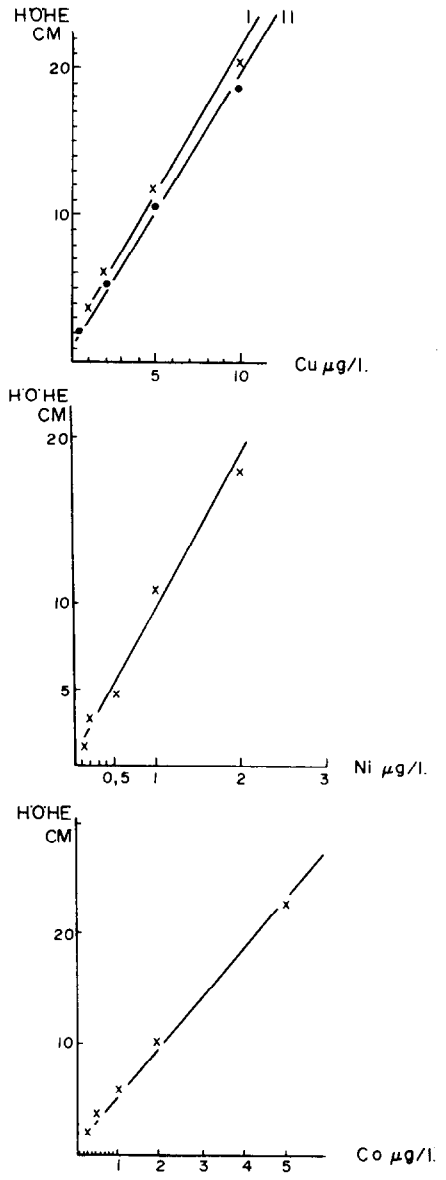


Abb. 7. Eichkurven zur Bestimmung von Cu, Co und Ni.

Gehalte von Kobalt, Kupfer und Nickel in organischer Matrix.

Bei einem Anreicherungsvolumen von 50 ml konnten Konzentrationen von 0,4 µg/l. Kupfer, 0,2 µg/l. Kobalt und 0,1 µg/l. Nickel quantitativ erfaßt werden. Eine weitere Erniedrigung der Bestimmungsgrenzen läßt sich ohne zusätzlichen Arbeitsaufwand durch Erhöhung des Anreicherungsvolumens erreichen. Die limitierenden Faktoren sind dabei die Adsorptionserscheinungen und die Blindwertprobleme.

Soll eine Umweltprobe auf ihren Gehalt an Metallspuren untersucht werden, so ist es notwendig, eine "Blindwert"-Messung *ohne* vorangehende Chelatbildung durchzuführen, um mögliche Störungen durch organische Spurenbestandteile zu erkennen. Tritt ein Blindwertsignal auf, lassen sich die

unerwünschten Begleitkomponenten auf einer weiteren Vorsäule *vor* der Derivatisierung abtrennen. Danach werden die Metallspuren chelatisiert, angereichert und bestimmt.

Als Anwendungsbeispiele seien zwei Flußwasser-Analysen aufgeführt.

Donauwasser. Die Probenahme erfolgte in einem Quarzkolben, Entnahmeort war Ulm. Es wurden jeweils drei Bestimmungen aus drei Proben vorgenommen.

Cu^{2+}	$3{,}9 \pm 0{,}2$ µg/l.
Ni^{2+}	$2{,}4 \pm 0{,}2$ µg/l.
Co^{2+}	n.n., $< 0{,}2$ µg/l.

Rißwasser. Die Probenahme erfolgte wie vorher, Entnahmeort war Biberach.

Cu^{2+}	28 ± 2 µg/l.
Ni^{2+}	$2{,}5 \pm 0{,}1$ µg/l.
Co^{2+}	n.n., $< 0{,}2$ µg/l.

Auffallend ist der hohe Cu^{2+}-Gehalt in der Riß. Der Durchschnittwert relativ stark beanspruchter Gewässer wie Rhein, Ems, Donau und Weser liegt bei 3–6 µg/l.[23]

Das beschriebene Anreicherungs- und Bestimmungsverfahren schließt eine Kontamination der Probe mit den zu bestimmenden Elementen weitgehend aus. Probenahme, Derivatisierung, Anreicherung und Bestimmung erfolgen in einem geschlossenen System. Es werden nur sehr geringe Mengen an Derivatisierungs- und Pufferreagenzien benötigt, deren Verunreinigungen mit den zu bestimmenden Metallen leicht zu kontrollieren sind.

Die Verwendung von Glas und Teflon erfordern einen Druckabfall von weniger als 35 bar, was durch die damit verbundene Verwendung von relativ grobkörnigem Packungsmaterial zu begrenzten Trennleistungen der analytischen Säule führt. Erste Vorversuche zeigten jedoch, daß durch geeignete Packungsmethoden auch mit 5-µm Material ein Druckabfall von weniger als 35 bar bei einer Säulenlänge von 15 cm und einem Fluß von 0,6 ml/min zu verwirklichen ist. Die Trennung von sieben Metallchelaten des Tetramethyldithiocarbamats wurde beschrieben.[24]

Durch die Anwendung anderer Chelatbildner, bei denen dieser kein Störsignal erwarten läßt, kann die Trennung auf andere pH-Bereiche und auch andere Metallionen angewendet werden. Als spezifisches Reagenz für die Kupferbestimmung wurde das Dinatriumsalz der Bathocuproindisulfonsäure verwendet. Vor der eigentlichen Chelatbildung ist dabei eine Reduktion von Cu^{2+} zu Cu^{+} erforderlich. Anreicherung und Bestimmung erfolgte unter den gleichen Bedingungen wie bei Kupferdiäthyldithiocarbamat.[17]

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Summary—Cobalt, copper and nickel can be determined in the lower µg/l. range by preconcentration of their diethyldithiocarbamates from water on reversed-phase material. The enrichment step is done on a precolumn (length 20 mm, diameter 2 mm) of LC 5-Phenyl Reversed-Phase material. As the chelates are eluted directly onto the analytical column, the whole sample can be used and any contamination by handling can be easily controlled. The chelates are separated by high-pressure liquid chromatography on the same reversed-phase material with methanol/water (3:1 v/v) as eluent. It is necessary to use glass or Teflon for constructing the analytical system, to avoid ligand exchange, memory and contamination phenomena. With a 50-ml sample, the determination can be done in the 0.2–10 µg/l. range. The limiting factors are adsorption phenomena. Below the 0.2 µg/l. level this source of systematic error cannot be easily controlled.

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THE DETERMINATION OF OXYGEN IN POWDERED TUNGSTEN CARBIDE BY REDUCING FUSION: SUPPRESSION OF PRIMARY CARBON DIOXIDE, SELECTION OF WORKING CONDITIONS AND ADSORBED-MOISTURE EFFECTS

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Summary—A method for the reducing fusion determination of oxygen in powdered tungsten carbide is described. It is shown that, as theory predicts, tungsten carbide may evolve its oxygen content not only as carbon monoxide but also as perceptible quantities of carbon dioxide. An easy suppression of this primary carbon dioxide, and thus of any error associated with it made when computing the sample oxygen content, is obtained by the simple expedient of dropping the samples into the crucible a few seconds after having started the instrument analytical cycle, *i.e.* when the crucible is already at very high temperature. Criteria followed in the selection of the sample fusion conditions and their possible application to the analysis of massive tungsten carbide samples are discussed. The very large effect of the adsorbed moisture content on the results is made evident, and practical means (preliminary water desorption from samples kept at 200° under high vacuum) to overcome it are suggested.

Because of its primary importance for the hard metals industry, powdered tungsten carbide (WC) received attention in the last year in the Community Reference Bureau (BCR)† programme, as a candidate reference material for oxygen determination. The reducing fusion determination of oxygen in WC is not new, and references to it, though not abundant, may be found in the relevant literature.[1–4] These papers, because of their more general character, pay only marginal attention to this determination, and because of our interest in it, in our capacity as a BCR-collaborating laboratory, a re-investigation was decided on, aimed at finding the best operating conditions to be used with the commercial impulse-heating inert-gas fusion instrument (LECO TC 36) available in our laboratory.

Together with an evaluation and assessment of some practicable sample-fusion conditions, this paper will show some peculiarities of this determination which render it rather interesting from a more general point of view.

EXPERIMENTAL

All the experiments were done on powdered WC having an average particle size of about 2 μm; unless specified, apart from the time spent in preparation of the samples for analysis, the material and the analytical samples were constantly kept in a desiccator over anhydrous magnesium perchlorate until the time of analysis.

Table 1 gives the main characteristics of the instrument used for this work. Further information pertinent to the discussion which will follow, is that the standard crucible referred to has an inner surface area of *ca.* 5.9 cm^2 and a capacity of *ca.* 1.4 cm^3 and in operation it stands in a furnace cavity of volume *ca.* 7 cm^3. The crucible leans with its bottom against an electrode and with its mouth against a hollow counter-electrode which also acts as the supply line for the helium stream, which probably behaves, because of its direction, like a sort of gaseous lid which favours contact of the evolved gases with the crucible walls. The crucible reaches its working temperature within a few seconds from the start of the degassing time.

For the sake of completeness it is recalled that whereas the instrumental nitrogen blank is usually negligibly small, the oxygen blank, measured on different outgassed crucibles that are either empty or contain *in-situ* outgassed bath materials such as those which will be described later, amounts in average to *ca.* 6.5 $\pm$ 1 μg of oxygen (evolved entirely as CO; see also Fig. 1, later). It is nevertheless common practice in the authors' laboratory to use as a blank for a given analytical sample the individual value measured with the pertinent single-use crucible, rather than an average value.

In favourable operating conditions the instrument has a conservative detection limit of *ca.* 1 ppm oxygen or nitrogen; its range extends to an upper limit which is mainly set by the smallest mass of sample still meaningfully usable; the response linearity of the overall system extends to at least 500 μg of oxygen or nitrogen.[5]

RESULTS AND DISCUSSION

In reducing fusion procedures, the oxygen content of the material under examination is always calculated on the assumption that the oxygen has been evolved wholly as CO; this is true for most metals, but cases may exist where oxygen is partially evolved as some other chemical species, especially CO_2.[6] If this fact is not given due heed, the result of the determination is obviously liable to error.

† Community Reference Bureau, Directorate General XII, Commission of the European Communities, 200 rue de la Loi, Brussels, Belgium.

Table 1. Essential features of the impulse-heating, inert-gas fusion apparatus LECO TC 36

Simultaneous determination of N, O.
Unlidded single-use graphite crucible heated directly through the Joule effect; working temperature fixed at *ca.* 2150–2250°C (corresponding to a current of 1.02–1.08 kA flowing through the empty crucible); sample degassing time fixed at 30 sec; helium carrier-gas at *ca.* 160 cm^3/min with flow direction opposite to that of the gases coming from the crucible.
Measurement of the extracted N_2 and CO by gas chromatography as N_2 and CO_2, after oxidation of any evolved H_2 and CO to H_2O and CO_2 in a CuO reactor followed by H_2O removal in an "anhydrone" reactor: helium carrier-gas at *ca.* 160 cm^3/min, 30–60 mesh silica gel column (18 × 0.25 in.) at 50°C, thermal conductivity cell, electronic signal integration, digital read-out, provision for recorder attachment.
Home-made calibration system[5] with known and fixed amounts of pure N_2 and CO_2, instead of the usual manufacturer's calibration system with metals of certified N and O content.

Table 2A. Free-energy variations for reactions supposed to occur simultaneously within the analytical sample

	ΔG°, *cal* (Ref. 7)	Temperature (T) validity range, *K*
$0.5WO_2 + WC = 1.5W + CO$	$48200 - 39.65T$	298–1500
$1.5W + 1.5C = 1.5WC$	$-13650 + 0.60T$	298–2000
$2CO = C + CO_2$	$-40800 + 41.70T$	298–2000
$0.5WO_2 + 0.5C + CO = 0.5WC + CO_2$	$-6250 + 2.65T$	298–1500

Equilibrium constant k for the overall reaction (extrapolated to 2273 K): $k = \exp[-(\Delta G^\circ/RT)] = 1.052$
Ratio P_{CO_2}/P_{CO}, assuming ideal behaviour, excess of carbon, WO_2 dissolved in WC: $P_{CO_2}/P_{CO} = k(X_{WO_2})^{1/2}$ where X_{WO_2} is the mole fraction of WO_2 in WC.

Table 2B. Examples of calculated amounts of oxygen extracted from the analytical sample as CO and as primary CO_2

O content of WC, *ppm*	X_{WO_2}	P_{CO_2}/P_{CO}	O as CO, *ppm*	O as CO_2, *ppm*
100	6.12×10^{-4}	0.0260	95	5
500	3.06×10^{-3}	0.0582	448	52
1000	6.12×10^{-3}	0.0823	859	141
2000	1.23×10^{-2}	0.1164	1622	378

Remark. On the assumption that the oxygen extraction will conform to the theoretical predictions, an instrument based on the measurement of the extracted CO measures the amount given by column 4; an instrument which is based on the measurement of CO_2 after oxidation of CO to CO_2, measures the amount given by column 4 *plus* half of that given by column 5.

A simplified thermodynamic approach, such as that shown in Table 2, indicates indeed tnat a not negligible equilibrium ratio CO_2/CO may occur within a WC sample submitted to high temperature in a graphite crucible. The trend shown by the data of Table 2 must be regarded as only an indication of a possible behaviour, which should be experimentally verified. The actual ratio could be substantially different from that calculated, not only because of the oversimplified model used, but also because the so-called primary CO_2, once it has left the sample, comes into contact with the hot walls of the graphite crucible, where it tends to give CO through the well-known reaction $CO_2 + C = 2CO$, which in practice is shifted completely to the right at temperatures higher than *ca.* 1100°. Reconversion of any CO_2 into CO will be a function of contact time, temperature, surface area and volume of the crucible, and thus dependent on the working conditions and instrument used.

As Fig. 1A shows, appreciable quantities of primary CO_2 are indeeed evolved from the WC under study when the samples are dropped into the crucible under the customary sample-dropping conditions, *i.e.* at room temperature before starting the analytical cycle (which corresponds to sample degassing and measurement of the extracted gases). The chromatograms presented as well as any measurements of primary CO_2 (see later) were obtained by removing the CuO reactor from the instrument (see Table 1) and replacing it by a tube filled with glass-wool and kept at room temperature, so as to avoid the oxidation of any extracted CO. As Fig. 1B shows, the simple expedient of dropping the samples into the crucible 5 sec after having started the analytical cycle, *i.e.*, at an already very high crucible temperature, has the effect of supressing any primary CO_2 formation, thus leading to a correct oxygen contant determination.

It must be noted that the behaviour depicted is not peculiar to the sample-fusion conditions used for Figs. 1A and 1B, which are given only as an example, but was found to occur under all the conditions which will be described next.

Preliminary experiments showed the unsuitability, at least for our instrument, of using pure nickel or iron as bath materials for dissolving WC in the degassing step of the process (cracks on crucibles, bad dissolution, some evidence of getter effects *etc.*). Thus, after some other experiments, the sample-fusion conditions submitted to final examination were those summarized in Table 3, leading to the results pre-

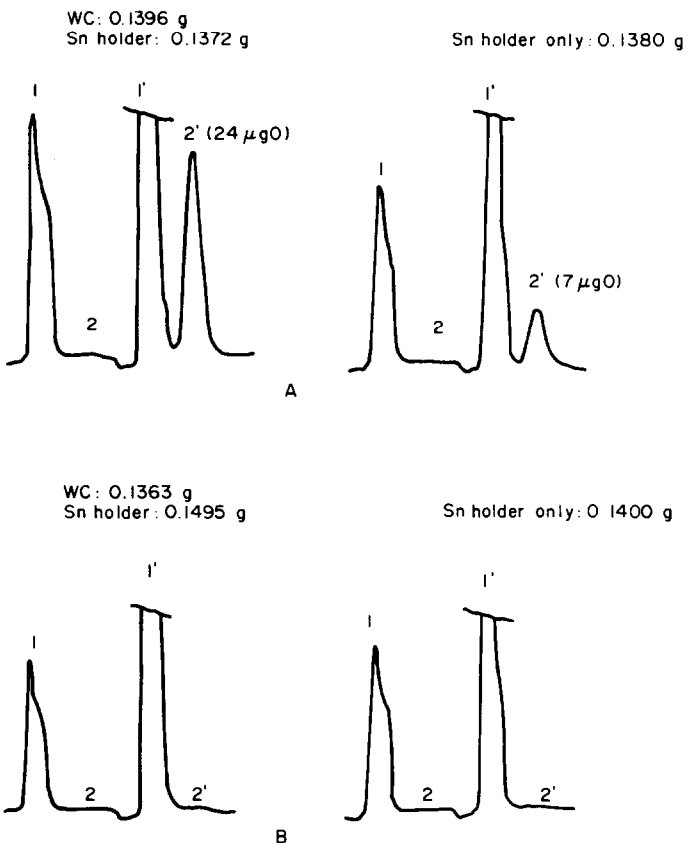

Fig. 1. Extraction of primary CO_2 from WC samples and Sn holders. 1 and 1': $H_2 + N_2 + CO$; 2 and 2'; primary CO_2 (if any). 1 and 2: crucible blank; 1' and 2'; materials analysis. A—sample dropped into empty crucible before start of analytical cycle. B—sample dropped into empty crucible 5 sec after start of cycle.

sented in Table 4, all of which were obtained by dropping the samples into the crucible 5 sec after starting the analytical cycle.

Although a certain difference appears between the Pt/WC and the Sn/WC results, the agreement may be considered satisfactory: it is believed that the difference should be ascribed to some small error made in determining the oxygen content of the Pt and Sn holders (*ca.* 18 and 230 ppm, respectively), rather than to some inefficiency in the oxygen extraction. However, although all the fusion conditions examined appear satisfactory for the analysis of this particular WC, the remarks expressed in Table 4 indicate that only conditions 3, 4 and 6 could perhaps be fully practicable for the analysis of massive WC samples (possibly with small alterations: for instance the use of Pt or Sn holders could prove to be useless for massive samples and, for condition 4, Sn could be placed and outgassed directly in the crucible, with an obvious improvement in the analysis blank).

For the results to be consistent, the WC oxygen content calculated by summing up that determined

Table 3. Sample-fusion conditions examined for oxygen determination in WC

1. WC contained in a home-made Pt holder (Pt/WC ~ 1/1) and dropped for reduction in its single-use empty outgassed crucible.
2. Same as 1, execpt for the crucible, which contained an *in-situ* outgassed Ni 75/Sn 25 bath (bath/WC ~ 5–10/1).
3. Same as 1, except for the crucible, which contained an *in-situ* outgassed Fe 75/Sn 25 bath (bath/WC ~ 5–10/1).
4. WC contained in a home-made Sn holder (Sn/WC ~ 1/1) and dropped for reduction in its single-use empty outgassed crubible.
5. Same as 4, except for the crucible, which contained an *in situ* outgassed Ni 75/Sn 25 bath (bath/WC ~ 5–10/1).
6. Same as 4, except for the crucible, which contained an *in situ* outgassed Fe 75/Sn 25 bath (bath/WC ~ 5–10/1).

Remark. WC weight always within the range 0.12–0.16 g. Holders made from Pt or Sn foils.

Table 4. Results obtained under the conditions given in Table 3, expressed as *ppm* oxygen

1 Pt/WC	2 Pt/WC Ni/Sn bath	3 Pt/WC Fe/Sn bath	4 Sn/WC	5 Sn/WC Ni/Sn bath	6 Sn/WC Fe/Sn bath
1971	1946	1970	1991	1956	2054
1967	1991	1970	2050	2065	1978
1968	1951	1970	1978	1967	2030
1936	1979	1964	1976	2028	1999
1946	1981	1985	2062	2079	1998
$\bar{X} \pm s$ 1958 ± 16	1970 ± 20	1972 ± 8	2011 ± 41	2019 ± 56	2012 ± 30

All samples dropped into crucible 5 sec after start of the analytical cycle (high temperature). $\bar{X}$ = mean value; s = standard deviation.

Remarks on fusion conditions, after crucible visual inspection:

1. Sample not completely reacted with Pt; considering the WC particle size (~2 μm), at least partial CO diffusion–extraction from the solid, possibly prevented by larger dimensions, could occur.
2. Sample fairly well dissolved in the bath, which only slightly sticks to the crucible.
3. Sample very well dissolved in the bath, which is not stuck to the crucible (buttonlike shape).
4. Sample wholly reacted with Sn to give a non-brittle mass silver-coloured throughout its bulk.
5. Sample sometimes not dissolved in the bath, which sticks only slightly to the crucible; considering the frequently incomplete dissolution, CO extraction could occur by the same mechanism as under 4.
6. Sample very well dissolved in the bath, which is not stuck to the crucible (buttonlike shape).

under the customary sample dropping conditions (which includes half the oxygen from primary CO_2) and that from half the primary CO_2 directly measured, should equal that determined by dropping the samples at an already high crucible temperature, in which case any primary CO_2 formation is fully suppressed.

Table 5A shows, however, a very remarkable difference between the two results, which was believed to be produced, despite the desiccator storage conditions, by some water adsorbed on the material. Such water, which desorbs at low temperatures, is likely to be less efficiently converted into CO and some CO_2 if the samples undergo a progressive heating from a crucible standing at room temperature. The Karl Fischer method proved the astonishing presence of *ca.* 900 ppm of water which, though apparently enormous in view of the WC storage conditions, is not so large considering the particle size (~2 μm) of the WC studied. In fact, assuming perfectly cubic particles, it corresponds to a water surface-cover of *ca.* 0.5 μg/cm² and probably less in fact, since the true surface is certainly greater than the geometrical one calculated.

With these indications as guidelines, the weighed samples were submitted to water desorption for 3 hr in a high vacuum at 200°, then cooled in the same vacuum and immediately analysed. Sample exposure to ambient air before analysis was certainly reduced to less than a minute.

The results, presented in Table 5B, were in excellent agreement, thus showing that water was the cause of the previous discrepancy. Considering that a desorption period of 20 hr at 200° did not appreciably alter the results of Table 5B, we regard them as a good

Table 5. Comparison of results, *ppm*, (A) without and (B) with sample water-desorption treatment

A Sn/WC			B Pt/WC Fe/Sn bath		
a	*b*	*c*	*a*	*b*	*c*
1679	101	1991	1485	13	1499
1693	97	2050	1492	17	1522
1728	71	1978	1497	29	1531
1744	132	1976	1510	23	1532
1685	135	2062	1541	14	1563
$\bar{X}$ 1706	107	2011	1505	19	1529
$\bar{X}_a + 0.5\bar{X}_b$ 1759			1515		

a = sample dropped into crucible before starting the analytical cycle (room temperature).

b = same as *a* and with CuO reactor removed from the instrument in order to measure any primary CO_2.

c = sample dropped into crucible 5 sec after start of the analytical cycle (high temperature).

Table 6. Percentages of the oxygen content of various materials evolved as primary CO_2

Material (pieces, unless specified)	Approximate oxygen content, *ppm*	Oxygen as primary CO_2 (as % of content)
Pd (Heraeus)	13	~9
Pt (Heraeus)	5	~8
Pt (foil, Heraeus)	18	~25
Sn (Merck)	15	~16
Sn (foil, BDH)	230	~18
Ni (Merck)	50	~4
Fe (Pierchimica)	700	~1
Steel (501–660 LECO)	30	~6
Steel (501–551 LECO)	300	~2
Cu (deox, 18 BCR)	70	~2
Cu (ind, BCR)	190	~2
Cu (ETP, 22 BCR)	140	~2

Remark. All determinations carried out without using any auxiliary metal bath or flux. Sample weight in general in the range 0.5–1 g.

estimate of the true oxygen content of the WC examined.

It can be concluded that under our operating conditions, whilst the formation of primary CO_2 from the WC studied is easily perceptible but perhaps negligible at the oxygen level measured, adsorbed water unexpectedly has a very significant effect on the results, and this might be expected to hold for all fine powders. It may be suggested that, all other conditions remaining identical, a WC with a lower oxygen content will produce even smaller quantities of primary CO_2, while a WC with a larger particle size will give less pronounced adsorbed water effects (assuming a constant density of water surface-cover, the concentration of adsorbed water should be inversely proportional to the linear particle size).

As complementary information, Table 6 gives the fraction of the oxygen of various materials that will be evolved as primary CO_2, as measured under our operating conditions.†

† This work was carried out within the framework of the JRC–METRE programme. The paper was presented at the Conference "The Analysis of Non-Metals in Metals", West Berlin, 10–13 June 1980.

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BIS(4-HYDROXYPENT-2-YLIDENE)DIAMINOETHANE AS A REAGENT FOR COPPER

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Summary—A rapid, simple extractive spectrophotometric method for the determination of copper, based on the formation of a purple complex between the metal and bis(4-hydroxypent-2-ylidene)diaminoethane is described. The properties of the reagent and some of its colour reactions with other metal ions are reported. The copper complex has an absorption maximum at 540 nm and its molar absorptivity in chloroform is 1.36×10^3 l.mole^{-1}.cm^{-1}. Beer's law is valid up to 384 μg/ml with an optimum working range of 36–334 μg/ml. Job's method and the molar ratio method indicate 1:2 (metal/ligand) composition for the complex. The effects of pH, time, temperature, order of addition of reagents, reagent concentration, and interference from various ions are reported. The method is applied to the direct determination of copper in copper-base alloys in the presence of zinc and some other metals without prior separation.

Belcher *et al.*[1] have studied the gas chromatographic, thermogravimetric and mass spectral characteristics of some tetradentate β-ketoamine complexes which they had briefly noted previously.[2] These complexes were first prepared by Combes,[3] investigated by Morgan,[4] and comprehensively reviewed by Holm *et al.*[5]

Hitherto these compounds have not found much use either as extractants or as spectrophotometric reagents, although as quadridentate ligands they have been of great interest in co-ordination chemistry. In this paper the use of bis(4-hydroxypent-2-ylidene)diaminoethane (BHPDE) as a photometric reagent for the rapid determination of copper(II) in copper-base alloys in the presence of associated elements such as zinc and iron is described.

EXPERIMENTAL

Reagents

All reagents and solvents were of analytical grade. All aqueous solutions were prepared with distilled demineralized water.

BHPDE solution, 0.1*M*. Freshly prepared.

Copper nitrate solution, 0.1*M*. Standardized by titration with EDTA, and diluted as required.

Buffer solutions. Clark and Lubs solutions[6] were used for pH 1–10, and those for pH 11–13 were made as described by Kolthoff *et al.*[6]

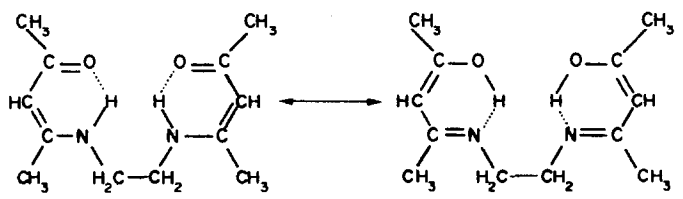
Synthesis of the reagent

Ethylenediamine (6.01 g; 0.1 mole) was added gradually with stirring to acetylacetone (20.02 g; 0.2 mole) in a 250-ml beaker, both liquids having been chilled in an ice–salt mixture for about 30 min. If the ethylenediamine solidified during cooling it was just liquified by very gentle heat. The reaction was exothermic and initially a whitish solid was formed, but as more ethylenediamine was added more heat was generated and the reaction mixture became hot and deep golden yellow. The reaction mixture was stirred intermittently until it had cooled sufficiently for crystallization to begin, leading to formation of a pale cream cake. The product was recrystallized twice from carbon tetrachloride, to yield whitish crystals (94% yield; m.p. 108–109°; found C 64.3%, H 9.0%, N 12.6%; calculated for $C_{12}H_{20}O_2N_2$: C 64.3%, H 8.9%, N 12.5%).

Properties of the reagent

BHPDE is moderately soluble in water, and readily soluble in methanol, ethanol, acetone and chloroform.

The bands (cm^{-1}) in the infrared spectrum (KBr discs) are assigned to the stretching vibrations of –NH– involved in –N–H...O hydrogen-bonding (3160 s) and >C=O (1605 vs). The ultraviolet spectrum of a methanolic solution of the reagent shows absorbance maxima at 322 nm (log ε = 4.53) and 302 nm (log ε = 4.48), where ε is in l.mole^{-1}.cm^{-1}. Table 1 summarizes the chemical shifts (δ, ppm) of the protons in BHPDE dissolved in various solvents, while Scheme 1 gives one possible tautomeric equilibrium. Some other tautomeric species are possible but the



Ketoamine Hydroxyimine

Table 1. Proton NMR data for BHPDE in various solvents (chemical shifts in ppm)

Solvent	Methyl a	Protons b	Methine proton	Methylene bridge protons	Amino proton	Hydroxyl proton
CCl_4	1.93	1.98	4.9	3.4, 3.6	6.2	10.8(br)
$CDCl_3$	2.0	2.05	5.2	3.4, 3.6	6.5	10.5(br)
MeOH-d_4	1.90	1.95	5.05	3.5, 3.65	—	10.77(br)
D_2O	1.73	1.78	4.95	3.33	5.83	—
DMSO-d_6	1.83	1.88	4.99	3.33, 3.5	—	10.63(br)

br = broad.

proton NMR data are rationalized in terms of these two forms. The NMR data indicate that BHPDE exists as a tautomeric mixture of the ketoamino and hydroxyimino forms in CCl_4 and $CDCl_3$, and as the hydroxyimine in methanol and DMSO-d_6, and as the ketoamine in D_2O.

A 0.1*M* aqueous solution of the reagent gave a pH value of 8.6. Protonation was investigated by titration with 0.1*M* hydrochloric acid. The protonation constant, log K_p, was found to be 3.5. The single pK value may be interpreted as revealing simultaneous protonation of the two identical >N–H sites in the ligand (ketoamine form) to give H_2L^{2+}.

Reactions of BHPDE

The reactions of the reagent with 37 metal ions were studied at various pH values ranging from 1 to 13. Li, Na, K, Hg(II), Pb, Bi, Ca, Mg, Sr, Al, Sb(III), In, Rh(III), La, Gd, Nd, Pr, Eu, Er, Lu, Hf, Pt(IV), Zr, V(V), Ta and Mo(VI) gave no colour reactions under the conditions of the tests, although colourless solid lanthanide complexes could be prepared under certain conditions. The most important colour reactions are summarized in Table 2.

Determination of copper

In aqueous medium. Place 6 ml of buffer solution (pH 6) in a 50-ml standard flask. Add 6 ml of 0.1*M* BHPDE followed by 1.0 ml of copper solution containing 0.2–7 mg of Cu(II), giving a final Cu(II) concentration of 4–140 μg/ml. Shake and dilute to the mark with distilled water. Measure the absorbance in 1-cm cells at 540 nm against a reagent blank. This procedure is for copper(II) solutions with concentrations not exceeding 144 μg/ml since at higher concentrations some of the complex is precipitated.

By extraction into chloroform. Place 10 ml of buffer solution (pH 6) in a 50-ml separating funnel. Add 25 ml of 0.1*M* BHPDE followed by 1.0 ml of Cu(II) solution containing 0.2–18 mg of Cu(II). Dilute to about 50 ml with distilled water and extract with 50 ml of chloroform by vigorous shaking for 2 min. Measure the absorbance at 540 nm against a chloroform blank prepared in the same way.

Table 2. Characteristics of qualitative colour reactions of BHPDE with some cations

Cation	pH	Colour of precipitate (ppte) or solution (soln)
Cu(II)	2–12	purple ppte.
Ni(II)	6–12	brown ppte.
Co(II)	8–12	golden yellow soln.
Zn(II)	6–7, 11–12	white ppte.
Pd(II)	1	red ppte.
	2–12	yellow ppte.
Cr(III)	1–12	greenish soln.
Fe(II)	1–12	golden yellow soln.
Fe(III)	1–13	wine red soln.
Au(III)	1–6	blue-black soln.
U(VI)	1–2	deep golden yellow soln.

In copper-base alloys. Dissolve 0.20 g of alloy in 5 ml of nitric acid (1 + 1) (plus a few drops of concentrated hydrochloric acid for bronzes). Evaporate nearly to dryness and dissolve the residue with 150 ml of distilled water, filtering off any precipitate and collecting the filtrate and washings in a 250-ml standard flask. Make up to the mark with distilled water. Analyse aliquots of this solution by the chloroform extraction method.

By differential spectrophotometry

Dissolve about 0.45 g of pure copper (accurately weighed) in 15 ml of dilute nitric acid (1 + 1). Transfer the solution to a 50-ml standard flask and dilute to the mark with distilled water and mix. Pipette 5 ml into a separating funnel containing 20 ml of buffer solution (pH 6) and 25 ml of 0.3*M* BHPDE and shake vigorously for 2 min with 50 ml of chloroform. Use the chloroform extract as the reference solution.

Dissolve about 0.60 g (accurately weighed) of alloy (Cu > 80%) or 0.90 g (Cu 55–80%) in 15 ml of dilute nitric acid (1 + 1). In the case of bronzes add a few drops of conc. hydrochloric acid to aid dissolution. Evaporate on a water-bath to 5–10 ml, then dilute to about 30 ml with distilled water and filter off any precipitate. Make up the filtrate and washings to volume in a 50-ml standard flask. Treat 5 ml of this solution in the same way as the reference solution, and measure the absorbance of the extract at 540 nm against the reference. Translate the absorbance into concentration difference from a calibration curve, and add this concentration to the reference concentration.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of aqueous solutions of the complex, the reagent and copper nitrate, all at pH 6, are shown in Fig. 1. The maximum absorbance of the complex in the visible region is at 540 nm, and the absorbance of the reagent is very small.

Effects of experimental variables

pH. The absorbance–pH graph for the copper(II) complex (at 540 nm) is shown in Fig. 2. The absorbance was maximal and constant over the pH range 3–12. A pH of 6 was chosen for further work. Extraction was found to be quantitative at all pH-values studied.

Reagent concentration. Maximum absorbance was obtained with at least a 5-fold molar excess of reagent relative to copper(II). Using a greater concentration of the reagent up to 10-fold excess did not alter the absorbance values. It was therefore decided to use at least a 5-fold excess of reagent. The order of addition of the reagents is immaterial.

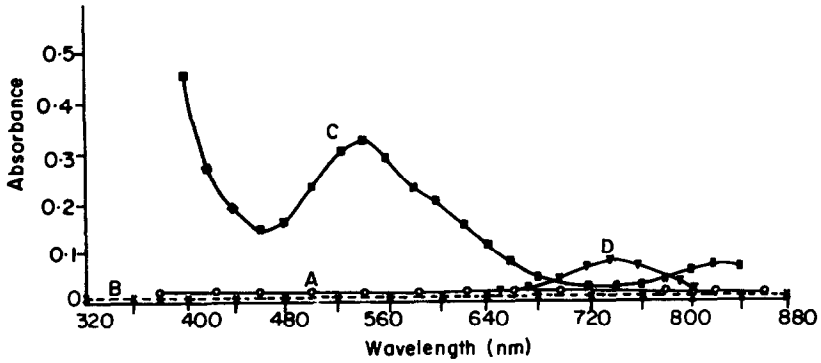


Fig. 1. Absorption spectra. A—reagent (0.033*M*) blank *vs.* water; B—copper nitrate (0.0033*M*) *vs.* water; C—copper complex *vs.* reagent blank (copper 0.0033*M*, BHPDE 0.033*M*); D—copper nitrate (0.02*M*) *vs.* water.

Time. The stability of the colour was studied at room temperature (~25°). Maximum absorbance was obtained after 10 min and remained constant for at least 1 hr. A development time of 10 min was therefore chosen.

Temperature. Absorbance measurements were made at intervals of 1°. The highest absorbance was obtained at 15° (0.580) and the lowest absorbance was obtained at 35° (0.520). Constant absorbance was obtained between 21 and 29°. All determinations were carried out at room temperature (~25°).

Calibration range, sensitivity, and precision

In aqueous solution, Beer's law is obeyed up to 144 μg/ml (144 μg/ml corresponds to 0.24 absorbance). Precipitation occurs at higher concentrations. At 540 nm the molar absorptivity in aqueous medium is 1.02×10^3 l.mole^{-1}.cm^{-1}. In chloroform, Beer's law is obeyed up to 384 μg/ml with an optimal working range of 36–334 μg/ml (Ringbom plot). At 540 nm, the molar absorptivity in chloroform medium is 1.36×10^3 l.mole^{-1}.cm^{-1}. The relative standard deviation, calculated from ten determinations (100 μg/ml) is ±1.2%.

Interferences

The effects of some ions, particularly those which often accompany copper in copper-base alloys, were studied by extracting with chloroform (50 ml) 50 ml of solution containing 10 ml of buffer solution (pH 6), 25 ml of 0.1*M* BHPDE, and 2.6 mg of Cu(II) and varied amounts of the foreign ion, and measuring the absorbance at 540 nm against a chloroform blank obtained in the same way. The tolerance limits (w/w ratio of interferent to copper, for 1% error) are given in Table 3.

The reagent offers promise for determination of copper in brasses and bronzes, since zinc does not interfere, and the iron content of these metals is too low to cause interference.

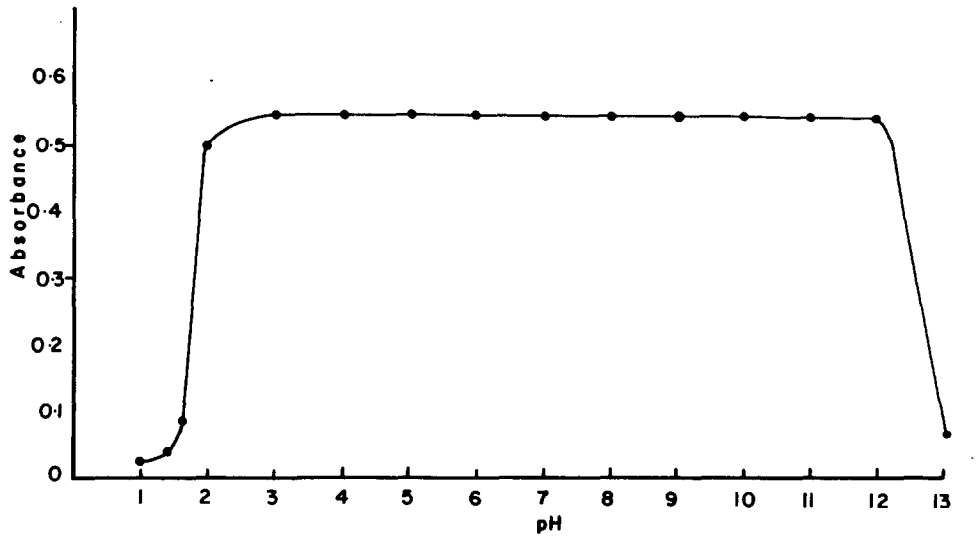


Fig. 2. Variation of absorbance with pH: 10 ml of buffer, 25 ml of 0.1*M* reagent and 2 ml of 0.1*M* copper nitrate diluted to 50 ml with water and extracted with 50 ml of chloroform.

Table 3. Effect of foreign ions on the determination of Cu(II) (2.60 mg/50 ml)

Species added	Limiting ratio to copper
Ni^{2+}	1.9
Co^{2+}	1.8
Cr^{3+}	1.3
Fe^{3+}	0.6
Pd^{2+}	3.0
Ag^{+}	9.0
Hg^{2+}	8.7
Zn^{2+}	2.1
Al^{2+}	8.6
Sb^{3+}	2.0
Mn^{2+}	2.2
Sn^{2+}	5.0
Pb^{2+}	7.9
Mg^{2+}	8.0
Li^{+}	11.0

Composition of the complex

Job's method[8,9] and the mole-ratio method[10] showed the complex to be 1:2 Cu:BHPDE. By Harvey and Manning's method[11] the instability constant was found to be 3.52×10^{-8}.

The complex was isolated by use of a concentration of Cu(II) greater than 150 μg/ml in aqueous medium at pH 6. Its m.p. is 147°. Microanalysis revealed a 1:2 stoichiometry, confirming the previous results.

Analysis of alloys

The extraction procedure was applied to various standard samples, both by simple and by differential spectrophotometry, the differential method being used in order to exploit the comparatively low molar absorptivity. The optimum concentration of the reference solution was found to be 0.9 mg of copper per ml, and solutions containing 0.9–1.28 mg/ml give a linear calibration plot. The interference studies were repeated with 40 mg of foreign ion per 50 ml. Iron(III) caused 5.4% increase in the absorbance but the other ions did not interfere. Reduction of the amount of iron(III) to 10 mg eliminated the interference. Results for various alloys are shown in Table 4.

Conclusion

Although hundreds of reagents for the colorimetric determination of copper are known, comparatively few are well suited for the purpose. For the determination of macro amounts of copper such as in copper-base alloys, the use of an ultrasensitive method has the disadvantage of requiring great dilution before the spectrophotometric measurements. Zinc, aluminium, tin, iron, lead and all other elements associated with copper in these alloys do not interfere in the present method at the concentrations in which they occur. This method is of moderate sensitivity, rapid, and

Table 4. Determination of copper in copper-base alloys

Alloy	Nominal composition, %	Cu found, %	Standard deviation (10 replicates), %
NBS-C 1118 (Aluminium brass A)	75.1Cu (21.9 Zn (2.8 Al)	75.0	0.2
NBS-C 1119 (Aluminium brass B)	77.1 Cu (20.5 Zn) (2.1 Al)	77.0	0.1_4
NBS-C 1120 (Aluminium brass C)	80.1 Cu (18.1 Zn) (1.5 Al)	80.1	0
NBS-63C (Phosphor bronze bearing metal)	80.5 Cu (9.4 Pb) (9.0 Sn) (0.1 Zn)	80.3 (80.4)*	0.2_5
BCS-Manganese brass B	58.8 Cu (33.9 Zn) (1.0 Mn) (0.9 Fe) (1.6 Al) (1.8 Sn) (0.8 Pb) (1.0 Ni)	58.6 (58.7)*	0.3_4
BCS-183/1 (bronze)	84.8 Cu (5.0 Sn) (5.2 Zn) (3.5 Pb)	85.0 (84.7)*	0.2_4

* By differential spectrophotometry.

practically free from interferences, and therefore no prior separations are necessary. The reagent is very easily synthesized and purified and the starting materials for the synthesis are reasonably cheap.

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A NEW CATALYTIC METHOD FOR DETERMINATION OF COPPER IN BLOOD SERUM

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Summary—A sensitive, accurate and fast catalytic method is proposed for determination of copper in blood serum, based on a new catalytic reaction—oxidation of 1,3,5-trihydroxybenzene by H_2O_2. The method permits copper to be determined down to 1 ng/ml in a sample of 0.10 ml of blood serum with a sufficient accuracy and precision even in sera with abnormally low copper assay. Twenty samples can be analysed per hour by a very simple procedure without separation of the metal to be determined.

The copper assay of blood serum is usually determined photometrically by various methods. The main disadvantage of these methods is the relatively large volumes (1–5ml) of the serum sample, due to the low normal copper content (70–160 μg per 100 ml) and the insufficient sensitivity of the photometric reactions. The atomic-absorption (AA) methods used lately for the same purpose offer some advantages, mainly reduction of the time for a single determination, but not of the sample volume, when the most widely used flame variant of AA is applied.

The relatively large sample volume needed for this determination poses serious problems in clinical practice, especially in pediatrics or when the copper level in blood must be determined very often.

It has already been shown that catalytic methods can be applied successfully in clinical laboratory practice. Till now, however, attempts to develop a catalytic procedure for determination of copper in serum have not succeeded because of either low sensitivity[1] or low selectivity[2] of the reactions used. Recently a sensitive catalytic method for determination of copper in blood serum (based on hydrogen peroxide oxidation of sulphanilic acid) was proposed from our laboratory.[3] The method permits a very small serum sample to be analyzed (0.10 ml) but its sensitivity, measured by the slope of the calibration curve, is not very high, which also decreases the accuracy of the determination. The determination limit is enough for estimation of the copper assay when it is in the normal range, but when it is abnormally low (< 70 μg per 100 ml) larger volumes of serum must be taken for the determination. Another shortcoming of the method is the fact that the substrate solution must be prepared daily, because it is oxidized to a measurable extent when kept for a longer period. For that reason we pursued our attempts to develop a sensitive and selective method for copper determination in blood serum, which at the same time would be more sensitive and suitable for practical application.

To this end, we tried to apply the catalytic oxidation of some polyphenols by hydrogen peroxide. A study on a large number of such compounds as substrates has shown that 1,3,5-trihydroxybenzene (THB) is the most promising. THB has not previously been studied as a substrate for copper determination. The literature mentions only that THB can be oxidized catalytically by aerial oxygen,[4] but neither this reaction nor the oxidation with hydrogen peroxide has been studied till now.

The aim of the present work was to study the catalytic oxidation of THB with hydrogen peroxide in the presence of Cu(II) and to apply the results obtained to development of a catalytic procedure for determination of the copper assay in blood serum.

EXPERIMENTAL

Reagents

The reagents used were of analytical-reagent grade and the solutions were prepared with water redistilled in quartz. The glassware used was pretreated with conc. nitric acid and rinsed with redistilled water.

The stock hydrogen peroxide solution was 0.20*M*, and was standardized by permanganate titration. It has been shown that the action of the peroxide in the reaction studied is not influenced by the presence of stabilizers and hence stabilized solutions were used, which kept at constant concentration for at least a week.

The THB solution ($4.23 \times 10^{-2}M$) was prepared by dissolving 0.3429 g in 50 ml of water at 50–60°, and cooling. The solution was kept in the dark and could be used for at least three days when stored at room temperature, and for a week when stored in a refrigerator.

The Cu(II) solution (0.1000*M*) was prepared by dissolving the pure (99.999%) metal in nitric acid (1 + 1), evaporating the solution to a moist residue and diluting to volume. The working solutions (concentration $1–5 \times 10^{-5}M$) were prepared by dilution. When polyethylene vessels are used for storage, the concentration of these solutions does not vary for at least 10 days.

Phosphate buffer solutions were used, prepared from $Na_2HPO_4.2H_2O$ and KH_2PO_4.

Procedure

When the tangent method was used, the solutions of the reagents and the catalyst were put in the separate sections of the reaction vessel[5] and buffer solution was added to give a total volume of 10.0 ml. When the interfering ions

and the activators were studied their solutions were added to the solution of the catalyst. The reaction vessel was kept in a thermostat for 10 min, the reagents were mixed by shaking and part of the solution was transferred to a thermostatically controlled 10-mm cell in a Spécol photometer. The absorbance A was monitored as a function of time and the results were expressed graphically. The tangent method of kinetic analysis was used for the initial period of the reaction, where the absorbance *vs.* time graph is linear.[5] The slope of the graph, $dA/dt = \tan \alpha$, was used as a measure of the reaction rate. When the fixed-time method[5] was used for determination of the reaction rate, test-tubes were used instead of the special reaction vessels. In this case the solution of the substrate was used as the reagent that starts the reaction and is added last to the vessel containing all the other reagents.

RESULTS AND DISCUSSION

Kinetic studies

The reaction substrate does not absorb in the visual part of the spectrum, having an absorption band at ~205 nm, a shoulder at 225 nm and a quintet band at ~260 nm, due to the phenyl ring.

The catalytic oxidation of THB with hydrogen peroxide in the presence of Cu(II) leads in the first few minutes to the formation of a colourless intermediate, which absorbs in the ultraviolet, and this is then converted into a stable orange-reddish product with an absorption band at 480 nm. In the absence of the catalyst the reaction system shows a small but measurable absorption which is taken into account as a blank during the measurements.

As the initial absorption in the ultraviolet is more intense than that in the visible region, we first studied the reaction kinetics by using the absorbance at 360 nm. The ultraviolet absorption, however, is due to the intermediate product and the kinetic curves A *vs.* t are linear for only a very short time, thus decreasing the reproducibility of the results. For that reason, in the further experiments we used the absorbance at 480 nm, *i.e.*, that of the final stable reaction product. In this case the lower determination limit is somewhat higher, but the kinetic curves show a good linear section in the range 7–14 min after the start of the reaction and the reproducibility is much better.

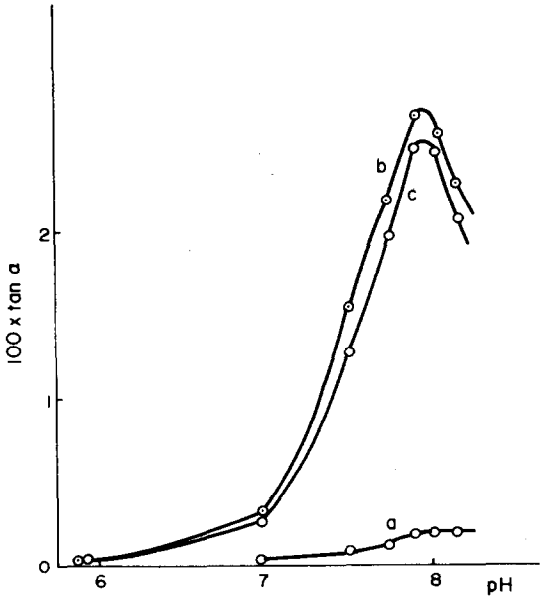

Fig. 1. Dependence of the reaction rate on the acidity. $C_{THB} = 1.5 \times 10^{-3} M$; $C_{H_2O_2} = 7.8 \times 10^{-3} M$; $C_{Cu(II)} = 5.0 \times 10^{-7} M$; $40.0 \pm 0.1°C$; $\mu = 0.07$; *a*—uncatalysed reaction; *b*—in the presence of the catalyst; *c*—catalytic reaction ($c = b - a$).

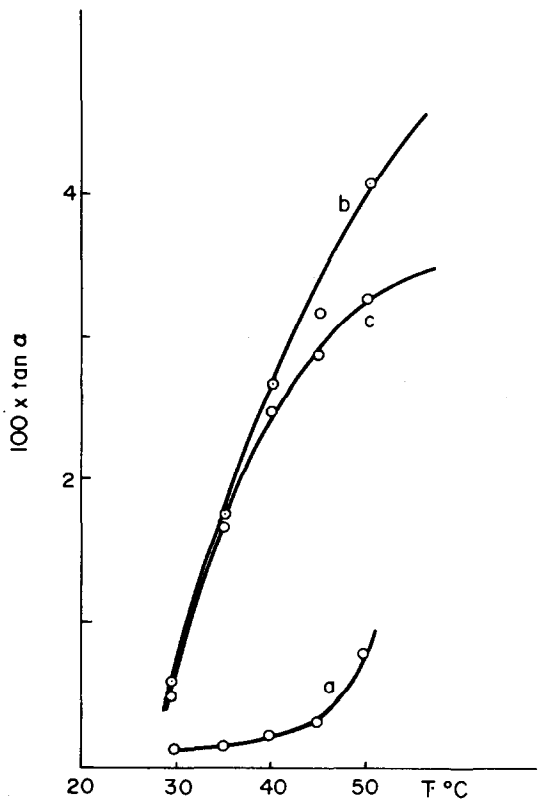

Fig. 2. Dependence of the reaction rate on temperature. $C_{THB} = 1.5 \times 10^{-3} M$; $C_{H_2O_2} = 9.0 \times 10^{-3} M$; $C_{Cu(II)} = 5.0 \times 10^{-7} M$; pH 8.00; $\mu = 0.07$; *a*—uncatalysed reaction; *b*—in the presence of the catalyst; *c*—catalytic reaction ($c = b - a$).

To find the optimum reaction conditions for determination of Cu(II) the influence of reagent concentrations, acidity and temperature on the reaction rate was studied. The results shown in Figs. 1–4 are the averages from 3–5 replicates, the absorbances being measured at 480 nm.

The reaction rate is maximal at pH 8.0. This dependence on acidity is characteristic of the catalytic reactions of Cu(II), as it has already been shown that unsaturated copper(II) hydroxy-complexes, formed in the pH range 7–9, are the species of Cu(II) with highest catalytic activity, at least in redox reactions catalysed by Cu(II).[6] The difference in the optimum pH value (but still in the pH-range 7–9) when the substrate or the oxidant is changed is connected with the different pK_a values and concentrations of the reagents used. The dependence shown in Fig. 1 indicates that the reaction system must be buffered as well

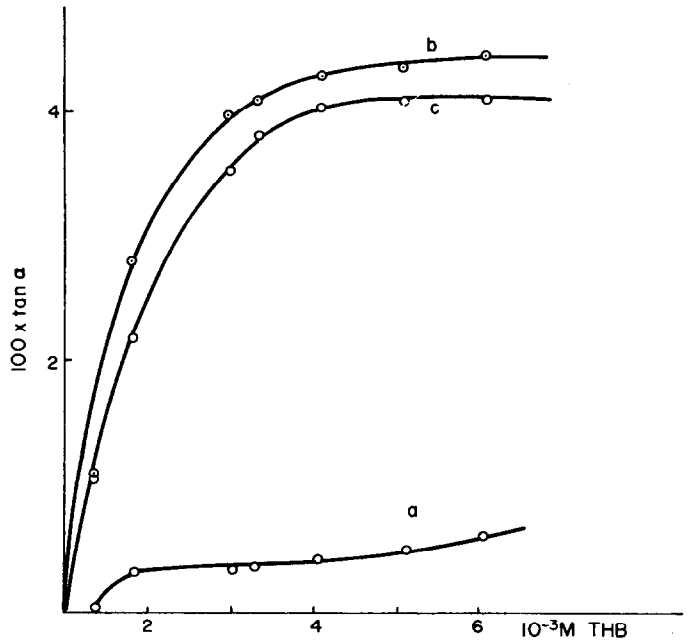

Fig. 3. Dependence of the reaction rate on the THB concentration. $C_{H_2O_2} = 2.1 \times 10^{-2} M$; $C_{Cu(II)} = 5.0 \times 10^{-7} M$; 40.0 ± 0.1°C; pH 8.00; $\mu = 0.07$; *a*—uncatalysed reaction; *b*—in the presence of the catalyst; *c*—catalytic reaction ($c = b - a$).

as possible. It was found that phosphate buffers are the best for this purpose and at the same time give a higher reaction rate than other buffer systems.

It can be seen from Fig. 2 that higher sensitivity can be obtained at higher temperature, as the increase in the absorbance of the blank is low enough in comparison with that of the catalytic reaction. However, at temperatures of about 50° and higher the reproducibility is not good, owing to the catalytic decomposition of hydrogen peroxide which takes place to a measurable extent in this temperature range. Hence all further experiments were carried out at 40.0 ± 0.1°.

The reaction rate ceases to change with concentrations of the substrate and the oxidant (Figs. 3 and 4) at higher concentrations. From the analytical point of view the existence of a plateau in the curve of reaction rate *vs.* concentration is very convenient as small variations in the reagent concentrations in the plateau region practically do not influence the results.

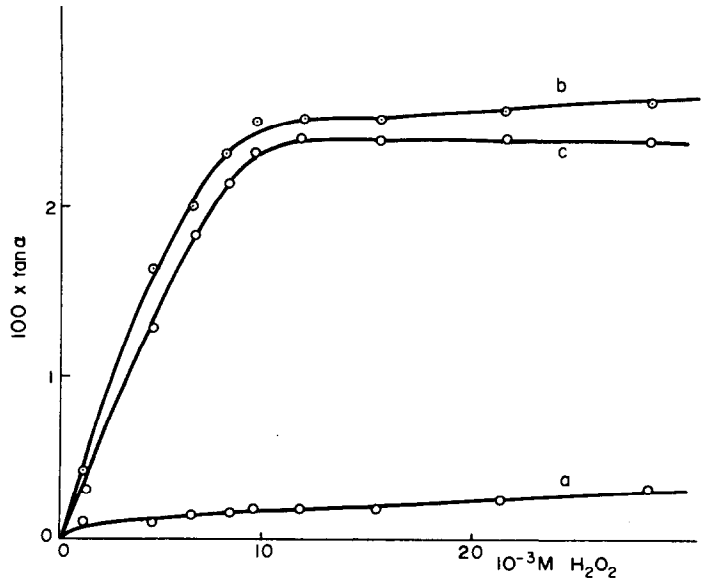

Fig. 4. Dependence of the reaction rate on the H_2O_2 concentration. $C_{THB} = 1.5 \times 10^{-3} M$; $C_{Cu(II)} = 5.0 \times 10^{-7} M$; 40.0 ± 0.1°C; $\mu = 0.07$; pH 8.00; *a*—uncatalysed reaction; *b*—in the presence of the catalyst; *c*—catalytic reaction ($c = b - a$).

Under the optimum reaction conditions thus determined ($C_{THB} = 4.5 \times 10^{-3}M$; $C_{H_2O_2} = 2.0 \times 10^{-2}M$; pH 8.0, realized with phosphate buffer solution with $\mu = 0.133$; temperature 40.0 ± 0.1°) a linear relation exists between the reaction rate and the catalyst concentration in the range 3×10^{-8}–$3 \times 10^{-6}M$. The determination limit is 1.9 ng of Cu(II) per ml, estimated by the 3*s*-criterion.

Activators and interfering ions

We have tried to increase further the sensitivity of the reaction by using nitrogenous heterocyclic ligands with conjugated π-bonds, as it has already been shown[6] that the bonding of the catalyst with such ligands usually increases the catalytic activity. Amongst the 14 derivatives of quinoline and pyridine which were studied in this respect, only pyridine, 1,10-phenanthroline, 2,2′-bipyridyl and 5-methyl-1,10-phenenthroline show an activation effect and even with these it is small. Thus in the presence of pyridine the determination limit for copper(II) is lowered from 1.9 ng/ml only to 1.0 ng/ml. However, this result is to be expected, because THB, as an oxygen-containing ligand, could hardly compete with the nitrogen-containing activators for co-ordination with the catalyst. The pyridine and quinoline derivatives which tend to form more stable complexes with Cu(II) occupy the co-ordination sphere of the catalyst practically completely and prevent the bonding of the substrate into the inner co-ordination sphere of Cu(II).

The dependence of the reaction rate on the concentration of the activators shows a maximum, thus indicating that the catalytic activity in the presence of the activator is due to the formation of co-ordinatively unsaturated complex or complexes between the activator and the catalyst.[6]

In spite of the fact that pyridine activates the catalyst to only a low extent, it was applied in the analytical procedure, because otherwise the results for copper are low by about 25–30%. Most probably this is because the copper is partly bonded in catalytically inactive complexes with some of the low molecular-weight compounds which remain in the serum after its deproteination, such as hydroxy- and aminocarboxylic acids. In the presence of pyridine, the copper(II) is converted into catalytically active complexes with it and in this case the total amount of copper can be determined by the catalytic method.

The possible interference of some ions usually present in serum or which could be introduced by the reagents was studied. It was found that iron(III) does not interfere when present in concentrations at least five times the upper limit of its normal content in serum, while alkali and alkaline-earth metal ions, Zn^{2+}, Cl^-, phosphates *etc.* do not interfere at concentrations at least two orders higher than their normal content in blood serum. Cobalt(II) interferes at lower concentration (3×10^{-2} μg/ml), than any other species, but its level in serum is much lower and therefore it also should not interfere (Table 1).

Table 1. Influence of interfering ions

Interfering ion, A	[A],*M*	B = [A]:[Cu(II)]
Ag(I)	6.0×10^{-4}	2.0×10^{3}
Ca(II)	5.6×10^{-5}	180
Mg(II)	7.1×10^{-3}	2.3×10^{4}
Zn(II)	6.5×10^{-5}	220
Mn(II)	1.0×10^{-3}	3.3×10^{3}
Co(II)	6.0×10^{-7}	2
Ni(II)	2.0×10^{-5}	70
Hg(II)	9.5×10^{-6}	30
Fe(III)	1.0×10^{-5}	30
Al(III)	1.0×10^{-3}	3.3×10^{3}
Mo(VI)	5.0×10^{-6}	20
Cl^-	5.5×10^{-2}	1.8×10^{5}
I^-	2.0×10^{-4}	670
SO_4^{2-}	1.4×10^{-2}	4.8×10^{4}
PO_4^{3-}	2.0×10^{-1}	6.7×10^{6}

[A] = the limiting concentration, at which the change in absorbance caused by the ion does not exceed 3 standard deviations; B = the ratio [A]:[Cu(II)] below which the ion does not interfere. Tested with 50 μg of copper per 100 ml.

On the basis of the reaction studied a simple and sensitive catalytic method was developed for determination of copper in blood serum. The serum sample necessary for the analysis is small enough (0.10 ml), and the total volume of the reaction system is 4.70 ml. The fixed-time method is used because of its simplicity, which makes the procedure more suitable for application in clinical laboratories.

Procedure

Add 0.100 ml of 2*M* hydrochloric acid to 0.100 ml of the serum to be analysed, in a dry test-tube. After 10 min add 0.200 ml of trichloroacetic acid and after another 10 min centrifuge the solution for 10 min at 4000 rpm. Transfer 0.200 ml of the clear supernatant to a dry test-tube, neutralize with 0.40 ml of 1*M* sodium bicarbonate and shake to remove the carbon dioxide. Add 0.50 ml of 0.2*M* hydrogen peroxide, 0.50 ml of 6.20*M* (50%) pyridine and 2.60 ml of phosphate buffer (pH 8.00). The order of addition of the reagents must be followed strictly. Keep the reaction system in a thermostat at 40.0° for 10 min, add 0.50 ml of $4.23 \times 10^{-2}M$ THB and put the test-tube in the thermostat again. Measure the absorbance at 480 nm in a 10-mm cell 35 min after addition of the THB solution. Read the copper assay in μg per 100 ml from a calibration curve obtained in the same way, except that standard copper(II) solutions ($C_{Cu^{2+}}$ = 50, 100, 150, 200 and 250 μg per 100 ml) are used instead of the serum.

The method can be applied for serial analysis, in which case the THB solution is added at 1-min intervals to the series of test-tubes containing the deproteinated samples and all other reagents. In this variant of the procedure about 20 analyses can be performed per hour.

Analytical data and statistical treatment

Twenty serum samples were analysed according to the procedure and by the photometric method with bis-cyclohexanone–oxalylhydrazide,[7] five replicate analyses being performed for every sample by each procedure. The results are given in Table 2.

Table 2. Comparison of the catalytic and the photometric methods

Sample no.	Cu, μg/100 ml $\bar{x}_{kin}$	$\bar{x}_{ph}$	Δx	s^2_{kin}	s^2_{ph}	F_{exp}	t_{exp}
1	132	129	3	17.75	4.00	4.44	1.44
2	126	128	2	36.50	12.00	3.04	0.64
3	119	114	5	12.50	20.25	1.62	1.95
4	129	123	6	16.50	31.50	1.91	1.94
5	127	121	6	9.00	28.50	3.17	2.19
6	150	154	4	13.75	2.50	5.50	2.23
7	104	98	6	25.50	8.25	3.09	2.31
8	135	130	5	6.00	25.00	4.17	2.01
9	149	153	4	8.50	6.75	1.26	2.29
10	136	136	0	4.50	5.75	1.28	0.00
11	103	105	2	9.25	5.50	1.68	1.16
12	113	114	1	15.75	35.25	2.24	0.31
13	199	203	4	28.00	60.75	2.17	0.95
14	218	223	5	12.00	12.00	1.00	2.28
15	134	131	3	4.75	7.75	1.63	1.90
16	117	112	5	19.25	45.50	2.36	1.47
17	108	108	0	1.25	3.00	2.40	0.00
18	111	106	5	17.50	10.50	1.67	2.11
19	152	148	4	14.00	29.00	2.07	1.36
20	139	136	3	7.00	4.75	1.47	1.82

First, by the Cochran test, the hypothesis of homogeneity of the variance of both methods was tested:

$$G_{kin} = \frac{s^2_{max}}{\sum_{i=1}^{20} s_i^2} = \frac{36.50}{279.25} = 0.1307$$

$$G_{ph} = \frac{s^2_{max}}{\sum_{i=1}^{20} s_i^2} = \frac{60.75}{358.50} = 0.1695$$

$G_{0.05}(4, 20) = 0.1921$ and hence $G_{kin}, G_{ph} < G_{tab}$.

It can be concluded therefore that the reproducibility of both methods is the same in the whole concentration range.

By using the F-test it can be shown that there is no significant difference in the reproducibility of both methods:

$$F_{exp} = \frac{s^2_{ph}}{s^2_{kin}} \quad \text{or} \quad \frac{s^2_{kin}}{s^2_{ph}}$$

so that $F_{exp} < 1$; $F_{0.05}(4, 4) = 6.388$.

It can be seen from Table 2 that for all samples $F_{exp} < F_{tab}$.

In order to test the results for the existence of a systematic error the t-test was used:

$$t_{exp} = \frac{|\bar{x}_{kin} - \bar{x}_{ph}|}{\sqrt{s^2\left(\frac{1}{n_{kin}} + \frac{1}{n_{ph}}\right)}}$$

where

$$s^2 = \frac{(n_{kin} - 1)s^2_{kin} + (n_{ph} - 1)s^2_{ph}}{n_{kin} + n_{ph} - 2} = \frac{4s^2_{kin} + 4s^2_{ph}}{8}$$

$$t_{0.05}(8) = 2.31$$

From Table 2 it is evident that for all samples $t_{exp} \leqslant t_{tab}$ and therefore no systematic error exists in the procedure used.

The kinetic method was also compared with the AA method in its flame variant.[8] For this purpose ten serum samples were analysed by both methods, 5 determinations being performed for every sample by each procedure. The results are presented in Table 3.

Table 3. Comparison of the catalytic and the AA methods

Sample no.	Cu, μg/100 ml $\bar{x}_{kin}$	$\bar{x}_{AA}$	Δx	s^2_{kin}	s^2_{AA}	F_{exp}	t_{exp}
1	162	157	5	4.00	19.50	4.87	2.30
2	210	211	1	4.75	6.50	1.37	1.33
3	169	165	4	12.00	5.00	2.40	2.17
4	156	158	2	4.25	3.75	1.13	1.58
5	121	119	2	2.25	4.50	2.00	1.72
6	110	106	4	6.25	9.50	1.52	2.26
7	159	156	3	2.75	6.00	2.18	2.27
8	105	101	4	18.00	5.50	3.27	1.84
9	121	121	0	8.00	5.50	1.45	0.00
10	113	115	2	23.50	4.25	5.53	0.89

Table 4. Results (μg/100 ml) obtained by the standard-addition method

Sample no.	Cu assay, $\bar{x}$	Cu added	Total Cu expected	Total Cu found, $\bar{x}$	Δx
1	162	24	186	188	2
2	98	11	109	112	3
3	201	22	223	222	1
4	51	28	79	80	1
5	57	28	85	83	2
6	54	28	82	79	3
7	56	28	84	78	6
8	77	28	105	110	5

Here again the Cochran test was used for the same purpose:

$$G_{kin} = \frac{23.50}{85.75} = 0.2740;$$

$$G_{AA} = \frac{19.50}{70.00} = 0.2785;$$

$G_{0.05}(4,10) = 0.3311$ and therefore $G_{kin}, G_{AA} < G_{tab}$.

As $F_{0.05}(4,4) = 6.388$ and $t_{0.05}(8) = 2.31$ it is evident from Table 3 that for all samples $F_{exp} < F_{tab}$ and $t_{exp} < t_{tab}$.

As the AA method is the more reliable as a method for comparison we calculated by the least-squares method the regression line for the results obtained by the kinetic and the AA method. The data for the slope of the regression line and the intercept thus obtained are $b = 0.98$ and $a = 4.00$. In order to decide whether these values differ significantly from the theoretical values $\beta = 1$ and $\alpha = 0$, we calculated the confidence ellipse for a and b. Here a joint estimation of a and b was carried out, because they are not independent parameters. The following equation[9] was used for the purpose:

$$N(\alpha - a)^2 + 2(\Sigma x_i)(\alpha - a)(\beta - b) + (\Sigma x_i^2)(\beta - b)^2 = 2FS_R^2,$$

where $N = 10$ is the number of samples analysed, x_i is the analytical result obtained by the AA method for the ith sample and $S_R^2 = 7.909$ for $f = N - 2 = 8$ degrees of freedom is the variance characteristic of the deviation of the results from the regression line.

The calculation of F according to this equation gives the value $F_{calc} = 2.006$. The random quantity F has a Fisher distribution with $f_1 = 2$ and $f_2 = N - 2$. Its tabulated value for the significance level 0.05 is $F_{0.05}(2,8) = 4.459$. Therefore, $F_{calc} < F_{tab}$ and the hypothesis that a and b do not differ significantly from the theoretical values $\alpha = 0$ and $\beta = 1$ is proved for the significance level 0.05.

On these grounds we consider that the kinetic method has no systematic errors, either dependent or not dependent on the Cu(II) concentration.

Finally, the accuracy of the method was tested by the standard-addition method. For this purpose the copper content of eight samples was determined by the catalytic method (five determinations for each sample), and after addition of a known amount of copper(II) the total assay was determined again with five analyses by the catalytic method. The results thus obtained are presented in Table 4.

The comparison of the catalytic method with the photometric and AA methods shows that the reproducibility and the accuracy of the catalytic method are of the same order as those of both the other methods. The higher sensitivity, however, expressed either by the determination limit or as the slope of the calibration curve, is higher for the catalytic method, which together with its simplicity shows good promise for application in clinical laboratory practice.

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MECHANISM OF THE COPPER(II)-CATALYSED PEROXIDE OXIDATION OF *m*-AMINOBENZOIC ACID AND 1,3,5-TRIHYDROXYBENZENE USED FOR ANALYTICAL PURPOSES

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Summary—The mechanism of two indicator reactions, used for catalytic determination of copper—the oxidation of *m*-aminobenzoic acid and of 1,3,5-trihydroxybenzene by H_2O_2—was studied. It was found that, depending on the reaction conditions (reagent concentrations, acidity, temperature) the reactions can proceed according to two reaction mechanisms: (*a*) on the basis of a mixed-ligand catalyst–substrate–oxidant complex, or (*b*) through a radical mechanism with the formation of free radicals in the substrate oxidation. Direct experimental and kinetic data are shown, supporting the two reaction pathways.

The successful application of catalytic methods in analytical chemistry[1] is connected with investigations on the kinetics and mechanism of the indicator reactions used. Information in this field is very valuable in the search for new catalytic reactions and also facilitates maximization of the sensitivity and selectivity, when catalytic analytical methods are developed.[2]

It has already been shown that the oxidation of *m*-aminobenzoic acid (AB) and 1,3,5-trihydroxybenzene (THB) is catalysed by copper(II) and on that basis catalytic methods have been proposed for determination of copper(II) in the concentration range 1 ng/ml–1 μg/ml.[3,4] The aim of the present work was to study the mechanism of these catalytic reactions.

EXPERIMENTAL

The reagents used, AB, THB, hydrogen peroxide, pyridine and nicotinic acid, were of analytical-reagent grade. Phosphate and borate buffers were used. All solutions were prepared in redistilled water. The standard solutions of copper(II) were obtained by dissolving copper metal (99.999% pure) in nitric acid (1 + 1), evaporating to a moist residue and diluting. The concentration of the stock solution was determined electrogravimetrically.

A Specol spectrophotometer with an additional ZV amplifier and a Sprecord UV-VIS spectrophotometer, both with controlled-temperature cuvettes, were used, as well as a standard EPR spectrometer.

The analytical procedures have already been described.[3,4]

RESULTS AND DISCUSSION

Oxidation of m-*aminobenzoic acid with hydrogen peroxide in the presence of Cu(II) as catalyst*

The kinetic data obtained[3] for the dependence of the reaction rate on the substrate concentration give two plateaux on the graph, leading to the assumption that the reaction proceeds by two pathways. In the substrate concentration range up to the end of the first plateau (up to $4.0 \times 10^{-3} M$) the catalytic reaction is quenched completely in the presence of radical traps, such as methylmethacrylate and acrylonitrile (10–15% v/v). In the second substrate concentration range ($C_{AB} = 7 \times 10^{-3} M$) the presence of radical traps has practically no effect on the course of the reaction—the reaction rate decreases by less than 10%. It might be concluded therefore that in the first concentration range the reaction proceeds through a free-radical mechanism.

It is well known from the literature (see Sichev[5] for example) that the system H_2O_2–Cu(II) can serve as a generator of short-lived and very active hydroxyl radicals OH·. On the other hand OH· very rapidly and easily attacks arylamines[6,7] with the formation of ArNH· radicals, further transformation of which leads to the final reaction products.[2]

There also exists another possibility for the radical mechanism—a direct one-electron oxidation of the substrate by the catalyst, with further formation of the final reaction products and regeneration of the catalyst. Such a possibility must be ruled out, however, because when only Cu(II) and the substrate are present, no measurable oxidation of the substrate and reduction of Cu(II) takes place.

We have tried to estimate directly the formation of ArNH· radicals in the reaction system, by use of the EPR technique at room temperature, but unfortunately did not succed in obtaining signals from radicals, most probably because of their short life in these conditions. It is well known that when the nitrogen atom of the amino group is not sterically hindered, the radicals obtained are generally very active, with a short life, and EPR signals from them can hardly be obtained at room temperature, owing to their low stationary concentration.[7] The strong influence of radical traps on the reaction, however, is good evidence for the participation of radicals in the reaction in the first concentration range.

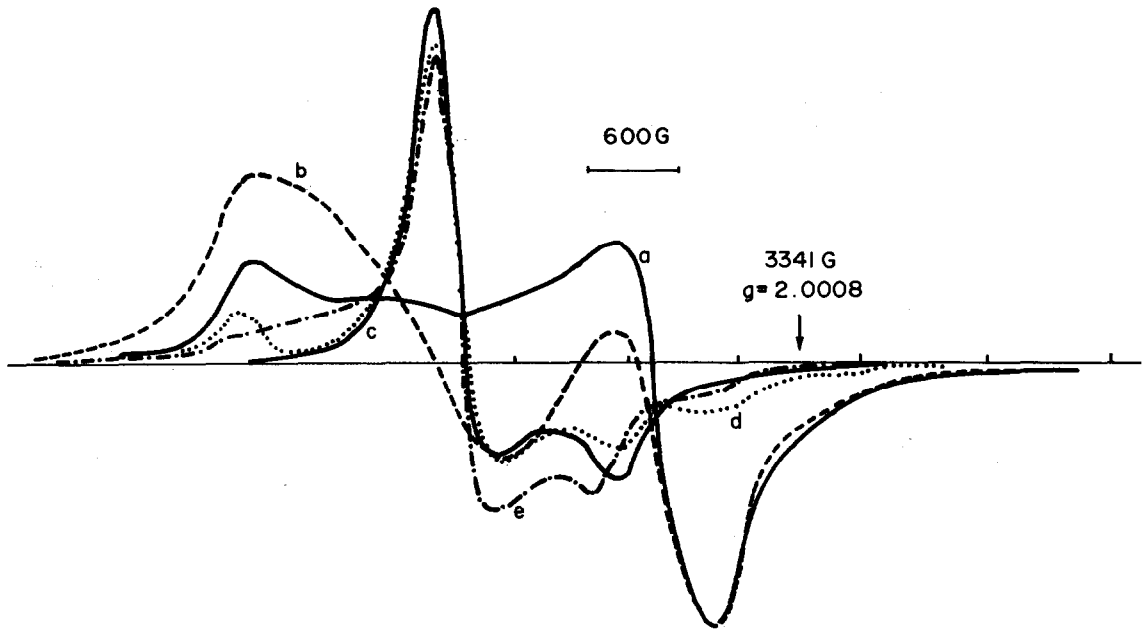

Fig. 1. EPR spectra of Cu(II) at pH 7 with: (*a*) AB; (*b*) AB + H_2O_2; (*c*) NA; (*d*) AB + H_2O_2 + NA; (*e*) AB + NA; $C_{Cu(II)} = 5 \times 10^{-3}M$; $C_{AB} = C_{NA} = 1 \times 10^{-2}M$; $C_{H_2O_2} = 2M$.

The experiments show that in the same concentration range there is a marked difference (10–40%) in the reaction rate in the presence of phosphate or borate buffers, the reaction rate being higher in the latter. This can be ascribed to the deactivation of the free radicals to stable products by the phosphate ions. This assumption was confirmed by direct EPR experiments on the second reaction studied, stated below.

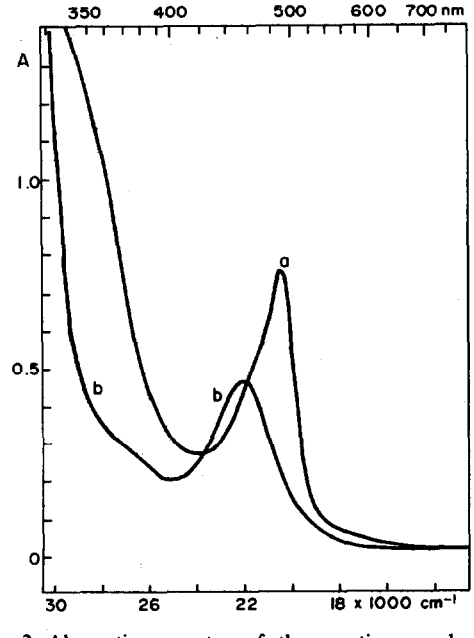

Fig. 2. Absorption spectra of the reaction product in the visible region of the spectrum at: (*a*) pH 8.0; (*b*) pH 2.0; $C_{THB} = 4.5 \times 10^{-3}M$; $C_{H_2O_2} = 2.0 \times .10^{-2}M$; $C_{Cu(II)} = 4.2 \times 10^{-7}M$; $C_{py} = 0.62M$.

As mentioned above, in the concentration range which corresponds to the second plateau the reaction rate is not influenced to a significant extent by the presence of radical traps. This means that in these conditions ($C_{H_2O_2} = 3.0 \times 10^{-2}M$, $C_{AB} \geqslant 8.5 \times 10^{-3}M$, 40°) the reaction proceeds mainly by a non-radical pathway, most probably through formation of a ternary catalyst–substrate–oxidant complex. This assumption is supported by the EPR spectra, showing that in these reaction conditions in the pH range 3–9 the substrate is co-ordinated to Cu(II) and in the presence of hydrogen peroxide a ternary complex is formed (Fig. 1). The form of the EPR signals indicates axial symmetry of the complexes thus formed.

The addition of the activator nicotinic acid, used in the analytical procedure,[3] changes the EPR parameters of the complex (Fig. 1), indicating that nicotinic acid also participates in the complex responsible for the reaction path. This fact correlates with the literature data[8] on the formation of a stable copper(II) complex with the ligand.

The results obtained show that the radical mechanism is operative in the analytical procedure.[3]

Oxidation of 1,3,5-trihydroxybenzene by hydrogen peroxide with Cu(II) as catalyst

During the catalytic oxidation of THB with hydrogen peroxide at pH 8, after an induction period of several minutes an orange product appears in the solution. The absorption spectrum of the reaction system show bands at 273 and 480 nm and a shoulder at 440 nm (Fig. 2). When the solution is acidified after the formation of the orange product the absorption band at 480 nm disappears and the band at 440 nm

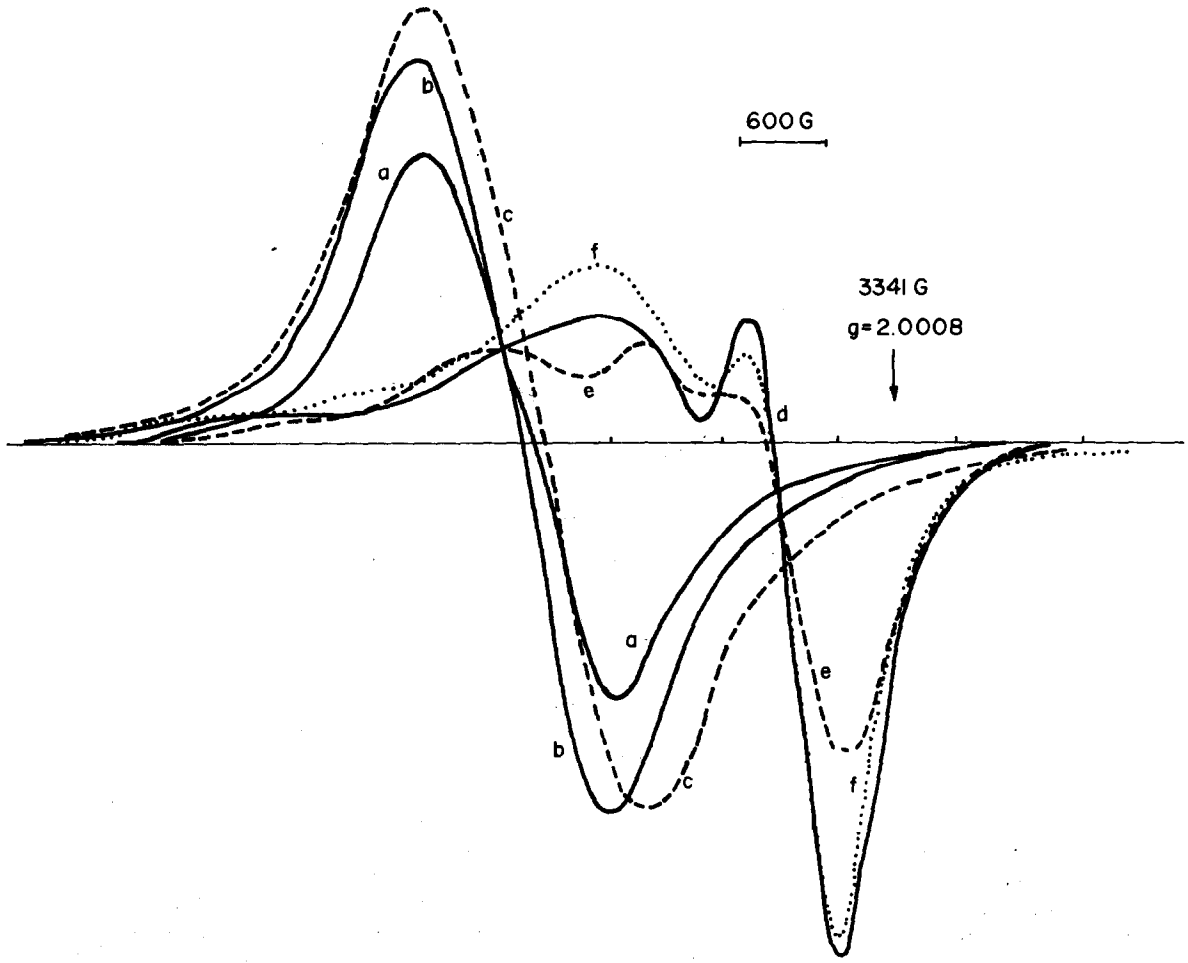

Fig. 3. EPR spectra of Cu(II) at pH 8.0 with: (*a*) H_2O_2; (*b*) THB; (*c*) THB + H_2O_2; (*d*) THB + H_2O_2 + py; (*e*) THB + py; (*f*) py; $C_{Cu(II)} = 5 \times 10^{-3}M$; $C_{THB} = 1 \times 10^{-2}M$; $C_{H_2O_2} = 2M$; $C_{py} = 0.80M$.

increases in intensity. At the same time the band at 273 nm is shifted to 267 nm and a shoulder at 293 nm also appears. Adjustment of the pH back to 8 regenerates the initial spectrum. This shows that the orange product exists in two forms—alkaline (with bands at 273 and 480 nm) and acidic (bands at 267 and 440 nm and a shoulder at 293 nm) in protolytic equilibrium.

If the catalytic reaction is carried out in the presence of radical traps the reaction rate practically does not change. An EPR study gives no indication of the presence of free radicals, but instead shows complex formation between Cu(II) and THB, or formation of a ternary Cu(II)–THB–H_2O_2 complex when all the reagents are present (Fig. 3). According to the EPR data the complexes have high symmetry.

The addition of pyridine, which is used as an activator in the reaction, results in further change in the EPR spectrum of the complex. The EPR data show (Fig. 3) that this is connected with additional coordination of pyridine to the catalyst and not with a substitution reaction. The form of the signal in this case indicates axial symmetry of the mixed-ligand complex.

When the reaction is done in more alkaline medium (pH $\geqslant$ 10) a blue substance appears as an intermediate, which remains unchanged for 2–3 hr if the reaction system is cooled to room temperature. The electronic spectrum of this intermediate shows an intense absorption band at 350 nm and a broad less intense one at 560 nm (Fig. 4,*a*,*b*). The final reaction product formed in such alkaline medium at 40° is a yellow substance, with a spectrum which differs from that of the orange product obtained at pH 8 (Fig. 4,*c*).

The blue intermediate can also be obtained if the catalytic oxidation of THB is done with aerial oxygen instead of hydrogen peroxide. In this case, however, this intermediate is obtained at much lower rate and in lower concentrations.

The high pH value of the solution in which the blue intermediate is obtained, and the position and the form of the absorption bands, lead to the assumption that the bands are due to free radicals, stabilized in the strongly alkaline solution.[9] This was confirmed by the EPR spectra of the blue solutions, showing signals from free radicals (Fig. 5, *a*,*b*). When the substrate: oxidant ratio and the sodium hydroxide concentration

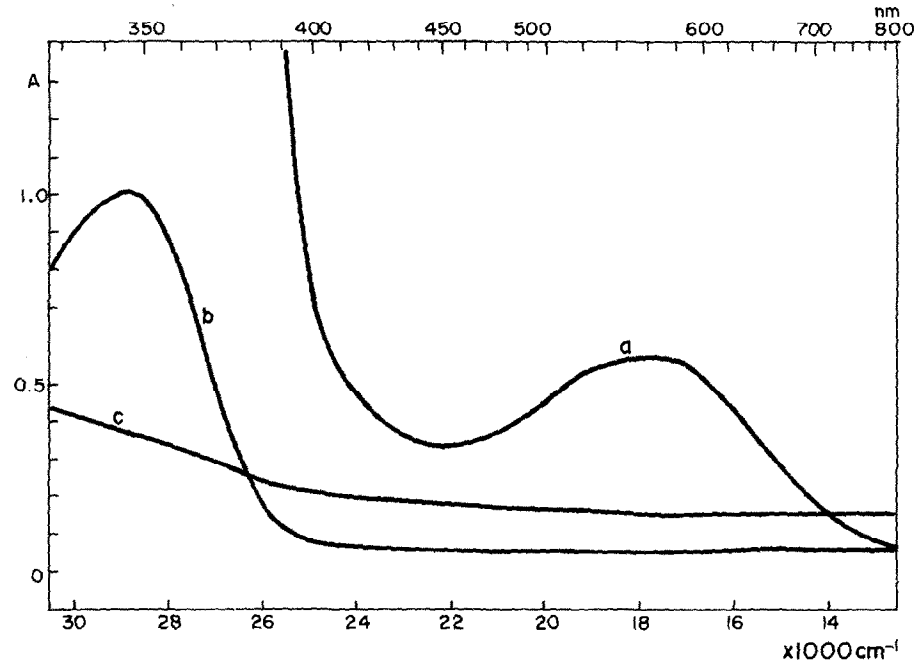

Fig. 4. Absorption spectra of the reaction system at pH > 10. $C_{THB} = 4.5 \times 10^{-3}M$; $C_{H_2O_2} = 2.0 \times 10^{-2}M$; $C_{Cu(II)} = 4.0 \times 10^{-7}M$; $C_{NaOH} = 0.27M$; (*a*) the intermediate product; (*b*) the solution from (*a*) after 100-fold dilution; (*c*) the final reaction product.

(pH $\geqslant$ 10) are varied, different EPR signals from radicals are obtained, only one of them showing a partly resolved hyperfine structure (Fig. 5,*a*).

The addition of acrylonitrile, as a radical trap, to the blue solution results in its decolorization and at the same time in disappearance of the EPR signals and also of the absorption band at 350 nm. Also taking into account the fact that the blue substance is sorbed to a high extent by an anion-exchange resin (Wofatit ES, chloride form) it is evident that the blue intermediate obtained in a strongly alkaline medium is a free phenoxy radical, present mainly in anionic form.

When an excess of Cu(II) is added to the blue solution, the EPR signals sharply decrease in intensity, the absorption bands at 350 and 560 nm disappear and cuprous oxide is formed in the reaction system. This fact can be connected with the literature data[7] that phenoxy radicals can be easily oxidized by Cu(II), which is reduced to Cu(I).

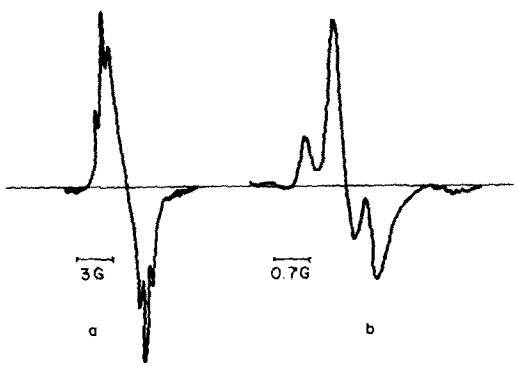

Fig. 5. EPR spectra of the free radicals obtained from THB at pH $\geqslant$ 10.

The addition of phosphates also decreases the intensity of the EPR signals and of the absorption bands at 350 and 560 nm. This is in accordance with the fact that in the reaction conditions for oxidation of AB proceeding through a radical mechanism, the phosphate buffer also diminishes the reaction rate. Evidently, the phosphate ions act as traps for the active radicals, formed through the substrate oxidation in both reactions.

The fact that at pH 8 the reaction proceeds through formation of an intermediate catalyst–substrate–oxidant complex is also supported by the analysis of the kinetic data obtained for the reaction in these conditions.[4]

The data reported here indicate the successive coordination of the oxidant and the substrate to the catalyst, followed by a redox process in the complex, *i.e.*,

$$M + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} MA \qquad (1)$$

$$MA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} MAB \qquad (2)$$

$$MAB \xrightarrow{k_5} M + P \qquad (3)$$

where M is the catalyst, A hydrogen peroxide and B the substrate, present at this pH-value as H_2L^-, and P is the final reaction product.

If the complexation processes (1) and (2) are fast, compared with (3), which is usual for Cu(II)-catalysed reactions, the overall reaction rate, expressed as the formation rate of the final reaction product P, is $V = k_5[MAB]$.

The dependence of the reaction rate on pH and the reagent concentrations can be derived by the steady-state method.[10] The steady-state condition for the intermediates MA and MAB is

$$\frac{d[MA]}{dt} = 0 \quad \text{and} \quad \frac{d[MAB]}{dt} = 0 \tag{4}$$

which, expressed in terms of the scheme (1)–(3), leads to

$$[MA] = \frac{k_1[M][A] + k_4[MAB]}{k_2 + k_3[B]} \tag{5}$$

and

$$[MAB] = \frac{k_3}{k_4 + k_5}[MA][B]. \tag{6}$$

The introduction of (5) into (6) gives

$$[MAB] = \frac{k_1 k_3[M][A][B]}{(k_4 + k_5)(k_2 + k_3[B]) - k_3 k_4[B]} \tag{7}$$

Using the substitutions $[M] = K'C_M$; $k_1k_3K' = K_1$; $(k_4 + k_5)k_2 = K_2$ and $(k_4 + k_5)k_3 - k_3k_4 = K_3$ we obtain

$$[MAB] = \frac{K_1 C_M[A][B]}{K_2 + K_3[B]} \tag{8}$$

From the equilibria

$$H_2O_2 + H_2O \rightleftharpoons HO_2^- + H_3O^+ \ (K_a)$$

$$H_3L + H_2O \rightleftharpoons H_2L^- + H_3O^+ \ (K_{a(1)})$$

[A] and [B] can be expressed in terms of $[H_3O^+]$ and the total concentrations $C_{H_2O_2}$ and C_{H_3L}:

$$[A] = [H_2O_2] = \frac{C_{H_2O_2}[H_3O^+]}{K_a + [H_3O^+]}$$

$$[B] = [H_2L^-] = \frac{K_{a(1)}C_{H_3L}}{K_{a(1)} + [H_3O^+]}$$

When [A] and [B] are introduced into (8) and $[H_3O^+] \gg K_a, K_{a(1)}$ it is transformed into

$$[MAB] = \frac{K_1 K_{a(1)}}{K_2}\frac{C_M C_{H_2O_2} C_{H_3L}}{[H_3O^+]}$$

$$= KC_M C_{H_2O_2} C_{H_3L} 10^{pH}$$

which for fixed total concentrations of the reagents leads to

$$V = \text{const.}\ 10^{pH} \tag{9}$$

At high pH-values, where $[H_3O^+] \ll K_a, K_{a(1)}$, we obtain

$$V = \text{const.}\ 10^{-pH} \tag{10}$$

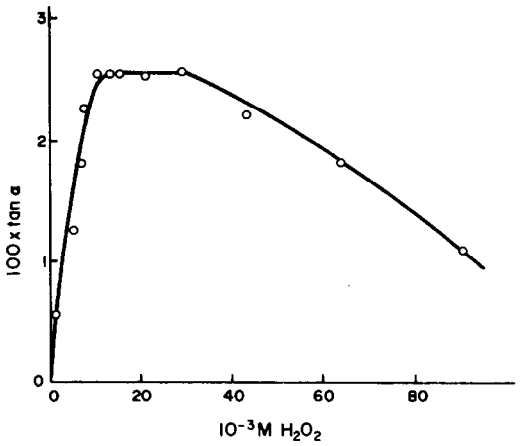

Fig. 6. Dependence of the catalytic reaction rate on the H_2O_2 concentration. $C_{THB} = 1.5 \times 10^{-3}M$; $C_{Cu(II)} = 5.0 \times 10^{-7}M$; 40.0 ± 0.1°C; pH 8.0; $\mu = 0.07$.

Equations (9) and (10) are in good agreement with the experimental data, that at low pH-values the reaction rate increases exponentially and at higher pH it decreases in the same way.[4]

The dependence of the reaction rate on the reagent concentrations also follows from the kinetic equation (8) and corresponds to the experimental data found.[4] The reaction rate must depend linearly on the substrate concentration when this is low, but be independent of it when it is high. A linear dependence for low oxidant concentrations also follows from (8), while at high concentrations the reaction rate should decrease slowly, because of the formation of co-ordinatively saturated peroxide complexes of Cu(II).

In the kinetic study of the dependence of the reaction rate on the peroxide concentration we have found that the reaction rate *vs.* $C_{H_2O_2}$ graph shows a plateau.[4] The study of this dependence at higher peroxide concentrations has shown that it is linear up to a peroxide concentration of $10^{-2}M$, then gives the plateau and at higher oxidant concentrations decreases as predicted (Fig. 6), because A competes with B for M, so that reaction (2) is hindered because M is mainly present as the inactive MA_2.

The present investigations on the reaction mechanism have shown that under the conditions used for the analytical application of the reaction it proceeds through an intermediate complex of the catalyst–substrate–oxidant type and is not connected with the formation of free radicals from the substrate. However, changing the reaction conditions, especially the acidity of the solution, can change the reaction mechanism, and at pH ≥ 10 the reaction goes through the formation of radicals from the substrate, and these are then transformed by further processes into a new reaction product.

This new reaction pathway is also favoured if the reaction is carried out at higher temperature. It was found[4] that at temperatures ≥ 50° the catalytic decomposition of hydrogen peroxide becomes measurable and in this case the OH· radicals first formed

participate in the formation of the phenoxy radicals—the intermediates from which the new reaction product is obtained. The existence of a new pathway in this case was confirmed also by the activation energy of the catalytic reaction, measured for the temperature ranges 20–40° and 50–65°. In the first range the activation energy is $E_a = 32.8 \pm 0.2$ kcal/mole, for the second it is only 5.4 ± 0.4 kcal/mole, in accordance with the values given[5] for the homogeneous catalytic decomposition of hydrogen peroxide.

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SHORT COMMUNICATIONS

DETERMINATION OF SUBMICROGRAM QUANTITIES OF IRON(III) BY A CATALYTIC POLAROGRAPHIC METHOD

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(*Received 8th January* 1980. *Accepted* 22 *May* 1980)

Summary—A simple and sensitive polarographic method has been developed for the determination of submicrogram quantities (0.01–0.16 μg/ml) of iron(III) based on the catalytic polarographic reduction of bromate in presence of resacetophenone isoniazid hydrazone. A 200-fold ratio of Mg^{2+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+} to Fe^{3+} does not interfere. However, Cu^{2+} and Cd^{2+} interfere if they are present in more than 20-fold ratio to Fe^{3+}. Ti^{4+}, V^{5+}, Mo^{6+} and W^{6+} interfere even at 5-fold ratio. There is no interference from F^-, Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^- or NO_2^- even when present in large excess, but EDTA, CN^-, SCN^-, $C_2O_4^{2-}$, citrate and tartrate suppress the wave to a marked extent.

During polarographic investigations on resacetophenone isoniazid hydrazone (RPINH), it was noticed that at pH 5.0 iron(III) (at submicrogram level) gave a wave with a current maximum (peak) at about −0.1 V *vs.* SCE in the presence of potassium bromate and the hydrazone. The investigations were carried out in the pH range 4.0–6.0 and very little change in the quantitative behaviour was observed over the pH range 4.5–5.5. Hence 5.0 was chosen as the optimum. Iron(III) did not exhibit any appreciable reduction current at the selected potential in the absence of bromate and the hydrazone, neither did the hydrazone or bromate undergo polarographic reduction under the conditions chosen. This suggests that the presence of all three components is essential for the appearance of the current peak. Buffered iron(III)–bromate solutions do not appear to have been investigated polarographically, though Rao and Rao[1] have reported observing the catalytic polarographic reduction of bromate by iron(III) in potassium chloride and dilute sulphuric acid solutions. The peak current noticed in the present studies increased with increase in the iron(III) concentration. The possibility has been explored of determining submicrogram quantities of iron in solution by this catalytic approach, and the results are communicated here.

EXPERIMENTAL

All chemicals used were of analytical-reagent grade. Stock solutions of ammonium ferric sulphate ($1 \times 10^{-5}M$), potassium bromate ($0.5M$) and acetate–acetic acid buffer (pH 5.0) were prepared in doubly distilled water. A methanolic solution of RPINH ($1 \times 10^{-4}M$) was used.

A recording polarograph, a Lingane type H-cell and a digital pH-meter were employed in the studies.

Procedure

Different volumes of the iron(III) solution, 2.5 ml of the potassium bromate solution, 5 ml of the methanolic RPINH and 10 ml of buffer were transferred to 25-ml standard flasks and made up to the mark with doubly distilled water and mixed. The polarograms were recorded after deoxygenation of the solution by passage of nitrogen for 15 min.

RESULTS AND DISCUSSION

Typical polarograms of RPINH (A); RPINH + $KBrO_3$ (B); Fe(III) + $KBrO_3$ (C) and Fe(III) + $KBrO_3$ + RPINH (D) at pH 5.0 are shown in Fig. 1. The peak current varies linearly with the concentration of iron(III) over the range 0.01–0.16 μg/ml. The reagent should be present at a concentration not less than five times and not more than about 100 times that of the iron. A large excess of bromate is necessary. The species reduced polarographically is one involving iron(III); the species has not been identified unequivocally. Variation in the height of the mercury column has no effect on the peak current. Surfactants such as gelatin and Triton X-100 do not suppress the peak. The limiting current of the wave is much larger than would be expected for a diffusion-controlled wave. These facts confirm that the wave is of catalytic nature and may be due to the catalytic reduction of bromate by the iron(II) species formed at the electrode by the reduction of the iron(III) species. The mechanism of the process can

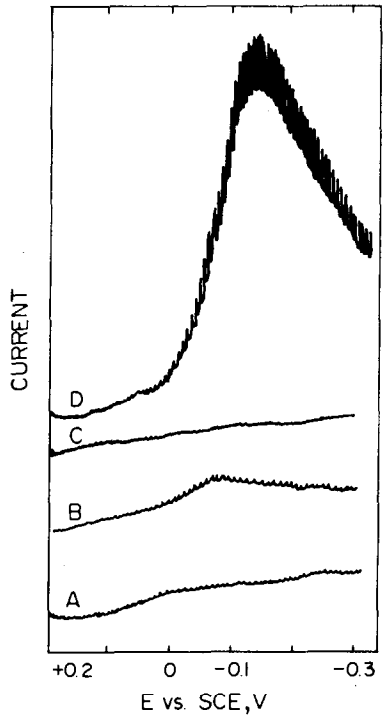

Fig. 1. Polarograms of A—RPINH + buffer; B—RPINH + $KBrO_3$ + buffer; C—$KBrO_3$ + Fe(III) + buffer; D—$KBrO_3$ + Fe(III) + RPINH + buffer. [Fe(III] = 16 × $10^{-7}M$; [RPINH] = 2 × $10^{-5}M$; [$KBrO_3$] = 5 × $10^{-2}M$; pH 5.0.

therefore be proposed as

$$Fe^*(III) + e^- \longrightarrow Fe^*(II) \quad (1)$$

$$Fe^*(II) + \text{bromate} \xrightarrow[\text{(fast)}]{[H^+]} Fe(III) \quad (2)$$

The considerable magnitude of the peak current in the presence of the hydrazone is probably due to activation of step (2).

* Species not known.

A similar observation was made by Chikryzova *et al.*[2] in their studies on the molybdenum(VI)–potassium chlorate system in the presence of carboxylic acids.

Interference studies

Magnesium, calcium, chromium(III), manganese(II), cobalt(II), nickel, zinc and aluminium do not interfere when present even in 200-fold ratio to iron. Copper(II) and cadmium interfere when present in more than 20-fold ratio to iron. Titanium(IV), vanadium(V), molybdenum(VI) and tungsten(VI) interfere even at 5-fold ratio to iron. High concentrations of fluoride, chloride, bromide, iodide, sulphate, nitrate and nitrite do not interfere, but thiocyanate, cyanide, oxalate, tartrate, citrate and EDTA suppress the wave to a marked extent.

Application

The method has been used for the determination of iron in maize leaves, after ashing of the plant material according to Parkes *et al.*[3] The iron content found was 0.09%, in agreement with the reported[4] value of 0.1%.

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INTRACAVITY SPECTROSCOPY WITH A PHOTON DETECTOR BASED ON STEPWISE ATOM PHOTO-IONIZATION

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Summary—A method of measuring atomic-absorption factors by means of a photon detector based on stepwise atom photo-ionization is proposed for the first time. This method can be widely used in laser analytical spectroscopy.

Though intracavity laser spectroscopy (ICLS) has been known for about 10 years[1] and its merits and advantages in analytical measurements[2–4] are obvious, it has so far not been extensively used in analytical practice. Such a slow introduction is explained by the absence of a simple, accurate method to detect the absorption lines in the broad-band laser radiation spectrum.

The atomic-absorption factor of different media is generally recorded by three methods: (1) with high-resolution spectroscopic equipment,[2–5] (2) with resonance monochromators,[6–8] and (3) with dual-beam systems.[8] A new and simple method based on the stepwise atom photo-ionization phenomenon[9] is suggested in this paper for the measurement of atomic-absorption factors. It is based on the following principle.[10] Suppose we have a cell containing atomic vapour (*e.g.*, lithium). To ionize these atoms we can irradiate the cell with laser radiation of wavelengths λ_1, λ_2, λ_3 (Fig. 1). At sufficiently high atom concentration in the cell and with sufficient laser power at wavelengths λ_2 and λ_3, any light quantum of wavelength λ incident on the cell will be absorbed and the atom thus excited will be ionized. It is not difficult to detect the resultant ions, with secondary-emission multipliers using avalanche ionization of buffer-gas atoms in the cell (as in the Geiger–Müller counter[11]) or with a Langmuir probe. Highly excited atoms can be ionized not only by the action of light, but also by a pulsed electrical field,[12] or by collisional ionization by a buffer gas having a higher partial pressure.[13] By using such detectors based on stepwise atom photo-ionization (SAPD) we can effectively detect absorption of narrow wavebands of light quanta involved in atomic and molecular transitions.

In our experiments we first demonstrated the possibilities of such photon detectors by measuring the intracavity absorption of the resonance line of lithium in a flame.

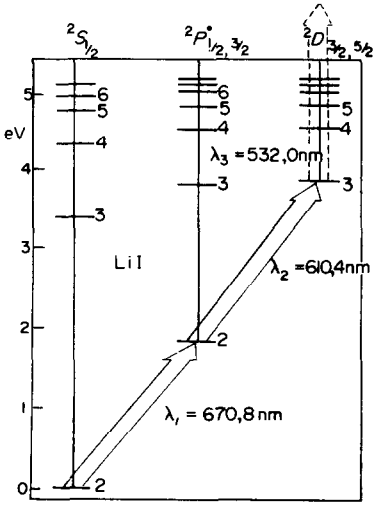

Fig. 1. Scheme of lithium atom ionization.

EXPERIMENTAL

The experimental set-up is shown in Fig. 2. The second harmonic of an Nd^{3+}:YAG laser (1) type LTIPCH-7 with pulse duration 10 nsec and power 200 kW, was used to pump two dye-lasers, one (2) tuned to wavelength λ_1 and the other (3) to λ_2.

A 10-cm acetylene–air slot-burner from a C-302 atomic-absorption spectrometer was placed inside the resonator of the first laser. The resonator length was 45 cm. Standard lithium solutions were introduced into the acetylene flame. The radiations of both dye-lasers and a part of the second harmonic of the Na^{3+}:YAG laser were directed into the centre of a propane–butane flame (4) into which a 10-μg/ml lithium solution was nebulized. The second burner flame served as the SAPD cell for the lithium resonance absorption line. Lithium ions formed in the propane–butane flame by the laser radiation were detected with a 0.5-mm nichrome wire probe kept at −600 V by a d.c. source (5). The probe signal was fed to a broad-band UIS-2M spectrometric amplifier (6) and then to the S-17 oscillograph

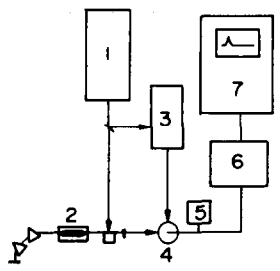

Fig. 2. Block-scheme of the experimental set-up

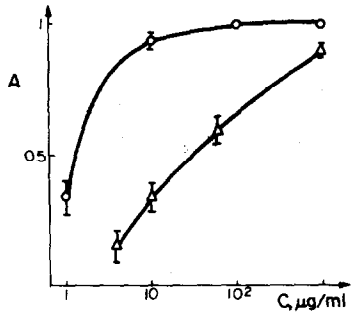

Fig. 3. Analytical curves: ○—intracavity absorption, △—single-pass absorption. $A = \int E\nu(1-e^{-k\nu l})\,d\nu/\int E\nu d\nu$ (*i.e.*, the total absorption factor).

(7). The pulse amplitude was measured visually on the oscillograph. Initially it was assumed that the second harmonic Nd^{3+}:YAG radiation directed into the burner flame (4) would ionize lithium atoms in the excited $^2D_{3/2,\ 5/2}$ state, but the first experiments showed that the signal magnitude was not at all affected even when the pulse energy density was increased to 67 mJ/cm² (2×10^{17} quanta/cm²). This shows that it is collisional ionization of the lithium $^2D_{3/2,\ 5/2}$ state that is taking place, though it is 1.5 eV below the ionization potential.

RESULTS AND DISCUSSION

By introducing standard lithium solutions into the flame of a burner located inside the resonator, we measured the absorption factors (A) of the lithium resonance line and plotted the analytical curves (Fig. 3). Such a curve was also plotted for single-pass absorption, with the burner placed outside the resonator. From the figure it is seen that ICLS is superior to single-pass absorption for lithium concentrations up to about 10 μg/ml.

In subsequent experiments we tested our atomic-absorption analysis scheme by irradiating the SAPD cell (4) with only a broad-band laser. When 1-mg/ml lithium solution was introduced into the cell, we observed a signal due to collisional ionization of the lithium $^2P^0_{1/2,\ 3/2}$ state. This signal was three orders of magnitude lower than the signal obtained with the two-step laser radiation. Introduction of such a concentrated solution into the flame gives rise to a large number of thermally produced ions and increases the noise, which worsens the measurement accuracy. Also in this case the signal value is non-linearly dependent on the laser radiation intensity. Hence it was difficult to make quantitative measurements when a broad-band laser was used. Nevertheless, it is not difficult to design an SAPD for transitions from the ground state of atoms or molecules without use of any additional laser, for example by using a gas-discharge atom source (hollow-cathode lamps, electrodeless discharge lamps, *etc.*) as a detector, or thermally heated cells with atomic vapours of different elements. The possibility of doing this is shown by the experiments on the optogalvanic effect.[13]

In our experiments the sensitivity of detection of low lithium concentrations in the flame was limited by the fluctuations in the ionization signal caused by instability of the laser power. Stabilization of the power and use of integrated electronics should greatly improve the limits of detection.

The advantage of the SAPD is that differential methods of measurement can be easily realized in practice for weak absorption lines. For example, at high and fairly uniform density of the atomic vapour in the SAPD cell, photons with energy corresponding to the line centre will be absorbed almost entirely in the surface layer of the cell. Photons of frequencies corresponding to the wings of the absorption line will be absorbed more or less uniformly over the whole length of the cell. Measurement of the ion concentration distribution over the whole length of the cell will give information on the width and depth of radiation penetration into the cell. The characteristics of this ion distribution will depend only on the distribution of spectral power intensity in the radiation analysed, and not on the total intensity.

It should be noted that the SAPD can be used not only to record the absorption coefficient, but also to detect weak radiation of atomic and molecular fluorescence. It can also be applied successfully for detecting a weak Raman line which coincides with the absorption frequency of some atom,[14] and also as an effective narrow-waveband detector of infrared radiation.[15]

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DETERMINATION OF A TRACE AMOUNT OF BROMINE IN ROCKS BY ION EXCHANGE CHROMATOGRAPHY AND DIRECT POTENTIOMETRY WITH AN ION-SELECTIVE ELECTRODE

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Trace halogens in sedimentary rocks have so far been considered to be promising as indicators for the depositional environment of sedimentary rocks.[1] Moreover, halogens are said to be useful for geochemical prospecting.[2] However, the use of these elements for such purposes has received little attention mainly because of the lack of reliable methods of determination. For determination of bromine in rock samples, neutron-activation analysis (NAA) has been the only reliable method.[3] However, NAA at low levels is inherently poor in precision and not suitable for routine analyses of geological samples. Recently, we reported determination of chlorine in silicate rocks by ion-exchange chromatography and direct potentiometry with an ion-selective electrode.[4] In the present work, the same technique has effectively been applied to the determination of bromine in geochemical samples.

EXPERIMENTAL

Reagents and apparatus

Redistilled water and guaranteed-reagent grade inorganic chemicals were used throughout. Dowex 1 X-10 (100–200 mesh, Cl-form) was converted into the NO_3-form. An Orion Model 94-35 bromide electrode and Model 90-02 double-junction reference electrode were used, and an Orion Model 701/digital pH-meter combined with a Hitachi QPD 53 type recorder was also used. The chromatographic column was 7 mm in bore and 8 cm long. The apparatus for the ion-exchange chromatography is shown in Fig. 1.

Procedure

A powdered sample (0.500 g) is fused in a platinum crucible with 4.00 g of sodium carbonate at 980–1000° for 30 min and the fusion cake is dissolved in *ca.* 30 ml of water. The resulting solution is neutralized by adding 5.5 ml of concentrated nitric acid, and digested for 2–3 hr at 60–70°. The solution is filtered through a porosity-4 glass filter, and the volume of filtrate adjusted to 50.0 ml. A standard solution is prepared in the same way, with silica (0.500 g) and a known amount of bromide (added as sodium bromide solution by microsyringe). About 10 ml of the sample solution is transferred onto the column at the injection port by syringe. Bromide is eluted from the column with 0.5*M* sodium nitrate[5] at a flow-rate of 1.5 ml/min (obtained by adjusting the height of the eluent reservoir). The effluent, which contains interfering ions such as chloride and iodide, is discharged at the ejection port. Bromide is determined by comparing the peak heights of the chromatograms of sample and standard.

RESULTS AND DISCUSSION

Although an ion-selective electrode is selective for a particular ion, interfering ions should be removed in ultratrace analysis. In this work, ion-exchange chromatography was used for this purpose. A selective chromatogram of bromide was obtained by chromatographic separation and discharge of interfering ions such as chloride and iodide. Chloride, bromide and iodide could be quantitatively separated under the conditions described. As large amounts of chloride and trace amounts of iodide, silver and mercury interfered in the bromine determination the effluent containing these interfering species* was dis-

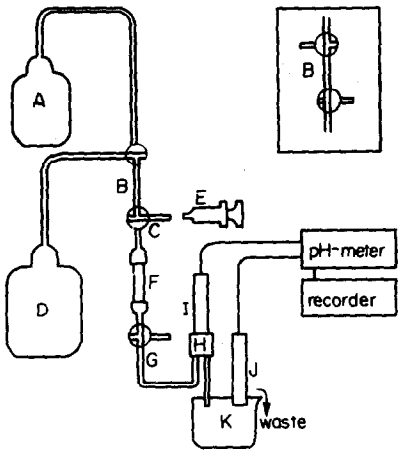

Fig. 1. Schematic diagram of chromatographic system. A—eluent reservoir, B—sample-charging column (1.0 ml), C—injection port, D—bottle for excess of sample during sample-charging, E—syringe, F—chromatographic column, G—ejection port, H—flow-through cap, I—bromide electrode, J—reference electrode, K—beaker (100 ml) bridging column outlet and reference electrode.

* The average levels of silver and mercury in rocks (<0.1 ppm) are too low for precipitation or formation of their bromide. For example, with 1 ppm of silver present in the rock sample, precipitation would occur only if the bromide content exceeded about 44 ppm.

Table 1. Reproducibility test

Sample	Br found, *ppm*	$\overline{X}$, *ppm*	Standard deviation, *ppm*	Br found by NAA, *ppm*
Siltstone	4.6, 4.3 4.3, 4.3 4.0	4.3	0.2	5.4

Table 2. Accuracy test

Sample	Mean Br found,* *ppm*	Br found by NAA, *ppm*
Siltstone	3.0 (2)	2.9
Shale	8.7 (2)	9.0
Shale	11.4 (2)	8.9
Pond sediment†	13.9 (5)	15.2, 16.8

* Number of determinations is given in brackets.
† A standard reference material issued by National Institute for Environmental Studies.

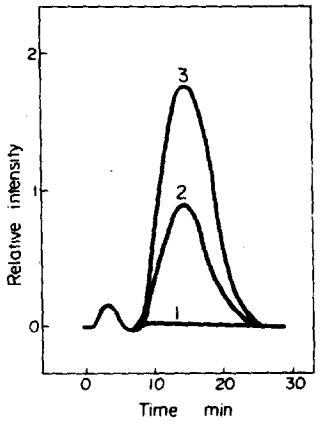

Fig. 2. Selective chromatograms of bromide standards: 1—blank; 2—40 μg/l.; 3—80 μg/l.

charged from the system at the ejection port. The eluate fraction containing only bromide was identified by means of the retention time and passed into the bromide-selective detector. Selective chromatograms of bromide standards are shown in Fig. 2.

The calibration curve obtained by plotting the peak height on the chromatogram against bromide concentration was linear over the range 20–200 μg of bromine per litre. The linearity is in agreement with expectation.[6] Reproducibility was tested by using siltstone, and a value of 4.3 ppm was obtained as the average of 6 determinations, the coefficient of variation being 4% (Table 1). An accuracy test based on various geochemical samples is shown in Table 2. Taking the poor precision of NAA into account, fairly good agreement of the results obtained by our experiment with those of NAA can be seen. The present method is regarded as well applicable to geochemical studies.

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FLUORIMETRIC DETERMINATION OF TRACE QUANTITIES OF MERCURY AS AN ION-ASSOCIATION COMPLEX WITH RHODAMINE 6G IN THE PRESENCE OF IODIDE

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Summary—A procedure for the indirect fluorimetric determination of mercury(II) is described, based on selective extraction of the ion-association complex formed between triiodomercurate(II) and Rhodamine 6G and subsequent release of the fluorescent Rhodamine 6G. The calibration curve is linear up to 1 ppm of mercury(II). The few interferences are easily overcome.

Three fluorimetric procedures based on ion-association complexes have been reported for determination of trace quantities of mercury. Those based on extraction of the tetrachloromercurate(II)–Rhodamine B complex into benzene–ether mixture from acid medium[1] and the extraction of the tetrabromomercurate(II)–Butylrhodamine B complex into benzene[2] are quite sensitive, but interference studies have not been reported. The procedure[3] based on the reduction in fluorescence intensity of Rhodomine 6G by complexation with tetraiodomercurate(II) is subject to interferences.

During an investigation of this last complex[4] we found that addition of small amounts of oxygen-containing solvents such as acetone caused complete dissociation of the ion-association complex. This has been made the basis of a sensitive and selective method for indirect fluorimetric determination of mercury(II).

EXPERIMENTAL

Apparatus

A Carl Zeiss PMQ II spectrophotometer with a ZFM 4 fluorescence attachment provided with a 250-W mercury vapour lamp was used. Slit-widths of 1 mm for the excitation filter and 0.06 mm for the emission monochromator were employed for the fluorescence measurements with 10 × 10 × 45 mm quartz cells with polished bottoms.

Reagents

Standard mercury(II) solution (2 *ppm*). Prepare a 1000-ppm solution of mercury(II) by dissolving 0.3385 g of mercury(II) chloride in water and making up to 250 ml. Dilute appropriately to obtain a 2-ppm solution.

Potassium iodide solution (100 *ppm*).

Acetate buffer (*pH* 4.8), 0.5 *M*.

Sodium chloride solution (3%).

Rhodamine 6G solution (0.005%).

Solvent mixture. Thiophene-free benzene and cyclohexane in 2:1 ratio.

Isobutyl methyl ketone (*IBMK*)

Recommended procedure

Transfer a portion of sample solution containing up to 15 μg of mercury(II) into a 60-ml separating funnel. Add, with mixing, 2 ml each of acetate buffer, sodium chloride and potassium iodide solutions followed by 2.5 ml of Rhodamine 6G solution. Dilute to about 15 ml with water and shake gently for 2–3 min with 5 ml of the benzene–cyclohexane solvent mixture. Discard the aqueous phase. Mix 2.5 ml of the organic extract with 5 ml of IBMK and measure its fluorescence intensity at 560 nm, using a 365-nm excitation filter. Subtract the blank reading and establish the concentration of mercury by reference to a calibration graph prepared by applying the procedure to 0–7.5 ml of 2-ppm mercury(II) solution in place of the sample solution.

RESULTS AND DISCUSSION

Preliminary studies indicated that at low concentrations of iodide (0.5m*M*), only extraction into benzene is selective. The precision of the results was found to improve when a 2:1 mixture of thiophene-free benzene and cyclohexane was used for the extraction. The complex extracted was readily broken up by the addition of acetone, methanol, IBMK or n-butanol; IBMK was chosen because of its low volatility.

Figures 1 and 2 present the excitation and emission spectra of the fluorescent species recorded with an Aminco-Bowman spectrofluorimeter with a 250-W xenon source. There are three excitation maxima at 388, 348 and 525 nm (in order of increasing prominence), and one emission maximum at 560 nm. As a mercury vapour lamp was to be used as the excitation source, a 365-nm filter was used.

Effect of experimental variables

The fluorescence intensity is unaffected by pH over the range 1–7. The influence of the potassium iodide and Rhodamine 6G concentrations is shown in Figs 3 and 4. On the basis of these studies, 2 ml of a 100-ppm solution of potassium iodide and 2.5 ml of 0.005% Rhodamine 6G solution are chosen as optimal. The fluorescence intensity is unaffected by changes in the ionic strength of the aqueous phase. As the presence of an electrolyte assists in rapid phase separation, 2 ml of 3% solution of sodium chloride are added to the aqueous phase. Two minutes of

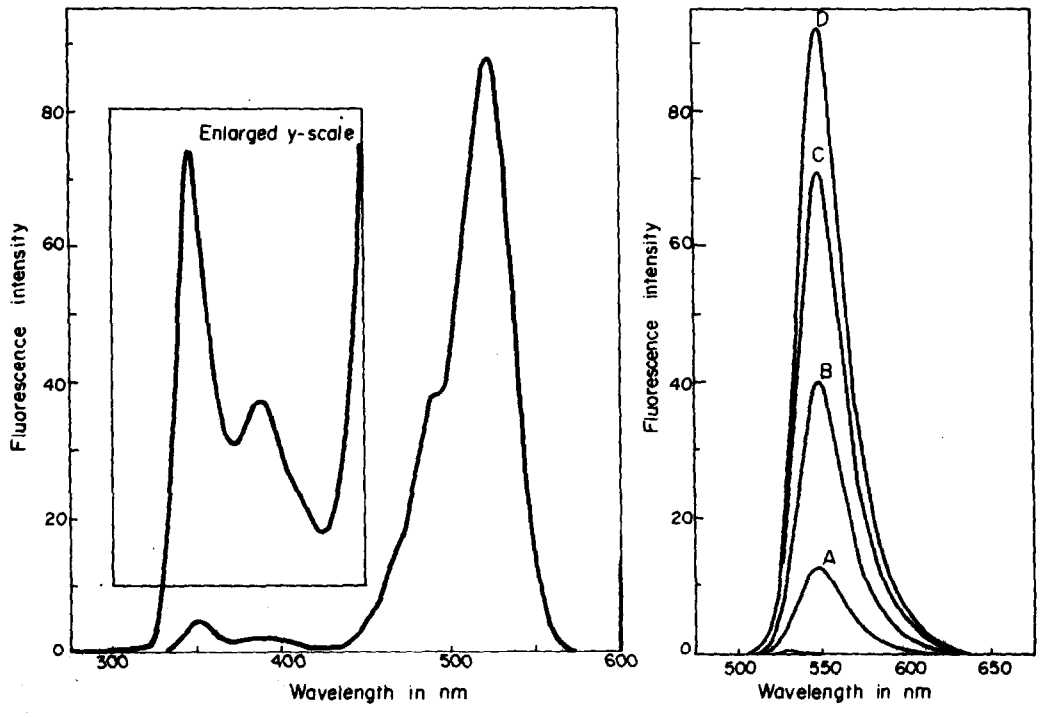

Fig. 1. Excitation spectra (uncorrected): 9 μg of Hg(II) treated as in procedure.

Fig. 2. Emission spectra: (A) 0, (B) 3, (C) 6 and (D) 9 μg of Hg(II).

shaking suffices for complete extraction and slight changes in the phase-volume ratio do not affect the fluorescence intensity. The fluorescence intensity of the liberated Rhodamine 6G is indefinitely stable, and varies linearly with mercury(II) concentration in the range 1–15 μg in 15 ml of aqueous phase. From 25 determinations at 15 μg of mercury(II), the relative standard deviation is 3%.

Effect of diverse ions

The effect of 1 mg each of Li^+, Ag^+, Cu^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, BO_3^{3-}, Tl^+, Tl^{3+}, Al^{3+}, Ce^{3+}, La^{3+}, Th^{4+}, Pb^{2+}, Sn^{2+}, Sb^{5+}, Bi^{3+}, $H_2AsO_3^-$, AsO_4^{3-}, VO_3^-, PO_4^{3-}, NO_2^-, NO_3^-, Cr^{3+}, SO_3^{2-}, $S_2O_3^{2-}$, SeO_3^{2-}, TeO_3^{2-}, $Cr_2O_7^{2-}$, MoO_4^{2-}, WO_4^{2-}, Mn^{2+}, Cl^-, Br^-, ClO_4^-, IO_3^-, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Pd^{2+}, Pt^{4+} and SCN^- ions on the determination of 15 μg of mercury(II) was examined. Only Ag^+, Bi^{3+}, MoO_4^{2-}, Sn^{2+}, $H_2AsO_3^-$ and $S_2O_3^{2-}$ were found to interfere. Bi^{3+} and MoO_4^{2-} were masked by the addition of 2 ml of 0.05*M* EDTA before addition of the other reagents. Interference of Sn^{2+}, $H_2AsO_3^-$ and $S_2O_3^{2-}$ was overcome by oxidation with bromine water and removal of excess of bromine by boiling. The only serious interference was from Ag^+ and it was eliminated by centrifuging to

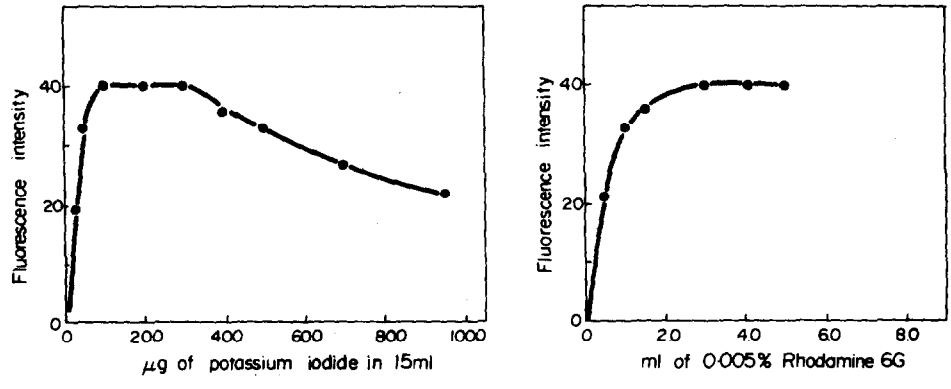

Fig. 3. Effect of potassium iodide concentration: 15 μg of Hg(II) treated as in procedure, with KI concentration varied.

Fig. 4. Effect of Rhodamine 6G concentration: 15 μg of Hg(II), 200 μg of KI and 0–5 ml of 0.005% Rhodamine 6G.

Table 1. Analysis of brine and chlor–alkali plant liquid wastes*

Sample	Mercury added†, μg	Fraction taken for analysis, ml	Total mercury found μg/25 ml		Recovery, %
			Proposed method	Dithizone method	
Weak brine	—	4.0	395	400	—
	200	2.0	590	—	98
	300	2.0	710	—	102
Cell wash water	—	5.0	138	140	—
	100	5.0	240	—	100
	200	2.0	345	—	101
Hydrogen cooler water	—	4.0	275	280	—
	100	3.0	380	—	100
	300	2.0	575	—	99
Combined effluent water	—	5.0	110	108	—
	100	5.0	208	—	100
	200	2.0	310	—	101

* Collected at M/s Dhrangadhra Chemical Works, Arumuganeri, Tirunelveli District, Tamil Nadu.
† To 25 ml of sample before making up to 100 ml.

separate the silver iodide formed, before the addition of Rhodamine 6G.

Analysis of brine and chlor–alkali plant liquid waste

Early results showed that when the procedure was applied to solutions containing 1–10% sodium chloride and spiked with mercury(II), there was no loss of mercury. It was therefore decided to apply the method to the analysis of brine and liquid wastes discharged by a chlor–alkali plant, for total inorganic mercury content.

The liquid samples were pretreated, as described elsewhere,[6] to convert all forms of mercury into mercury(II), and suitable fractions of the treated solution were analysed. Samples to which known amounts of mercury(II) were added were also analysed to establish the recovery. The results along with those obtained for the same samples by the dithizone procedure[7] are presented in Table 1, and show that the method is reliable for the determination of mercury in these samples.

CONCLUSIONS

The method reported offers a rapid and reliable means for the trace determination of mercury by spectrofluorimetry. Even though it is less sensitive than the method based on the mercury-induced oxidation of thiamine to fluorescent thiochrome,[7] it is rapid and hence may find useful applications when low concentrations of mercury are to be determined on a routine basis. It is suitable for analysis of trade effluents.

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POTENTIOMETRIC TITRATION OF THALLIUM(I) WITH SODIUM TETRAPHENYLBORATE, USING ION-SELECTIVE ELECTRODES*

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Summary—Thallium(I) has been determined by potentiometric titration with sodium tetraphenylborate. The titrations were monitored with a double-junction reference electrode and various liquid-membrane and solid-state ion-selective electrodes. The largest end-point breaks were obtained with the liquid-membrane fluoroborate and nitrate electrodes. The cyanide electrode yielded the largest break of the solid-state electrodes tested. Although the magnitude of the end-point break for the cyanide electrode is considerably less than for the above-mentioned liquid-membrane electrodes, routine use of this electrode is preferred because no conditioning is required for the solid-state electrodes. Precision was satisfactory for all electrodes investigated. Thallium(I) can be titrated with tetraphenylborate at any pH from 1.3 to at least 10.2. Other cations that can be precipitated with tetraphenylborate interfere.

Thallium(I) has previously been determined by potentiometric titration with sodium tetraphenylborate (NaTPB).[1-3] The indicator electrodes were polarized silver[1,3] or graphite electrodes[3] or a liquid-membrane lead electrode[2] which is no longer commercially available. In this paper we report the use of various solid-state and liquid-membrane ion-selective electrodes (ISEs) as sensors for this determination. A polarizing current is not required. The determination was of interest to us (*a*) for use as a comparison method in developmental work on other methods for thallium (I), and (*b*) standardization of NaTPB,. The amounts of thallium to be determined varied from 1 to 20 mg.

EXPERIMENTAL

Reagents

The titrant was approximately 0.01*M* sodium tetraphenylborate (made from "Baker Analyzed" reagent). It was filtered through Whatman No. 42 paper one day after preparation. The thallous nitrate was 99.9995% pure (Alfa-Ventron puratronic), suitable as a primary standard.

Apparatus

The titration system was controlled by a Tektronix 4051 graphics system, as previously described.[4] Potential differences were monitored with a double-junction reference electrode (salt bridge 0.1*M* ammonium fluoride) and the following Orion indicator electrodes: 93-05 fluoroborate, 93-07 nitrate, 93-19 potassium, 93-20 calcium, 93-32 bivalent cation, 94-06 cyanide, 94-16 silver/sulfide, 94-53 iodide, and 94-82 lead electrodes.

Stirring was provided by a magnetic stirrer. The stirring motor was separated from the titration vessel by a water-cooled plate and an earthed aluminium plate.

Procedure

Samples were pipetted into a 50-ml beaker containing a Teflon-covered stirring bar and diluted to 25 ml with distilled water before titration. In all titrations the titrant was added at 0.33 ml/min. Titrations were performed at room temperature (23 ± 1°).

Titration endpoints were calculated according to Savitsky and Golay.[5] A convolute was used for a third-order second derivative based on 25 points. The zero-crossing was found by linear interpolation near the change of sign.

RESULTS AND DISCUSSION

According to Schmidt[1] the solubility of thallium(I) tetraphenylborate is less than 10^{-6} mole/l. The direct titration of Tl(I) with NaTPB yielded sharp potentiometric end-points which were detected with polarized silver electrodes. Siska and Pungor[3] used either a polarized graphite or a polarized silver electrode for the same purpose. Lal and Christian[2] have used a liquid-membrane lead electrode (Orion 92-82) for the potentiometric titration of several univalent cations, including Tl(I), with NaTPB, but this electrode is no longer commercially available. We have found that polarized electrodes are not necessary for the potentiometric titration of Tl(I) with NaTPB, and indeed many commercially available ISEs can function as sensors.

Data for the standardization of approximately 0.01*M* NaTPB with Tl(I), monitored with various ISEs are presented in Table 1. Of the liquid-membrane electrodes tested, the nitrate and fluoroborate ISEs yielded the largest potentiometric breaks. The cyanide ISE yielded the largest break of the solid-state ISEs tested. The highest precision was obtained with the nitrate and cyanide electrodes, although the

* This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore Laboratory under contract number W-7405-ENG-48.

Table 1. Standardization of approx. 0.01*M* NaTPB; comparison of electrodes (pH 4.9–5.7)

Electrode	Normality, mean	Standard deviation	Number of replicates	Mean end-point break, *mV*
Fluoroborate	0.009099	0.00004	4	340
Nitrate	0.009565	0.00001	5	360
Bivalent cation	0.009372	0.00005	4	155
Silver/sulfide	0.009480	0.00007	4	75
Lead(II)	0.009773	0.00001	4	60
Iodide	0.009721	0.00003	4	95
Cyanide	0.009600	0.00001	4	130
Potassium	0.009481	0.00005	4	170

other electrodes also gave good precision (as judged from the standard deviations). The calcium ISE could also be used as end-point sensor but not enough determinations were done for statistical evaluation. With the fluoroborate electrode titrations are feasible over the pH range from 1.3 to 10.2, but for a series of titrations the results are best if the pH does not vary by more than ±1. The results in Table 1 and 2 were obtained for titrations at pH 4.9–5.7. Typical titration curves are shown in Fig. 1. With most of the electrodes smooth S-shaped curves were obtained. The lead electrode, however, yielded an inverted V-shaped curve (Fig. 1), similar to the curves obtained in the non-aqueous titration of weak organic acids, with two polarized platinum electrodes.[6,7] Although the silver/sulfide electrode normally yielded an S-shaped curve (Fig. 1C), when a small polarizing current was applied an inverted V-shaped titration curve was obtained (Fig. 1F). For the inverted V-shaped curves it is possible to calculate end-points by two methods:

(1) the usual calculation of the maximum change in emf with titrant increment, and

(2) the point of maximum emf according to Shain and Svoboda.[6]

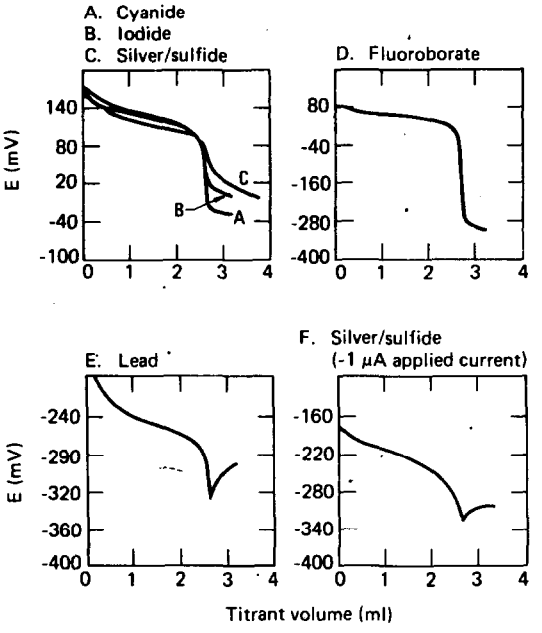

Fig. 1. Titration curves for 0.025 mmole of thallous nitrate with 0.01*N* NaTPB, with various ion-selective electrodes.

Both methods can be programmed into a computer and thus can easily be used for each determination. It is, of course, mandatory to standardize the titrant according to the method to be used in the final determination.

We have already pointed out[8,9] that when liquid-membrane electrodes are used the first several titrations of each day do not yield end-point breaks as large, and potentiometric breaks as sharp, as subsequent runs. For this reason, it seems preferable to use solid-state ISEs such as the cyanide electrode, although smaller end-point breaks are obtained.

Table 2 presents statistics for the determination of 1–20 mg of thallous nitrate with Na-TPB, with the cyanide ISE.

Vytras[10] has used a Crytur 19-15 valinomycin potassium electrode for the standardization of NaTPB with Tl(I). It is noteworthy that our original Orion 93–19 potassium electrode yielded extremely small potential breaks, of the order of 10 mV, but a new one gave a much more acceptable value of 170 mV.

Cations precipitated by NaTPB will interfere in the determination of Tl(I). We have also attempted to

Table 2. Statistics for the titration of Tl(I) *vs.* 0.01*M* NaTPB, with the cyanide electrode (pH 4.9–5.7)

$TlNO_3$ taken, *mg*	$TlNO_3$ recovered, *mg*	Mean recovery, %	Standard deviation, %	Number of replicates
1.334	1.333	100.0	0.5	4
4.00_2	4.06_4	101.6	0.2	4
6.67	6.67	100.0	0.2	5
13.34	13.36	100.2	0.2	5
20.01	19.98	99.9	0.1	4

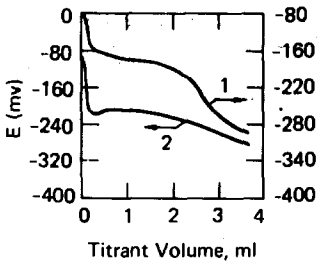

Fig. 2. Titration curves for 0.025 mmole of (1) Cs(I) and (2) Rb(I) with 0.01*M* NaTPB, by use of the fluoroborate ISE.

determine cesium and rubidium by titration with NaTPB. Rubidium yielded very shallow and small potentiometric breaks. Cesium yielded somewhat larger breaks (about 100 mV) and steeper titration curves when the fluoroborate ISE was used. Typical titration curves for Cs(I) and Rb(I) are presented in Fig. 2. Although Cs(I) can be estimated, the titration curve is not sufficiently steep for accurate determination. Use of the cyanide ISE did not improve the titration curves significantly.

We have attempted to use sodium cyanotriphenylborate as titrant for the potentiometric titration of Tl(I). Precipitation did not occur until near the estimated end-point, and no usable titration curves were obtained.

We plan to do further work on the potentiometric titration of organic compounds such as alkaloids with NaTPB, using various ion-selective electrodes.

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A NEW INDICATOR REACTION FOR KINETIC DETERMINATION OF TRACES OF COBALT

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Summary—The reduction of dissolved oxygen by SO_3^{2-} in feebly alkaline media is catalysed by Co^{2+} ions. By use of an electrochemical sensor for oxygen, the optimum analytical conditions for determining traces of the catalyst have been established. At 25° the optimum conditions are pH = 8.25 and $[SO_3^{2-}] \leq 1.25 \times 10^{-3} M$. Under these conditions cobalt may be determined in the range $1–7 \times 10^{-7} M$.

We have observed that the sulphite reduction of oxygen dissolved in water is catalysed by Co^{2+} ions. With a view to using this as an indicator reaction for the kinetic determination of traces of Co^{2+} the following factors have been investigated: the influence of pH on the catalysis; the influence of sulphite and cobalt concentration on the reaction rate; the range of linearity of the calibration curve; interferences.

The reaction is found to be very sensitive (detection limit $< 10^{-7} M$) and has good selectivity.

EXPERIMENTAL

Apparatus

The apparatus is shown in Fig. 1. The cell is kept at $25 \pm 0.5°$ by water circulating from the thermostat. The oxygen-sensor (type LCCA, supplied by the Analytical Chemistry Research Laboratory, Babes-Bolyai University, Cluj-Napoca, Romania) has a lead anode, silver cathode and 0.1*M* sodium hydroxide as electrolyte. Contact with the reaction solution was by means of a polyethylene membrane permeable to oxygen. Any similar type of oxygen sensor[5–17] could equally well be used.

Reagents

These were prepared from analytical-grade reagents and doubly distilled water. Working solutions were prepared by dilution of the stock $10^{-3} M$ cobalt solution and $2.38 \times 10^{-2} M$ sulphite solution. Buffers were made from 0.2*M* sodium tetraborate and 0.1*M* sodium hydroxide.

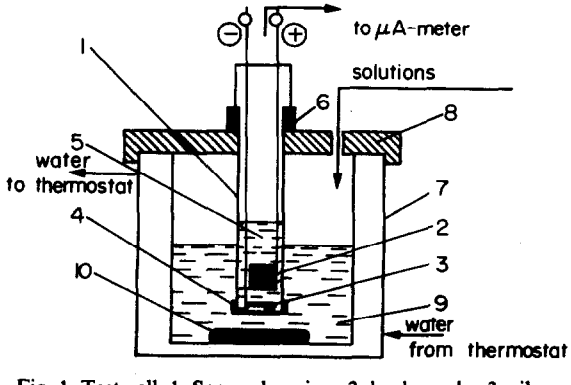

Fig. 1. Test cell. 1, Sensor housing; 2, lead anode; 3, silver cathode; 4, membrane; 5, 0.1*M* sodium hydroxide; 6, coupling; 7, thermostatic jacket; 8, Plexiglas cover; 9, reaction mixture.

Procedure

The reaction mixture, consisting of 25 ml total of buffer and water, is placed in the cell and saturated with air by bubbling air through it. The catalyst solution is then added and stirred in (stirring rate ~60 rpm). The sulphite solution is then added, the sensor inserted and the timer started. The stirring is stopped at intervals and the microammeter readings are recorded as a function of time.

An inert atmosphere in the cell is not necessary because the reduction reaction between sulphite and oxygen is much faster than the dissolution of aerial oxygen in the solution.

The sensor current is a linear function of the oxygen concentration, and the analytical signal used for the kinetic measurements is the difference (ΔX) between the current when the sensor is exposed to air saturated with water vapour (X_0) and that when it is immersed in the reaction medium (X). ΔX can be expressed either in μA or in scale divisions. It varies with time because of the reaction

$$2SO_3^{2-} + O_2 = 2SO_4^{2-}$$

the rate of which is dependent on pH, and on the concentrations of sulphite and catalyst. X_0 is practically constant at constant temperature.

RESULTS AND DISCUSSION

Influence of pH on the catalytic action of Co^{2+}

When the reducing agent is added to the reaction medium containing dissolved oxygen, the sensor signal begins to decrease as a consequence of lowering of the oxygen concentration; hence ΔX increases, at a rate which is significantly accelerated in the presence of Co^{2+} but only within a rather narrow pH range (7.7–8.5), Fig. 2. This influence of Co^{2+} is also characteristic for other redox reactions[18] (*o*-diphenols, *o*-hydroxyazo-derivatives as reductants and hydrogen peroxide or sodium perborate as oxidants) except that the pH at which the catalytic activity is maximal is much higher (>11).

To explain the strong catalytic action of Co^{2+} in alkaline medium some investigators consider $CoOH^+$ to be the catalytically active species, which is then

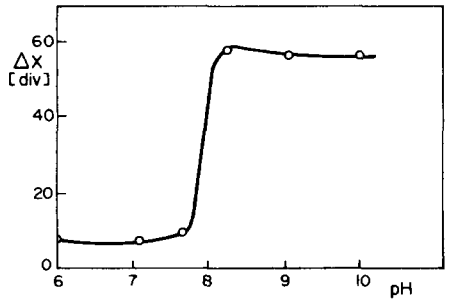

Fig. 2. Influence of pH on the catalytic action of Co^{2+} at 25°C: $[SO_3^{2-}] = 9.52 \times 10^{-4}M$; $[Co^{2+}] = 4 \times 10^{-7}M$; $t = 5$ min.

able to form complexes easily with the oxidizing agent.[19]

In alkaline media, the hydrolysis of SO_3^{2-}

$$SO_3^{2-} + 2H_2O \rightleftharpoons H_2SO_3 + 2OH^-$$

is strongly shifted towards the left, so the reducing agent remains in solution, which results in increasing the reaction rate.

Influence of SO_3^{2-} concentration

The influence of SO_3^{2-} concentration on the reaction rate was studied by the constant time method, in which $[SO_3^{2-}]$ is varied and for each concentration ΔX is measured at the same time interval after the start of the reaction. Figure 3 shows the dependence of ΔX (measured after 5 min reaction time) on SO_3^{2-} concentration. The curve has three portions with different slopes; ΔX slowly increases with sulphite concentration in the range 0–1.5 $\times 10^{-3}M$, more rapidly within the range 1.5–3.8 $\times 10^{-3}M$ and then remains practically constant. The first two portions have different slopes because the sensor signal depends on both chemical and physical processes and will be a function of the rate of the dominant process. The value of ΔX remains constant at sulphite concentrations higher than 3.8 $\times 10^{-3}M$ because the whole of the oxygen dissolved in the reaction medium is reduced during the 5-min interval, at a cobalt concentration of 1 $\times 10^{-7}M$.

To widen the range of catalyst concentration that can be investigated, the rate of the uncatalysed reaction should be as low as possible. For this reason, the concentration of SO_3^{2-} chosen was 9.52 $\times 10^{-4}M$, within the first linear range.

Influence of Co^{2+} concentration

The Co^{2+} ion considerably catalyses the reduction of oxygen by sulphite. Under the optimum conditions of pH and reducing agent concentration, the catalytic effect becomes detectable at a cobalt concentration of about $10^{-7}M$ (about 5 ng/ml). The sensitivity is comparable to that of the reactions with *o*-diphenols and *o*-hydroxyazo-derivatives.

For the calibration curve the quantities $1/t$ (proportional to the reaction rate) and Co^{2+} concentration were taken as the variables. The t values for different catalyst concentrations were determined by the fixed concentration method. The consumption of oxygen in the reaction between sulphite and oxygen is accompanied by increase in ΔX. Hence a fixed value of ΔX implies that the consumption of dissolved oxygen remains constant.

The equation $1/t = f([Co^{2+}])$ might be expected to be rather complicated, being determined by both chemical and physical processes. The main processes to be considered are the half-cell reactions in the sensor, the SO_3^{2-}/O_2 reaction, and oxygen diffusion through the sensor membrane. Generally, the half-cell reactions are faster than the other two processes and are found not to be affected by the Co^{2+} concentration. Hence the time required for the sensor signal to change by a fixed amount will depend only on the rate of reaction between SO_3^{2-} and dissolved oxygen and on the rate of diffusion of O_2 from the reaction medium into the sensor.

In the case of the uncatalysed reaction and for small amounts of catalyst the SO_3^{2-}/O_2 reaction is slow compared to the diffusion rate, and thus is the rate determining step. Hence the reaction rate and $1/t$ will be a roughly linear function of the catalyst concentration (Fig. 4, branch AB). At high catalyst con-

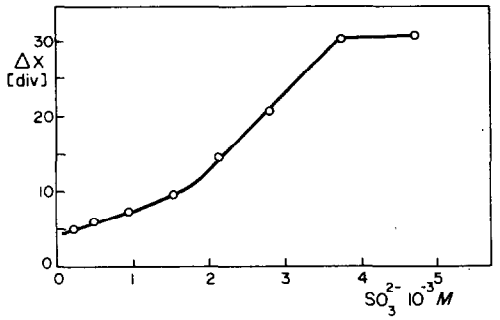

Fig. 3. Influence of SO_3^{2-} concentration on ΔX at 25°C; pH = 8.25; $[Co^{2+}] = 1 \times 10^{-7}M$; $t = 5$ min.

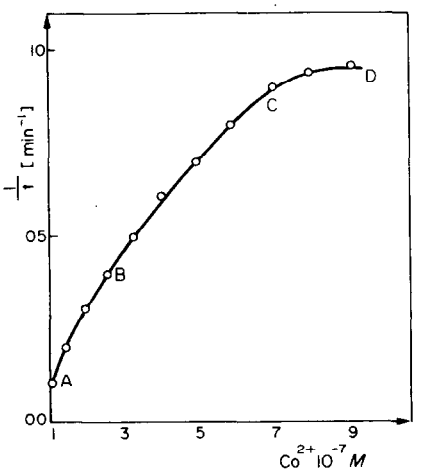

Fig. 4. Influence of Co^{2+} concentration on $1/t$ at 25°C; pH = 8.25; $[SO_3^{2-}] = 9.52 \times 10^{-4}M$; $\Delta X = 50$ divisions.

Table 1. Interferences

	Zn^{2+}	Cd^{2+}			Ni^{2+}		
Concentration, M	1×10^{-4}	1×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-5}	2×10^{-4}	6×10^{-4}
$X_i - X_0$,* divisions	0.5	1	8	12.5	1	3	5

* Difference between ΔX in presence (ΔX_i) and absence (ΔX_0) of interferent.

centrations, on the other hand, the rate of the SO_3^{2-}/O_2 reaction is high and the overall rate will be determined by the rate of oxygen diffusion, and $1/t$ becomes independent of $[Co^{2+}]$ (Fig. 4, branch CD). The range BC corresponds to the transition range, where the redox reaction rate is comparable to the diffusion rate. Consequently, the sensor signal will depend on both rates and within this transition range the rate of the overall process ($1/t$) does not vary linearly with catalyst concentration. However, we found empirically that the relation $1/t = f(\sqrt{[Co^{2+}]})$ is linear over this range.

A statistical treatment of the data obtained gave for the calibration curve at pH 8.25 the equation

$$1/t = a + b(10^7\,[Co^{2+}])^{1/2}\ min^{-1}$$

(from 9 results, 95% probability level, $a = -0.807 \pm 0.029$, $b = 1.017 \pm 0.034$, variance $= 1.12 \times 10^{-4}$ min^{-2}). The cobalt concentration range of application of the equation is $1\text{–}7 \times 10^{-7} M$ since at higher concentrations the reaction rate rapidly tends towards a constant value, irrespective of the catalyst concentration.

Interferences

Since an intended application of the work was the determination of Co^{2+} in presence of Zn^{2+}, the interference of the latter was investigated, as well as that of other ions such as Cd^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, MoO_4^{2-}, VO^{2+}, CN^-, tartrate, citrate, ammonia and EDTA. ΔX was measured 3 min after the start of the reaction in the presence and absence of the species investigated. The results are given in Table 1.

Zinc in concentrations up to $10^{-4} M$ is seen not to show catalytic activity, but Cd^{2+} and Ni^{2+} act as catalysts at concentrations of only 10 and 100 times the Co^{2+} concentration, respectively.

Other ions, such as Cu^{2+}, Fe^{3+}, Mg^{2+}, MoO_4^{2-}, VO^{2+}, do not interfere. The first three do not catalyse the reaction even when present at the concentration corresponding to the solubility product of the hydroxide.

Cyanide, tartrate, citrate, ammonia and EDTA inhibit the catalytic action of Co^{2+} even at concentrations comparable to the Co^{2+} concentrations because they form stable complexes with Co^{3+}.

Fe^{2+} interferes not by catalytic action but by its reduction of dissolved oxygen.

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CYCLIC VOLTAMMETRIC DETERMINATION OF ADRENALINE IN THE PRESENCE OF COPPER(II) IONS

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Summary—Low concentrations of adrenaline (10^{-5}–$10^{-4}M$) have been found to give rise to quite marked current peaks in the cyclic voltammograms recorded for ammoniacal solutions containing a copper(II) salt at around $10^{-4}M$. Fast sweep-rates (20 V/sec) are generated by a cathode-ray polarograph and applied to a DME. Peak heights are proportional to the adrenaline concentration, the procedure is rapid and simple, and the reproducibility is good.

Adrenaline belongs to a group of compounds known as catecholamines which play a particularly important role in the regulation of physiological processes in living systems. Catecholamines serve as carriers for the nervous system, influencing the constriction of blood vessels and controlling tissue metabolism by increasing the levels of glucose and lactic acid. Polymerization of oxidized forms of catcholamines, taking place in living organisms, leads to the formation of melanin-biopolymers, regarded as biological transducers of photon and phonon energy as well as regulators of trace-element concentrations.

Adrenaline may be determined by fluorometric,[1] colorimetric,[2] or radioisotopic[3] methods. These methods are characterized by the long and complicated procedures needed in sample preparation and determination, or the necessity of employing expensive apparatus and reagents. For these reasons the application of an electrochemical method, characterized by short measurement time and relatively simple experimental procedure, might bring significant advantages in this field.

Polarographic analysis for the determination of adrenaline was first proposed by Wiesner,[4] who determined the adrenochrome produced by enzymatic oxidation of adrenaline. This compound is reduced reversibly at the dropping mercury electrode (DME), with $E_{1/2}$ values (in Britton–Robinson buffers) lying in the range from −0.082 V at pH 4.5 to −0.200 V at pH 6.81. The oxidation of adrenaline with iodine to iodoadrenochrome and its subsequent reduction at the DME has also been employed for analytical purposes. According to Henderson and Freedberg[5] the $E_{1/2}$ of the reduction wave for iodoadrenochrome in an acetate buffer at pH 4.5 is +0.03 V.

Electrochemical detection of some catecholamines after separation on an alumina chromatographic column was proposed by Kissinger *et al.*,[6] Hashimoto and Mamyama,[7] and Mayer and Jiang.[8] Oxidation currents for these compounds were recorded, a silicone-impregnated graphite electrode being used.

The effects of the adsorptive enrichment of a layer of *o*-diphenols on the electrode in oscillopolarography and the possibility of using this phenomenon for the determination of small amounts of these compounds in the presence of certain cations (and *vice versa*) have been reported by Matysik.[9] A preliminary report describes a method for the determination of adrenaline and noradrenaline in the presence of the UO_2^{2+} ion.[10] The smallest concentration detectable by this method was 0.5 μg/ml, but the qualitative differentiation of these compounds was not possible.

In this study, advantage has been taken of the tendency of aromatic organic compounds with two –OH groups in the *ortho*-position to cause unusually large effects in oscillopolarography in the presence of many cations, to develop a determination of adrenaline by rapid cyclic voltammetry, with the Cu(II) as the accompanying cation.

EXPERIMENTAL

Measurements were taken with a Telpod OP-3 oscillopolarograph. The oscillopolarographic method is based on the $i = f(E)$ relationship established for symmetrical triangular polarization of the electrode. A DME was used in a three-electrode system with a saturated calomel electrode as reference.

The supporting electrolyte was a $1M$ aqueous solution of ammonium nitrate and ammonia at pH 8.5, and the adrenaline concentrations ranged from 0.1×10^{-4} to $2.0 \times 10^{-4}M$. The solutions were not deoxygenated. Measurements were made at 20°. The Cu(II) concentration in the solution was $0.51 \times 10^{-4}M$.

RESULTS AND DISCUSSION

On the cyclic voltammetric curves recorded for ammoniacal solutions of copper(II) there appeared two cathodic peaks (c.p.) and two anodic peaks (a.p.).

The potentials for the peak maxima were as follows:

E_{1st} cp = −0.142 V, E_{2nd} cp = −0.456 V

E_{1st} ap = −0.292 V, E_{2nd} ap = −0.552 V

According to Grochovskaya[11] the electrode processes for the reduction of copper(II) in ammoniacal buffers correspond to the reactions:

(1) $Cu(NH_3)_4^{2+} \rightleftharpoons Cu(NH_3)_2^{2+} + 2NH_3$

(2) $Cu(NH_3)_2^{2+} + e \rightarrow Cu(NH_3)_2^{+}$

(3) $Cu(NH_3)_2^{+} + e^- + Hg \rightarrow Cu(Hg) + 2NH_3$

The addition of adrenaline to an ammoniacal buffer containing Cu(II) produces a significant increase in the height of the reduction and oxidation peaks of the Cu(II) and Cu(I) (Fig. 1).

The greatest increase in the current was observed for the first pair of peaks, with the changes proportional to the adrenaline concentration from $0.13 \times 10^{-4}M$ to $1.83 \times 10^{-4}M$. The potentials of the peak maxima also changed, reaching the following values:

E_{1st} cp = −0.276 V, E_{2nd} cp = −0.406 V

E_{1st} ap = −0.255 V, E_{2nd} ap = −0.389 V.

The shift in the E_{1st} cp of Cu(II) towards negative potentials seems to be connected with the complexing action of adrenaline. Since adrenaline undergoes specific adsorption within the whole interval of the electrode polarization, complex formation would be expected to be facilitated in the layer adjoining the electrode. The direct dependence of increase in the

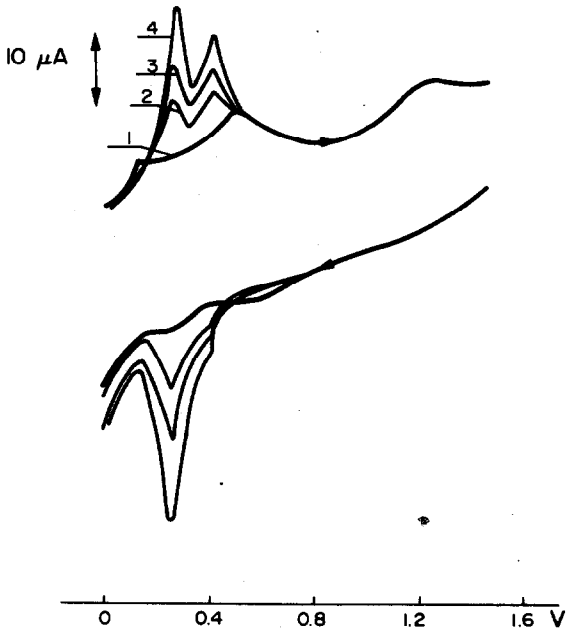

Fig. 1. Chronovoltamperograms of solutions containing Cu^{2+} in the presence of adrenaline. Sweep rate: 20 V/sec. (1) $0.51 \times 10^{-4}M$ Cu^{+2} in supporting electrolyte; (2) solution (1) $+2.62 \times 10^{-5}M$ adrenaline; (3) solution (1) $+0.52 \times 10^{-4}M$ adrenaline; (4) solution (1) $+0.79 \times 10^{-4}M$ adrenaline.

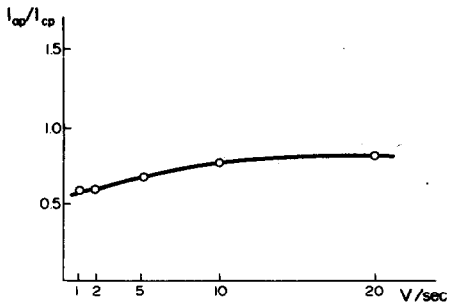

Fig. 2. The relationship between I_{ap}/I_{cp} and potential-sweep rate. $[Cu^{+2}] = 0.51 \times 10^{-4}M$. [Adrenaline] = $1.048 \times 10^{-4}M$.

reduction current for the copper on $V^{1/2}$ indicates that transport of the depolarizer to the DME surface is diffusion-controlled. Additional confirmation is provided by the dependence of the ratio I_{ap}/I_{cp} on the rate of change of the DME polarization (Fig. 2), which is one of the criteria determining the mechanism of the electrode process.[11] The course of this function shows that the secondary reaction is irreversible.

The height of the reduction peak of the Cu(II)–adrenaline complex increases directly with the adrenaline concentration, which makes the effect useful for routine analytical work. In comparison with other analytical methods the proposed method is very simple and quick: the time of measurement in the case of pure or previously isolated substances is only several seconds. This means that problems connected with the poor stability of catecholamines are minimized, and that the reduction current can be read virtually instantaneously. The precision would seem to be as good as, if not better than, that obtained with fluorimetry or colorimetry: peak currents of 70 μA showed a variation of 0.1–0.2 μA.

Interferences from other complexing agents are not as serious as might be expected, since *o*-diphenols generally form very stable complexes with heavy metals. The appropriate stability constants for the Cu(II)–adrenaline system[12] are $K_1 = 10^{11.4}$ and $K_2 = 10^{10.7}$. In addition, the concentration of free adrenaline is relatively high near the electrode since it is strongly adsorbed on it. The method is very specific for the *o*-diphenol group, but it is not possible to distinguish polyphenols in all cases. Because our method is simple, quick and accurate, but may in some cases be less sensitive or selective than other methods, we think it is more suitable as a quality-control method for pharmaceutical products. However, heavy metals, *o*-diphenol compounds and high concentrations of proteins interfere.

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ANALYTICAL DATA

2-(3'-SULPHOBENZOYL)PYRIDINE THIOSEMICARBAZONE AND PHENYL THIOSEMICARBAZONE AS SPECTROPHOTOMETRIC ANALYTICAL REAGENTS

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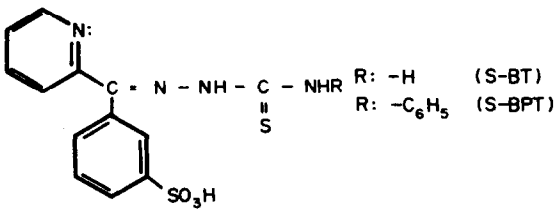
Summary—The synthesis, characteristics, properties and metal-ion interactions of 2-(3'-sulphobenzoyl)-pyridine thiosemicarbazone and phenyl thiosemicarbazone have been studied. These substances exhibit three pK values in aqueous solution, and form water-soluble chelates with high molar absorptivities, which may be used in analysis, especially the iron(II) chelates (λ_{max} 620–630 nm). The behaviour of the iron–reagent systems has been investigated.

Thiosemicarbazones and phenylthiosemicarbazones react as chelating ligands with transition-metal ions by bonding through the sulphur and hydrazine nitrogen atoms,[1] although in a few cases they behave as terdentade ligands and bond through an additional co-ordinating group.

The principal difficulty in use of these reagents is their low solubility in aqueous media. We have therefore synthesized sulphonated 2-benzoylpyridine and its thiosemicarbazone (S-BT) and phenylthiosemicarbazone (S-BPT). This paper describes the synthesis, characterization, acid dissociation costants and analytical applications of these reagents.



EXPERIMENTAL

Reagents

Analytical-reagent grade chemicals were used throughout. All metal-ion solutions were standardized. The pH-4.7 buffer solution was made by dissolving 56.0 g of sodium acetate and 25.0 ml of glacial acetic acid in water, and diluting to 1 litre.

Preparation of S-BT and S-BPT

2-Benzoylpyridine was sulphonated by the procedure of Bradsher *et al.*[2] A 43.5% yield of 2-(3'-sulphobenzoyl)pyridine was obtained. For synthesis of the thiosemicarbazone, a solution of 1.45 g of 2-(3'-sulphobenzoyl)pyridine in 75 ml of water was mixed with a solution of 0.5 g of thiosemicarbazide in 5 ml of water and refluxed for 2 hr, then cooled to room temperature. The yellow crystals that separated were collected and washed with small portions of hot water and dried in a vacuum desiccator (m.p. >325°, dec.). For the synthesis of the phenylthiosemicarbazone, 0.74 g of 2-(3'-sulphobenzoyl)pyridine was dissolved in 30 ml of ethanol–water (4:1) and mixed with 0.47 g of phenylthiosemicarbazide, then refluxed etc. as above. Yellow crystals were obtained (m.p. >325°).

Elemental analysis gave C 43.9%, H 3.5%, N 15.7% for S-BT; $C_{13}H_{12}N_4O_3S_2.H_2O$ requires C 44.06%, H 3.98%, N 15.81%. For S-BPT analysis gave C 55.6%, H 3.9%, N 13.6%; $C_{19}H_{16}N_4O_3S_2$ requires C 55.33%, H 3.91%, N 13.58%. The difference between the experimental and theoretical results for hydrogen in S-BT is attributed to the errors arising from use of an automatic C, H, N analyser.

Reactions with metal ions

Solutions were prepared in 25-ml standard flasks, with different concentrations of metal ion, 5 ml of reagent solution in dimethylformamide, 10 ml of buffer solution and diluted to volume with distilled water. Absorbances were measured against a reagent blank.

RESULTS AND DISCUSSION

The infrared spectra of S-BT and S-BPT were obtained (KBr discs), and the bands were assigned. The ultraviolet spectra of the reagents in various solvents were measured and the results are summarized in Table 1. These compounds exhibit intense $n \rightarrow \pi^*$ bands between 250 and 350 nm, which are highly characteristic of these systems; the wavelengths of

* Reprint requests

Table 1. Ultraviolet spectra of the reagents in common solvents

Solvent	S-BT		S-BPT	
	λ_{max}, nm	ϵ_{max}, $l.mole^{-1}.cm^{-1}$	λ_{max}, nm	ϵ_{max}, $l.mole^{-1}.cm^{-1}$
Water	318	2.05×10^4	325	2.41×10^4
Ethanol	335	1.77×10^4	340	2.40×10^4
Acetone	350	1.50×10^4	352	2.10×10^4
Methyl isobutyl ketone	355	1.15×10^4	365	1.47×10^4

Table 2. Dissociation constants of the reagents in aqueous medium (0.1*M* KCl, 25°C)

Reagent	pK_1	pK_2	pK_3
S-BT	−3.25	3.73	11.45
S-BPT	−3.50	3.65	10.49

these bands are found to decrease (blue-shift) with increase in the polarity of the solvent.[3,4]

Acid–base behaviour

Both reagents are tribasic acids, showing three ionization steps. The ultraviolet spectra of aqueous solutions at different pH values in acid medium are shown in Fig. 1 (a,b); the reagents slowly hydrolyse in alkaline medium, and no isosbestic point is observed; because sulphonic acids are strong, the ultraviolet spectra in various perchloric acid media have been recorded (Fig. 2, a,b). The equilibria are marked by isosbestic points on the absorption spectra.

The values of pK_2 and pK_3 were calculated from the variation of absorbance with pH, by the Stenström and Goldsmith[5] and Sommer[6] methods. The p*K* values shown in Table 2 are the arithmetic means of the values obtained from measurements at three different wavelengths; pK_2 is due to deprotonation of the nitrogen atom in the pyridine ring and pK_3 to loss of the proton of the –SH group.

For determination of pK_1 for dissociation of the sulphonic acid group, the procedure described by Davis and Geissmann[7] was used. It consists essentially in finding the Hammett acidity at which the compound is 50% ionized. The acidity functions (H_0) have been determined for perchloric acid solutions by Yates *et al.*[8]

The wavelengths 315 and 350 nm were chosen, close to the points of maximal difference between the molar absorptivities of the essentially non-ionized and practically completely ionized compounds. The inflection point of the plot of H_0 *vs.* D, where $D = A_{350} - A_{315}$, is given in Fig. 3 for both com-

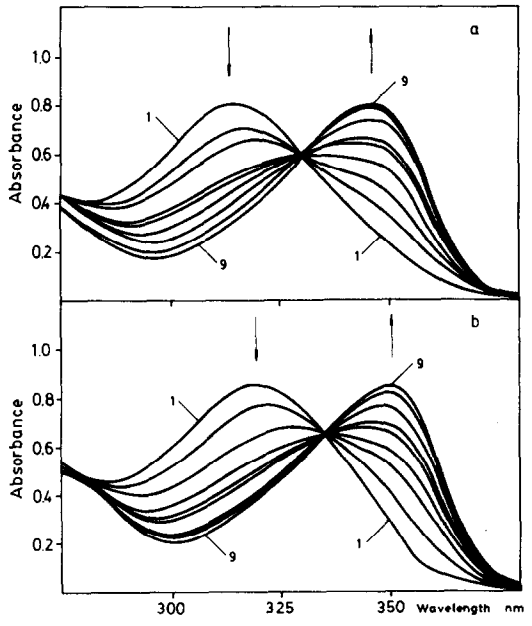


Fig. 1. Absorption spectra of both reagents at different pH values. (a) S-BT, $4.0 \times 10^{-5}M$; pH 1, 6.70; 2, 3.81; 3, 3.72; 4, 3.48; 5, 3.39; 6, 3.27; 7, 3,01; 8, 2.63; 9, 2.42. (b)-S-BPT, $4.0 \times 10^{-5}M$; pH 1, 4.30; 2, 3.78; 3, 3.57; 4, 3.42; 5, 3.32; 6, 3.23; 7, 2.99; 8, 2.67; 9, 2.39.

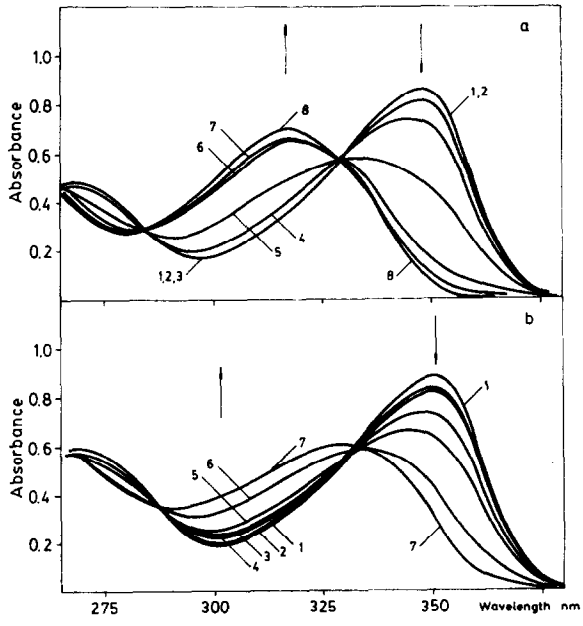


Fig. 2. Absorption spectra of both reagents at different concentrations of perchloric acid. (a) S-BT, $4.0 \times 10^{-5}M$; $HClO_4$% w/w: 1, 3.6; 2, 16.7; 3, 30.4; 4, 41.2; 5, 46.9; 6, 51.6; 7, 53.4; 8, 56.0, (b) S-BPT, $4.0 \times 10^{-5}M$; $HClO_4$% w/w: 1, 3.6; 2, 30.4; 3, 41.9; 4, 45.0; 5, 48.8; 6, 51.6; 7, 56.0.

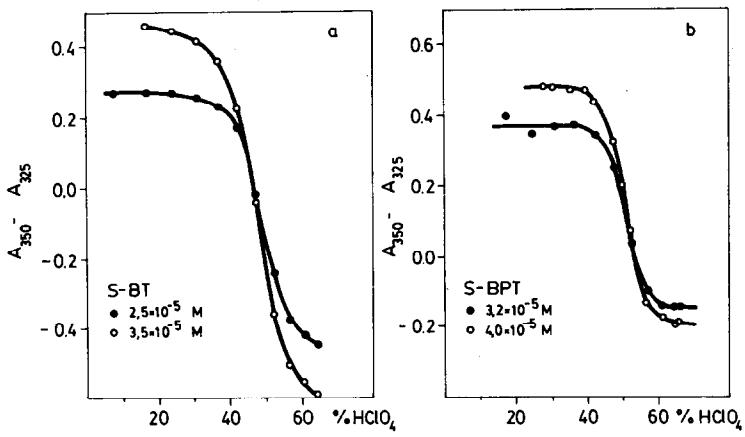


Fig. 3. Determination of pK_1 for both reagents.

pounds. Results obtained for pK_1 are also shown in Table 2.

Reactions with metal ions

The reaction of S-BT and S-BPT with 40 cations at concentrations up to 100 ppm and at various pH values, was investigated. As(III) and (V), Sn(II) and (IV), Pt(IV), Al, Cr(III), Be, U(VI), Th, La, Ce(III), W(VI), Mo(VI), alkali and alkaline-earth metal ions do not react. The most sensitive reactions are those of Co(II), Fe(II) and (III), Ni, Cu(II), Bi, Hg(II), Zn and Cd.

The absorption spectra of the chelates were recorded. The absorption maxima appeared between 370 and 410 nm, except for the green Fe(II) complex (λ_{max} 620 and 630 nm) for S-BT and S-BPT, respectively.

The optimal pH-ranges for formation of the chelates, the stoichiometric ratios, and other analytical parameters were studied, and the results are shown in Table 3. The metal-ligand ratios are the same for S-BT and S-BPT as for picolinaldehyde phenylthiosemicarbazone and related compounds, which act as terdentate ligands with Fe(II) and (III), Co(II) and Ni, and form octahedral complexes; in certain conditions, with Cu(II), they may act as bidentate chelating agents. All the complexes studied are retained on an anionic ion-exchanger resin, in weakly acid or basic media.

Study of iron–reagent systems

Simple kinetic studies on the green iron(II) complexes confirmed that the presence of ascorbic acid was necessary to keep the iron reduced in both complexes. The initial green colour formed on mixing iron(II) and S-BT or S-BPT in the absence of ascorbic acid, slowly changed to yellow at pH > 5 and the new spectra were identical with those obtained by mixing iron(III) with the reagents. The rate of oxidation was found to be first-order with respect to iron(II). The influence of pH on the oxidation rate was also studied and the results are shown in Fig. 4 and is probably attributable to the decrease in the potential of the O_2/O^{2-} system.

However, at pH > 11.5, the yellow iron(III) complexes were slowly reduced to the green iron(II) chelates; these reductions were also pH-dependent (Fig. 4). This behaviour is similar to that described for picolinaldehyde thiosemicarbazone,[9] and can be attributed to the reducing properties of these reagents in basic medium.

Table 3. Photometric characteristics of the complexes in solution

	S-BT				S-BPT			
Cation	Optimal pH	λ_{max}, nm	ϵ_{max}, $l.mole^{-1}.cm^{-1}$	M:R	Optimal pH	λ_{max}, nm	ϵ_{max}, $l.mole^{-1}.cm^{-1}$	M:R
Fe(II)	5.0–12.0	620	0.90×10^4	1:2	4.5–12.0	630	0.93×10^4	1:2
Fe(III)	5.0–8.0	380	1.24×10^4	1:2	5.0–8.0	405	3.95×10^4	1:2
Co(II)	2.0–10.5	370	1.20×10^4	1:2	4.0–8.5	405	2.90×10^4	1:2
Ni(II)	6.0–10.0	400	2.00×10^4	1:2	5.0–8.5	390	3.44×10^4	1:2
Cu(II)	5.0–11.0	390	1.03×10^4	1:1	5.0–9.5	400	2.12×10^4	1:1
Zn(II)	9.0–10.5	390	2.94×10^4	1:2	6.0–9.0	410	3.69×10^4	1:2
Cd(II)	6.0–10.0	385	3.10×10^4	1:2	5.0–7.0	400	3.20×10^4	1:2
Hg(II)	7.0–9.0	365	1.05×10^4	1:2	5.0–8.5	375	1.85×10^4	1:2

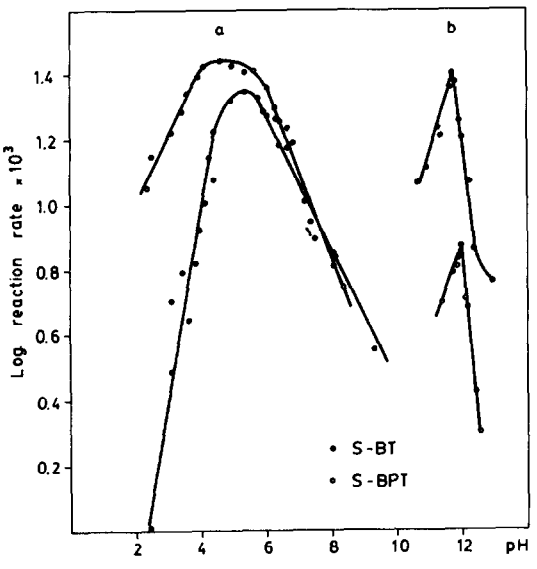


Fig. 4. Log of reaction rate ($\times 10^3$) *vs.* pH for iron(II) and (III) complexes. (a) Oxidation of green iron(II) complexes; 33°C; 4.25 ppm iron. (b) Reduction of iron(III) complexes; 33°C; [iron]: ● 8 ppm, ○ 4 ppm.

CONCLUSION

S-BT and S-BPT both possess the thioazomethine (thioferroin) grouping, which is selective for iron(II). The wavelength of maximal absorption (600–650 nm) is less likely to be subject to interference than that of ferroin itself.

Other reagents with this functional group had previously been studied, but gave uncharged iron chelates which were insoluble in aqueous medium, so that extraction into organic solvents was necessary for spectrophotometric determination. Introduction of the sulphonate group makes it possible to use the reagents for determination of iron(II) in aqueous medium.

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HARVEY DIEHL

BRUNO JASELSKIS*, ALTA HEFLEY, WILLIAM HOYLE and GERALDINE M. HUITINK

November, 1980 will be a banner month for analytical chemists as Dr Harvey Diehl, Iowa State University's Distinguished Professor in Sciences and Humanities, celebrates his 70th birthday. Dr Diehl, an analytical chemist, will be greeting his 70th year with the same intellectual vigor and wisdom with which he has met the challenges of chemistry for so many years. As stated by T. W. Higginson (Atlantic Essays, 1871), "Great men are rarely isolated mountain peaks, they are summits." In the world of analytical chemistry, none can dispute the monumental stature of Harvey Diehl. In pure chemistry, Dr Diehl's innovative approaches to research repeatedly expressed profound creativity and ingenuity; in teaching, Professor Diehl's enthusiasm and individuality provided students with a consistent impetus for discovery, which has served to shape the future of analytical chemistry; as a member of the society of man, Harvey Diehl remains a dynamic integrator of humanitarian concern and scientific excellence.

We share Dr Diehl's joy in celebrating his birthday and honor his contributions to chemistry and society with this special dedication issue of *Talanta*.

Harvey Diehl was born in Detroit on 2 November, 1910. His father, an insurance salesman, maintained the Germanic tradition of love and pride in individual achievement for the good of mankind and infused this same spirit into young Harvey. At age 18, Harvey Diehl entered the University of Michigan where he received both his B.S. (1932) and his Ph.D. (1936) in chemistry under the expert tutelage of Hobart Hurd Willard. While still a graduate student, Harvey assembled and organized Willard's voluminous lecture notes on advanced quantitative analysis, which later merited publication under the joint authorship of Willard and Diehl.

While being indoctrinated in the ways of chemistry by Willard, the young Diehl met Helen Louise Clark and, in 1936, culminated his pursuits with both a Ph.D. in chemistry and a "degree" in matrimony. Thus, Dr Diehl began his career as a dedicated chemist and husband, father, and family man—with his love extending beyond his personal family to include the family of graduate students that he directed.

After attaining the Ph.D., Diehl served as an instructor of chemistry at Cornell University (1936–37) and Purdue University (1937–39), then joined the faculty of the Iowa State College of Agriculture and Science, now known as Iowa State University, as an Assistant Professor of Chemistry. Iowa State provided an ideal atmosphere for Professor Diehl's teaching and research interests. In 1947 Dr Diehl became a full professor and tenured member of the faculty.

For over 40 years Professor Diehl has dedicated himself to the instruction of Iowa State students. He will soon be retiring from this regimen of constant teaching, but will continue his research and his remarkable publication efforts. The more than forty years of teaching and research have left their mark on both Dr Diehl and the world of chemistry, but the joy of stimulating and molding young minds has not been dimmed by the passage of years.

Dr Diehl's colleagues in chemistry have recognized him with many awards. These include: the prestigious Fisher Award in Analytical Chemistry (ACS, 1956); the Iowa Gold Medal Award of the Iowa Section of the ACS (1961); and the status award of "Distinguished Professor in Science and Humanities" (Iowa State, 1961). In addition, in 1965 the first John Anderson Wilkinson Teaching Award in chemistry was bestowed upon Professor Diehl by Iowa State, and in 1966 the Anachem Award was presented for contributions to analytical chemistry through teaching, research and service.

Throughout his many years at Iowa State, Harvey Diehl was a member and officer of many professional organizations, serving as Chairman of the Ames Section and the Division of Analytical Chemistry of the American Chemical Society. He also served on the editorial boards of *Talanta* and *Analytical Chemistry*. He is currently a member of the American Chemical Society; American Association for the Advancement of Science, Iowa Academy; the Society for Analytical Chemistry; Fibonacci Association; Sigma Xi; Phi Lambda Upsilon; and Alpha Chi Sigma (chemical fraternity).

Harvey Diehl has not limited himself to academic and professional activities, but also has been a consultant to many industrial organizations. For many years he was associated with the late G. F. Smith in the development and application of novel analytical reagents and in the use of perchloric acid. He was a consultant to and a member of the Board of Directors of the Lithium Corporation of America; was associated with the Office of Scientific Research and Development as an Office Investigator (1940–43) and directed projects concerning oxygen-carrying com-

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pounds and cobalt chelates. He is presently a consultant to the Hach Chemical Company.

During World War II Diehl and Willard and their students conceived the idea of an informal research conference to meet during the Christmas holidays. They formed the nucleus of what would eventually come to be known as the Midwest Universities Analytical Chemists Conference (MUACC), an organization that has since grown in both size and stature and now convenes over 100 analytical professors for an intense day and a half session once a year.

It would not be correct to praise the scientist, Harvey Diehl, without giving due consideration to his contribution to education. A quiet yet dynamic man, Harvey Diehl is a complex individual whose strong convictions and idealistic philosophy are reflected in every aspect of his life. Nowhere is this more strongly reflected than in his teaching.

Dr Diehl's impressive, indeed almost encyclopedic mind, has enabled him to devote his full attention to individual students while retaining his knowledge of all the particular details of his academic, professional and personal roles in life. Harvey Diehl is, and was, concerned with each of his students, always finding the time to converse with any who sought his advice. He maintains a unique ability to communicate with all levels in the intelligence spectrum, exhibiting an uncanny ease in both guiding the freshman through difficult chemical concepts and in analyzing the most penetrating questions and concepts discussed in a faculty or graduate seminar. "The progress of mankind throughout all time is the direct result of individual effort... it is the individual that stays awake thinking about the problem, collecting data, sorting the wheat from the chaff." This is Harvey Diehl's philosophy, and this is the attitude he has tried to exemplify. To his graduates, Harvey Diehl is not only seen as a primary research director, but also as the man perhaps most instrumental in shaping their careers. He shows interest and concern for his students, whether they be in the classroom, laboratory, or in a casual encounter. His constant interest is best exemplified by his usual query of "What have you learned today?"—a question Diehl students will not soon forget.

Never was there a time when a student would feel unwelcome in Diehl's office (which, most of the time, would be "equipped" with a 4-litre beaker of boiling, distilled water, tea bags, and a 500-g bottle of reagent grade sucrose). Harvey would never consider limiting the topics of conversation to just chemistry; literature, world news, camping and history were frequent subjects in which he and his students could "Diehl-ite." Yet, Professor Diehl retained his professionalism. His students were allowed to be open and proceed at their own pace, yet no-one was allowed to take advantage of this freedom. In fact, Diehl's own enthusiastic, inspiring approach to research could occasionally reach such a demanding pace that his graduates eventually found it worthwhile to construct a buzzer alarm warning of his approach! Dr Diehl's love for his students is perhaps best reflected by his compilation of texts on the various areas of analytical chemistry. In fact, to expedite the publication of these texts, Diehl founded his own publication company. The Oakland Street Science Press is the direct result of Diehl's dedication to chemical education.

There is perhaps one interest which could occasionally divert Harvey Diehl's attention from chemistry. This is his love for the outdoors. He built, mostly with his own hands, his home in Ames and his "cabin" in the Colorado Rockies. Each generation of his graduates was involved in some manner in the building process by helping to stack the boards, by pouring the concrete in the driveway or basement, by loading the trailer with a 400-lb bell or bricks to be transported to Colorado. Thus, his home and the cabin became places where many of his friends and students came to visit. The love of nature has taken Diehl, his family and his students to many places. He has crossed the Continental Divide on foot many times, canoed from Wisconsin to Hudson Bay and vacationed in the Rockies. Needless to say, the paths of life chosen by Dr Diehl have been both many and wondrous.

HARVEY DIEHL THE SCIENTIST

Diehl's contributions as a scientist have been significant and numerous. His various research interests, which have covered coordination and chelate ring compounds, vitamin B_{12}, analytical reagents used in the determinations of iron, copper, calcium, magnesium, cobalt, and beryllium, uses of perchloric acid, controlled potential separations, coulometry and electrochemical methods for the redetermination of the Faraday constant, have led to over 150 publications, three textbooks, and a number of monographs.

Diehl's research activity can be grouped into four broad areas of interest: (1) the continuation and expansion of his doctoral work on cobalt chelates, the development and characterization of new gravimetric and colorimetric reagents, and the application of the ethylenediaminetetra-acetic acids, (2) the study of electrogravimetric and coulometric methods of analysis and the design of controlled cathode electrolysis apparatus, (3) the development and characterization of metallochromic reagents, and (4) the high-precision electrochemical measurements dealing with the determination of the Faraday constant. Each of these areas has proven to have useful analytical applications contributing to the understanding of chemistry.

Diehl initially worked on metal chelate chemistry, particularly cobalt chelates, which he studied as a graduate student with H. H. Willard, and then expanded his research to include the development and application of organic analytical reagents and oxygen-carrying cobalt compounds. These latter research studies were conducted during World War II. At the end of the war these resulted in a series of declassified publications.

In the early 1950s, scientists throughout the world were excitedly exploring the world of vitamins. Vitamin B_{12}, cobalamine, was of particular interest. Many chemists were busily engaged in determining the chemistry and structure of this elusive vitamin. To a great extent, the elucidation of the structure of this vitamin depended on the preparation of the crystalline, partially degraded vitamin B_{12} or the red acid fragment. Diehl's work in such a hotly pursued area cannot go unmentioned, despite the fact that the final chemistry and the structure of this vitamin was provided by Nobel Prize recipients Sir Alexander Todd and Dorothy Hodgkin.

As a graduate student and in the years that followed, Diehl was interested in the analytical applications of perchloric acid. A method for the determination of potassium in silicate rocks was developed jointly with Willard and Liggett in 1942, using perchloric acid for the distillation of fluorosilicic acid. The separation of the insoluble potassium perchlorate from soluble metal perchlorates was achieved by extraction with ethyl acetate. Also, a rapid method for the determination of silicon in organosilicon compounds was developed, using perchloric and nitric acid oxidation and gravimetric determination of silicon dioxide (1946). The use of hot perchloric acid for the dissolution of copper sulfide ores was introduced in 1949. This method has been used, without incident, for thirty years by the undergraduates at the Iowa State University in the quantitative analysis laboratory.

The use of perchloric acid for wet ashing was investigated extensively by varying the temperature and the concentration of the perchloric acid, and the addition of other mineral acids such as sulfuric, phosphoric and iodic. These observations were reported by the late G. F. Smith and Diehl. Procedures were developed for the oxidation of coal, ion-exchange resins, alkaloids, synthetic fibers, rubber, as well as bones and other materials containing fats. A collection of these procedures with the eye-catching title, "Perchloric Acid Liquid Fire Reactions," was published by Diehl and Smith in 1960. The selective usage of 50% perchloric acid with solid iodic acid was used to isolate spores in coal. Diehl's interest in the use of perchloric acid has prevailed throughout his career. One wonders what he will do with perchloric acid next week.

Diehl's interest in electrogravimetric methods of analysis was sparked early in his career. His many subsequent contributions to electrochemistry were based on his pioneering work in the design of apparatus for electrogravimetric analysis with controlled, graded cathode potential. This work facilitated electrogravimetric separations using three-electrode systems which followed the potential between the cathode and reference electrodes. Although he was the first to design an automatic, graded, controlled potential apparatus and to apply it to the separation of silver from copper in the analysis of bronzes. Diehl maintained his interest in pure chemistry and remained a chemist rather than an instrument designer.

Dr Diehl's early research efforts were followed by research concerning the application of polarographic methods of analysis of cobalt and cerium compounds, the analysis of vitamin B_{12}, and the use of ion-selective electrodes. His Fisher Award address at the Dallas ACS meeting (1956) to a great extent summarized this early exhaustive research and stimulated the practical development of ion-selective electrode technology.

Diehl pursued not only the synthesis of new analytical reagents, but also their characterization and application in conjunction with the late G. F. Smith and his students, Clifford Hach, the late Charles Banks (another Fisher Award recipient) and others. In particular, this work was extended from the precipitation of colored metal complexes (dimethylglyoxime and dioxime compounds) to colorimetric, spectrophotometric, and fluorometric determinations of metals with detection limits which challenge the sensitivity of atomic-absorption spectrophotometry and nuclear methods.

Diehl's early interest in organometallo complexes is apparent in his monograph dealing with the application of dioximes to solving analytical problems, followed by work published on dimethylglyoxime and the preparation and use of 1,2-cyclohexanedioxime as a reagent for determining nickel, palladium and ferrous iron. The synthesis of 2,2′-bipyridyl and 1,10-phenathroline (Fritz and Blau), and the subsequent characterization and application of 2,2′-bipyridyl as a colorimetric reagent for iron and of 1,10-phenanthroline as a high-potential oxidation–reduction indicator for the ferrous–ferric couple, generated a great interest in tailoring derivatives of these reagents for specific uses. Out of this interest emerged three new metallochromic reagents for iron.

The synthesis of 4,7-diphenyl-1,10-phenathroline by F. H. Case in the early 1950s provided new methods for determining small amounts of iron in municipal waters, metallic copper, and urine. The versatility of these reagents was increased by sulfonation and the preparation of sulfonated derivatives.

The synthesis of 2,4,6-tripyridyl-*s*-triazine (TPTZ) by F. H. Case and E. Koft enabled Diehl and his students to characterize and apply this reagent to the determination of iron in limestone, silicates, refractory materials, wine and sea-water. Both bathopenanthroline and TPTZ iron complexes are formed in slightly acidic media; however, there was a need for a reagent which would form iron complexes in basic media. Such a reagent, namely, phenyl 2-pyridyl ketoxime, was found to fulfill this requirement and was applied to the determination of small amounts of iron in strongly alkaline solutions and in the presence of metallic iron. The description and application of these reagents have been summarized by Diehl and Smith in a monograph.

Concurrent with the work being done with iron reagents, similar investigations were carried out with copper reagents such as cuproine, neocuproine and bathocuproine. Because of the similarity of these compounds to the iron reagents, Diehl and Smith published a monograph describing their applications. Subsequent work with phenyl 2-(6-methylpyridyl) ketoxime and with 6-methyl-2-pyridinecarboxamide oximes resulted in suitable reagents for the determination of copper.

Another area which attracted Diehl's interest was that of the utilization and application of a versatile compound then being produced by Bersworth Chemical Company. This attraction was the result of a 3-drum delivery of this reagent to the Iowa State University Chemical Storeroom. Shortly thereafter, in 1950, Diehl and his associates published papers on the "Versenate" titration for the total hardness of water and the determination of the ratio of calcite to dolomite in carbonate rocks. It was during this period that (ethylenedinitrilo)tetra-acetic acid, alias ethylenediaminetetra-acetic acid (EDTA), alias 'Versene", was making itself famous as a "universal" reagent for metal ion chelation. Its primary use at that time was the determination of the sum of calcium and magnesium. There was a very definite need for a reagent or reagents to serve as indicators for the detection of the end-points. Schwarzenbach and Biedermann used Eriochrome Black T as an indicator for the combined determination of calcium and magnesium. Unfortunately, this indicator was not stable, requiring fresh solutions every few days. Diehl and his students spent considerable time and effort trying to find a replacement indicator for the calcium–magnesium titration. They were eventually successful and the indicator "calmagite" provided characteristics similar to Eriochrome Black T but with excellent stability. However, the determination of calcium and magnesium in combination was not the ultimate goal. There was a need to assay these important metals independently. During the search for a reagent to assay for total magnesium in the presence of calcium, work was conducted to find reagents which showed specificity for calcium alone and magnesium alone. This search proved fruitful. The determination of calcium in the presence of magnesium by using a newly synthesized reagent, "Calcein", was applied in 1956. The publication of this research initiated an avalanche of approximately 50 papers dealing with the application of Calcein, in which Calcein emerged not only useful as an indicator for the titration of calcium with EDTA but also as a fluorimetric reagent that could directly measure the amount of calcium present in a sample by its fluorescence intensity. Further research succeeded in providing the synthesis of Statocalcein and Calcein Blue and its methyl and other derivatives. Magnesium determination by a fluorometric method was achieved with *o,o'*-dihydroxyazobenzene. The work on the calcium and magnesium determinations, the applications of the new reagent, and characterization of the reactions involved were carried out by Diehl and his students. An overview of metallochromic indicators was presented at the 1966 Detroit Anachem meeting.

HARVEY DIEHL AND THE FARADAY CONSTANT

In the mid-sixties, high-precision coulometry, *i.e.*, with a relative error of less than 1–3 parts in 10^5, was made possible by developments in electronics and the apparatus made available by Leeds and Northrup Co. These developments made it possible for Diehl's group to tackle the problem of primary standard ammonium hexanitratocerate which has been discussed in the literature for many years. This work with high-precision coulometry kindled an interest in Diehl to use the electron as the ultimate primary standard reference material; the electron could be the standard reference for all acid–base and oxidation–reduction analyses. The basis for this was expressed verbally by Michael Faraday himself in 1832 and the three laws of electrolysis were later expressed mathematically by Diehl as

$$G = \frac{ItM}{Fn}$$

where G is the number of grams of material, It is the number of coulombs (I = current in A, t = time in sec), M/n is the electrochemical equivalent weight and F is the proportionality constant known as the Faraday.

However, before chemists could routinely use high-precision coulometry, that is, the electron, as their sole primary standard, the Faraday constant had to be evaluated to a high degree of accuracy. It is interesting to note that of the fundamental constants, *i.e.*, the velocity of light, Avogadro's number, Planck's constant, *etc.*, only the Faraday constant was *not* known to a very high degree of certainty. The physicists were content with their ability to determine the fundamental constants affecting their work; they could measure time, mass, frequency, *etc.* to better than 1 part in 10^7. Only the chemists could not measure the Faraday constant to better than 6 parts in 10^5 because it was based on a chemical reaction, and even the "accepted" number was highly disputed.

The work on the Faraday constant at Iowa State began in early 1969. During a lecture in a graduate-level electrochemistry course, Diehl described the importance of the Faraday constant and the difficulties of high-precision coulometry. This discussion sparked the beginning of an intensive investigation and search for suitable compounds for the redetermination of the Faraday. After several months of research on a variety of compounds which were thought to be suitable for the project, it was announced one afternoon during one of Diehl's frequent "tea parties" that 4-aminopyridine was to be the compound of choice. The choice of 4-aminopyridine as a primary standard was

based on a number of important features: (1) it could be purified by sublimation, (2) it had a high melting point, allowing the determination of impurities by the freezing-point depression, (3) it was a sufficiently strong base to give a good acid–base end-point, and (4) it contained only three elements, carbon, hydrogen and nitrogen, and for these the isotope ratios are readily determined. Thus, pure 4-aminopyridine was used for titration by generation of either hydronium or hydroxide ions. A current efficiency of 100% at the anode for the generation of hydronium ions was achieved on the basis of hydrazinium ion reaction:

$$N_2H_5^+ \rightarrow N_2 + 5H^+ + 4e$$

Also, coulometrically generated hydroxide was used to back-titrate excess of perchloric acid added to 4-aminopyridine. In this manner high-precision measurements were performed in establishing the Faraday with an uncertainty of a few parts per million.

In performing these measurements it was realized that the greatest uncertainty was that of the measurement of mass. Thus, suitable procedures had to be developed, using either a spherical float of stainless steel or a hollow, evacuated stainless-steel cylinder. This allowed measurement of the density of air well enough to make the error from buoyancy correction only about 1 ppm. The mass in vacuum was determined by using a vacuum weighing bottle, *i.e.*, by weighing the evacuated bottle alone and then with the sample. These studies also revealed that platinum weights were inferior to stainless steel because of the higher adsorption of gases on the platinum surface.

The work on the Faraday continues at ISU and has led to many instrumental improvements and interesting sidelights. It also cast some doubt on the ability to measure mass to a high degree of accuracy and may ultimately lead to changing the basis of the Periodic Table from carbon-12 to silicon-28. Furthermore, this work is characteristic of Diehl's perseverance, exactness and dedication to follow research projects to their ultimate frontiers in the best tradition of T. W. Richards and H. H. Willard.

Under Harvey Diehl's tutelage, 60 students have earned the M.S. degree in chemistry and 47 the Ph.D. One of Diehl's life goals has been achieved by producing four more Ph.D.s than his mentor, H. H. Willard. The majority of Dr. Diehl's students have gone into industry and risen to responsible positions. Twenty-one of his students have entered academia and 17 of these have remained in teaching and developed independent research programs. One of Diehl's students, the late Charles V. Banks, was himself a professor at Iowa State who, in addition to being a Fisher Award recipient, had seen 17 of his students receive the Ph.D. and 22 receive the M.S. Dr. Banks's demise was a sad occasion for chemistry and his presence is sorely missed. Another of Harvey's students, William F. Koch, is presently working with the National Bureau of Standards.

We are sure that Dr. Diehl is as proud of his academic chemistry offspring as they are of their chemistry parent.

Charles V. Banks, Iowa State University
Edward B. Buchanan, Jr., Iowa University
Albert L. Caskey, Southern Illinois University
Thomas N. Dobbelstein, Youngstown State University
Amparo M. Escarilla, Utica College
Chris Wyatt McGowan, Tennessee Technological College
Laurie Grennan, University of Tennessee
Geraldine M. Huitink, Indiana University—South Bend
Bruno Jaselskis, Loyola University of Chicago
Byron G. Kratochvil, University of Alberta
Frederick Lindstrom, Clemenson University
Richard Markuczewski, Iowa State University
Rodney L. Osen, Hamlin University
Donald P. Poe, University of Minnesota, Deluth
David L. Pringle, Colorado Northern University
John K. Senne, Trinity College
Gerald I. Spielholtz, Herbert-Lehman College

In surveying the almost 45 years of chemistry to which Harvey Diehl has devoted himself, one is left with a feeling of having been exposed to a dynamic, individualistic teacher and an inquisitive, meticulous, and persevering researcher. It has been these qualities that have made Dr. Diehl the successful teacher and researcher that he is. Diehl's vast energy has been felt not only within the academic world but also in industry and in society.

These few pages and occasional glimpses into Professor Diehl's career do not purport to present an adequate picture of this complex person who has accomplished so much and solved so many difficult problems in such a short time span. With the help of his family, particularly his wife, Helen, and his students, whether they be graduate or undergraduate, he may assuredly exclaim: "*Exegi monumentum aeri perennius regalique situ pyramidum altius...*" (Horatii Carminum XXX).

THE M.S. AND Ph.D. STUDENTS OF HARVEY DIEHL

Master of Science

1.	Nathaniel C. Fick	1938
2.	Galen Porter	1939
3.	Arnold J. Veraguth	1939
4.	Robert C. Parker	1940
5.	John C. Kaskie	1941
6.	Morris Friedkin	1941
7.	Russell E. Carr	1942
8.	Francis T. Lee, Jr.	1943
9.	Charles V. Banks	1944
10.	Richard Brouns	1945
11.	Johanna Henn	1947
12.	John Mathews, Jr.	1949
13.	Robert J. Bever	1949
14.	John N. Mandas	1951

15.	John P. Butler	1952
16.	Richard A. Murie	1952
17.	John I. Morrison	1952
18.	John L. Ellingboe	1953
19.	Frederick H. Lohman	1953
20.	Bruno Jaselskis	1954
21.	Russell Craig	1955
22.	Robert Burr	1955
23.	Albert Caskey	1955
24.	Peter F. Collins	1957
25.	Ana Isabel Pellecher	1957
26.	David E. Blair	1958
27.	H. Whitney Wharton	1958
28.	Amparo E. Escarilla	1959
29.	Byron G. Kratochvil	1959
30.	Fred C. Trusell	1959
31.	Carl L. Hanson	1960
32.	Rodney L. Olsen	1960
33.	Anthony D. Pietrzykowski	1961
34.	Albert L. Baetz	1962
35.	Alta J. Hefley	1965
36.	Geraldine M. Huitink	1965
37.	David L. Pringle	1965
38.	Thomas N. Dobbelstein	1966
39.	John R. Pemberton	1966
40.	John W. Knoeck	1966
41.	Ilga Birze	1967
42.	Frederick W. Hartford	1967
43.	John J. Contario	1968
44.	Charles R. Peters	1969
45.	Linda Ann Freytag	1971
46.	James A. Gaunt	1971
47.	Donald P. Poe	1972
48.	James M. Gorum	1974
49.	William F. Koch	1974
50.	Dennis B. Martin	1974
51.	Theresa Walter Michels	1975
52.	Chris Wyatt McGowan	1976
53.	Samir G. Gharfeh	1977
54.	James Steven Gibson	1977
55.	Michael C. Hadka	1977
56.	Kathryn Johnson Bush	1978
57.	Danny Shek-cheong Hui	1978
58.	Eugene Shi-jik Khoong	1978
59.	Peter Kai-cheung Lo	1978
60.	John W. Furry	1980

Doctor of Philosophy

1.	Donald E. Howe	1942
2.	Lawrence M. Liggett	1943
3.	George M. Harrison	1944
4.	Charles V. Banks	1945
5.	Eugene M. Sallee	1948
6.	Richard Brouns	1948
7.	John M. Brierly	1953
8.	John P. Butler	1954
9.	Richard A. Murie	1955
10.	Frederick H. Lohman	1955
11.	Bruno Jaselskis	1955
12.	John L. Ellingboe	1956
13.	Edward B. Buchanan, Jr.	1959
14.	Peter F. Collins	1959
15.	Frederick J. Lindstrom	1959
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THE INFRARED SPECTRA AND STRUCTURES OF THE THREE SOLID FORMS OF FLUORESCEIN AND RELATED COMPOUNDS

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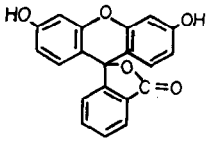
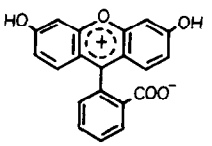
Summary—The three solid forms of fluorescein, yellow, red and (newly isolated) colourless, have been shown to differ distinctly in structure. By spectroscopic methods, principally infrared absorption, the colourless form has been assigned the lactone structure, the red solid the *p*-quinone structure, and the yellow solid a zwitterion structure with a positive charge distributed over the oxygen-bearing ring.

Although fluorescein was first prepared by Baeyer[1] over 100 years ago, a definitive assignment of structures to the various solid forms of the compound has not been made. The fluorescence of fluorescein has, of course, attracted a great deal of attention and the many papers dealing with it range from mere descriptions and applications to involved theoretical treatments. Of the papers concerned strictly with structure, excluding the early papers of Bayer, E. Fischer,[2] O. Fischer,[3] and others, in which the basic features of the composition and structure were established, we single out as the two principal papers those of Orndorff and Hemmer[4] published in 1927, and Zanker and Peter,[5] 1958, and two papers dealing with the infrared spectrum, Davies and Jones,[6] 1954, and Sklyar and Mikhailov,[7] 1966.

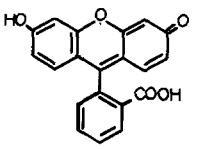
Orndorff and Hemmer cleared away the faulty experimental observations regarding the extent of hydration, the existence of an orange solid form in addition to the yellow and red solids, various misconceptions on the composition and basic structure, and, largely on the basis of the absorption of ammonia gas, an experiment now known to be faulty, they made assignments of structure: the lactone form (**I**) to the yellow solid, the *p*-quinonoid form (**III**) to the red solid. These are the structures found in the textbooks of organic chemistry to this day although Fieser and Fieser[8] and Karrer[9] present them with reservations.



I Lactone structure, colourless form



II Zwitterion structure, yellow form



III p-Quinonoid structure, red form

The Zanker and Peter[5] work deals principally with the various cationic, neutral and anionic forms of fluorescein in solution. This paper is particularly relevant to the present discussion in that renewed attention was paid to the colourless form of fluorescein present in such non-polar solvents as dioxan, and it was recognized that this colourless form is probably the lactone (**I**). The Davies and Jones[6] and Sklyar and Mikhailov[7] papers are sadly incomplete, but this is quite understandable as they were done at a time when the use of infrared spectrometry in organic structure work was just developing.

On the basis of new experimental work, we believe the solid forms of fluorescein to have the following structures: the colourless, newly isolated solid, the lactone structure (**I**); the yellow solid, the zwitterion structure (**II**); the red solid, the *p*-quinonoid structure (**III**). Without attempting to document each error here, we believe we have avoided certain faults which have affected adversely one or another of the various earlier studies. Mistaking the disodium salt (uranine) for fluorescein itself has been avoided by converting the commercial material into the various free solid fluoresceins and determining the equivalent weight by potentiometric titration with alkali. The very considerable amounts of the heavy metals, iron, mercury, zinc, and aluminium, present in commercial fluorescein,[10] which seriously affect the physical and chemical properties, have been eliminated by conversion of the fluorescein into diacetylfluorescein and hydrolysis back again to fluorescein. A colourless fluorescein has been isolated in the solid state by freeze-drying a solution of fluorescein in dioxan. The three solid forms of

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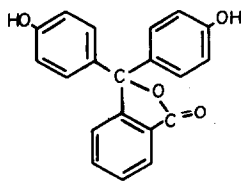
fluorescein, colourless, yellow and red, have been shown by X-ray diffraction powder patterns to be definite and individual species. Although some useful information was obtained from the mass spectra of the three solid forms, the assignments of structure just made are based principally on the infrared spectra.

COLOURLESS FLUORESCEIN—THE LACTONE STRUCTURE

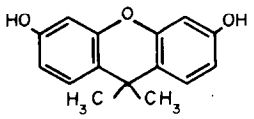
As early as 1908, it was predicted[11] that a colourless form of fluorescein should exist, in which the lactone ring is present. Indeed, in 1914, colourless forms were prepared,[3] in the form of "addition compounds" containing pyridine and quinoline. It is curious that in their classic paper, in 1927, in which they straightened the chemistry of fluorescein, Orndorff and Hemmer[4] completely ignored the colourless solids and in the end assigned the lactone structure to the yellow solid. Probably, they simply did not care to cope with the additional complexity of the addition compounds and the further complication that in the presence of water the colourless form changes into the yellow form with ease. Indeed, the colourless form is never observed in solution except in anhydrous solvents.

The assignment made here of the lactone structure to the colourless solid is based on analogy with related chemistry and evidence from the infrared spectrum and the mass spectrum.

In the lactone form of fluorescein (**I**), all three of the benzene rings are benzenoid, the carbon atom in the 9′-position is attached to the other parts of the molecule by four single bonds, no chromophore group is present, and the compound should be colourless. Phenolphthalein (**IV**), similar in all respects to fluorescein except that the bridging oxygen atom is absent, is colourless. Another similar but simpler molecule is 3,6-dihydroxy-9,9-dimethylxanthene (**V**) which is also colourless. An apparent exception is fluorescin, the reduced form of fluorescein, which should be colourless but is pale yellow; fluorescin is very easily oxidized, however, and the yellow colour is undoubtedly caused by contamination with fluorescein. The lower stability of the lactone of fluorescein in comparison with phenolphthalein is probably a result of (1) increased strain in the lactone ring of fluorescein resulting from the forced planar character of the xanthene ring, and (2) the zwitterion structure (**II**) being stabilized by the oxygen bridge.



IV Phenolphthalein



V 3,6-Dihydroxy-9,9-dimethylxanthene

The infrared spectrum of colourless solid fluorescein is marked by a prominent absorption band at 1730 cm^{-1}, not present in the spectra of the red and yellow solids. This band falls in the region, 1745–1715 cm^{-1}, in which a band appears in the spectra of substituted phthalides and of phenolphthalein and its derivatives, compounds all known to contain the lactone ring. The band results from absorption by the carbonyl group (carbon–oxygen stretching frequency) and differs in position from that of carbonyl groups in free carboxylic acids and in carboxylate anions.

In the mass spectrum of the colourless form of fluorescein, the parent peak is absent when low-heat volatilization is used and becomes visible only after the input of considerable heat. In the mass spectra of the yellow and red forms of fluorescein, on the other hand, the parent peaks are easily observed, even at low heat. The weak appearance of the parent peak in the mass spectrum of the colourless fluorescein is characteristic of compounds with a five-membered lactone ring substituted at the γ-carbon atom. Besides the weak parent peak, the mass spectrum of the colourless form also contains a medium–weak peak at $m/e = 237$ which does not appear in the mass spectra of the yellow and red forms. It also contains a peak at $m/e = 88$ and one at $m/e = 55$, owing to the presence of dioxan. In all other respects, the mass spectrum of the colourless form is identical to that of yellow or red fluorescein.

RED FLUORESCEIN—THE *p*-QUINONOID STRUCTURE

The *p*-quinone structure (**III**) was assigned to the red solid form of fluorescein by Orndorff and Hemmer;[4] the present work places the assignment on a firmer basis.

The *p*-quinone structure contains a highly conjugated chromophore and exists in various resonance forms. Concentrated alkaline solutions of fluorescein and the solid disodium salt have the same colour as red solid fluorescein; the same *p*-quinone structure is present in all three cases. Alkaline solutions of phenolphthalein and the solid dipotassium salt are highly coloured because of the same *p*-quinone chromophore, known, of course, in literally hundreds of such compounds.

The infrared spectrum of red solid fluorescein is marked by a prominent absorption band at 1711 cm^{-1}, in the region of absorption by carbonyl groups (1800–1600 cm^{-1}) and more specifically in the region of the frequencies corresponding to a free (non-

dissociated) carboxylic acid group. The frequency of the carbonyl absorption band of the quinone function, normally observed at 1685–1626 cm^{-1}, is presumably shifted to lower frequency because of the high conjugation of the quinone group, and merged with the broad aromatic absorption band at 1600–1590 cm^{-1}. Such shifts to low frequencies for highly conjugated systems are well-known.

The mass spectra of red and yellow fluorescein are identical. The yellow form is converted into the red when heated, and probably such a change occurs in the mass spectrometer during the excitation of the yellow form. The mass spectra are characterized by peaks caused by decarboxylation and loss of carbon monoxide, oxygen and hydroxyl groups; these degradation patterns are consistent with a structure containing carboxylic acid, quinone, ether, and phenolic functions.

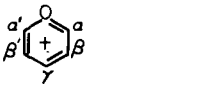
YELLOW FLUORESCEIN—THE ZWITTERION STRUCTURE

The zwitterion structure (II) proposed here for the yellow solid form of fluorescein was postulated by Zanker and Peter[5] as a transient intermediate in the conversion of colourless fluorescein in solution in dioxan into the yellow form on the addition of water. Zanker and Peter were interested only in the forms of fluorescein in solution and considered an aqueous solution of fluorescein to consist of a mixture of the lactone and *p*-quinone forms; their own evidence really indicated that in pure water only the coloured form was present and Lindqvist[12] showed that the equilibrium between the two is established very rapidly.

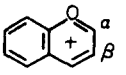
The proof that the yellow solid form is the zwitterion (II) is based on (1) the amphoteric nature of the material as reflected in the exceptionally high melting point, the low solubility, and the unusual, highly acidic character of the replaceable hydrogen atoms; (2) evidence from the infrared spectrum; and (3) analogy with the pyrylium compounds. The zwitterion structure offers neat explanations for hitherto unexplained phenomena observed in the chemistry of compounds related to fluorescein.

The infrared spectrum of the yellow form is marked by three features which differentiate the yellow solid from the red and colourless forms. First, no absorption band appears in the region 1800–1600 cm^{-1} characteristic of the carbon–oxygen stretching vibration of the carbonyl group; the presence of either a free (non-dissociated) carboxylic acid group and of a lactone group is thus ruled out. Secondly, a prominent band appears at 1536 cm^{-1}, not present in the spectra of the red and colourless forms but present in a class of compounds known as the pyrylium salts; by analogy then, fluorescein contains a six-membered, oxygen-containing ring carrying a positive charge, that is, yellow fluorescein is a zwitterion. The positive charge on the ring must then be balanced by a negative charge on the carboxyl group, that is, the latter is present as a carboxylate anion, and the hydrogen atom must then be present as a second phenolic group, and thus the two resorcinol rings of the fluorescein molecule are both benzenoid in character. Finally, the two absorption bands characteristic of the carboxylate group appear in the spectrum of yellow fluorescein, at 1596 cm^{-1} and at 1461–1450 cm^{-1}, but both bands are merged with or superimposed on major bands corresponding to aromatic carbon–carbon stretching vibrations; a detailed study, however, indicates that both bands are present.

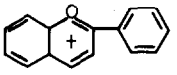
The zwitterion structure postulated poses two problems of great interest: the position of the positive charge on the six-membered, oxygen-containing ring, and the source of the yellow colour. Two oxonium structures are possible: one in which the positive charge is located on the bridging oxygen atom, the other in which it is located on the carbon atom in the 9-position, that is, the 9-carbon atom is a carbonium ion. The central, heterocyclic ring in the zwitterion structure of fluorescein is similar to that in the highly coloured pyrylium salts. Benzopyrylium and flavylium salts, which include the naturally occurring anthocyanin dyes responsible for much of the colour in the plant world, have been studied extensively. The literature in this field is enormous, a fair part of it being devoted to the study of distribution of the positive charge on the atoms of the ring.



VI Pyrylium cation



VII Benzopyrylium cation



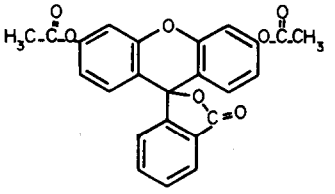
VIII Flavylium cation

An excellent review of the possible structures of the pyrylium salts is that of Hill.[13] The location of the positive charge on the ring was studied by Föhlisch and co-workers[14,15] by NMR, infrared, and ultraviolet spectrometry. In a review of the chemistry of the pyrylium salts, Balaban *et al.*[16] use the common notation of the positive charge on the oxygen atom; they emphasize, however, that this is only a matter of convenience, because all the chemistry of the pyrylium salts is that of substances with the positive charge at either the α- or the γ-position. In another study, Shriner and Moffett[17] concluded that benzopyrylium salts are quite different from the common oxonium salts obtained from ethers or γ-pyrones; they are more similar to the carbonium-type salts derived from triphenylcarbinols. Recently, Martensson and Warren[18] performed molecular orbital calculations on the pyrylium ring and concluded that the positive charge cannot be assigned to any particular

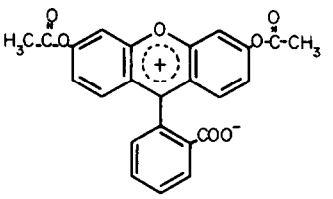
atom; rather, it exists in an aromatic-like distribution over the entire ring, although the α- and γ-carbon atoms definitely carry a partial positive charge, δ^+. A charge distribution of this type explains the unusual stability of the pyrylium ring in which the four electrons of the α-, α'-, β-, β'-carbon atoms and the unshared electron-pair of the oxygen atom provide the six π-electrons for an aromatic system. Reasoning by analogy then, the aromaticity and uniform charge distribution have been adopted for fluorescein in this work and incorporated into the zwitterion structure for the yellow form.

The yellow colour of the zwitterion structure of fluorescein is a consequence of the positively charged pyrylium-type ring portion of the molecule. Such structures are always coloured, and compounds which possess these structures are widespread in nature, accounting for the colours of flowers and fruit. The colours are very pH-dependent, changing from yellow, orange, or red on the acid side to purple or blue on the alkaline side. That the yellow colour is derived from the positive charge of the ring and not from the negative charge of the carboxylate group is seen from the behaviour of fluorescein in strongly acidic solutions, in which the protonated fluorescein has the structure of a positively charged ring and a free carboxylic acid group. Such solutions are invariably yellow. In addition, the well-known carbonium ion structures of simple triphenylmethane compounds are also usually yellow or orange.

The zwitterion structure of the yellow form is useful in explaining not only the colour but also other characteristics of fluorescein and its derivatives. A puzzling observation about diacetylfluorescein was made by Hefley[10] and confirmed during the course of the present work. During thin-layer chromatography on silica gel with a methanol–benzene mixture as mobile phase, diacetylfluorescein (no matter how pure) yields two spots. Incomplete acetylation of the original fluorescein and partial hydrolysis of the diacetylfluorescein were ruled out by repeating the acetylation and by repeated recrystallizations, and by the deliberate addition of free fluorescein as a tracer and proof of purification. It is now proposed that diacetylfluorescein, a colourless compound, can under certain conditions exist both as the lactone and as the yellow zwitterion form, structures **IX** and **X** respectively. Two spots would then be expected on the chromatography plates. Such opening of the lactone



IX Diacetylfluorescein colourless



X Diacetylfluorescein yellow

ring would also account for the slight yellowish discoloration of diacetylfluorescein caused by exposure to moist air. It explains also the yellow form of diacetylfluorescein prepared by Nagase *et al.*[19] by adding a few drops of sulphuric acid to the acetylation mixture during the preparation of diacetylfluorescein.

EXPERIMENTAL

Materials

Diacetylfluorescein. Commercial fluorescein, 200 g, was mixed with 100 g of anhydrous sodium acetate and added to 1 litre of acetic anhydride. The mixture was refluxed for approximately 4 hr. The hot mixture was then poured into approximately 5 litres of demineralized water. The tan, curdy precipitate which formed was allowed to settle overnight, then filtered off and washed with approximately 50 ml of ethanol. The material was dissolved in hot benzene and a portion of the benzene evaporated. Upon cooling of the light yellow solution, a precipitate was formed, lighter in colour than the starting material. The recrystallization from benzene was repeated twice, 177 g of an off-white powdery product being obtained: m.p. 203.5–205.5°; reported 199.5° (Dolinsky and Jones[20]), 200° (Orndorff and Hemmer[4]), 200–202° (Nagase *et al.*[19]), 205–206° (Liebig[21]).

Attempts to recrystallize more than very small amounts of diacetylfluorescein from ethanol as described by Dolinsky and Jones[20] and by Hefley[10] were impracticable; the solubility in hot ethanol is simply too low and the recrystallized material obtained was little improved in either colour or melting point.

Yellow fluorescein. Purified diacetylfluorescein was suspended in 95% ethanol containing sodium hydroxide in 2:1 mole ratio to the compound and the mixture refluxed for 20 min. The hot mixture was then filtered, and the filtrate diluted with demineralized water, cooled to room temperature, and treated with glacial acetic acid added dropwise. The yellow precipitate which formed was filtered off, washed with copious amounts of demineralized water, and dried at 110–120° for 2 hr; equivalent weight (potentiometric titration with 0.1*N* sodium hydroxide) found: 167.5, 165.1, 166.0, average 166.2; calculated (two replaceable hydrogen atoms): 166.15. The material did not melt below 300°, but between 260° and 280° was completely converted into the red form.

Red fluorescein. Purified diacetylfluorescein was refluxed with ethanolic sodium hydroxide as above. The hot alkaline solution was poured into hot dilute hydrochloric acid. The red precipitate formed was filtered off, washed with demineralized water, and dried; equivalent weight found: 166.1; calculated 166.15. The material did not melt at temperatures up to 300°.

Red fluorescein was also prepared by heating yellow fluorescein in a sublimation apparatus at about 250°. No significant sublimation occurred, but after several days the yellow powder was converted into red crystals of apparently regular shape. Inspection under the microscope, however, revealed that the crystals were highly twinned and unsuitable for a single-crystal X-ray investigation.

Colourless fluorescein. Approximately 5 g of red fluorescein were dissolved in 300 ml of dioxan, slight warming being necessary to effect complete dissolution and a yellowish solution resulting. On freeze-drying, an almost colourless residue was obtained, with only the slightest hint of yellow; equivalent weight (by potentiometric titration in water with 0.1*N* sodium hydroxide) found: 191.2; calculated for $C_{20}H_{12}O_5.\frac{1}{2}C_4H_8O_2$ 188.2. Calculated from equivalent weight found: 0.57 mole of dioxan per mole of fluorescein. Loss on drying in vacuum at 60° for 60 hr 1.7%; equivalent weight found after drying, 184.2 and 188.2, corresponding to the presence of 0.41 and 0.50 mole of dioxan, respectively, per mole of fluorescein. Behaviour on heating on a microscope slide: above 120°, an increasingly yellowish discoloration, the colour being deep yellow at 175°; at 180°, softening; at 182–185°, melting to an orange-brown liquid; at 186°, small islands of solid material beginning to form, followed by complete solidification into orange-red crystals at 200°; 270°, red centres appearing and at 285° conversion into red crystals complete; no further change up to 300°.

4',5'-Dimethylfluorescein. 4',5'-Dimethylfluorescein was synthesized from 2-methylresorcinol and phthalic anhydride by the method of Hefley.[22] The impure material obtained was refluxed with pyridine containing acetic anhydride. The mixture was cooled, diluted with demineralized water, and treated with hydrochloric acid. The diacetyldimethylfluorescein thus precipitated was filtered off, washed with cold 2% hydrochloric acid, and recrystallized twice from ethanol; m.p. 241.5–242.5°. The diacetyl derivative was then hydrolysed by refluxing with potassium hydroxide in ethanol. The mixture was filtered while hot, and the filtrate diluted with demineralized water and treated with diluted hydrochloric acid (1 + 1). The precipitated 4',5'-dimethylfluorescein was a reddish orange powder; equivalent weight found: 181.8; calculated (two replaceable hydrogen atoms) 181.15. The compound did not melt at temperatures up to 300°; at 325° charring began and at 350° carbonization was complete.

Hefley[22] did purify her 4',5'-dimethylfluorescein by conversion into the diacetyl derivative but failed to characterize completely this derivative, the 4',5'-dimethylfluorescein, and the 4',5'-dimethylcalcein prepared subsequently. The pyridine–acetic anhydride method of acetylation is better than the acetic anhydride–anhydrous sodium acetate procedure of Hefley.

3,6-Dihydroxy-9,9-dimethylxanthene. 3,6-Dihydroxy-9,9-dimethylxanthene was synthesized by the condensation of resorcinol and acetone in the presence of anhydrous zinc chloride, the procedure of Hanousek.[23,24] The very impure product, otherwise difficult to purify, was converted into the diacetyl derivative by refluxing with pyridine and acetic anhydride. The diacetyl derivative was isolated as a light brown powder; m.p. 153–155°, reported 151.5–152.5° (Hanousek[23]). Hydrolysis yielded 3,6-dihydroxy-9,9-dimethylxanthene as almost colourless needles; m.p. 263–265°, reported 260° (Hanousek[23]) and 266° (Hanousek[24]).

Phenolphthalein. Commercial phenolphthalein (J. T. Baker Chemical Company) was used without further purification.

X-Ray diffraction patterns

The X-ray powder patterns of the three forms of fluorescein were obtained by Professor Donald L. Biggs of the Department of Earth Sciences at Iowa State University, Ames, Iowa, on a Geiger-Müller counter instrument, the Norelco Diffractometer Type 42266, with a copper target (Cu $K_{\alpha 1}$ with $\lambda = 1.54050$ Å). National Bureau of Standards Tables[25] were used to obtain the *d*-values (which were rounded off to three significant figures) from the observed diffraction angles.

The *d*-values are listed in Table 1. It is well established that the pattern for each polymorphic form of a substance is specific and can be used as a means of identification.[26] The *d*-values in the table are sufficiently different and distinctly unique to each form of fluorescein. Thus there is no question that the characteristic differences in colour are associated with specific differences in the crystal structure and are not caused by impurities or by particle-size effects. The claim that the yellow form is amorphous whereas the red form is crystalline[7] is also refuted. The X-ray diffraction patterns of all three forms are sharp, indicating a definite crystalline structure. For non-crystalline or amorphous substances, the pattern does not consist of sharp peaks but of one or a few broad diffuse halos.[26]

Table 1. Interplanar *d*-spacings (Å) of the three forms of fluorescein from X-ray diffraction patterns

Yellow	Red	Colourless
7.31 s	8.26 s	7.57 m
5.55 vw	7.43 s	7.49 m
5.29 m	6.60 m	5.48 w
4.74 s	5.60 s	5.04 w
4.50 m	5.29 s	4.58 m
4.29 w	4.87 s	4.39 s
4.05 s	4.79 s	4.34 s
3.83 s	4.74 s	3.84 s
3.61 vs	4.39 m	3.66 s
3.52 m	4.09 vw	3.49 m
	3.86 s	3.39 w
	3.85 s	3.37 w
	3.68 m	3.35 w
	3.66 m	3.18 m
	3.48 s	3.02 w
	3.37 vs	2.67 w
	3.14 vs	

Strength of peak: vs—very strong; s—strong; m—medium; w—weak; vw—very weak.

Mass spectroscopy of the solid forms of fluorescein

Mass spectra were obtained of the three forms of fluorescein, and of diacetylfluorescein, 4',5'-dimethylfluorescein, and dioxan on an Atlas CH_4 mass spectrometer.

The mass spectra of yellow and red fluorescein are identical both in the number and location of the *m/e* peaks and in their relative intensity. Thus it is apparent that application of heat for vaporization of the sample converted the yellow fluorescein into the red form, as expected from the observations of Orndorff and Hemmer,[4] confirmed in the present work.

In the mass spectra the parent peak, *P*, at $m/e = 332$ agrees with the molecular weight of 332.30 for fluorescein, $C_{20}H_{12}O_5$. A distinct $P - 1$ peak at $m/e = 331$ is also observed. The spectrum is characterized by the following *m/e* peaks: 304 weak; 288 and 287 very strong; 272 and 271 strong; and 259 and 258 medium–weak. There are evidently two different pathways for the fragmentation of fluorescein, consistent with the *m/e* peaks in the pattern. In the less favourable pathway, the peak at $m/e = 304$ corresponds to loss of CO ($P - 28$); subsequent decarboxylation gives rise to the peaks around $m/e = 259$ ($P - 28 - 45$). In the more favourable pathway, the very strong peaks at $m/e = 288$ and 287 correspond to decarboxylation ($P - 44$ or 45), and then the subsequent loss of O or OH results in the peaks at $m/e = 272$ and 271. The degradation patterns are characteristic of compounds containing phenolic, ketone, carboxylic, and ether functions.[27] The pattern at lower *m/e* values becomes too complex for

analysis; there are prominent peaks at $m/e = 202$ and 143, but no fragmentation sequence was found to explain them.

For the colourless form of fluorescein, the mass spectrum is similar to that of the red and yellow forms, except that many of the peaks are weaker. The parent peak is absent and becomes visible only after application of 200 units of heat in the source (as compared with 85 and 95 units for yellow and red fluorescein, respectively) and subsequent ionization at 70 eV. Such weak appearance of the parent peak is typical of 5-membered lactone rings with the γ-carbon substituted[28] and is consistent with the assignment of the lactone structure for the colourless fluorescein. After the vaporization and ionization, the degradation pattern becomes almost identical with that of yellow and red fluorescein, the main exceptions being a medium–weak peak at $m/e = 237$ (not present in the spectra of yellow and red fluorescein and probably characteristic of the lactone structure only) and a distinct peak at $m/e = 88$, followed by one at $m/e = 58$ indicating the presence of dioxan, as expected. For confirmation, a mass spectrum of dioxan was obtained. The prominent parent peak at $m/e = 88$ and the strong peak at $m/e = 58$ ($P - 30$), corresponding to the loss of an OCH_2 group, are characteristic of that class of compounds. Thus the mass spectrum of colourless fluorescein is another proof that dioxan is present in the species isolated.

The mass spectrum of diacetylfluorescein is also instructive. The parent peak, P, at $m/e = 416$ confirms the molecular weight of diacetylfluorescein, $C_{24}H_{16}O_7$. The next prominent peak at $m/e = 330$ corresponds to the loss of two CH_3CO groups ($P - 2 \times 43$); a weaker peak at $m/e = 273$ corresponds to the loss of only one CH_3CO group ($P - 43$). The loss of OR groups is typical of esters.[28] Subsequent peaks at $m/e = 304$, 288, 272 and 259 indicate a degradation pattern similar to that of the parent compound, fluorescein. An additional peak at $m/e = 314$ may be due to the loss of oxygen from the fragment corresponding to $m/e = 330$.

Thus the mass spectra are indicative of the easy convertibility of yellow fluorescein into red fluorescein by application of heat. The mass spectrum of colourless fluorescein points to an initial lactone structure which, upon ionization, follows a degradation pattern essentially similar to that of the other two forms. The presence of dioxan in the colourless form of fluorescein isolated was also confirmed. The mass spectrum of the diacetyl derivative is typical of an ester of that type, the ester group splitting off first and then the fragmentation pathway of the parent compound being followed.

The mass spectrum of 4',5'-dimethylfluorescein has more peaks than any of the other fluoresceins, but the assignment of a fragmentation pattern is straightforward. A very intense peak at $m/e = 360$ is the parent peak P which confirms the molecular weight of 360 for fluorescein substituted by two methyl groups, $C_{22}H_{16}O_5$. The loss of both methyl groups gives rise to a moderate peak at $m/e = 330$ ($P - 2 \times 15$); subsequent decarboxylation results in the strong peak at $m/e = 286$ (330 − 44). In another pathway, the molecule is first decarboxylated, then loses two methyl groups in succession, accounting for the very strong peaks at $m/e = 316$ ($P - 44$), 301 (316 − 15), and 286 (301 − 15), respectively. A moderate peak at $m/e = 272$ is the result of loss of CH_2 from the $m/e = 286$ fragment (286 − 14). Subsequent loss of OH or CO gives rise to the very weak peaks at $m/e = 255$ (272 − 17) and 244 (272 − 28) respectively. The degradation pattern starting at $m/e = 226$ is too complex for analysis, however.

Infrared spectroscopy of the three forms

The infrared spectra were obtained with a Beckman IR8 spectrophotometer. The samples were prepared by suspending the solid in a minimum amount of ether, smearing the suspension over the surface of a sodium chloride plate, and allowing the solvent to evaporate rapidly. The residue was a strongly adhering thin film, the colour of which resembled that of the original sample, showing that no significant conversion of one form of the fluorescein into another had occurred.

The potassium bromide pellet technique proved unsatisfactory. The prepared pellets were often mechanically too brittle to permit normal handling. On other occasions, blotches of moisture and discoloration were visible within the pellet. Potassium bromide is very hygroscopic; the effect of this on infrared spectra has been studied previously. Because the state of fluorescein is affected by the presence of water and the exclusion of moisture proved very difficult, the use of the potassium bromide pellet method was abandoned.

The nujol mulling technique and the use of other organic liquids was considered and abandoned, principally because the particular solid form assumed by fluorescein depends greatly on the liquid present or from which the solid is formed. In addition, the absorption band of the carbonyl group, the band of particular significance in this study, is known to be sensitive to the effects of solvents.[29]

The infrared spectrum of 3,6-dihydroxy-9,9-dimethylxanthene, a compound used for comparative purposes, was obtained by the potassium bromide pellet method with a Perkin-Elmer Model 237B infrared spectrophotometer.

The infrared spectra obtained from the solid films of the three forms of fluorescein are marked in general by broad bands, low absorption, and not as much detail as found in spectra commonly obtained for compounds in solution. The disadvantages of solid film spectra are well-known.[29] Crystal orientation in a preferred direction, variations with particle size, method of preparing the sample, molecular association, and polymorphism all affect the quality of the spectra. Additional difficulties are encountered because of adsorption effects and interactions of alkali halide matrices with compounds bearing carboxylic or phenolic groups. Despite the difficulties, sufficiently good spectra were obtained on films in the present work to permit assignments of structures to each of the three solid fluoresceins.

The characteristic absorption bands of the infrared spectra are listed in Tables 2 and 3.

DISCUSSION

For convenience, the infrared absorption bands of fluorescein are best grouped into four regions: 1800–1600 cm^{-1} characteristic of the carbonyl group; 1600–1350 cm^{-1} characteristic of the aromatic skeletal carbon–carbon stretch; 1300–1100 cm^{-1} characteristic of the carbon–oxygen stretch; 900–650 cm^{-1} characteristic of the aromatic carbon–hydrogen bending vibrations. In the last three regions, the infrared spectra of all three forms of fluorescein are very similar. In the carbonyl region, however, major differences occur.

At 1800–1600 cm^{-1}, no band at all is present in the spectrum of yellow fluorescein, one at 1711 cm^{-1} is present in that of red fluorescein, and one at 1730 cm^{-1} in that of colourless fluorescein. In the spectrum of yellow fluorescein, a band is present at 1536 cm^{-1} which does not appear in the other two spectra. The interpretations placed on these bands are: (1) the band at 1711 cm^{-1} indicates the presence of a free carboxylic acid group and consequently the *p*-quinone structure for red fluorescein; (2) the band at

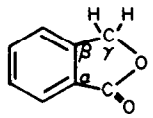
Table 2. Major infrared absorption bands of fluorescein

Form	Carbonyl-type frequencies, cm^{-1}				
	Lactone	Carboxylic acid	Carboxylate	Quinone	Others
Yellow	none	none	—[a]	—[b]	1536
Red	none	1711	—[b]	—[c]	none
Colourless	1730 plus 1700–1760 shoulder	none	—[b]	—[b]	none
	Aromatic skeletal carbon–carbon stretching frequencies, cm^{-1}				
Yellow	1595–1572[d]	1461–1450 plus 1430 shoulder[e]			1370
Red	1600–1590[f]	1465–1455			1382
Colourless	1610–1590	1460–1450			1385
Disodium salt[g]	1575[d,f]	1460[e]			1380
Disodium salt[h]	1580[d,f]	1460[e]			1398

[a] Expected, but see note d.
[b] None expected and none observed.
[c] Expected, but see note f.
[d] Contains asymmetric carboxylate stretching bands.
[e] Contains symmetric carboxylate stretching band.
[f] Contains conjugated carbonyl band.
[g] Data from Freytag.[30]
[h] Data from Davies and Jones.[6]

1730 cm^{-1} indicates the presence of a lactone ring and consequently the lactone structure (I) for colourless fluorescein; (3) the band at 1536 cm^{-1} indicates the presence of an oxygen-containing pyrylium-type ring and consequently the zwitterion structure (II) for yellow fluorescein.

The absorption of a normal, saturated, five-membered lactone ring (as in I) generally falls[31] in the frequency region 1780–1760 cm^{-1}, and it is such a frequency that Davies and Jones[6] and Sklyar and Mikhailov[7] searched for unsuccessfully in the spectra of yellow and red fluorescein. Phthalide (XI), generally considered as a model compound for the lactone ring, has a carbonyl absorption band[32] at 1776 cm^{-1}. However, substitution on the carbon atom in the



XI Phthalide

γ-position results in a considerable variation in the frequency of the lactone band. Phthalides in which a hydroxyl or chloride group replaces one of the hydrogen atoms on the γ-carbon atom and a disubstituted phenyl ring replaces the other have carbonyl frequencies ranging[33] from 1802 cm^{-1} to 1757 cm^{-1}. Other substituents can decrease the frequency considerably. In the related compound carrying a methyl group on the γ-carbon atom and a hydroxyl group on the aromatic ring in position 7, the carbonyl frequency appears[34] at 1727 cm^{-1}. For several phthalides with various substituents at the γ-position, the carbonyl absorption bands range[35] from 1745 cm^{-1} to as low as 1715 cm^{-1}. In the closed, lactone form of fluorescein, the carbon atom in the γ-position can be considered as being substituted by two phenyl-like groups with hydroxyl groups in the *p*-positions; the carbonyl frequency can be expected to be decreased. A group of similar compounds, the phthaleins, for which the lactone structure is undisputed because they are colourless in the solid neutral form, do have such lowered frequencies;[36] for phenolphthalein the

Table 3. Minor infrared absorption bands of fluorescein

Form	Carbon–oxygen stretching frequency region, cm^{-1}						
Yellow	1315–1280br		1255w	1229m	1202m	1159m	1112m
Red	1310w	1290w	1262m	1234m	1210m	1180w	1108m
Colourless	1315vw	1285w	1250w	1232vw/sh	1206vw	1180–70w	1110m
	Aromatic carbon–hydrogen bending frequency region, cm^{-1}						
Yellow	871m	—	845m	821 + 810sh	788w	752m	716w/sh	694w/sh
Red	none	858w	841m	—	780vw/br	—	720w	700w
Colourless	none	—	845m	—	785vw	754m	—	—

br—broad; w—weak; vw—very weak; m—medium; sh—shoulder.

band appears at 1725 cm^{-1}, for cresolphthalein at 1700 cm^{-1}, and for thymolphthalein at 1725 cm^{-1}. Davies and Jones[6] found the carbonyl band of phenolphthalein at 1740 cm^{-1}. Thus, the assignment of the band at 1730 cm^{-1} in the spectrum of colourless fluorescein to the carbonyl group of a lactone ring is most reasonable, despite the objection raised by Sklyar and Mikhailov[7] that such a frequency is too low for a five-membered lactone ring.

In their work, Sklyar and Mikhailov[7] assigned to the lactone ring the unusually strong absorption band at 1760 cm^{-1} in the spectrum of diacetylfluorescein (**IX**). On the basis of the discussion above, however, this assignment seems unwarranted: the position and intensity of the band in diacetylfluorescein indicate rather that the absorption is due to the acetate group. Aromatic esters absorb[37] at 1760 cm^{-1}. Rao[29] reports a band at 1761 cm^{-1} for acetate esters of the type RCOOAr. Thus 1730 and not 1760 cm^{-1} is the frequency associated with the lactone ring of structure I of colourless fluorescein.

In the work of Davies and Jones,[6] the carbonyl band of fluorescein was observed at 1729 cm^{-1}. Although they did not mention which coloured form of fluorescein was used in their study, it can be presumed that during the preparation of the sample by deposition of a solid film from a solution of an unspecified solvent they prepared the closed lactone form. Indeed, the rest of their spectrum is similar to that obtained for the colourless form in this work. Thus, it is not surprising that they assigned the closed lactone form, "the classical formula" in their own words, as the structure of fluorescein (yellow or red).

In the present work, there appears in the spectrum of colourless fluorescein, on the 1730-cm^{-1} band, a sizeable shoulder at 1770–1760 cm^{-1}. Colourless fluorescein carries 0.5 mole of dioxan, the solvent from which it was formed, per mole of fluorescein and it would be convenient to assign this shoulder to dioxan. In the infrared spectrum of dioxan there appear[38] a very weak band at 1725–1720 cm^{-1} and much stronger bands at 868 and 888 cm^{-1}. Rao[29] found bands for dioxan in the region 1750–1700 cm^{-1} which were variable and which he attributed to impurities. In the spectrum of colourless fluorescein, obtained in this work, no bands appear at 868 and 888 cm^{-1}. The shoulder at 1770–1760 cm^{-1} thus presents a problem. A plausible explanation is that dioxan and fluorescein form an "adduct" or "addition compound" of such a nature that the normal vibration of the carbonyl group of the lactone ring is distorted.

The band found at 1711 cm^{-1} in the spectrum of red fluorescein is typical of aromatic carboxylic acids. For benzoic acid the band appears at 1690 cm^{-1}, for fluorescin (the reduced form of fluorescein), it is[30] at 1706 cm^{-1}, and for Rhodamine B, a dye analogous to red fluorescein, at 1715–1705 cm^{-1}.[36]

Davies and Jones[6] pointed out that the presence of a carboxylic acid function in the fluorescein molecule should result in absorption in the 3500–2500 cm^{-1} region. Besides this, the region should also contain bands due to the phenolic hydroxyl group and the aromatic carbon–hydrogen stretch band at 3100–3000 cm^{-1}.[28] However, no significant absorption bands are found in the entire region 3500–2000 cm^{-1} in any of the three spectra. The very weak and broad band at about 3200–3000 cm^{-1} observed in the spectrum of red fluorescein in this work is too small for inclusion in the discussion. This lack of absorption is attributed to the nature of infrared spectra obtained on solid films deposited on sodium chloride plates.

A more serious concern is the apparent lack of an absorption band corresponding to the quinone carbonyl group of red fluorescein (**III**). The carbonyl absorption bands for quinone-type compounds are generally found[29] at 1685–1626 cm^{-1}, but no absorption band in this region appears in the spectrum of red fluorescein. It is known that the frequency of the carbonyl group is decreased considerably whenever the carbonyl group is conjugated with double bonds.[37,39] Conjugation of a carbonyl group with carbon–carbon double bonds and phenyl groups results in delocalization of the π-electrons, thus causing the absorption to be shifted to lower frequencies.[28] A decrease in the frequency of the carbonyl group absorption as a function of conjugation can be seen in many of the compounds tabulated by Szymanski.[40] In some of the compounds, such as the tetrasubstituted (methyl or tert.-butyl)biphenyl-1,4'-diones, the carbonyl absorption bands appear at 1604 and 1597 cm^{-1}, respectively. In structure **III**, assigned to red fluorescein, a very high degree of conjugation of the quinone results in a decrease of the frequency of the carbonyl absorption and a merging with the broad aromatic absorption band at 1600–1590 cm^{-1}. In the infrared spectra of the dipotassium salt of phenolphthalein and the disodium salt of fluorescein, in both of which the same quinone group is present as in **III**, the absorption bands appear at 1572 and 1580 cm^{-1} respectively,[6] again indicating a merging of the carbonyl absorption with the aromatic absorption band.

In the zwitterion structure **II**, assigned to the yellow form of fluorescein, a carboxylate group is present. This assignment was made because in the spectrum of yellow fluorescein no absorption band appears in the region 1800–1600 cm^{-1} and thus neither a lactone ring nor a carboxylic acid group can be present in the molecule. The carboxylate anion is characterized by two absorption bands, a strong one at 1600–1560 cm^{-1}, corresponding to the asymmetric stretching vibrations, and a weaker one at 1430–1400 cm^{-1}, corresponding to the symmetric stretching vibrations. A major absorption band, that associated with the aromatic carbon–carbon stretch, is present in the spectrum of each of the three solid forms of fluorescein: in the yellow at 1595–1572 cm^{-1}, in the red at 1600–1590 cm^{-1}, in the colourless at 1610–1590 cm^{-1}. For yellow fluorescein this band appears at

lower frequency than for the red and colourless forms; the shift to lower frequency can be attributed to a merging of this band with that of the asymmetric stretch of the carboxylate group. The merging of the asymmetric stretching band of the carboxylate group and the aromatic carbon–carbon stretch was also observed by Davies and Jones[6] in the spectra of the disodium salt of fluorescein and of the dipotassium salt of phenolphthalein, in both of which the carboxylate anion is surely present. They attempted to explain this phenomenon in terms of a resonating oxygen system with centres in which the oxygen frequencies are coupled. The symmetric stretching vibration of the carboxylate group, at 1430–1400 cm^{-1}, is weak and of much less use for diagnostic work than the asymmetric stretching vibration; sometimes it is not seen at all, especially in complex molecules for which other strong absorption bands lie nearby. This may be the case for yellow fluorescein, although the band may simply be buried under the 1461–1450 cm^{-1} band, especially since there appears to be a weak shoulder at about 1430 cm^{-1}.

There is present in the spectrum of yellow fluorescein a sharp, well-defined band at 1536 cm^{-1}. This band had been observed previously, by Freytag[30] and Sklyar and Mikhailov,[7] but no importance was attached to it. The band does not appear in the spectrum of either red or colourless fluorescein. The structure proposed above for yellow fluorescein contains a central, six-membered, oxygen-containing ring carrying a positive charge. This ring structure is identical with that in a class of compounds known as the pyrylium salts. The pyrylium salts constitute an important family of compounds which include the anthocyanin dyes, the yellow, red, and blue colouring matter of flowers, fruits and plants. Prominent in the family are the benzopyrylium and flavylium salts (**VII** and **VIII**). The infrared spectra of pyrylium ring compounds are characterized by two absorption bands, one at 1650–1615 cm^{-1}, attributed[41,42] to a symmetric carbon–oxygen stretching vibration with a frequency stated by various authors to be 1540–1530 cm^{-1} (Ribereau-Gayon and Josien[41]), 1552 cm^{-1} (Tsubomara[43]), 1548–1520 cm^{-1} (Balaban *et al.*[44]), and 1560–1530 cm^{-1} (Arnold[45]). In the spectrum of yellow fluorescein, the symmetric stretching band is probably merged with the strong broad band resulting from the aromatic carbon–carbon stretch at 1595–1572 cm^{-1}, but the unique band at 1536 cm^{-1} can be used with certainty as support for the assignment of structure **III** to yellow fluorescein.

Except for the well-defined band at 1536 cm^{-1} in the spectrum of yellow fluorescein, the infrared spectra of all three forms are very similar at frequencies below 1600 cm^{-1}. The major bands at about 1600, 1450 and 1380 cm^{-1} in each spectrum are associated with skeletal aromatic carbon–carbon stretching vibrations and can be found in many aromatic compounds. For example, in the spectrum of 3,6-dihydroxy-9,9-dimethylxanthene (**V**), a compound which is very similar to the xanthene portion of the molecule of fluorescein, the bands at 1620 and 1450 cm^{-1} are among the most prominent, Table 4. In the 1380–1370 cm^{-1} region, the bands are strong in the spectra of the three fluoresceins but weak in the spectrum of 3,6-dihydroxy-9,9-dimethylxanthene. In the spectra of phenolphthalein (**IV**) and of its dipotassium salt, the bands appear at 1366 cm^{-1} and at 1367 cm^{-1}, respectively.[6] Possibly these bands can be attributed to vibrations in the phthalate portion of the fluorescein molecule.

The absorption bands in the spectra of the three forms of fluorescein in the region of the carbon–oxygen stretching frequencies (1300–1100 cm^{-1}) and aromatic out-of-plane bending frequencies (900–650 cm^{-1}) associated with adjacent carbon–hydrogen bonds[28] are presented in Table 3. Again, no significant difference between the various forms can be observed. The presence of a moderate absorption band at 754 cm^{-1} in the spectrum of the colourless

Table 4. Infrared absorption bands of 3,6-dihydroxy-9,9-dimethylxanthene

Band, cm^{-1}	Intensity	Assignment of vibration
1620	strong	Aromatic skeletal carbon–carbon stretch
1502	medium	Aromatic skeletal carbon–carbon stretch
1450	very strong	Asymmetric methyl bending plus aromatic skeletal carbon–carbon stretch
1379	very weak	Symmetric methyl bending
1350	weak	Unassigned, but probably associated with carbon–oxygen vibrations
1322	weak	
1330	weak	
1166	very strong	Carbon–oxygen stretch of phenolic group
1120	medium	Carbon–carbon in-plane strain plus methyl rocking deformation
1000	medium	Symmetric carbon–oxygen–carbon stretch
852	weak	Carbon–hydrogen out-of-plane bending, aromatic
813	weak	Carbon–hydrogen out-of-plane bending, aromatic

form and at 752 cm^{-1} in that of the yellow form and the absence of an absorption band in this region in that of red fluorescein, may be attributed to the similar symmetry of the colourless and yellow forms, whereas such symmetry is lacking in the red form owing to the quinone structure at one end of the molecule and the phenolic structure at the other. A strong absorption band at 750 cm^{-1} is found in the spectrum of xanthene[36] which also has a symmetry similar to that of the colourless and yellow forms of fluorescein.

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LABORATORY MICROCOMPUTER SYSTEM FOR THE DEVELOPMENT OF MICROCOMPUTER-CONTROLLED ANALYTICAL INSTRUMENTATION

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Summary—Researchers in analytical procedures can acquire greater control over their experimental parameters, procedures and the mathematical operations used to manipulate and evaluate their data, by incorporating microcomputer technology into their laboratory instrumentation. A hybrid microcomputer system has been assembled to aid in the development of microcomputer-controlled analytical instrumentation and to enhance performance of microcomputer-controlled instrumentation through improved logic sequencing. Fundamental hardware modules, including the central processing, memory and input/output are specified. These modules are described in terms of the hardware system features necessary for effective programming capabilities. Included are those dealing with burning of programs on erasable programmable read only memory and with the types and sizes of memories required. Software elements are described in terms of system programming features required for creating and incorporating the desired logic sequencing into analytical instrumentation. Although language selection and installation are not examined in complete detail, some problems encountered in this area are discussed.

The advent of microcircuits and the programming languages needed to make them functional, is a major technological advance giving expanded capabilities for the conduct of scientific investigations. This new technology, which has given rise to microprocessors and microcomputers, is affecting laboratory research in two main ways. First, microcomputers are being used by instrument manufacturers to replace discrete logic in their products and to add new automated features to their instruments. In many cases it has been possible for them to automate routine laboratory procedures such as instrument calibration and to incorporate data-processing into the instrument itself. Secondly and more importantly, microcomputers are enabling scientists to explore new experimental approaches in their laboratory investigations. Microcomputer technology gives researchers expanded powers to define and control their experimental parameters. It gives them additional techniques for processing and analysing results in the laboratory. The design of experiments will be characterized by a new degree of complexity as the chemist is required to consider not only the chemical system and the instrument used to measure that system, but also the hardware and software features of the microcomputer system used in the controlling and measuring mechanisms.

Ideally, a measurement and data-processing system which is cheap and reliable is required. Microcircuits, especially microcomputers, have laid the foundation for the creation of such laboratory instrumentation. Formerly, the more complex changes in experimental and instrumental design demanded similarly complex changes involving rewiring and replacement of hard-wired logic components. This was time-consuming and required a greater knowledge of electronics than that usually possessed by chemists. However, microcomputer technology now provides a new method by which instrumental and experimental parameters can be altered. With a microcomputer support-system and microcomputer-controlled instrumentation, it is possible to alter parameters by software programming. Microcomputer instrumentation offers other advantages in terms of increased reliability. Once programs have been properly developed and implemented in firmware their reliability may be improved by reduction in human operative error. Another gain from their use for certain dedicated instruments is the reduction in size and power consumption.

Various types of computers are being used on-line for a number of purposes. As defined by Perone and Jones[1] some of these applications are described as passive, *i.e.*, there is no active control of the experiment by the computer. Other applications are characterized as active, in which the computer is significantly involved in control of the experiment and/or

† Reprint requests.

experimental parameters by feed-back. The last category includes the following applications: automation, real-time computer iteration, iterative optimization, user interaction, and design of instrumentation. In relation to the last application, a number of workers in the field of instrumentation have used computers in their design activities.[2–6] However, for the most part these researchers have applied minicomputers to this task. Scientific investigations in which microcomputers have been incorporated into instruments are being reported at an increasing rate,[7–10] but no work has been reported in which a microcomputer system has been used to develop and implement both the hardware and software aspects of microcomputer-controlled analytical instrumentation. The purpose of this paper is to describe a hybrid microcomputer system which has been assembled and is being used in this manner.

THE MICROCOMPUTER SYSTEM

Design requisites

The development of this microcomputer system is based on a number of design requisites. First, the system needs to have large memory and storage capacities accessible through a variety of peripheral devices. Secondly, the system should not have as its primary purpose the direct control of on-line experimentation, but should be capable of some control for test purposes. Thirdly, the system should be able to give the data output in whatever form (optical, audio, hard copy *etc.*) is deemed appropriate by the researcher for his specific experimental problems. Fourthly, the system should have the capability for burning on the EPROMs (erasable programmable read only memories) compatible with the instrumentation into which they will be inserted. Fifthly, the system should provide the necessary language capabilities for writing programs needed for both the system itself and for any instrumentation which is being developed and implemented by the system.

System configuration

As indicated in Fig. 1, our microcomputer system consists of three functional modules. These are the central processing unit (CPU), and memory and input/output (I/O) devices. The circuit cards for all three modules are housed in an IMSAI® 8080 microprocessing unit mainframe (IMSAI Manufacturing Corp., San Leandro, Calif., 94577, U.S.A.). Each circuit card is terminated with 100 edge-tab card connectors. Each connecting tab is identically defined on every card and on the back plane of the central bus system. The commonality of the architecture of the circuit cards allows them to be connected to any plug on the back plane. This feature is referred to as the S-100 bus. Three distinct buses, the data bus, the control bus and the address bus, are included within the framework of the S-100 bus. The channelling of signals on the bi-directional data bus provides a mechanism for the transmission of data to service all portions of the microcomputer system. The presence of the S-100 bus gives the researcher three options which are advantageous to him. First, the S-100 bus facilitates modular expansion and upgrading of the system, as new and more powerful cards which are

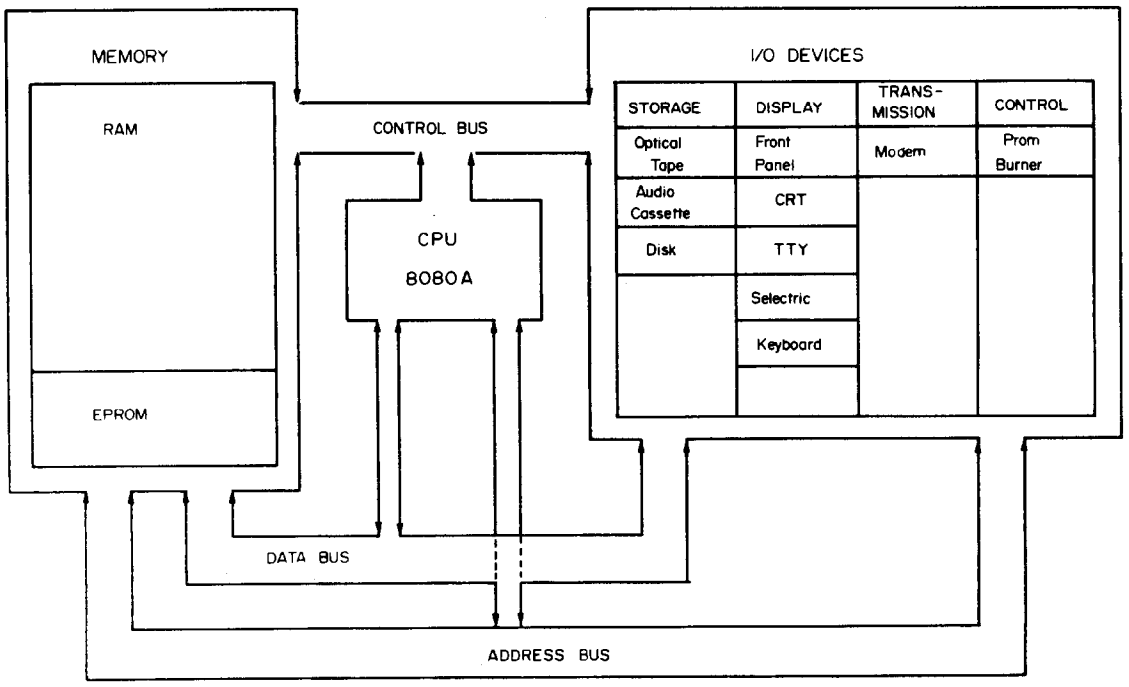

Fig. 1. Configuration of microcomputer system.

compatible with the S-100 bus become commercially available. Secondly, as experimental design changes are made in hardware, the S-100 bus allows the system to be easily altered to meet changing needs. Thirdly, the common bus feature permits almost total interchangeability of the circuit cards between microcomputers embodying the same bus. The exchangeability of cards we have termed companion compatibility. It is a flexible manoeuvre which greatly lessens the time and effort spent in tracing chip and card malfunctions as well as interconnection failures.

Central processing unit

The system in this laboratory has been built around an IMSAI® 8080 microprocessing unit board upon which the Intel® 8080A microprocessor chip (Intel Corp., Santa Clara, CA 95051, U.S.A.) is resident. The central processing module also houses the timing system and certain interface circuitry for memory and I/O devices. It is connected to other modules through a bi-directional data bus, a uni-directional control bus and a uni-directional address bus.

The microprocessor chip is the nucleus of the central processing unit (CPU). The distribution of logic within a given microprocessor chip varies according to the chip designer and manufacturer. For a hybrid system of this type the microprocessor architecture is selected by the choice of chip and once a particular chip is chosen the researcher is committed to the architecture of that component. At the time this system was developed the 8080A was selected as the most suitable chip for a number of reasons. First, it allowed for the use of a very convenient architectural feature of the system, the S-100 bus. The use of this bus made available to us a very wide selection of options and peripherals which could be readily obtained. Secondly, an extensive variety of software programs, including system programs, have been written for the 8080A and these are also easily obtained. Thirdly, several literature sources provide good documentation for the chip. Fourthly, further-developed compatible devices were anticipated and have become available during the development of the system.

The 8080A chip itself consists of an 8-bit parallel central processor unit containing the following functional sections: the register array and address logic, the arithmetic and logic section (ALU), the instruction register and control section, and a bi-directional tri-state data bus buffer. The register section is made up of a static random access memory (RAM) array arranged as six 16-bit registers which include the program counter, the stack pointer, six pairs of 8-bit general purpose registers (B,C; D,E; H,L) capable of being addressed singly or in pairs. The last feature of the register section is a non-addressable register pair (W,Z) for internal storage use only. Rapid switching of the program environment is achieved through the 16-bit stack pointer which controls the addressing of an external stack in memory. When the pointer receives the correct instruction any portion of the external memory can be used on a last-in/first-out basis. As a result, subroutine nesting is possible. The pointer also makes it feasible to store and retrieve the contents of the program counter, condition flags, data registers and accumulator from the external stack.[11,12]

Memory

The active memory is limited by the 16 address lines to 64 kbyte of memory. It is possible to utilize a port as a control which will effectively act as a 17th (and upward) address line. The control port operates to select which one of several 64 kbyte of memory, known as memory pages, will be active. This process is referred to as memory paging. The 16 remaining address lines can select any byte from the 64 kbyte of information contained on that page. Such an operation, in reality, still has only 64 kbyte of memory active at any given time. The remaining pages are held as a rapid-access storage facility. Rapid access to peripheral storage devices is less expensive and obviates the need to implement memory-paging in our microcomputer system. Rather than utilize a full 64 kbyte of random access memory (RAM) we have found it more desirable to include 4 kbyte of programmable read-only memory (PROM) as a non-volatile self-initiating memory system. This size of memory has provided adequate space for the installation of all the software necessary to accomplish our present purposes, and is believed to be adequate for our expected applications.

All RAM employed in our apparatus is of the static type. Dynamic RAMs have not been used, because although direct memory accessing (DMA) is not implemented at present it is planned as a future addition. Dynamic RAMs have not operated well in systems containing DMA because the refresh cycle does produce interference although it is supposed not to.

I/O peripherals

The system supports an extended array of I/O peripheral devices which, on the basis of function, can be categorized as follows: display, storage, communication, and control. At the present time the display and storage devices have been fully implemented in the system. A data-communication device or modem for connection to the campus maxi-computer is planned for future expansion. Currently, the only external control functions implemented are those controls necessary for the PROM burner.

Display peripherals include the front panel, a cathode ray tube (CRT) and a keyboard, a teletype (TTY) with punched-paper tape capabilities and an IBM Selectric Typewriter. Each of the display peripherals has an input device associated with it.

The front panel utilizes an IMSAI® CPA front panel control board, Rev. 4, which provides a set of 8 address-programmed input switches and 8 address-data switches. These allow the operator to enter desired information (programs, data and addresses)

onto the bi-directional data bus. In addition, 6 control-function switches allow the usual operator commands of run/stop, examine, examine next, single step *etc.* The single-step functional control is the primary reason for having a front panel in the system. A panel of this type gives the researcher single bit-by-bit control over the microcomputer. This is essential for the examination of both hardware and software problems that arise either in the system or the microcomputer-controlled instrumentation developed and implemented with it. A panel is not needed in the microcomputer-controlled instrument itself, provided that a good systems monitor is installed in the instrument's microcomputer. This, of course, is based upon the assumption of fundamental companion compatibility between the system and the instrumentation developed with it.

The CRT is a TV-15 monitor (Ball Brothers Research Corp., Miratel Division, St. Paul, Minnesota 55112, U.S.A.). It is interfaced through a CRT driver or video card, Polymorphic Systems Video Terminal Interface (VTI) Card (Polymorphic Systems, Galeta, CA 93017, U.S.A.) which is memory-mapped with a self-contained 256 byte memory. It is capable of displaying 16 lines of 64 characters on the CRT. The character font (No. MC65751A) includes upper and lower case alphabetical letters, a complete set of numbers, a set of Greek letters, certain mathematical symbols and certain selected ASCII (American Standard Code for Information Interchange) control characters. The character font is program-replaceable with a special set of 64 graphics-plotting characters which allow the production of limited graphics. The VTI also has a latched 8-bit parallel input port, making it possible to interface the CRT, keyboard and CPU. The keyboard itself is an ASCII Keyboard B70-4753 (Cherry Electrical Products Corp., Waukegan, IL 60085, U.S.A.). Program segments of 16 or even 32 lines read from the CRT are not sufficient to carry out the writing and examination of a reasonably complex program. Therefore, two hard copy devices, a Teletype (TTY) ASR/33 (Teletype Corp., Skokie, IL, U.S.A.), and an IBM Selectric Typewriter Model 735 (IBM, Minneapolis, Minnesota 55401, U.S.A.), have been included in the system to produce print-outs of programs and data. Programs can be displayed in either assembly language or the ASCII representation of the hexadecimal value of any individual word. The TTY provides storage in the form of punched-paper tapes and although the TTY is a slow device in operation, it is a reliable input and output mechanism, capable of withstanding much laboratory abuse. The IBM Selectric Typewriter provides a more sophisticated type of display and through software programming output data can be presented in a number of ways. A variety of easily exchanged character fonts is also obtainable. The Selectric is interfaced to the CPU through a Type Away Selectric I/O Writer Interface Card (Micromation Incorporated, San Francisco, CA 94133, U.S.A.). A hard copy graphics generator has been considered for the system, but it is our opinion that this function could best be implemented in a higher level computer or a mainframe computer, with which the microcomputer system would be placed in communication.

A floppy disk system, an audio-cassette tape recorder and an optical tape reader, along with the punched-paper tape from the TTY described above, constitute the long-term storage mechanisms for the system. These involve the storage of programs and data on disks, magnetic tapes and paper tapes (listed in order of decreasing speed of access). The floppy disk system is a full-size single-density system Discus I (Thinker Toys, Berkley, CA 94710, U.S.A.). There is an 8-drive capacity S-100 controller on board with buffer and serial interface. This allows the operator to read and record the contents of a disk into active memory. A cheap but slow-access mechanism is afforded by maintaining long-term storage on cassette tapes. A Centrex Model KD-12 audio tape recorder (Pioneer Electronics of America, Long Beach, CA 90810, U.S.A.) is employed in the system and has been very useful in terms of the storage of critical software, especially lengthy system and utility programs on back-up tapes. The system is equipped with an optical tape reader, the OP-80A Paper Tape Reader (Oliver Audio Engineering, Glendale, CA 91203, U.S.A.), which is interfaced through one of the parallel ports of the microcomputer. This device makes it possible to insert programs rapidly from paper tapes.

The PROM burner or PROM Setter Module (Szerlip Enterprises, Harbor City, CA 90710, U.S.A.), in conjunction with the EPROM socket unit, is used to burn programs onto chips which are then inserted into the microcomputer-controlled instrument. PROM burners are differentiated in terms of the types of PROMs which they will burn. Some of the more commonly used PROMs are the 1702, the 2708 and the 2716. The capacities of the last two PROMs are much greater than that of the 1702. However, in many instruments requiring no more than 256 bytes of non-volatile memory, the 1702 has been chosen by designers as an appropriate chip. The burning process for the 1702, unfortunately, is more complicated than that for any of the more recent PROMs. Nevertheless, at the present time this particular burning capability is needed to implement instruments containing this chip. Both 1702s and 2708s can be burned on this system. Burn capability for the 2716 is available, but requires plug modification for implementation.

SOFTWARE

Software plays the key role in addressing and manipulating hardware logic. To create the application programs needed for dedicated instrumentation it is necessary to pick the right language level and work with it in order to sequence instructions effectively. Essentially, three levels of programs are needed to

maintain and utilize the microcomputer system.[13] At the first level are those programs which constitute the operating system. This includes programming for the control of I/O, allocation of memory and management of other tasks such as memory-mapped display and bootstrap start. The next level contains utility programs for PROM programming, text editing, language translation and debugging. The third level is made up of specific application programs.

The systems program installed is an executive-type which utilizes a slightly modified version of Polymorphic's Poly 88® and the disk operating system (DOS) know as CP/M (Digital Research, Pacific Grove, CA 93950 U.S.A.). The Polymorphic monitor provides the interrupt-control capabilities for the system as well as the video display and other inputs and outputs with the exception of the DOS. It also establishes the files and appropriate headers used for cassette tape I/O. To supplement this latter function the monitor can ascertain the reliability of the cassette data by means of a check sum. The initial bootstrap start program is contained on ROM and serves the purpose of calling the polymorphic monitor which in turn allocates the various portions of RAM and enables the interrupt control. The monitor handles the I/O tasks (*i.e.*, information exchange rate, bits/sec) such as USART timing or baud-rate generator control. In addition, it handles the memory-mapped video display which allows for the display of the CPU register contents as well as a selectable 64-byte window into memory. While the primary purpose of CP/M is control of the DOS, once this program is installed it operates as an executive filter which places the appropriate I/O under software control. A number of transient or utility programs, including an editor, assembler and dynamic debugger tool (DDT), are incorporated in the CP/M software package to facilitate the writing of programs for itself and for dedicated microcomputers in specialized instrumentation.

Editor

To the chemist involved in a large amount of programming, the text editor is probably the most important of the utility programs. It is through the editor program that the programmer enters into memory, in the appropriate format, the information desired for the writing of his individual application programs. It should be noted here that the use of the word "text" should be construed as representing all the various computer languages as well as languages of choice, such as English, French or German. The editor is not limited solely to the English language.

There is no standard text editor and editors differ in quality. Some editors are character-oriented, some are line-oriented, while others are interval-oriented. Whether the researcher is writing his own programs or modifying programs from other sources, it is advantageous in terms of time and effort for him to become proficient with one particular form of text editing, format and correcting. It is also important to have available a text editor which can furnish the techniques for simplifying the overall task of programming. The principal editor currently installed in our system is the TSC Text Editing System-SL80-10 (Technical Systems Consultants, Inc., West Lafayette, IN 47906, U.S.A.). This is a line-oriented editor. With this editing program it is possible to write a series of subroutines that separately and individually accomplish very specific purposes. This series of subroutines can then be concatenated by the editor to form a larger operating program. By so doing the programmer not only saves time, but also increases reliability, because he can make use of tested subroutines in other programs. In addition to editing microcomputer languages, the fact that the editor is capable of being used to edit ordinary language text aids in the handling of a wide variety of programs and provides a supplementary service for the writing of final reports. A second complementary program, the TSC Text Processing System-SL80-11 (Technical Systems Consultants, Inc., West Lafayette, IN 47906, U.S.A.), has been installed in our system as a subroutine under control of the text editor. The processing system provides for the output of text and numerical data in an appropriate and controllable format.

Assembler, interpreter and compiler programs nearly always come with text editors embodied as part of their content. This duplication of the editing feature occupies a considerable amount of memory space when the programs listed above are installed simultaneously. For the most part these additional editors are not necessary in our microcomputer system and are being removed. It is possible to replace them by linking the principal or common editor to those utility programs which require the editing capability. With these deletions a considerable amount of memory space has been saved. In addition, program reliability is again improved because the use of a common editor requires the researcher to become proficient with only a single set of manipulative commands. As a result, fewer errors in programming occur.

Assemblers

The language used by the microcomputer is object code machine language. If no form of computer assistance is available, that object code is very difficult to write and to write looping programs in object code that extend over 20–30 lines and are error-free requires a great deal of time and skill. To alleviate this problem a number of assemblers which convert assembly-level mnemonics source code into machine-level object code have been installed in our system at various times. Program development has been aided through the use of the following assemblers: Processor Technology, IMSAI, Intel, Polymorphic, CP/M and Diskate. Although assemblers come in wide variety, the differences between them lie not in the final result, the object code, but rather in format of the assembly language or source code. In general, there

are two major types of formats, that of Intel and that of Processor Technology, into which the others may be fitted. When the researcher is writing his own programs, the particular form selected is a matter of personal preference and transfers between formats can be accomplished without a great deal of difficulty by hand insertion. However, if the programmer wishes to obtain source code in machine-readable form from other researchers or from commercial sources, then format assumes greater importance, because a microcomputer incorporating one type of format will not be able to translate received code that is in a different format. This is the major reason for installing more than one assembler. Other differences in assemblers occur in their implementation of pseudo operations and in their macro capabilities. All of them, however, support the standard of the pseudo instruction with the exception of the "if" command and only the Diskate assembler implements that instruction. Another significant variation in assemblers is in the size of the program that the assembler is capable of handling, either by virtue of its label table or by its maximum permissible memory. Nearly all of the assemblers permit the assembly of programs approximately 1000 lines in length. Non-disk operating systems are generally limited by the lack of available memory. CP/M has a built-in label table of only 256 labels. Diskate does not suffer from this disadvantage and programs of over 500 labels and approximately 4000 lines have been successfully assembled on our system. Both of these latter assemblers are capable of handling either the Intel or Processor Technology format. In the case of CP/M format, selection is automatic. For Diskate, the type of format to be dealt with must be previously selected. Many assemblers have an editor associated with them. For the most part we have found them to be inefficient in comparison to the TSC 8080 Editor, and we have, therefore, removed them where possible to conserve memory space.

Language selection and translation

Language selection is a far more critical problem for the researcher who is writing software for a microcomputer than it is for one who is programming for a large computer. The principal reason is the limited memory space associated with microcomputers. Consequently, because of the resultant efficient use of memory space, programming has commonly been done in machine language. As microcomputer programs have evolved, becoming longer and more sophisticated, machine-language programming has become very costly. Because of difficulties encountered in writing complex programs for data manipulation and number crunching, particularly the latter, high-level languages have been adapted for use on the microcomputers. In higher-level languages individual statements are translated into larger blocks of machine language. A number of these languages, including FORTRAN, BASIC, PASCAL and MUMPS (medical) are available. Some may be purchased, while others are publicly available.

The higher-level languages used in the past for large-computer programming make more efficient use of the programmer's time. Because so many commands and words are in English, programming is easier and more efficient in the higher-level languages. However, these languages do present certain inherent disadvantages when used for microcomputer programming. They require more memory space and more time for compilation and execution. In some instances we have dealt with the problem of compilation by cross-compiling from source code to assembly language on the central campus computer. However, debugging the resultant program was time-consuming. For some tasks the researcher may hand-code certain parts of a program in assembly language and write other program parts in a high-level language, or choose to make use of macro-assembly language.

When programming for dedicated application purposes, consideration must also be given to the manner in which the high-level language is converted into object code, which can be by one of three software programs involving the compiler, the interpreter or the interpretive compiler. The compiler operates upon high-level language source-code and generates machine-readable code from it. Compiling and executing processes are accomplished in two separate steps and the resulting compiled program can be directly transferred to a microcomputer in a dedicated application without consideration of any specialized operating code. The interpreter program, on the other hand, is one in which the translation and execution processes are performed simultaneously. It operates on a higher-level language source-code on a line-by-line basis, generating machine-readable code. Because the conversion into machine-readable code is accomplished at the same time as the program is operating, the interpreter must be resident in the memory of the operating microcomputer. Thus when an interpreter-based language program is transferred to a dedicated or slave microprocessor, not only the program but also the interpreter itself must be transferred. Generally, interpreter-based language programs are slower in operating programs than are machine-readable programs.

Intermediate between these two levels of compiler and interpreter is a third type of program, partially compiled to a simplified interpreter state. Such programs require the presence of a simplified interpreter in the memory of the operating system, as in a dedicated microcomputer.

At present two forms of BASIC have been installed in our microcomputer system for programming. Direct interactive calculations are handled easily with the interpreter form of BASIC. In this form the interpreter scans the line of code and performs a set of operations based upon instructions and data stored in tables by previous instructions. This procedure saves time in translation, but has the disadvantage that no

machine code is saved for later repeat execution. The principal advantage of the interpreter form is the ease with which program errors can be corrected. Results of each line of code can be printed and the error source pinpointed without difficulty. The trade-off for this feature however, is the need for added memory space. The source code, data, tables and the interpreter program must all be resident in the memory at the same time. In addition, programs run more slowly with this form, because the code within a loop must be reinterpreted on each pass.

For those researchers intending repeated operations of the same program with different numbers, the compiler form of BASIC will be faster and more applicable. However, straight compiler forms of BASIC are not readily available. Most BASIC is in the compiler-to-interpreter or intermediate form, and requires that the simplified version of the interpreter be in memory.

Although other higher-level language programs can be purchased they have not as yet been implemented in the system as described. A FORTRAN program is available which is compiled in machine-readable code and this form appears to offer certain advantages, especially in terms of ease of programming. However, this has not been totally evaluated and it must always be remembered that high-level language programs require more time for execution and more memory space. The memory-space requirements become important in those cases where dedicated microcomputers contain built-in limited read-only-memory (ROM) space.

PROM burner program

The PROM burner program is very important for this type of system. A set of 6 main programs and several subroutines necessary for the PROM burner to write and read EPROMs are supplied with the burner itself. Information relating to locations and lengths for the EPROMs and memory must be entered into the microcomputer. The test program is conveniently arranged so that when errors are found, both the address and the error will be printed out in sexadecimal notation.

Dynamic debugging tool

Another significant program is the dynamic debugging tool (DDT). It is a program which contains features requisite to the examination, testing and modification of application programs. It is capable of providing a general program in machine-level language. If started at the appropriate place, it will provide assembly-level mnemonics corresponding to the machine-level language. The C3 is converted into mnemonic JMP together with its corresponding address. This program, which is often called a disassembler, to indicate that it is the inverse of an assembler, is a very convenient method of examining programs. Labels are not present and the addresses are given as addresses rather than labels. A command is available to provide for any changes to be made directly in machine language when an assembly mnemonic is provided. A display command will give a hexadecimal memory dump together with the ASCII equivalent of any memory location. A file of commands is also available to set any block of memory to a constant value, generally "00", which is advantageous if the operator does not know the actual length of the program with which he is working. A "go" command initiates execution of the program under consideration and is capable of exercising break-point options as set by the operator. Additional subroutines, such as a move subroutine, capable of transferring a block of information from one location to another in RAM, a read program which serves as an input, and a substitute program which will substitute a desired memory byte for one which was already present, are included in the DDT. A trace program allows visual display of all the CPU registers. The memory mnemonics for each step of a program can be shown during the run. It is possible with a modification of this program to display the register contents of only the last addressed state of the program rather than the entire program. In addition, it is possible to examine, modify and change any of the registers in the CPU. In short, the DDT is a program which attempts to provide those functions which an operator programmer must have to develop application-type programs.

Memory diagnostic program

As large blocks of memory are acquired, the probability of having a malfunctioning byte increases dramatically. It is necessary to have a method of detecting the address of a bad byte. The memory diagnostic (MD) program accomplishes this. It does so by writing a discrete pattern into the memory and then reading it back and comparing it with the written block. Only if a difference occurs is an output to the screen or hard copy made, and in that case the address, the correct information and the incorrect information are displayed. Interpretation based upon this information will easily locate the malfunctioning component. The program will search through and check 16000 bytes of memory in a matter of a few minutes.

Application programs

Application programs designed to meet specific experimental purposes are the product of the type of microcomputer system described here. The programs developed will usually be quickly transferred out of the system. A few programs are retained for processing numerical data received from separate microcomputers, functioning as dedicated controllers and data-collectors at the experimental level. In our laboratory this system is being used to develop a high repetition-frequency square-wave polarograph.[14]

CONCLUSION

The microcomputer system described has been designed to implement and develop microcomputer-controlled analytical instrumentation through a variety of techniques. The system has functioned well on projects currently under development. The applications of systems of this type, which are planned for use in small laboratories, are not limited solely to the development of instrumentation. Our experience with the system has shown that it is adaptable to numerous research and educational tasks, either alone, or in combination with other microcomputers or minicomputers.

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A MICROCOMPUTER-CONTROLLED SQUARE-WAVE POLAROGRAPH

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Summary—A dedicated microcomputer has been developed for control and operation of a square-wave polarograph. Microcomputer control allows a high degree of variation in the experimental parameters, such as square-wave frequency, gate delay times, gate aperture, drop-time, the amplitude of the square wave, and the magnitude of the staircase rise, but still maintains the required coherency in the signals so that an appropriate separation between the faradaic and capacitance currents is achieved. The variation is achieved through software control. Measurement is accomplished by the application of a multiplicity of square waves to each "tread" of a staircase voltage, thus providing a true square-wave polarogram.

The principal obstacle to increasing the sensitivity of polarographic methods of analysis is the separation of the capacitance charging current and the faradaic current. A number of instrumental variations have been developed to improve the discrimination between these two forms of current. One of these variations, differential pulse polarography, has gained wide acceptance as a method of analysis for trace concentrations of several analytes.[1–6] However, as Osteryoung pointed out, one disadvantage of the pulse-type method is the long drop-time and the correspondingly slow potential scans required.[7] In an attempt to overcome this time restriction Blutstein and Bond[8] have developed a fast-sweep mode of differential-pulse polarography but the method is limited by the poorer peak resolution at fast sweep-rates, as a consequence of lack of separation of the charging and faradaic currents. A more recent attempt by Bond and Grabaric[9] utilized computerized instrumentation in an attempt to correct mathematically for the background charging current.

Still another approach to the charging-current problem is square-wave polarography, first reported by Barker.[10,11] Since then many square-wave polarographs have been constructed. Hamm[12] developed a simplified instrument, but its sensitivity was not as great as that of Barker's. Hamm's design was later improved by Geerinck *et al.*[13] Rosset[14] described a more compact version of the Barker instrument, and Taylor[15] modified a Sargent XXI Polarograph. Buchanan and McCarten[16] used operational amplifiers to control the potential, and later modified this instrument[17] to improve the signal-to-noise ratio and sensitivity, determining lead at the 2 ng/ml level.[18] Liddle[19] described a square-wave polarograph incorporating a positive feedback loop. The instrument was subsequently modified to operate in conjunction with a computer. This had the advantage of placing a number of polarographic functions under programmable control. Kalvoda and Holub[20] similarly constructed a square-wave polarograph utilizing operational amplifiers along with integrated logic. With this reversibly reducible substances could be determined at concentrations as low as $10^{-6}M$. Barker *et al.*[21] briefly described a multi-mode polarograph. Sturrock and Carter[22,23] used solid-state circuitry, and with a thin mercury-film rotating disk electrode measured concentrations as low as $10^{-8}M$ for certain metals amenable to stripping analysis. Ramaley and Krause[24,25] were the first to employ a staircase scanning-potential instead of a ramp. In a technique which they called square-wave voltammetry they imposed a square-wave signal on a staircase scanning-potential. Operating with a hanging mercury drop electrode they achieved detection limits of $4.7 \times 10^{-8}M$ and $2.5 \times 10^{-8}M$ for cadmium and indium respectively. Later, Christie *et al.*[26] extended the theory of square-wave voltammetry with the dropping mercury electrode, and verified it experimentally with the detection of cadmium at the $7 \times 10^{-8}M$ level.[27] Similarly, Ramaley[28,29] reported the application of this technique with the dropping mercury electrode.

Formerly, the instrumentation required for square-wave polarography was extremely complex owing to the necessity for sophisticated timing and gating circuitry. The recent development of relatively cheap microcomputer systems has made it possible to design a square-wave polarograph with instrumental parameters under microprocessor control, resulting in construction of an instrument with simple hardware and operational diversity achieved with appropriate software. Two distinct sets of functions can be implemented in software; those for experimental control and those for data processing.

This paper reports on the design of such a square-wave polarograph.

† Reprint requests.

INSTRUMENTATION

A generalized block diagram of the instrument is shown in Fig. 1. The electrical signals applied to the cell are derived from the microcomputer through the potentiostat, which minimizes the cell resistance. The current through the cell is returned to the potentiostat, as the current monitor is an integral part of the potentiostat circuitry. The current signals are then passed to the analogue-signal processor which receives additional directives from the central microcomputer through the digital timing and interface card. This combination of signals is used for effective separation of the Faradaic and capacitative components of the current. The potentiostat also receives directives from the microcomputer through the digital-timing interface card and thus these signals and signals going to the analogue processor are maintained in a state of strict coherency. The microcomputer allows the facile change of the experimental parameters by a change in software, which is easier than changing the hardware.

The microcomputer system

The microcomputer was assembled in our laboratory from a number of commercially available units. It is housed in an aluminium cabinet and has no front panel controls except a simple on/off switch and a reset switch. The microcomputer section is itself powered with an IMSAI* microcomputer power supply (IMSAI Manufacturing Corp., San Leandro, CA 94577) which provides the usual ± 16 V as well as $+8$ V. The microcomputer back-plane consists of a locally produced mother board which is wired to utilize an S-100 bus configuration (*i.e.*, circuit cards with 100 edge-tab connectors). The organization of the S-100 bus allows for easy arrangement and ready exchange of the circuit boards. The heart of the module is a central processor unit (CPU) card which utilizes the Intel 8080A microprocessor chip. The board incorporates several support components, including a crystal-controlled clock, a systems controller and enough EPROM (erasable progammable read-only memory) to provide the system bootstrap loader (back-up reset program). Other microcomputer functions are implemented by commercially-available circuit cards. The following cards are incorporated into the system: 4 kbyte of random access memory (RAM), 16 kbyte of EPROM, and a video card (Solid State Music, Santa Clara, CA95050). Provisions for keyboard input to the microcomputer as well as cassette recorder input for longer programs have been combined and placed on a single locally-produced circuit card which also provides vectored interrupt, and certain output capabilities such as an RS-232 serial interface for possible modem applications and current-loop outputs to allow a hard-copy to be printed on a teletype (TTY). The video card provides 16 lines of 64 characters to a cathode-ray tube and maintains its own on-board memory of approximately 1000 bytes for screen display. The microcomputer described is a general-purpose microcomputer and is limited only by the extent of the memory inserted. Additional memory up to 64 kbyte can be added. As indicated by the dotted lines in the block diagram, the interface circuit card may be considered as a part of the microcomputer, but it may also be considered as the input controller for the square-wave polarograph. The details of this circuit card are described below.

Timing and control functions. The control linkage between the microcomputer and the square-wave polarograph is through the digital timing and interface card in the mainframe of the microcomputer. A diagram of this circuit card is given in Fig. 2. The purpose of this circuit card is to generate a series of coherent timing signals which will define the various time-periods required for the proper operation of the square-wave polarograph. The time-periods involved are as follows:

(1) Drop-time—the time between initiation of the drop and activation of the knock-off pulse.
(2) Strobe period—the time between initiation of the drop and the time at which sampling is commenced.
(3) Half-period of the square wave—the time between two successive zero values of the applied square-wave voltage.
(4) Cycle delay period—the time interval between the most recent zero value of the applied square-wave voltage and the opening of the sampling gate.
(5) Gate aperture—the length of time that the sampling gate is open for measurement.

In addition, the card provides other specialized signals such as the polarography stop signal, the square-wave amplitude selection-signal and the digitized potential data for the digital-to-analogue converter (DAC). The timing and interface board is accessed by the CPU through the S-100 bus. As all circuit cards attached to the S-100 bus receive all the signals, there must be on-card controls which will identify the signals that are directed to a particular card. The card is addressed as a series of ports by the CPU. Some of the components (*e.g.*, 8253 counters) require a bidirectional bus for operation, and as the S-100 bus is unidirectional an on-card conversion is required. A series of octal buffers (U1–U3), a magnitude comparator (U-4) and a decoder (U5) take care of most of the work of board-selection and data-line multiplexing.

Theory of operation. The basis of the coherent timing signals required for the operation of the square-wave polarograph is the 8253 programmable interval-timer which receives its timing pulse from the buffered and divided clock-line of the CPU. The single signal source ensures the coherency of the signal. The purpose of the two 8253 chips (A) and (B) is to divide, under software control,

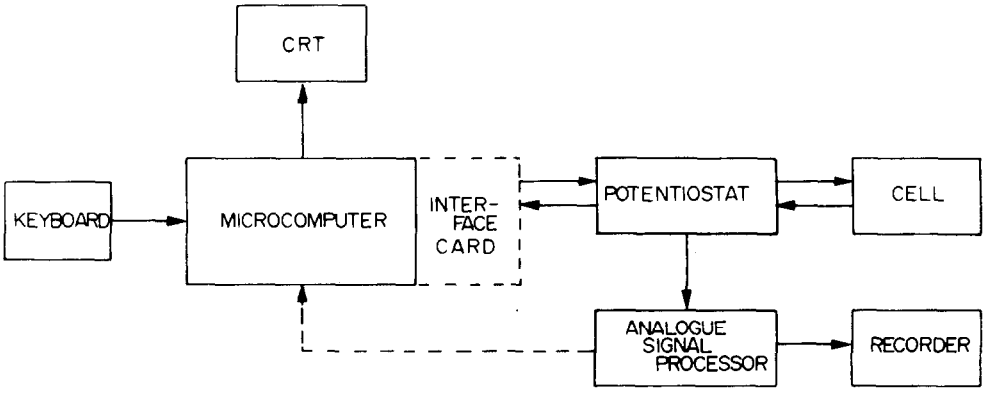

Fig. 1. Block diagram of square-wave polarograph.

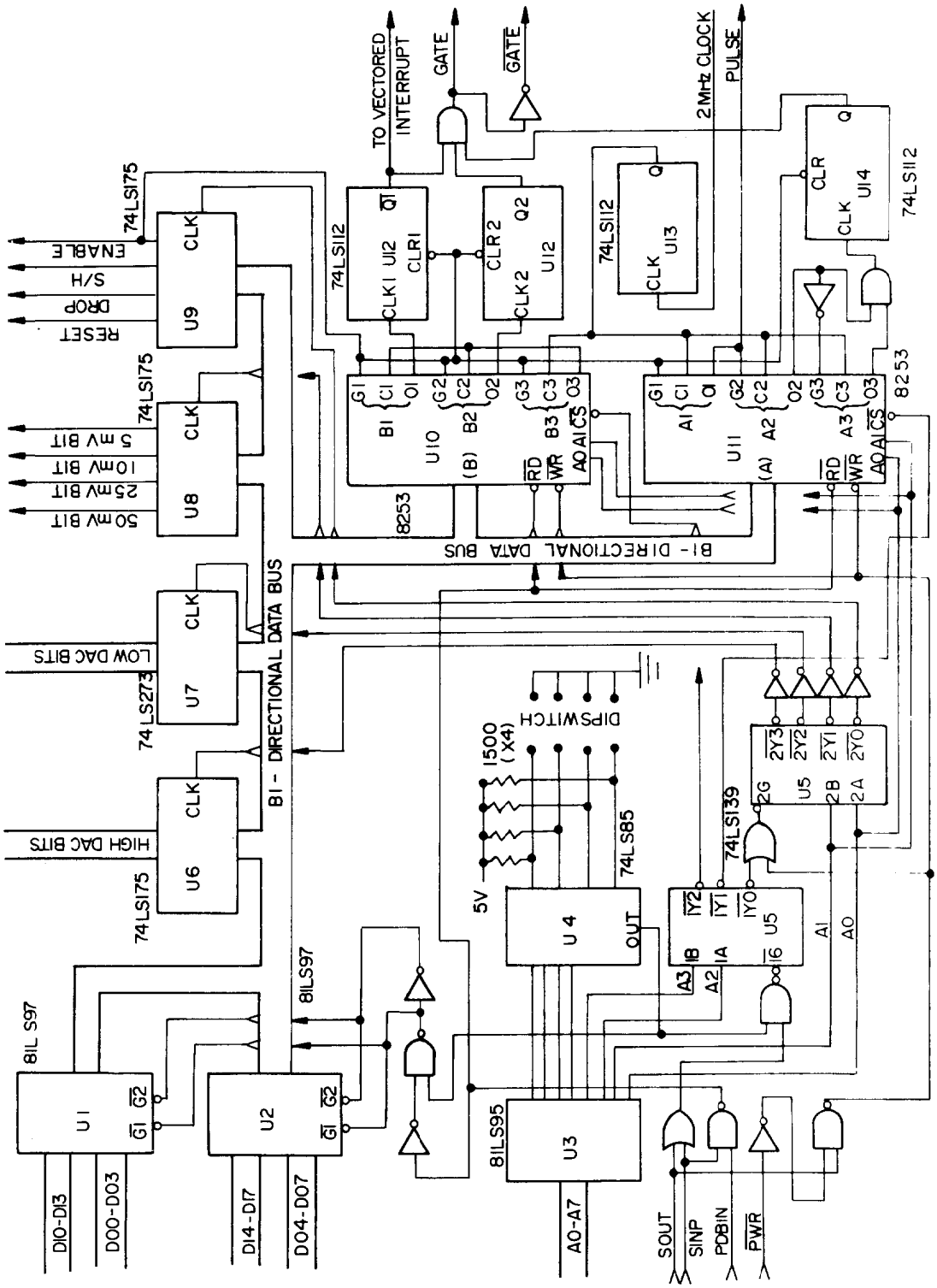

Fig. 2. Timing and control card.

a 1-MHz signal into a series of lower frequency signals. Each 8253 consists of three separate 16-bit presettable down-counters. The appropriate mode of counting is selected under software control. Each counter section of the 8253 receives its count number from the CPU at the beginning of a polarographic run. A number corresponding to the half-period of the square-wave (in μsec) is entered on the first 8253 (A). Upon a signal from the CPU through the control port, the counter A1 is enabled and begins its count-down. When zero is reached a pulse is sent to an edge-triggered flip-flop located on the analogue input card, causing change of its state. This series of transitions constitutes the square-wave signal to the polarograph. Coincident with the triggering of the flip-flop a signal is sent to the enable gate of counter A2 on the 8253. When A2 reaches zero a pulse is transmitted through an AND gate to an edge-triggered flip-flop and sets it. The same pulse, after inversion, is transmitted to the enable gate of counter A3 on the 8253. When A3 reaches zero it transmits a pulse through the AND gate to clear the flip-flop. In its set state, this flip-flop serves as the sampling-gate aperture signal. Thus with three counters, the first timing the half-period of the square-wave, the second timing the delay until the opening of the gate and the third timing the gate aperture, we have completely defined a series of signals that will coherently control the square-wave transitions and the opening and closing of the sampling gate.

Two other timing signals have much longer durations than those above. These are the drop-time and the strobe period, defined above. To facilitate the timing for these two signals, counter B3 on chip 8253 (B) is used to convert the 1-MHz clock pulse into a 1-kHz clock pulse. The output of B3 is then utilized as the driver pulse for B1 and B2. When counter B1 is enabled by the enabling port, it starts to count down and on reaching zero triggers a flip-flop. The flip-flop then sends a signal through an AND gate to prevent further sampling of the cell, and also a signal to the interrupt control regulator of the CPU, which then initiates a series of events resulting in dislodging of the drop and in reset of all requisite timers. Counter B2 received its enable pulse from the enabling port at the same time as B1. On reaching zero B2 transmits a signal which enables sampling to occur. Thus B1 controls the drop-time and B2 the strobe period.

An octal and a Quad D type flip-flop are combined to serve as a 12-bit latch to hold the digital data for the DAC. The loading of the low-order 8 bits and the high-order 4 bits is accomplished separately, as each is addressed as a separate output port.

Analogue input. The components that deal mainly with the analogue circuitry are considerably more susceptible to noise than the digital components are. Hence it was considered advisable to separate physically the analogue sections from the microprocessor containing the digital components. Accordingly, the analogue sections are housed in a separate box and shielded from the digital section.

It is not possible to isolate the two sections completely, so a separate board (the analogue input board, Fig. 3). which contains the hybridized circuitry involved in the conversion between the digital and analogue sections of the instrument was constructed. A signal from counter A1 is used to trigger a change of state in an edge-triggered flip-flop each time the counter passes zero. This series of transitions generates a square wave that is biased positive to zero. An RC network is employed to remove the bias and the zero-centred signal is fed to a tapped attenuator. Any tap can be selected by the closure of a relay, to provide a preselected square-wave amplitude. The choice is accomplished by a signal from the CPU through the amplitude-control port on the digital interface card. A second flip-flop, with its transitions locked to those of the square-wave generator, is used to generate signals that will be used by the current-gating section as square-wave sign indi-

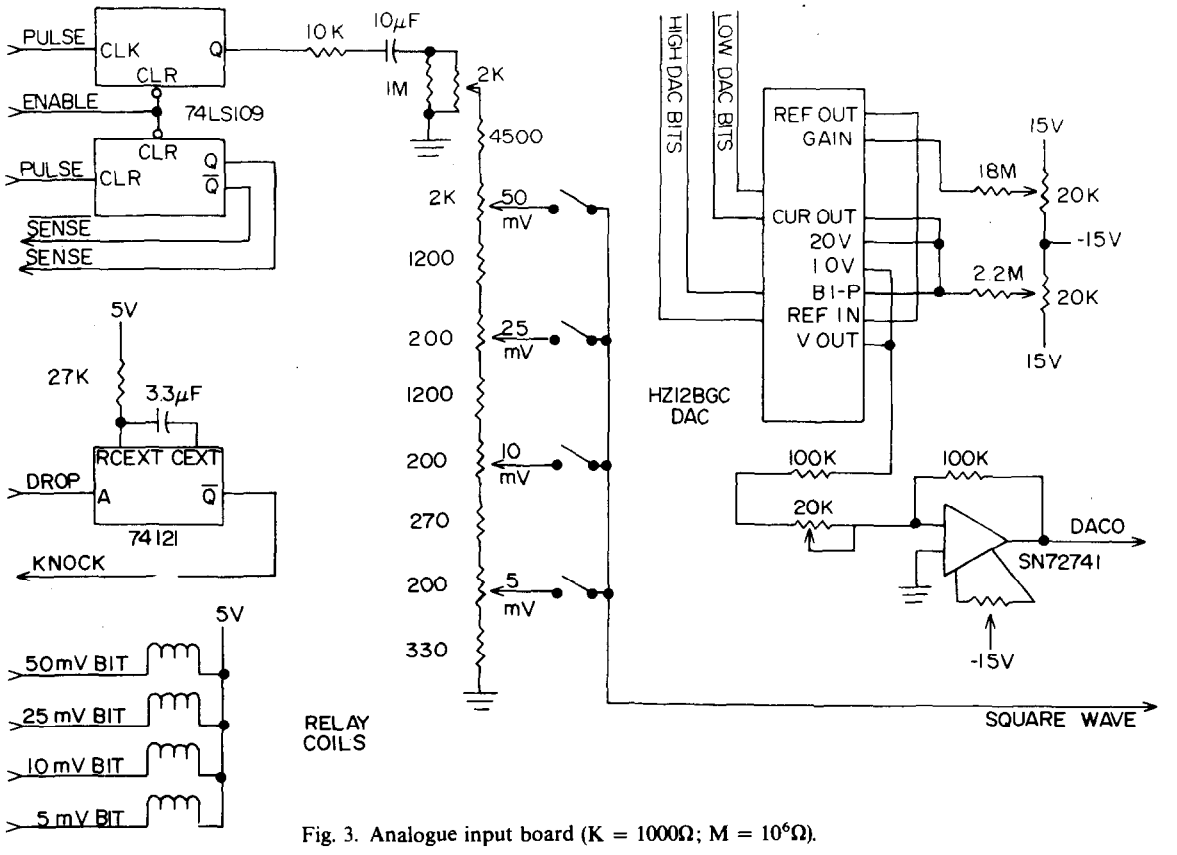

Fig. 3. Analogue input board (K = 1000Ω; M = 10^6Ω).

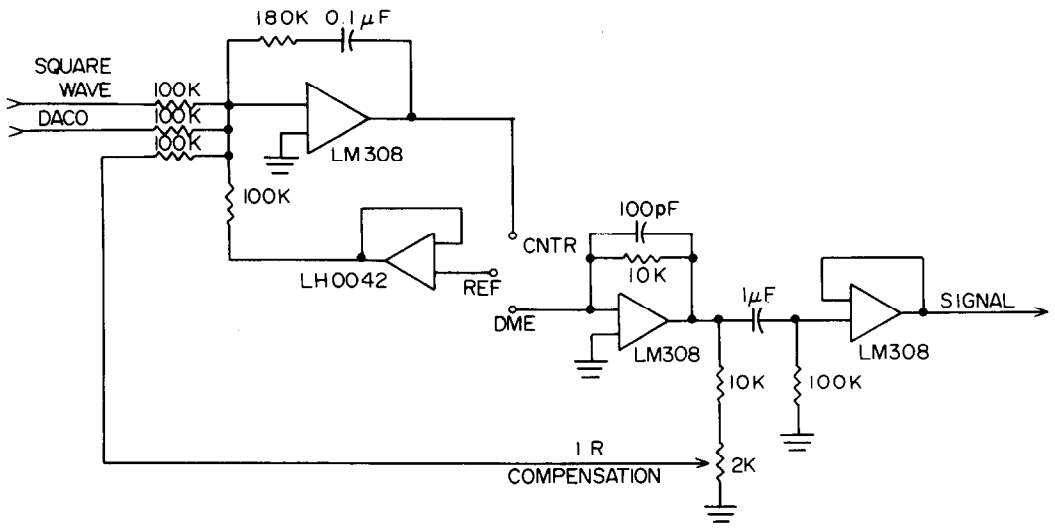

Fig. 4. Potentiostat board (K = 1000Ω).

cators. The drop-knocker pulse from the digital interface card triggers a monostable multi-vibrator (sometimes called a one-shot vibrator) the output of which provides a pulse of known duration to the base of a transistor which in turn controls the current through the solenoid employed as a drop-knocker. For electrical-noise and mechanical reasons the solenoid and its relay are kept in a separate box, located adjacent to the dropping mercury electrode. The input circuit board also contains the DAC (and its associated circuitry), which provides the staircase voltage employed in lieu of the usual ramp. The control signals for the DAC come from the digital interface card as twelve separate lines. Because a 1-bit change in the digital input results in a 1.22-vV output, an active attenuator is employed to reduce the step to precisely 1 mV.

Potentiostat. The circuitry of this is displayed in Fig. 4. Its purpose is to provide an interface potential between the electrode and the solution which is independent of the solution resistance, and to provide a means of measuring the current flow across that interface. The potentiostat is of the usual variety, and consists of an operational amplifier (the working amplifier) and a feedback amplifier (the follower). The current is measured by means of the current follower. The circuit is optimized to provide a fast response to the square-wave signal and its associated current signal. Since the small but finite separation between the reference electrode and the working electrode prevents the potentiostat from totally compensating for cell resistance, additional compensation is provided to deal with this uncompensated cell resistance. An RC circuit is used following the current amplifier to remove the d.c. components of the signal.

Analogue signal processor. The circuitry on this board is displayed in Fig. 5. The a.c. component of the current signal is brought to the analogue-signal processing board

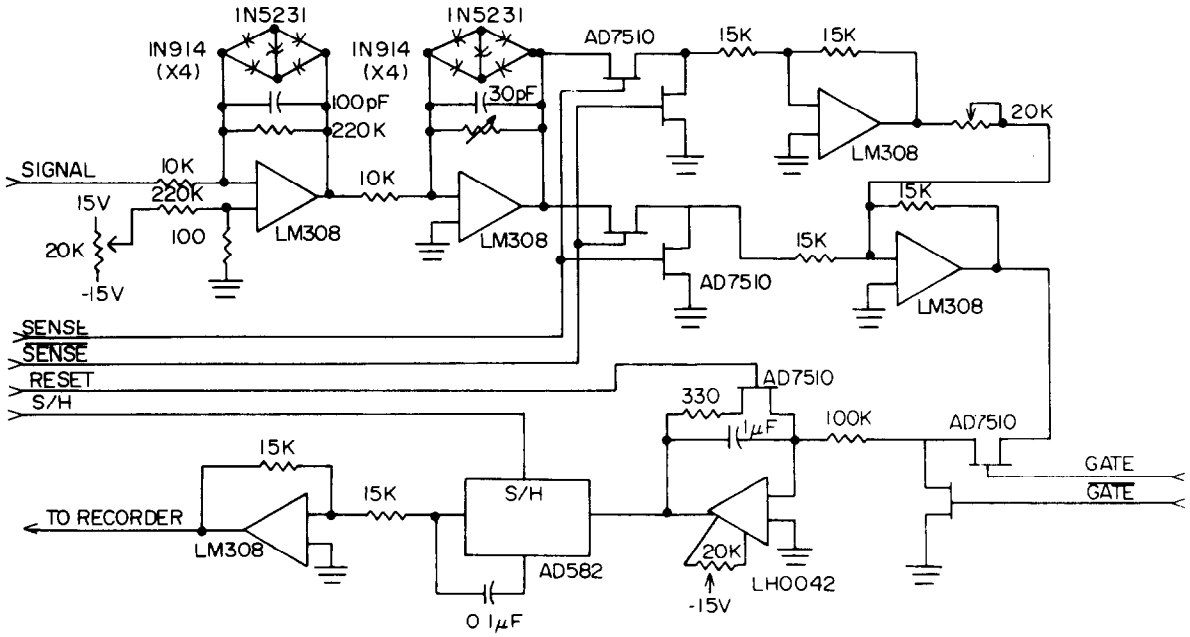

Fig. 5. Analogue-signal processor board (K = 1000Ω).

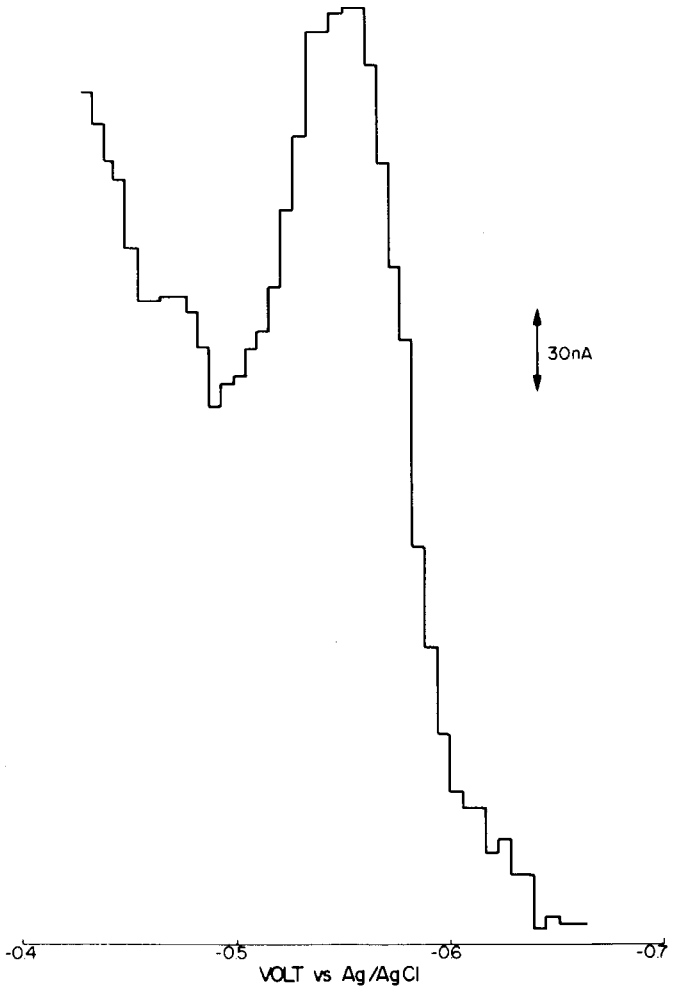

Fig. 6. Representative polarogram.

where it is first amplified approximately 400-fold by a two-stage amplifier. It is necessary to include a limiter on each amplifier, to limit the output of the very large capacitance-current spike. After amplification, the resultant signal is led to a sign-sensitive rectifier wherein the signal resulting from the negative-going square-wave peaks is inverted and added to the signal from the positive-going peaks. Finally, the signal is sent to a gated integrator, the gate signal of which is generated on the timing and interface board. The opening of the gate for signal transmission is permitted from the start of the strobe period until just before the removal of the drop, but the gate is open only when directed by the gate-aperture signal. The integrated signal is stored in a sample-and-hold circuit for digitization and/or analogue display on a strip-chart recorder. On completion of storage in the sample-and-hold circuit the integrator is reset to zero.

Software. Several programs are required for the operation of the square-wave polarograph. System programming for the microcomputer itself is a modified form of the Poly 88 (PolyMorphic Systems, Santa Barbara, CA 93111) monitor program. The operational program for the square-wave polarograph is written in assembly language and assembled on a separate microcomputer. The resultant object-code versions of both the systems program and the operating program, with the exception of the sections for changeable experimental parameters which, of necessity, are maintained in a small section of RAM (called the scratch pad memory), are burned onto EPROMS and maintained in high memory. Hence, the operating software is in reality firmware. The remainder of the RAM is kept free for the eventual storage of polarographic data. Only the operating parameters for the particular experiment need to be entered from the keyboard.

RESULTS AND DISCUSSION

A representative polarogram is shown in Fig. 6. This polarogram, typical of the quality achieved, is for $1.5 \times 10^{-7}M$ cadmium nitrate in $0.4M$ potassium nitrate supporting electrolyte adjusted to pH 3.5 with dilute nitric acid. The instrumental operating parameters were established as: (1) square-wave amplitude, 50 mV; (2) square-wave half-period, 4000 μsec; (3) square-wave cycle delay, 500 μsec; (4) gate aperture, 3225 μsec; (5) drop-time 4 sec; (6) strobe-period, 1 sec. The potential was scanned between -0.400 and -0.700 V *vs.* an Ag/AgCl electrode, at a rise of 5 mV per drop.

The usual oscillations associated with the dropping electrode are absent and only the integrated current that is held by the sample-and-hold circuit is

presented to the input of the strip-chart recorder. As a consequence the current signal appears as a series of discrete steps for each drop. The presence of the cadmium peak on a slope indicates that separation between faradaic and capacitance current has not been completely achieved. Further improvement may well be obtained by numerical analysis of the data once the digitization is completed.

The instrument differs from that described by Turner *et al.*[27] in that a multiplicity of square waves is employed for each "tread" on the staircase voltage. The same number of waves is measured for each of the staircase treads and thus an averaged value rather than a single value is obtained. In addition, the d.c. components of the current are removed with the low-pass filter before summation in the integrator.

It is our intention to utilize the collected data as feedback into the microcomputer and, in turn, to use the feedback data to optimize the parameters of an experiment. We have not yet had time to attempt this, nor have we tried any computer optimization of the type indicated by Bond and Grabaric[9] (which was mainly mathematical correction of data). Even in its analogue form, however, the device provides a powerful analytical tool for the determination of trace level components. The output of the data onto a strip-chart recorder provides a display which at present provides more meaningful information than a column of digitized information. While it is intended to proceed with digitization and subsequent feedback for experimental optimization, it is felt that the instrument in its present form could be useful to other analysts.

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2,4,6-TRIS{2'[4'-(*p*-SULFOPHENYL)PYRIDYL]}-*s*-TRIAZINE: A NEW ANALYTICAL REAGENT FOR THE SPECTROPHOTOMETRIC DETERMINATION OF IRON

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Summary—The sulfonated analog (TPPTZ-S) of 2,4,6-tris[2'-(4'-phenylpyridyl)]-*s*-triazine has been synthesized and its analytical utility investigated. The characteristics of the iron(II) complex of this ligand, including optimum conditions of formation, adherence to Beer's law and relative stability are described. Procedures for the quantitative determination of trace amounts of iron with TPPTZ-S are given. The analytical applicability of the reagent is illustrated by the successful determination of iron in *E. coli* and various beverage and beer samples.

The analytical utility of ferroin-type complexes is well known. The systematic studies of Smith, Diehl and Schilt in conjunction with the synthetic work of Case have yielded hundreds of compounds incorporating the methine chromophore. Their efforts have produced a series of oxidation–reduction indicators covering the range 0.97–1.33 V and a variety of tailor-made organic molecules for analytical use. Notable among these are bathophenanthroline,[1] terrosite,[2] PPDT,[3] neocuproine, bathocuproine,[4] Snyder's reagent[5] and 4,4'-dihydroxy-2,2'-biquinolyl.[6] Bathophenanthroline, terrosite and PPDT are very sensitive towards iron(II), with molar absorptivities of 2.24×10^4, 3.02×10^4 and 2.87×10^4 $1.mole^{-1}.cm^{-1}$, respectively, while neocuproine and bathocuproine are predominantly reagents for copper. Snyder's reagent (4,7-dihydroxy-1,10-phenanthroline) and 4,4'-dihydroxy-2,2'-biquinolyl were designed for determining iron and copper in strongly alkaline solutions, pH >13.

Systematic studies have shown that substitution of methyl, ethyl and phenyl groups in positions *para* to the nitrogen atoms of the methine groups have the greatest effect on the molar absorptivity, also that substitution in the 2,9-positions in 1,10-phenanthroline reduces the chelation of iron(II), but retains the complexing ability of the same reagent toward copper(I), thus allowing for a built-in selectivity for the determination of copper in the presence of iron. As would be expected, phenyl substitution in the positions *para* to the nitrogen atoms of the active functional groups increases the sensitivity towards iron(II) to a greater extent than would methyl or ethyl substitution in the same position.

Table 1 lists a number of well-known analytical reagents, the corresponding phenyl derivatives and the molar absorptivities for their respective iron(II) complexes. From Table 1, it can be seen that phenylation produces a small bathochromic shift and a marked increase in the molar absorptivity. For the lower molecular-weight reagents and their phenyl derivatives, which present no solubility problem, the molar absorptivity of the phenyl derivative complex is approximately twice that of the parent reagent complex. The analytical utility of the higher molecular-weight chromogens, *e.g.*, terrosite, which are very sensitive and selective toward ferrous, iron, is restricted by their limited solubility. In fact 2,4,6-tris[2'-(4'-phenylpyridyl)]-*s*-triazine (TPPTZ) is so insoluble in water and in most organic solvents that a cellosolve–water–ethyl alcohol solution was necessary for determination of the molar absorptivity.[8] TPPTZ gives a molar absorptivity that is only 1.1×10^3 more than that of the TPTZ complex. The solubility sets the upper limit of the free ligand concentration, of course, and if non-aqueous solvents are used there are solubility problems associated with the iron, and thus there is a severe constraint on the ability of the metal and ligand to interact, in spite of the very favorable formation constant.

TPPTZ is not the only phenanthroline-related compound that has a low solubility; in fact, most of the higher molecular-weight reagents do. To overcome this problem, Trinder[9] sulfonated bathophenanthroline, and found that the sulfonated derivative retained its chromogenic properties, with the added advantage of solubility in water. Subsequent work by Zak, Diehl, and others, produced sulfonated derivatives of a number of reagents. Some sulfonated derivatives of reagents such as bathophenanthroline,[10,11] bathocuproine, PDT[12] and terrosite[13,14] have been isolated in the solid form and their analytical utility documented. The most sensitive iron reagent to date ($\epsilon = 3.22 \times 10^4$ $1.mole^{-1}.cm^{-1}$), has also been converted into the sulfonic acid form (2,4'BDTP-S).[15] All these derivatives were found to retain the chromogenic properties of the parent compounds, with some

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Table 1. The effect of increased aromaticity on selected iron(II) chelates

Reagent	Complex λ_{max}, nm	Complex ϵ, $l.mole^{-1}.cm^{-1}$	Phenyl derivative	Complex λ_{max}, nm	Complex ϵ, $l.mole^{-1}.cm^{-1}$	Reference
2,2′-bipyridyl	522	8.65×10^3	4,4′-diphenyl-2,2′-bipyridyl	552	2.11×10^4	7
1,10-phenanthroline	510	1.11×10^4 (a)	4,7-diphenyl-1,10-phenanthroline	533	2.24×10^4 (b)	1
2,2′,2″-terpyridyl	552	1.25×10^4	2,6-bis(2-pyridyl)-4-phenyl pyridine	569	2.20×10^4	2
			2,6-bis(4-phenyl-2-pyridyl)-4-phenyl pyridine (terosite)	583	3.02×10^4 (d)	2
3-(2′-pyridyl)-5,6-diphenyl-1,2,4-triazine	555	2.40×10^4	3-(4-phenyl-2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PPDT)	561	2.87×10^4	7, 3
2,4,6-tris(2′-pyridyl)-s-triazine	593	2.26×10^4 (a)	2,4,6-tris(4-phenyl-2-pyridyl)-s-triazine	605	2.52×10^4 (e)	8
(TPTZ)	595	2.41 ± 10 (c)	(TPPTZ)	605	3.05×10^4 (f)	this work

(a) Water
(b) Ethanol–water solution
(c) Nitrobenzene
(d) Chloroform–ethanol solution
(e) Cellosolve–water–ethanol solution
(f) Water–ethanol–chloroform solution

minor deviations. As can be seen from Table 2, sulfonation does not generally decrease the sensitivity of the reagent towards iron, and indeed, as is the case for some of the larger molecules, the molar absorptivity may increase, possibly on account of electronic effects.

Thus, it was of interest to see whether the sensitivity of TPPTZ ($\epsilon = 2.52 \times 10^4$) would be improved, as well as the solubility, through sulfonation to give TPPTZ-S. The TPPTZ-S was successfully synthesized, even though an earlier attempt by Kiss had failed.[16] It proved to be a very sensitive and selective iron chromogen, the molar absorptivity of the iron(II) complex being 2.98×10^4 $l.mole^{-1}.cm^{-1}$ at 607 nm. TPPTZ-S is more sensitive than PPDT and TPTZ but not as sensitive as 2,4′-BDTP-S. The analytical utility of TPPTZ-S is illustrated by the successful determination of iron in *Escherichia coli* and beer.

EXPERIMENTAL

Reagents

2,4,6-Tris{2′-[4′-(p-sulfophenyl)pyridyl}-s-triazine (TPPTZ-S). The synthesis of the reagent is discussed below. A 0.001*M* solution of TPPTZ-S was prepared by dissolving 0.1237 g of reagent in 100 ml of iron-free water.

Iron standards. A stock solution of iron was prepared by dissolving electrolytic iron in 5 ml of sulfuric acid (1 + 1) and diluting with iron-free water. A known weight of stock solution was diluted to give known concentrations as needed.

Hydroxylamine hydrochloride solution. Prepared by dissolving 10 g of the salt in 90 ml of water. Iron is removed by treating with excess of bathophenanthroline and extracting with isoamyl alcohol.

Buffers. A 1*M* acetic acid–1*M* sodium acetate (pH 4.8) and a 1.0*M* tartrate buffer prepared by dissolving 23 g of sodium tartrate in 100 ml of iron-free water.

All other chemicals were reagent-grade, and were checked for iron contamination. To minimize blank correc-

Table 2. The effect of sulfonation on the iron(II) chelates of selected ferroin type reagents

Compound	Unsulfonated λ_{max}, nm	Unsulfonated ϵ, $l.mole^{-1}.cm^{-1}$	Sulfonated derivative λ_{max}, nm	Sulfonated derivative ϵ, $l.mole^{-1}.cm^{-1}$	Reference
4,7-diphenyl-1,10-phenanthroline	533	2.24×10^4	535	2.21×10^4	11
3-(2′-pyridyl)-5,6-diphenyl-1,2,4-triazine	555	2.40×10^4	562	2.79×10^4	12
3-[2′-(4-phenyl)pyridyl]-5,6-diphenyl-1,2,4-triazine	561	2.87×10^4	563	3.07×10^4	15
2,6-bis(5′,6′-diphenyl-1′,2′,4′-triazin-3-yl)pyridine	468 580	1.80×10^4 1.40×10^4	470 580	1.82×10^4 1.41×10^4	15
2.4-bis(5′,6′-diphenyl-1′,2′,4′-triazin-3-yl)pyridine	563	3.22×10^4	565	3.22×10^4	15
2,6-bis[2′-(4-phenyl)pyridyl]-4-phenylpyridine	583	3.02×10^4	583	3.02×10^4	14
2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline	479	1.39×10^4*	483	1.23×10^4*	11

* Copper complex.

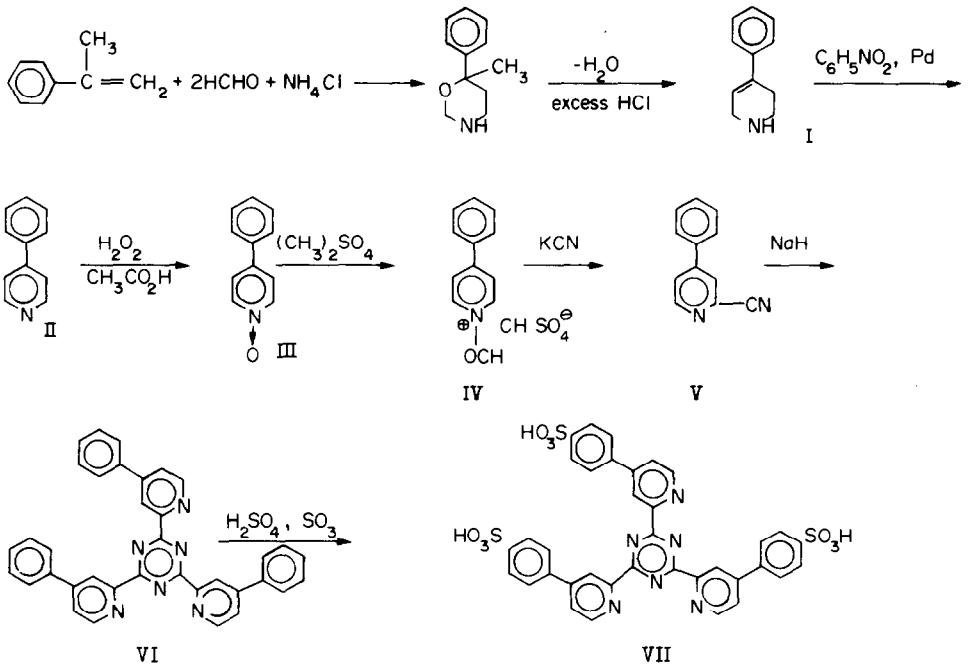


Fig. 1. Synthesis of TPPTZ-S

tions for the wet-ashing procedures, doubly distilled perchloric acid (G. F. Smith Chemical Co.) and nitric acid which had been redistilled from an all-glass still and stored in a borosilicate glass bottle, were used.

Apparatus

A GCA/McPherson EU-700 series spectrophotometer was used, with 1-cm glass cells. A Hach model 8596 expanded-range pH-meter with a Beckman combination pH electrode was used for the measurement of pH. Organic matter was destroyed before the determination of iron, by wet-ashing the samples in 125-ml borosilicate Erlenmeyer flasks equipped with a reflux head (similar to G. F. Smith catalogue item No. 181).

Synthesis of TPPTZ-S

The flow chart for the synthesis of this reagent is given in Fig. 1.

4-Phenylpyridine (**II**).[17,18] To 216 g of ammonium chloride 668 g of 37% aqueous formaldehyde were added. The temperature of the mixture was raised to 65°, with stirring. To this homogeneous solution, 236 g of α-methylstyrene were slowly added with vigorous stirring. The temperature was maintained below 75° by external cooling. After the exothermic reaction was controlled, the temperature was sustained at 60–65° for 1 hr, then the solution was allowed to cool to 35°, and 500 ml of methanol were added. The mixture was stirred for 1 hr more at room temperature, and allowed to stand for 36 hr. The methanol was removed under reduced pressure, and the residue dissolved in 600 g of concentrated hydrochloric acid. The resulting mixture was stirred at 90–95° for 3 hr, cooled, and poured into 800 ml of water. The solution was extracted with toluene, and made basic with 19*M* sodium hydroxide. The amine was extracted with toluene, dried over potassium carbonate and distilled under reduced pressure (1.0 mmHg) to yield 145 g (45%) of 4-phenyl-1,2,3,6-tetrahydropyridine (**I**), b.p. 95–110°, n_D 1.5880. This compound was then dehydrogenated. Two methods were used.

Method A. A mixture of 88 g of 4-phenyl-1,2,3,6-tetrahydropyridine (**I**), 5 g of 10%-palladinized alumina and 450 g of nitrobenzene was stirred for 6 hr at 135°. The reaction was carried out with the removal of water, in an atmosphere of nitrogen. Excess of hydrochloric acid was added to the cooled mixture; the solution was then filtered and extracted with toluene. The aqueous layer was made basic with 10*M* sodium hydroxide, and the amine extracted with toluene and dried over potassium carbonate. After removal of the solvent, the product was distilled to give 61 g (72%) of 4-phenylpyridine (**II**), b.p. 95–100° (1.2 mmHg). The colorless solution solidified upon cooling, and after two recrystallizations from heptane melted at 74–76° (literature value 77–78°).

Method B. A mixture of 145 g of 4-phenyl-1,2,3,6-tetrahydropyridine (**I**) and 5 g of 10%-palladinized alumina catalyst was stirred for 20 hr at 200° in an atmosphere of nitrogen. The cooled mixture was diluted with ethyl alcohol, filtered and distilled. The fraction boiling at 95–110° (1.5 mmHg) was collected, and after recrystallization from heptane gave a melting point range of 69–71°. The yield of 4-phenylpyridine by this method was 64 g (45%).

4-Phenylpyridine-N-oxide (**III**).[19] A mixture of 28 g of 4-phenylpyridine, 130 ml of glacial acetic acid and 20 ml of 30% hydrogen peroxide was heated in a water-bath at 70–80° for 12 hr. After 3 hr, an additional 20 ml of 30% hydrogen peroxide was added. The solution was evaporated under reduced pressure (40–50 mmHg) and the residue made strongly alkaline with a concentrated solution of sodium carbonate. The amine oxide was extracted with chloroform (200 ml) and dried with magnesium sulfate. The removal of the solvent yielded 28 g (91%) of 4-phenylpyridine-*N*-oxide (**III**), m.p. 149°, which was used in the next step without further purification.

4-Phenyl-2-cyanopyridine (**V**).[16,20] To 28 g of crude 4-phenylpyridine-*N*-oxide 20.6 g of dimethyl sulfate were slowly added. After the initial reaction had subsided, the temperature was raised to 90–95° and kept there for 2 hr. Following cooling, the salt was dissolved in 150 ml of water, and the solution slowly added to 32 g of potassium cyanide (in 200 ml of water). The mixture was stirred for 3 hr at 25–30°, and the resulting suspension was filtered by suction and the product washed several times with cold water. The yield of crude product was 21 g (71%) after air-drying. The 4-phenyl-2-cyanopyridine was purified by

distillation under reduced pressure, the fraction boiling at 120–140° (2 mmHg) being collected. The solid product was dissolved in hot acetone, and allowed to recrystallize from petroleum ether (60–90°). After drying, the yield was 16 g (54%) of white powder, m.p. 98–100° (literature value 100°).

2,4,6-Tris[2'-(4'-phenyl)pyridyl]-1,3,5-triazine (**VI**).[21] A mixture of 4.2 g of 4-phenyl-2-cyanopyridine (V) and 0.1 g of sodium hydride was heated at 120–130° for 6 hr in an atmosphere of nitrogen. The unreacted starting material was extracted with hot petroleum ether (60–90°). The residue was dissolved in chloroform, and the solution passed through a column of alumina (basic form). Removal of the solvent and crystallization from benzene yielded 1.6 g (38%) of 2,4,6-tris[2'-(4'-phenyl)pyridyl]-*s*-triazine, m.p. 241–243°. Recrystallization from benzene produced 1.1 g (28%) of light tan solid, m.p. 242–244° (literature value 244–245°).

2,4,6-Tris{2'-[4'-(p-sulfophenyl)pyridyl]}-s-triazine (**VII**).[22] To 1.1 g of 2,4,6-tris[2'-(4'-phenyl)pyridyl]-*s*-triazine (**VI**) 30 ml of 30% fuming sulfuric acid were added. The mixture was heated at 220–230° for 3 hr, cooled, diluted with iron-free water and made alkaline with concentrated ammonia solution. After removal of the excess of ammonia by heating on a water-bath, the water was distilled off under reduced pressure. The residue, without removal from the flask, was extracted with five 300-ml portions of boiling 95% ethanol. The combined ethanol fractions were filtered through a coarse fritted glass funnel. The salt was converted into the acid by passage of the ethanol solution through a cation-exchange resin (Dowex 50W-X8), hydrogen form, that had been purified by washing with concentrated hydrochloric acid and then washed with demineralized water until the effluent was free from chloride. Removal of the solvent and recrystallization from water produced 1.21 g (67%) of water-soluble compounds (dec. >300°).

The free acid was converted into the ammonium salt by neutralization with ammonia solution, and evaporation to dryness. The sodium salt was prepared by neutralizing the acid, bringing the pH of the solution to 9.5 and evaporating the water (without boiling).

Determination of iron

Transfer a sample containing 1–20 μg of iron into a 10-ml standard flask. Add the following reagents in the order given, with mixing after each addition: 1 ml of 10% hydroxylamine hydrochloride solution, 1 ml of $1.2 \times 10^{-3}M$ ligand and 4 ml of 1*M* acetate buffer (pH 4.8). (The *minimum* amount of ligand added should be at least in 4-fold molar ratio to the amount of iron expected). Dilute to volume with iron-free water, and measure the absorbance at 607 nm after 20 minutes. Use a reagent blank and a suitably prepared calibration curve or empirical equation to convert absorbance into concentration.

Interference study

Solutions made up to contain 1 ppm of iron, and known amounts up to 1 mg of various substances were subjected to the procedure above. Absorbance readings differing from the expected value by 3% or more were assumed to be indicative of an interference by the substance added.

Complex formation

The stoichiometry of the complex was determined by the mole-ratio and continuous-variation methods in the usual way.

Determination of iron in beer and soft drinks in presence of copper

Accurately transfer 20 ml of degassed beer into a 125-ml Erlenmeyer flask, and evaporate nearly to dryness. To the cooled flask add 10 ml of a 2:1 mixture of concentrated nitric and 70% perchloric acid. Heat the solution gently until the evolution of brown fumes terminates. Continue heating at a higher temperature, until dense white fumes of perchloric acid fill the flask, and the solution turns clear. After cooling, add 2 ml of iron-free water, rinsing the condenser and the sides of the flask, then 1 ml of 10% hydroxylamine hydrochloride solution, 1 ml of $1.2 \times 10^{-3}M$ TPPTZ-S, and 3 ml of 1*M* tartrate buffer. Adjust the pH of the solution to 2.8–3.1 with concentrated ammonia solution (pH-meter). Cool the solution and transfer it to a 10-ml standard flask. Dilute to volume and measure the absorbance at 607 nm after 30 min. Prepare a reagent blank and standards, following the same procedure. Convert the absorbances into concentrations with a calibration curve.

Determination of iron in biological samples

Transfer weighed samples of bacteria, containing from 2 to 15 μg of iron, into a 125-ml Erlenmeyer flask. Add 4 ml of 1:1 mixture of concentrated nitric and 70% perchloric acid to the digestion flask. Heat gently until the vigorous evolution of brown fumes subsides, and continue to add concentrated nitric acid in 1-ml portions, until two successive additions produce no additional brown fumes. Continue heating at a higher temperature until dense white fumes of perchloric acid fill the flask, and the solution is concentrated to about 1 ml. Cool, rinse the condenser head with about 2 ml of iron-free water, and boil briefly to remove any chlorine produced during the digestion. The final volume should be about 1 ml. Neutralize the excess of perchloric acid with concentrated ammonia solution and transfer the solution to a 10-ml standard flask. Add 5 ml of 1*M* acetate buffer (pH 4.8), 1 ml of 10% hydroxylamine hydrochloride solution, 1 ml of $1.2 \times 10^{-3}M$ TPPTZ-S and dilute to volume. Measure the absorbance at 607 nm after 20 min. Carry a reagent blank and standard through the same procedure.

RESULTS

The absorption spectrum of the reagent and the blue iron(II) complex are shown in Fig. 2; the spectra are comparable to those for TPPTZ published by Diehl.[8] Beer's law is followed for concentrations of Fe(II) from $2 \times 10^{-6}M$ to about $3 \times 10^{-5}M$, a slight improvement over the behaviour of the unsulfonated ligand. The molar absorptivity ($\epsilon = 2.98 \times 10^4$ $1.\text{mole}^{-1}.\text{cm}^{-1}$) is independent of pH from 3 to 7.5, but the complex forms more rapidly at pH 3–6. Color stability is excellent, no change being observed during several days. Continuous-variation and molar-ratio studies show the iron(II) species to be the bis-complex, $Fe(TPPTZ\text{-}S)_2^{n-}$, with a conditional formation constant (pH 4.8, $\mu = 0.4$, and 26.6°) $\log \beta_2 = 10.75$.

The ferrous complex of TPPTZ-S is readily soluble in aqueous media. The rate of formation of the complex is relatively slow, maximum color formation at optimum pH being attained in about 10 min. The effects of various ions on the color formation are summarized in Table 3. As would be expected, many of the transition metal ions form complexes with TPPTZ-S, but only that of iron(II) is colored. Thus if a sufficient amount of reagent is added, these ions should not interfere. Ions which successfully compete with TPPTZ-S for complexation with iron, such as cyanide, nitrite and oxalate, do interfere with the determination of iron. The tolerance levels listed in

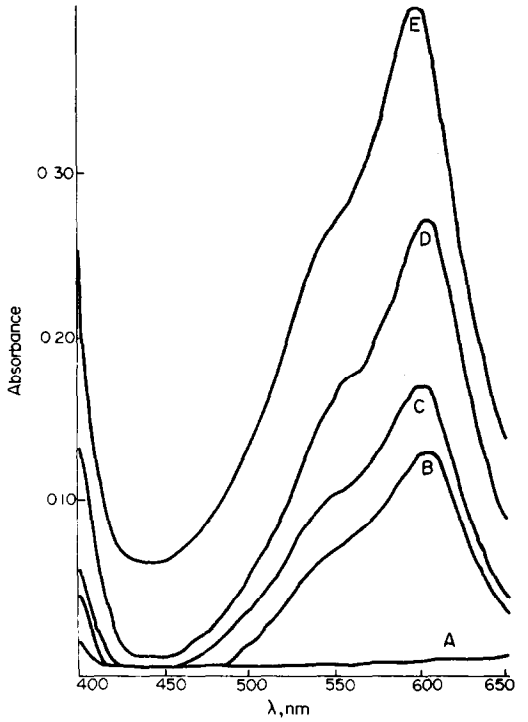


Fig. 2. Absorption spectra of TPPTZ-S and its iron(II) complex in aqueous media. A—blank; B—4.20 × $10^{-6}M$ Fe(II); C—5.55 × $10^{-6}M$ Fe(II); D—9.10 × $10^{-6}M$ Fe(II); E—1.33 × $10^{-5}M$ Fe(II).

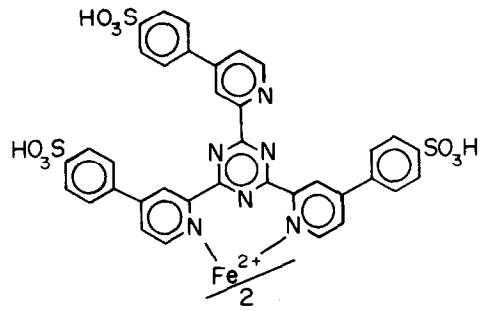
Table 3 for the various ions are based on the quantity of reagent specified in the recommended procedure. The maximum quantity tested was 100 ppm; higher levels are tolerated if the concentration of reagent is increased.

The versatility of TPPTZ-S was tested by determining iron in various brands of beer, a soft drink and in *Escherichia coli*. The results of these determinations are compiled in Tables 4 and 5. The procedure employed for the determination of iron in the soft drinks and beer samples required very careful pH adjustment in order to minimize interference due to copper. A tartrate buffer is used so that copper can be masked as the tartrate complex, but tartrate can also form iron(II) and iron(III) complexes, so a pH of 2.5–3.1 has to be used to minimize this side-reaction with iron, and this is not only near the lower end of the useful pH-range for use of TPPTZ-S, but also reduces the efficency of tartrate as a masking agent for copper. The relative standard deviations of these replicate determinations are somewhat larger (4.0, 7.2, 7.8 and 28%) than normal. This is a result of the relatively low iron concentrations, the small volumes employed (10 ml) and the relatively high reagent blanks due to difficulties in cleaning up the tartrate buffer. The utility of TPPTZ-S for determination of iron in samples where no copper interference is expected was demonstrated. Results obtained for the determination of iron in bacteria, with an acetate buffer, yielded a relative standard deviation (1.2 and 2.2%) of the order expected for a spectrophotometric determination.

Prior oxidation of the organic matter was accomplished by wet oxidation with a nitric and perchloric acid mixture. The wet oxidation does not need careful temperature control and is more rapid than dry-ashing. It also eliminates the iron losses due to volatility, that may occur in dry-ashing; a 99.5% recovery of iron from spiked samples was found even when the excess of nitric and perchloric acids was removed by evaporating the final solution nearly to dryness.

DISCUSSION

TPPTZ-S has been synthesized and purified by successive extractions. It exhibits marked sensitivity as a reagent for iron(II) and is readily soluble in water, thus enhancing its use for the rapid and convenient determination of iron. From its formula and spectral characteristics, the ferrous complex of TPPTZ-S most likely has the same structure as the unsulfonated complex. The structure of $Fe(TPPTZ\text{-}S)_2^{2-}$ may be depicted in fully protonated form as:



The relative difficulty of synthesis of the parent compound (TPPTZ) will probably retard the wide-

Table 3. Effect of various ions on the determination of iron by recommended procedure

No interference from 100 ppm of the following ions:
NH_4^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}*, Ba^{2+}*, Zn^{2+}, Cd^{2+}, Ag^+*, Al^{3+}, Bi^{3+}*, Sn^{2+}, Pb^{2+}*
Cl^-, Br^-, ClO_4^-, $C_2H_3O_2^-$, CO_3^{2-}, SO_4^{2-}, NO_3^-, PO_4^{3-}, BO_3^{3-}, $C_6H_5O_7^{3-}$, phthalate, tartrate
Interfering ions and approximate tolerance levels in ppm:
Cr^{3+} (25), Co^{2+} (5), Ni^{2+} (5), Cu^+ and Cu^{2+} (0.2)
NO_2^- (17), CN^- (13), $C_2O_4^{2-}$ (44)

* After centrifugation of precipitate.

Table 4. Selective determination of iron in refreshments

Sample	Iron added, μg	Iron found, μg	Original, *ppm*
Mountain Dew	4.27	8.18	0.196
	4.50	7.95	0.173
	0.00	4.00	0.200
	0.00	4.10	0.205
			$\bar{X} = 0.196$
			$S = 0.015$
Draft beer	0.00	11.5	0.230
	0.00	10.9	0.218
			$\bar{X} = 0.224$
			$S = 0.0065$
Hudepohl, bottled	3.16*	5.45	0.115
	4.43*	7.30	0.146
	0.00†	4.10	0.082
	0.00*	1.68	0.084
			$\bar{X} = 0.107$
			$S = 0.030$
Schlitz Light, canned	4.57	8.05	0.174
	4.53	7.65	0.156
	0.00	3.73	0.186
	0.00	3.23	0.162
			$\bar{X} = 0.170$
			$S = 0.0135$

$\bar{X}$ = mean; S = standard deviation.
* 20-ml sample.
† 50-ml sample.

spread analytical use of the sulfonated reagent, even though the sulfonation step is very simple. TPPTZ-S has the advantage that it can be easily isolated in pure form by extraction of the free acid, a characteristic lacking in the preparation of many other sensitive iron reagents. During evaluation of the potential utility of the sulfonated material, the sensitivity of TPPTZ was re-evaluated and the iron(II) complex was found to have a molar absorptivity of 3.05×10^{-4} $1.mole^{-1}.cm^{-1}$ at 605 nm in a chloroform–methanol–water mixture as the solvent. The $Fe(TPPTZ)_2^{2+}$ complex can also be readily extracted as its perchlorate from a water–methanol mixture (~50% saturated with perchlorate) into chloroform.

Unfortunately TPPTZ-S proved not to give a higher sensitivity for iron(II) and is only one more of a number of highly sensitive iron reagents. Since TPPTZ-S does not form a colored complex with copper, it cannot be used for the occasionally required simultaneous determination of iron and copper. The

Table 5. Determination of iron in bacteria grown in iron-rich media

Sample	Fe added, μg	Fe found, *ppm*		Recovery, %
		Total	Original	
A	0.0	2.94	2.94	
	0.0	2.92	2.92	
	0.0	3.08	3.08	
	0.0	3.05	3.05	
	2.48	3.17	2.92	98.0
	5.17	3.48	2.96	99.3
			$\bar{X} = 2.98$	
			$S = 0.069$	
B	0.0	1.34	1.34	
	0.0	1.36	1.36	
	2.55	1.64	1.38	101.5
	4.44	1.79	1.35	99.3
			$\bar{X} = 1.36$	
			$S = 0.017$	Mean 99.5

$$\text{Recovery} = \frac{\text{Total Fe found } (\mu g) - \text{original Fe } (\mu g)}{\text{Fe added } (\mu g)} \times 100\%$$

Final volume = 10.00 ml
Sample size = 5 mg of freeze-dried *E. coli*.

only foreseeable use of this interesting compound appears to be for determination of iron in the presence of cyanide. The procedure described will tolerate approximately 17 ppm of cyanide, whereas other iron reagents such as PPDT are severely restricted by cyanide, even at the sub-ppm level.

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CALCIUM COMPLEXES DETERMINED BY POLAROGRAPHY

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Summary—A d.c. polarographic technique has been developed which allows measurement of the concentration of calcium ions in aqueous solutions. By applying a potential scan at a rate of 25 mV/sec to a 1.5 sec/drop dropping mercury electrode, peak-shaped waves are obtained for calcium at m*M* concentrations in tetrabutylammonium hydroxide supporting electrolyte. The square root of the peak height is directly proportional to the calcium concentration over the range 0.1–1.0 × 10^{-3} *M*. In the presence of the sequestrants ethylenediaminetetra-acetic acid (EDTA), nitrilotriacetic acid (NTA) and tripolyphosphate (TP), indications of distinct calcium complexes were seen by noting the positions of slope changes in plots of peak height *vs.* mole ratio of calcium to sequestrant. The species found were CaEDTA, $Ca_3(NTA)_2$, $Ca_2(TP)_3$ and Ca_3TP. All of these are readily acceptable complexes at the high pH of the experimental conditions.

Professor Diehl is a remarkable teacher and experimenter. From him I learned the merits of giving something a try, of pushing classical approaches beyond established bounds to see what might happen. His enthusiasm for laboratory experimentation was contagious. The first question—does the experiment work?—that is the important one. In industry we add—is the technique useful? Then, comes the last question—what is the theory behind the successful and useful experiment?

The work reported here results in "yes" to the first two questions: it works, and it is useful. The third question remains unanswered as other duties prevented our pursuing the theory. What is important is that Professor Diehl's philosophies encouraged giving it a try and I am reporting the fascinating results of doing just that.

To help get clothes clean, water hardness must be overcome. Calcium hardness is especially troublesome and the search for effective, economical and ecologically acceptable sequestrants for calcium takes a good deal of our time. Evaluating potential sequestrants is also time-consuming as few of them have useful spectroscopic properties to follow when they are exposed to calcium. Also, calcium is not easy to monitor at the pH values and ionic strengths used in practical laundry work. The aim of our work was to establish the ratios of calcium to sequestrant as a function of varying concentrations of each and to do so at realistic pH values—above 9 or so.

EXPERIMENTAL

We had been investigating the application of polarography to many materials we use and decided to try it with calcium. To get the reduction wave, we needed supporting electrolyte of the tetra-alkylammonium variety.[1] We chose polarographic grade tetrabutylammonium hydroxide (TBA^+OH^-), 0.05*M*, which resulted in a pH of about 11, not too far away from laundry pH values. To avoid leaky bridges from an S.C.E. reference electrode, we used an isolated mercury pool anode. This was achieved by placing an approximately 10 mm tall and 15 mm diameter glass vial filled with fresh mercury in the bottom of the 100 or 150-ml beaker used as the polarographic sample container. This vial was positioned so as not to be contaminated by the mercury falling from the dropping electrode. Electrical connection was made through a platinum wire sealed through glass and submerged in the mercury. All solutions were deaerated with nitrogen for 5 min before the polarography.

Standard solutions of calcium were prepared by dissolving known weights of primary standard calcium carbonate in a slight excess of hydrochloric acid and diluting to volume.

A succinct description of the polarography of calcium has been published.[2] Calcium polarograms on our Sargent XXI polarograph were normal under conventional scan characteristics, which meant that they had large maxima. We could not use the published methods for suppression of these maxima (*e.g.* addition of multivalent cations such as La^{3+} or using partially non-aqueous solvents) because to do so would cause confusion in the complexation studies we wanted to follow. We found a way around this as follows. We increased the rate of applying the potential to the D.M.E. from the conventional 1–2 mV/sec to 25 mV/sec. Originally we connected an Erector Set motor, complete with gear-box, to the external shaft of the "Initial" voltage 3-turn helipot on the polarograph. One particular gear-box setting resulted in a rate of 25 mV/sec which gave very satisfactory looking polarograms. This rate is consistent with that recommended by Ross *et al.*[3] for rapid potential scans with a hanging mercury drop electrode. Because this led to polarograms meeting our needs, no attempt was made to determine whether this rate was optimal. With this established, we replaced the Erector Set motor with a 1.5-rpm Hurst reversible synchronous motor. In this mode, neither the "span" setting function nor the slide-wire bridge of the Sargent XXI polarograph is used.

With this rate of potential scan, our scan interval from −1.5 to −2.8 V *vs.* the Hg pool compressed our polarograms into less than 2 in. of chart. By connecting a spit motor from a home charcoal grill (later a more sophisticated 5-rpm Hurst reversible synchronous motor was used) to the last shaft before the paper-drive roll of the Sargent XXI recorder, we could display our 1.3-V span over $5\frac{1}{2}$ in. of chart paper, which was more satisfactory.

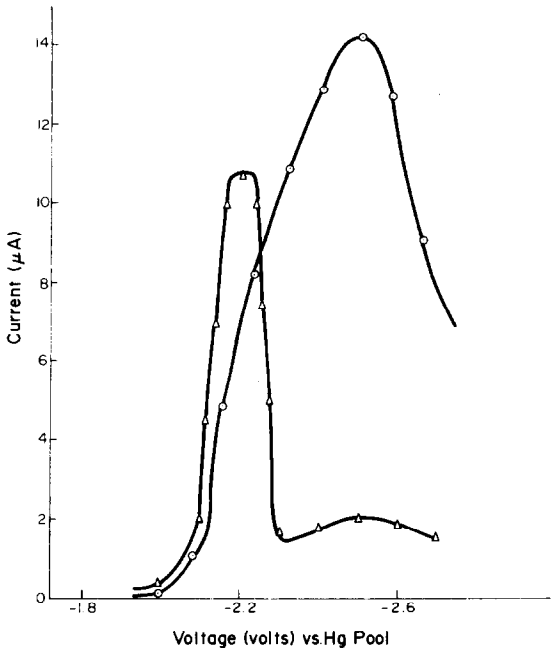

Fig. 1. Comparison of calcium polarograms for 1 m*M* Ca^{+2} in 0.1*M* TBA^+ OH^- by conventional and rapid scan techniques. ⊙ Drop-time 1 sec, rapid scan at 25 mV/sec. △ Drop-time 6 sec, normal scan at 4 mV/sec.

The main factors which made the system work were as follows.

1. Isolating the mercury pool anode in a small glass vial submerged in the polarographic cell.
2. Cleaning the dropping mercury electrode after each polarogram by a 5-min immersion in 1*N* hydrochloric acid with a fast flow of the mercury, followed by a 5-min "rest" in demineralized water before the next polarogram.
3. Storing the dropping mercury electrode in demineralized water overnight, with the mercury dropping slowly.
4. Using solutes in either their free acid form, or converting them into the tetrabutylammonium salt by ion-exchange.

POLAROGRAPHY RESULTS

Using this rapid scan approach, we found that no reduction waves for the supporting electrolytes or for calcium-free solutions of any of the sequestrants to be studied occurred in the potential range of interest, from −1.5 to −2.8 V, *vs.* Hg pool.

With calcium present, we found peaks reminiscent of polarographic maxima. Figure 1 compares the polarograms for 1 m*M* Ca^{2+} in 0.1*M* TBA^+OH^-, obtained by conventional polarography and by the rapid scan technique. The polarographic peak current from the rapid scan is about 7 times that for the plateau (post-maximum) current obtained by conventional scan. Figure 2 shows a family of such peaks for

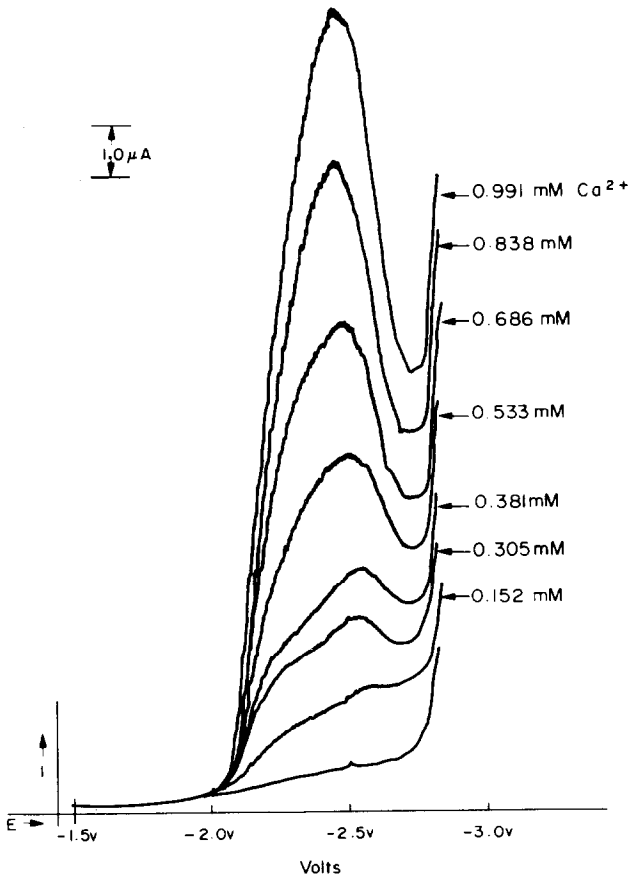

Fig. 2. Family of polarographic curves for varying concentrations of calcium.

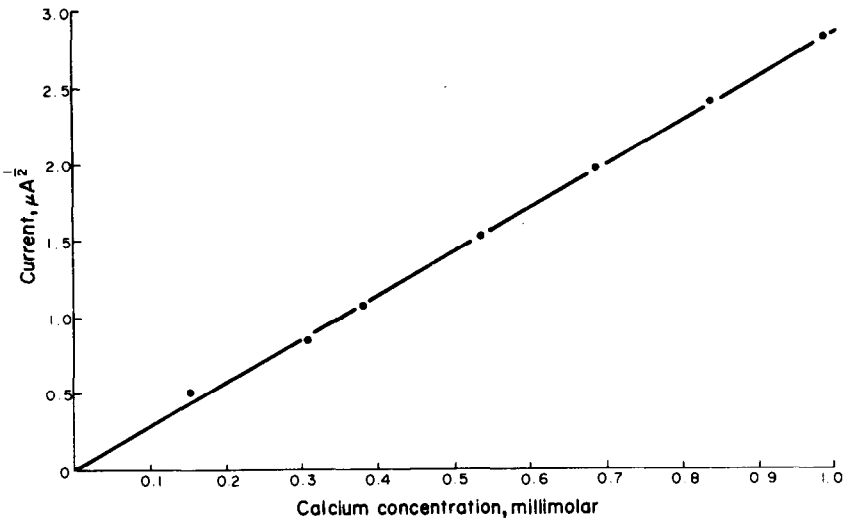

Fig. 3. Relationship of polarographic current and calcium concentration.

varying concentrations of calcium. The range is from about 1m*M* down to about 0.15m*M* with well-defined peaks for 0.3m*M* and above. The "tick" at −2.5 V in the background (bottom) polarogram was imposed by the operator (by slight instantaneous finger pressure on the cable driving the recorder pen) to serve as a marker. The peak for calcium is at about −2.5 V and currents were measured at this potential (hence the "tick") if the calcium concentration was too low for a distinct peak to occur.

The polarographic peak current is not a linear function of concentration. Figure 3 shows a linear relationship for calcium concentration *vs.* the square root of the peak current. This is a new current/concentration relationship. As seen in Table 1, all other forms of polarography—conventional d.c., a.c., stripping and oscillographic—have a direct proportionality between concentration and current. Only our technique stands out and only in this characteristic. Note that D.M.E. droptimes are not really different from those used in the other techniques and even our potential-scan rate is within the range used in stripping analysis.[3]

The reproducibility of the $i_p^{1/2}$ *vs.* concentration plot is impressive. During the 2-month period over which we used this system the slope varied by only about ±5%: 2.8–3.1 × 10^3 $\mu A^{1/2}.l.mole^{-1}$. Blank values were 0.94 $\mu A^{1/2}$ without sequestrants and 0.99 $\mu A^{1/2}$ with sequestrants (but no calcium), and the average deviation was only 0.03 $\mu A^{1/2}$. Note that all peak heights must be converted into $\mu A^{1/2}$ before the usual arithmetic of subtracting blank from sample is done.

The upper limit of calcium concentration is about 1.0–1.3m*M*. Above 1.5m*M*, an opalescence appears in the solutions [$Ca(OH)_2$?] and the current/concentration relationship departs from linearity in a predictable direction: it flattens out. We did not pursue the lower limit of applicability as that was not relevant to our need.

Summarizing this work on calcium solutions, we have peaks in the polarograms like those found in oscillographic polarography but the current/concentration relationship is unique.

Sequestrant effects

The sequestrants we used included ethylenediaminetetra-acetic acid (EDTA), nitrilotriacetic acid (NTA), and tripolyphosphoric acid (TP). The sequestrants were prepared as follows. EDTA: from H_4EDTA dissolved in water with the aid of TBA^+OH^- and standardized by titration with standard calcium solution to the Calmagite end-point in

Table 1. Typical polarographic characteristics

Type	Potential-scan, *mV/sec*	Drop-time, *sec*	Current/concentration relationship	Reference
Conventional d.c.	1–2	2–5	$i_d \propto C$	4
Alternating current	1–2	3–5	$i_p \propto C$	5
Stripping analysis (hanging Hg drop)	10–50		$i_p \propto C$	3
Oscillographic	10^3–10^5	3–5	$i_p \propto C$	6, 7
Rapid scan	25	2	$(i_p)^{1/2} \propto C$	this work

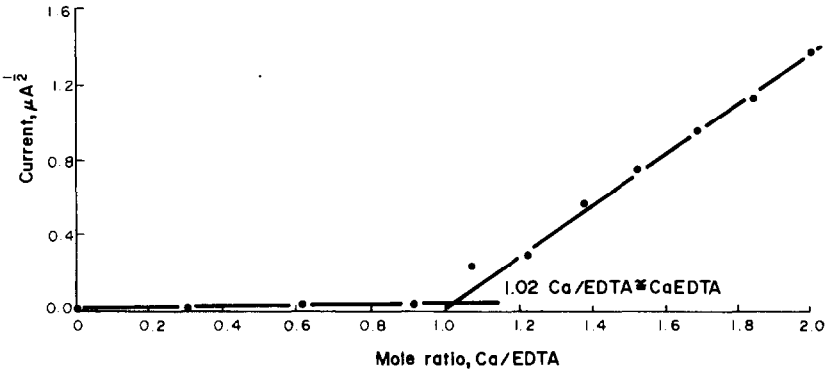

Fig. 4. Mole-ratio plot of calcium and EDTA *vs.* polarographic current.

pH 10 ammonia buffer. NTA: H_3NTA standardized by titration with standard alkali. TP: carefully purified $Na_5P_3O_{10}$ was converted into $(TBA)_5P_3O_{10}$ by ion-exchange. Polarograms of 1m*M* solutions of these sequestrants in 0.05*M* TBA^+OH^- supporting electrolyte, obtained by the rapid scan technique, showed them to be free of interfering materials such as alkali metal cations, *i.e.*, no polarographic waves or peaks were seen.

When a sequestrant is added to a solution of calcium, the polarographic peak height decreases. No additional peaks arise in the potential range we looked at, from −1.5 to −2.8 V. This means that the sequestrant removes calcium from solution by complexation, leaving less calcium available to be polarographically reduced.

Professor Diehl always encouraged his students to draw on techniques in one field for possible application in another. We did just that. Using the common spectrophotometric technique for establishing the ratio of metal to sequestrant, or more commonly the ratio of metal to coloured analytical reagent, we used a mole-ratio method for calcium and various sequestrants. This technique of using plots of the polarographic diffusion currents *vs.* mole-ratio of metal to ligand has been reported previously.[8] Figure 4 shows the result for calcium and EDTA. In this example we kept the EDTA concentration constant at 0.5m*M* and added increasing amounts of calcium. Peak currents were negligible until the molar amount of calcium added equalled that of the EDTA present. Peaks for calcium reduction then appeared and increased in height as more calcium was added. The inflection in this plot indicates the formation of the usual 1:1 Ca:EDTA complex. The slope of the plot at mole ratios above 1 is the same as would be found for calcium alone. (At mole ratio 2.0, the $i_p^{1/2}$ is $\sim 1.4\ \mu A^{1/2}$ and the total calcium concentration is twice that of the EDTA or $2 \times 0.5 = 1.0$m*M*; the free calcium is therefore 0.5m*M*, and the slope is 2.8×10^3

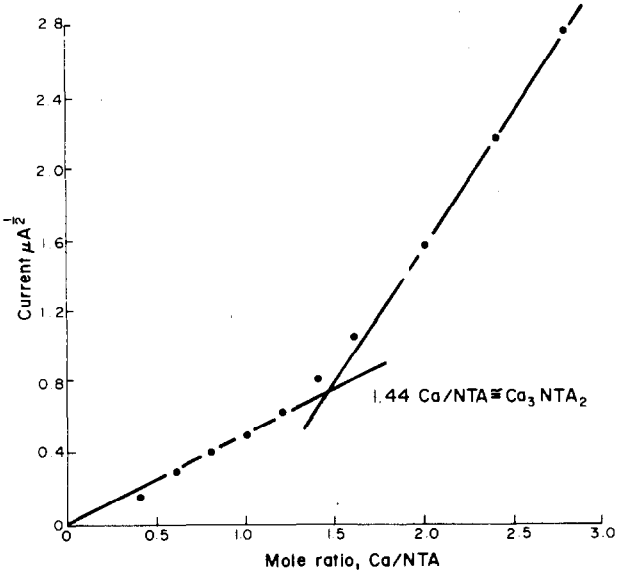

Fig. 5. Mole-ratio plot of calcium and NTA *vs.* polarographic current.

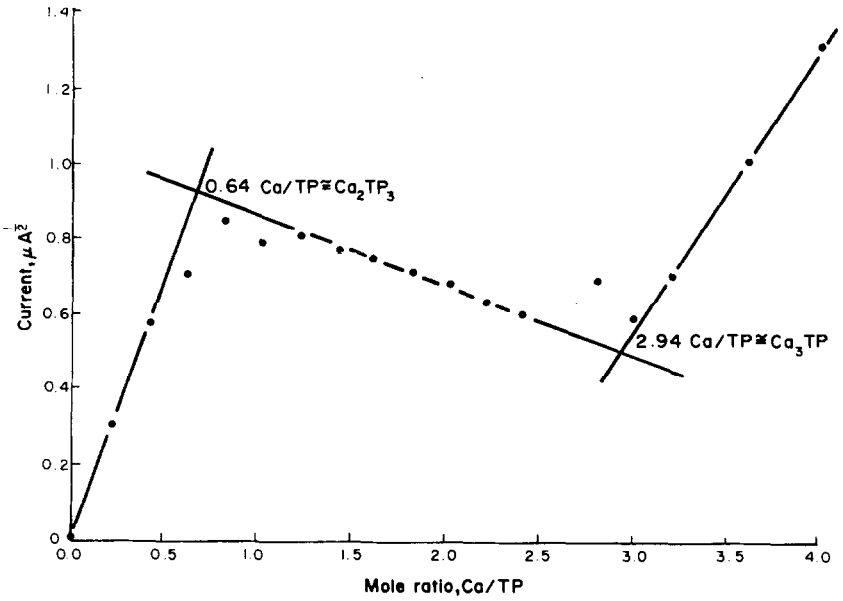

Fig. 6. Mole-ratio plot of calcium and tripolyphosphate (TP) *vs.* polarographic current.

$\mu A^{1/2}.l.mole^{-1}$ consistent with that for sequestrant-free calcium in Fig. 3 a,) Note that until we reach the 1:1 Ca:EDTA ratio, the mole ratio plot is very flat. The interpretation of this is quite straightforward; nothing reducible is present at concentrations that are detectable under these polarographic conditions. At this point the concentration of uncomplexed calcium is $\sim 10^{-7} M$.

If we look at the plot for NTA in Fig. 5, we see something different. The experimental conditions were the same as with EDTA. The mole-ratio plot inflection suggests the presence of the complex $Ca_3(NTA)_2$. Such a complex is tenable at the pH involved, since the NTA would be totally ionized. Again, the slope beyond this is like that for calcium alone. The earlier slope is interesting. Qualitatively it suggests that this Ca–NTA complex is weaker than the Ca–EDTA complex. The polarographic peak shapes and position indicate that we are still measuring free calcium ions, so what we are probably doing is measuring the free calcium in equilibrium with the Ca–NTA complex.

It we reverse the experimental mole-ratio system we get similar results. In other words, if calcium is present initially and increasing amounts of EDTA or NTA sequestrant are added, the peak heights decrease predictably. The peak currents decrease to background level with EDTA and near to background with NTA. For the system with the common detergent sequestrant, tripolyphosphate (TP), the same experimental conditions as before were used. Our interpretation of the mole-ratio plot shown in Fig. 6 leads to two Ca–TP complexes, *viz.* $Ca_2(TP)_3$ and Ca_3TP. Both of these [like the $Ca_3(NTA)_2$ complex noted earlier] are rational at the pH-11 conditions used. It should be recalled that this technique uses a strongly buffered system, unlike those systems (used to study metal complexes) in which the hydrogen ion concentration is used to follow the complex formation. The steep initial slope in this Ca–TP system indicates a very weak Ca–TP complex. The diminishing slope then appearing suggests that a stronger complex involving a lower TP:Ca ratio is forming. Finally, the slope beyond the 3:1 Ca–TP complex again mimics that of free calcium. The situation is probably more complicated than is indicated by this simplified interpretation. For example, the peak we see may be due to a combination of reduction of free calcium and of a Ca–TP complex which is more readily reducible or has a higher diffusion current than free calcium. Exactly what is happening is not totally clear, however.

Conclusion

We had fun with this system. We stretched classical experimental conditions a bit, we drew on techniques common to other analytical methods, and we ended up with something which worked and which was useful. Unfortunately, time was not available to develop a full understanding or the theory of the system.

Professor Diehl really started all this with his creative, innovative and enthusiastic willingness to experiment. I am indebted to him for that since it has affected my approach to industrial analytical chemistry immensely.

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STUDIES OF 7-HYDROXYBENZO-4-PYRONES

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Summary—Spectral data and rates of hydrolysis in 0.7*M* potassium hydroxide are reported for a series of nine 7-hydroxybenzo-4-pyrones differing in substitution at positions 2 and 3. The results reveal that 2,3-dimethyl substitution produces a molecule, 7-hydroxy-2,3-dimethylchromone, sufficiently fluorescent and resistant to alkaline hydrolysis to warrant its use as the fluorescent moiety in a metallofluorescent indicator of the Calcein Blue type.

Metallofluorescent indicators consisting of a fluorescent molecule bonded to methyleneiminodiacetic acid (half of the EDTA molecule) have properties that are, to a large extent, governed by the fluorescent moiety. The spectral properties of Calcein Blue, for example, are determined by its fluorophore, 4-methylumbelliferone. Likewise, the fluorescence loss exhibited by alkaline solutions of Calcein Blue is the result of pyrone-ring opening of 4-methylumbelliferone.

While its spectral properties render Calcein Blue an excellent indicator for titrimetric EDTA determinations of metal ions,[1] susceptibility to attack by hydroxide ion precludes the use of Calcein Blue in spectrofluorimetric determinations of metal ions that form weak metal ion–indicator compounds, determinations which must be performed at or above pH 10.5 and which require a constant fluorescence signal.

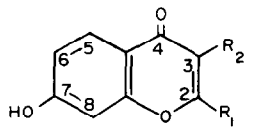
4-Methylumbelliferone is a 7-hydroxybenzo-2-pyrone. Might, then, molecules containing the 7-hydroxybenzo-4-pyrone nucleus be more resistant to attack by alkali? Scheppers[2] found that introduction of a phenyl group at position 2 of 7-hydroxybenzo-4-pyrone produces a molecule, 7-hydroxyflavone, with low susceptibility to attack by hydroxide ion and low relative fluorescence. 2-Methyl-3-phenyl substitution produces a molecule, 7-hydroxy-2-methylisoflavone, also resistant to attack by alkali but exhibiting significantly higher relative fluorescence. This molecule has been used as the fluorescent moiety in the indicator, Isocein, and Isocein has been employed in spectrofluorometric determinations of calcium.[3]

One is led to wonder what pattern or patterns of substitution at positions 2 and 3 of 7-hydroxybenzo-4-pyrone may serve to produce molecules suitable for use as metallofluorescent indicators. In addition to addressing this question, the present paper presents basic information on the absorbance, behaviour as an acid, and fluorescence as a function of pH for a series of nine 7-hydroxybenzo-4-pyrones.

EXPERIMENTAL

Reagents

Synthesis. The following compounds were prepared.



7 – Hydroxybenzo – 4 – pyrone

I R_1=H, R_2=H (7-hydroxychromone)
II R_1=CH_3, R_2=H (7-hydroxy-2-methylchromone)
III R_1=H, R_2=CH_3 (7-hydroxy-3-methylchromone)
IV R_1=CH_3, R_2=CH_3 (7-hydroxy-2,3-dimethylchromone)
V R_1=H, R_2=C_6H_5 (7-hydroxyisoflavone)
VI R_1=CH_3, R_2=C_6H_5 (7-hydroxy-2-methylisoflavone)
VII R_1=C_6H_5, R_2=C_6H_5 (7-hydroxy-2,3-diphenylchromone)
VIII R_1=C_6H_5, R_2=H (7-hydroxyflavone)
IX R_1=C_6H_5, R_2=CH_3 (7-hydroxy-3-methylflavone)

References to the preparation, melting point and elemental analysis are furnished in Table 1.

Purity was checked by thin-layer chromatography: ethanolic solutions of the compounds were spotted on silica gel plates and a toluene–methanol–ethyl acetate–acetic acid–chloroform mixture in ratio 80:20:15:10:5 was used as developing solvent. The developed plates yielded one fluorescent spot when irradiated with long-wavelength ultraviolet light.

All chemicals were of reagent grade quality and were used without further purification. All water used was distilled and then demineralized by passage through Amberlite MB-1.

Procedures

Absorption spectra. Absorption spectra were obtained at intervals of 0.5 pH unit over the range 3.5–12, with a Cary Model 14 Recording Spectrophotometer. Stock solutions were prepared by dissolving 3.11×10^{-4} mole of each compound in 100 ml of 95% ethanol. Volumes of 250 ml of

Table 1. Melting point, elemental analysis and literature reference to preparation of 2- and 3-substituted 7-hydroxybenzo-4-pyrones

Compound	m.p. (lit.), °C	Elemental analysis % (theoretical) Carbon	Hydrogen	Reference
I	227–228 (218)	67.2 (66.67)	3.9 (3.73)	5
II	255 (253–254)	68.0 (68.18)	4.5 (4.58)	6, 2
III	240–242 (244)	68.2 (68.18)	4.7 (4.58)	7
IV	273–275 (265)	69.4 (69.46)	5.4 (5.30)	8
V	208–210 (210)	75.7 (75.62)	4.5 (4.23)	9
VI	242–243.5 (240)	76.2 (76.18)	4.7 (4.79)	10
VII	262–264 (270–271)	80.4 (80.24)	4.6 (4.49)	10
VIII	240.5–243 (240)	75.5 (75.62)	4.4 (4.23)	6, 2
IX	286–287 (278)	76.3 (76.18)	4.8 (4.79)	8

stock solution of compounds VII–IX were transferred to 25-ml standard flasks with a Micro Metric SB 2 microburette and a model S5Y syringe and were diluted to the mark with appropriate buffers of constant ionic strength. Additional absorbance measurements were made on solutions of pH equal to the estimated $pK_a \pm 0.0$, 0.2, 0.4 and 0.6. Acid dissociation constants were extracted from the data by means of the equation

$$pK_a = pH - \log \frac{(A_{mix} - A_{HA})}{(A_{A^-} - A_{mix})}$$

A_{HA}, A_{A^-} and A_{mix} being the absorbance values for solutions containing the phenol form, the phenolate form and a mixture of phenol and phenolate forms of the compounds, at a pH near the pK_a value.

Fluorescence spectra. Fluorescence excitation and emission spectra of all the compounds except VII (which does not fluoresce under the conditions employed) were obtained over the pH range 3.5–12.0 at 0.5 pH intervals and in 0.7*M* potassium hydroxide, with an Aminco-Bowman Spectrofluorometer equipped with a Mosely XY Recorder. Spectra were not corrected for variations in the emission characteristics of the lamp and response characteristics of the photomultiplier. The test solutions were prepared by adding appropriate volumes of stock solution to 25-ml standard flasks and diluting to the mark with an appropriate buffer, and were of constant ionic strength. Fluorescence emission resulting from selective excitation of the phenolate form of the compounds was substituted in the equation

$$pK_a = pH - \log \frac{(F_{mix} - F_{HA})}{(F_{A^-} - F_{mix})}$$

to determine acid dissociation constants. The subscripts HA, A^- and mix refer to the species defined above.

Rates of hydrolysis. Rates of 7-hydroxybenzo-4-pyrone hydrolysis in 0.7*M* potassium hydroxide (at 25.5 ± 0.1°) were determined by monitoring the decrease in fluorescence at appropriate time intervals and wavelengths on a Turner model 110 fluorometer. The filters used for chromones and isoflavones were: primary, 110–811 (7–60); secondary, 110–827 (3), plus a 10% neutral density filter. Filters used for flavones were: primary, 110–811 (7–60); secondary, 110–826 (2A–15). A flow-through cell assembly was used for the study of 7-hydroxychromone and 7-hydroxyisoflavone. Apparent first-order rate constants were calculated from slopes of plots of the logarithms of the difference between the fluorescence emission at any time, F, and the final fluorescence, F_∞, *vs.* time, in accordance with the equation for an apparent first-order reaction

$$\log|F - F_\infty| = \frac{-kt}{2.303} + \log|F_0 - F_\infty|.$$

The rate of hydrolysis of non-fluorescent 7-hydroxy-2,3-diphenylchromone in 0.7*M* potassium hydroxide at 25.5 ± 1° was obtained by recording the change in absorbance at 264.1 nm as a function of time, on a Beckman DK-2A spectrophotometer. The apparent first-order rate constant was calculated from slopes of plots of the logarithms of the difference between the absorbance at any time, A, and the final absorbance, A_∞, *vs.* time in accordance with the equation

$$\log|A - A_\infty| = \frac{-kt}{2.303} + \log|A_0 - A_\infty|.$$

The slopes were determined by linear regression analysis. Each study was repeated at least four times and at different concentrations of the benzo-4-pyrone to ensure reproducibility. The hydrolyses were, in most cases, followed for 5.5 half-lives.

RESULTS AND DISCUSSION

Absorption data

Wavelengths of maximum absorbance are listed in Table 2. In both acid and alkaline solution, the long-wavelength absorption maxima of compounds VII, VIII and IX appear at longer wavelengths than those of compounds I–VI. This is attributed to conjugation of the 2-phenyl and carbonyl groups. Addition of a second phenyl group (compound VIII) or of a methyl group (compound IX) at position 3 forces the 2-phenyl group out of its planar arrangement with the benzo-4-pyrone system, reducing the degree of conjugation and shortening the wavelength of maximum absorbance.

Introduction of a 2-methyl group produces a slight hypsochromic shift of the long-wavelength band (compare compounds I and II, III and IV, V and VI). The presence of either a 3-methyl group or a 3-phenyl group alone has little, if any, effect on wavelengths of maximum absorbance.

In addition to the band near 300 nm present in the spectrum of the acid form of each of the compounds, there are shorter-wavelength absorption maxima. All bands shift to longer wavelengths with increasing pH and upon neutralization of the phenolic proton. Acid dissociation constants are given in Table 3.

Table 2. Absorption maxima and molar absorptivities of 2- and 3-substituted 7-hydroxybenzo-4-pyrones at pH 4.5 and 10.5

Compound	pH 4.5		pH 10.5	
	Absorption maximum, *nm*	Molar absorptivity, $l.mole^{-1}.cm^{-1}$	Absorption maximum, *nm*	Molar absorptivity, $l.mole^{-1}.cm^{-1}$
I	297.6	1.17×10^4	334.5	1.07×10^4
	247.8	1.81×10^4	307.0(sh)	6.46×10^3
	240.8	1.52×10^4	254.0	2.23×10^4
II	294.5	1.30×10^4	336.0	1.35×10^4
	249.0	1.65×10^4	299.2(sh)	5.79×10^3
	242.6	1.50×10^4	255.7	2.59×10^4
III	298.4	1.27×10^4	335.7	1.21×10^4
	249.3	1.45×10^4	305.5(sh)	7.40×10^3
	243.0	1.32×10^4	257.6	2.43×10^4
IV	296.1	1.62×10^4	333.7	2.03×10^4
	250.9	1.27×10^4	302.0(sh)	1.47×10^4
	244.8	1.29×10^4	259.6	3.22×10^4
V	301.3	1.31×10^4	337.4	1.29×10^4
	242.6	2.89×10^4	309.4(sh)	9.00×10^3
			262.5	3.13×10^4
VI	296.9	1.57×10^4	334.9	1.58×10^4
	241.7(sh)	2.48×10^4	303.0(sh)	8.62×10^3
	230.1	2.75×10^4	259.3	3.19×10^4
VII	307.6	8.68×10^3	349.2	1.37×10^4
	245.5	1.25×10^4	312.2	1.00×10^4
			264.1	3.14×10^4
VIII	310.9	2.96×10^4	360.6	1.43×10^4
	250.2	2.38×10^4	305.2(sh)	8.00×10^3
			266.1	3.25×10^4
IX	306.1	1.64×10^4	346.7	1.50×10^4
	246.0	1.38×10^4	309.3	9.42×10^3
			261.5	3.12×10^4

Table 3. Acid dissociation constants obtained from absorbance and fluorescence data

Compound	pK_a	
	Absorbance	Fluorescence
I	7.23 ± 0.03	7.23 ± 0.05
II	7.38 ± 0.01	7.39 ± 0.03
III	7.42 ± 0.02	7.39 ± 0.05
IV	7.61 ± 0.02	7.64 ± 0.06
V	7.32 ± 0.02	7.28 ± 0.05
VI	7.49 ± 0.03	7.42 ± 0.02
VII	7.27 ± 0.05	non-fluorescent
VIII	7.16 ± 0.05	7.19 ± 0.04
IX	7.44 ± 0.07	7.44 ± 0.06

Fluorescence data

Wavelengths of maximum fluorescence excitation and emission are listed in Table 4. Maxima in the excitation spectrum of each compound correspond to those in the absorbance spectrum and like them shift to longer wavelengths at higher pH.

The fluorescence emission of compounds I–VI increases and shifts to shorter wavelengths with increasing pH whereas the fluorescence emission of compounds VIII and IX increases with no concomitant change in wavelength on pH increase. In all cases fluorescence data yield the same pK_a values as determined absorptiometrically and correspond to ground-state ionization of the 7-hydroxy group.

It is presumed that the phenolate ion is responsible for the fluorescence at high pH. Because the wavelength of fluorescence emission does not change over the pH range 3.5–12.0 and in 0.7*M* potassium hydroxide solution for compounds VIII and IX, the phenolate ion must be responsible for the fluorescence, indicating that in the excited state the phenol is more acidic than it is in the ground state. This behaviour parallels that of 4-methylumbelliferone and is typical of phenols.

Compounds I–VI fluoresce at longer wavelengths in acid solution than in alkaline solution. This behaviour is unexpected and may be attributed to photo-tautomerism of the phenol following excitation.

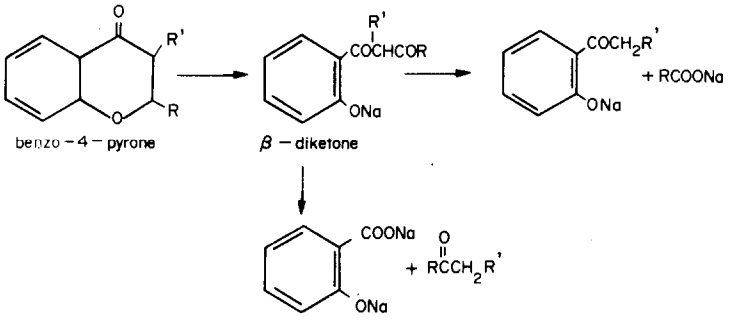
Hydrolysis study

Attack on benzo-4-pyrone by hydroxide ion occurs at position 2 and initially produces a β-diketone. Pro-

Table 4. Wavelengths of maximum fluorescence excitation and emission at pH 4.5 and 10.5

Compound	Fluorescence excitation maxima, *nm*		Fluorescence emission maxima, *nm*	
	pH 4.5	pH 10.5	pH 4.5	pH 10.5
I	240, 300	251, 338	479	471
II	242, 295	250, 332	475	460
III	240, 300	251, 332	475	469
IV	249, 301	252, 333	479	460
V	249, 302	260, 339	489	479
VI	245, 298	255, 333	485	465
VII	Non-fluorescent		Non-fluorescent	
VIII	261, 319	268, 360	537	536
IX	254, 309	258, 349	528	528

longed treatment with alkali results in a mixture of four products.[4]



The decrease in fluorescence, corresponding to pyrone ring-opening and measured at appropriate wavelengths and time intervals, provided a convenient means of monitoring rates of hydrolysis. Under the experimental conditions employed, hydrolyses were found to follow first-order kinetics with respect to the benzo-4-pyrone. The hydrolyses did not appear to be affected by dissolved oxygen or carbon dioxide, similar values for the rate constants being obtained in solutions purged with ultrapure nitrogen and in untreated solutions. Apparent first-order rate constants for the alkaline hydrolyses are listed in Table 5. These vary from $0.0989 \pm 0.0006\ min^{-1}$ for the most rapidly hydrolysed compound, 7-hydroxyisoflavone, to $0.0416 \pm 0.0007\ day^{-1}$ for 7-hydroxy-3-methylflavone, and correspond to half-lives of 6.98 ± 0.04 min and 16.6 ± 0.3 days, respectively.

The susceptibility to alkaline hydrolysis is consistent with inductive effects. The mild electron-donating ability of a 3-methyl group retards hydrolysis by decreasing the probability of nucleophilic attack by hydroxide ion while the electron-withdrawing capacity of a phenyl group at the same position enhances the probability of nucleophilic attack. The rate of attack by hydroxide is also sensitive to steric effects and varies with substitution at position 2 in the order: $—H > —CH_3 > —C_6H_5$. Compounds with substituents at both the 2- and 3-positions were found to be the most resistant to hydrolysis.

While 7-hydroxy-3-methylflavone (IX) is least prone to attack by hydroxide ion, $t_{1/2} = 16.6$ days, the low relative fluorescence precludes its use as the fluorophore in a metallofluorescent indicator. Substitution of methyl groups at positions 2 and 3 produces a molecule, 7-hydroxy-2,3-dimethylchromone (IV), having a relative fluorescence approaching that of 4-methylumbelliferone and a high resistance to alkaline hydrolysis ($t_{1/2} = 9.80$ days in $0.7M$ potassium hydroxide). This molecule has been condensed with formaldehyde and iminodiacetic acid to form a metallofluorescent indicator. Studies of this indicator are under way.

Table 5. Apparent first-order rate constants and half-lives for 7-hydroxybenzo-4-pyrones differing in substitution at positions 2 and 3; solvent $0.7M$ potassium hydroxide (25.5 ± 0.1°C)

Compound	k	$t_{1/2}$
I	$0.0701 \pm 0.0007\ min^{-1}$	9.80 ± 0.05 min
II	$0.108 \pm 0.0004\ hr^{-1}$	6.37 ± 0.04 hr
III	$0.137 \pm 0.0014\ hr^{-1}$	5.02 ± 0.05 hr
IV	$0.0704 \pm 0.0007\ day^{-1}$	9.80 ± 0.09 days
V	$0.0989 \pm 0.0006\ min^{-1}$	6.98 ± 0.04 min
VI	$0.0594 \pm 0.00002\ hr^{-1}$	11.6 ± 0.04 hr
VII	$0.202 \pm 0.005\ day^{-1}$	3.41 ± 0.09 days
VIII	$0.0239 \pm 0.003\ hr^{-1}$	28.9 ± 0.3 hr
IX	$0.0416 \pm 0.0007\ day^{-1}$	16.6 ± 0.3 days

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POTENTIOMETRIC TITRATIONS OF METAL IONS IN ACETONITRILE WITH POLYAMINE LIGANDS

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Summary—Ethylenediamine, diethylenetriamine, and triethylenetetramine were investigated as titrants for metal ions in acetonitrile. Copper(II) gave the best titration plots; cobalt(II), manganese(II), nickel(II), iron(III) and magnesium(II) also gave results acceptable for analytical determinations. Platinum, silver–silver(I), mercury–mercury(II), carbon, and a copper ion-selective electrode were studied as indicating electrode systems; of these platinum gave the sharpest and largest inflections. The mechanism by which platinum responds as an indicating electrode in these complexation titrations is unclear.

Complexing titrants which have only nitrogen atoms as ligands, such as polyamines, are more selective in their interactions with metal ions than are reagents such as EDTA which possess both nitrogen and oxygen donor atoms. Reilley and Sheldon[1] used the selectivity of triethylenetetramine for titrations of metals such as copper, zinc, mercury, and cadmium in the presence of aluminium, bismuth, lead, and the alkaline earths. They emphasized the need when selecting titrants and titration conditions to consider the competitive equilibria of metal ion hydrolysis, hydrogen-ion contributions from buffers or added acid, and complexing effects by buffers. The potentiometric titration of copper, cadmium and zinc with triethylenetetramine and tetraethylenepentamine with a silver indicating electrode has been investigated by Hulanicki *et al.*[2]

In aprotic non-aqueous solvents the competing equilibria to be considered in complex formation with metal ions are simpler than in water. This is because neither solvolysis, corresponding to the formation of metal hydroxides in water, nor protonation of coordination sites on the ligand by protons from the solvent, can occur. The absence of these two processes means that buffers, with the attendant complications of proton contribution and auxiliary complex formation, are unnecessary. In short, there is little need to consider conditional formation constants.

The major disadvantage of aprotic solvents as media for complexation titrations is the low solubility of most metal salts owing to poor solvation of the anions. The most soluble salts are those of large, symmetrical anions of low charge-density such as perchlorate, tetrafluoroborate, and hexafluorophosphate, anions not usually encountered in systems of analytical interest. Common ions such as chloride, nitrate, or sulphate must therefore be exchanged for species such as perchlorate if adequate analytical concentrations of the metal salts are to be obtained.

Despite the inconvenience of this conversion step, it appears worthwhile to assess the scope of metal ion titrations with chelating ligands in aprotic media for possible analytical use. In this study acetonitrile was selected as solvent since it is a poor hydrogen-ion donor or acceptor, is readily available, has a moderately high dielectric constant, is relatively non-toxic, and has a convenient liquid range and low viscosity. As titrants, the polyamines ethylenediamine, diethylenetriamine, and triethylenetetramine were selected because of the solubility of the ligands and their metal complexes in acetonitrile, and because of the stability of some of the ligand–metal complexes.[3]

Potentiometric titration is preferred so that the stoichiometry and extent of the reactions can be followed. For this purpose both the mercury and silver indicating electrodes used in aqueous complexometric work were investigated. It was found that platinum also functioned as an indicating electrode for several of the titration systems.

EXPERIMENTAL

Chemicals

Ethylenediamine (en), diethylenetriamine (dien), triethylenetetramine (trien) and dimethyl sulphoxide (DMSO) (J.T. Baker) were purified by distillation under reduced pressure. Acetonitrile (Matheson, Coleman, and Bell) was dried overnight over calcium hydride and then distilled.

The hydrated perchlorate salts of copper(II), zinc(II), manganese(II), nickel(II), iron(III), cobalt(II) and chromium(III) (G. F. Smith Chemical Co.) were converted into the corresponding dimethyl sulphoxide solvates either by the procedure of Selbin, Bull and Holmes[4] or of Cotton and Francis.[5] All were converted readily and analysed as reported except for the copper(II) salt, which, when prepared according to the published method,[5] precipitated as a pale blue salt that gave an analysis corresponding to the pentasolvate rather than the reported tetrasolvate. Analysis gave C 18.5%, H 4.6%, Cu (iodometric titration) 9.7%; $Cu(ClO_4)_2.5DMSO$ requires C 18.39%, H 4.63%, Cu 9.73%.

As the dimethyl sulphoxide solvates of the perchlorates of magnesium, calcium, and strontium have not been reported previously, details of their preparation are given here.

$Mg(ClO_4)_2.6DMSO$. To a saturated solution of anhydrous magnesium perchlorate in dry acetone was added an

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equal volume of dimethyl sulphoxide. Upon addition of ethanol a white precipitate formed; this was filtered off, washed with ethanol, and dried overnight under vacuum at room temperature. Analysis gave C 20.7%, H 5.2%, Mg (EDTA titration) 3.50%; $Mg(ClO_4)_2.6DMSO$ requires C 20.83%, H 5.20%, Mg 3.52%.

$Ca(ClO_4)_2.6DMSO$. A slurry of 20 g of calcium carbonate in 50 ml of water was treated with 70% perchloric acid in 1-ml portions until evolution of carbon dioxide ceased and a pH of 1 was obtained. Water was removed under vacuum until solid calcium perchlorate appeared and little liquid remained. The material was dissolved in a minimum amount of acetone, the solution was filtered, an equal volume of dimethyl sulphoxide added, and the mixture shaken. Upon addition of diethyl ether a heavy white precipitate formed. After cooling in ice, the precipitate was filtered off, washed twice with ether, and dried overnight under vacuum at room temperature. Analysis gave C 19.7%, H 4.9%, Ca (EDTA titration) 5.6$_4$%; $Ca(ClO_4)_2.6DMSO$ requires C 20.36%, H 5.12%, Ca 5.66%.

$Sr(ClO_4)_2.6DMSO$. This material was prepared in the same way as the calcium salt except that the white precipitate obtained was dissolved in acetone and reprecipitated with ether before drying overnight under vacuum. Analysis gave C 18.5%, H 4.6%; $Sr(ClO_4)_2.6DMSO$ requires C 19.08%, H 4.80%.

Titration procedure

For the complexometric titration studies, approximately 40 mg of each DMSO-solvated metal perchlorate were dissolved in 40 ml of dry acetonitrile and titrated with approximately 0.06*M* amine in dry acetonitrile. An 80-ml glass cell fitted with a Teflon lid containing openings for the burette tip, electrodes, and nitrogen inlet was used as a titration vessel. The indicating electrode was either mercury–mercury(II) amine, silver–silver ion, or platinum, and the reference electrode was silver–0.01*M* silver nitrate in acetonitrile.

For the mercury electrode one of the arrangements described by Reilley and Schmid[6] was employed. One drop of a $10^{-3}M$ solution of mercury(II)–en or mercury(II)–trien was added to the solution to be titrated to provide the mercury(II) ions necessary for the electrode couple. For the silver–silver ion indicating electrode a silver wire, along with a drop of 0.008*M* silver nitrate in acetonitrile added to the solution to be titrated, was used, as described by Fritz and Garralda for complexometric titrations in water.[7] All titrations were done with the platinum indicating electrode, except where indicated, and on a Metrohm E436 automatic recording titrator at a titrant delivery rate of about 0.25 ml/min. The reference electrode and the solution being titrated were separated by a glass junction[8] and a glass frit, with a bridge solution of 0.1*M* lithium perchlorate in acetonitrile between them. Aerial oxidation and water absorption were minimized by starting the titration immediately upon dissolution of the samples, and by carrying out all titrations under a blanket of dry nitrogen.

RESULTS AND DISCUSSION

Titrations in acetonitrile of hydrated metal salts of the form $M(ClO_4)_n.xH_2O$ with the polyamine ligands did not give useful end-points, apparently because of formation of metal hydroxides and protonation of the polyamines by water. Ethylenediamine and the other polyamines are quite basic in acetonitrile and can be titrated quantitatively with perchloric acid in dioxan. For example, large potential breaks are observed with a glass electrode at 1:1 and 2:1 ratios of H^+ to en, indicating the appreciable base strength of both nitrogen atoms in this system. Therefore the titration system must be kept water-free if useful results are to be obtained.

Titrations with ethylenediamine

Results of titrations of a series of metal ions with en are summarized in Table 1. The key features are that cobalt(II) and copper(II) show sharp inflection points at 2:1 ratios of en to metal, while manganese(II), nickel(II) and magnesium(II) show smaller inflections at 3:1 ratios (Fig. 1). For copper(II), a set of titrations in which $Cu(ClO_4)_2.4CH_3CN$ was used as the sample in place of the DMSO solvate gave identical titration curves to those in which DMSO was present, showing that DMSO does not affect the shape of the curves. Calcium(II), strontium(II), chromium(III), and silver(I) do not yield titration breaks, but zinc gives two, a small sharp break at a ratio of en to zinc of about 1.85:1 and a more drawn-out break at a ratio somewhat over 3:1. Both 2:1 and 3:1 complexes of en with zinc have been reported as existing in water–ethanol mixtures,[9] but at high ethanol concentrations, of the order of 80%, only the 3:1 complex is seen. In acetonitrile the behaviour of iron(III) is similar to that of zinc(II), but the break at the 3:1 ratio is sharper. The largest potential change is observed with copper(II). The 2:1 stoichiometry observed here parallels the behaviour of copper(II) with en in water;[10,11] this parallel behaviour is not seen with cobalt(II), which forms with en in water 1:1, 2:1, and 3:1 complexes of roughly equal stability, but shows only a 2:1 break in acetonitrile.

Several titrations of mixtures were investigated to assess the potential selectivity of en as a titrant; the results are summarized in Table 1.

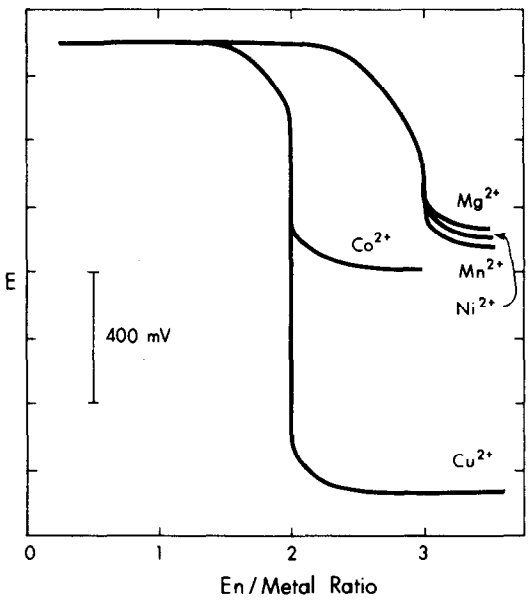

Fig. 1. Relative magnitude of breaks at the inflection points for the potentiometric titration of several metal ions with ethylenediamine in acetonitrile.

Titrations with dien and trien

Table 2 summarizes the results of titrations of a series of metal ions with dien, and Table 3 with trien. With dien copper(II) shows breaks at ratios of both 1:1 and 2:1, while with trien only a 1:1 break is seen. In both cases the curves are sharp and stiochiometric. The only other ions of those studied that react stoichiometrically with dien are magnesium(II), cobalt(II), and manganese(II), all of which produce small but well-defined inflections at 2:1 ratios (Fig. 2). No metals other than copper gave stoichiometric breaks with trien. The lack of stoichiometry can be attributed in the case of trien to difficulty in selecting reproducible end-points from the drawn-out, asymmetric titration plots, but for dien the non-stoichiometry must be attributed to some other cause since the inflections are generally sharp.

The mechanism by which platinum functions as an indicating electrode in these systems has not been established. Measurements of the potentials of a series of solutions of varied copper(II) concentrations in acetonitrile with a platinum indicating electrode and a silver–silver nitrate reference electrode pair were not reproducible, nor were similar measurements on solutions with varied concentrations of trien. Pretreatment of the platinum with nitric acid or acidic iron(II) sulphate had no effect on its response. In one titration of copper with dien the platinum was replaced by a graphite rod pretreated with molten wax to decrease its porosity;[12] two sharp breaks of 120 and 300 mV were obtained at stoichiometries of 1:1 and 2:1.

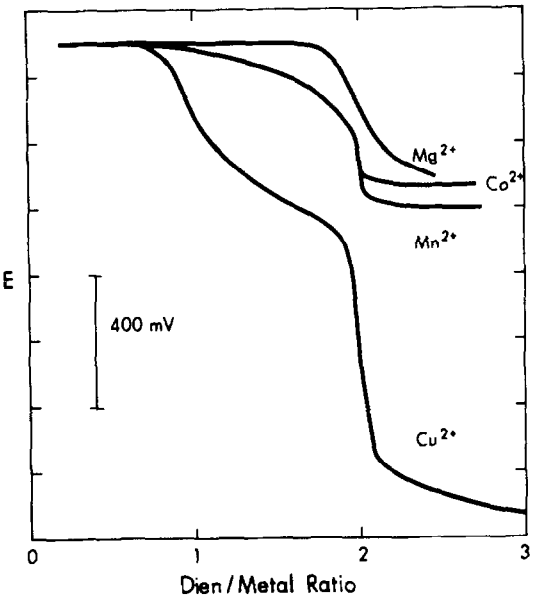

Fig. 2. Relative magnitude of breaks at the inflection point for the potentiometric titration of several metal ions with diethylenetriamine in acetonitrile.

However, no useful break of the kind observed with platinum was seen when the graphite electrode was used for the titration of magnesium with dien. A solid-state copper ion-selective electrode (Orion Model 94-29) was also tested. Titrations of copper(II) with en gave a single inflection of about 450 mV at

Table 1. Summary of titrations of $M(ClO_4)_n$. DMSO salts with en in acetonitrile

Species titrated	en:metal ratio at inflection point	Approximate potential change at inflection point, *mV*
Cu^{2+}	2.00_2, 2.00_7, 2.00_6, 2.00_0	960 very sharp
Co^{2+}	1.99_9, 2.00_1, 1.99_9, 2.00_1	240 sharp
Zn^{2+}	1.83, 1.86, 1.86	80 sharp
	3.00, 3.28, 3.15	140 drawn-out
Mn^{2+}	2.99_9, 3.00_1, 3.00_0	120 fairly sharp
Fe^{3+}	~1.8, 1.8, 1.8, 1.8	slight inflection
	2.99_7, 3.00_1, 3.00_1, 3.00_3	120 fair
Ni^{2+}	~2.5, 2.5, 2.5	slight inflection
	3.00_9, 3.00_4, 3.00_0	90 sharp
Mg^{2+}	2.99_8, 3.00_1, 3.00_2	80 fair to poor
Cu^{2+a}	2.03_3, 2.00_0, 1.99_5, 2.00_0	950 very sharp
H^{+b}	0.500	160 sharp
	1.00_0	200 sharp
Cu^{2+}, Ca^{2+}, Sr^{2+}, Cr^{3+}	2.00_1, 2.00_6, 2.00_1	600 very sharp (only Cu^{2+} titrated)
Cu^{2+}, Zn^{2+}	end-point 3–7% early for total	600 sharp
Cu^{2+}, $AgNO_3$	2.01_4	120 fair, ppte (only Cu^{2+} titrated)
Fe^{3+}, Cr^{3+}	3.00_2, 3.00_3, 2.99_8	
	2.99_7, 2.99_8	130 fair (only Fe^{3+} titrated)
Mn^{2+}, Cr^{3+}	3.06_1, 3.06_5, 3.05_7	130 drawn-out (only Mn^{2+} titrated)
Ni^{2+}, Cr^{3+}	3.08_6 2.83_3	190 fairly sharp (only Ni^{2+} titrated)
Mg^{2+}, Ca^{2+}, Sr^{2+}	no inflection	
Mn^{2+}, Ca^{2+}	no inflection	

[a] Cu^{2+} added as $Cu(ClO_4)_2 . 6CH_3CN$
[b] H^+ added as $HClO_4 . 2H_2O$ (70% aqueous acid).

Table 2. Summary of titrations of $M(ClO_4)_n . xDMSO$ salts with dien in acetonitrile, with platinum as indicating electrode

Species titrated	dien:metal ratio at inflection point	Approximate potential change at inflection point, *mV*
Cu^{2+}	1.00_0, 1.00_3, 1.00_6	330 very sharp
	2.00_0, 1.98_8, 2.00_6	630 very sharp
Zn^{2+}	2.00_1, 2.03_9, 2.01_7 2.12_1	360 very sharp
Fe^{3+}	1.69, 1.68, 1.68	300 very sharp
Ni^{2+}	2.02, 2.03, 2.04	220 very sharp
Mg^{2+}	2.00_3, 2.00_1, 2.00_0 1.99_8	200 sharp
Mn^{2+}	1.98, 2.00_3, 2.01	140 very sharp
Co^{2+}	1.99_7, 2.04, 1.98	100 sharp
Ca^{2+}	2.16, 2.13, 2.14	60 poor, drawn-out
Ca^{2+}, Mg^{2+}	2.01_2 for total	180 fairly sharp (both titrated)

Table 3. Summary of titrations of $M(ClO_4)_n . xDMSO$ salts with trien in acetonitrile

Species titrated	trien:metal ratio at inflection point	Approximate potential change at inflection point, *mV*
Cu^{2+}	1.00_8, 1.00_1, 1.00_0	840 very sharp
Zn^{2+}	1.07, 1.13, 1.08	400 fair break
Co^{2+}	1.08, 1.07	140 fair break
Fe^{3+}	1.23, 1.44, 1.39	120 poor break
	1.66, 1.88, 1.84	100 poor, drawn-out
Ni^{2+}	1.09, 1.09, 1.09	120 poor, drawn-out
Mn^{2+}	1.05, 1.07, 1.07	60–80 fairly sharp
Ca^{2+}		~100 too drawn-out for use
Mg^{2+}		~90 too drawn-out for use

Table 4. Summary of titrations of $M(ClO_4)_n . xDMSO$ salts with en in acetonitrile, with Hg/en indicating electrode

Species titrated	en:metal ratio at inflection point	Approximate potential change at inflection point, *mV*
Cu^{2+}	1.99_8, 1.99_8, 1.99_0, 2.00_0	360–560, very sharp
Co^{2+}	2.00_3, 1.99_9, 2.00_1	280 very sharp
Mn^{2+}	~3	drawn-out, not useful
Ni^{2+}	3.00_2, 3.00_3	130, good
Mg^{2+}	no inflection	drawn-out
Ca^{2+}	no inflection	

Table 5. Summary of titrations of $M(ClO_4)_n . xDMSO$ salts with en in acetonitrile, with Ag/Ag^+ indicating electrode

Species titrated	en:metal ratio at inflection point	Approximate potential change at inflection point, *mV*
Cu^{2+}	1.99_8, 2.00_0, 2.00_0	180, sharp
Cu^{2+}	1.98_8, 2.00_0 (only silver wire, no Ag^+ added)	200, sharp
Co^{2+}	1.99_2, 1.91_6	240, sharp
Co^{2+}	1.99_8 (only silver wire, no Ag^+ added)	220, very sharp
Mn^{2+}	~3	120, poor, hard to locate the end-point
Ni^{2+}	3.00_1, 2.99_8, 3.00_3	150, sharp
Mg^{2+}	~3	poor, drawn-out
Ca^{2+}	no inflection	gradual potential change of about 60 mV throughout titration

ratios of en to copper(II) of 2.13_5, 2.14_3, 2.14_8, and 2.14_5 (four consecutive titrations). Although the end-point could be located reproducibly, it was not stoichiometric. Replacement of the silver–silver nitrate reference electrode with an aqueous saturated calomel electrode in one titration of nickel(II) with en was also done to test whether trace amounts of silver ion were diffusing into the cell solution and affecting the potential at the end-point. However, results were unchanged from those obtained with the silver reference electrode. Also, addition of silver nitrate to the titration cell in one titration of copper(II) with en gave a precipitate and a reduction in the size of the end-point break from 1 V to 120 mV.

Results of titrations of a series of metal ions with en, either the mercury–en or silver–silver ion system being used as indicating electrode and the silver–silver ion couple as reference electrode, are summarized in Tables 4 and 5. As with the platinum indicating electrode, copper(II) shows the sharpest and largest potential breaks. Cobalt(II) titrations also show sharp inflection points at 2:1 ratios of en to cobalt(II) in the case of the mercury electrode, but at less than 2:1 for the silver indicating electrode. Similar sharp potential breaks, but at a 3:1 ratio of ligand to metal, are seen with both electrodes for the titration of nickel(II). With both electrodes manganese(II) shows drawn-out potential breaks at about 3:1 ratios which are not analytically useful. Calcium(II) does not yield titration breaks with either electrode, while magnesium(II) shows a poor, drawn-out break at about a 3:1 ratio with the silver indicating electrode, but does not show an inflection with the mercury electrode.

Tables 6 and 7 summarize the results of titrations of a series of ions with trien, with the mercury–trien or silver–silver ion indicating electrode and a silver–silver ion reference electrode. The only system studied that gave a break at a clean, stoichiometric 1:1 ratio was magnesium(II) with the mercury–trien indicating electrode. All the other systems showed breaks at ratios of more than 1:1 even though some of the inflections were fairly large or sharp.

The silver–silver ion indicating electrode did not provide satisfactory end-points for any of the metal-ion titrations. All the systems investigated showed drawn-out, poor breaks at ratios of more than 1:1, an exception being manganese(II), which showed fair through non-stoichiometric single inflections.

Titration of aqueous solutions of manganese(II) perchlorate or tetraphenylborate with an ethanolic solution of trien has been reported by Chiswell[13] to yield a precipitate of $[Mn_2trien_3]X_4.nH_2O$. A 1:1 Mn(II)–trien salt was reported to be obtained upon mixing anhydrous manganese perchlorate and trien in equimolar amounts in ethanol and allowing the solution to crystallize over a period of weeks. These results indicate that end-points at ratios of both 1:1 and greater than 1:1 might be expected for titrations of manganese(II) and perhaps of other metals with trien. This is seen in the manganese(II)–trien titration using a mercury–mercury(II) trien indicating electrode; two small breaks are observed (Table 6), the

Table 6. Summary of titrations of $M(ClO_4)_n.xDMSO$ salts with trien in acetonitrile, with Hg/trien indicating electrode

Species titrated	trien:metal ratio at inflection point	Approximate potential change at inflection point, *mV*
Cu^{2+}	1.1, 1.1, 1.1	360 (1st) asymmetric
	1.4, 1.3, 1.4	180 (2nd) asymmetric
Co^{2+}	1.09_3, 1.08_9, 1.09_5, 1.09_0	240 sharp
Mn^{2+}	1.06_5, 1.05_4	120 (1st), good
	1.29, 1.29_5	80 (2nd), fair
Ni^{2+}	1.10_6, 1.09_6, 1.11_3 with a second break, drawn-out, at less than 2:1 ratio	90, good
Mg^{2+}	1.00_0, 1.00_2, 1.00_0, 1.00_6, 1.02_2	150, good
Ca^{2+}	~1.2	drawn-out, not calculable

Table 7. Summary of titrations of $M(ClO_4)_n.xDMSO$ salts with trien in acetonitrile, with Ag/Ag^+ indicating electrode

Species titrated	trien:metal ratio at inflection point	Approximate potential change at inflection point, *mV*
Cu^{2+}	~1	200 (1st), poor
	~1.4	120 (2nd), drawn-out
Co^{2+}	1.21_1	120, poor
Mn^{2+}	~1.1	100, poor
Ni^{2+}	~1.1, 1.1, 1.1	800, poor
Mg^{2+}	1.10_3, 1.08_7, 1.25_0, 1.05_4	180, fair
Ca^{2+}	~1	drawn-out

first at trien to manganese ratios of about 1.05:1 and the second at ratios of about 1.29:1. Only single breaks, however, are seen with the platinum and silver–silver ion indicating electrodes, at about 1.05:1 and 1.14:1 ratios.

In summary, a range of metal–ligand titrations can be performed in an aprotic solvent such as acetonitrile. Potentiometric titrations are possible in several cases through use of a platinum indicating electrode, which was found to be superior to the other indicating electrodes investigated, including mercury, silver, carbon, and the copper ion-selective electrode. The mechanism by which platinum functions as an indicator is not clear. In general the results, though of experimental interest, do not indicate that acetonitrile offers any analytical advantage over water as a solvent for metal–ligand titrations.

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COULOMETRICALLY GENERATED COPPER(II) IN ACETONITRILE AS AN ANALYTICAL OXIDANT

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Summary—Copper(II) was generated at 100% current efficiency from solutions of copper(I) perchlorate in acetonitrile. Micromole quantities of ferrocene and several alkyl-substituted ferrocenes were determined with high precision and accuracy. Hydroquinone and several thiols, on the other hand, could not be determined because of the lack of an acceptor for the protons produced upon oxidation.

Copper(II) in acetonitrile is a useful reagent for the determination of oxidizable compounds that are insoluble in or react with water.[1-11] Acetonitrile solutions of copper(II) are fairly stable, but require periodic standardization. Ferrocene has been proposed as a primary standard for this purpose because it is soluble in acetonitrile, can be purified readily, is stable on storage, and is oxidized quantitatively to the ferricinium ion by copper(II).[4]

Constant-current coulometry has several advantages over volumetric titrimetry. Chief among them are the elimination of the need for preparation, storage, and standardization of titrant solutions, and the ability to generate electrochemically exceedingly small quantities of chemically reactive species with accuracy and precision. Accordingly an investigation of the conditions necessary for the quantitative generation of copper(II) from solutions of copper(I) in acetonitrile was undertaken, with the determination of ferrocene and several alkyl-substituted ferrocenes as sample systems. Previous work had assessed the direct titrations of these compounds with copper(II).[5] Several alkyl-substituted ferrocene derivatives have been titrated by coulometric oxidation of copper(I) tetrafluoroborate in acetonitrile, with biamperometric end-point detection at an applied potential of 20–50 mV.[12] However, relative standard deviations ranging from 1 to 3% on samples of 6–20 μmole were reported, much larger than would be expected on the basis of the direct potentiometric titrations. Exploratory work on the use of electrogenerated copper(II) in acetonitrile for the oxidation of several other compounds, including hydroquinone, tetramethylbenzidine, and selected thiols is also described briefly.

EXPERIMENTAL

Reagents

Commercial acetonitrile (Matheson, Coleman and Bell) was used either as received or after purification by the method of O'Donnell *et al.* with one modification.[4]

Copper(I) perchlorate was prepared by reaction of copper metal with copper(II) perchlorate in acetonitrile.[14] It was recrystallized once from purified acetonitrile and dried under vacuum. Analysis for copper by EDTA titration gave a purity of 99.8% $CuClO_4.4CH_3CN$.[15] n-Butyl-, amyl-, tert.-butyl-, and di-n-butyl derivatives of ferrocene were obtained from Arapahoe Chemicals, and used as received. Ferrocene (Arapahoe Chemicals) was recrystallized twice from heptane and sublimed once.[4] Tetramethylbenzidine (Eastman) was recrystallized from acetonitrile. Thiourea (Baker and Adamson) and dodecanethiol (Matheson, Coleman and Bell) were used as received. Thiophenol (Terochem Laboratories) was distilled under vacuum. Hexamethylenetetramine (J. T. Baker) was purified by sublimation under reduced presure.

Solutions of approximately 0.02*M* copper(I) perchlorate were prepared by dissolution of $CuClO_4.4CH_3CN$ in either commercial or purified acetonitrile.

Apparatus

A constant-current coulometer, designed and built in the electronics shop of the Department of Chemistry, University of Alberta,[16] provided current levels of 0.5, 5, or 20 mA. A Fisher Model 520 Accumet pH-meter was used to follow the potential difference between the indicating electrodes.

The titration vessel was a borosilicate H-cell with a 150-ml anode compartment and a 60-ml cathode compartment (Fig. 1). The compartments were separated by an anion-exchange membrane (American Machine and Foundry, AMF A104-EC).[17] Samples were introduced as approximately 1-g portions of 1–10m*M* solutions with a hypodermic syringe through a rubber serum cap. The quantity of sample taken for each titration was obtained by weighing the syringe to the nearest mg on a top-loading balance before and after sample introduction. The potential of the solution in the anode compartment was followed with a platinum wire indicating electrode and a reference electrode consisting of a silver wire immersed in 0.01*M* silver perchlorate in acetonitrile in a glass tube with a glass junction[18] at one end. The reference compartment was separated from the anode solution by a second longer tube with a fine glass frit. This tube contained 0.1*M* lithium perchlorate in acetonitrile as a bridge solution to prevent ferrocene oxidation by silver ions leaking through the first frit. For each set of titrations about 50 ml of an acetonitrile solution 0.14*M* in acetic acid and 0.04*M* in lithium perchlorate were placed in the cathode compartment, and about 70 ml of 0.02*M* $CuClO_4.4CH_3CN$ in the anode

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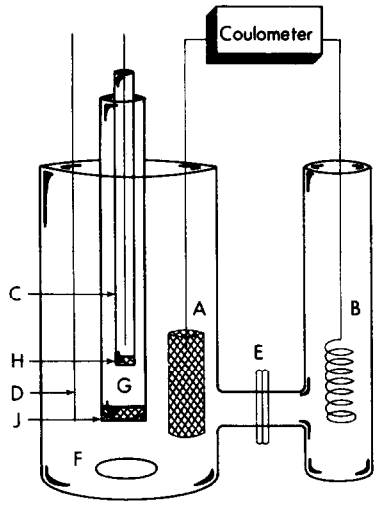

Fig. 1. Coulometric cell and electrodes. A, platinum gauze anode; B, platinum wire cathode; C, Ag/0.01*M* $AgNO_3$ in acetonitrile, reference electrode; D, platinum indicating electrode; E, anion-exchange membrane; F, magnetic stirring bar; G, 0.1*M* lithium perchlorate in acetonitrile; H, glass junction; J, fine frit.

compartment. About 0.2 ml of distilled water was added to the solution of copper(I) to prevent the oxidation potential of the copper couple from becoming so high that the ferricinium ion was oxidized further.[5] The generating electrodes in both the cathode and anode compartments were spirals of platinum wire. To minimize oxidation by air of the substances being titrated, nitrogen was passed first through a wash bottle containing acetonitrile, then through the cell solution for a few minutes before the titration. During titrations the nitrogen inlet was raised so that the gas passed over the surface of the solution.

Procedure

A small portion of sample solution was injected into the anode compartment and the solution deaerated. Then copper(II) was coulometrically generated at a constant current of 5 mA and the course of the titration was followed potentiometrically. When the potential reached a predetermined value at or near the inflection point of the potential break, the current was stopped. This first sample was considered as a blank. A second portion of sample solution, weighed to the nearest mg, was then injected into the same solution, deaerated, and copper(II) generated until the same potential was obtained. The time was again recorded and the next sample portion added. Typically 7–9 samples could be titrated successively in a single portion of analyte solution before the potential change at the end-point became too drawn-out to be useful.

For the potentiometric titrations an 80-ml cylindrical cell with a Teflon lid was used as a titration vessel. A platinum flag indicating electrode and a silver–0.01*M* silver nitrate in acetonitrile reference electrode were used. The reference electrode was separated from the cell solution by the same bridge as described for the coulometer cell. A Metrohm potentiograph E-436 automatic recording titrator delivered titrant by means of a 5-ml syringe burette at a rate of 0.25 ml/min. Aerial oxidation was minimized by starting each titration immediately upon dissolution of the sample, and by passing argon through the solution during the titration. Magnetic stirring was used.

RESULTS AND DISCUSSION

The initial work was done with carefully purified acetonitrile. However, it was found that titrations of ferrocene in purified and in commercial acetonitrile gave results that agreed within 0.5 part per thousand (ppt), and so commercial acetronitrile was used in subsequent work.

Results of titrations of ferrocene and four substituted ferrocenes with coulometrically generated copper(II) are given in Table 1. Relative standard deviations ranged from 1 to 4 ppt. The results, which ranged from 99.9 to 100.6% (assuming the ferrocene to be 100% pure), can be considered satisfactory. For the ferrocene derivatives, which were analysed as received, the results indicated purities ranging from 97.3 to 99.9%, with n-butyl- and tert.-butylferrocene appearing to be the purest. Acetylferrocene and benzoylferrocene give too small a change in potential to be determinable with copper(II), confirming earlier work.[5]

Table 1. Results of coulometric titrations of ferrocene and ferrocene derivatives with copper(II) in acetonitrile*

Compound	Potential at end-point, *mV*	No. of runs	Mean purity found, %	Relative standard deviation, %
		In purified acetonitrile		
Ferrocene	317	7	99.8_6	0.2_9
	319	9	100.0_6	0.2_3
	320	10	100.0_9	0.2_1
	320	9	100.1_0	0.4_0
	320	9	100.1_1	0.1_1
	320	8	100.0_6	0.2_9
		In commercial acetonitrile		
Ferrocene	246	7	100.0_8	0.3_0
	246	8	100.0_2	0.3_5
	246	8	100.0_2	0.1_8
n-Butylferrocene	239	9	99.6_6	0.4_8
Amylferrocene	234	8	99.1_7	0.3_1
tert.-Butylferrocene	245	8	99.9_0	0.3_1
Di-n-butylferrocene	244	9	97.3_0	0.3_6

* All titrations done at 5-mA current on samples of 1–10 μmole.

Increasing the quantities of ferrocene determined to twice the levels used to obtain the data of Table 1 did not affect the precision or accuracy of the determinations, nor did use of a current level of 20 instead of 5 mA.

Potentiometric investigation of thiol and hydroquinone oxidation

Extension of the coulometric generation of copper(II) to the determination of several other compounds directly titratable with copper(II) was also investigated. Among the substances studied were hydroquinone, thiourea, 1-dodecanethiol, and thiophenol. All gave small, drawn-out breaks unsuitable for analytical use.

Acetonitrile is a very weak Brønsted base, and in pure acetonitrile the oxidation of compounds such as hydroquinone and thiophenol is inhibited by the absence of a sufficiently strong base to accept the hydrogen ions produced.[2] Most Brønsted bases are also Lewis bases of some strength relative to copper(II), however, and the addition of compounds such as ammonia or pyridine results in undesirable complexation of copper(II). A number of bases were surveyed in an attempt to find one that could be used as a proton scavenger in acetonitrile; of those investigated hexamethylenetetramine (HMT) showed the most promise. Addition of excess of HMT to solutions of thiophenol before titration with copper(II) resulted in sharp potential changes of several hundred mV at the end-point, but the position of the end-point varied with the amount of HMT present, and tended to be about 2% early. To investigate the reasons for the early end-points, potentiometric titrations with copper(II) of thiophenol, 1-dodecanethiol, and hydroquinone (H_2Q) in the presence of varying amounts of HMT were performed. Titrations of 2:1 mixtures of thiophenol and HMT in 0.1*M* lithium perchlorate with copper(II) showed two potential breaks. The first break had an inflection of about 80 mV, was smaller than the second break, and appeared at a copper(II): thiophenol mole ratio of less than 0.5. The second break had a height of about 180 mV and appeared at a copper(II): thiophenol mole ratio of about 1 (0.998 and 0.996). A precipitate appeared before the first break was reached and dissolved before the second break started. The precipitate is thought to be the copper(I) salt of the thiol anion. The two breaks, then, are probably caused by formation of the thiol precipitate, followed by dissolution and oxidation of the precipitate according to

$$4RSH + 2Cu^{2+} + 4HMT \rightarrow RSSR + 2CuSR(s) + 4H_2MT^+$$

$$2CuSR + 2Cu^{2+} \rightarrow RSSR + 4Cu^+$$

When the amount of HMT was increased to a level equivalent to that of thiophenol, the end-point came at a copper(II): thiophenol mole ratio of 1.15. When HMT was added to the copper(II) titrant solution in 1:1 mole ratio, and the titration was done 2 hr after mixing, a large sharp break of about 640 mV was obtained at a copper(II):thiophenol mole ratio of 0.942. No precipitate appeared in this titration. This is as expected on the basis of the discussion above, for there is no excess of HMT to react with RSH to allow formation of CuSR. The reaction in this case can be written as

$$2CuHMT^{2+} + 2RSH \rightarrow 2Cu^+ + RSSR + 2H_2MT^+$$

Repeating the last titration 24 hr after mixing of the HMT with copper(II) gave an end-point corresponding to a copper(II): thiophenol mole ratio of 1.16, and the potential break was not as sharp. This indicates that there is a slow reaction between copper(II) and HMT which results in copper(II) not being available for oxidation of the thiophenol. Solutions of copper(II) in acetonitrile are blue, but turn dark green on addition of HMT. The reaction possibly is formation of a stable copper(II) complex with HMT. Adam and Přibil report that copper(II) is readily extracted from aqueous solutions (well buffered with HMT) into 1*M* chloroform solution of phenylacetic acid.[19] The chloroform extract is an intense green. There is evidence that the extractable species is a copper(II)–HMT–phenylacetate complex, formed by way of a copper(II)–HMT–sulphate precursor in the aqueous phase.[20] Mel'nichenko and Gyunner[21] observed that copper(II) chloride and HMT do not react in water, but form a dark brown precipitate in methanol, with the composition $5CuCl_2 . 3HMT$. If a complex forms between copper(II) and HMT in acetonitrile the stability constant is small, for potentiometric titrations of HMT with copper(II) show no inflection at 1:1 or 2:1 HMT–copper(II) ratios.

The only other metal–HMT complex reported is that of silver; Job[22] and Pawelka[23] report the stability constant for the complex in aqueous solution as 3.1×10^3 and 3.75×10^3 respectively. Overall, the addition of HMT to either the copper(II) titrant or to the solution being titrated does not appear useful from an analytical point of view.

When 1-dodecanethiol in 0.1*M* lithium perchlorate was titrated with a 1:1 mixture of copper(II) and HMT, the titration curve showed a potential break of 280 mV at a copper(II): 1-dodecanethiol mole ratio of 1.41. Again the reason for a large amount of copper(II) being required is probably reaction of a portion of the copper(II) with HMT.

Titrations with copper(II) of mixtures of hydroquinone and HMT in a ratio of 1:1.96 in 0.1*M* lithium perchlorate showed a potential break of 400 mV at a copper(II): hydroquinone mole ratio of 1.997. When the amount of HMT was increased to a level equivalent to exactly double the amount of H_2Q the stoichiometry and size of break did not change. When the H_2Q:HMT ratio was changed to 1:1.06 and 1:1.60 the end-points came at copper:H_2Q mole ratios of 1.15 and 1.68 respectively, while the potential breaks became larger and sharper. Increasing the amount of

HMT to give an H_2Q:HMT ratio of 1:2.04 or even 1:4.1 resulted in a positive error at the end-point of about 7% relative, although the quality of the end-point and the precision of replicate runs was excellent.

In summary, it has been shown that copper(II) can be produced with 100% current efficiency from solutions of copper(I) in acetonitrile, and that micromole quantities of ferrocene and some alkyl-substituted ferrocenes can be determined with high precision and accuracy by constant-current coulometric generation of copper(II). The scope of the coulometric procedure for the determination of other substances appears more limited than does the direct titration.

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SPECTROPHOTOMETRIC DETERMINATION OF MICROAMOUNTS OF TOCOPHERYL ACETATE (VITAMIN E) IN MULTI-VITAMIN CAPSULES

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Summary—Micro amounts of vitamin E in multi-vitamin tablets are extracted from aqueous EDTA medium with petroleum ether and are determined after transesterification, by use of iron(III) in the presence of Ferrozine. Vitamin E is determined in concentrations of 40–200 μg per 25 ml of final solution with a relative precision of about 1–2% when the standard addition method is used.

At least seven different types of vitamin E have been identified, namely, α-, β-, γ-, δ-, ε-, ζ- and η-tocopherol. McBride and Evans[1] identified α-, γ- and δ-tocopherol by a rapid voltammetric method in their work on estimation of tocopherols and antioxidants in oils and fats, but the peak of β-tocopherol was superimposed on that of γ-tocopherol. An Analytical Methods Committee Panel[2] found that although γ- and η-tocopherols were inseparable by two-dimensional paper chromatography, and so were β- and ε-tocopherols, α-tocopherol gave a separate spot.

The chemical determination[3,4] of tocopherols in food and other materials is rather complicated. The general method involves the extraction of the lipids containing tocopherols, removal of the interfering substances and determination of the tocopherols. Most procedures follow the Emmerie and Engel method,[5] which is regarded as the most suitable for the determination of purified tocopherols. The method we adopt in this work avoids the conventional complicated procedures, because of the absence of lipids in the multi-vitamins being analysed.

We have used iron(III) in the presence of 3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,3-triazine, Ferrozine, for the determination of vitamin E. Since the molar absorptivity of the $Fe(II)(Ferrozine)_3^{4-}$ complex at 562 nm is 2.80×10^4 l.mole^{-1}.cm^{-1} as compared to 1.12×10^4 for the $Fe(II)phen_3^{2+}$ complex, the use of Ferrozine enhances the sensitivity and enables microamounts of vitamin E to be determined. In the procedure described in this paper, a method of analysis for vitamin E in multi-vitamin capsules containing iron and other minerals is developed. Although our method is similar to that of Tsen,[6] in his determination of tocopherol with 4,7-diphenyl-1,10-phenanthroline, bathophenanthroline, our method shows distinct differences. Ferrozine is more sensitive than 1,10-phenanthroline or bathophenanthroline and is also readily soluble in water. We have adopted a suitable method for eliminating interferences.

EXPERIMENTAL

Reagents

All reagents were of analytical grade.

Ferrozine. A 0.012*M* solution was prepared by dissolving in 250 ml of distilled water 1.531 g of Ferrozine (purchased from the Hach Chemical Company). The Ferrozine solution was then stored in a dark bottle in a refrigerator.

Iron(III) solution. Prepared by dissolving 0.723 g of ferric ammonium sulphate, $FeNH_4(SO_4)_2.12H_2O$ (Baker Analyzed), in about 2.5 ml of concentrated perchloric acid plus 2 drops of concentrated nitric acid and sufficient water. The solution was gently boiled until perchloric acid fumes appeared and then transferred into a 500-ml standard flask and made up to the mark. The flask was covered with aluminium foil and stored in a dark area.

Buffer. A buffer solution, 0.3*M* and pH 3.4, was prepared by adding sodium hydroxide pellets to 0.3*M* trichloroacetic acid until the pH was 3.4.

d-α-Tocopherol standard solution. A stock solution was prepared by dissolving 0.1769 g of *d*-α-tocopheryl acetate (Eastman Kodak) in 100 ml of methanol. It was stored in a refrigerator and renewed monthly. Dilute solutions were prepared by taking 250, 200, 100, 50 and 25 μl aliquots of the stock solution and placing them in 25-ml standard flasks.

Orthophosphoric acid. One ml of orthophosphoric acid (sp. gr. 1.75) was diluted to 150 ml with absolute ethanol.

EDTA solution. 0.0077*M*. Anhydrous disodium ethylenediaminetetra-acetate (0.287 g) dissolved in 100 ml of demineralized water.

Procedure

Transesterification. Tocopheryl acetate was converted into tocopherol by a modification of the procedure described by Sheikh *et al.*[7] and Campbell *et al.*[8] Standards were prepared by taking 25, 50, 100, 200 and 250 μl portions of stock solution in 25-ml standard flasks, adding a drop of sulphuric acid (to act as a catalyst) and 20 ml of methanol to each, covering the necks of the flasks with aluminium foil perforated to permit the escape of methyl acetate and excess of methanol, and heating the flasks at 70–80° in a water-bath for 90–105 min; within this period,

the flask contents were reduced almost to dryness. The flask was then flushed with carbon dioxide before the end-product of the transesterification was dissolved in 15 ml of methanol, and the reagents were added in the order: 3 ml of $3.03 \times 10^{-3}M$ iron(III), 1.0 ml of $0.012M$ Ferrozine, 0.5 ml of trichloroacetate buffer of pH 3.4 and 1.0 ml of the dilute orthophosphoric acid. The absorbance was measured 2 min after the addition of the iron(III) solution. The calibration curve was drawn in the usual way.

Samples. A multi-vitamin solution was prepared according to the method suggested in a collaborative study by the AOAC.[4] Capsules were ground in a mortar into a very fine powder and an exact amount of this was weighed into a 100-ml standard flask, and dissolved and diluted to volume with $7.7 \times 10^{-3}M$ EDTA. Suitable (5 or 10 ml) aliquots of the EDTA–vitamin solution were taken for analysis. The tocopheryl acetate was extracted with two 10-ml portions of petroleum ether (b.p. 35–60°). The resulting combined extract was evaporated almost to dryness and the residue was thoroughly washed with methanol into a 25-ml standard flask. This solution was then acidified, transesterified and analysed by the procedure given above for the standards.

For the standard addition method three separatory funnels were used. To the first was added a 5-ml aliquot of the EDTA–vitamin solution. A mixture of a 5-ml aliquot of the EDTA–vitamin solution and 0.100 ml of standard solution (measured with a micropipette) was placed in the second. The third contained 100 μl of the standard solution alone. The tocopheryl acetate in each solution was extracted with two 10-ml portions of petroleum ether and the extracts were treated as just described for the samples. The standard addition method was repeated with 10-ml aliquots.

The tocopheryl acetate content in mg/g of dry multi-vitamin (I.U.), is calculated from the standard addition method results as follows: I.U. = [(mg of vitamin E standard taken) $\times A_{unk}/(A_{unk+std} - A_{unk})]/w$ where A_{unk} is the absorbance of the unknown, $A_{und+std}$ is that of the unknown with the standard added, and w is the weight of dry multi-vitamin (in g) in the aliquot taken for analysis.

RESULTS AND DISCUSSION

The oxidation of α-tocopherol to the quinone by iron(III) and the complexation of the resulting iron(II) with Ferrozine is summarized in the reactions below:[7,9]

As shown in Table 1, we obtained a molar absorptivity of 5.35×10^4 l.mole^{-1}.cm^{-1}, indicating that a 2-electron change was involved in the reaction.

The rate of the reaction is indicated by the rate of colour development, which depends on the rate of reduction of iron(III) by tocopherol and the rate of combination of iron(II) with Ferrozine. To optimize conditions, we have investigated a number of parameters, such as time, pH, interference, transesterification, catalysis, photochemical reaction and order of adding reagents.

Effect of time

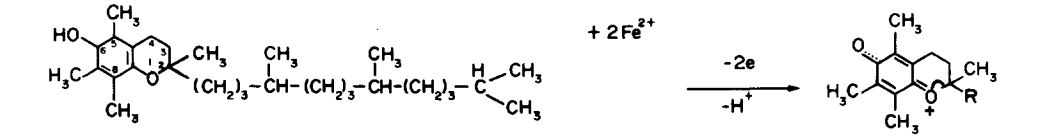
A preliminary investigation showed that the oxidation of tocopherol to the quinone is complete within approximately 30–60 sec after addition of the reagents. Development of the iron(II)–Ferrozine colour is rapid and in the presence of phosphoric acid the colour intensity remains constant for more than 30 min.

Effect of pH

In his work on Ferrozine, Stookey[10] established that $Fe(Ferrozine)_3^{4-}$ is stable in aqueous solution between pH 4 and 9. Our investigation showed that the reaction of tocopherol with iron(III) in the presence of Ferrozine will proceed in acidic buffers. We found that a gradual decrease in absorbance occurs if the pH is higher than 5.6. At pH > 7 the solution produced brown gelatinous precipitates of the ferric complex. We have also observed that the absorbance of $Fe(II)(Ferrozine)_3^{4-}$ is generally maximal at pH 3.2.

Catalyst effect

The transesterification process is so slow that a catalyst is essential to make the reaction go faster. We add a drop of concentrated sulphuric acid to the reagents in each flask, and the sulphuric acid has been found to be a very satisfactory catalyst, but Rehberg[11]



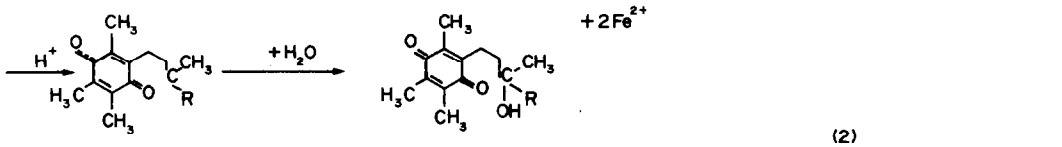


Table 1. Standard curve data for absorbance of Fe(II)(Ferrozine)$_3^{4-}$ as a function of d-α-tocopherol concentration at 23.4°C

Tocopherol		Absorbance		
μM	*ppm*	Experimental*	Calculated†	Std. devn§
Blank		0.025	0.009	0.0000
1.87	0.88	0.100	0.109	0.0014
3.74	1.77	0.204	0.209	0.0037
7.48	3.53	0.404	0.409	0.0034
14.97	7.07	0.810	0.810	0.0010
18.71	8.84	1.015	1.010	0.0100
Correlation coefficient§				0.999997
Molar absorptivity§			5.35×10^4 l.mole^{-1}.cm^{-1}	
Intercept§, blank absorbance				0.0089

* Based on the average absorbance for all the trials at each concentration.
† Based on the equation for the best straight line.
§ Based on a total of 24 trials.

suggested *p*-toluenesulphonic acid as a better alternative.

Order of adding reagents

We have observed that the reagents must be added in the order given, to ensure maximum reproducibility. In particular, the phosphoric acid must be added last; otherwise iron(III) is complexed and the results are low.

Prevention of photochemical reaction of ferric iron

Tocopherol is photochemically active and easily oxidized by air. Tsen[6] has made several suggestions for dealing with this, including determination under dim artificial light in a darkened room and the use of a brown glass flask in the determination. In our work, the reagent flasks are flushed with carbon dioxide (generated from solid CO_2) before the final dilution to volume. The addition of 1 ml of dilute orthophosphoric acid, as suggested by Tsen,[6] stabilizes the colour. A darkened environment for the reaction is provided by covering the flasks with aluminium foil.

Elimination of interferences

To eliminate interference of any metals present in the multi-vitamin preparations, the sample solution is prepared in EDTA medium, to complex the metal ions. The interference of vitamin C, a component of the multi-vitamins which is capable of reducing iron(III), can be eliminated by extracting the tocopheryl acetate with petroleum ether, a solvent in which vitamin E, but no other vitamin, is highly soluble. The effect of errors arising in the transesterification can be reduced by use of the standard addition method. Since all solutions are prepared in the same manner, experimental errors for the solutions of vitamin extract or the extract containing the standard increment are minimized.

Precautious in transesterification

During transesterification, the standard flasks containing the methanol–tocopherol solution should be gradually heated in the water-bath. If the flasks are immersed directly in a hot bath, spurting will occur,

Table 2. Standard addition method: absorbance of Fe(II)(Ferrozine)$_3^{4-}$ as a function of the concentration of d-α-tocopherol in multi-vitamin capsules

	Net absorbance* for different trials				
Solution	1	2	3	4	
5-ml aliquot†	0.205	0.201	0.205	0.207	
5-ml aliquot + 0.100 ml of standard solution	0.543	0.540	0.552	0.554	
0.100-ml aliquot of standard§	0.347	0.349	0.349	0.350	
Tocopheryl acetate calculated‡, *mg/g*	8.83	8.63	8.60	8.68	$\bar{x} = 8.68 \pm 0.10$

* Corrected for blank. Absorbance was determined on 25 ml of final solution, containing 3 ml of $3.05 \times 10^{-3}M$ iron(III), 1 ml of 0.012*M* Ferrozine, 0.5 ml of 0.3*M* acetate buffer of pH 3.4 and 0.04*M* phosphoric acid.

† Aliquots were taken from 100 ml of solution in $7.76 \times 10^{-3}M$ EDTA, containing 0.1058 g of multi-vitamin capsules. Reaction temperature was 25.5°C.

§ $1.63 \times 10^{-3}M$ standard, containing 0.770 mg of tocopheryl acetate per ml was used for standard additions.

‡ Amount of tocopheryl acetate (mg/g) in the dry multi-vitamin was calculated as follows: $x = \{[\text{mg of vitamin E standard}] \cdot A_{unk}/(A_{unk+std} - A_{unk})\}/(\text{g of multi-vitamin taken})$. $x = [0.077 \times 0.205/(0.552 - 0.205)]/[0.1058/(100/5)]$.

with a consequent loss of some of the contents. Transesterification should be continued until the solution in each flask is almost evaporated to dryness. Incomplete transesterification results in a low absorbance value.

Micro amounts of vitamin E in multi-vitamin tablets can be determined with iron(III) in the presence of Ferrozine with a relative standard deviation of 1.1% when the standard addition method is used, as shown in Table 2. In this manner the errors incurred in the extraction and, in particular, the transesterification, are minimized. Best precision is obtained when the concentration of vitamin E is in the range 40–200 μg/25 ml of the final solution being measured, so that the absorbance is in the range 0.3–1.2.

The results for vitamin E are expressed in terms of International Units, IU, *i.e.*, as mg of vitamin E per gram of dry multi-vitamin powder, as described by AOAC.[12]

The procedure described permits the determination of micro amounts of vitamin E in the presence of mineral salts and large amounts of vitamin C.

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THE PROBLEM OF LEAD IN MEXICAN POTTERY

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Summary—Much public attention has been focused in the United States on utilitarian Mexican pottery as a source of lead poisoning. Our work demonstrates that, if a firing temperature of at least 1150° is used, lead-glazed earthenware is made safe for the storage and preparation of foods. Examination by d.c. arc emission spectroscopy and X-ray diffraction shows that the lead then remains in crystalline form. An exchange-equilibrium for lead between solutions and earthenware material is postulated.

During the last decade the use of Mexican pottery has led to reported cases of lead poisoning. The public has been alerted to this danger by articles in popular magazines like *Good Housekeeping*[1] and *Reader's Digest*[2] as well as by television broadcasts. The articles described how a California family was almost fatally poisoned after using Mexican pottery over a 3-year period. Recognizing this lead toxicity problem, the United States Food and Drug Administration determined the lead content of randomly chosen Mexican pottery. The results indicated the presence of high levels of lead in the pottery.[3]

Lead chromate and lead oxide have been primary components of the glaze in pottery materials. The lead imparts low surface tension and low viscosity over wide ranges of temperature. These properties result in greater smoothness, lustre, brilliance, and a more fool-proof glaze that will cover up blemishes more readily and be freer from defects.

Although the United States Food and Drug Administration also found toxic levels of lead in pottery from Japan, Italy, France, Portugal, and even the United States, the United States public seems to have focused on the lead content of Mexican pottery. This may be due to the greater probability of importing Mexican pottery because of the proximity of Mexico and the comparatively low cost of Mexican goods.

Poisoning as the result of ingesting lead along with food is not new. The decline of the Roman Empire has, at least in part, been attributed by some historians to the steady erosion of mental faculties and general health among the ruling elite, caused by ingesting food prepared and served in lead-based pewter and lead-glazed ceramics as well as water obtained from lead-lined reservoirs and aqueducts.[4]

Lead poisoning is a serious medical problem which can result in severe sickness and even death. It has been estimated that a dose of 0.5 g absorbed by the human body can be fatal. Accumulation and subsequent toxicity can occur if more than 0.5 mg of lead is absorbed per day over a period of time.[5] Under normal conditions, not more than 5–10% of the ingested lead is absorbed from the digestive system into the body[6] but higher values of 16 and even 40% have been reported.[7,8] According to these data, a person drinking half a litre of fluid daily, containing 10 ppm lead, will be in danger of lead poisoning. At present, under the food additive provisions of the Food, Drug and Cosmetic Act, an upper limit has been set on the amount of lead which may migrate from product to food. A concentration of 7 ppm lead obtained after leaching with a 4% acetic acid solution for 24 hr is considered violative.[9,10]

So far, work on the leaching of lead from pottery by the usual liquid and solid cooking ingredients has yielded insufficient and inconclusive results.

This paper reports the results of experiments to find the amounts of lead leached from pottery, as a function of contact time and number of times of extraction. Further, we have shown that the temperatures at which the pottery is "fired" changes the crystalline structure of the lead compound and alters the solubility considerably. Firing at high temperature may provide a solution to the anthropological problem of maintaining a safe pottery craft in Mexico.

EXPERIMENTAL

Apparatus

Qualitative analyses were performed with a Bausch & Lomb 1.5 m emission spectrograph with a d.c. arc source. A standard spectrum was used for comparison. Quantitative analyses were performed with a Perkin–Elmer model 303 atomic-absorption spectrophotometer. The source was a Perkin–Elmer lead hollow-cathode Intensitron lamp. An air–acetylene flame was used. The acetylene pressure was 9 psig and flowmeter setting 5.5 (2.0 l./min); the air pressure was 30 psig and flowmeter setting 7.5 (20.0 l./mn). The 283.3-nm resonance line and slit position 4 giving a slit-width of 1 mm (0.7-nm spectral band-pass) were used for

absorbance measurements. Settings with unstated units refer to the manufacturer's arbitrary scales on the Perkin-Elmer 303. The visible flame resulting from these acetylene and air flow-rates was about 2.5 in. high, with a $\frac{1}{8}$-in. blue reducing zone.

The flowmeter settings, burner position, rate of solution uptake and the positioning of the source were each adjusted to give maximum absorbance when a solution containing lead was aspirated. The slit position was as suggested for lead determination by the manufacturer.

A Norelco–Philips diffractometer was used to obtain the X-ray powder patterns of the fired glaze samples.

The electric furnace was a Lindberg heavy duty type 59344.

Reagents

All reagents were of analytical grade. All water used was demineralized.

All pottery used was produced in one folk community in the Valley of Puebla in the Central Highlands of Mexico.

Acetic acid solution, 4%. Dilute 83 ml of glacial acetic acid to 2 litres with water.

Standard lead solutions. Weigh accurately 0.810 g of analytical reagent grade lead nitrate. Dissolve it in 4% acetic acid solution and transfer to a 1-litre standard flask and dilute to volume. The lead content of this solution is about 500 ppm. Prepare a fresh solution every 2 weeks. From it prepare a series of standards in the range 5–25 ppm. These solutions should be prepared every 2 days.

Procedures

Leaching of lead into 4% acetic acid solution. A 4% acetic acid solution was allowed to sit in the pottery ware, a small pot or dish, for a 24-hr period. The solution was then transferred to a standard flask, made up to volume and analysed for lead content by atomic-absorption spectroscopy. This procedure was repeated with fresh 4% acetic acid solution after each 24-hr period, for 20 consecutive days.

Effect on food prepared in pottery vessels. Individual experiments were performed by cooking tomato, chicken or coffee with water for 2 hr at below the boiling point, in the pottery sample. The volume was kept constant by addition of water as required. The solids were then separated by decantation and filtration from the supernatant liquid, and washed with water, the washings being added to the filtrate. The solids were oxidized with a mixture of hot nitric acid and perchloric acid. The resulting clear solutions were transferred to 100-ml standard flasks, made up to volume and analysed for lead content by atomic-absorption spectroscopy. The filtrates were similarly diluted and analysed.

To monitor the exchange of lead between pottery and food material, 2.6 mg of lead nitrate were added to the mixtures before the cooking process. A control experiment was carried out in a Coors 6A porcelain evaporating dish.

Reactivity of lead in glaze as a function of firing temperature. To determine any effect of firing temperature on the leaching of lead from pottery glaze, a small pitcher or cup was placed in the electric furnace at 600° for 2 hr. It was then allowed to cool and 4% acetic acid solution was left in it for 24 hr. The solution was then analysed for lead by atomic-absorption spectroscopy. The procedure was repeated with the same pottery sample at temperatures increased by 50° each time.

After the firings at 700° and 1200°, chips of glaze were scraped from the samples and their X-ray powder patterns obtained with the Norelco–Philips diffractometer over the range $2\theta = 5$–$80°$.

RESULTS AND DISCUSSION

Leaching of lead

Instead of the standard test for lead, usually run after one extraction, 20 runs were made on each of four different pottery samples, to simulate more closely the multiple and continued use the vessels would normally receive. It was found that after six successive extractions, the amount of lead leached had dropped sharply. For the four runs illustrated in Fig. 1 there is an average drop of 71% in the amount of lead leached before the curves level off. In the most striking case, the concentration of lead in the extract dropped from 4400 to 600 ppm, but this last value is still far above safe health levels.

The levelling off suggests that some pottery which could not meet the upper limit of 7 ppm when subjected to a single standard test might become safe after a period of repeated use.

Effect of foods

As can be seen from Table 1, after foods placed in water have been cooked in the pottery, lead appears in the solids at dangerously high levels of concentration, but it appears that in the majority of cases the liquid would be safe to take. The lead is apparently reacting with the protein molecules of the foods and is thus trapped in the solids. A process of exchange-equilibration may be taking place.

In certain experiments, lead was added to the solutions before the cooking process. These additions of known quantities of lead made it possible to follow any exchange of lead between solids, solution, and pottery. In the initial work described in Table 1, there was no apparent indication of loss of lead to the pottery. However, according to the results of experiments summarized in Tables 2 and 3, loss of lead to the

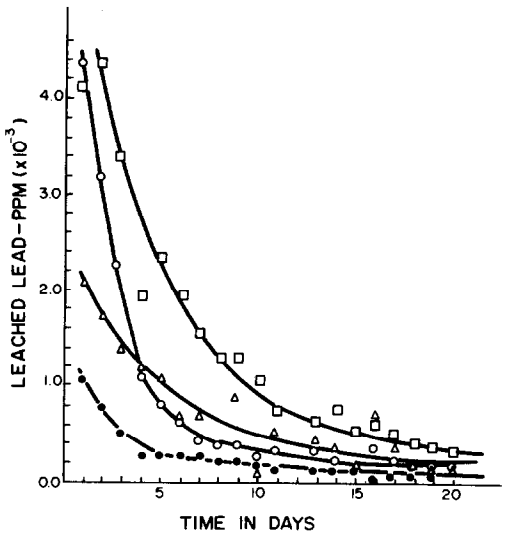

Fig. 1. Lead leached as a function of time of contact of pottery with 4% acetic acid solution.

Table 1. Food and water cooked for 2 hr in Mexican pottery

Sample	Food, g	Liquid, ml	Lead found: In liquid mg	In liquid %*	In liquid ppm	In solid mg	In solid %*	In solid ppm	Total lead, mg
Tomato									
Bowl A	9.47	50.3	0.20	50	4.0	0.20	50	21	0.40
Bowl B	9.19	41.3	0.20	29	4.8	0.49	71	53	0.69
Pitcher C	6.29	39.1	0.40	28	10.2	1.03	72	163	1.43
Pitcher D	7.09	32.5	0.12	24	3.7	0.37	76	52	0.49
Bowl A	4.10	50.0	0.64	67	12.8	0.32	33	78	0.96
Bowl B	4.05	39.4	0.33	59	8.4	0.23	41	56	0.56
Pitcher C	3.63	40.9	0.28	55	6.8	0.23	45	63	0.51
Pitcher D	2.01	35.3	0.25	56	7.1	0.20	44	99	0.45
Chicken									
Bowl A	6.4	50	0.04	36	0.8	0.07	63	11	0.11
Bowl B	5.4	50	0.05	17	1.0	0.23	82	43	0.28
Pitcher C†	3.8	42	1.4	48	32	1.5	52	389	2.9
Pitcher D†	5.8	38	0.88	30	23	2.0	68	344	2.9
Coffee									
Bowl A†	1.80	55.0	0.62	17	11.2	3.05	83	1654	3.67
Bowl B†	1.70	55.0	0.65	21	11.8	2.37	78	1394	3.02
Pitcher C	1.60	42.3	0.10	33	2.3	0.20	66	125	0.30
Pitcher D	1.70	39.4	0.09	39	2.3	0.14	60	82.3	0.23

* Expressed as fraction of total lead recovered.
† Liquid contains 2.6 mg of lead (added as lead nitrate).

Table 2. Tomato and water plus 2.6 mg of added lead cooked for 2 hr in Mexican pottery

Sample	Tomato, g	Liquid, ml	Lead found: In liquid mg	In liquid %	In liquid ppm	In solid mg	In solid %	In solid ppm	Total lead found, mg
Bowl A	3.20	56.8	1.6	76	27.6	0.5	24	147	2.1
Bowl B	4.38	37.5	1.4	70	36.8	0.6	30	139	2.0
Pitcher C	4.20	40.8	1.5	58	36.3	1.1	42	251	2.6
Pitcher D	2.40	41.5	1.1	65	27.3	0.6	35	260	1.7

pottery did occur. It is therefore postulated that the lead undergoes an exchange-equilibration between the solids, liquid and the pottery, since the total mass of lead recovered was less than the amount added. The theory of an exchange-equilibration of lead is supported by the observation that all the lead added can be accounted for when tomato is cooked with water in the Coors evaporating dish, Table 4. It is not unreasonable to suggest that lead diffuses into the pottery and reacts by co-ordinating with available sites in the clay material. The evaporating dish is not porous.

An exchange-equilibration may also have existed in the experiments summarized in Table 1. In general the mass of lead appearing in the solids plus liquid is approximately equal to the amount added plus the amount extracted in the corresponding experiments without added lead, but there are exceptions in both directions, indicating the possibility of gain or loss by exchange.

It was found that the pottery is safe for the storage as well as for the boiling of water. The data to support this are summarized in Table 5.

Reactivity as a function of firing temperature

There is a definite relationship between the temperature of firing in the preparation of the pottery and the amount of lead leached by a 4% acetic acid

Table 3. Water (2.6 mg of lead added) boiled for 2 hr in Mexican pottery

Sample	Water, ml	Lead found: mg	%	ppm
Bowl A	55.0	0.98	37	18
Bowl B	55.0	0.90	35	16
Pitcher C	45.8	0.35*	14*	8*
Pitcher D	45.5	0.80	31	18

* Leakage.

Table 4. Tomato and water plus 2.6 mg of added lead cooked for 2 hr in a porcelain evaporating dish

			Lead found					
			In liquid			In solid		
	Tomato, *g*	Liquid, *ml*	*mg*	%	*ppm*	*mg*	%	*ppm*
Dish 1	4.80	55.0	1.6	61	29.1	0.98	39	204
Dish 2	6.78	55.0	1.5	61	27.3	1.1	39	162

solution. The data presented in Table 6 show what happened when two pottery samples, a cup and a pitcher, were subjected to 2-hr firings in the electric furnace in the 620–1300° range at 50° intervals.

The fall-off in amount of lead leached is due to the effect of the firing and not to the successive leachings. This was established by firing two new pottery samples at 700°, cooling, leaching for 24 hr, refiring at 1200° and leaching again. The concentrations of lead in the leach solutions were 908 ppm (700°) and 0.1 ppm (1200°) for a cup and 1580 ppm (700°) and 2.0 ppm (1200°) for a pitcher.

The pottery evidently becomes safe for storage and cooking purposes if fired at temperatures above 1150°. Mexican potters fire their wares for 10–12 hr in wood-burning fireplaces where the temperatures do not exceed about 850°. The pottery is then cooled overnight, the entire process thus taking about 24 hr. We consider that firing at 1150° might well solve this problem of safety from leaching of lead.

Table 5. Mexican pottery containing water

Sample	Treatment	Lead found, *ppm*
Bowl A	Standing for 24 hr	0
Pitcher C		0
Bowl B	Boiling for 2 hr	2.9
Pitcher D		2.9

Table 6. Lead leached after firing at increasing temperature

Firing temperature, °C	Lead leached, *ppm* Cup	Pitcher
—	905	1956
650	745	335
700	535	219
750	179	165
800	139	45.3
850	56.0	43.9
900	19.0	41.3
950	17.1	15.0
1000	13.0	12.9
1050	6.2	9.9
1100	2.4	10.4
1150	1.3	4.5
1200	0.12	0.91
1250	0.11	0.72
1300	Glaze melted	

As the firing temperature increased, the glaze changed colour, attaining a metallic lustre at about 1000°. The hardness of the glaze also increased with firing temperature. From an easy-to-file material at the low end of the temperature range, the hardness increased in striking fashion toward the high end.

The glaze contains lead chromate and lead oxide. Qualitative analyses show lead still present after firings at 700 and 1200° and in identical amounts. The change in the amount of lead leached and in the physical properties of the glaze suggests a change in crystalline structure as well as a change in composition of the lead compound. The X-ray powder patterns indicated that the glazes are crystalline in nature, but, since considerable amounts of clay were present, only four extremely strong peaks were observed. Any others that may have been present were obscured by the relatively high background from the clay. It is not yet possible to make any statement concerning the exact change in structure with temperature.

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BIS-[2[(TETRAHYDRO-2H-PYRAN-2-YL)THIO]PHENYL]DIAZINE: A NEW COLORIMETRIC REAGENT FOR MERCURY

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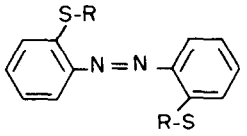
Summary—The synthesis of bis-[2-[(tetrahydro-2H-pyran-2-yl)thio]phenyl] diazine is reported and its potential as a spectrophotometric reagent for Hg^{2+} is explored. The dye reacts in 1:1 stoichiometry with Hg^{2+} and the product of this reaction, which is extracted into chloroform, has a molar absorptivity of 3.20×10^3 l.mole^{-1}.cm^{-1} at 525 nm and obeys Beer's law in the range 0–50 ppm Hg^{2+}.

The *o,o'*-dihydroxyazo compounds such as Eriochrome Black T, Calmagite and Calcon have long been recognized as metallochromic indicators for complexometric titrations with EDTA. In addition, some of these dyes, as well as *o,o'*-dihydroxyazobenzene, have been utilized as direct fluorometric or colorimetric reagents for calcium and magnesium.[1] Little, however, has been reported regarding the syntheses and uses of the corresponding *o,o'*-dimercaptoazo analogues.

There have been several papers which discuss the effect of the replacement of a hydroxy substituent by a mercapto group in several common organic reagents. For example, the sulphur analogue of 8-hydroxyquinoline, 8-mercaptoquinoline, has been prepared and found to precipitate a number of metal ions such as Cu^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+} and Hg^{2+}. All of these precipitates can be extracted into a variety of organic solvents, which permits colorimetric determination of these ions.[2] Gravimetric methods for the determination of Pd^{2+} and Ni^{2+} with 8-mercaptoquinoline have been reported.[3] Furthermore, a number of the complexes of 8-mercaptoquinoline have been found to be more stable than their hydroxy counterparts.[4] Studies of other mercapto compounds such as *o*-aminothiophenol and 1-phenazinethiol have shown that these compounds also form more stable chelates than their oxygen analogues with a number of metal ions.[5,6]

Based on the fact that metal chelates formed with ligands containing sulphur and nitrogen are frequently more stable than those containing oxygen and nitrogen and that azo-dyes have found such wide use in analytical chemistry, the investigation of *o,o'*-mercaptoazo compounds and their derivatives seemed attractive. Among the few references to the preparation and reactions of *o*-mercaptoazo compounds are a series of papers by Burawoy *et al.*,[7–9] who prepared a large variety of compounds having the general structure (shown below) where R is $-CH_3$, $-H$, $-CH_2\phi$, $-Br$, $-Cl$, $-I$, $-ClO_4$, $-SCN$, $-CN$. The dimercapto compound, R = H, was reported by Burawoy to form a black precipitate with Cu^{2+}. The dimethyl thioether compound, R = $-CH_3$, has been prepared and also found to form a complex with Cu^{2+} that is soluble in water–ethanol mixtures.[10]



This paper reports the synthesis of a new compound, which has the same structure as that above but where R is tetrahydropyran. Also reported are the results of the preliminary investigations of the reaction of this dye with Hg^{2+}, which indicate that it may be well suited for the colorimetric determination of Hg^{2+} in the ppm range.

EXPERIMENTAL

Apparatus

Spectral data necessary for the identification of all organic products were obtained with a Varian T60-A nuclear magnetic resonance spectrometer or a Perkin–Elmer 727B infrared spectrometer. Spectral data needed for the study of the absorption properties of bis-[2[(tetrahydro-2H-pyran-2-yl)thio]phenyl]diazine (PTPD) and its Hg^{2+} complex in the ultraviolet–visible region were obtained with a Varian Superscan III recording UV-visible spectrophotometer. Additional data was gathered with a Beckman DU spectrophotometer.

Reagents

PTPD was synthesized by using 2-chloronitrobenzene, lithium tetrahydridoaluminate, and 2,3-dihydropyran. Sodium sulphide nonahydrate was either recrystallized before use or solutions prepared from the unrecrystallized salt were filtered before use. The benzene and diethyl ether used in the lithium tetrahydridoaluminate reduction were reagent grade and dried over sodium.

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A stock solution was prepared that was $9.95 \times 10^{-3}M$ in mercury(II) nitrate and contained 5 ml of concentrated nitric acid per litre. It was standardized by titration with $0.01056M$ potassium thiocyanate with ferric alum as indicator.[11] The solution for the mole-ratio study was prepared by diluting 5.00-ml of the Hg^{2+} stock solution and 5.00 ml of concentrated nitric acid to volume in a 500-ml standard flask. The mercury solutions for the Beer's law study were prepared by diluting 1.00 ml of the stock solution to volume in a 100-ml standard flask and then diluting appropriate volumes of this solution to 10.00 ml to give the desired concentration of Hg^{2+}. All other materials were reagent grade and used without further purification.

Synthesis of PTPD

This was accomplished by a series of reactions starting with the conversion of 2-chloronitrobenzene into di-2-nitrophenol disulphide,[12] followed by the reduction of the disulphide to 2-nitrobenzenethiol.[13] This thiol was used to prepare tetrahydro-2-[(2′-nitrophenyl)thio]-2H-pyran and the final step was reduction of this nitropyran to PTPD with lithium tetrahydridoaluminate.

Tetrahydro-2-[(2′-nitrophenyl)thio]-2H-pyran was prepared by slowly adding 4.00 g (0.026 mole) of 2-nitrothiophenol to 4.7 ml (4.4 g, 0.052 mole) of 2,3-dihydropyran to which no more than two drops of concentrated hydrochloric acid had been added. This mixture was heated under gentle reflux for 1 hr, cooled to room temperature and then placed in a freezer at −15° for 2 hr. The resulting yellow-brown solid was washed with cold absolute ethanol to give 3.6 g (63% yield) of tiny pale yellow needles, with an uncorrected m.p. of 54–56° (lit.[14] 60–61°). The NMR and infrared spectra of this material were consistent with the structure of tetrahydro-2-[(2′-nitrophenyl)thio]-2H-pyran.

Bis-(2-[(tetrahydro-2H-pyran-2-yl)thio]phenyl)diazine was prepared by adding 3.0 g (0.013 mole) of tetrahydro-2-[(2′-nitrophenyl)thio]-2H-pyran in 100 ml of anhydrous benzene to a stirred suspension of lithium tetrahydridoaluminate (1.00 g, 0.025 mole) in 100 ml of anhydrous diethyl ether at a rate just sufficient to maintain reflux without heating. The mixture was then heated under reflux for 3 hr. About 75 ml of the organic solvents were removed by distillation, and the excess of lithium tetrahydridoaluminate was hydrolysed by the dropwise addition of $0.05M$ sulphuric acid. The mixture was filtered and the organic layer of the filtrate isolated. The organic solvent was stripped by use of a rotary evaporator and the resulting tar taken up in the minimum amount of hot acetone. Water was then added until the cloud-point was reached, the solution was just clarified with additional hot acetone, and then cooled. The crude orange-red powder, m.p. 172–177°, was recovered by vacuum filtration and recrystallized from absolute ethanol to give 0.75 g (30% yield) of tiny orange needles, m.p. 185–186°. The NMR and infrared spectra were consistent with the structure of PTPD. Calculated for $C_{22}H_{26}N_2O_2S_2$: C, 63.74%; H, 6.32%; N, 6.76%; S, 15.47%; found: C, 63.3%; H, 6.2%; N, 6.4%; S, 15.3%.

Properties and reactions of PTPD

PTPD is virtually insoluble in water and all reactions of PTPD with metal ions were therefore carried out by shaking a chloroform solution of the dye with an aqueous solution of various metal ions. The ions studied for possible reaction with PTPD were Zn^{2+}, Ni^{2+}, Fe^{2+}, Cd^{2+}, Hg_2^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+}, Pb^{2+}, Hg^{2+}, and Ag^{+}.

To determine the optimum pH for the extraction of Hg^{2+}, 1.00-ml portions of $1.0 \times 10^{-2}M$ mercury(II) nitrate were diluted to volume in 100-ml standard flasks with nitric acid of various concentrations from $1M$ to $10^{-5}M$ and their pH-values (0–5) checked. Aliquots (10.00 ml) of each Hg^{2+} solution were then added to Erlenmeyer flasks and each treated with 10.00 ml of a $1.00 \times 10^{-4}M$ PTPD solution in chloroform. The flasks were stoppered and shaken by hand for 1 min. The chloroform layer was separated and its absorbance at 525 nm measured *vs.* water-saturated chloroform as reference.

The stoichiometry of the reaction between Hg^{2+} and PTPD was investigated by taking 10.0-ml aliquots of $9.95 \times 10^{-5}M$ mercury(II) nitrate, adding increasing volumes of $3.85 \times 10^{-4}M$ PTPD solution in chloroform plus additional chloroform as needed to bring the total organic phase volume to 10.0 ml. The flasks containing the mixtures were stoppered and shaken on a mechanical shaker for 20 min. The chloroform layer was separated and its absorbance at 525 nm measured *vs.* water-saturated chloroform as reference.

Adherence to Beer's law was verified by diluting various volumes of $9.95 \times 10^{-5}M$ mercury(II) nitrate to 10.0 ml in 50-ml Erlenmeyer flasks, adding 10.00 ml of $4.46 \times 10^{-4}M$ dye solution, stoppering the flasks and shaking them for 30 min with a mechanical shaker. The chloroform layer was separated and its absorbance at 525 nm measured *vs.* the PTPD solution.

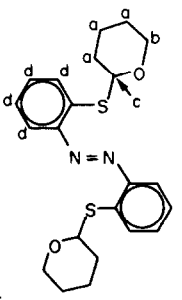
RESULTS AND DISCUSSION

PTPD is relatively soluble in chloroform, n-hexanol, n-octanol and ethyl acetate, but only moderately soluble in acetone. The dye is only slightly soluble in cold ethanol, but its solubility is considerably higher in hot ethanol. PTPD appears to be insoluble in water, acetonitrile, and 1,4-dioxan. The solubility data are only qualitative, based mostly on observation of the colour of the solvent after equilibration with the dye. No quantitative studies of solubility were undertaken.

Both the NMR and infrared data are consistent with the proposed structure of PTPD, which is the expected product from the lithium tetrahydridoaluminate reduction of tetrahydro-2-[(2′-nitrophenyl)thio]-2H-pyran. The important features of the NMR spectrum are given in Fig. 1.

The ultraviolet–visible spectrum of PTPD in chloroform has three absorption maxima, at 250 nm ($\epsilon = 8.70 \times 10^3$ l.mole^{-1}.cm^{-1}), 324 nm ($\epsilon = 8.10 \times 10^3$) and 410 nm ($\epsilon = 6.20 \times 10^3$).

When originally synthesized, PTPD was intended to be an intermediate in the synthesis of bis-(2-mercaptophenyl)diazine or *o,o′*-dimercaptoazobenzene. The 2,3-dihydropyran was to be used as a blocking group for the sulphur during coupling and to prevent



a 1.80δ, multiplet
b 3.90δ, complex multiplet
c 5.45δ, broad triplet
d 7.4δ, multiplet

Fig. 1. The proposed structure and NMR assignments for PTPD. The theoretical integration ratios consistent with the proposed structure are 6:2:1:4, the actual integration ratio were 6:1.85:0.92:4.

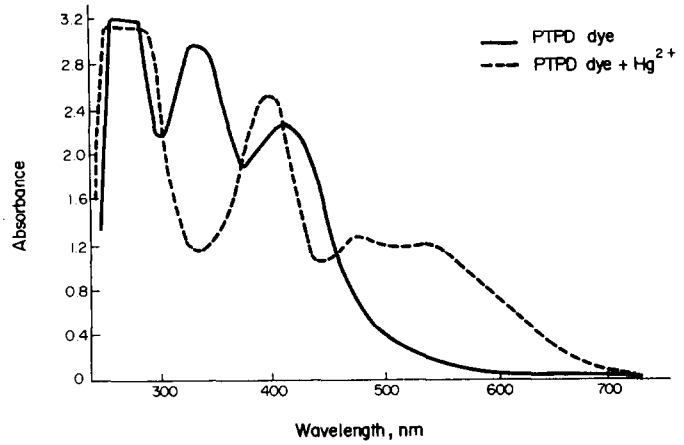


Fig. 2. Comparison of the ultraviolet spectra of PTPD before and after equilibration with an excess of Hg^{2+}. The concentration of the dye was $3.55 \times 10^{-4}M$ and the solvent was chloroform.

air oxidation of the mercaptan to the disulphide. A number of metal ions were tried to see whether they might effect the cleavage of the pyran blocking group. Of the metal ions tested by shaking their aqueous solution with a chloroform solution of PTPD (these included Zn^{2+}, Cd^{2+}, Hg_2^{2+}, Hg^{2+}, Cu^{2+}, Ba^{2+}, Pb^{2+} and Ag^{+}) only Hg_2^{2+}, Hg^{2+} and Ag^{+} showed any evidence of reaction. Ag^{+} formed a pink precipitate at the interface of the aqueous and chloroform layers, while Hg_2^{2+} changed the orange colour of the chloroform layer to amber. The reaction between PTPD and Hg^{2+} was readily apparent as the orange colour of the chloroform layer changed to purple.

The spectral characteristics of PTPD changed under the influence of Hg^{2+}. Figure 2 shows the absorption spectra. The reaction results in bathochromic shifts of both the 324 nm and 410 nm absorption bands of the dye, the 324 nm band to 392 nm ($\epsilon = 7.00 \times 10^3$ $l.mole^{-1}.cm^{-1}$) and the 410 nm band to about 500 nm (with considerable broadening). The broad band shows two weak maxima, one at 470 nm ($\epsilon = 3.50 \times 10^3$) and the other at 530 nm ($\epsilon = 3.40 \times 10^3$). The ultraviolet–visible spectra of PTPD and its mercury complex in n-hexanol, n-octanol, ethyl acetate and ethanol are all quite similar except for the fine structure in the 250-nm region. The change in the spectrum when Hg_2^{2+} was reacted with the dye was not significant and may have been due to traces of Hg^{2+} in the Hg_2^{2+} solution, from disproportionation of Hg_2^{2+} to Hg and Hg^{2+}, which is known to occur slowly.[15]

In an ethanolic solution of PTPD, both Ag^{+} and Hg_2^{2+}, as well as Hg^{2+}, caused an immediate colour change, whereas Cd^{2+}, Ba^{2+} and Pb^{2+} showed no colour change. All of the ethanol solutions produced precipitates within about an hour. The precipitates from the first three solutions were reddish-purple and were taken to be the complexes, while those from the last three were orange and were taken to be PTPD.

The reaction between PTPD in chloroform and metals in aqueous solution to form a chloroform-soluble species appears to be highly specific for Hg^{2+}. For this reason the reaction was investigated further for possible analytical application as a colorimetric method for Hg^{2+}.

The effect of pH of the aqueous phase on the formation of the Hg^{2+}–PTPD complex is shown in Fig. 3, where it is seen that the pH of the aqueous phase must be less than 3 for extraction to be quantitative. The influence of pH in this reaction is not surprising, as studies of cleavage of the pyranyl group from a variety of aromatic thiopyranyl compounds in acidic water–dioxan mixtures with Hg^{2+} as catalyst have shown that the acidity is involved in the kinetics of the reaction.[16] Possible explanations are elimination of competitive formation of hydroxy complexes of Hg^{2+}, or protonation of the pyranyl oxygen atom, with increase in polarity of the sulphur–carbon bond, thereby facilitating the mercuration of the thioacetal sulphur atom.

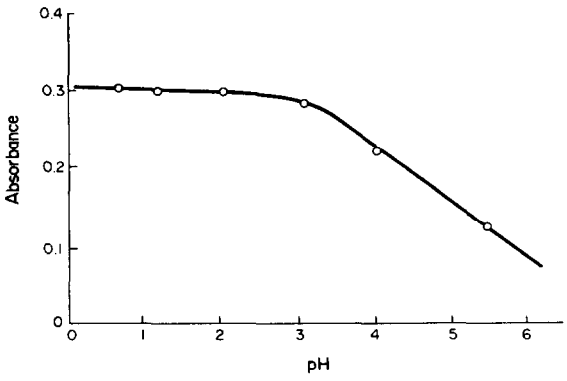


Fig. 3. The effect of the pH of the aqueous Hg^{2+} phase on the formation and subsequent extraction of the Hg^{2+}–PTPD complex. Solutions were obtained by shaking 10.0 ml of buffered $1.0 \times 10^{-4}M$ Hg^{2+} with 10.0 ml of $1.00 \times 10^{-4}M$ PTPD in chloroform. Absorbance read at 525 nm.

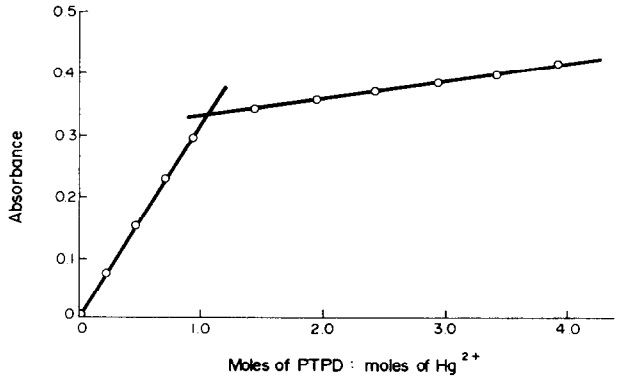


Fig. 4. The mole-ratio study of Hg^{2+} and PTPD. Solutions were prepared by equilibrating 10.0 ml of $9.95 \times 10^{-4} M$ Hg^{2+} with 10.0 ml of a chloroform solution containing various amounts of PTPD.

During the preparation of buffers for the pH study, it was found that buffers containing chloride or acetate had a deleterious effect on the Hg^{2+}-PTPD complex, presumably because of formation of non-dissociated complexes between Hg^{2+} and chloride or acetate ions. This would indicate that these anions must be avoided in the development of any analytical procedure. For this reason nitrate was used in the rest of this work.

A mole-ratio study was used to determine the stoichiometry of the reaction. The results are shown in Fig. 4 in which it can be clearly seen that the dye and Hg^{2+} react in a 1:1 ratio. This study was repeated a number of times as earlier runs gave ratios as high as 1.3:1. However, successive recrystallizations of the PTPD lowered the ratio to a constant 1:1. This illustrates the importance of knowing the exact concentration or purity of the reactants used for studies of this type, as previously pointed out by Diehl.[17] The slight increase in absorbance after the mole ratio of 1:1 is reached is due to the slight absorbance of PTPD at 525 nm.

The absorbance of the complex at 525 nm, this wavelength being chosen because of the flat region in the absorption band of the complex, and the low absorbance of PTPD, is linearly related to the concentration of Hg^{2+}. A slight advantage might be gained by using a wavelength of 540 nm. A Beer's law plot is linear for Hg^{2+} concentrations up to 50 ppm. From the slope of the curve the molar absorptivity in terms of Hg^{2+} concentration is 3.20×10^3 $l.mole^{-1}.cm^{-1}$. Although the molar absorptivity is low, it should be possible to extract and thereby concentrate low concentrations of Hg^{2+} and extend the method to the ng/ml level. Work is currently under way to explore the effect of the dilution of the aqueous phase on the reaction.

Even though the stoichiometry of the reaction between PTPD and Hg^{2+} is 1:1, the exact nature of the reaction product or complex is not yet known. What is known with certainty is that the Hg^{2+} is extracted from the aqueous layer. This has been verified by both the titration of the aqueous phase with thiocyanate after successive additions of the dye and also by the identification of Hg emission lines in the arc spectrum of the solid isolated from the chloroform phase after equilibration. The fate of the PTPD is not so clear. Although previous work would indicate that the pyranyl group should be cleaved and converted into 5-hydroxypentanol under the influence of Hg^{2+}, no evidence of this reaction product has been found. The NMR spectrum of the chloroform layer after reaction with Hg^{2+} shows a loss of the signal at 5.45δ and the concomitant development of a new signal at about 4.9δ, as well as some changes in the aromatic region. Unfortunately it is impossible to decide clearly from the integrated spectrum whether the pyranyl group is cleaved and the Hg^{2+} is complexed with what would then be the anion of *o,o'*-dimercaptoazobenzene, or whether the pyranyl group remains attached to the sulphur atom and is somehow also involved in the co-ordination of the mercury ion. Additional work is planned to resolve this problem.

CONCLUSION

From the discussion above it can be concluded that PTPD is an attractive candidate as a reagent for the spectrophotometric determination of Hg^{2+}. The dye is reasonably easy to synthesize and purify. The most favourable characteristic of PTPD is its high specificity toward Hg^{2+}, as the only other metal ion yet found to react with it is Ag^+ and the product of this reaction is a precipitate which is not extractable into chloroform. The reaction with Hg^{2+} is reasonably rapid and is stoichiometric. In addition, the absorbance of the complex formed adheres to Beer's law over a wide range of concentration. Perhaps the only drawback is the relatively low molar absorptivity of the complex; however, the ability to extract and concentrate the mercury complex may outweigh this disadvantage.

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MIXED-LIGAND COMPLEXES OF IRON AND HYDROXY-1,10-PHENANTHROLINES

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Summary—The half-wave potentials of all of the possible tris-complexes formed between iron(II) and 1,10-phenanthroline (A), 4-hydroxy-1,10-phenanthroline (B), and 4,7-dihydroxy-1,10-phenanthroline (C) have been measured at a rotated platinum electrode. Values on the hydrogen scale are 1.06 V for $A_3Fe(II)$, 0.78 for A_2B, 0.58 for A_2C, 0.60 for AB_2, 0.39 for B_3, 0.39 for ABC, 0.21 for AC_2, 0.22 for B_2C, 0.06 for BC_2, and −0.10 for C_3 at pH 11.0, 25°, and ionic strength 0.2. Half-wave potentials of the parent binary complexes are constant from pH 9 to 13 and are equal to the conditional reduction potentials. The mixed complexes are stable from pH 10 to 12, and form a redox indicator system with a continuous range of accessible potentials from −0.10 to +1.06 V *vs.* NHE.

The introduction of tris(1,10-phenanthroline)iron(II) (ferroin) as a reversible, high-potential redox indicator in 1931[1] was followed by intense study of the iron(II) 1,10-phenanthroline complexes as redox indicators. Several reviews[2–4] are available which discuss their oxidation–reduction chemistry. It is notable that almost all the potentials reported fall between 0.84 V for the 3,4,6,7-tetramethyl-1,10-phenanthroline complex[5] and 1.26 V for the 5-nitro-1,10-phenanthroline complex.[6] Two exceptions are 0.71 V for the 3-carbethoxy-4-hydroxy-1,10-phenanthroline compound[7] and −0.1 V for the 4,7-dihydroxy-1,10-phenanthroline complex.[8–10]

The last named phenanthroline has several properties which are unique in the class of 1,10-phenanthrolines. It forms a stable coloured complex with iron(II) in highly alkaline solutions, and has been used as a spectrophotometric reagent for iron in such media.[11] In addition the iron(III) tris-chelate is stable indefinitely in mildly alkaline solutions,[12] a property possessed by no other iron(III)-phenanthroline complex at any pH. The stability of the iron(III) derivative and the extremely low reduction potential of the iron(III,II) chelate couple have resulted in the use of the iron(II) derivative as a reagent in the spectrophotometric determination of dissolved oxygen[9] and as a redox indicator in the titration of sodium dithionite with potassium ferricyanide.[10]

The development of additional iron–phenanthroline complexes with potentials in the intermediate range from −0.1 to +0.8 V seems highly desirable. Although a large number of redox indicators with potentials in this range are already available, such as the indophenols and indigosulphonates,[13,14] these compounds are characterized by multielectron redox reactions with varying degrees of irreversibility. In addition the potentials of the latter compounds vary with pH, and in most cases the oxidized form is the highly coloured species. In contrast the iron–phenanthrolines undergo rapidly reversible single-electron reactions, the potentials are independent of pH, and the reduced forms are the highly coloured species.

The low reduction potential of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple is apparently a direct result of the powerful electron-donating effect of the hydroxy groups, which leads to stabilization of the iron(III) derivative. Thus one would look to other hydroxy-1,10-phenanthrolines in the search for low-potential indicators. To date at least 39 substituted 1,10-phenanthrolines incorporating the hydroxy group have been synthesized;[15] only a few of these have been studied in detail, and in general their synthesis is rather difficult. On the other hand, available ligands can be used to prepare mixed-ligand complexes which have properties intermediate between those of the respective binary complexes. This approach has been used by Taylor and Schilt,[16] who found that the reduction potentials of the mixed-ligand complexes of iron and various substituted 1,10-phenthrolines were approximately equal to the weighted averages of the reduction potentials of the corresponding binary complexes. In that study the range of potentials involved was 0.87–1.28 V. In this paper we report potentials for all the possible tris complexes formed by mixing iron(II) with 1,10-phenanthroline, 4-hydroxy-1,10-phenanthroline and 4,7-dihydroxy-1,10-phenanthroline, covering a range of potentials from −0.10 to 1.06 V.

EXPERIMENTAL

Apparatus

The instrumentation used for obtaining current–potential curves at a rotated platinum electrode is described in the accompanying paper.[17]

Reagents

4-Hydroxy-1,10-phenanthroline and 4,7-dihydroxy-1,10-phenanthroline were prepared according to Snyder and Freier[18] with minor modifications.[19] 4-Hydroxy-1,10-phenanthroline was recrystallized three times from water,

yielding the anhydrous reagent (99.9% pure by potentiometric titration). 4,7-Dihydroxy-1,10-phenanthroline was recrystallized twice from 6*M* hydrochloric acid, yielding a crystalline compound corresponding to the formula $C_{12}H_8N_2O_2 . 0.31$ HCl. Solutions of these two phenanthrolines were prepared by adding 10*M* sodium hydroxide dropwise to an aqueous suspension of the crystals until dissolution was complete. Solutions of 1,10-phenanthroline were prepared by adding the minimum required amount of concentrated sulphuric or hydrochloric acid dropwise to an aqueous suspension of the solid. Solutions of iron(II) were prepared by dissolving $FeSO_4 . 7H_2O$ (99.9% pure by titration with standard dichromate) in deaerated 1*M* sulphuric acid. Ammonia buffers were prepared by adding 5*M* ammonium chloride to 5*M* ammonia to give pH 10.

Electrode pretreatment

At the beginning of each day, the rotated platinum electrode was soaked in sulphuric acid–dichromate solution for 5 min and rinsed thoroughly with distilled water. Before each run, the potential was kept at the foot of the oxygen evolution wave for 2 min, then at the foot of the hydrogen wave for 2 min, then at +0.3 V relative to the hydrogen wave for 2 min, according to a procedure suggested by Adams.[20]

Preparation of the mixed complexes and electrolysis procedure

Solutions of the binary complexes were prepared, containing 5.0×10^{-5} mole of iron(II) and 5.0×10^{-4} mole of ligand in a total volume of 50 ml. Solutions of the mixed complexes contained 5.0×10^{-5} mole of iron(II) and 2.5×10^{-4} mole of total ligand in 50 ml. The typical procedure is illustrated here for bis(1,10-phenanthroline)-(4-hydroxy-1,10-phenanthroline)iron(II). 1,10-Phenanthroline monohydrate (0.0335 g, 1.69×10^{-4} mole) was dissolved in a few ml of dilute hydrochloric acid and transferred to the electrolysis cell. Then 0.0162 g (0.83×10^{-4} mole) of 4-hydroxy-1,10-phenanthroline was added directly to the electrolysis cell, followed by 10 ml of 5*M* ammonia buffer and enough water to give a total volume of 50 ml. The pH was adjusted to 11.0 by dropwise addition of 10*M* sodium hydroxide, the solution purged with nitrogen for 20 min, a final pH-adjustment made, and a current–potential curve obtained. Next 0.50 ml of 0.100*M* $FeSO_4$ was added, the solution was stirred and left for 40 min to come to equilibrium, a final pH-adjustment was made, and a current–potential curve for the mixed complexes was obtained. Potentials were scanned from 0.6 or 0.7 V *vs.* SCE to that for the hydrogen-evolution wave and back again. Scans initiated at more positive potentials showed almost complete suppression of anodic currents. The ionic strength of the solutions was approximately 0.2.

Reversibility studies

The reversibility of the oxidation of tris(4-hydroxy-1,10-phenanthroline)iron(II) was studied by cyclic voltammetry at a stationary platinum electrode. The equipment and general procedure are described in the accompanying paper.[17] Cyclic voltammograms were obtained at pH 11.0 and 25°, in ammonia–ammonium chloride buffer (ionic strength = 0.02) and dilute sodium hydroxide (ionic strength = 0.1) media, in presence and absence of 1.0×10^{-3}*M* tris(4-hydroxy-1,10-phenanthroline)iron(II).

RESULTS AND DISCUSSION

In this discussion the following symbols will be used: A = 1,10-phenanthroline, B = 4-hydroxy-1,10-phenanthroline, C = 4,7-dihydroxy-1,10-phenanthroline, *a*, *b*, *c* = stoichiometric coefficients for A, B, C respectively. In the formation of a stable mixed-ligand complex $A_aB_bC_cFe(II)$, the sum $a + b + c$ is equal to 3, where *a*, *b*, and *c* can take integral values from 0 to 3.

The formation of $A_3Fe(II)$ is normally considered complete over the pH-range 2–9. In the presence of sodium dithionite, or in the absence of oxygen, formation of this complex as determined from its absorbance at 510 nm is quantitative up to pH 11, with only a slight decrease in absorbance occurring at pH 12 and 13. Previous investigations have shown that the optimum conditions for formation of $B_3Fe(II)$ and $C_3Fe(II)$ are from pH 11 to 2*M* sodium hydroxide[20] and from pH 9 to concentrated sodium hydroxide solution,[12] respectively. Spectrophotometric measurements of solutions of the mixed-ligand complexes indicated that maximum stability of the complexes is obtained from pH 10 to 12. Therefore pH 11 was selected as the optimum pH for study of the mixed complexes. This pH represents the upper limit of stability of tris(1,10-phenanthroline)iron(II) in the presence of excess of phenanthroline. In order to ensure complete formation of the mixed complexes in solutions containing 1,10-phenanthroline, a 5:1 molar ratio of ligands to iron was used.

Half-wave potentials of the binary complexes

The half-wave potential of the $A_3Fe(III,II)$ couple is 1.06 V (all potentials will be quoted *vs.* NHE) from pH 0 to 11 at ionic strength = 1, and is equal to the conditional reduction potential, as demonstrated in the accompanying paper.[17]

The reduction potential of the $B_3Fe(III,II)$ couple was recently reported as 0.39 V, with no supporting data.[19] The $E_{1/2}$-values obtained in this work at pH 9, 11, 13 are all 0.39 V. A potentiometric measurement of the reduction potential was not attempted because

Table 1. Cyclic voltammetry of tris(4-hydroxy-1,10-phenanthroline)iron(II) at pH 11

Electrolyte	Ionic strength	Scan-rate, *mV/sec*	ΔE_p, *V**	$E_{1/2}$, *V vs. SCE*†	i_a/i_c§
NH_3, NH_4Cl	0.02	5	0.060	0.135	0.83
NH_3, NH_4Cl	0.02	100	0.080	0.130	0.74
NaOH	0.1	5	0.067	0.151	0.92
NaOH	0.1	50	0.080	0.140	1.19

* ΔE_p = difference between anodic and cathodic peak potentials.
† $E_{1/2}$ was calculated as the mid-point between the peak potentials.
§ i_a = anodic peak current, i_c = cathodic peak current.

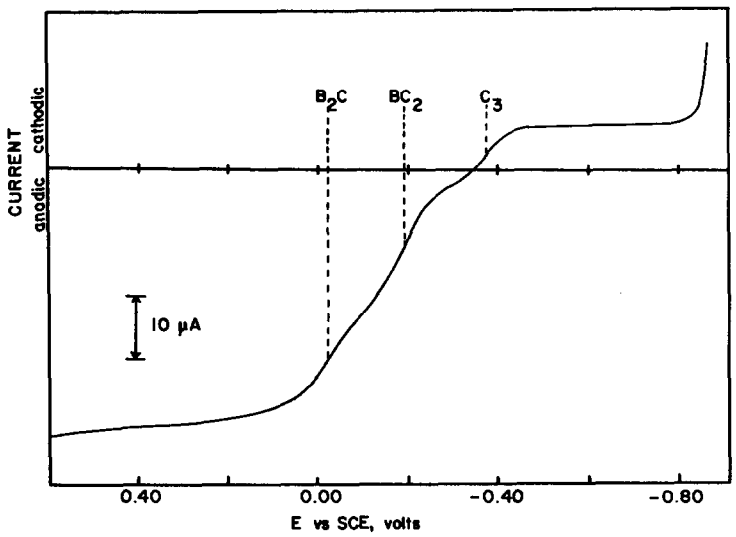

Fig. 1. Linear-scan voltammetry of $BC_2Fe(II)$ system in pH 11 ammonia buffer at a rotated platinum wire electrode: $1.00 \times 10^{-3} M$ total iron added, scan-rate 4 mV/sec.

a stable iron(III) tris-chelate is not formed under these conditions. In order to relate $E_{1/2}$-values to the reduction potential, cyclic voltammetric studies at a stationary platinum electrode were performed. A summary of the results appears in Table 1. Background currents due to surface reactions of the platinum electrode were approximately equal to the faradaic currents for oxidation and reduction of the complex, making measurements of peak potentials and currents subject to considerable uncertainty. In the ammonia buffer system the peak separation indicates that the electrode reaction is reversible at a scan rate of 5 mV/sec. The calculated $E_{1/2}$ is therefore equal to the conditional reduction potential, 0.38 V, in good agreement with $E_{1/2} = 0.39$ V determined by linear scan voltammetry at the rotated platinum electrode.

The reduction potential of the $C_3Fe(III,II)$ couple has been reported three times previously as -0.13, -0.11, and -0.06 V.[8-10] The ionic strength was not given in any of these reports. The $E_{1/2}$-values found at ionic strength = 0.16 in this work were -0.10 V from pH 9 to 13. Potentiometric titration of a solution of sodium dithionite and $C_3Fe(II)$ at pH 10.6, 25° and ionic strength = 1.0 gave $E^0 = -0.11$ V.

Because of the good agreement with potentiometric and cyclic voltammetry results we conclude that the $E_{1/2}$-values found are equal to the conditional reduction potentials of the binary complexes, and that the reduction potentials do not vary over the pH range 9–13. Presumably the $E_{1/2}$-values reported below for the mixed complexes are also equal to the conditional reduction potentials.

Half-wave potentials of the mixed complexes

Current–potential curves for solutions of $BC_2Fe(II)$ and for ABCFe(II) are shown in Figs. 1 and 2 as

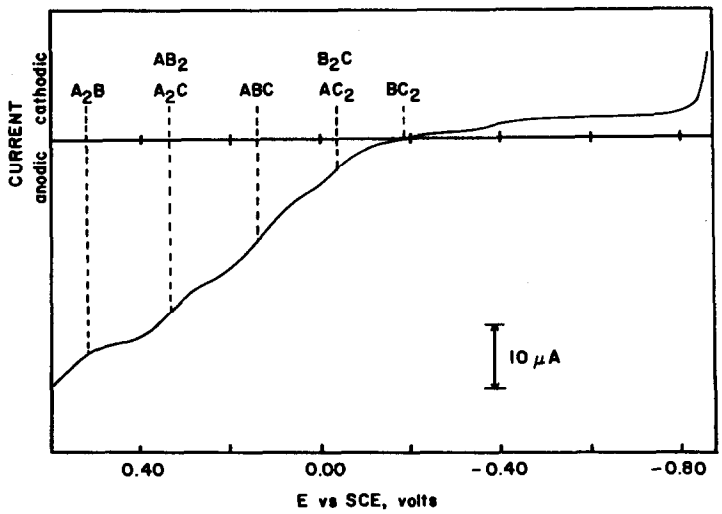

Fig. 2. Linear-scan voltammetry of ABCFe(II) system in pH 11 ammonia buffer at a rotated platinum wire electrode: $1.00 \times 10^{-3} M$ total iron added, scan-rate 4 mV/sec.

Table 2. Half-wave potentials of the mixed-ligand complexes at pH 11

Molar ratio L:Fe			$E_{1/2}$, V vs. NHE			
A	B	C	calculated	observed	$\Delta E_{1/2}$, V*	Species observed†§
3	0	0		1.06		1.0 A_3
2	1	0	0.84	0.78	−0.06	1.0 A_2B: 0.5 AB_2: 0.0 A_3‡
2	0	1	0.67	0.58	−0.09	1.0 A_2C: 0.2 AC_2: 0.2 C_3(III)
1	2	0	0.61	0.60	−0.01	1.0 AB_2: 0.7 A_2B: 0.1 B_c
0	3	0		0.39		1.0 B_3
1	1	1	0.45	0.39	−0.06	1.0 ABC: 0.7 (A_2C + AB_2): 0.6 (AC_2 + B_2C): 0.2 A_2B: 0.1 C_3(III)
1	0	2	0.29	0.21	−0.08	1.0 AC_2: 2.6 A_2C: 0.1 AC_2(III)
0	2	1	0.23	0.22	−0.01	1.0 B_2C: 0.5 B_3: 0.3 BC_2
0	1	2	0.06	0.06	0.00	1.0 BC_2: 0.8 B_2C: 0.2 C_3: 0.3 C_3(III)
0	0	3		−0.10		1.0 C_3: 0.2 C_3(II)

* $\Delta E_{1/2} = E_{observed} - E_{calculated}$.

† Relative magnitude of the diffusion current for each observed species is given, normalized so that the value for the desired species equals 1.0.

§ All species indicated are the iron(II) derivatives, except for those followed by a romal numeral III, used to indicate the iron(III) derivatives.

‡ It is likely that some A_3Fe(II) was present, but could not be observed (see text).

examples. The curves for each mixed-ligand system showed two or more separate waves. The assignments in Table 2 were made by matching observed half-wave potentials with weighted averages calculated from the $E_{1/2}$-values of the binary complexes by means of the equation:

$$E_{\frac{1}{2},calc} = \frac{a(1.06) + b(0.39) + c(-0.10)}{3}.$$

In making the assignments the magnitudes of the diffusion currents and the probable equilibrium concentrations of individual species were also considered. In all except the AC_2 system the largest diffusion current corresponded to the complex in which the ligand-to-ligand ratio matched the ratio in which the ligands were added to the system. Appearance of the waves at the predicted potentials in several different systems lends validity to the assignments.

Some significant differences between the observed and calculated half-wave potentials, listed as $\Delta E_{1/2}$ in Table 2, are apparent. In every case the observed value is equal to or less than the calculated value, in contrast to the findings of Taylor and Schilt.[16] They determined the reduction potentials of 12 mixed-ligand iron–phenanthroline complexes, incorporating the methyl, chloro, phenyl, sulpho and unsubstituted derivatives of 1,10-phenanthroline, and found that the reduction potentials agreed with the weighted averages within 0.01 V, except when 5-nitro-1,10-phenanthroline was present as a ligand. To explain this observation, the calculated and observed potentials for ternary complexes in which at least one molecule of 1,10-phenanthroline is present are summarized in Table 3. The calculated values agree with the experimental results within 0.01 V for all complexes involving ligands which form binary complexes with reduction potentials within 0.19 V of that of the tris(1,10-phenanthroline)iron(III, II) couple. Only when the 5-nitro, 4-hydroxy, or 4,7-dihydroxy substituted phenanthrolines are present do the observed values differ significantly from the calculated values. These three have substituents which are either strongly electron-accepting or electron-donating, and form binary complexes with reduction potentials which differ by more than 0.20 V from 1.06 V. Thus it appears that the weighted average provides a good prediction of the reduction potential of a mixed-ligand iron–phenanthroline complex when the ligands are similar in electronic character. Such agreement is not expected, however, when two ligands are present which have substantially different electronic properties.

The relative magnitudes of the diffusion currents for the individual mixed complexes are listed in Table 2 as an indication of the equilibrium concentrations. Conversion of these values into concentrations is hindered by the inability to prepare a standard solution of a single mixed complex. Furthermore, there is no assurance that true equilibrium conditions were obtained, especially for systems in which ligand C was present. Trace amounts of oxygen leaking into the system would immediately be reduced by C_3Fe(II), causing a continuous disturbance of the equilibrium. The detection of C_3Fe(III) in some systems demonstrates this to be the case. Other species, particularly BC_2Fe(II), B_2CFe(II), and AC_2Fe(II) might be oxidized if C_3Fe(II) is absent.

Several cases in Table 2 deserve special comment. In the A_2B system, some A_3Fe(II) was probably present, but was not observed because of the onset of oxidation of water. In the ABC system five separate waves were observed, shown in Fig. 2. Assignments were made on the basis of $E_{1/2}$-values observed in simpler systems, but it was impossible to distinguish between A_2CFe(II) and AB_2Fe(II), or between AC_2Fe(II) and B_2CFe(II). It was also assumed that negligible amounts of the binary iron(II) derivatives would be formed in the ABC system, and the wave at 0.39 V was regarded as due entirely to ABCFe(II), even though the potential corresponds exactly to $E_{1/2}$ of B_3Fe(II).

Table 3. Calculated and observed reduction potentials (V) of ternary iron–phenanthroline complexes

Complex*	Binary complexes†		Ternary complexes§		
	E_L	$E_L - 1.06$	E_{calc}	E_{obs}	$E_{obs} - E_{calc}$
$(5\text{-}NO_2)_2A$	1.28	0.22	1.21	1.24	+0.03
$(5\text{-}NO_2)A_2$	1.28	0.22	1.13	1.09	−0.04
$(5\text{-}SO_3)_2A$	1.23	0.17	1.17	1.16	−0.01
$(5\text{-}Cl)_2A$	1.16	0.10	1.13	1.13	0.00
$(5\text{-}Cl)A_2$	1.16	0.10	1.09	1.10	+0.01
$[4,7\text{-di}(\phi SO_3)]A_2$	1.09	0.03	1.07	1.07	0.00
$(5\text{-}\phi)A_2$	1.08	0.02	1.07	1.07	0.00
$(5\text{-}CH_3)A_2$	1.01	−0.05	1.04	1.04	0.00
$(5\text{-}CH_3)_2A$	1.01	−0.05	1.02	1.02	0.00
$(4,7\text{-di}CH_3)A_2$	0.87	−0.19	1.00	0.99	−0.01
$(4,7\text{-di}CH_3)_2A$	0.87	−0.19	0.93	0.93	0.00
$(4\text{-}OH)A_2$	0.39	−0.67	0.84	0.78	−0.06
$(4\text{-}OH)_2A$	0.39	−0.67	0.61	0.60	−0.01
$(4,7\text{-h}OH)A_2$	−0.10	−1.16	0.67	0.58	−0.09
$(4,7\text{-di}OH)_2A$	−0.10	−1.16	0.29	0.21	−0.08

* The iron(III,II) complex couples are designated by the ligands only, with the following abbreviations: A (1,10-phenanthroline), 5-NO_2 (5-nitro-1,10-phenanthroline), 5-Cl (5-chloro-1,10-phenanthroline), 5-SO_3 (1,10-phenanthroline-5-sulphonic acid), 4,7-di(ϕSO_3) (4,7-diphenylsulphonato-1,10-phenanthroline), 5-ϕ (5-phenyl-1,10-phenanthroline), 5-CH_3 (5-methyl-1,10-phenanthroline), 4,7-diCH_3 (4,7-dimethyl-1,10-phenanthroline, 4-OH (4-hydroxy-1,10-phenanthroline), 4,7-diOH (4,7-dihydroxy-1,10-phenanthroline).

† E_L is the reduction potential of the binary complex with the substituted ligand.

§ E_{calc} is the reduction potential of the mixed complex predicted from the weighted average. E_{obs} is the observed reduction potential of the mixed complex. Values for 4-OH and 4,7-diOH are from this work; others are taken from reference 16.

CONCLUSION

The possibilities for application of the mixed complexes as redox indicators are limited by the fact that several mixed complexes will always exist in equilibrium with the desired complex. Perhaps the ABC-Fe(II) system, in which at least six individual mixed complexes exist in equilibrium, with a continuous range of accessible potentials from 0.2 to 0.8 V, will lead to some novel applications. A solution of the ABCFe(II) system could serve as a wide-range redox indicator system, in which absorbance is a measure of the reduction potential of a solution, much the same as the colour of an acid–base indicator shows the pH. Another possibility is the use of the mixed complexes as electrode mediators in coulometric titrations. Finally, it should be possible to extend the upper potential limit of this system by incorporating another ligand such as 5-nitro-1,10-phenanthroline in the mixed-ligand complex.

The reduction potential of tris(4-hydroxy-1,10-phenanthroline)iron(II) has not been previously reported except as a passing note in a recent paper.[19] Its reduction potential of 0.39 V is significantly different from any previously reported for the binary iron–phenanthroline complexes, and it may find use as a redox indicator as well as a reagent in the spectrophotometric determination of traces of oxidants and reductants.

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TETRA-ARYLBORATES AS NMR SHIFT-REAGENTS

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Summary—The efficiency of substituted tetraphenylborates as reagents for inducing shifts in NMR spectra has been examined.

In 1969 it was found that tris-(2,2,6,6-tetramethyl-3,5-heptanedionato)europium(III), $Eu(thd)_3$, and its bipyridyl adduct produced shifts in the NMR spectrum of cholesterol monohydrate.[1] The shift produced by the unsolvated $Eu(thd)_3$ was found to be four times that produced by the bipyridyl adduct.[2] However, such lanthanide shift-reagents were found to have limitations. These were:

(1) the compound under investigation had to possess a functional group with an unshared electron pair capable of co-ordination with a metal shift-reagent;
(2) the compound must be stable in the presence of acids; thus, some of the shift reagents could not be used because of their sensitivity to acids;
(3) polar compounds which are soluble in solvents containing oxygen could not be used.

In an attempt to overcome these limitations, tetraphenylborate has been used as a short-lived counterion in the preparation of systems involving phosphonium,[3] arsonium,[4] stibonium,[4] pyridinium[5] and quaternary ammonium[6] centres. Tetraphenylborate was also used to resolve diastereomers.[7] These studies were performed by making an equimolar solution of tetraphenylborate and the onium centre in chloroform or methylene chloride, or by precipitation of the onium tetraphenylborate from methanolic solution. In most cases, the latter method produced the largest shifts.

It was postulated[3] that as a charged boron atom approaches the onium centre the protons of the cation assume a position above the phenyl rings of the anion and the aromatic ring currents of the phenyl rings shift the α proton resonances upfield. It was observed that normal proton shifts for the phosphonium centres were 70 Hz. These shifts decreased when substituents on the cation hindered the approach of the anion. The β- and γ-proton resonances were shifted about 20% of the α-shift by the same anion. Protons further down the chain exhibited only small shifts.

Schiemenz has postulated[3] a new effect based on ring current as the controlling factor. This effect operates in addition to, or instead of, the ordinary anion effect. Normally, the magnitude of the upfield shift and the shift of the methyl proton signals relative to the benzyl-proton signals should give an indication of the controlling effect. Changing the anion may produce a benzyl-proton shift twice that produced for the methyl protons in the same molecule if the anion effect is in control. If ring current is a controlling factor, this is no longer true, because the methylene protons, which are the same distance from the onium centre, should be in roughly the same position above the phenyl ring as the methyl protons. Results for methylphosphonium tetraphenylborate and the corresponding benzyl salts have been compared and the effect shown to be about the same for both salts.[3]

Another interesting result[3] is the direction of the shifts for the β- and α-proton signals. In the ethyl halides, the β-proton signals are shifted downfield while the α-proton signals are shifted upfield by the electronegative halogen atom. The same phenomena are observed in the anion effect. Whenever α-proton signals move upfield, β- and δ-proton signals shift downfield. However, the tetraphenylborate anion shifts all proton signals upfield. This would be expected if the aromatic ring current plays the most prominent role.

In this study we report the change in shift magnitude that occurs when different tetraphenyl- and substituted tetraphenylborates are used.

EXPERIMENTAL

To a 5-mm NMR tube was added 1 ml of deuterochloroform ($CDCl_3$) containing a few milligrams of (methoxymethyl)triphenylphosphonium chloride. An NMR spectrum was then obtained and used as a standard. For homocyclic tetra-arylborates and *S*-heterocyclic tetra-arylborates, a few milligrams of the caesium tetra-arylborate were added. A precipitate of caesium chloride was immediately noted and a spectrum obtained and integrated for proton ratio. The integration was used to obtain the concentration ratio of the tetra-arylborate to the onium centre.

This process was repeated until the shift became constant or the solution was saturated with tetra-arylborate. For *N*-heterocyclic tetra-arylborates, a few hundred μg of tetramethylammonium tetra-arylborates were added and mixed. A spectrum was subsequently obtained and integrated. The concentration ratio of tetra-arylborate to the onium centre was calculated as the ratio of proton integration, I_{N-CH}/I_{O-CH}. This process was repeated until the solution was saturated with tetra-arylborate.

All spectra were taken on a Varian EM 360–60 MHz instrument and were run at a spectrum amplitude of 1200, filter 0.1, RF power of 0.05 MHz, sweep time of 2 min, sweep width of 10 ppm and end of sweep at 0 ppm.

RESULTS AND DISCUSSION

Table 1 contains the maximum proton shifts for the methylene and methoxy proton signals and their ratios. From these data it appears that the system shows little dependence on the structure of the tetra-arylborate used, since the ratio of methylene to methoxy shift lies consistently between 3.13 and 4.00. An indication of the shift ability of the individual tetra-arylborates may be seen in Table 2, which shows the shift at a 1:1 concentration ratio of tetra-arylborate to (methoxymethyl)triphenylphosphonium chloride. From these results it appears that tetraphenylborate or tetrakis(3-fluorophenyl)borate is the best shift reagent.

In the study of *N*-heterocyclic tetra-arylborates, the upfield shifts of methylene and methoxy proton signals were also found. No comparison with tetramethylammonium tetraphenylborate was made, because this experiment was limited by the solubility of the tetramethylammonium salts in deuterochloroform. Pyrazole tetra-arylborate exhibits a shift pattern similar to that of tetraphenylborate. However, imidazole and benzimidazole tetra-arylborates show an upfield shift which is not linear with concentration ratio (Fig. 1).

In order to explain the behaviour of the borates, it is necessary to consider the ring currents of the aryl groups. No values of ring currents are available. The only relevant data available are Benson and Flygare's values for experimental diamagnetic anisotropies and the calculated anisotropies obtained without consideration of the ring currents.[12] These values are presented in Table 3. The term $X_{zz} - \frac{1}{2}(X_{xx} + X_{yy})$ is a criterion established for the aromaticity or delocalization of the perpendicular π-electrons in the ring systems. The term $(X_{xx} + X_{yy})$ describes the anisotropies of the magnetic susceptibility in the molecular plane. This term also reflects the amount of asymmetry produced in response to the magnetic field. As the expression $(X_{xx} + X_{yy})$ becomes more negative, the response becomes more symmetrical. As the term $X_{zz} - \frac{1}{2}(X_{xx} + X_{yy})$ becomes more negative, the ring current is more easily excited by the magnetic fields perpendicular to the ring.

Benson and Flygare have pointed out that the open structures propene, dimethyl sulphide, dimethyl ether and isoprene have lower anisotropies than the respective ring analogues cyclopropene, ethylene sulphide, ethylene oxide and cyclopentadiene. They attributed the change in anisotropies to non-local effects whenever the carbon hybridization was the same. This is not the case for three-membered ring molecules. However, in isoprene and cyclopentadiene, the hybridization is about the same, implying that the difference in the anisotropies is due to non-local effects in the cyclopentadiene, caused by ring formation. Benson and Flygare's conclusions were that the anisotropies in the ring molecules listed in Table 3 must be described in terms of both local and non-local contributions. From the isoprene cyclopentadiene study, the local effects and the ring current contribute roughly an equal amount to the anisotropies. They concluded that their work tends to support the modified ring-current theories.

From Schiemenz's postulation, the ring current is considered to be the controlling factor in the shift ability of tetraphenylborate. It would then follow from Benson and Flygare's values that tetraphenylborate would act as a better shift reagent than the unsubstituted thiophene ring borates. Our results support this postulate. The comparison of the behaviour of the substituted phenylborates with thiophenes indicates that the ring-current postulate of Schiemenz does not hold true in this case. For example, according to Benson and Flygare's data, the order of shift ability for tetraphenylborate, tetrakis(*m*-fluorophenyl)borate and tetrakis(2 and 3-thienyl)borates should be in decreasing magnitude from tetraphenylborate to tetrathienylborate. However, our experimental data

Table 1. Observed NMR shifts of (methoxymethyl)triphenylphosphonium chloride by caesium tetra-arylborates

Borate	Maximum methylene proton shift, *ppm*	Methoxy proton shift, *ppm*	Ratio of methylene to methoxy shift
Tetraphenylborate	1.9	0.53	3.6
Tetrakis(2-thienyl)borate[8]	1.2	0.37	3.2
Tetrakis(3-thienyl)borate[8]	1.5	0.40	3.7
Tetrakis(5-bromo-2-thienyl)borate[9]	1.7	0.40	4.3
Tetrakis(2,5-dimethyl-3-thienyl)borate[8]	0.5	0.13	3.9
Cyanotriphenylborate[9]	1.5	0.43	3.5
Cyanotri(4-chlorophenyl)borate[9]	1.2	0.30	4.0
Tetrakis(3-chlorophenyl)borate[10]	1.6	0.50	3.2
Tetrakis(3-fluorophenyl)borate	1.5	0.43	3.5

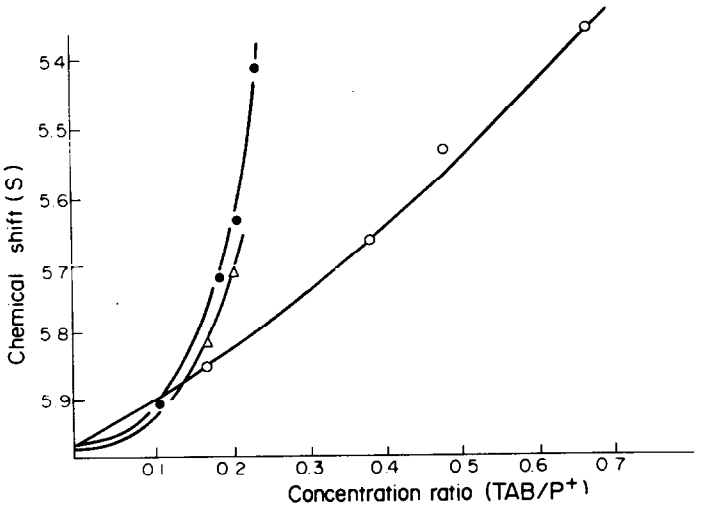

Fig. 1. The upfield shift of the α-proton signal of methoxymethyl triphenyl phosphonium employing *N*-heterocyclic tetra-arylborates; ● tetrakis(1-pyrazolyl)borate, △ tetrakis(1-imidazolyl)borate, ● tetrakis(1-benzimidazolyl)borate.

show that the tetrakis(*m*-fluorophenyl)borate and tetrakis(2 and 3-thienyl)borates switch positions. The shift was not significantly different between the tetrakis(2 and 3-thienyl)borate and tetrakis(*m*-fluorophenyl)borate, but the difference in Benson and Flygare's data for fluorobenzene and thiophene is 8.2, which is significant when contrasted with the 1.8 shift differential between benzene and fluorobenzene and the 9.6 shift differential between benzene and thiophene. The 8.2 difference in the ring current between thiophene and fluorobenzene, when compared to the similar shift ability, led us to believe that other factors in addition to the ring currents must be involved to produce these shifts.

In the lanthanide shift-reagents, it was noted that not only is the shielding ability important, but also the angle at which the reagent approaches the compound.[2] It is probable that the same is true for the tetra-arylborate shift reagents and that molecular geometry may contribute to the lack of agreement between ring-current values and the shifts actually observed. The structures of these short-lived counter-ions were not elucidated either by Schiemenz or in the present study. Schiemenz observed that phosphoniumtetraphenylborate salts gave NMR spectra which exhibited shifts greater than the shift observed in an equimolar solution of the reagents. Therefore, it appears that some other geometry is present in solution.

In the thiophene borates, it appears that the ring current may be the controlling factor, because tetrakis(5-bromo-2-thienyl)borate was slightly less efficient and tetrakis(2,5-dimethyl-3-thienyl)borate was much less efficient than tetrakis(2 and 3-thienyl)borates. Thus, Schiemenz's postulate of ring current seems to hold true. Benson and Flygare's work suggests that anything that localizes electrons, such as a substituent on a ring, lessens the ring current. Our data indicate that the substituted thiophenes depend mostly on the ring current for their shift ability. The angular dependence in the thiophene series would seem to be of less importance than that in the phenyl series.

In the case of the cyanotriarylborates, our results suggest that the ring current will be changed and the

Table 2. Observed NMR shifts of (methoxymethyl)triphenylphosphonium chloride at 1:1 concentration ratio to caesium tetra-arylborates

Borate	Observed methylene shift, in *ppm*	Concentration ratio at which shift stops increasing*
Tetraphenylborate	1.90	0.68
Tetrakis(3-chlorophenyl)borate	1.60	0.70
Tetrakis(3-fluorophenyl)borate	0.94	1.50
Cyanotriphenylborate	1.50	0.93
Cyanotri(4-chlorophenyl)borate	0.75	1.90
Tetrakis(2-thienyl)borate	1.14	1.10
Tetrakis(3-thienyl)borate	1.10	1.50
Tetrakis(5-bromo-2-thienyl)borate	0.94	1.90
Tetrakis(2,5-dimethyl-3-thienyl)borate	0.67	0.67

* Ratio of tetra-arylborate to (methoxymethyl)triphenylphosphonium chloride.

Table 3. Magnetic susceptibility anisotropies[8]

Compound	$X_{zz} - \frac{1}{2}(X_{xx} + X_{yy})$, 10^{-6} erg.gauss^{-2}.mole^{-1}
Benzene	−59.7
Fluorobenzene	−58.3 ± 0.8
Pyridine	−57.4 ± 0.7
Thiophene	−50.1 ± 0.1
Pyrrole	−42.4 ± 0.5
Furan	−38.7 ± 0.5
Cyclopentadiene	−34.4 ± 0.3
Cyclopropene	−17.0 ± 0.5
Ethylene sulphide	−15.4 ± 0.4
Ethylene imine	−10.9 ± 0.7
Ethylene oxide	−9.4 ± 0.4
1,3-Cyclohexadiene	−7.4 ± 2.2
Cyclobutanone	−2.1 ± 1.0
Trimethylene oxide	+16.8 ± 0.07
Trimethylene sulphide	+22.8 ± 1.0
Isoprene	−18.0 ± 1.1
Propene	−6.3 ± 0.4
Dimethyl sulphide	+3.5 ± 0.7
Dimethyl ether	+4.6 ± 0.5

angle of the borate's approach will be different. Both factors may influence the shifts observed. The cyanotriaryl(*p*-chlorophenyl)borate was much less effective than either tetraphenylborate or cyanotriphenylborate. Consequently, other cyanotriarylborates would not be expected to react any better than their tetra-arylborate analogues.

In the *N*-heterocyclic tetra-arylborates, there is a marked difference of shift ability between pyrazole and imidazole or benzimidazole tetra-arylborates. This suggests that the ring current and approach angle are affected by the non-boron-bonded nitrogen in the ring.

The future of tetra-arylborates as proton-shift reagents will depend on the range of their application. Tetraphenylborate complexes are the best of the tetra-arylborates for shift ability. However, more significant effects can be produced by the use of higher frequencies or by the use of different aromatic anions. For example, Hellwinkel[13] reported that *p*-toluenesulphonate was inferior to tetraphenylborate, but that $P(C_{12}H_8)_3$ was 50% more effective.

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KINETICS OF DISSOCIATION OF MAGNESIUM AMINOCARBOXYLATE COMPLEXES

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Summary—The kinetics of dissociation of magnesium aminocarboxylate complexes of the EDTA type have been examined and the results used to determine the rate constants for formation of the complexes and to find the rate-determining steps in the mechanism. The results indicate that loss of the first water molecule from the hydration sheath of the magnesium ion and formation of the first metal-ligand bond is the rate-determining step.

When the formation rates of metal complexes are compared for EDTA, DPTA, DBTA and DCTA*, two types of behaviour are noted.[2] Nickel, cobalt(II), copper(II), zinc and cadmium react with all the ligands at nearly the same rate to form complexes, but calcium, strontium and lead react with the ligands at rates in the order EDTA≫ DPTA > DBTA > DCTA. The metal ions in the first of these groups all have fairly slow characteristic water-loss rates (10^4–10^7 sec^{-1}) and are formed from main group elements which form strong metal to amino-nitrogen co-ordinate covalent bonds; those in the second group all have very rapid water-loss rate constants (10^7–10^{10} sec^{-1}) and come from main group elements which form only weak metal–amino-nitrogen bonds. Magnesium is a main group element having ions which form only weak bonds to amino nitrogen-donors but also have a small water-loss rate constant. Examination of the dissociation kinetics should indicate whether the behaviour is governed by the water-loss rate or by equilibrium or periodic table relationships.

The dissociation rate constants of many metal complexes of EDTA and related polyaminocarboxylates have been measured and mechanisms have been proposed. From such observed dissociation rate constants and formation equilibrium constants, formation rate constants can be computed and compared with predictions based upon Eigen's model for complex ion formation.[1] This model originally dealt with unidentate ligands and assumed that aqueous metal ions first form outer-sphere complexes with aqueous ligand molecules or ions. The metal ion of such an outer-sphere complex then loses a solvated water molecule and the ligand moves from the outer sphere to the inner sphere to form the complex ion. Three types of metal ion behaviour were identified. Type 1 metal ions are those from which water loss is very rapid—so rapid that formation of the outer-sphere complex is rate-determining. Type 2 metal ions have a slower water-loss rate so that formation of the outer-sphere complex may be regarded as at equilibrium before the rate-determining water loss. Type 3 metal ions are those which have extremely slow water-loss rate, so slow that hydrolysis of co-ordinated water (base-catalysed) becomes a preferred pathway for vacating a co-ordination site.

Complexation by multidentate ligands must involve formation of the first metal–ligand bond in the same way as for unidentate ligands and also appropriate rotation of the non-bonded portions of the ligand molecule to achieve the correct position for subsequent bonding. The difficulty of performing such rotations will presumably depend on the degree of steric hindrance and should be in the order DCTA > DBTA > DPTA > EDTA, but be largely independent of the identity of the metal ion bonded. If the rotation is fast compared to the loss rate for the first water molecule, formation of the first bond will be rate-determining, but slower rotation can shift the rate-determining step to formation of the second bond.

The reactions were monitored by polarimetry in this work since the ultraviolet absorption spectra of these complexes do not provide enough information about the extent of reaction. Each of the following reactions was studied at a variety of pH and concentration values.

$$d,l\text{-DCTA} + \text{Mg–}l\text{-DPTA} \rightarrow \text{Mg–}d,l\text{-DCTA} + l\text{-DPTA} \quad (1)$$

$$d,l\text{-DCTA} + \text{Mg–}d\text{-DBTA} \rightarrow \text{Mg–}d,l\text{-DCTA} + d\text{-DBTA} \quad (2)$$

$$d\text{-DCTA} + \text{Mg–meso-DBTA} \rightarrow \text{Mg–}d\text{-DCTA} + \text{meso-DBTA} \quad (3)$$

$$d\text{-DCTA} + \text{Mg-EDTA} \rightarrow \text{Mg–}d\text{-DCTA} + \text{EDTA} \quad (4)$$

Jensen[3] and Margerum[4] have measured the dissociation kinetics of Mg–DCTA by related methods.

* Abbreviations used: EDTA = ethylenediaminetetra-acetic acid; DPTA = 1,2-diaminopropanetetra-acetic acid; DBTA = 2,3-diaminobutanetetra-acetic acid; DCTA = *trans*-1,2-diaminocyclohexanetetra-acetic acid.

† Reprint requests.

EXPERIMENTAL

Optically active DCTA, DBTA and DPTA as well as meso-DBTA were prepared as described previously.[2,5] All other reagents were reagent grade and were used as received. Nitric acid or tetramethylammonium hydroxide (TMAOH) was used to adjust pH, and the DCTA served as buffer. The solution pH was constant to ± 0.03 over the first 2–3 half-lives of a reaction. All ligand solutions were prepared by dissolving the appropriate tetra-acid in distilled demineralized water with the addition of about twice as many moles of TMAOH. Magnesium complexes were formed by mixing stoichiometric amounts of standard magnesium nitrate and ligand solution. TMAOH was used to avoid the presence of alkali metal ions which complex these ligands and possibly affect the reaction kinetics.[6] The temperature was maintained at 25.0° by a water-bath with water circulating from a thermostatic tank. Solution ionic strength was controlled only by the DCTA and in most cases was $>0.1M$.

The reactions were monitored on a Perkin–Elmer Model 141 polarimeter equipped with a transmitting potentiometer allowing strip-chart recording of optical rotation (α) *vs.* time. The exchange reactions of racemic DCTA with magnesium complexes of optically active DPTA and DBTA were monitored at 365 nm and the reactions of optically active DCTA with magnesium complexes of EDTA and meso-DBTA were monitored at 345 nm. This latter wavelength required that the instrument be modified with a xenon discharge source and monochromator as described previously.[7] The reagents were mixed externally and transferred to constant-temperature cells as rapidly as possible. Half-times of reactions ranged from 1 to 90 min.

The molar rotations of the magnesium complexes at 365 nm are as follows: Mg–*l*-DPTA([α] = -3.82 deg.l.mole^{-1}.cm^{-1} at pH 9.86 and $-1.78°$ at pH 11.26); Mg–*d*-DBTA([α] = $+1.69$ deg.l.mole^{-1}.cm^{-1} at pH 8.19 and $+0.70$ at pH 12.98).

Pseudo first-order rate constants (k_{obs}) were calculated from the slopes of plots of $-\ln(\alpha_t - \alpha_e)$ where α_t and α_e are the rotations observed at time t and at equilibrium respectively.

Plots of k_{obs} *vs.* concentration of incoming ligand, at constant pH, give a slope of k_2 and intercept of k_1 [see equation (8)]. These values are tabulated for all systems in Table 1. Values of k_H^{MgL}, k_d^{MgL} and k_{OH}^{MgL} are obtained from the pH-dependence of k_1 either by plots of k_1 *vs.* [H$^+$] or [OH$^-$] or by non-linear least-squares fit of k_1 and [H$^+$], where k_d^{MgL} is the dissociation rate constant. Resolved values of these terms are compiled in Table 2.

Values of the formation rate constants of both the unprotonated and monoprotonated ligands are calculated from equations (5) and (6) and are included in Table 3.

$$L^{4-} + Mg^{2+} \rightleftharpoons MgL^{2-}$$

$$K_{MgL} = \frac{[MgL^{2-}]}{[Mg^{2+}][L^{4-}]} = \frac{k_L^{Mg}}{k_d^{MgL}}$$

$$k_L^{Mg} = K_{MgL} k_d^{MgL} \qquad (5)$$

$$HL^{3-} + Mg^{2+} \rightleftharpoons MgL^{2-} + H^+$$

$$K_{eq} = \frac{[MgL^{2-}][H^+]}{[HL^{3-}][Mg^{2+}]} = \frac{k_{HL}^{Mg}}{k_H^{MgL}}$$

$$= \frac{[MgL^{2-}]}{[L^{4-}][Mg^{2+}]} \times \frac{[L^{4-}][H^+]}{[HL^{3-}]}$$

$$= K_{MgL} K_4$$

$$k_{HL}^{Mg} = K_{MgL} K_4 k_H^{MgL} \qquad (6)$$

Also included in Table 3 are values of the rate constants calculated according to Eigen through equation (7).

$$k_f = K_{OS} k_{Mg}^{H_2O} \qquad (7)$$

where K_{OS} is the stability constant of the outer-sphere complex. Values of 50 and 13 for K_{OS} for $Mg^{2+} + L^{4-}$ and $Mg^{2+} + HL^{3-}$ respectively[4] and a characteristic water-loss rate constant[9] of 1×10^5 for Mg^{2+} are used in these calculations.

RESULTS

Experimental conditions and rate constants obtained are presented in Table 1. All the reactions are shown to proceed according to the rate law

$$\text{rate} = k_1 [MgL] + k_2 [MgL] [DCTA]$$
$$= k_{obs} [MgL] \qquad (8)$$

where k_1 is the observed dissociation rate constant, k_2

Table 1a. Reaction conditions and k_{obs} values for Mg–*l*-DPTA + DCTA in the presence of TMAOH

pH	[Mg–*l*-DPTA], $10^{-3}M$	$[DCTA]_T$, $10^{-3}M$	$k_{obs} \times 10^3$
8.18	6.50	62.5	15.6
8.90	5.39	53.8	5.11
9.18	5.39	53.8	3.06
9.42	5.39	53.8	2.22
9.46	6.49	62.3	2.19
9.62	5.39	53.8	2.53
9.86	5.39	53.8	2.05
10.12	5.39	53.8	1.51
10.18	5.39	53.8	1.23
10.19	5.07	55.1	1.31
10.30	5.39	53.9	1.48
10.50	5.07	55.0	0.928
10.53	5.39	53.8	1.06
10.65	5.39	53.9	1.17
10.64	5.27	120.7	1.19
10.65	3.54	145.8	1.40
10.79	5.39	53.8	0.995
11.26	5.05	54.8	0.821
11.82	4.00	38.2	0.793
11.88	4.00	38.2	0.93
11.94	4.00	38.2	0.874
12.06	4.00	38.2	1.08
12.22	4.00	38.2	1.40
12.44	4.00	38.2	2.05
12.58	4.00	38.2	2.52
12.62	2.90	54.6	2.46
12.62	1.50	73.5	2.15
12.86	4.00	28.1	3.68
12.98	4.00	38.2	3.66

Table 1b. Reaction conditions and k_{obs} values for Mg–*l*-DPTA + DCTA in the presence of KOH

pH	[Mg–*l*-DPTA], $10^{-3}M$	$[DCTA]_T$, $10^{-3}M$	$k_{obs} \times 10^4$
9.19	5.27	51.7	3.28
9.30	5.27	51.7	5.32
9.45	5.01	49.2	3.67
9.77	5.26	51.6	2.61
10.06	5.27	51.7	2.48
10.35	5.24	51.4	1.48
10.15	5.26	51.6	2.35
10.56	5.28	51.8	2.42
10.62	5.11	50.1	3.60
10.79	5.28	51.8	1.37

Table 1c. Reaction conditions and k_{obs} values for Mg–*d*-DBTA + DCTA in the presence of TMAOH

pH	[Mg–*d*-DBTA], $10^{-3}M$	$[DCTA]_T$, $10^{-3}M$	$k_{obs} \times 10^3$
7.65	4.96	51.9	4.39
7.92	4.96	51.9	2.46
7.93	4.66	83.3	2.83
7.93	4.66	116	3.10
7.92	4.66	149	3.61
8.05	6.90	345	3.99
8.19	4.97	52.0	1.52
8.18	6.90	138	2.05
8.18	6.90	172	2.24
8.18	6.90	207	2.56
8.65	4.96	51.9	0.688
8.63	5.01	90.2	0.799
8.64	5.01	125	0.843
8.63	5.01	160	0.914
8.90	4.96	51.9	0.407
9.31	4.97	52.0	0.184
9.31	4.97	124	0.360
9.31	4.97	174	0.412
9.32	4.97	224	0.467
9.73	4.96	52.0	0.142
10.75	4.97	52.0	0.0507
12.98	4.42	46.3	0.0318

Table 1d. Reaction conditions and k_{obs} values for Mg–EDTA + *d*-DCTA in the presence of TMAOH

pH	[Mg-EDTA], $10^{-4}M$	$[d\text{-DCTA}]_T$, $10^{-3}M$	$k_{obs} \times 10^2$
9.22	7.61	7.57	1.75
9.50	7.60	7.49	1.22
9.46	7.64	11.3	1.77
9.46	7.61	16.9	2.76
9.48	7.59	22.5	4.24
9.84	7.61	7.58	1.09
9.79	5.70	7.50	1.10
9.86	3.80	7.50	1.03
9.80	3.04	7.50	1.50
9.75	7.64	11.3	1.16
9.75	7.67	15.1	1.65
9.77	7.79	26.7	2.68
10.05	7.61	7.50	1.01
10.20	7.64	11.3	1.30
10.22	7.62	17.0	1.53
10.24	7.59	22.5	1.75
10.36	7.61	7.58	0.925
10.77	7.61	7.58	1.05
10.97	7.61	7.50	1.23
11.16	7.62	7.51	1.39

Table 1e. Reaction conditions and k_{obs} values for Mg–meso-DBTA + *d*-DCTA in the presence of TMAOH

pH	[Mg–meso-DBTA], $10^{-4}M$	$[d\text{-DCTA}]_T$, $10^{-3}M$	$k_{obs} \times 10^3$
9.15	7.60	7.57	7.93
9.30	7.60	7.57	8.79
9.56	7.60	7.57	7.23
9.93	7.61	7.57	7.15
10.27	7.61	7.58	6.65
10.39	7.61	7.58	5.52
10.55	7.61	7.58	5.00
10.88	7.61	7.58	4.50
10.91	7.61	7.58	4.33
11.66	7.62	7.59	3.93

Table 2. Values of the total dissociative and total ligand-dependent rate constants for Mg–*d*-DBTA + DCTA and Mg–EDTA + *d*-DCTA at the various pH values employed

pH	Mg–*d*-DBTA + DCTA $k_1 \times 10^4$	$k_2 \times 10^3$	$k_2([H^+]/K_4) \times 10^{-2}$
7.92	18.5	11.5	17.0
8.18	10.1	7.39	6.01
8.64	5.96	2.01	0.566
9.31	1.22	1.63	0.0983
pH	Mg–EDTA + *d*-DCTA $k_1 \times 10^3$	k_2	$k_2([H^+]/K_4) \times 10^{-3}$
9.47	(0.00)	1.61	6.71
9.75	1.24	0.964	2.11
10.22	8.46	0.402	0.298

is an observed ligand-dependent rate constant, and the incoming ligand is always in >10-fold excess over MgL. The emphasis in this work was on the value of k_1, which can be further resolved into three terms:

$$k_1 = k_H^{MgL}[H^+] + k_d^{MgL} + k_{OH}^{MgL}[OH^-] \quad (9)$$

involving an intrinsic, non-catalysed dissociation rate constant (k_d^{MgL}), a proton-catalysis constant (k_H^{MgL}) and a hydroxide-catalysis constant (k_{OH}^{MgL}). Not all the terms were observed for each system. The value of k_2 is also expected to be pH-dependent because the unprotonated incoming ligand is expected to react at a different, and much greater, rate than the protonated species.[2]

In order to calculate k_L^{Mg} and k_{HL}^{Mg}, values of K_4 and K_{MgL} for each ligand are necessary. These values should be obtained under the conditions used for the kinetic experiments (*i.e.*, 25° and 0.5*M* TMA^+). Literature values must be corrected to correspond to these conditions. Most importantly, the presence of 0.1*M* K^+ in the measurements of K_{MgL} must be corrected for because of the complexation of potassium by these ligands.[5,10,11] The value used for K_{MgL} is the appropriate literature value[11] multiplied by $(1 + K_{KL}[K^+])$.

The rate constants for formation of the magnesium complexes from the unprotonated ligands show values which are all very similar to each other, with a value of about 3×10^7 $l.mole^{-1}.sec^{-1}$. The consistency of these values implies that the loss of the first water molecule is the rate-determining step. The value 3×10^7 is about six times larger than is expected from $k^{-H_2O} = 1 \times 10^5$ and $K_{OS} = 50$. Our interpretation is that the small magnesium ion allows a closer approach of the fully dissociated anion and accordingly the K_{OS} value is actually larger than that for other doubly charged but larger cations.

The rate constants for the formation of a magnesium complex from the protonated ligand (Table 4) agree well for DPTA with the value expected from the characteristic water-loss rate constant and an outer-sphere stability constant of 50. The values for DBTA and DCTA are considerably lower than the DPTA

Table 3. Dissociation and formation rate constants for magnesium complexation

	k_d, sec^{-1}	K^*_{MgL}	k_L^{Mg}
DPTA	1.12×10^{-3}	3.51×10^{10}	3.93×10^{7}
d,l-DBTA	3.0×10^{-5}	9.84×10^{11}	2.95×10^{7}
DCTA	7.7×10^{-5}†	2.00×10^{11}	1.54×10^{7}
Eigen mechanism prediction			5.0×10^{6}

* Values are chosen from reference and corrected for potassium ion concentration.
† Value reported by Larsen and Jensen[3]

Table 4. Proton-catalysed dissociation rate constants and formation rate constants with protonated ligands

	k_H^{MgL}	K_{MgL}	K_4*	k_{HL}^{Mg}	$k_{HL}^{Mg} \times \alpha$ non-chelated
DPTA	3.19×10^{6}	3.51×10^{10}	9.77×10^{-12}	1.09×10^{6}	7.3×10^{6}
d,l-DBTA	1.42×10^{5}	9.84×10^{11}	4.90×10^{-13}	6.85×10^{4}	3.1×10^{6}
DCTA	7.70×10^{6}	2.00×10^{11}	8.13×10^{-14}	1.25×10^{5}	1.2×10^{7}
Eigen mechanism prediction					1.3×10^{6}

* From references 5,10,11.

value. If, however, the fraction of the protonated ligand in an open, or non-chelated, form is considered (Table XI in reference 2), the formation rate constants are all very similar and agree quite closely with the value predicted from the Eigen mechanism, 1.3×10^6 $l.mole^{-1}.sec^{-1}$. This assumes that the closed, or chelated-proton, form of the ligand is much slower to react than is the open form. Again, the DPTA value is about six times greater than the Eigen mechanism predicts. The minor differences in values from DPTA to DCTA are probably not significant when the uncertainties in the calculations are considered.

The general conclusion is similar in each case, the relatively slow water-loss rate for magnesium allowing the formation of the first metal–ligand bond to be be rate-determining.

Rate data from magnesium–EDTA could not be satisfactorily resolved into the various terms described. This is principally due to the rapidity of these reactions and the very small changes in optical rotation (*ca.* 0.040°) characteristic of these reactions. In addition, apparently all the terms in equations (8) and (9) apply to the Mg–EDTA substitution reaction.

Approximate resolved values for attack of H_2DCTA on Mg–EDTA and Mg–*d*-DBTA are 2.7×10^3 and 0.58 $l.mole^{-1}.sec^{-1}$ respectively and for attack of HDCTA on these same complexes are 0.15 and 1.0×10^{-3} $l.mole^{-1}.sec^{-1}$. Attack by the unprotonated ligand, $DCTA^{4-}$, was not observed.

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INVESTIGATION OF SELECTED FERROIN-TYPE COMPOUNDS AS FLUOROMETRIC REAGENTS

DETERMINATION OF ZINC WITH 2-(4-METHYL-2-PYRIDYL)-5(6)-PHENYLBENZIMIDAZOLE

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Summary—In a search for new fluorometric reagents for trace metal determinations, forty different ferroin-type compounds and their silver, lead and zinc chelates were tested for luminescence in aqueous–ethanol solutions at various pH values. Twelve formed fluorescent chelates of potential analytical utility, and the results revealed some correlation between ligand structure and fluorescence. After identification of the most sensitive fluorometric reagent for zinc among those tested, a spectrophotofluorometric method for the determination of zinc was developed. Proper control of pH and reagent concentration allows determination of nanogram amounts of zinc.

Although several different ferroin-type ligands have been recommended as fluorometric reagents for trace metal determinations,[1] no systematic search has been made among the many new ferroin-type compounds to discover other promising fluorometric reagents. With samples of most of these compounds available to us from previous studies, we decided to undertake such a study. Representative compounds were selected and tested for fluorometric properties as free ligands and as silver, lead, and zinc chelates. Those that fluoresced most strongly were tested spectrofluorometrically to identify the most sensitive one for use as a fluorometric reagent for zinc. The results are reported in this paper.

The compounds tested are listed below, with references to their synthesis and chelation properties.

I 2-(4-Methyl-2-pyridyl)-benzimidazole[2,3]
II 2-(4-Methyl-2-pyridyl-5(6)-phenylbenzimidazole[2,3]
III 2-(1-Isoquinolyl)-benzimidazole[4,5]
IV 2-(1-Isoquinolyl)-5(6)-phenylbenzimidazole[4,5]
V 2-(3-Isoquinolyl)benzimidazole[4,5]
VI 2-(3-Isoquinolyl)-5(6)-phenylbenzimidazole[4,5]
VII 2-(2-Pyridyl)-2H-imidazo[4,5-h]quinoline[2,3]
VIII 2-(3-Isoquinolyl)-3H-imidazo[4,5-*h*]quinoline[4,5]
IX 3-(2-Pyridyl)-5-phenyl-6-(2-pyridyl)-1,2,4-triazine[5,6]
X 3-(3-Isoquinolyl)-5,6-diphenyl-1,2,4-triazine[5,6]
XI 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine[7,8]
XII 3-(4-Methyl-2-pyridyl)-5-phenyl-1,2,4-triazine[9,10]
XIII 3-(4-Phenyl-2-pyridyl)-5-phenyl-1,2,4-triazine[9,10]
XIV 5,6,7,8-Tetrahydro-3-(2-pyridyl)-1,2,4-benzotriazine[11]
XV 3-(2-Thiazolyl)-1,2,4-triazine[11,12]
XVI 3-(2-Pyridyl)-1,2,4-triazine[11,12]
XVII 3-Pyrazyl-5,6-diphenyl-1,2,4-triazine[13,14]
XVIII 3-Pyrazyl-5,6-bis(2-pyridyl)-1,2,4-triazine[13,14]
XIX 3-(2-Pyridyl)-5,6-dimethyl-1,2,4-triazine[11,12]
XX 3-(2,2′,6-Bipyridyl)-5,6-diphenyl-1,2,4-triazine[16,17]
XXI 3-(4-Phenyl-2-pyridyl)-5-phenyl-6-benzoyl-1,2,4-triazine[9,18]
XXII 3-(4-Methyl-2-pyridyl)5-phenyl-6-benzoyl-1,2,4-triazine[9,18]
XXIII 3-(2-Pyridyl)-5,6-bis(2-pyridyl)-1,2,4-triazine[7,8]
XXIV 2,4-bis(5,6-Diphenyl-1,2,4-triazin-3-yl)pyridine[12,19]
XXV 2,4-bis(5,6-Dimethyl-1,2,4-triazin-3-yl)pyridine[12,19]
XXVI 2,6-bis(5,6-Diphenyl-1,2,4-triazin-3-yl)pyridine[12,19]
XXVII 2,6-bis(5,6-Dimethyl-1,2,4-triazin-3-yl)pyridine[12,19]
XXVIII 2,3-bis(2-Pyridyl)pyrido[2,3-*f*]quinoxaline[20,21]
XXIX 2,6-bis(2-Pyridyl)dipyrido[2,3-*f*:3′,2′-*h*]quinoxaline[20,21]

XXX Tetrapyrido(2,3-*a*:3′,2′-*c*:2″,3″-*h*:3‴,2‴-*j*)phenazine[20,21]
XXXI Dipyrido[2,3-*a*:2′,3′-*h*]phenazine[13,14]
XXXII 3-(3-Pyridazyl)-5,6-diphenyl-1,2,4-triazine[13,14]
XXXIII 5-Methyl-2-(2-pyridyl)pyrimidine[13,15]
XXXIV 3-(2-Pyridyl)-5-methyl-1,2,4-triazoline[11,12]
XXXV 3-(2-Thiazolyl)-5-(2-pyridyl)-1,2,4-triazoline[11,12]
XXXVI 2-Pyridylhydrazone of di(2-pyridyl) ketone[22,23]
XXXVII Azine from 2-pyridinecarboxaldehyde and picolinamide hydrazone[24]
XXXVIII Azine from 2-pyridinecarboxyaldehyde and *N*-2-pyridylbenzamide hydrazone[24]
XXXIX 3-(4-Methyl-2-pyridyl)-9H-indeno-[1,2-*e*]-1,2,4-triazin-9-one[9,25]
XXXX 3-(4-Phenyl-2-pyridyl)-9H-indeno-[1,2-*e*]-1,2,4-triazin-9-one[9,25]

EXPERIMENTAL

Reagents

Standard solutions of zinc, lead, and silver were prepared by dissolving known amounts of the pure metals in nitric acid followed by dilution to volume with distilled water.

Solutions of the test compounds were prepared by dissolving weighed amounts in sufficient 95% ethanol to obtain the desired concentrations.

The pH 3 and pH 4 buffers were prepared by adding sufficient 1*M* sodium acetate to 1*M* acetic acid. The pH 5 buffer was prepared by adding sufficient glacial acetic acid to 1*M* sodium acetate. The pH 7 buffer was 1*M* ammonium acetate. The pH 9 buffer was prepared by adding sufficient concentrated ammonia solution to 1*M* ammonium chloride. The pH 11 buffer was prepared by diluting concentrated ammonia solution. All pH measurements and adjustments were made with a Corning Model 7 pH meter with glass and saturated calomel electrodes.

A 500-ppm quinine sulphate stock solution was used to prepare fresh 1-ppm and 5-ppm solutions of quinine, as needed, by dilution with 0.05*M* sulphuric acid.

The $1.6 \times 10^{-4}M$ solution of MPPB was prepared by dissolving 11.4 mg of 2-(4-methyl-2-pyridyl)-5(6)-phenylbenzimidazole in 250 ml of 95% ethanol.

Apparatus

Fluorescence measurements were made with an Aminco-Bowman Model 4-8202 spectrophotofluorometer equipped with an Aminco Ratio Photometer and an XY-recorder. Fused quartz cells of 1.30-cm optical path were used.

Test procedures

Visual tests for luminescence were performed as follows. Mixtures of 0.1 ml of 0.003*M* metal ion, 0.2 ml of 0.01*M* test compound, and 0.3 ml of buffer were prepared, and portions were transferred to a spot-plate and examined for luminescence when irradiated by an ultraviolet lamp (Ultraviolet Products Model UVSL-25 Mineralight with both 254 and 366 nm tubes) in a darkroom. Colours were recorded and relative intensities estimated on a scale of 1–10. Any turbid samples were treated with additional ethanol to give clear, non-scattering solutions.

Spectrophotofluorometric measurements were performed on samples prepared by mixing 1.00 ml of $1.00 \times 10^{-3}M$ zinc nitrate, 1.00 ml of 0.01*M* test compound, and 2 ml of appropriate buffer, in the order cited, in a 10-ml standard flask and diluting to volume with ethanol. After adjustment of the meter multiplier setting and sensitivity to give a reading of 100 for 5.00-ppm (or 1.00-ppm, when appropriate) quinine sulphate solution, the relative fluorescence of the sample solution was measured, the optimum wavelengths for excitation and emission for each having been found previously. The intensities of the zinc chelates could thus be compared, even though uncorrected for instrumental parameters that vary with wavelength.

Recommended procedure for determination of zinc

Pipette 2.00-ml samples (Zn 5–200 ng/ml) of unknown and standards into separate 10-ml standard flasks, add 5.00 ml of $1.6 \times 10^{-4}M$ MPPB to each and dilute to volume with the pH 4 buffer. Set the fluorometer to read 100 relative intensity with the most concentrated zinc standard thus prepared, using an excitation wavelength of 353 nm and an emission wavelength of 432 nm. Measure the relative fluorescences of the other solutions at the same wavelength and sensitivity settings. Construct the calibration curve to determine the unknown zinc concentration. Because of non-linearity of the calibration, at least four different standard zinc samples, covering the concentration region of interest, should be carried through the procedure to construct the calibration curve.

RESULTS AND DISCUSSION

Compounds I–VIII and XXVIII–XXXI fluoresced both as free ligands and as metal chelates. Fluorescent colours and relative intensities for these are compiled in Table 1. None of the other test compounds or metal chelates displayed visually detectable fluorescence. These results indicate that ferroin-type ligands which incorporate a benzimidazole, quinoline, quinoxaline or phenazine moiety afford good prospects as fluorometric reagents for metal ions. Consistent with previous conclusions,[26,27] compounds with fused-ring and rigid planar structures are more commonly fluorescent than those that have a relatively high degree of flexibility.

Because the most intensely fluorescent products observed were those of zinc with compounds I–VIII, these were examined spectrofluorometrically to determine which would serve best as a sensitive fluorometric reagent for zinc. Compound II, hereafter referred to as MPPB, proved to be the most sensitive; VII was next best, but less than one-half as sensitive as MPPB.

In connection with our observation that MPPB in particular or benzimidazole derivatives in general impart intense fluorescence to zinc chelates, it is interesting to note that a similar conclusion was reached by Ryan *et al.*[28] in studying zinc chelates of some *N*-heterocyclic hydrazones.

The effects of pH on the fluorescence of MPPB and its zinc chelate are evidence by the data compiled in Table 2. Maximum differences in fluorescence between the two, and thus optimum sensitivity for zinc, are obtained in the pH range 2–6.

Table 1. Colours and intensities of luminescence as a function of pH*

Compound	Metal chelate	pH 3	5	7	9	11	Compound	Metal chelate	pH 3	5	7	9	11
I	—	V-4	Br-1	Br-1	Br-1	Br-1	VII	—	B-5	Gy-4	Gy-3	Y-4	Y-3
	Zn^{2+}	B-7	V-6	V-6	V-2	V-2		Zn^{2+}	G-5	Gy-3	Br-3	Gd-3	B-5
	Pb^{2+}	B-9	B-6	V-4	V-4	V-3		Pb^{2+}	G-6	G-3	Gd-3	Y-2	Br-2
	Ag^{+}	B-7	V-1	V-2	V-3	V-3		Ag^{+}	G-6	Gy-2	Gd-2	Y-5	Br-5
II	—	B-10	G-8	P-7	Y-7	P-7	VIII	—	V-2	V-1	V-1	Y-3	Y-3
	Zn^{2+}	B-10	B-10	B-10	V-7	V-7		Zn^{2+}	Br-5	Y-5	G-8	G-8	G-9
	Pb^{2+}	G-10	P-10	P-10	P-10	P-10		Pb^{2+}	O-6	V-5	V-5	V-4	V-5
	Ag^{+}	G-10	V-4	P-5	P-7	P-10		Ag^{+}	O-6	V-3	V-3	P-5	V-3
III	—	B-9	V-8	V-8	V-8	V-8	XXVIII	—	B-2	V-3	V-3	V-3	V-3
	Zn^{2+}	B-10	B-8	B-6	V-5	V-4		Zn^{2+}	B-5	V-4	V-4	V-4	V-4
	Pb^{2+}	B-7	V-7	V-8	V-9	V-9		Pb^{2+}	B-6	V-4	V-4	V-4	V-4
	Ag^{+}	B-6	V-7	V-7	V-7	V-7		Ag^{+}	B-6	V-4	V-3	V-3	V-3
IV	—	G-6	B-5	B-5	B-4	B-4	XXIX	—	Gd-3	Gd-3	Gd-3	Gd-3	Gd-3
	Zn^{2+}	G-9	B-9	B-9	B-8	G-8		Zn^{2+}	G-6	Br-5	Br-5	Br-5	G-6
	Pb^{2+}	B-7	B-7	B-7	B-7	B-6		Pb^{2+}	G-7	Br-5	Br-5	Br-5	G-6
	Ag^{+}	B-7	B-8	B-8	B-8	B-8		Ag^{+}	G-7	Br-5	Br-5	Br-5	G-6
V	—	B-8	V-7	V-4	V-4	V-4	XXX	—	Gd-2	Gd-1	Gd-1	Gd-1	Gd-1
	Zn^{2+}	B-9	V-8	P-7	P-6	G-10		Zn^{2+}	G-4	G-4	G-4	G-2	Br-1
	Pb^{2+}	V-9	V-7	V-6	V-6	V-6		Pb^{2+}	G-2	G-1	G-1	Br-1	Br-1
	Pb^{2+}	V-9	V-7	V-6	V-6	V-6		Ag^{+}	G-2	G-1	G-1	Br-1	Br-2
	Ag^{+}	V-10	V-7	V-6	V-6	V-6	XXXI	—	B-2	V-3	V-3	V-3	V-3
VI	—	B-7	B-7	B-6	B-5	B-5		Zn^{2+}	G-4	B-6	B-4	B-3	B-3
	Zn^{2+}	B-9	B-8	B-8	B-8	B-5		Pb^{2+}	G-4	B-5	B-5	B-5	B-4
	Pb^{2+}	B-10	B-9	B-8	B-8	B-8		Ag^{+}	G-2	B-2	B-1	B-3	B-3
	Ag^{+}	B-9	B-7	B-7	B-7	B-7							

* Colours observed in dark on excitation with ultraviolet lamp: V = violet; B = blue; G = green; Y = yellow; O = orange; P = pink; Br = brown; Gd = gold; and Gy = grey. Intensities estimated on scale of 10, where 1 = lowest and 10 = highest intensity observed.

Table 2. Excitation and emission wavelengths and relative intensities of fluorescence of II and its zinc chelate

pH	Free ligand λ_{ex}	λ_{em}	R.I.	Zinc chelate λ_{ex}	λ_{em}	R.I.	R.I.†	ΔR.I.‡
0.8	350	483	69.3	350	479	72.2	69.3	+2.9
1.7	348	500	55.1	352	442	68.8	32.2	+36.6
2.8	352	441	33.0	353	436	69.6	32.2	+37.4
3.6	346	414	26.5	353	432	65.6	23.3	+42.3
4.4	349	406	23.8	353	432	53.0	17.4	+35.6
5.2	344	407	29.4	355	432	50.0	18.5	+31.5
6.0	344	402	29.5	355	431	48.0	17.4	+30.6
7.0	348	398	21.4	352	432	32.2	11.9	+20.3
7.8	346	399	22.0	346	407	24.1	19.1	+5.0
8.8	346	402	23.4	346	402	24.6	23.4	+1.2
10.2	346	402	25.3	345	402	26.0	25.4	+0.6
11.1	346	401	28.9	346	401	29.4	29.0	+0.4
12.2	346	400	29.6	345	400	31.9	29.6	+2.3

* The zinc concentration is $2 \times 10^{-5} M$; the 2-MPPB concentration is $8 \times 10^{-5} M$; the solutions are 50% in ethyl alcohol; λ_{ex} is the primary excitation wavelength (nm); λ_{em} is the emission wavelength (nm); a 1-ppm quinine sulphate solution in $0.1N$ H_2SO_4 has relative intensity (R.I.) 8.8; the pH is that of the aqueous buffer before addition to the analyte solution.

† Relative intensity of 2-MPPB at the excitation and emission wavelengths of its zinc chelate.

‡ ΔR.I. is equal to the relative intensity of the zinc chelate minus the relative intensity of 2-MPPB free ligand measured at the same wavelengths as the zinc chelate.

Table 3. Accuracy and precision of fluorometric measurements by the recommended procedure

n	Zinc, ng/ml μ*	Zinc, ng/ml $\bar{X}$†	Mean error %	Std. devn, ng/ml	Rel. std. devn.%
5	10.0	10.4	4.0	1.3	12.7
5	20.0	20.4	2.0	2.3	11.1

* True mean
† $\bar{X}$ Observed mean.

Table 4. Effect of various ions on the determination of 10-ng/ml zinc solution by the recommended procedure

Ions tolerated at 100 ppm:
NH_4^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Pb^{2+}, Cl^- citrate, acetate, NO_3^-, ClO_4^-, SO_4^{2-}, OH^-

Ions tolerated at 1 ppm:
Al^{3+}, Cr^{3+}, Mn^{2+}, I^-

Ions tolerated at 10 ng/ml:
Cd^{2+}, Ag^+, Cu^+, Hg^{2+}, Ni^{2+}, Fe^{2+}

Co^{2+} interferes at 10 ng/ml

For accurate fluorometric determination of zinc with MPPB it is necessary to reproduce precisely both the amount of MPPB taken and the final volume of solution prepared for measurement. This is because the excess of free ligand also fluoresces. It also absorbs fluorescent emission from the zinc chelate. Use of a suitably prepared calibration curve, although non-linear, empirically corrects for both effects. The optimum total concentration of MPPB for maximum sensitivity was found to be approximately $8 \times 10^{-5} M$. Below this level there may be insufficient ligand available for formation of zinc chelate; above it, the greater the excess of MPPB, the more the fluorescence is diminished by the inner filter effect.

Replicate determinations of zinc in known solutions gave the results and statistical quantities listed in Table 3. Accuracy and precision are comparable to those of most fluorometric procedures, while sensitivity is one or two orders of magnitude greater than that using 3-hydroxyquinoline[29] or benzoin.[30]

Study of the effects of added substances yielded the results presented in Table 4. The method is relatively free from interferences, but best suited for samples which contain only trace quantities of transition metal ions.

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ANALYTICAL DATA

EFFECT OF pH ON THE CONDITIONAL REDUCTION POTENTIAL OF THE TRIS(1,10-PHENANTHROLINE)IRON(III,II) COUPLE

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Summary—The half-wave potential for reduction of tris(1,10-phenanthroline)iron(II) at a rotated platinum electrode is constant from pH 0 to 11. The value is 1.06 V at 25.0° and ionic strength 1.0 and is equal to the conditional reduction potential of the tris(1,10-phenanthroline)iron(III,II) couple, as demonstrated by cyclic voltammetry.

The commonly accepted value of the conditional reduction potential for the reaction

$$(phen)_3Fe(III) + e^- \rightarrow (phen)_3Fe(II) \qquad (1)$$

where phen = 1,10-phenanthroline, is 1.06 V, measured in 1*M* sulphuric acid.[1] George *et al.*[2] measured the reduction potential for reaction (1) at different ionic strengths in 0.0045*M* nitric acid medium and obtained a value of 1.120 V at zero ionic strength and 25°. They observed an increase in potential with decreasing ionic strength, as predicted by theory. Others have reported that the reduction potential increases from 0.76 V in 8*M* sulphuric acid to 1.12 V in 0.005*M* sulphuric acid.[3] Similar trends have been observed for the iron derivatives of various substituted 1,10-phenanthrolines, and for the bipyridyl, terpyridyl and mixed cyano-bipyridyl complexes of iron, ruthenium and osmium.[3–5] It has been noted that the shift in potential for these systems is greater than can be explained by ionic strength effects,[4] and several authors have suggested that a specific interaction with hydrogen ion is involved.[6,7] No data are available for the reduction potential for reaction (1) at pH above 2.5, and the impression left by the literature is that even more positive values might be obtained in neutral or basic solution. This investigation was undertaken to determine whether this is the case.

EXPERIMENTAL

Reagents

1,10-Phenanthroline monohydrate was used as received. The reagent grade $FeSO_4 \cdot 7H_2O$ was found to be 99.9% pure by titration with dichromate. Distilled demineralized water and reagent grade chemicals were used to prepare the solutions.

Apparatus

Current–potential curves were obtained with a rotated platinum electrode consisting of a length of 19-gauge platinum wire sealed into a 6-mm glass tube (partially filled with mercury for electrical contact) and projecting for 7 mm. This was inserted into a 200-ml cell through a Teflon cover with holes for glass and saturated calomel electrodes, an isolated auxiliary electrode and a nitrogen inlet. The cell was partially immersed in a water-bath at 25.0 ± 0.1°. The platinum electrode was rotated at 600 rpm. Current–potential measurements were made with a Beckman Electroscan 30 and a Fluke model 8030A multimeter. A Corning model 112 pH-meter and glass-electrode assembly was calibrated against NBS standard buffers.

Current–potential curves

Unbuffered solutions of $5.0 \times 10^{-3}M$ tris(1,10-phenanthroline)iron(II) were prepared by dissolving 0.0695 g (0.25 mmole) of $FeSO_4 \cdot 7H_2O$ and 0.150 g (0.75 mmole) of 1,10-phenanthroline monohydrate in 50 ml of dilute hydrochloric acid containing enough potassium chloride to give an ionic strength of 1.0. The pH was adjusted with small volumes of dilute hydrochloric acid or sodium hydroxide solutions in 1.0*M* potassium chloride. An atmosphere of nitrogen was maintained over neutral and basic solutions to prevent pH changes due to absorption of carbon dioxide. Some solutions were deoxygenated, but this had no effect on the voltammograms in the potential range of interest. After adjustment of pH, the solution was allowed to stand for 15 min, then the potential was scanned once from +1.15 V *vs.* SCE to the cathodic limit and back again. Before each scan the electrode was pretreated by being kept for 2 min at the potential corresponding to the foot of the cathodic limiting wave, then for 2 min at 1.15 V. The electrode was soaked in dichromate–sulphuric acid before each series of scans.

For reversibility studies, cyclic current–potential curves were obtained for a quiescent solution, with a platinum disc electrode (area about 24 mm^2), shielded with a short length of 7-mm glass tubing attached to the end of the electrode, as described by Laitinen and Kolthoff.[8]

RESULTS AND DISCUSSION

Rationale for using half-wave potentials

The classical potentiometric method for the measurement of reduction potentials is not well-suited to the iron–phenanthroline system because of the instability of the iron(III) derivative. It has been possible to obtain reliable data for highly acidic solutions by rapid addition of enough oxidant to react with exactly half of the iron(II) complex.[4] The oxidized form dissociates at a rate which increases with increasing pH,[9] and undergoes instantaneous reduction in alkaline solution,[4] so a potentiometric measurement of the reduction potential in neutral or basic solutions would be extremely difficult. These difficulties, coupled with the fact that the couple is usually used as a redox indicator in highly acidic media, probably account for the lack of reduction potential data at high pH. The iron(II) complex, however, is stable indefinitely from pH 2 to 9, so measurement of half-wave potentials for solutions containing only the reduced form avoids the problem of unstable solutions. For a reversible electrode process which involves only dissolved species, $E_{\frac{1}{4}}$ is given by the relation

$$E_{\frac{1}{4}} = E^{o\prime} + \frac{RT}{nF} \ln \left(\frac{D_o}{D_r}\right)^{\frac{1}{4}}$$

where D_o and D_r represent the diffusion coefficients of the oxidized and reduced forms, and $E^{o\prime}$ the conditional reduction potential. For the system under investigation the diffusion coefficients have not been measured, but D_o and D_r would be expected to be nearly equal, leading to $E_{\frac{1}{4}} \cong E^{o\prime}$. It will be shown later that, where potentiometric measurements have been made, the values obtained by potentiometry and voltammetry are identical.

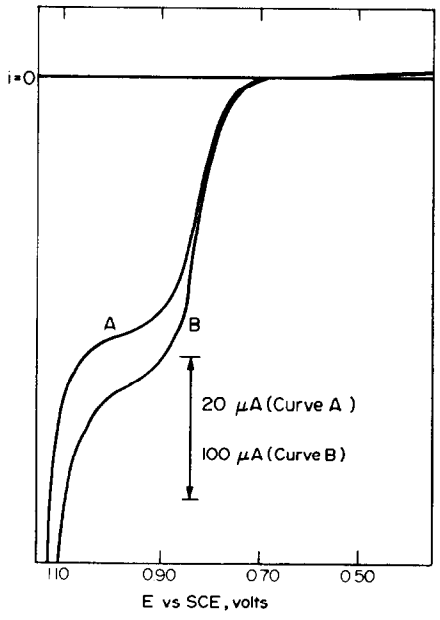

Fig. 1. Current-potential curves at rotating platinum electrode for (A) $1.0 \times 10^{-3}M$ tris-(1,10-phenanthroline)-iron(II) in 1.0*M* KCl–0.10*M* phosphate at pH = 7.05, and (B) $5.0 \times 10^{-3}M$ tris(1,10-phenanthroline)iron(II) in 1.0*M* KCl at pH = 6.73. Scan direction from + to −, scan-rate = 10 mV/sec.

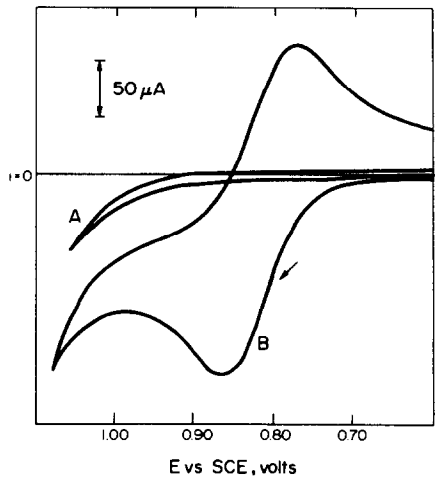

Fig. 2. Cyclic voltammograms at a stationary platinum electrode; 0.10*M* phosphate–1.0*M* KCl at pH 7.0 (A) without and (B) with $5.0 \times 10^{-3}M$ tris(1,10-phenanthroline)-iron(II) present. Scan-rate = 100 mV/sec.

Reversibility studies

The reversibility of an electrode reaction is best evaluated by cyclic voltammetry at a stationary electrode, for which Nicholson and Shain have developed the theory for a variety of cases.[10] For a one-electron transfer the electrode reaction is reversible if the separation between the cathodic and anodic peaks is 0.058 V, and if the cathodic and anodic peak currents are equal. In this study the half-wave potentials were taken from current–potential curves obtained at an ionic strength of unity. Under these conditions the anodic wave for oxidation of water is too close to that of the iron(II) complex (Fig. 1) for suitable cyclic voltammetric data to be obtained. However, the oxidation of water is shifted to more positive potential in phosphate buffer, but the iron–phenanthroline wave is not shifted at all, making it possible to obtain good cyclic voltammagrams in the presence of phosphate. Note that in Fig. 1 the effect of phosphate is deemphasized because the current span and concentration are greater for curve B than for curve A.

Representative cyclic voltammograms are shown in Fig. 2. The ratio of the cathodic- and anodic-peak currents was 1.00 and 0.96 for scan-rates of 100 and 50 mV/sec, respectively. At slower scan-rates the ratio decreased significantly. The peak separation (ΔE_p) and anodic-peak potential ($E_{p,a}$) varied with scan-rate (V), as shown in Table 1. A plot of the rate of shift of potential ($\Delta E_{p,a}/\Delta \log V$) as a function of scan-rate, Fig. 3, suggests that the electrode reaction is a rever-

Table 1. Variation of peak separation and anodic peak potential with scan-rate

V, mV/sec	ΔE_p, *V*	$E_{p,a}$, *V vs. SCE*
500	0.19	0.89
200	0.114	0.874
100	0.093	0.864
50	0.082	0.856
20	0.071	0.851
10	0.066	0.848
5	0.060	0.844

sible charge-transfer either preceded by a homogeneous first-order chemical reaction or followed by an irreversible catalytic reaction.[10] In connection with this we note that the limiting current at the rotated platinum electrode for solutions containing phosphate was about 75% of that obtained in the absence of phosphate, indicating that phosphate may be involved in the coupled reaction.

A possible explanation of these observations is given by the sequence of reactions

$$RP \rightleftharpoons R + P \quad (2)$$

$$R \rightleftharpoons O + e^- \quad (3)$$

$$O \rightarrow D \quad (4)$$

where R = tris(1,10-phenanthroline)iron(II), P = phosphate, O = tris(1,10-phenanthroline)iron(III), and D = decomposition product. According to this scheme, an ion-pair is initially present, which must dissociate [reaction (2)] before the reversible heterogeneous electron-transfer [reaction (3)]. Following oxidation a relatively slow decomposition of the iron(III) complex occurs [reaction (4)]. This last reaction is responsible for the small peak-current ratios obtained at slow scan-rates. The favourable current ratios obtained at faster scan-rates (50 and 100 mV/sec) suggest that no significant decomposition should occur at a rotated electrode, and that reaction (4) would not affect the reversibility of the electrode reaction at a rotated electrode, regardless of scan-rate. Reaction (2) results in a shift in anodic-peak potential with scan-rate, but this effect disappears for scan-rates <5 mV/sec. Therefore, the overall electrode reaction will appear completely reversible at a rotated electrode if the scan-rate does not exceed 5 mV/sec. Also, the cyclic voltammogram obtained at this scan-rate will provide an accurate measure of the conditional reduction potential.

Half-wave potentials

Under the conditions of reversibility defined above, the half-wave potential in 0.10*M* phosphate and 1.0*M* potassium chloride (ionic strength = 1.2) at pH 7.0 and 25.0° is 1.06 V *vs.* NHE. This value, which is regarded as the conditional reduction potential, was taken from the cyclic voltammogram scanned at 5 mV/sec. Results for other conditions of pH and supporting electrolyte were taken from single-sweep voltammograms at a rotated platinum electrode. All curves obtained in the absence of phosphate were similar to Curve B in Fig. 1, independent of pH. The proximity of the wave for oxidation of water made exact determinations of the half-wave potentials difficult, but our calculations show that errors from this source would produce uncertainties of ≤ 5 mV in the value of the half-wave potential. The error in measuring the applied potential was also about ± 5 mV, so the total error in the half-wave potentials is not more than ± 10 mV.

Half-wave potentials obtained at various pH-values are listed in Table 2. The potential is constant within experimental error over the entire pH-range from 0 to 11, regardless of the presence of phosphate. The slopes obtained for *E vs.* $\log[i/(i_l - i)]$ where i = cur-

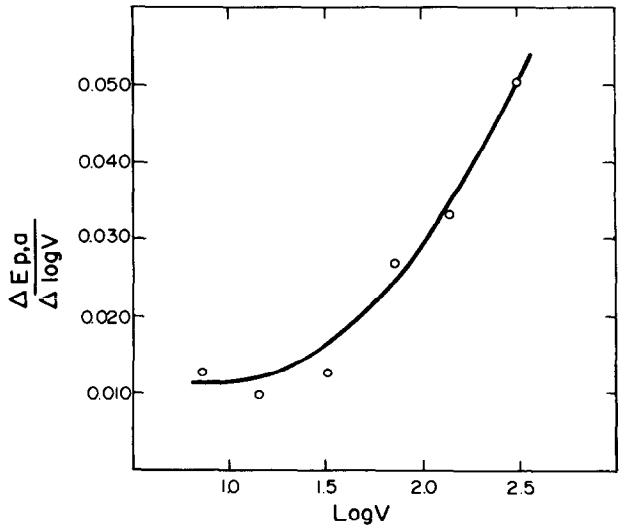

Fig. 3. Rate of shift of potential as a function of scan-rate.

Table 2. Half-wave potentials at various pH-values*

pH	Ionic strength	Electrolyte	$E_{\frac{1}{2}}$ *vs.* NHE, *V*	Slope, *V*†
0	1.0	1.0*M* HCl	1.06	
1	1.0	0.9*M* KCl + 0.1*M* HCl	1.064	0.0690
3.0	1.0	1.0*M* KCl + 10^{-3}*M* HCl	1.060	0.0618
5.0	1.0	1.0*M* KCl + HCl	1.066	0.0620
6.7	1.0	1.0*M* KCl	1.064	0.0644
8.1	1.0	1.0*M* KCl + NaOH	1.051	0.0620
11.0	1.0	1.0*M* KCl + NaOH	1.053	0.0591
7.0	1.2	1.0*M* KCl + 0.10*M* phosphate	1.064	0.0580
9.0	1.3	1.0*M* KCl + 0.10*M* phosphate	1.065	0.0585
11.1	1.4	1.0*M* KCl + 0.10*M* phosphate	1.061	0.0598

* Potentials were measured with SCE reference. *E vs.* NHE = *E vs.* SCE + 0.245 V. Temperature was 25.0 ± 0.1°C.

† For plot of *E vs.* $\log[i/(i_l - i)]$.

rent and i_l = limiting current, indicate good reversibility. The overall average value (1.0*M* potassium chloride medium) is 1.060 ± 0.005 V (90% confidence level), in excellent agreement with the value obtained by cyclic voltammetry under conditions of reversibility at pH 7, and with the accepted potentiometric value in 1*M* sulphuric acid (ionic strength = 1.1, calculated from pK_a = 1.06 for bisulphate[11] at ionic strength = 1.0).

CONCLUSION

Considering the good agreement among the various methods of measurement, we conclude that the conditional reduction potential of the tris(1,10-phenanthroline)iron(III,II) couple is 1.06 V over the entire pH-range from 0 to 11 for ionic strength 1.0 at 25°. While this result does not rule out the possibility of a specific interaction with hydrogen ions at higher acid concentrations, it allows us to confidently disregard any effects of pH at hydrogen-ion concentrations lower than 1*M*, except for one. As noted earlier, tris(1,10-phenanthroline) iron(III) may undergo fairly rapid decomposition at high pH, making observation of the conditional reduction potential experimentally difficult except in highly acidic media.

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HYDROXIDE COMPLEXES OF LANTHANIDES—III*

GADOLINIUM(III) IN PERCHLORATE MEDIUM

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Summary—From the precipitation borderline in the pM′–pH diagram, determined experimentally under CO_2-free conditions, the stability constants of the mononuclear and polynuclear species of gadolinium hydroxide have been established. The values found are $\log^*\beta_1 = -7.3$, $\log^*\beta_2 = -14.6$, $\log^*\beta_3 = -21.9$, $\log^*\beta_{4,3} = -19.0$ and $\log {}^*K_{s0} = 17.0$. They refer to fresh precipitates, prepared at room temperature in sodium perchlorate medium with an ionic strength of 1.

In previous papers[1,2] the determination of the hydrolysis constants of cerium(III) and samarium(III) was described. In this paper the hydrolysis of gadolinium(III) will be discussed. The hydrolysis constants published (see Table 1) show a fair diversity, in some cases because of neglect of the presence of polynuclear hydroxide complexes, but mostly because of differences in experimental conditions.[1] Additionally, the need for exclusion of CO_2 has not always been recognized as important in studying lanthanide hydrolysis. In the unbuffered media near pc_H 7 we are dealing with, the pc_H is very sensitive to even trace amounts of hydrogen ions. The absorption of carbon dioxide by the solution and the formation of small amounts of carbonate will release hydrogen ions, and this influences the pH. Concerning the hydrolysis constants, it should be noted that concentration constants can only be found if pc_H can be measured directly or if the measured pa_H can conveniently be converted into concentration units. Values determined for concentration constants always depend on the method used for calibrating the pH equipment. It is a pity that this is not always clearly indicated in publications. It may lead to misinterpretation by up to about 0.7 pH units. For accurate determinations calibration in pc_H units is preferable.

Our experiments with Gd(III), done in sodium perchlorate medium (I = 1.0, 23°), gave reproducible results when fresh precipitates were formed under nitrogen in a glove-box. This is in accord with our previous experience.[1,2]

THEORY

In previous publications[1,2,3] it has been shown that a borderline of precipitation in the pM′–pH diagram can be estimated by straight-line segments for which the following equation holds:

$$pM'_{max} = (np - q)pH - (p \log^* K_{s0} + \log^* \beta_{q,p} + \log p) \quad (1)$$

The envelope curve, which has to coincide with the borderline, can be described by

$$[M']_{max} = \sum_p \sum_q 10^{-(np-q)pH + (p\log^* K_{s0} + \log^* \beta_{q,p} + \log p)} \quad (2)$$

When the envelope curve has been fitted to the experimental points, the stability constants and the probable composition of the hydroxide complexes can be found from the position of the corresponding straight-line segments and their points of intersection. This has been done previously for cerium[1] and samarium.[2] These papers should be consulted for more detailed discussion of the theory.

EXPERIMENTAL

All manipulations were performed in a large glove-box, which was kept permanently under nitrogen in order to obtain a CO_2-free atmosphere. Access to the box was gained through an air-lock.

The pc_H measurements were made with a glass–calomel electrode system and a Radiometer pH-meter. If a calomel electrode is inserted in a concentrated perchlorate solution the porous liquid-junction contact clogs, because of precipitation of potassium perchlorate. In the samarium(III) experiments,[2] this was prevented by filling the calomel electrode with a concentrated solution of sodium chloride. During subsequent experiments it turned out that use of an electrode filled with potassium chloride in combination with a bridge filled with saturated ammonium nitrate solution (11.1M) has some advantages over the calomel electrode filled with sodium chloride [less drift, less noise, small liquid-junction potentials and negligible changes of these potentials when a calibration solution is replaced by an unknown solution of the same ionic strength (I = 1.0)]. Electrical contact between the solution to be measured and

* Part II: *Talanta*, 1979, **26**, 1105.

Table 1.

Ref.	$\log^*\beta_1$	Polycomplex	$\log^*\beta_{q,p}$	$\log^*K_{s0}$	Medium	Method
3	−7.2				sulphate, 0.05*M*	pH determination
3	−8.9				sulphate, 0.001*M*	pH determination
4				21.7	I = 0.1, T = 20°, 2 min aging	precipitation titration
5	−9.65				$NaClO_4$, 0.3*M*	potentiometric titration
6	−7.1				(H, Li)ClO_4, I = 0.1, 25°	solvent extraction and radiochemical indicator
6	−7.5				(H, Li)ClO_4, I = 0.5 25°	solvent extraction and radiochemical indicator
7	−8.27				NaCl + $NaClO_4$, I = 0.05 25°	potentiometric titration
8	−8.20				$LiClO_4$, 3*M*, 2 days aging, 25°	coulometry and pH
9	−9.2	$Gd_2(OH)_2$	−14.23	17.5 (I = 0)	$NaClO_4$, 3*M*, 25°	potentiometric titration, graphical
10				15.1	$NaClO_4$, 3*M*, 25°, aged precipitate	
11	−8.3				I = 0.05	empirical relation
11	−9.5	$Gd_2(OH)_2$	−13.8		I = 3	empirical relation

the bridge solution was made through an asbestos-filled capillary. The pH-measuring equipment was calibrated in pc_H units by means of dilute buffers in sodium perchlorate medium (I = 1.0). The following buffer solutions were used: 0.0100*M* perchloric acid standardized against iodate, to which pc_H 2.00 can be assigned; 0.025*M* acetate +0.025*M* acetic acid, to which pc_H 4.60 can be assigned; 0.0500*M* Na_2HPO_4, titrated exactly to half-neutralization with perchloric acid (thus making the concentrations of HPO_4^{2-} and $H_2PO_4^-$ equal), to which pc_H 6.26 can be assigned.

The assigned values lie on a straight line on a pc_H–mV diagram. The response efficiency of the glass electrode was 99%. This is all in accordance with the investigations of Bates[14,15] and McBryde[16,17] on the calibration of pH-equipment in pc_H units. The pc_H values assigned to the buffers are deduced from investigations by Ellilä[18] and Baes *et al.*[19–21] in combination with experimentally determined corrections to be made because of the change from potassium chloride to sodium perchlorate medium. The pc_H can be measured to ±0.02, which is satisfactory in comparison with the error in the experimental results.

Procedure

Prepare the gadolinium solutions by dissolving commercial 99.9% pure Gd_2O_3 in 1*M* perchloric acid by heating. Perform the experiments with 10^{-2}*M* gadolinium stock solutions except for the range pGd′ < 2, for which a 0.1*M* solution has to be used.

Add carbonate-free 50% sodium hydroxide solution dropwise to a 100-ml portion of the gadolinium stock solution with vigorous stirring until pc_H 1 is reached. Add dilute (carbonate-free) alkali until pc_H 4 is reached and cool the solution to room temperature. Add dilute alkali until precipitation starts. Separate precipitate and solution by decantation after exactly 30 min of aging. Centrifuge the decanted solution in order to collect residual and colloidal particles. Withdraw aliquots from the tube, measure the pc_H, acidify to pc_H 1 and keep the solution for later determination of Gd. Take a second 100-ml portion, proceed as previously, but adjust the pc_H to a slightly higher value (+0.2) after precipitation has started. Separate precipitate and solution after 30 min and continue as described before.

Repeat the procedure for another portion, increasing the pc_H by ~0.2 again. Proceed until the whole pc_H range of interest has been covered.

Take the acidified solutions out of the glove-box and determine the gadolinium contents by photometric titration with EDTA at pc_H 5.8 with Xylenol Orange as the indicator (wavelength 575 nm).

Special attention must be paid to keeping the ionic strength at 1.0 during the precipitation stage, all reagent solutions being made up in sodium perchlorate to I = 1.0.

RESULTS AND CONCLUSIONS

The experimental results are plotted in Fig. 1. No experiments were done for pGd′ < 1 because of the uncertainty in the corrections to be made for ionic strengths > 1 (see also refs. 1 and 2).

The borderline of the precipitation region of Gd consists of two nearly straight parts connected by a bend ranging from pc_H 7.3 (pGd′ 4.0) to pc_H 8.5. Regression analysis of the steep part up to pGd′ = 4.0 shows that the slope can be estimated as 4.7 ± 0.3. This leads to the conclusion that a polynuclear hydroxide complex must be present with a charge of at least +5. Only speculative remarks can be made about the real identity of the polycomplex species. By arguing in the same way as in the samarium paper,[2] it can be deduced that $Gd_3(OH)_4^{5+}$ is the most likely polycomplex formed and that polycomplexes with other charges are very unlikely. A slope of exactly 5 can be assigned to the straight-line segment that accounts for the steep part of the envelope curve. The following set of equations can be given for the straight-line segments (Table 2).

If equation (3a) is assigned to the steep part of the borderline (ranging up to pGd′ 4.0) the best fit to the experimental points is obtained with the equation

$$\text{pGd}' = 5\,pc_H - (32.5 \pm 0.1) \qquad (4)$$

Hence

$$3\log^*K_{s0} + \log^*\beta_{4,3} = 32.0 \pm 0.1 \qquad (5)$$

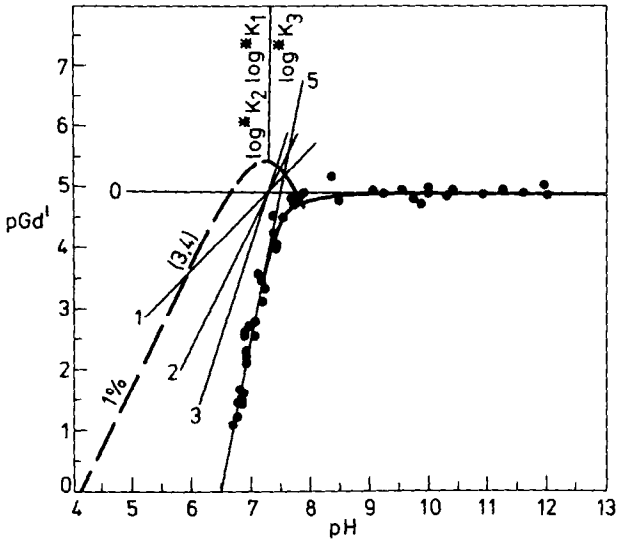

Fig. 1. The solid curve [the borderline of precipitation of $Gd(OH)_3$] was constructed with the values given in Table 3. The dashed line is the borderline for 1% polycomplexation. The numbers 0, 1, 2, 3 and 5 near the straight lines correspond to the slopes of the straight-line segments approximating the exact envelope curve [equations (3a)–(3e)]. The dots denote the experimental results.

Table 2.

Equation number	p	q	Slope $(np - q)$	Equation for the borderline segment
3a	3	4	5	$pGd' = 5pc_H - (3 \log^*K_{s0} + \log^*\beta_{4,3} + 0.5)$
3b	1	0	3	$pGd' = 3pc_H - \log^*K_{s0}$
3c	1	1	2	$pGd' = 2pcH - (\log^*K_{s0} + \log^*\beta_1)$
3d	1	2	1	$pGd' = pc_H - (\log^*K_{s0} + \log^*\beta_2)$
3e	1	3	0	$pGd' = -\log^*K_{s3} = -(\log^*K_{s0} + \log^*\beta_3)$

$$^*\beta_{q,p} = \frac{[Gd_p(OH)_q][H^+]^q}{[Gd^{3+}]^p}; \quad ^*\beta_{q,1} \equiv {}^*\beta_q \text{ and } [Gd(OH)_3]_{max} = {}^*K_{s3} = {}^*\beta_3{}^*K_{s0}.$$

Equation (3e) can be assigned to the horizontal part of the borderline. The best fit corresponds to

$$pGd' = 4.90 \pm 0.06 \tag{6}$$

from which follows

$$\log^*K_{s0} + \log^*\beta_3 = -4.90 \pm 0.06 \tag{7}$$

Equations (5) and (7) can be substituted in equation (2). With the equation

$$[Gd']_{max} = 10^{-5pc_H+32.5} + 10^{-4.9} \tag{8}$$

derived from equation (2) by omitting the other terms, a good fit to the experimental points making up the bend in the borderline is obtained, and it turns out to be the best fit which can be achieved. A similar situation was found with samarium.[2] Analogous conclusions can be drawn.

(*a*) The other straight-line segments corresponding to equations (3b)–(3d) do not contribute to the envelope curve.

(*b*) The limiting positions of these line-segments correspond to $p^*K_1 = 7.3$, $p^*K_2 = 7.3$ and $p^*K_3 = 7.3$. The p^*K_i values cannot exceed these values as there would then be a contradiction with (*a*), and the envelope curve would exhibit a worse fit to the experimental points.

(*c*) Lower p^*K_i values lead to an increase in the size of the polycomplex region indicated in Fig. 1 by the 1% polycomplex borderline.[2,12,13] As hydrolysis does not become appreciable at 10^{-2}–$10^{-3}M$ concentrations until pH values above 5.5 are reached, it follows that the interval in which the three p^*K_i values can be varied is very small. The upper limit values which correspond to a minimum extent of the polycomplex region are likely to be the best estimations.

Table 3.

$\log^*\beta_1 = -7.3$	$\log^*\beta_{4,3} = -19.0$
$\log^*\beta_2 = -14.6$	
$\log^*\beta_3 = -21.9$	$\log^*K_{s0} = +17.0$

Substitution of the above-mentioned upper limits in equations (5) and (7) finally completes the set of hydrolysis constants (Table 3). This set can be regarded as useful for analytical practice.

DISCUSSION

The differences between our results and those listed in Table 1 can be attributed to the neglect of the occurrence of polyhydroxide formation,[3–8] the neglect of sulphate (or other anion) interactions,[3] the use of too simple an extraction model,[6] different ionic strengths, different aging times of the precipitate, different or undefined pH scales for calibration, and the non-exclusion of carbon dioxide.

It has been concluded that $Gd_3(OH)_4^{5+}$ is the predominant species in the polycomplex region. Although this has not been established unambiguously, it is likely from the steepness of the precipitation borderline as far as $pGd' = 4.0$ that $Gd_2(OH)_2^{4+}$, assumed by others,[9,11] is not formed to a measurable extent.

Baes and Mesmer[11] report the possibility of formation of $Gd(OH)_4^-$ species and give a value $p^*K_4 = -9.2$ on the basis of the increased solubility reported by others.[22–25] We could find no experimental evidence that the solubility increases in the pc_H region 9.2–12.0.

Further experiments on ytterbium are in preparation.

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OXYMERCURIC METHOD OF ORGANIC ANALYSIS IN NON-AQUEOUS SOLUTIONS

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Summary—A new method of non-aqueous titration based on the oxymercuration of organic compounds is presented. A kinetic investigation of the oxymercuration of olefin and acetylene bonds has permitted the optimal conditions for quantitative determination to be found. The selectivity and determination are given.

Non-aqueous titration is very versatile.[1] At first it was used only for the analysis of substances insoluble in water, but its use has been greatly extended owing to realization that non-aqueous solvents can change equilibrium constants and reaction rates; the last factor is very important for organic analysis.

The application of acid–base titration in non-aqueous solution is described in a number of monographs.[2–5] The search for new non-aqueous titration reagents which give addition and complexation reactions useful in functional organic analysis, has led to the investigation of aqueous and non-aqueous solutions of mercury salts. The tendency to form stable complexes and insoluble compounds, and the participation of mercury ions as catalysts or reagents in hydrocarbon transformations, have led to the development of sensitive and selective oxymercurimetric methods in organic analysis.

However, a purely empirical approach to working out a non-aqueous titration is not the best in organic analysis; it is superior to consider the thermodynamics and kinetics of the reaction. Mercury salts show great activity with respect to addition, redox and complexation reactions in non-aqueous solvents (alcohols, acetic acid, *etc.*), cationic mercury complexes or mercury ions with low solvation being the reactive species.[6]

DETERMINATION OF UNSATURATED COMPOUNDS

Olefins

The reaction rate of the electrophilic oxymercuration of olefins is determined by the electron density of the double bond and the nature of the solvated complexes of the reagents. In alcohols the reaction observed is

$$>C{=}C< \; + \; HgX_2 \; + \; ROH \rightarrow \; >\underset{OR}{\underset{|}{C}}{-}\underset{HgX}{\underset{|}{C}}< \; + \; HX \quad (1)$$

with formation of the alkenemercurium cation in the rate-determining step.

The influencing of the solvent on reaction rate is not simple. It is not only determined by the polarity of the medium: for example, the logarithms of the rate constants of the non-catalysed oxymercuration of cinnamic acid in methanol (dielectric constant[4] $\epsilon = 32.6$), ethylene glycol ($\epsilon = 37.7$)[4] and acetonitrile ($\epsilon = 37.5$)[4] are -1.79, -2.15 and -2.92. The reaction rate is also connected with the degree of solvation of the substrate, reagent and transition state ($\neq$), by the relation

$$\log k^S = \log k^{H_2O} + \underset{H_2O \to S}{\log \gamma_t}(HgX^+) + \underset{H_2O \to S}{\log \gamma_t}(>C{=}C<) - \underset{H_2O \to S}{\log \gamma_t}\left(\begin{matrix}>C{=}C< \\ \searrow\swarrow \\ HgX^+\end{matrix}\right)^{\neq} \quad (2)$$

where γ_t is the change in activity coefficient on transfer from water to solvent S.

The solvation of the electrophilic reagent HgX^+ delays this reaction; the stronger the binding of the ligands in the inner co-ordination sphere of the mercury solvato-complexes, the more difficult wll be the co-ordination of the cationic mercury complex with the olefin. The reaction rate of (1) is higher in alcohol and glycol media. An ethylene glycol–ethanol mixture provides an average rate for solvation of the reagents and a high reaction rate, which allows direct oxymercurimetric titration of olefins instead of the usual procedures (titration of the acetic acid or excess of the mercury salt) (Fig. 1, Table 1).

Mercuric salts react with olefins according to reaction (1). The ability of mercurous salts to undergo dismutation gives the possibility of using them for titration of olefins. Depending on the character of the substituents the reaction of olefins with mercury(I) salts can result in mono- (3) or di-mercuration (4)

$$>C{=}C< \; + \; Hg_2X_2 \; + \; ROH \rightarrow \; >\underset{OR}{\underset{|}{C}}{-}\underset{HgX}{\underset{|}{C}}< \; + \; Hg \; + \; HX \quad (3)$$

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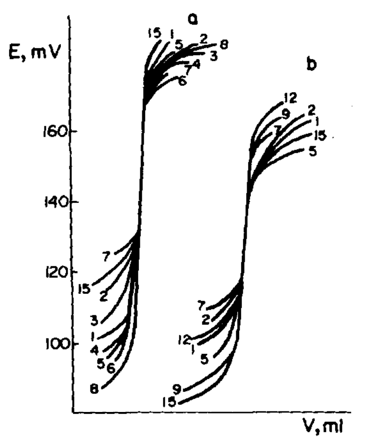

Fig. 1. Titration curves of olefins in ethylene glycol–ethanol mixture with $Hg_2(NO_3)_2$ (a) and $Hg(OAc)_2$ (b) (0.1*N*). The numbers correspond to the compounds listed in Table 1.

$$>C{=}C< + 2Hg_2X_2 \rightarrow >\underset{HgX}{C}{-}\underset{HgX}{C}< + Hg + HgX_2 \quad (4)$$

It takes 20 min to determine olefins by titration with mercury(II) acetate; the lower limit of determination is $9 \times 10^{-4}M$. In the titrations with mercury(I) the potential of the Pt-electrode equilibrates more slowly (the titration takes 30 min), but the potential jump (Fig. 1) at the equivalence point is larger (up to 150 mV). Except for phenylacetylene, compounds with acetylene bonds do not react; 1 mole of phenylacetylene consumes 2 moles of mercuric or mercurous salt. The acetylenes react more slowly than the olefins with mercury salts and other electrophilic reagents, and for the triple bond to react a large excess of mercury salt is needed, in a solvent with low solvating capacity.

Substituted acetylenes

The selective determination of acetylene bonds is based on reactions that are selective under certain conditions for triple bonds but not for double bonds.

The products of acetoxymercuration of substituted acetylenes

$$R{-}C{\equiv}C{-}R' + Hg(OAc)_2 \rightarrow R{-}\underset{OAc}{C}{=}\underset{HgOAc}{C}{-}R'$$

are characterized by spectral maxima in the region of 260–279 nm, whereas the mercurated olefins are characterized by another spectral absorption region (250–252 nm). The lower limit of spectrophotometric determination of substituted acetylenes after oxymercuration in acetic acid is $6 \times 10^{-5}M$ (Table 2). The presence of an olefin in 10–20-fold ratio to acetylene does not interfere.

Determination of double bonds of different activity

The kinetic investigations[8–10] indicate the possibility of differential determination of different types of olefin unsaturation: double bonds at the end of side-chains on aromatics react with mercury(II) quickly and quantitatively, but double bonds associated with electronegative groups react extremely slowly in the absence of the catalyst ($HClO_4$) (Table 3).

The kinetics of oxymercuration of olefin mixtures (Fig. 2) and calculations by the logarithmic extrapolation method[11] make it possible to analyse olefin mixtures. The vertical region of the curve corresponds to oxymercuration of the fast-reacting olefin, the slope to that of the slowly-reacting olefin and the horizontal region to the end of the reaction. Extrapolation of these regions permits determination of the total olefin content and the concentration of the less active olefin. The determination limit for the less active olefin, the

Table 1. Direct potentiometric titration of olefins

No.	Determined compound	Titration with Hg(II) *n*	taken, *mg*	found, *mg*	Titration with Hg(I) *n*	taken, *mg*	found, *mg*
1	Styrene	1	8.8	8.9 ± 0.1	2	10.5	10.8 ± 0.2
2	Allylbenzene	1	11.4	11.9 ± 0.5	2	7.7	7.8 ± 0.2
3	Cinnamic acid	1	11.7	11.9 ± 0.3	2	12.3	12.5 ± 0.2
4	4-Sulphocinnamic acid	1	47.2	47.6 ± 0.4	1	46.3	46.7 ± 0.4
5	*m*-Nitrocinnamic acid	1	25.1	25.4 ± 0.3	1	43.5	44.4 ± 0.2
6	Methacrylic acid	1	41.9	42.1 ± 0.3	1	11.8	12.0 ± 0.2
7	Acrylic acid	1	67.0	67.6 ± 0.6	2	7.1	7.3 ± 0.1
8	Methyl cinnamate	1	33.5	33.2 ± 0.3	2	24.9	24.7 ± 0.2
9	Ethyl cinnamate	1	52.2	51.6 ± 0.6	2	20.8	21.2 ± 0.4
10	Propyl cinnamate	1	15.7	15.9 ± 0.2	2	49.2	48.8 ± 0.4
11	Butyl cinnamate	1	14.6	14.9 ± 0.3	1	48.7	48.4 ± 0.3
12	Pentyl cinnamate	1	16.7	16.5 ± 0.2	1	49.8	50.3 ± 0.4
13	Hexyl cinnamate	1	23.2	22.8 ± 0.4	1	50.8	51.0 ± 0.2
14	Allyl alcohol	1	5.1	5.2 ± 0.1	2	6.2	6.3 ± 0.1
15	Vinyl butyrate	1	11.4	11.1 ± 0.2	1	50.7	50.9 ± 0.2
16	Vinyl acetate	1	49.5	50.0 ± 0.5	1	67.2	67.6 ± 0.4

n = the number of moles of mercury salt added per mole of unsaturated compound.

Table 2. Spectrophotometric determination of acetylenes

No.	Compound determined	λ_{max}, nm	ϵ_{max}, $l.mole^{-1}.cm^{-1}$	Taken, mM	Found, mM
1.	Butynediol	260	515	0.236	0.230 ± 0.005
	$HOH_2C-C \equiv C-CH_2OH$			2.360	2.358 ± 0.004
				1.200†	1.180 ± 0.011
				2.400†	2.370 ± 0.009
2.	Propargyl alcohol	260	424	0.374	0.365 ± 0.007
	$HC \equiv C-CH_2OH$			3.740	3.690 ± 0.004
				1.074†	1.071 ± 0.004
				3.580†	3.571 ± 0.014
3.	Propargylic acid	261	283×10^3	0.144	0.1400 ± 0.0033
	$HC \equiv C-CH_2COOH$			0.867	0.870 ± 0.004
				0.0296†	0.0280 ± 0.0016
				0.2960†	0.310 ± 0.005
4.	Phenylacetylene	263	9.80×10^3	0.0648	0.0652 ± 0.0004
	$HC \equiv C-C_6H_5$			0.216	0.214 ± 0.003
				0.0204*	0.0215 ± 0.0009
				0.1428*	0.1494 ± 0.0059
5.	Diphenylacetylene	279	3.32×10^4	0.1000	0.1001 ± 0.0013
	$H_5C_6-C \equiv C-C_6H_5$			1.000	0.940 ± 0.010
				0.0112*	0.0111 ± 0.0002
				0.0560*	0.0551 ± 0.0016
6.	Propargyl chloride	260	824	0.154	0.1490 ± 0.0036
	$HC \equiv C-CH_2Cl$			1.540	1.552 ± 0.009
				0.630†	0.630 ± 0.011
				2.100†	2.040 ± 0.097
7.	Propargyl bromide	260	3.26×10^3	0.0847	0.0842 ± 0.0004
	$HC \equiv C-CH_2B_2$			1.183	1.179 ± 0.004

* In the presence of 10-fold amount of styrene.
† In the presence of 10-fold amount of acrylic acid.

Table 3. Kinetic characteristics of olefin oxymercuration

No.	Mixture: Component A	Component B	Solvent	k, min^{-1}: A	B	k_A/k_B	τ, min	$[B]_{min}$, %
1.	Styrene	Cinnamic acid	MeOH	0.131*	0.0161*	8.1	25	18
2.	Styrene	Methyl cinnamate	MeOH	0.131*	0.0416	3.2	20	45
3.	Styrene	Ethyl cinnamate	MeOH	0.131*	0.0333	3.9	21	35
4.	Styrene	Methacrylic acid	MeOH	0.131*	0.0173*	7.6	25	18
5.	Styrene	Methyl methacrylate	MeOH	0.131*	0.00021*	620	75	—
6.	Styrene	Methyl methacrylate	MeOH	0.131*	0.0219	6.0	24	22
7.	Cinnamic acid	Methyl cinnamate	MeOH	0.180	0.0416	4.3	17	32
8.	Cinnamic acid	Ethyl cinnamate	MeOH	0.180	0.0333	5.4	18	25
9.	Acrylic acid	Cinnamic acid	MeOH	0.666*	0.0161*	41	9	—
10.	Acrylic acid	Methacrylic acid	MeOH	0.666*	0.0173*	38.5	9	—
11.	Acrylic acid	Methacrylic acid	EG + $CHCl_3$ (1:1)	0.438*	0.0096	45.6	14	—
12.	Methacrylic acid	Methyl methacrylate	MeOH	0.0173*	0.00021*	86.5	120	—
13.	Styrene	Cinnamic acid	EG	0.154*	0.00708*	21.8	30	—
14.	Styrene	Ethyl cinnamate	EG + EtOH (2:1)	0.216*	0.057	3.8	14	35
15.	Cinnamic acid	Ethyl cinnamate	EG + EtOH (2:1)	0.346	0.057	6.1	10	30

* Non-catalysed oxymercuration; in the other cases the data correspond to catalysis by $10^{-4}M$ $HClO_4$.

error and the reaction time are also shown in Table 3. The error for a 10^{-2}–$10^{-3}M$ olefin mixture does not exceed 2.4%. Instead of logarithmic extrapolation the method of proportional equations[11] can be used, which accelerates the determination but complicates the calculations. This method is used for separate determination of residual olefin content in co-polymers of styrene and methyl methacrylate.

EXPERIMENTAL

The solvation of mercury ions was studied by polarography at the rotating Pt-electrode with $1M$ lithium perchlorate as supporting electrolyte. The potentiometric curves of olefins were determined with Pt and (Tl/TlCl) electrodes. The olefin was dissolved in ethanol (50 ml), and an aliquot (5 ml) was mixed with 25 ml of ethylene glycol and titrated with aqueous $0.1M$ mercury(II) acetate or mercury(I) nitrate.

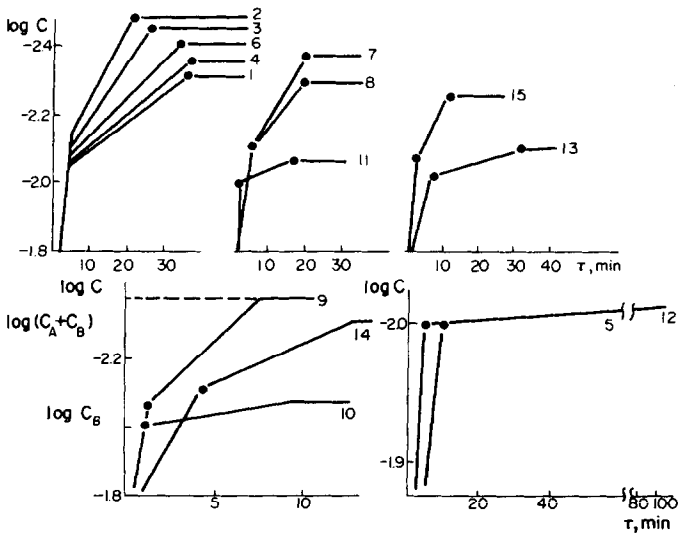

Fig. 2. Kinetic oxymercuration curves of olefin mixtures. The numbers correspond to the compounds in Table 3.

The acetylenes were determined spectrophotometrically. The acetylene sample (1×10^{-5}–1×10^{-2} meq) was placed in a 50-ml flask and 25 ml of 0.1*M* mercuric acetate in acetic acid were added. After 30 min the absorbance was measured at the absorption maximum (Table 2) against 0.05*M* mercuric acetate in acetic acid. A calibration curve was prepared similarly.

The differential kinetic olefin determination was carried out[8–10] at 25 ± 0.1°. The reaction mixture consisted of 180 ml of 0.1*M* mercuric acetate mixed with 20 ml of a solution of 0.2–2 meq of olefin in the appropriate solvent. Every 2 min an aliquot (*e.g.*, 1 or 10 ml) was taken out and added to 20 ml of 20% hexamine solution and the free mercury(II) titrated with EDTA (0.01 or 0.05*M* respectively). Alternatively a 1-ml aliquot was withdrawn, mixed with 1 ml of 0.05*M* EDTA and 15 ml of pH-10 ammonia/ammonium chloride buffer and diluted to volume in a 100-ml flask, then a 10-ml aliquot was shaken in a separating funnel with 10 ml of 0.01% dithizone solution in carbon tetrachloride and the absorbance of the organic phase was measured at 500 nm.[12] The results were plotted as $\log C_{Hg}$ *vs.* time.

For analysis of mixtures of olefins two samples were used. A methanol solution of the sample was prepared, of known total concentration (6–10 mg/ml), and 5-ml aliquots were placed in each of two flasks. To the first flask 15 ml of 0.1*M* mercuric acetate in methanol were added, the mixture was stirred magnetically for 2–3 min, then 20 ml of 20% hexamine solution were added and the free mercury was determined by titration with 0.05*M* EDTA (v_1 ml). A blank titration was performed similarly (v_2 ml). Then the number of meq of aromatic side-chain terminal double bonds was given by $(v_2 - v_1) \times$ EDTA molarity. The second sample was treated similarly except that 1 ml of 0.05*M* perchloric acid in methanol was added as catalyst. If the EDTA titration in this case was v_3 ml, the number of meq of double bonds conjugated with electronegative groups was given by $(v_3 - v_1) \times$ EDTA molarity. The precision was about 3%.

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THE MECHANISM OF INTERACTION BETWEEN MOLYBDOGERMANIC ACID AND THE BASIC DYE CRYSTAL VIOLET

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Summary—On the basis of light-absorption studies on solutions of Crystal Violet (CV) molybdogermanate in acetone, the optimal pH conditions for quantitative formation of molybdogermanic acid (MGA) have been determined. Di, tri-, and tetra-salts of MGA have been formed and isolated. It has been shown that formation of the higher salts is favoured by lowering the acidity, but this increases also the amount of the solid co-product CV–isopolymolybdate. To overcome this inconvenience the surplus molybdate ions are masked by adding oxalate ions, thus allowing the separation of the corresponding solid tetra-salt up to pH = 6.5; the molar absorptivity of this compound in acetone solution is very high (4.2×10^5 l.mole^{-1}.cm^{-1}).

It is of great interest to study the composition and separation conditions of the compounds of basic dyes with various heteropoly acids, with a view to establishing their mechanism of formation and also elucidating the conditions favouring increase of the analytical sensitivity for the determination of the element forming the heteropoly acid.

So far there are few examples of basic dyes being used as reagents for molybdogermanic acid (MGA).[1–6] Among the triphenylmethane dyes the highest sensitivity is given by Brilliant Green (molar absorptivity 1.93×10^5 l.mole^{-1}.cm^{-1}).[2] The formation of CV–MGA compounds (CV = Crystal Violet) has been studied in fairly acidic solutions (0.6–0.7*M* nitric acid), and the di-salt of MGA obtained.[2] This paper presents a detailed investigation of the interaction of CV and MGA over a wide range of acidity and concentration of the reacting components, with the aim of establishing the optimal conditions for the formation and separation of more highly substituted solid salts of MGA, and thus increasing the analytical sensitivity of the determination of germanium.

EXPERIMENTAL

Reagents

A 0.005*M* solution of Ge(IV) (pH 7.2) was prepared by dissolving the appropriate weight of (especially pure) GeO_2 in distilled water by adding small portions of sodium hydroxide solution, and was further diluted as required.

Reagents used were sodium molybdate (pure), 0.024 and 0.012*M* solutions, CV (pure) 0.1% aqueous solution, sodium oxalate (pure) aqueous solution, nitric acid (especially pure, s.g. 1.41), acetone (pure). All the solutions were kept in polyethylene bottles.

Preparation and separation of CV–MGA compounds

To a solution [containing a definite amount of Ge(IV)] in a conical centrifuge tube, a definite amount of molybdate was added followed by nitric acid until the optimum acidity (referred to as the initial acidity, pH_i) required for the quantitative formation of MGA was reached, and the volume was made up to 5 ml with distilled water.

The solution was stirred and left for 10–15 min for maximum formation of MGA. Then the optimum acidity (called the final acidity, pH_f) for the separation of CV–MGA was established, and a certain amount of oxalate solution (if necessary) and the reagent dye were added and the volume was brought to 10 ml with distilled water. After mixing, and formation of the considerable amount of precipitate, the mixture was centrifuged, the solution carefully decanted and the pH of the solution measured. The precipitate was washed in a test-tube with 2 ml of water, then separated by centrifuging and dissolved in 10 ml of acetone. The degree of combination of Ge(IV) in MGA and then in CV–MGA was estimated from the absorbance of the acetone solution.

A blank test was performed to check the formation of CV–isopolymolybdate salts.

The absorbance *A* of the solutions was measured at 595 nm, in 1-mm cells. Solid compounds were separated by centrifuging for 1–2 min at 3000 rpm.

RESULTS AND DISCUSSION

The optimal conditions for the formation of MGA

In establishing the optimal acidity for the formation of MGA some authors[2] have used the light-absorption properties of MGA and tried to find the conditions under which the yellow colour of MGA is maximal.

However, it is known that, depending on the acidity and the concentration of molybdate, various isomers of MGA (α- and β-MGA) are formed, which differ in their stability and spectral characteristics.[7–9] Therefore the maximum development of the yellow colour

* Lost in the post; copy received 17 June 1980.

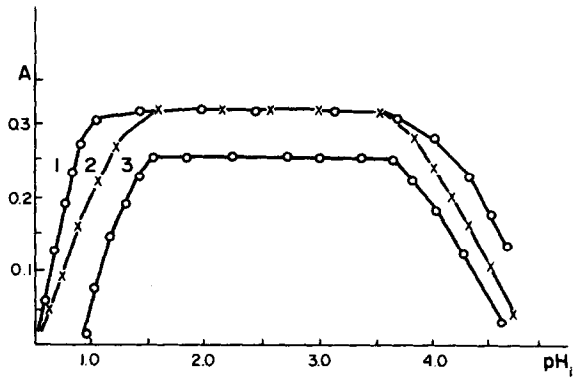

Fig. 1. Dependence of yield of MGA on acidity at various initial concentrations of molybdate ion. [Ge(IV)] = $1 \times 10^{-5}M$; [CV] = $1.2 \times 10^{-4}M$; $pH_f = 0.8$; [MoO_4^{2-}]: *1*, $2.4 \times 10^{-3}M$; *2*, $1.2 \times 10^{-3}M$; *3*, $0.7 \times 10^{-3}M$.

of MGA does not unequivocally indicate the quantitative formation of MGA. Consequently it is more convincing to investigate the formation of MGA not on the basis of its own light-absorption characteristics, but on that of the light-absorption of the salt of MGA with CV.

It should also be taken into account that though its formation is very sensitive to the acidity of the medium, MGA (once formed) can exist in more acidic medium without being decomposed.[10,11] This allows the complete suppression of the formation of isopolymolybdates, by increasing the acidity to $pH_f = 0.8$, at which decomposition of MGA does not take place, and accordingly a quantitative yield of CV–MGA is ensured.

Figure 1 shows the dependence of absorbance on pH_i for acetone solutions of CV–MGA at various initial concentrations of molybdate ions (pH_i was measured in separate experiments).

The acidity intervals given in Fig. 1 show the optimal conditions for MGA formation.* The validity of the considerations above is confirmed and the acidity interval for the formation of MGA is clearly indicated (pH_i = 1.5–3.8 for $1.2 \times 10^{-3}M$ molybdate and pH_i = 1.2–3.8 for $2.4 \times 10^{-3}M$ molybdate).

Thus, the absorbance of the acetone solutions obtained under these optimal conditions is practically independent of the molybdate concentration and the acidity, in contrast to the behavior of MGA itself. Hence CV–MGA is of greater interest for analytical use.

The optimal conditions for separating CV–MGA compounds

Figure 2 shows the yield of CV–MGA compounds as a function of pH_f at constant pH_i. It shows that pH_f = 0.7–0.9 ensures maximal and practically constant absorbance for the acetone solutions.

The precipitates of CV–MGA are violet, characteristic of the singly-charged form of CV. If the pH is lowered the acetone solution decreases in absorbance, and the solid compound becomes green (acidity from pH_f 0.4 to 4*M* nitric acid). From the colour of the precipitate it can be concluded that under these conditions MGA interacts with the green singly-protonated doubly-charged form of CV. An acetone solution of the product is violet, however, and presumably, when the precipitate is dissolved in acetone, the doubly-charged form of CV changes into the singly-charged form. Solutions of CV–MGA, at acidities from pH 0.4 to 2.0*M* nitric acid, have practically constant molar absorptivity (1.8×10^5 l.mole^{-1}.cm^{-1}), which is indicative of the constancy of the composition of the CV–MGA product under these conditions.

It therefore appears that the system studied can give rise to compounds of various compositions. Figure 2 shows that the absorbance of an acetone solution of CV–MGA is a function of pH_f but is independent of it over two ranges, in the vicinity of $pH_f = 0.1$ and 0.8, the absorbance being greater at the higher pH_f value. For both these pH_f values the concentration ranges of the reactants that will give constant absorbance for the product have been determined. They also permit the pure CV–MGA compounds to be obtained. They are listed in Table 1.

Use of higher concentrations of the components leads to appreciable separation of CV-isopolymolybdates and hence to decrease in the yield of CV–MGA compounds.

The preparation of CV–MGA compounds at optimal acidity conditions for MGA

According to the results above the investigation of the interaction between MGA and CV at $pH_f > 1.0$ is impossible because of the simultaneous precipitation of CV–isopolymolybdates (Fig. 2), which have similar spectral characteristics to those of the CV–MGA compounds. Hence it is necessary to mask the free

* Estimated on the basis of $\epsilon = 4.2 \times 10^5$ l.mole^{-1}.cm^{-1}.

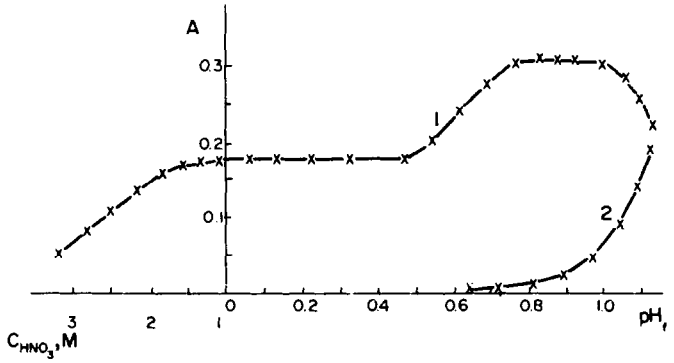

Fig. 2. Dependence of yield of (1) CV–MGA and (2) CV–isopolymolybdate on pH_f. [Ge(IV)] = $1 \times 10^{-5}M$; $pH_i = 2.5$; [CV] = $1.2 \times 10^{-4}M$; $[MoO_4^{2-}] = 1.2 \times 10^{-3}M$.

isopolymolybdate ions. Earlier this was done with oxalic acid at high acidity (0.6–0.7M nitric acid) which lowers the efficiency of the masking action. It was now found that the formation of the isopolymolybdates can be prevented by adding mineral acid to give $pH \leqslant 1.0$, without the need for the masking agent (Fig. 2).

In establishing the optimal acidity for the formation of MGA, it was shown that the quantitative formation of MGA is possible at relatively low acidity (pH_i 1.5–3.8). This makes it possible to form CV–MGA in this acidity range, with simultaneous masking of isopolymolybdates with oxalic acid. The effect of oxalate on the yield of CV–MGA as a function of pH is shown in Fig. 3.

It can be seen that CV–MGA is formed from a $1.2 \times 10^{-3}M$ sodium molybdate/0.01M oxalate solution in the acidity range pH_f 0.5–7.0, quantitatively at pH_f 1.5–2.5 or 4.0–6.0. The yield is lower at pH_f 2.5–4.0 because of the concurrent reactions of CV with both isopolymolybdates and MGA under these reaction conditions (Fig. 3, curve 1).

According to Alexeyeva,[12] the $HMo_2O_7^-$ ion occurs in this acidity range, and this (or some similar species) appears to form a precipitate with CV, causing a significant rise in the absorbance of the blank solutions. An eightfold increase in the oxalate concentration (Fig. 3, curve 2) does not affect the quantitative separation of CV–MGA in the pH range 2.0–5.5 but completely eliminates the formation of CV–isopolymolybdates. Unfortunately MGA is stable for only 15 min under these conditions. Therefore it must be prepared and separated during that interval of time.

The masking efficiency of the oxalate is mainly determined by the molybdate concentration. The yield of CV–MGA as a function of pH was studied at 0.08M oxalate concentration and fairly high concentrations of molybdate (Fig. 3, curve 3). Under these conditions the optimal range of acidity for formation of CV–MGA is expanded (pH 2.0–6.5).

At pH 4.5 and constant concentration of molybdate ($1.2 \times 10^{-3}M$) and oxalate ($1.0 \times 10^{-2}M$) the yield of CV–MGA was found to be quantitative with $(0.9–5.0) \times 10^{-4}M$ CV.

The correlation of these results with data obtained in the absence of oxalate indicates that the proposed variant of the method has markedly higher sensitivity. The acetone solutions of CV–MGA formed in presence of oxalate obey Beer's law over the Ge(IV) concentration range 1×10^{-7}–$1.6 \times 10^{-5}M$, the molar absorptivity being 4.2×10^5. This fact is unequivocally indicative of the change in composition of the CV–MGA compounds.

The composition of CV–MGA compounds

The composition of the principal compounds formed between CV and MGA was determined by Job's method (Fig. 4), at different final acidities: pH_f 0.1 (curves 3 and 4) and pH_f 0.8 (curves 1 and 2) and also at pH_f 4.0 in the presence of oxalate as masking agent (curves 5 and 6).

The maximum in the curves obtained at pH_f 0.1 corresponds to the ratio CV:MGA = 2:1. Therefore, in more acidic solutions (acidity between pH_f 0.4 and 2M nitric acid), according to the data in Fig. 2, the MGA salt precipitated contains the singly-protonated doubly-charged cationic form of the reagent dye (HCV^{2+}) (but see next paragraph). The formation of a compound with similar composition was reported

Table 1. The optimal reagent concentrations for the preparation of CV–MGA compounds

pH_f	$[MoO_4^{2-}]$, $10^{-3}M$	[CV], $10^{-4}M$	[Ge(IV)], $10^{-6}M$	Molar absorptivity, $l.mole^{-1}.cm^{-1}$ (595 nm)
0.8	0.8–1.8	1.1–2.2	0.2–16.0	3.2×10^5
0.1	0.8–4.8	1.1–3.0	0.3–18.0	1.8×10^5

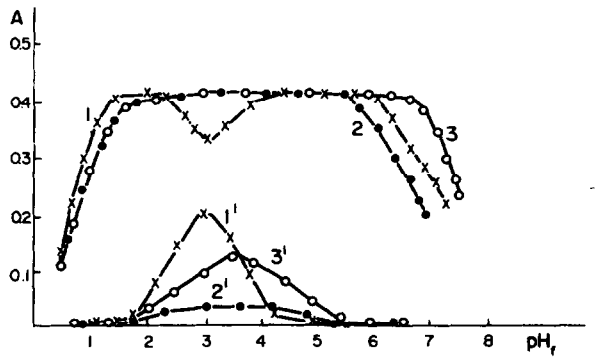

Fig. 3. Dependence of yield of CV–MGA (1, 2, 3) and CV–isopolymolybdate (1', 2', 3') on pH_f, for various initial concentrations of oxalate ion. [Ge(IV)] = $1 \times 10^{-5}M$; $pH_i = 2.2$; [CV] = $1.2 \times 10^{-4}M$; [Na_2MoO_4]: curves *1*, *2*—$1.2 \times 10^{-3}M$; curve *3*,—$6.0 \times 10^{-3}M$. [$Na_2C_2O_4$]: curve *1*,—0.010*M*; curves *2*, *3*—0.080*M*.

earlier,[2] in separation of CV–MGA compounds by flotation with organic solvent from 0.6–0.7*N* solutions of the acid. By the method proposed here, this compound is formed over appreciably wider ranges of concentration of the reactants.

At pH_f 0.8 the reacting ratio of MGA with CV is 1:3 (Fig. 4, curves 1 and 2). Therefore in the pH_f range 0.8–1.0, according to the data in Fig. 2, the trisubstituted acid salt of MGA is formed, containing singly-charged CV cations. This raises the question of the nature of the 2:1 CV:GMA species referred to in the last paragraph. Arguments based on protonation of germanomolybdate would lead us to expect $(CV^+)_2(H_2GMA^{2-})$ rather than the $(HCV^{2+})_2(GMA^{4-})$ which is postulated on the basis of the colour of the product. There seems to be no direct evidence either way.

If the acidity is further decreased and oxalate is added, the MGA interacts with the dye in 1:4 ratio (Fig. 4, curves 5 and 6), and the molar absorptivity of the acetone solution of the CV–MGA compound sharply increases (to 4.2×10^5 $l.mole^{-1}.cm^{-1}$).

The reliability of the composition established for the CV–MGA compounds is confirmed by the fact that the molar absorptivity of the compound formed at pH_f 0.1 is twice that of CV determined in acetone ($\epsilon_{CV} = 1.05 \times 10^5$ $l.mole^{-1}.cm^{-1}$), that of the pH_f-0.8 compound is $3 \times \epsilon_{CV}$ and that for the oxalate system is $4 \times \epsilon_{CV}$. The absorbance of the CV–MGA compounds is evidently solely due to their CV content.

The close correlation of the theoretically expected and experimental ϵ values, and also the correlation of the practically observed and theoretically estimated* sensitivity of the determination of Ge clearly indicates that under these experimental conditions the CV–MGA compounds are quantitatively formed and separated. Therefore determination of the molybdenum content of the precipitates obtained at a definite Ge concentration should enable us to define clearly the inner co-ordination sphere of the compounds.

The CV–MGA precipitate obtained under the conditions mentioned ([MoO_4^{2-}] = $1.2 \times 10^{-3}M$; [CV] = $1.2 \times 10^{-4}M$; acidity given in Table 2) and containing 0.10 μmole of Ge(IV), after centrifuging and washing with water was dissolved in the same test-tube in 2–5 ml of concentrated sulphuric acid. The solution was diluted to volume with water in a 25-ml standard flask, and its molybdenum content was determined by the thiocyanate method.[13] To avoid spectral interference by the reagent dye, the molybdenum thiocyanate was extracted with 10 ml of butyl acetate and its absorbance measured at 460 nm. A blank determination was also done. A single extraction was separately shown to be quantitative. The calibration curve was linear. The mean recovery of molybdenum was identical (2% relative error) irrespective of pH_f in the range 0.3–4.5 when pH_i was 2.5, and irrespective of pH_i (1.7–3.8) when pH_f was 0.7.

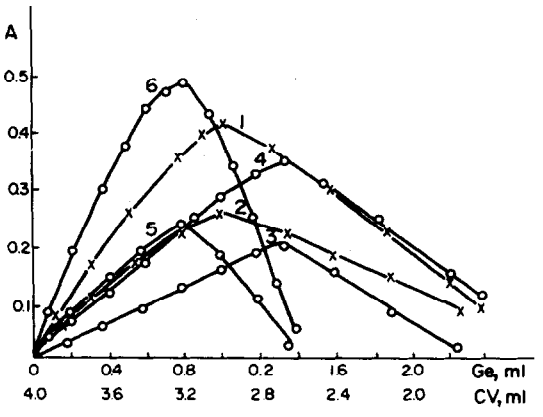

Fig. 4. Continuous-variations series for the system CV–MGA. $pH_i = 2.5$; [MoO_4^{2-}] = $1.2 \times 10^{-3}M$; pH_f: curves *1*, *2*,—0.8; curves *3*, *4*—0.1; curves *5*, *6*—4.0; [$Na_2C_2O_4$] = 0.04*M*. $\sum$[Ge(IV)] + [CV]: curves *1*, *4*, *6*—$8.0 \times 10^{-5}M$; curves *2*, *3*, *5*—$4.0 \times 10^{-5}M$.

* Note that if $pH_i = pH_f = 0.8$, MGA (and hence CV–MGA) will not be formed.

and corresponded to a ratio of Mo:Ge = 8:1 in the CV–MGA compounds.

It deserves attention that this is the stoichiometry of the MGA regardless of initial acidity in the range pH_i = 1.7–3.8. Thus in addition to the well-known existence of the 12-molybdogermanate and 11-molybdogermanate species in this acidity range,[14,15] it can be stated that 8-molybdogermanate is also present, which appears to be stabilized as a result of the precipitation.

Generalizing the results obtained, it can be concluded that under the reaction conditions described, molybdogermanic acid ($H_4GeMo_8O_{28}$) interacts with the dye reagent to form various ion-association compounds with different compositions, irrespective of the final acidity.

The tetrasubstituted salt of MGA, which gives intensely coloured acetone solutions ($\epsilon = 4.2 \times 10^5$ $l.mole^{-1}.cm^{-1}$), is used in a highly sensitive and simple method for the photometric determination of germanium.

Procedure

To the test solution containing 0.07–11.60 μg of Ge, add 0.5 ml of 0.024*M* sodium molybdate, adjust to pH 2.0–2.5 with nitric acid (the total volume must not exceed 9 ml at this point) and after mixing let the solution stand for 10 min. If the volume is less than 9 ml, bring it up to this value with distilled water, then add 0.5 ml of 0.2*M* sodium oxalate, mix, then add 0.5 ml of 0.1% CV solution and shake the mixture until a precipitate appears. Centrifuge at 3000 rpm, decant the liquid and dissolve the precipitate in 10 ml of acetone. Measure the absorbance at 595 nm. Determine the amount of Ge from a calibration curve.

The method has been used for determining Ge in various natural samples after its preliminary extraction and separation as $GeCl_4$.[16]

The results obtained were checked by the addition method, and the relative error was less than 3% at the $10^{-5}M$ level.

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APPLICATION OF AN AUTORANGING AMPLIFIER IN THE SIMULTANEOUS DETERMINATION OF TRACE HEAVY METALS BY ANODIC STRIPPING VOLTAMMETRY

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Summary—An autoranging amplifier with a gain of 1–1000 is described, together with its possible application in anodic stripping voltammetry. The performance of the amplifier is demonstrated by differential-pulse anodic stripping voltammetric analysis of stored sea-water, in the subtractive mode with two working electrodes. It is suggested that the autoranging amplifier could save considerable analysis time by eliminating the need for trial runs for gain adjustments and by relieving the operator from the need to change the recorder scale during the analysis.

Anodic stripping voltammetry (ASV) and in particular differential pulse ASV (DPASV) have recently received much attention because of their great sensitivity for several metals of environmental concern.[1–6] Advantages of the method are that little or no sample preparation is required, and that a number of species, notably Zn(II), Cd(II), Pb(II) and Cu(II), can be determined simultaneously.[7] Determination of each is based on measurement of the height of the current peak (i_p) recorded during the stripping phase, since this is proportional to the concentration.[8] The stripping current is normally transformed into a corresponding voltage signal and recorded on a strip-chart recorder. Simultaneous determination by ASV or DPASV of several metals at widely differing concentrations will necessitate changing the recorder scale during the analysis, which therefore requires the undivided attention of the operator, especially when the concentrations are unknown or cover a wide range. Consequently, the conventional procedure often involves one or more trial runs to determine the range changes required. This difficulty has been overcome by us by using the autoranging amplifier described here. It was found that in most applications the amplifier completely eliminated the need for a range change during a run, thereby saving considerable analysis time in two ways: no trial runs are required and the operator is free to attend to other tasks such as the preparation of the next sample.

Although at present used manually, the autoranging amplifier was designed to be used eventually in an automatic monitoring system, in which case, the device can simplify the data-acquisition system by making possible the use of a low-resolution, and hence low-cost, analogue-to-digital converter (ADC), even when an extremely wide dynamic range is expected. This can be achieved by reading into the data-acquisition system both the digitized signal and the gain of the autoranging amplifier. Since the gain range is from 1 to 10^3, the equivalent dynamic range of the system when a 10-bit ADC is used is about 20 bits.

Although originally designed and at present used for DPASV the autoranging amplifier could be useful in other instrumental methods which require the handling of large dynamic range signals, *e.g.*, gas chromatography.

Data compression could also be achieved by non-linear conversion, *e.g.*, by use of a logarithmic amplifier.[9] However, the autoranging amplifier has a number of practical advantages over a logarithmic amplifier. Since data are handled linearly there is no need for special (logarithmic) recording paper. Also, automatic data-handling is simpler because data-manipulation can be done directly without the need for an additional conversion back into linear form.

PRINCIPLE OF OPERATION

The autoranging amplifier (Fig. 1) comprises a programmable-gain amplifier and associated circuitry for automatically changing the gain of the amplifier when the output signal is outside a given amplitude range. The gain is increased or decreased in a stepwise manner when the output signal is smaller or larger than a predetermined level, respectively. This is accomplished by comparing the output signal to the

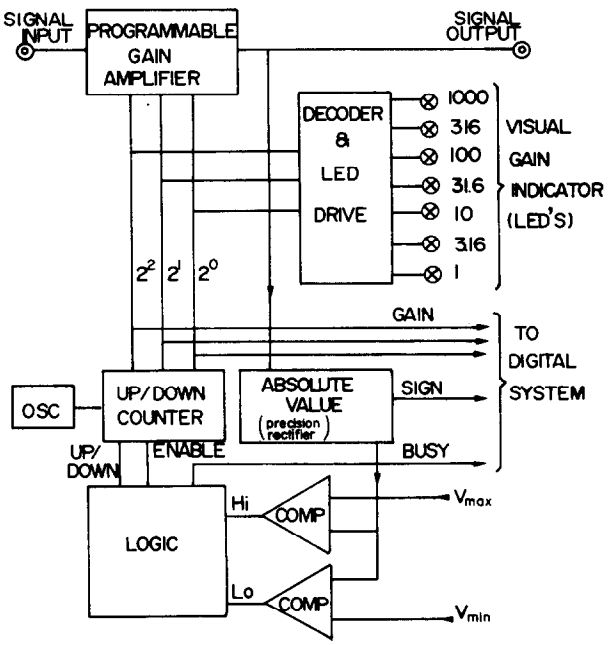

Fig. 1. Block diagram of the autoranging amplifier.

maximum (V_{max}) and minimum (V_{min}) levels permissible and applying a decrement or increment to an up-down counter whenever the output signal is outside the desired amplitude range. Since the state of the counter determines the gain of the amplifier, the output signal will always be maintained within the required range as long as the dynamic range of the device is not exceeded.

The gain increments are set so that each represents a factor of $\sqrt{10}$, *i.e.*, 3.16 for one step and of 10 for two steps. Since the lower limit (V_{min}) is set to approximately 0.3 V_{max}, the output signal will always be larger than 0.3 V_{max} except when the highest gain (1000) is reached and the input signal is smaller than 0.0003 V_{max}. If V_{max} is set to full scale (FS) on the strip-chart recorder, the recorded trace will thus always be kept in the region (0.3–1.0) FS except when the signal is too low, as indicated above, or larger than V_{max}.

Bipolar operation is made possible by basing the automatic gain adjustment system on the absolute value of the signal (Fig. 1). In the bipolar mode, the recorder pen is set to mid-scale for a zero-level signal and V_{max} is adjusted to 0.5 FS. The autoranging amplifier will then keep the trace on-scale except when the signal level exceeds $|V_{max}|$. For example, if the recorder FS is 10 V and V_{max} is 5 V, the autoranging amplifier will handle and permit the continuous recording of signals in the range ± 5 V with a maximum sensitivity of ± 5 mV. Furthermore, since each gain increment represents a factor of 3.16 the minimum shift of the recorded trace will be ± 0.3 FS except when the absolute amplitude of the signal is smaller than about 1.5 mV (corresponding to 0.3 FS at the highest sensitivity).

The voltage gain of the amplifier at any instant is displayed by LED indicators and is available as a TTL-compatible digital signal for use by the automatic data-handling system (Fig. 1). An additional line, BUSY, is used for indicating a transient state following a gain change, when the output data may not be valid. A complete circuit diagram of the autoranging amplifier is given in Fig. 2.

EXPERIMENTAL

Instrumentation

Measurements were made with a Ben-Gurion University (BGU) Model EI224 Polarographic Analyser equipped with a BGU Model IL06 Autoranging Amplifier and recorded with a Perkin-Elmer Model 56 strip-chart recorder. The polarographic analyser was developed by one of the authors (S.B-Y) and built at the Department of Electrical Engineering of BGU. It can be operated manually or under computer-control, and is capable of performing linear-sweep, pulse and differential pulse ASV. The option of using two working electrodes for subtractive ASV,[10] which enhances the stripping peaks by subtracting the background, is also built in.

When operating in the differential pulse mode, as applied here, the polarographic analyser uses a staircase waveform on which a pulse is superimposed (Fig. 3). This pulse form is similar to the one recently investigated by Turner *et al.*[12] and found to increase the sensitivity. In this work the subtractive mode was used, similar to the procedure described by Sipos *et al.*[10,11] except that we applied the final potential to the second working electrode for the last 10 sec of the plating time.

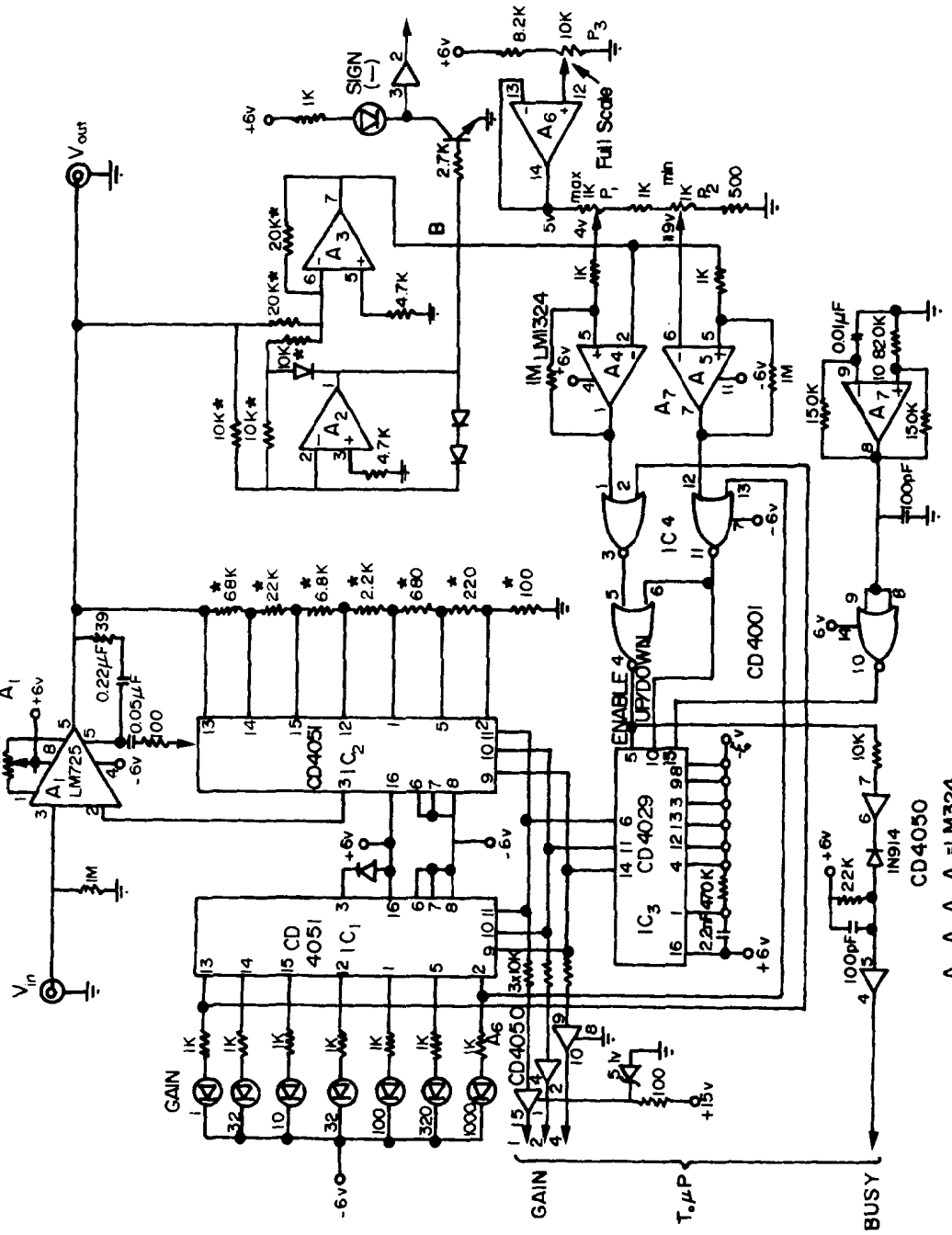

Fig. 2. Complete circuit diagram of the autoranging amplifier.

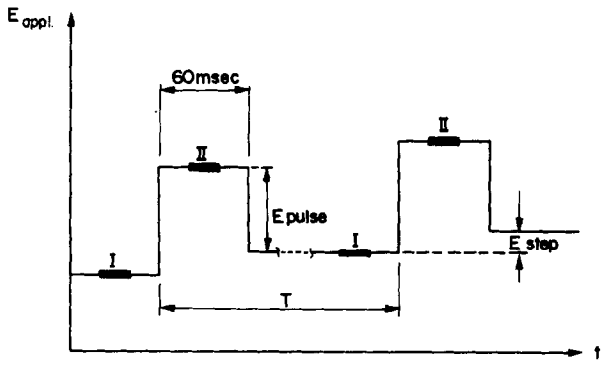

Fig. 3. Pulse shape used during the stripping phase of the DPASV analysis. Current samples are integrated over the periods marked by the heavier lines. The output signal of the polarographic analyser is proportional to the difference between the two integrated currents ($\int_{II} i\,dt - \int_{I} i\,dt$). In the present analysis $E_{step} = 10$ mV; $E_{pulse} = 80$ mV; $T = 640$ msec.

Electrodes and cell

Measurements were made in a 100-ml Teflon beaker fitted with a Teflon cover through which the electrodes and the tube carrying the purge gas (CO_2) were inserted. The solution was stirred with a Teflon-coated stirring bar.

A Radiometer type K-401 saturated calomel electrode equipped with a Coleman fibre liquid-junction (Perkin-Elmer C 003-0702) filled with 2*M* potassium chloride served as reference electrode and a coiled platinum wire was used as auxiliary electrode. The working electrodes were thin mercury-film electrodes (TMFE) deposited on glassy carbon (GC) 3 mm in diameter (Ringesdorff Werke S-10). Two electrodes were included in one housing to form a double TFME (DTFME) as required for the subtractive mode.[11] The DTFME was constructed by cementing two GC rods, 10 mm long and 3 mm diameter, into a 'Plexiglas' disc, 14 mm in diameter and 3 mm thick. The distance between the two GC surfaces was 5 mm. A coaxial cable was attached by a conductive expoxy resin to the other side of the GC rods, and the Plexiglas disc, with the GC pieces, was glued to a Plexiglas tube (outer diameter 15 mm, bore 8.5 mm, length 150 mm) to form the DTFME. Finally, the tube was filled with epoxy cement (Buchler No. 20-8130-032 and No. 20-8132-20) to reduce the chance of errors due to surface current leakages, and to fix the coaxial cables in place.

The electrode faces were polished with Hyprez Diamond Compounds of 6, 1, and 0.1 μm grade on a Hyprod Pellon Cloth (Engis Ltd.). The surface finish was checked for scratches by inspection through a reflected-light microscope.

Reagents and solutions

Gulf of Eilat (Red Sea) sea-water sampled and stored in 15-l. containers which had been cleaned with 1*N* nitric acid and rinsed several times with the water sampled. Before the ASV analysis the sea-water was spiked with Zn and Cu(II) to final concentrations of approximately $2.3 \times 10^{-10}M$ Zn and $2 \times 10^{-10}M$ Cu. The spike solutions were prepared from certified atomic-absorption standard reference solutions (Fisher Scientific Company). The plating solution was approximately $2 \times 10^{-5}M$ mercuric nitrate prepared by dissolving the salt (Baker Analyzed Reagent) in sea-water. Commercial CO_2 was bubbled through vanadium(II) chloride solution, and then through the sample during the entire analysis cycle, to remove dissolved oxygen.

Procedure

The Teflon cover, through which the electrodes were inserted, was placed on a 100-ml Teflon beaker containing the mercury plating solution and a potential of −1400 mV was applied to both working electrodes (WE) for 20 min. After the plating, the potential was changed to −100 mV for another 3 min to strip any trace metal deposited during plating. The electrodes were then rinsed with doubly distilled water and placed in the sample solution (stored sea-water). The solution was then aerated for 7 min, after which the ASV cycle was commenced. The analysis consisted of 5 steps: (1) application of −1400 mV to both working electrodes in a stirred solution for 110 sec; (2) the potential of WE_2 was changed to −100 mV for 10 sec while WE_1 was kept at −1400 mV; (3) a rest period of 30 sec for both working electrodes in unstirred solution at −1400 mV, and adjustment of the polarographic analyser to zero output current by setting of the current gain for WE_2; (4) scanning from −1400 to −100 mV ($E_{pulse} = 80$ mV, $E_{step} = 10$ mV, $T = 640$ msec) recording the difference in current from the two working electrodes; (5) stripping both working electrodes for 30 sec at −100 mV in stirred solution.

RESULTS AND DISCUSSION

The performance of the ASV system with an autoranging amplifier is demonstrated by the analysis of spiked sea-water (Fig. 4). Stored Gulf of Eilat sea-water was spiked with zinc and copper(II) to increase markedly the peak heights for these metals relative to those for cadmium and lead. Two ASV runs are shown: a normal run and one with the autoranging amplifier. In the conventional run, the gains of the voltammeter and recorder were kept constant during the analysis. The gain was initially adjusted to keep the large zinc and copper peaks on scale, as a result of which the much smaller cadmium peak is barely detectable and the somewhat larger lead peak is

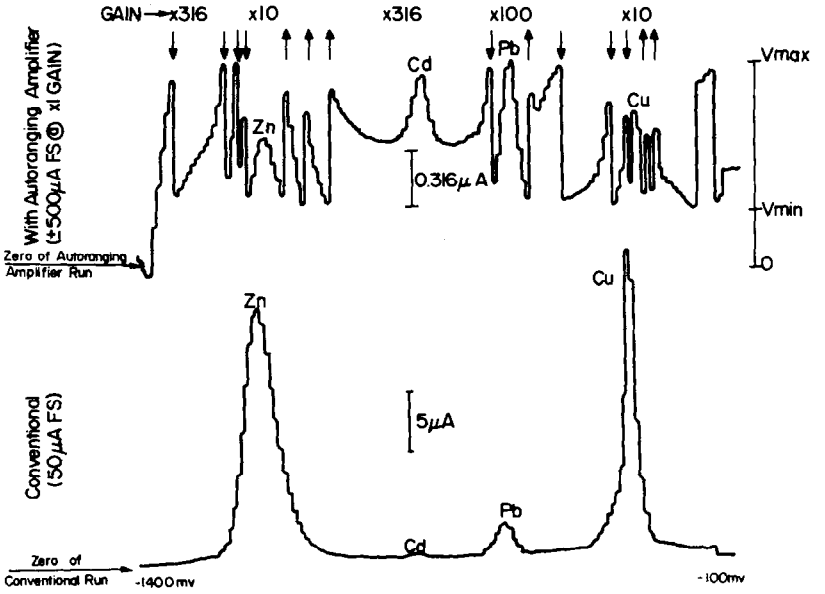

Fig. 4. SDPASV of stored sea-water. Lower trace: fixed-gain recording, upper trace: autoranging recording. Plating time: 2 min. Plating potential: −1400 mV. Pulse shape during stripping cycle: as in Fig. 2. Approximate concentrations: Zn = $2.3 \times 10^{-7} M$; Cd = $9 \times 10^{-11} M$; Pb = $2.4 \times 10^{-9} M$; Cu(II) = $2 \times 10^{-7} M$. See text for analytical procedure.

recorded with bad resolution. It should be pointed out in this connection that the subtractive mode is very effective in reducing the background current to very low levels (Fig. 4).

The upper trace of Fig. 4 was generated by repeating the analysis and using the autoranging amplifier. In this run, the recorder scale was set to a gain corresponding to a stripping current of 1000 μA FS. This gain is lower by a factor of 20 than the gain used in the conventional run. Also, the pen position was adjusted to mid-scale for zero input. V_{max} of the autoranging amplifier was set to a level corresponding to a current of approximately 350 μA. It should be noted that if V_{max} had been adjusted to a level corresponding to 500 μA, the trace would still have been kept on scale, and the resolution would have been somewhat better.

Although the trace generated by the autoranging amplifier seems at first complex, a closer examination reveals that it may be interpreted easily by following some simple rules. Fast pen movements are indicative of a change of scale; a trace step from a higher level to a lower level signals a decrease in gain (by a factor of $\sqrt{10}$) whereas a fast pen move from a lower level to a higher level is a result of a gain increase. Hence, once the initial gain factor is known, the gain for each section can easily be determined simply by following the gain changes as indicated by the steps. The absolute peak height is then determined by dividing peak height, measured on the chart, by the relevant gain factor. A similar approach is then used to determine the base-line level adjacent to the peak in quesion. The net peak height, that is the peak height above the base-line, is then obtained as usual by subtracting the absolute base-line level from the absolute peak height.

In the trace in Fig. 4, the smallest peak current (i_p) is 0.34 μA corresponding to a cadmium concentration of about $9 \times 10^{-11} M$, as determined by the standard addition method. The largest peak is approximately 26 μA, corresponding to a copper concentration of about $2 \times 10^{-7} M$. The ratio between the largest and the smallest peak currents is therefore about 75. To handle this dynamic range, the gain of the autoranging amplifier was switched from a gain factor of 316 for the smallest peak to a gain factor of 10 for the largest peak (Fig. 4). Since the inherent dynamic range of the present autoranging amplifier is larger, much larger peak-height ratios can be handled.

It should be emphasized, however, that although the autoranging amplifier simplifies the task of the operator by relieving him of the need to change scales during an analysis, the method does not in any way enhance the quality of the data or their readability. In particular, the amplifier will not enhance a small peak superimposed on the flanks of a larger peak. Nor will the amplifier circumvent the problem of a high base-line current. In such cases, the autoranging amplifier will not switch to a higher sensitivity, because it responds to the total signal and not just to the useful peak signal. Hence, the resolution of the system, when defined as the smallest detectable change in signal, is kept the same. However, the operational advantages of the autoranging amplifier could be crucial when large peak-height ratios are encountered.

The autoranging approach could also be useful in automatic systems. An alternative to this approach

would be a programmable-gain amplifier controlled by software. The two approaches are probably comparable when the analysis is controlled by a computer. However, the autoranging approach is more convenient to implement in simpler automatic systems which do not include a micro or minicomputer.

In this paper we have discussed only the problem of large dynamic range in the simultaneous determination of trace metals by ASV. This is but one of the many problems encountered when attempting to design an automatic ASV analyser. The problems of interpretation in view of the possible formation of intermetallic compounds and the problem of automatic calibration are just two additional examples of the many problems involved. Suggested solutions to these and other problems will be reported at a later stage of the present study.

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DETERMINATION OF WATER WITH A MODIFIED KARL FISCHER REAGENT

STABILITY AND THE MECHANISM OF REACTION WITH WATER

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Summary—It is shown that the water equivalent of the modified Karl Fischer reagent (standard Karl Fischer reagent in which dimethylformamide is substituted for methanol), depending on p*K*a and the concentration of the solvent used for preliminary titration, is not dependent on the water concentration being determined. Also discussed are different aspects of the stability of the Karl Fischer reagent and its modifications. On the basis of the literature data and the findings of this work, a mechanism of interaction between water and the modified Karl Fischer reagent is proposed: in the first stage of the reaction pyridine sulphodioxide is solvated with solvents containing active hydrogen (alcohols, organic acids and water). The lower the value of pK_a of the solvent, the greater the contribution of water to the pyridine sulphodioxide solvation reaction. The results of this work suggest that, especially in particular cases, the water equivalent of the Karl Fischer reagent and its modifications should be determined under the same conditions as the determination of water in the sample.

One of the current interests in analytical chemistry is aquametry, the determination of water, especially with the Karl Fischer reagent which is essentially a solution of iodine, sulphur dioxide and pyridine (Py) in methanol.[1] At present, among the many variations of the Karl Fischer reagent, the following find the widest application: (1) the standard reagent;[2] (2) the DMF modified reagent, *i.e.*, the standard reagent in which dimethylformamide (DMF) is substituted for methanol;[3] (3) the reagent based on methylcellosolve instead of methanol;[4] (4) the acetate reagent containing a mixture of salts, sodium acetate and iodide, instead of pyridine.[5]

The titration methods used include (1) direct titration;[1,6] (2) back-titration of excess of reagent, with a water solution in an appropriate organic solvent;[6] (3) Johansson's method (two-solution reagent) in which the sample is placed in a solution of sulphur dioxide in a mixture of pyridine and methanol.[7] or in a sodium acetate solution in acetic acid,[8] and titrated with a solution of iodine in methanol; (4) the "universal" method of titration with the modified Fischer reagent;[9] (5) coulometric titration of water with electrogenerated iodine.[10]

The Fischer reaction is a complex one-phase system in which the interaction with water is an ionic redox reaction but the side-reactions which lead to deterioration of the reagent (*i.e.*, to the reagent losing its stability) may proceed according to a free-radical mechanism.[11]

Initially, Mitchell and Smith[6] proposed a two-stage mechanism for the Fischer reaction:

$$Py.I_2 + Py.SO_2 + H_2O + Py \rightarrow Py.SO_3 + 2Py^{+}H.I \quad (1)$$

$$Py.SO_3 + ROH \rightarrow Py^{+}H.RSO_4^{-} \quad (2a)$$

$$Py.SO_3 + H_2O \rightarrow Py^{+}H.SO_4^{-}H \quad (2b)$$

The first stage of the reaction, formation of pyridine sulphotrioxide, is independent of the reagent composition, while the second stage proceeds differently depending on the nature of the solvent: reagents containing methanol, ethanol,[1,2] methylcellosolve[4] or any other alcohol react with water primarily by steps (1) and (2a), whereas in the absence of alcohols the reagents based on, for example, benzene,[2] DMF[3] or choloroform,[1,3] react by steps (1) and (2b).

Titration with the modified reagent in methanol or another alcohol does not decrease the water equivalent (WE) and is similar to titration with the standard reagent.[9] When the titration is done in alcohol, the WE of the modified reagent decreases almost twofold, which suggests that the reactions between pyridine sulphotrioxide and alcohol or water may be competing. This has been taken into consideration in proposing the "universal" method of titration with the modified reagent,[9] in which the WE and the water content of the sample should be determined under identical conditions in the same solvent, the latter being changed according to the nature of the compound analysed. Such a technique has made it possible for the modified reagent to be much more versatile than the alcohol-containing reagents.[9]

The modified reagent should preferably be used when an extremely accurate determination has to be made of water (particularly at concentrations below 1%) in compounds which release water when reacting with alcohols, such as aldehydes and ketones, organosilicon compounds containing silanol groups, strong

acids, and compounds insoluble in alcohol (*e.g.*, aromatic and heteroaromatic polymers, synthetic rubbers and their monomers[14]), as well as in some cases of aquametric functional analysis[15] and when some redox reactions are conducted in anhydrous media.[16] The DMF-based reagent is particularly suitable for work during the hot summer period when methanol-containing reagents are unstable because methanol evaporates.

Recently, the coulometric[17,18] and potentiometric[19] methods as well as a technique involving the use of a ring–disc system[20–22] were used in detailed studies of the kinetics and mechanism of the Fischer reaction. From the relationship between the logarithm of the Fischer reaction-rate constant (log K_3) and pH, it was shown[20,22] that pyridine is not a stoichiometric component of the reaction, but that its high buffer capacity ensures a constant pH of about 6 at which log K_3 is not dependent on pH. These data together with the relationship between log K_2 and log C_{H_2O} suggest[20,22] that when the standard Karl Fischer reagent is used, in the first stage of the reaction $Py.SO_2$ is solvated with methanol, yielding pyridinium monomethyl sulphite ($Py\overset{+}{H}.CH_3SO_3^-$) if $C_{H_2O} < 1M$. At $C_{H_2O} > 1M$, $Py.SO_2$ is hydrolysed, yielding pyridinium bisulphite ($Py\overset{+}{H}.HSO_3^-$). In the second stage of the reaction, $Py\overset{+}{H}.CH_3SO_3^-$ or $Py\overset{+}{H}.HSO_3^-$ is oxidized with iodine or tri-iodide (I_3^-) with addition of another water molecule. However, as was shown on the basis of coulometric measurements,[18] the first stage of the Fischer reaction yields only pyridinium bisulphite irrespective of the nature and concentration (up to 60% of DMF, dimethylacetamide or DMSO) of the solvent used for preliminary titration (the solvent being dehydrated beforehand in a titration cell) in determining trace concentrations (below 1%) of water.

In the present work, we have studied the dependence of the WE of a DMF-based reagent on the nature and concentration of the solvent used for preliminary titration, on the concentration of the water being determined, and on the titration conditions; we have also examined different aspects of the Karl Fischer reagent stability and the mechanism of the Fischer reaction.

EXPERIMENTAL

The apparatus and procedure for preparing the modified reagent have been described elsewhere.[1] Working solutions with WE ranging from 0.3 to 0.6 mg/ml were used. For preliminary titration, chemically pure solvents with a moisture content not exceeding 0.05–0.1% were used.

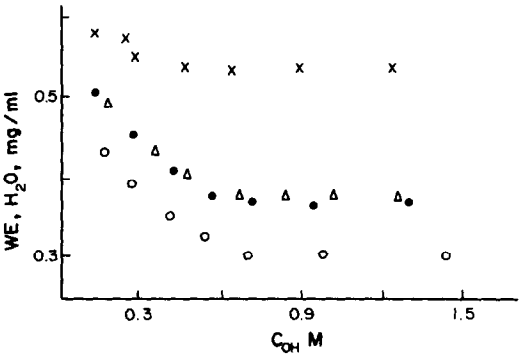

Fig. 1. WE of the modified reagent *vs.* concentration of the solvent used for preliminary titration (calculated as % w/w of solvent-OH group in the total volume of solution) (○) methanol, (●) propanol, (△) acetic acid, (×) tert-butanol.

Determination of the WE

A dry cell was coupled to the titrimeter and filled with 0.5–1 ml of DMF. The preliminary titration was done, then a weighed amount of water (1–2 mg) or sodium acetate trihydrate (2–4 mg) was added, and the water was titrated with the modified reagent. The end-point was determined biamperometrically by means of a current spike (i_{max}) corresponding to the appearance of traces of iodine in the solution.

In determining the WE of the modified reagent in the solvents under study, the preliminary titration was done in an appropriate solvent, and instead of a weighed amount of water or salt hydrate, 1 ml of DMF with a known water content (about 1 mg) was added. Then another 1 ml of DMF was added, and the WE was determined again, this procedure being repeated several times. Such a technique enabled us to reduce the concentration of the test solvent in the cell and to follow the changes in the WE of the modified reagent (Fig. 1).

RESULTS

Determination of the reaction rate with the modified reagent

To obtain comparative data on the reaction rate of water with the modified reagent, with various types of solvents in the preliminary titration, we proceeded on the assumption that if the reagent is added in equal amounts, the time intervals necessary for attaining the "apparent"* end-point of the titration will depend on the nature of the solvent. To this end, a simple experiment was conducted: preliminary titration was done with the solvent under examination, 1 ml of DMF containing a known amount of water was added, and the latter was titrated with the reagent added in equal amounts, the time needed to react the apparent end-point being registered. Given below are the total times of titration up to the stable end-point, averaged from 2 or 3 determinations; the repeatability is ±10 sec.

* Temporary attainment of i_{max} in the course of titration.

Solvent	Trifluoro-ethanol	Methanol	Ethanol	Propanol	Hexanol	Acetic acid	DMF
Time, *sec*	117	124	152	179	181	169	328

Dependence of the WE on concentration of the solvent used for preliminary titration

As can be seen from Fig. 1, the WE of the modified reagent depends on the nature of the solvent used for the preliminary titration, [when this contains active hydrogen (alcohols, organic acids)], and remains constant for each specific solvent while the latter is substantially in excess, *i.e.*, present in an amount two orders of magnitude greater than that of the water being determined. The WE starts increasing only when the amount of the solvent becomes commensurate with that of the water being determined. Experiments with methyl, propyl and butyl alcohols as well as acetic acid indicate that the WE of the modified reagent remains invariable if the solvent concentration (calculated as solvent OH-group in the volume already titrated) exceeds 0.5*M* (Fig. 1). On the other hand, when aprotic solvents are used, the WE of the modified reagent does not vary and remains constant if the solvent concentration does not exceed 15–30% (depending on the solvent polarity): at high concentrations of a solvent, e.g. acetone, pyridinium salts may precipitate, which will make it difficult to define the titration end-point.

Determination of water in an alcohol with the modified reagent

In order that the WE of the modified reagent should not depend on the composition and amount of the alcohol mixture to be analysed, it is important to establish whether taking an excess of methanol is sufficient for the preliminary titration (Fig. 1) or whether the mixture to be analysed should be used for the purpose.

An experiment with a methanol–butanol mixture (Fig. 2) shows that for determining its water content at least 1 ml of methanol should be used for the preliminary titration, which would make its concentration about 2.5*M* in 10 ml of the titrated solution. In this case, any alcohol taken in a quantity of 10–30% should produce practically no effect on the WE of the modified reagent, determined in methanol. If more alcohol is present in the mixture, the amount of methanol for preliminary titration should be increased accordingly or the analysis mixture itself be used and both the WE and the water content of the sample determined in it, just as in the case of "universal" titration with the modified reagent.[9]

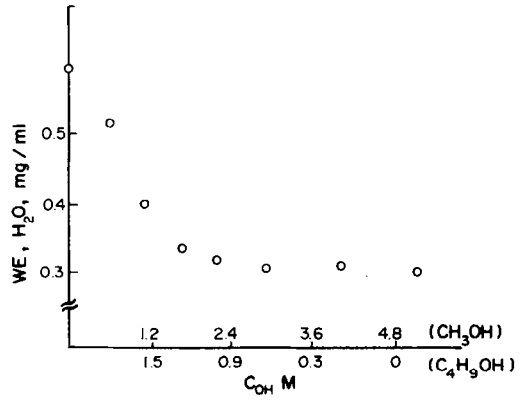

Fig. 2. WE of the modified reagent *vs.* alcohol ratio in the methanol–butanol mixture used for preliminary titration.

Dependence of the WE of the modified and standard reagents on the water concentration being determined

The dependence of the WE of the Karl Fischer reagent on the water concentration being determined (C_{H_2O}) has long been a matter of discussion and controversy.[6,20,23,25] Finding a solution to this problem has become urgent since titration with the Karl Fischer reagent is now a standard practice in water determinations in chemistry and chemical industry,[26] as well as in metrology and standardization of other water-measuring techniques.

A series of experiments was conducted to provide the solution. First, the repeatability of determining the WE (in the range 0.3–1 mg/ml) of the standard and modified reagents was checked, then the dependence of the WE on C_{H_2O}. The WE of the modified and standard reagents is shown to be independent of both the C_{H_2O} determined in the range 0.01–0.15*M* and of the volume titrated, and its repeatability, when the WE is averaged from 6 determinations, is 0.003–0.05 mg/ml. The dependence of the WE on C_{H_2O} may be primarily due to the high iodine content (C_{I_2}) in the Karl Fischer solutions used, and also to inadequate protection of the reagent against aerial moisture.[6,23,25] In the coulometric measurements, the variations in the WE with C_{H_2O} are probably due to the side-reactions occurring when electrogenerated iodine is used, particularly in determining trace concentrations of water. When the two-solution titration method is used,[7,8] the dependence of the WE on C_{H_2O} should first be established for both solutions 1 and 2 of the Karl Fischer reagent.[1,26] Otherwise, the analysis results may be erroneous, just as in the case of coulometric titration, because of the side-reactions leading to different changes in the WE during its determination and titration of the water in the sample under investigation. Side-reactions, including those involving iodine,[6,25] also take place in a freshly prepared Karl Fischer reagent but have ceased by the time it starts to be used.[6]

It has been established[17,19,20] that the Fischer reaction is first-order with respect to each component, *i.e.*, H_2O, $CH_3SO_3^-$ and I_2, with an overall third-order reaction-rate constant $K_3 = 8 \times 10^6\ l^2 \cdot mole^{-2} \cdot sec^{-1}$ when $Py^+H \cdot CH_3SO_3^-$ is oxidized with iodine. However, in dilute solutions of the Karl Fischer reagent,[8] the conversion of I_2 into I_3^- increases with C_{I^-}. The stability of I_3^- in methanol is sufficiently high ($K_{equiv} = C_{I_3^-}/C_{I^-}C_{I_2} = 2 \times 10^4$),[8] for the major portion of I_2 in the reagent which is ready for use (one day after preparation) to be in the form of I_3^-. The rate of oxidation of $Py^+H \cdot CH_3SO_3^-$ with I_3^-, as opposed to iodine, during titration of water with the standard Karl Fischer

reagent, decreases to $K_3 = 5 \times 10^2$ $l^2.mole^{-2}.sec^{-1}$, which in its turn reduces the effective rate constant of titration with the Karl Fischer reagent, and hence minimizes the side-reactions involving I_2 (or I_3^-).

In diluted Karl Fischer reagents with WE = 0.6–0.3 mg/ml and at low C_{I_2} = 0.06–0.03 mg/ml, C_{I^-}/C_{I_2} = 50–100. This provides for a high stability of the easily reversible redox pair I^-/I_2. The role of this relation in stabilizing the Karl Fischer reagent has been pointed out before[23,24] and it has been recommended to add iodide to it to enhance its stability.

In aquametric micromethods,[1] another essential factor providing for additional reagent stability and repeatability of WE and water content measurements is the "dilution effect,"† resulting in a better solubility of the sample or extraction of water from a sample insoluble in the medium of the Karl Fischer reagent.

The modified reagent is also more stable than the standard one[3,27] because of the absence of methanol from the reagent, and hence of the side-reactions in which it participates.[1,6,10]

DISCUSSION

Dependence of the WE of the modified reagent on acidity

It can be inferred from Table 1 and Fig. 1 that the WE of the modified reagent depends only on the nature and concentration of the solvent containing active hydrogen. For example, when excess of a saturated aliphatic alcohol is used in the preliminary titration, the WE increases with molecular weight of the alcohol up to butanol, but remains practically the same for further homologues up to octadecanol. The effect of primary and secondary alcohols on the WE of the modified reagent is similar, whereas the use of sterically hindered alcohols (diphenylcarbinol, tertiary alcohols) results in a sharp increase in the WE. The same regularity is observed when saturated aliphatic acids are used in the preliminary titration, except that, in this case, the changes in the WE are less significant (Table 1). It is logical to assume that such a change in the WE of the modified reagent is a function of the acidity of the solvents used for the preliminary titration. Since there is a certain discrepancy between the acid dissociation constants (pK_a) of solvents obtained by different authors by various methods and with different solvents,[28,29] it is impossible to establish a correlation between the variations in WE and the pK_a values of the solvents. However, the orders of acidity of saturated alcohols, for example, agree well. The values of pK_a can be correlated with the aid of Taft's parameters, and it should be borne in mind that ρ^* is 1.42 in water and 1.36 in isopropyl alcohol.[29] Since the Fischer reaction takes place in an anhydrous medium, the values of pK_a that we used for the alcohols were calculated from the dissociation reaction ($ROH \rightleftharpoons RO^- + H^+$) in isopropyl alcohol medium at 25°, with due account taken of the ρ^* and σ^* factors in accordance with the correlation equation:[30]

$$pK_a = pK_0 + (-\sigma^*)\rho^* \quad (3)$$

where pK_0 is a constant (−15.9) for the dissociation of saturated alcohols, ρ^* is a constant (1.36) for the series in isopropyl alcohol medium, and σ^* is Taft's

† A relationship between the weight of sample and the total volume of the solution in the titration flask.

Table 1. Dependence of the WE of the modified Karl Fischer reagent on the structure and pK_a of the solvent used for preliminary titration

Solvent	WE, *mg/ml* ($\bar{x} \pm 0.005$; n = 3 or 4)	log K_{WE}	pK_a
Trifluoroethanol	0.297	0	14.660
Methanol	0.301	≃0	15.900
Benzyl alcohol	0.304	≃0	15.609
Methylcellosolve	0.330	−0.921	5.985*
Ethanol	0.350	−0.658	16.036
Propan-1-ol and propan-2-ol	0.369	−0.479	16.056
Butylcellosolve	0.380	−0.390	16.070*
Butan-1-ol and butan-2-ol	0.450	−0.208	16.076
Pentan-1-ol–octadecan-1-ol	0.392–0.406	−0.208	16.076
Cyclohexanol	0.420	−0.111	16.101
tert.-Pentanol	0.440	0.001	16.123
tert.-Butanol	0.530	0.716	16.305
Diphenylcarbinol	0.560	1.267	16.450
Triphenylcarbinol	0.574	—	—
DMF, dimethylacetamide, pyridine, acetone, acetonitrile	0.574	—	—
Chloroacetic acid	0.325	—	2.85
Benzoic acid	0.357	—	4.20
Acetic acid	0.372	—	4.75
Propionic acid	0.382	—	4.87

* These values of pK_a were calculated from the corresponding values of WE (Fig. 3).

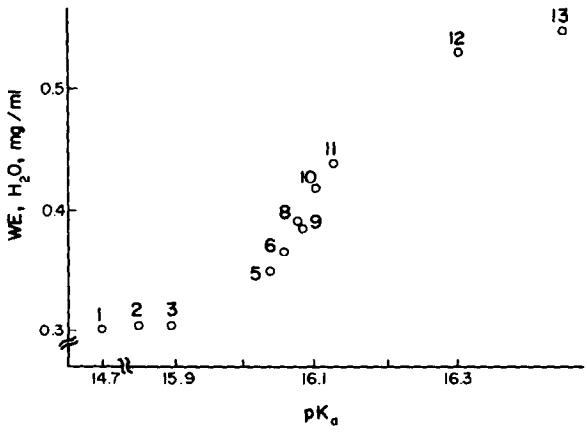

Fig. 3. WE of the modified reagent *vs.* pK_a of the alcohol used for preliminary titration. For solvents corresponding to the reference numerals see Table 1.

inductive constant representative of the effective electronegativity of a particular substituent.

The data of Table 1 were plotted as WE of the modified reagent *vs.* pK_a of the solvent (Fig. 3), from which it follows that the WE increases with pK_a. Hence, as the acidity of a solvent decreases, the participation of water in the sulphur dioxide hydrolysis reaction becomes greater, which, in turn, increases the total titration time, as has been mentioned above.

Mechanism of the Fischer reaction

When the modified reagent is used for titration and the preliminary titration is done with solvents free from active hydrogen, in the first stage of the Fischer reaction only water may react with the sulphur dioxide, yielding pyridinium bisulphite:

$$Py.SO_2 + H_2O \rightleftharpoons P\dot{y}H.HSO_3^- \qquad (4)$$

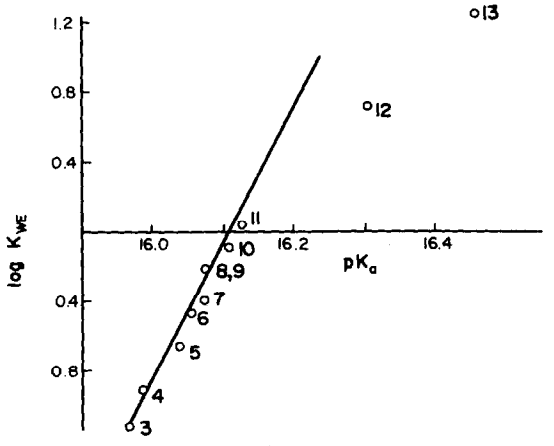

Fig. 4. Log K_{WE} *vs.* pK_a of the solvent used for preliminary titration (according to Table 1): $K_{WE} = x/(A_0 - x)$ where A_0 is the difference between the values of WE of the modified reagent, measured in DMF and in methanol and x is the difference in the values of WE of the modified reagent, measured in a particular solvent and in methanol.

The formation of $P\dot{y}H.HSO_3^-$ during coulometric titration of water is observed only at $C_{H_2O} > 1M$,[20] *i.e.*, when water is in great excess with respect to SO_2 and I^- and is probably independent of the nature of the solvent used. However, in titration with the standard or modified reagents, the Fischer reaction proceeds almost completely through formation of pyridinium monomethylsulphite

$$Py.SO_2 + CH_3OH \rightleftharpoons P\dot{y}H.CH_3SO_3^- \qquad (5)$$

Therefore, the use of any alcohol which is more acidic than methanol for the preliminary titration should not result in a lower WE. Indeed, as can be seen from Fig. 3 and Table 1, when trifluoroethanol is used for preliminary titration, the WE of the modified reagent remains practically the same as in methanol.

The observed decrease in the WE of the modified reagent when organic acids are used for the preliminary titration may be accounted for by the formation of mixed anhydrides:[31]

$$Py.SO_2 + RCOOH \rightleftharpoons P\dot{y}H.RC(=O)SO_3^- \qquad (6)$$

Hence, according to the data available in the literature[19,20] and our own findings, the first stage of the Fischer reaction involves solvation of sulphur dioxide with a solvent containing active hydrogen (alcohols, organic acids, water) and is a limiting step, the degree of this reaction being dependent on the pK_a of the solvent used since a decrease in this value is accompanied by a higher degree of hydration of $Py.SO_2$ to form pyridinium bisulphite.

The second stage of the Fischer reaction is a fast redox reaction[19,20,22] and seems to be less dependent on the nature of the solvent:

$$P\dot{y}H.RSO_3^- + Py.I_2 + Py + H_2O \rightarrow 2P\dot{y}H.I^- + P\dot{y}H.RSO_4^-, \qquad (7)$$

where

$$R = H, CH_3 \text{ or } RC = O.$$

A quantitative measure of the sensitivity of the Fischer reaction to the acidity of the hydroxy-containing solvent used for preliminary titration may be the slope of the curve representing log K_{WE} *vs.* pK_a of the solvent (Fig. 4 represents the data for saturated alcohols). Here, the distribution pattern of the points on the straight line represents the structural differences in the solvents: the deviation of diphenylcarbinol and tert-butanol from the average straight line may be explained by the contribution of steric factors in the solvation of $Py.SO_2$.[32]

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SHORT COMMUNICATIONS

PHOTOMETRIC DETERMINATION OF COPPER WITH *N*-(DITHIOCARBOXY)SARCOSINE AFTER PRECONCENTRATION WITH AMBERLITE XAD-2 RESIN

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Summary—The copper(II) chelate of *N*-(dithiocarboxy)sarcosine (DTCS) is sorbed on a column of Amberlite XAD-2 resin from a pH-7 phosphate solution (0.1*M*) and stripped with a pH-9.0 ammonia solution (0.2*M*) in 60% methanol. The absorbance of the eluted chelate is measured at 432 nm against water. Sodium nitrilotriacetate, sodium tripolyphosphate, and EDTA can be used to mask interfering metal ions other than mercury(II), the resulting complexes not being sorbed on the resin. The mercury(II) chelate of DTCS is also sorbed on and stripped from the column along with the copper chelate, but does not interfere in the photometric determination of copper, because it is colourless. The recovery of copper(II) is quantitative from test solutions (50–500 ml) of any salinity up to that of sea-water. Concentration factors of up to about 20 are obtained. The method is highly selective for copper and can be applied to its determination in sea-water.

N-(Dithiocarboxy)sarcosine (DTCS),[1] a water-soluble dithiocarbamate, is a very effective masking agent in the photometric determination of certain metal ions with various chromogenic reagents.[2–6] It is easily synthesized, and is fairly stable in air and aqueous media, in comparison with other water-soluble dithiocarbamates. A previous study[7] has shown that copper(II) reacts rapidly with DTCS to form a very stable chelate in the pH range 4–11; the molar absorptivity is 1.45×10^4 $l.mole^{-1}.cm^{-1}$ at 432 nm. When sodium nitrilotriacetate (NTA), sodium tripolyphosphate (TPP), and EDTA are used as masking agents, copper(II) can be determined selectively with DTCS.

In the present work, a method for concentrating copper was developed in order to determine trace amounts of copper photometrically with DTCS. Amberlite XAD-2 resin is a macroporous styrene–divinylbenzene copolymer which has been shown to be a versatile sorbent for a wide variety of organic substances,[8] mercury(II),[9] metal oxinates[10] and ferroin.[11] It was therefore chosen for concentration of the copper–DTCS chelate, which can be selectively sorbed on XAD-2 and eluted completely with a methanolic ammonia buffer.

EXPERIMENTAL

Reagents

Standard metal-ion solutions. Prepared by dissolving appropriate amounts of their salts in dilute hydrochloric acid, standardized by EDTA titration, and diluted as required.

DTCS solution (0.01M). Prepared by dissolving 1.00 g of the diammonium salt, synthesized by a procedure reported previously,[2,7] in 500 ml of water. The solution is stable for at least a month.

Phosphate buffer solution (*pH 7.0*). A 1.0*M* potassium phosphate solution adjusted to pH 7.0 with dilute potassium hydroxide solution.

Ammonia buffer solution (*pH 9.0*). A 0.5*M* ammonium chloride solution adjusted to pH 9.0 with dilute potassium hydroxide solution.

Wash-solution. Prepared by mixing 20 ml of DTCS solution with 50 ml of phosphate buffer and 430 ml of water.

All other reagents used were of analytical-reagent grade.

Column preparation

Amberlite XAD-2 resin, 20–60 mesh, was extracted for 8 hr with methanol in a Soxhlet extractor, then stored in a glass-stoppered Erlenmeyer flask containing methanol. The resin was packed in a glass tube (30 cm long, 1.0 cm bore) to give a resin bed about 10 cm long. The column was washed with 100 ml of 1.0*M* hydrochloric acid in 50% methanol and then 100 ml of 0.2*M* aqueous ammonia in 10% methanol. This washing sequence was repeated twice, then the methanol was removed with a large volume of demineralized water.

Preconcentration

To an aliquot of standard copper solution (and of another metal ion solution when necessary) in a 50-ml standard flask were added 2 ml of 0.05*M* NTA, 5 ml of 0.2*M* TPP, 1 ml of 0.01*M* EDTA, 5 ml of 1.0*M* phosphate buffer and 2 ml of 0.01*M* DTCS in that order. The whole was made up to volume with water, then transferred to a 100-ml separatory funnel attached to the top of the column, and loaded onto the column at a flow-rate of 3.0 ml/min by means of a peristaltic pump. The effluent (50 ml) was collected in a standard flask, then the column was washed with 50 ml of wash-solution at the same flow-rate. The sorbed chelate was then eluted with methanolic ammonia buffer at a flow-rate of 2.0 ml/min. The eluate

was collected with a fraction collector and the copper in it was determined by measuring the absorbance at 432 nm. Other metal ions were determined by atomic-absorption spectrometry.

RESULTS AND DISCUSSION

Effect of salt concentration on sorption of copper

The sorption of the copper–DTCS chelate on XAD-2 resin increases with increasing potassium or sodium phosphate buffer concentration up to 0.01*M*, and then remains constant and practically quantitative. Addition of potassium or sodium chloride as well as the buffer has no significant effect at chloride concentrations up to that in sea-water. As phosphate is effective in masking such metal ions as iron(III) and manganese(II), 0.1*M* phosphate solution was chosen as the sorption medium.

The sorbed chelate was partially desorbed by water (about 30% with 100 ml), but not by a reagent blank solution from which the masking agents are omitted. Hence 50 ml of wash-solution were used to wash the column retaining the chelate.

Retention of the masking agents on the XAD-2 is very unlikely (the amount of EDTA found in the effluent is in fair agreement with that initially present in the sorption solution). XAD-2 resin has essentially no affinity for inorganic anions. Free DTCS seems not to be sorbed on the resin.

Only the copper and mercury(II) chelates of DTCS are sorbed on the resin from the sorption solution.

Optimization of stripping conditions

Methanol gave only a 68% recovery when used for stripping the copper chelate from the resin, but addition of phosphate or ammonia buffers to the methanol improved the recovery. Ammonia buffer (pH 9.0) was the best as the DTCS chelates have higher stability in alkaline medium. Figure 1 shows the effect of the methanol concentration in the stripping solution. Recovery was quantitative with 60 ml of 0.2*M* ammonia in 50–80% methanol, the elution power increasing with methanol concentration, but when the methanol concentration was greater than 60%, air bubbles were rapidly formed in the column. Thus, the most suitable methanol concentration is about 60%.

The effect of the ammonia concentration was also investigated. Recovery of copper (determined by atomic-absorption spectrometry) is quantitative with

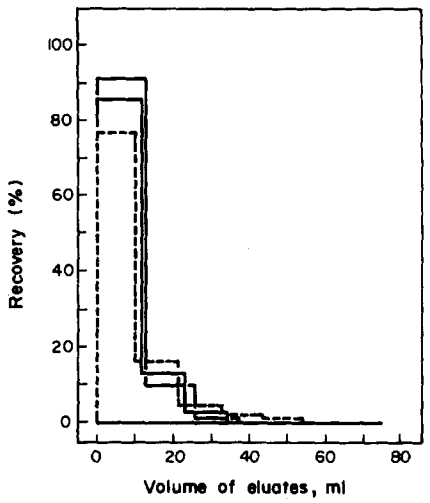

Fig. 1. Effect of methanol concentration in eluent on the recovery of copper–DTCS chelate. Flow-rate 2.0 ml/min. ---- 50% methanol, —— 60% methanol, —— 80% methanol.

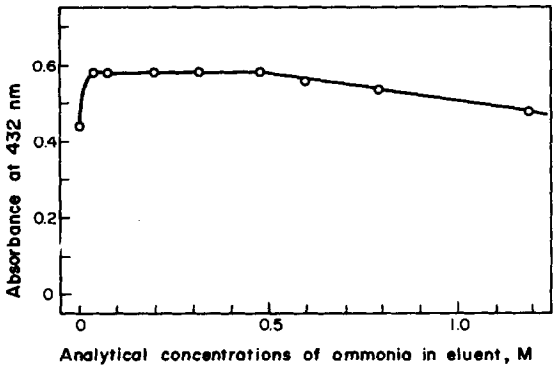

Fig. 2. Effect of the concentration of ammonia in the eluent (60% methanol) on the absorbance of copper–DTCS chelate at pH 9.0. Cu(II) taken; 65.0 μg.

ammonia concentrations above 0.04*M*, but the absorbance of the copper chelate at 432 nm is affected by the ammonia concentration as shown in Fig. 2 because of formation of copper ammine complexes, but not by the pH of the buffer in the range 8.2–9.5. The best eluent seems to be pH-9.0 ammonia buffer (0.2*M*) in 60% methanol, 25 ml of which will strip more than 98% of the sorbed chelate (Fig. 1).

The copper chelate in the eluate is stable for 8 hr, and the calibration curve is linear over the copper

Table 1. Effect of sample volume on the recovery of copper–DTCS chelate

Cu(II) taken, μg	Sample volume, ml	Number of runs	Average recovery, %	Coefficient of variation, %
26.0	50	3	100.0	0.3
	100	4	99.6	1.5
65.0	50	10	99.5	0.7
	100	3	99.2	0.8
	250	5	99.4	0.6
	500	8	98.1	0.7
	1000	3	89.2	1.1

Table 2. Effect of foreign ions on the recovery of copper-DTCS chelate (65.0 μg of Cu)

Foreign ion	Added, *mg*	Absorbance of the copper eluate at 432 nm	Foreign ion found in the copper eluate, *mg*
None	—	0.580	0
Mg(II)	21.5	0.580	0
Ca(II)	20.8	0.580	0
Al(III)	21.6	0.582	0
Cr(III)	10.8	0.580	0
	20.2	0.588*	0
Mn(II)	22.0	0.578	0
Fe(III)	21.6	0.580	0
Zn(II)	20.0	0.570	0
Co(II)	0.54	0.593	0
Ni(II)	0.53	0.588	0
Cd(II)	1.10	0.573	0
	20.5	0.580	0.11
Sn(IV)	9.9	0.580	0
	19.7	0.580	0.21
Pb(II)	2.06	0.585	0
	21.2	0.577	1.32
Hg(II)	1.90	0.577	1.90

* Ten ml of phosphate buffer were added.

concentration range 13–91 μg in 25 ml of eluate. The precision found was ±0.8 μg (95% confidence limits) for determination of 52.0 μg of copper in 50 ml of solution. The molar absorptivity is 1.40×10^4 $l.mole^{-1}.cm^{-1}$.

Concentration factor

Recoveries of copper (26.0 or 65.0 μg) from various volumes (50–1000 ml) of test solution were examined. As indicated in Table 1, recovery is good from volumes up to 500 ml, with good precision, but recovery from 1000 ml is poor.

Effect of foreign ions

The results obtained with foreign ions are summarized in Table 2.

Alkali and alkaline-earth metal ions, and aluminium(III) do not react with DTCS, so 20 mg or more of these will not interfere. Chromium(III), manganese(II), iron(III), zinc and tin(IV) form weak chelates with DTCS, but these are readily decomposed by NTA and TPP. Copper is easily separated from at least 20 mg of these metal ions, but when 20 mg of chromium are present, the solution becomes turbid on addition of phosphate buffer. This can be prevented, however, by adding more buffer and/or TPP.

Table 3. Recovery of copper added to sea-water (500 ml)

Added, μg	Found,* μg	Coefficient of variation, %
0	0.6	8.0
26.0	26.0	0.7
65.0	64.9	0.4

* Average of 5 determinations.

Cobalt(II), nickel, cadmium and lead can be masked, mainly with EDTA. Cobalt and nickel interfere seriously because both form strong, coloured DTCS chelates. Amounts of these ions larger than those indicated in Table 2 can be masked by increasing the amounts of EDTA and DTCS added. The EDTA complexes are not retained on the resin. Although the cadmium and lead DTCS complexes are partially sorbed on the resin (Table 2), the interference is only slight because the chelates are colourless. At least 20 mg of each can be tolerated.

Mercury(II) forms an extremely stable DTCS chelate, which is sorbed on XAD-2 resin and quantitatively eluted under the same conditions as copper, making separation impossible. Fortunately, the mercury chelate is colourless, so up to 2 mg of mercury can be tolerated.

Analysis of sea-water

To examine the applicability of this method, copper was determined in sea-water. The sample was filtered through a 0.45-μm Millipore membrane, and 500-ml portions were analysed alone and when spiked with 26.0 or 65.0 μg of copper(II). The pH was adjusted with TPP (pH 7.0) instead of phosphate buffer because the latter caused a white precipitate, probably of magnesium and calcium phosphates. The results are summarized in Table 3, and display good precision and accuracy.

Conclusion

The proposed method is simple and highly selective and can be applied at the 0.01 μg/ml level. A clear advantage is that copper can be concentrated from highly saline water (including sea-water) and separated from nearly all other metal ions. The method is

therefore useful for preconcentration before the use of methods such as electrothermal atomic-absorption spectrometry. The column is easily regenerated by washing with a large volume of water after the elution, and can be used repeatedly.

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INDIRECT POLAROGRAPHIC MICRODETERMINATION OF SULPHUR IN ORGANIC COMPOUNDS

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Summary—A polarographic method for the microdetermination of sulphur in organic compounds is described; it is based on oxygen-flask combustion to yield sulphate, which is then reacted with excess of barium dichromate solution in hydrochloric acid, followed by adjustment to pH 12, collection of the combined barium chromate and sulphate precipitate, and polarographic determination of the free chromate (equivalent to the sulphate originally present).

Several titration methods have been proposed, involving precipitation of barium sulphate, for determination of sulphur in organic compounds after conversion into sulphate.[1-5] The sulphuric acid produced in an oxygen-flask combustion of sulphur compounds can also be titrated with alkali.[5,6] An amplification procedure is based on reaction of the sulphate with excess of saturated barium bromate solution; the unreacted barium bromate is precipitated by addition of acetone, filtered off, redissolved in hot water, and treated with excess of iodide and acid, the iodine liberated being titrated with thiosulphate.[7]

Recently, it has been shown that trace amounts of inorganic and organic sulphur compounds can be determined by molecular emission cavity analysis by means of their S_2-emission measured at 384 nm.[8-10]

The present work offers an indirect polarographic method adapted from a procedure for determination in sulphate in sea-water,[11] based on addition of excess of barium dichromate solution in hydrochloric acid to precipitate barium sulphate, precipitations of the excess of barium as chromate by raising the pH with alkali, and polarographic determination of the free chromate (equivalent to the original sulphate).

EXPERIMENTAL

Apparatus

Any suitable polarograph, *e.g.*, a Metrohm E506 Polarecord, with a three-electrode electrolytic cell, the working electrode being a dropping mercury electrode (DME) connected with a tapping device for controlled drop-times of 0.4–0.6 sec. The reference electrode is Ag/AgCl, KCl and the auxiliary electrode a platinum wire.

Reagents

Analytical-grade reagents are used whenever possible. The 0.05% gelatin solution is prepared fresh weekly. Barium chromate is prepared by mixing 100 ml each of 1*M* barium chloride and 1*M* potassium chromate, collecting the precipitate on a porosity-4 sintered-glass crucible, and washing it several times with distilled water. It is then dried and stored. The barium dichromate solution is prepared by dissolving 0.333 g of barium chromate in 100 ml of 1*M* hydrochloric acid. Oxygen-free nitrogen is obtained by scrubbing the gas in a train of five bubblers, the first three containing pyrogallol in potassium hydroxide solution, followed by one containing concentrated sulphuric acid and one empty.

Procedure

Weigh accurately 2–6 mg of sample and wrap it according to Schöniger.[12] Place 10 ml of doubly distilled water in a 500-ml oxygen-combustion flask, fill the flask with oxygen and quickly add 1 ml of saturated bromine water. Light the paper fuse, and perform the combustion as usual. When the combusion is complete, shake the flask for 5 min, until the cloud of sulphur trioxide has completely disappeared. Open the flask and rinse down the stopper and gauze with about 10 ml of doubly distilled water. Place the flask on a hot-plate and boil gently until the volume of solution has been reduced to 2–4 ml.

Cool the flask under running water, add 3 ml of barium dichromate solution, stir for 1 min, then add 5 ml of concentrated ammonia solution to precipitate barium chromate. Filter off with a 12-ml porosity-4 sintered-glass crucible by suction into a small dry flask and wash the precipitate with 3 ml of 1*M* ammonia solution. Transfer the filtrate quantitatively into a 25-ml standard flask, add 1 ml of 0.05% gelatin solution, and make up to the mark with doubly distilled water. Mix thoroughly, place a portion in the polarographic cell, deoxygenate it by passage of the purified nitrogen, and rapidly record the d.c. polarogram of the cathodic reduction wave of chromate, with a starting potential of −0.2 V *vs.* the Ag/AgCl reference electrode and a sensitivity of 1 μA full-scale deflection.

Measure the height of the chromate wave and calculate the sulphur content with the aid of a calibration curve constructed by applying the precipitation and polarographic procedure to amounts of sulphuric acid solution containing 0.245–2.45 mg of H_2SO_4 (equivalent to 0.080–0.800 mg of sulphur).

RESULTS AND DISCUSSION

The polarographic behaviour of chromate has already been studied[10] and was used for the indirect determination of sulphate in different types of water.

† Reprint requests.

Table 1. Effect of volume of concentrated ammonia solution added

NH_3 solution, *ml*	i_d, *μA*	$E_{\frac{1}{2}}$, *V*
1	0.29	−0.31
2	0.29	−0.32
3	0.30	−0.34
4	0.31	−0.36
5	0.30	−0.36
6	0.25	−0.32

According to our investigation the cathodic chromate wave is best recorded at pH 12, readily attainable by addition of concentrated ammonia solution. The effect of the amount of concentrated ammonia solution added, on the $E_{\frac{1}{2}}$ and i_d of the chromate wave, is shown in Table 1. Use of 4–5 ml of concentrated ammonia solution is optimal.

The calibration curve is linear and passes through the origin. The calibration range of 0.245–2.45 mg of sulphuric acid per 25 ml is selected as it covers the expected sulphur content of 2–6-mg samples of most organic sulphur compounds.

Combustion of compounds containing chlorine, bromine, phosphorus and nitrogen leads to the production of HCl, HBr–Br_2, H_3PO_4, HNO_3 and nitrogen oxides in addition to H_2SO_4 from any sulphur present. Of these, bromine is added in the procedure to oxidize the sulphur combustion products, and the excess of bromine, HBr, CO_2 and oxides of nitrogen are completely eliminated in the boiling used to concentrate the absorption solution.

However, the effect of hydrochloric, nitric and phosphoric acid on the determination was examined by analysing solutions containing sulphuric acid alone and mixed with the other acids. Hydrogen chloride (3.7 mg) did not interfere in the determination of 2.45 mg of sulphuric acid but 6.3 mg of nitric acid caused −1% error and 9.8 mg of phosphoric acid −4% error.

Analysis of a variety of compounds gave satisfactory results (Table 2). The average absolute error for all the results was ±0.14% and the maximum error did not exceed ±0.4%. The method is simple and rapid; a single determination takes not more than 30 min, including the weighing. It can be applied to a wide range of organic compounds.

Table 2. The indirect polarographic microdetermination of sulphur in organic compounds

Compound	Sample weight, *mg*	Sulphur, % Theory	Found
Sulphonal	2.280		27.7
	2.845		28.1
	2.125	28.08	28.2
	3.425		28.1
	4.235		28.1
N-Acetyl-L-cysteine	4.975		19.3
	4.100	19.65	19.5
	3.845		19.6
	2.390		19.7
Sulphathiazol	2.325		25.1
	3.150	25.12	25.4
	4.235		25.1
	3.730		25.0
Sulphosalicylic acid	5.665		12.7
	6.370	12.61	12.6
	3.278		12.5
	4.775		12.7
8-Aminonaphthalene sulphonic acid	2.980		14.0
	3.850	14.36	14.5
	2.300		14.1
	3.125		14.3
Sulphanilamide	2.850		18.5
	3.865	18.62	18.7
	4.205		18.5
	4.765		18.4

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SEPARATION AND SPECTROPHOTOMETRIC DETERMINATION OF URANIUM(VI) BY EXTRACTION WITH TRI-n-OCTYLPHOSPHINE OXIDE AND BENZOPHENONE

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Summary—Uranium(VI) is separated by extraction from nitric acid medium into a molten mixture of tri-n-octylphosphine oxide and benzophenone at about 50°. The organic phase solidifies on cooling and is separated and dissolved in ethanol. The uranium(VI) in this solution is then determined spectrophotometrically with 1-(2-pyridylazo)-2-naphthol.

The liquid–liquid extraction of metal complexes by means of naphthalene (m.p. 80°) or benzophenone (m.p. 48°) has been developed in recent years.[1–5] The advantages are that a much smaller volume of solvent is needed, compared with liquid solvents such as toluene or cyclohexane, and upon cooling the organic phase easily separates out. Tri-n-octylphosphine oxide (TOPO) can be used by itself for the liquid–liquid extraction of uranium(VI) at elevated temperature, but it is difficult to separate the two phases, which detracts from its utility. Toluene or cyclohexane have therefore been used as diluents for the TOPO.[6–9] We have found that use of naphthalene as the diluent facilitates the separation of the phases, but the colour-reaction of uranium(VI) with PAN does not take place when applied to the extract dissolved in a polar organic solvent. Other diluents having low melting points have therefore been tested and benzophenone has been found to be satisfactory.

EXPERIMENTAL

Reagents

All reagents were of analytical grade. Standard uranium(VI) solution (1000 ppm) was prepared by dissolving 0.422 g of uranyl nitrate in demineralized water and diluting to 100 ml. Stock solutions of alkaline earth, rare-earth and transition metals (0.01 *M*) were prepared by dissolving appropriate amounts of the salts in 100 ml of 0.1 *M* nitric acid or hydrochloric acid. A 0.1% solution of PAN in 99.5% ethanol, 10% triethanolamine solution in 99.5% ethanol, and TOPO (Dojin Ltd.) were used as received, without further purification.

Procedure

Transfer a solution containing 0–100 μg of uranium(VI) into a 100-ml Erlenmeyer flask (with tightly fitting stopper), and adjust the acidity of the solution to 2 *M* in nitric acid. Add 100 mg of TOPO and 400 mg of benzophenone. Heat the flask on a water-bath at about 50° until the TOPO–benzophenone phase melts completely, then shake it vigorously for 2 min. Cool the mixture rapidly while stirring, and filter off the solid extract on paper. Wash the solid several times with water and transfer it to a 10-ml standard flask containing 1 ml of PAN solution and 2 ml of triethanolamine solution (both in ethanol). Dilute to the mark with ethanol. Mix well, and measure the absorbance at 555 nm against a reagent blank, in 1-cm cells. Prepare a calibration curve by applying the procedure to standard uranium solutions. If necessary, the uranium can be stripped by shaking the TOPO–benzophenone phase with 10 ml of 0.5 *M* sodium carbonate at about 50°.

RESULTS AND DISCUSSION

Absorption spectra and choice of solvent

Uranium(VI) forms a red complex with PAN in alkaline ethanol solution.[9] The absorption spectra of a solution containing 7.5 ppm of uranium(VI), measured against a reagent blank, and of a blank measured against ethanol, are shown in Fig. 1. The wavelength of maximum absorbance of the complex is 555 nm. Various organic solvents were used for dissolving the mixture of TOPO and benzophenone, such as ethanol, methyl isobutyl ketone, cyclohexane and dimethylformamide. The absorptivity of the complex was higher in ethanol than in the other three solvents and the colour was more stable.

Effect of amount of TOPO and benzophenone

The effect of variation in the amount of TOPO on the extraction of uranium(VI) was examined (Fig. 2). Uranium(VI) is extracted almost completely with 50 mg of TOPO. It was similarly found that when more than 200 mg of benzophenone is used, the TOPO phase is easily separated by cooling to room temperature.

The uranium is extracted almost completely if the nitric acid concentration is more than 0.1 *M*.

Calibration curve

Under the optimum conditions described above, the calibration curve is linear over the range 1–10 μg/ml. The molar absorptivity is 2.52×10^4 l.mole^{-1}.cm^{-1}. Ten replicate determinations of 50 μg of urani-

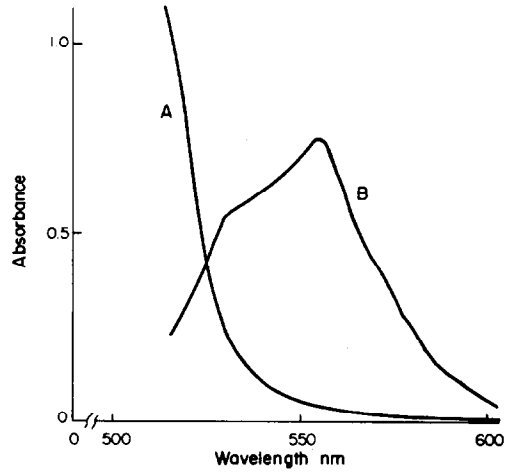

Fig. 1. Absorption curves. A: Reagent blank *vs.* ethanol; B: 7.5 ppm uranium *vs.* reagent blank.

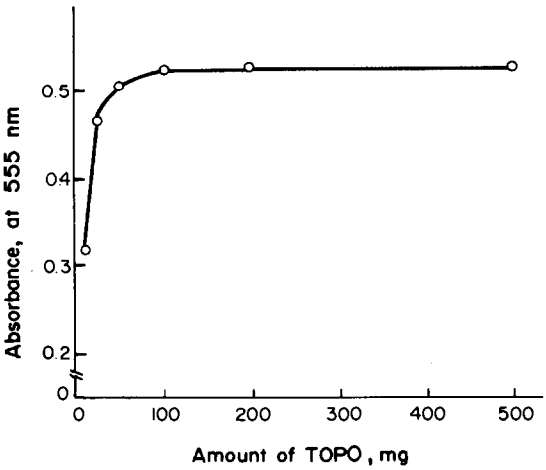

Fig. 2. Effect of TOPO on the extraction of uranium(VI) at 50°C. Conditions: U(VI) 50 μg; benzophenone 500 mg; [HNO_3] 2*M*; shaking time 2 min.

Table 1. Determination of uranium(VI) (50 μg) in the presence of 1 mg of various cations*

Cation	Absorbance	Cation	Absorbance
Mg^{2+}	0.530	Cr^{3+}	0.535
Ca^{2+}	0.530	Mn^{2+}	0.530
Sr^{2+}	0.528	Fe^{3+}	0.648
Ba^{2+}	0.528	Co^{2+}	0.568
Sc^{3+}	1.687	Ni^{2+}	0.618
Y^{3+}	0.531	Cu^{2+}	0.536
La^{3+}	0.541	Zn^{2+}	0.533
Ce^{3+}	0.544	Zr^{4+}	1.710
Nd^{3+}	0.552	Al^{3+}	0.516
V^{5+}	0.560	—	0.529

Table 2. Determination of uranium(VI) (50 μg) in the presence of 1 mg of various cations after stripping with 0.5*M* sodium carbonate*

Cation	Absorbance
Sc^{3+}	0.542
V^{5+}	0.521
Fe^{3+}	0.534
Co^{2+}	0.506
Ni^{2+}	0.514
Zr^{4+}	1.429
—	0.529

um gave a mean absorbance of 0.529 with a standard deviation of 0.010 (relative standard deviation of 1.9%).

Effect of other ions

Solutions containing 50 μg of uranium(VI) and various amounts of other metal ions were prepared and the uranium(VI) was determined. The results are given in Table 1. There was no interference from 1 mg of Mg, Ca, Sr, Ba, Y, La, Ce(III), Nd, Cr(III), Mn(II), Cu(II), Zn or Al, but Sc, V(V), Fe(III), Co(II), Ni and Zr gave considerable interference. If the uranium was stripped with 0.5*M* sodium carbonate, the interference of Sc, V(V), Fe(III), Co(II) and Ni was removed, but not that of Zr (Table 2). The method was designed for the separation of uranium(VI) from numerous other metal ions, and its determination. Since only zirconium seems to interfere, the method can be applied to a wide variety of mixtures containing traces of uranium.

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REDOX INDICATORS IN TITRATIONS WITH DICHLORAMINE-B

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Summary—Naphthidine, dimethylnaphthidine, dimethylnaphthidinedisulphonic acid, *o*-dianisidine, Quinoline Yellow, diphenylbenzidine and Amaranth are proposed as indicators in titrations of arsenic(III), iron(II), antimony(III), hydroquinone, hydrazinium sulphate, phenylhydrazine hydrochloride, semicarbazide hydrochloride and ascorbic acid with dichloramine-B. They give a very sharp colour change at the equivalence point. Arsenic(III) and iron(II) are suggested for standardization of dichloramine-B solutions. A potentiometric method for the determination of arsenic(III) and semicarbazide hydrochloride is described.

Only starch has been proposed as indicator for the standardization of dichloramine-B (DCB) by the iodometric method.[1] The present paper describes three new methods for the standardization of DCB solutions and simple but accurate methods for the determination of arsenic(III), iron(II), antimony(III), hydroquinone, hydrazinium sulphate, phenylhydrazine hydrochloride, semicarbazide hydrochloride and ascorbic acid with DCB, using naphthidine (N), 3,3′-dimethylnaphthidine (DMN), 3,3′-dimethylnaphthidinedisulphonic acid (DMNS), *o*-dianisidine (ODA), Quinoline Yellow (QY), diphenylbenzidine (DB) and Amaranth (AM) as indicators.

EXPERIMENTAL

Reagents

Indicator solutions. Solutions (0.1%) of N and DMN in glacial acetic acid, of DMNS,[2] QY and AM in water, of ODA in 0.5% sulphuric acid, and of DB in concentrated sulphuric acid were prepared and stored in amber bottles.

DCB solution. Recrystallized chloramine-B (30 g) was dissolved in distilled water (500 ml) and pure chlorine was bubbled through the solution for about 8 hr till saturation was reached. The white precipitate obtained was filtered off, washed well with water, dried on the filter by suction of air through it and kept for 24 hr in a blackened desiccator containing anhydrous calcium chloride. An approximately 0.1 *N* solution was prepared by dissolving 2.8 g of DCB in 500 ml of glacial acetic acid containing 50 ml of acetic anhydride. The solution was stored in an amber bottle fitted with an automatic burette and a guard-tube for the exclusion of moisture, standardized[1] and used immediately.

Reductants. A standard solution of arsenic(III) was prepared from recrystallized arsenic(III) oxide.[3] Approximately 0.1*N* solutions of iron(II),[4] antimony(III),[5] hydroquinone,[6] hydrazinium sulphate,[7] phenylhydrazine hydrochloride,[8] semicarbazide hydrochloride[8] and ascorbic acid[9] were prepared and standardized by the recommended methods. The solutions were stored in amber bottles and diluted as required.

Standardization of DCB solution with arsenic(III) and iron(II)

A mixture of 20 ml of 0.1–0.02*N* arsenic(III) or iron(II), 3 ml of 10% potassium bromide solution and 0.3 ml of QY, 0.1 ml of AM or 0.05 ml of DB for As(III), and 0.1 ml of ODA for Fe(II), was diluted to 40 ml with water for arsenic(III) or mixed with enough phosphoric acid to give a concentration of 2*M* at the end-point for iron(II), and titrated with 0.1–0.02*N* DCB to the appropriate colour change (disappearance of the yellow or red colour or appearance of a blue-violet or red colour).

Potentiometric titration of arsenic(III) and semicarbazide hydrochloride

A mixture of 5–20 ml of 0.1–0.02*N* arsenic(III) or 2–10 ml of 0.1–0.02*N* semicarbazide hydrochloride and 3 ml of 10% potassium bromide solution was diluted to 60 ml with water for arsenic(III) or mixed with enough sulphuric acid to give a concentration of 1*M* at the end-point for semicarbazide hydrochloride, and titrated potentiometrically with 0.1–0.02*N* DCB, a bright platinum gauze indicator electrode being used.

Titration of antimony(III), hydroquinone, hydrazinium sulphate, phenylhydrazine hydrochloride, semicarbazide hydrochloride and ascorbic acid

A mixture of 10 ml of 0.1–0.02*N* reductant, 3 ml of 10% potassium bromide solution and 0.1 ml of AM for antimony(III) and hydrazinium sulphate, 0.1 ml of AM or DB for phenylhydrazine hydrochloride, 0.2 ml of N for hydroquinone, 0.1 ml of AM for semicarbazide hydrochloride (added after 95% titration) was mixed with enough sulphuric acid to give a concentration of 1*M* (0.3*M* for phenylhydrazine hydrochloride) at the end-point and titrated with 0.1–0.02*N* DCB to the disappearance of the red colour or appearance of a blue-violet or pinkish red colour.

A mixture of 20 ml of ascorbic acid solution, 3 ml of 10% potassium bromide solution and 0.1 ml of any of the indicators was diluted with distilled water to 40 ml and titrated with 0.1–0.02*N* DCB.

RESULTS AND DISCUSSION

Standardization with arsenic(III)

QY, DB and AM give very sharp and correct end-points in 2.5–5.1*M* acetic acid medium, obtained by adding DCB in glacial acetic acid to the aqueous titrand solution. Higher acidities cause sluggish end-

points and lower acidities give higher DCB titres. QY gives a reversible change from yellow to colourless and DB and AM give irreversible changes from colourless to blue-violet and from red to colourless respectively. The end-point colour of DB is stable for 20 min.

A final concentration of 0.5–1.3% potassium bromide is satisfactory. Too high a concentration causes sluggish end-points. The volume of indicator recommended is 0.1–0.3 ml of QY, 0.05–0.15 ml of DB or 0.1–0.15 ml of AM. Too much indicator results in higher titres. The indicator correction is 0.03 ml of 0.01*N* DCB for 0.05 ml of DB or AM or 0.2 ml of QY. The sensitivity of the indicators decreases in the order DB > QY > AM.

The acetic acid and potassium bromide concentrations used for the potentiometric titration are the same as above, and changes in the concentrations have the same effects.

Standardization with iron(II)

Only ODA gives a sharp and reversible colour change (colourless → red) in the titration of iron(II) with DCB (in 1.5–3*M* phosphoric acid or 1.3–5.1*M* acetic acid containing 0.3–1.3% potassium bromide). The end-point colour is stable for 20–40 min. Lower acidities and concentrations of bromide give sluggish end-points and higher acidities and concentrations of bromide give higher titres.

Use of 0.1–0.3 ml of ODA in a total volume of 60 ml gives a satisfactory colour change (indicator correction 0.02 ml of 0.01*N* DCB per 0.1 ml of ODA). Higher concentrations of ODA give overshot end-points.

Comparison of standardizations

The results in Table 1 show satisfactory agreement between the standardization methods used. The titrations were done against primary-standard arsenic(III) and potassium dichromate indirectly through the agency of standardized ammonium iron(II) sulphate and sodium thiosulphate solutions. The arsenic(III) method is superior because it uses direct titration of a primary standard.

Other reductants

Antimony(III), hydrazinium sulphate and semicarbazide hydrochloride. AM gives a sharp colour change (which is irreversible from red to colourless) (0.5–2*M* sulphuric acid, 0.3–1.7% potassium bromide and 0.1–0.4 ml of AM; indicator correction 0.03 ml of 0.01*N* DCB for 0.1 ml of AM).

For semicarbazide hydrochloride the indicator is added after 95% of the titration has been completed.

Hydroquinone. N (0.2–0.4 ml) gives sharp reversible end-points (from light yellow to pinkish red) in 0.5–2*M* sulphuric acid containing 0.3–1.7% potassium bromide. The end-point colour is stable for 2–3 min. The indicator correction is 0.04 ml of 0.01*N* DCB for 0.2 ml of N.

Phenylhydrazine hydrochloride. DB gives a very sharp reversible change from colourless to blue-violet in 0.1–0.75*M* hydrochloric acid or 0.1–1*M* sulphuric acid containing 0.3–1.3% potassium bromide. The end-point colour is stable for 2–5 min. AM gives an irreversible change from red to colourless in the same acid range. The indicator correction is 0.03 ml of 0.01*N* DCB for 0.1 ml of DB or AM.

Ascorbic acid. N, DMN, DMNS, ODA, QY and DB give reversible end-points and AM an irreversible end-point in 3.2–7.7*M* acetic acid medium containing 0.3–1.5% potassium bromide. N, DMN, DMNS, ODA and DB change from colourless, QY from yellow to colourless, and AM from red to colourless. The end-points with N, DMN, DMNS, ODA and DB are stable for 7, 15, 30, 10 and 10 min respectively. Amounts of indicator suitable for titration of 0.1–0.05*N* ascorbic acid (60 ml) are 0.1–0.3 ml of N, DMN, DMNS, ODA, AM or DB and 0.2–0.6 ml of QY. Smaller amounts of indicator are suitable for titration of 0.02*N* ascorbic acid.

The indicator corrections are 0.06 ml of 0.01*N* DCB for 0.1 ml of AM or DB, and 0.04 ml for 0.1 ml of N, DMN, DMNS, ODA or QY. The sharpness of the end-points is in the order DMNS > N > ODA = QY = DB > DMN = AM.

The DCB titration can be used for determination of ascorbic acid in vitamin C tablets and injections. A synthetic mixture containing 315 mg of gum acacia, 250 mg each of citric acid, sodium alginate, stearic acid, sucrose, talc and gelatin, 270 mg of reserpin and

Table 1. Standardization of DCB solution

Iodometric method, *N*	Arsenic(III) method, *N*		Iron(II) method, *N*
	Indicator	Potentiometric	
0.1033	0.1029	0.1027	0.1031
0.1273	0.1274	0.1275	0.1277
0.1389	0.1392	0.1391	0.1389
0.05066	0.05067	0.05067	0.05068
0.05482	0.05484	0.05483	0.05485
0.05825	0.05822	0.05823	0.05825
0.02119	0.02115	0.02116	0.02118
0.02525	0.02525	0.02523	0.02526

Table 2. Titration of arsenic(III), iron(II), antimony(III), hydroquinone, hydrazinium sulphate, phenylhydrazine hydrochloride, semicarbazide hydrochloride and ascorbic acid with DCB

Reductant	Taken, *mg*	Found,* *mg*	Standard deviation, *mg*
Arsenic(III)	76.3	76.3	0.03
	9.11	9.13	0.04
	7.14	7.15	0.01
Iron(II)	113.7	114.0	0.05
	18.19	18.17	0.06
	9.14	9.16	0.04
Antimony(III)	60.2	60.2	0.06
	5.48	5.46	0.06
	2.41	2.43	0.05
Hydroquinone	110.5	110.4	0.08
	4.63	4.64	0.04
	1.12	1.13	0.03
Hydrazinium sulphate	32.26	32.29	0.02
	3.92	3.91	0.05
	1.34	1.33	0.05
Phenylhydrazine hydrochloride	36.23	36.27	0.08
	4.42	4.44	0.06
	1.46	1.47	0.09
Semicarbazide hydrochloride	28.14	28.19	0.04
	3.17	3.19	0.07
	1.12	1.13	0.08
Ascorbic acid	177.4	177.4	0.03
	2.83	2.82	0.01
	1.54	1.53	0.01

* Average of five determinations.

Table 3. Assay of vitamin C tablets and injections

Sample	Nominal content, *mg*	Ascorbic acid found, *mg*	
		B.P. method	Proposed method
Citravite (Pharmed)	500	500.0	498.9
Sukcee (IDPL)	500	497.8	495.5
Redoxon (Roche)	500	494.2	497.8
Sorvicin (East India)	500	493.8	495.7
Celin (Glaxo)	500	493.7	495.6
Chewcee (Cynamid)	500	496.9	501.5
Ascorbicin (Sarabhai)	500	509.1	510.4
Reclor (Sarabhai)	250	246.4	252.1
Redoxon (Roche)	500	508.6	505.1
Calcium Sandoz 10% + Vit C (Sandoz, India)	500	508.6	506.2

305 mg of starch did not interfere in the titration of 45 mg of ascorbic acid with DCB.

Results

Tables 2 and 3 show that satisfactory precision is obtained, and the results agreed well with those obtained by other methods.[5–10] Departure from the conditions given leads to incorrect results and may result in sluggish end-points.

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N-SUBSTITUTED PHENOTHIAZINES AS REDOX INDICATORS IN TITRATIONS WITH CHLORAMINE-T AND CHLORAMINE-B

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Summary—Profenamine hydrochloride, fluphenazine dihydrochloride, trifluopromazine hydrochloride, cyamepromazine maleate, perphenazine dihydrochloride and mepazine hydrochloride are proposed as redox indicators in the titration of hydroquinone, metol and ascorbic acid with chloramine-T and chloramine-B in sulphuric, hydrochloric and acetic acid media. They give a sharp reversible colour change at the equivalence point. A simple but accurate method for the determination of hydroquinone, metol and ascorbic acid is described. The conditional potentials and molar absorptivities of the indicators and redox potential of chloramine-B are reported.

In Chloramine-T (CAT) and chloramine-B (CAB) titrations starch is often used as indicator but has several disadvantages.[1] Some of the organic dyestuffs used as indicators act by being destroyed at the equivalence point. The lack of suitable reversible redox indicators for these titrations led us to investigate the use of *N*-substituted phenothiazines.

EXPERIMENTAL

Reagents

Indicator solutions. Aqueous solutions (0.2%) of profenamine hydrochloride (PFH), fluphenazine dihydrochloride (FPH), triflupromazine hydrochloride (TFPH), cyamepromazine maleate (CMM), perphenazine dihydrochloride (PPH) and mepazine hydrochloride (MH) were prepared and stored in amber bottles. PFH was dissolved in hot water (60°).

Potentiopoised solutions. Equimolar vanadium (V)–vanadium (IV) solutions in 0.00625–6.0*M* sulphuric acid were prepared.[2,3]

Buffer solutions. Acetate, phosphate and borax buffers were prepared.

Oxidants. Approximately 0.05*M* CAT was standardized by the usual methods. CAB was prepared by passing pure chlorine through benzenesulphonamide dissolved in 4*M* sodium hydroxide, for about 1 hr at 70°. The mass obtained was filtered off and recrystallized from water. A 0.05*M* solution was prepared and standardized iodometrically.[4]

Reductants. Approximately 0.05*M* hydroquinone, metol and ascorbic acid solutions were standardized, stored in amber bottles and diluted as required.

Determination of the conditional indicator potentials

The conditional potentials of the indicators were determined by the potentiopoised solution method[2,3] and Schilt's method[5] and are given in Table 1.

Determination of molar absorptivity of the indicators

The indicators were oxidized with ceric sulphate in phosphoric acid medium and the absorbances were measured at the absorption maxima against a reagent blank.

Determination of the redox potentials of CAB

Equimolar solutions (0.005*M*) of CAB and benzenesulphonamide in acetate, phosphate and borax buffers were prepared and their potentials measured.

Titration procedures

Hydroquinone. Transfer 20 ml of 0.01–0.05*M* hydroquinone, 8 ml of 10% potassium bromide solution, 1 ml of PFH, FPH, TFPH, CMM or PPH solution and enough sulphuric or hydrochloric acid to give a 0.3*M* concentration or acetic acid to give a 1*M* concentration at the end-point and dilute to 40 ml. Titrate with CAT or CAB to orange-red (add the indicator for acetic acid medium after 95% titration). For 0.0025–0.005*M* hydroquinone (10 ml)

Table 1. Determination of formal potentials (*mV*) of PFH, FPH, TFPH, CMM, PPH and MH

$[H_2SO_4]$, *M* / Indicator	Potentiopoised method			Schilt's method					
	0.009	0.075	0.20	0.25	0.50	0.75	1.00	1.25	1.50
PFH	—	899	—	834	831	825	822	817	811
FPH	—	—	921	904	879	874	871	865	853
TFPH	—	—	921	895	870	864	853	847	843
CMM	—	—	921	904	896	889	881	872	860
PPH	—	—	—	786	773	764	738	725	709
MH	808	—	—	802	796	782	771	762	754

Table 2. Titration of hydroquinone, metol and ascorbic acid with PFH, FPH, TFPH, CMM, PPH and MH as indicators

	Reductant taken, *mg*	Reductant found, *mg*		Standard deviation
		CAT method	CAB method	
Hydroquinone	112.3	112.4		0.06
	112.3		112.5	0.08
	55.4	55.5		0.08
	55.4		55.5	0.05
	2.23	2.24		0.02
	2.23		2.24	0.02
Metol	159.4	159.3		0.08
	159.4		159.1	0.09
	43.9	43.9		0.05
	43.9		43.9	0.06
	2.13	2.12		0.03
	2.13		2.12	0.02
Ascorbic Acid	166.3	166.4		0.04
	166.3		166.5	0.03
	38.5	38.4		0.02
	38.5		38.5	0.03
	2.11	2.12		0.02
	2.11		2.11	0.01

use 3 ml of 10% potassium bromide solution, 0.5 ml of indicator (except PPH in CAB titrations in hydrochloric acid media) adjust the acidity, dilute to 25 ml and titrate with 0.0025–0.005*M* CAT or CAB.

Metol. Dilute 0.01–0.05*M* metol solution (20 ml) and 8 ml of 10% potassium bromide to 40 ml with enough sulphuric, hydrochloric or acetic acid to give a concentration of 0.4*M* at the end-point. Titrate with CAT or CAB to orange-red, adding 1 ml of PFH, FPH, TFPH or CMM solution near the expected end-point (after 95% titration). For 0.0025–0.005*M* metol, add 3 ml of 10% potassium bromide solution, add the acid, dilute to 25 ml and titrate with CAT or CAB, using 0.5 ml of indicator.

Ascorbic acid. For 20 ml of 0.025–0.05*M* ascorbic acid, use 8 ml of 10% potassium bromide solution and 1 ml of PFH, FPH, TFPH, CMM, PPH or MH (PPH and MH added near the end-point), and dilute to 40 ml with enough acid to give a concentration of 0.5*M* hydrochloric or sulphuric acid or 1.5*M* phosphoric acid (do not use PPH and MH in phosphoric acid medium). Titrate with CAT or CAB to orange-red or pink.

Ascorbic acid in vitamin C tablets and injections. Prepare the solution of vitamin C tablets and injections by the procedure described earlier.[6] To 10 ml of the solution add 20 ml of 3*M* phosphoric or 1*M* sulphuric acid and 1 ml of PFH, FPH, TFPH or CMM solution and titrate with 0.025*M* CAT or CAB to pink or orange-red.

RESULTS AND DISCUSSION

PFH, FPH, TFPH, PPH and MH are highly soluble in water, giving colourless solutions. CMM gives a light yellow solution. The stability and mechanism of oxidation of the indicators are similar to those of *N*-substituted phenothiazines as described earlier.[7]

The molar absorptivities of oxidized PFH, FPH, TFPH, CMM, PPH and MH are 25.8, 21.4, 16.9, 24.2, 25.5 and 22.0 × 10^3 l. $mole^{-1}$. cm^{-1} at 512, 498, 496, 508, 530 and 510 nm respectively, indicating that the indicators should be very sensitive. Phosphoric acid

Table 3. Determination of ascorbic acid in vitamin C tablets and injections, with PFH, FPH, TFPH and CMM as indicators

Tablet or injection	Nominal amount of ascorbic acid, *mg*	Amount of ascorbic acid found, *mg*		
		CAT method	CAB method	B.P. method
Tablets				
Celin (Glaxo)	500	488.6	499.3	493.7
Redoxon (Roche)	500	499.4	498.7	494.2
Sorvicin (East India)	500	496.8	494.6	493.8
Ascorbicin (Sarabhai)	500	514.9	514.3	519.6
Suckcee (IDPL)	500	500.8	501.3	496.9
Chewcee (Lederle)	500	501.6	502.8	491.2
Citravite (Pharmed)	500	500.1	500.3	500.1
Injections				
Calcium Sandoz + Vitamin C (Sandoz)	500	500.0	500.4	506.5
Redoxon (Roche)	500	502.8	503.6	508.6

was used as the reaction medium to stabilize the coloured radical cation of the indicator. Ceric sulphate was chosen as oxidant because it is quantitatively reduced and gives an equivalent amount of coloured radical cation in the presence of large excess of the indicator.[8]

The redox potential of CAT has been reported to be 1.138 V in hydrochloric acid–sodium acetate medium at pH 0.65.[9] It is difficult to determine the redox potential of CAB in acid medium because CAB and benzenesulphonamide are only sparingly soluble. However, the solubility increases with pH. An equimolar CAB–benzenesulphonamide solution (0.005*M*) in various media was used to determine the redox potentials of CAB; the results were 1.258, 1.184, 0.891 and 0.619 V at pH 0.65, 3.95, 6.75 and 9.25 respectively.

Titration conditions

The permissible ranges of acidity vary widely, depending on the reductant being titrated and on the acid, titrant and indicator used. For economy we therefore present only some general conditions.

In hydrochloric acid medium, an acidity of 0.2–0.5*M* is suitable for all five indicators with both CAT and CAB as titrant for all three reductants. In sulphuric acid medium the corresponding acidity range is 0.3–0.5*M*. Acetic acid in the range 1–2*M* is suitable for hydroquinone, and in the range 0.3–1.3*M* for metol, but is not suitable for ascorbic acid. Instead, phosphoric acid (1–2*M*) is used for ascorbic acid (it complexes metal ions which might catalyse aerial oxidation of the ascorbic acid).

For all three reductants 1–2% of potassium bromide should be present, and 1 ml of 0.2% indicator (0.05 ml of 0.005*M* CAT or CAB, average correction) is recommended. PFH is in general the most sensitive of these indicators.

Assay of vitamin C tablets and injections

Titration with CAT and CAB has been successfully employed for the determination of ascorbic acid in vitamin C tablets and injections. The usual diluents and excipients such as oxalic, citric, tartaric, succinic, acetic and malic acids, glucose, fructose, sucrose and starch (up to 300 mg each) and gelatin, talc, stearic acid, alginic acid, reserpine, pulvis acaciae (up to 180 mg each) do not interfere in the determination of 40–45 mg of ascorbic acid in 1*M* sulphuric acid or 1.5*M* phosphoric acid. The results compare favourably with those obtained by the official method in the British Pharmacopoeia.[10]

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SPECTROPHOTOMETRIC DETERMINATION OF RUTHENIUM AND OSMIUM

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Summary—The optimum conditions for preparation of stable solutions of ruthenate and osmate, after alkaline fusion of ruthenium(IV) compounds, ruthenium metal and osmium metal in a silver crucible, have been determined. The molar absorptivities of ruthenate and osmate are 1.74×10^3 l.mole^{-1}.cm^{-1} at 465 nm (Ru) and 2.75×10^3 l.mole^{-1}.cm^{-1} at 340 nm (Os) in 2*M* sodium hydroxide. A differential spectrophotometric method has been developed for determination of ruthenium in ruthenium dioxide, lead ruthenite and bismuth pyroruthenate. Simultaneous spectrophotometric determination is proposed for ruthenium and osmium. The other platinum metals interfere seriously only when present in >1:1 w/w ratio to Ru.

Some ruthenium(IV) compounds (*e.g.*, ruthenium dioxide, lead ruthenite and bismuth ruthenite) have recently been utilized in electronics. Methods of precise determination of the ruthenium content of these compounds are needed. Since there are few suitable gravimetric and titrimetric methods for determination of macroamounts of ruthenium,[1] the orange ruthenate ion[2] (RuO_4^{2-}) has been used for the spectrophotometric determination, the precision being increased by use of differential absorbance measurements.[3,4] Spectrophotometric determination of ruthenate has been described before[5–7] but the precision was rather low. In this work the conditions for the differential spectrophotometric determination of ruthenium and osmium as RuO_4^{2-} and OsO_4^{2-} have been examined.

EXPERIMENTAL

Preparation of reference ruthenium solution

Weigh about 15 mg of pure ruthenium powder accurately on a semimicrobalance and place it in a 15-ml silver crucible. Fuse the ruthenium with 1 g of sodium hydroxide and 0.2 g of sodium peroxide at dull-red heat for 15 min. Dissolve the cooled melt in a small amount of water, transfer the solution to a 500-ml standard flask, make up to the mark with 2*M* sodium hydroxide and mix.

Preparation of the differential calibration curve

Prepare solutions containing about 20, 25 and 30 mg (accurately weighed) of ruthenium in 500 ml, as above. Measure the absorbances of 465 nm against the reference ruthenium solution, and plot the differential calibration curve.

Determination of ruthenium in ruthenium dioxide

Weigh accurately about 30 mg of ruthenium dioxide and fuse it with 1 g of sodium hydroxide at dull-red heat for 15 min in a silver crucible. Dissolve the cooled melt in a small amount of water and dilute to volume with 2*M* sodium hydroxide in a 500-ml standard flask. Measure the absorbance at 465 nm against the reference ruthenium solution.

Determination of Ru in lead or bismuth ruthenite

Fuse about 60 mg of the ruthenite (accurately weighed) with 1 g of sodium hydroxide in a silver crucible as above, but before adding the sample, coat the bottom of the crucible with sodium hydroxide by melting a fraction of the alkali in it and cooling. Dissolve the melt in a small amount of water, by heating for 15 min on a steam-bath, and make up to volume in a 500-ml standard flask with 2*M* sodium hydroxide. (In the case of bismuth pyroruthenite heat for 20 min in a water-bath at 50° and make the solution up to 500 ml with 4*M* sodium hydroxide.) Measure the absorbance of the solution at 465 nm against the reference ruthenium solution.

RESULTS AND DISCUSSION

Determination of ruthenium as ruthenate

The standard solutions of water-soluble, orange ruthenate (RuO_4^{2-}) are prepared from ruthenium metal powder of appropriate purity by fusion with a mixture of sodium hydroxide and peroxide. The absorption spectrum of ruthenate is shown in Fig. 1 (curve 1).

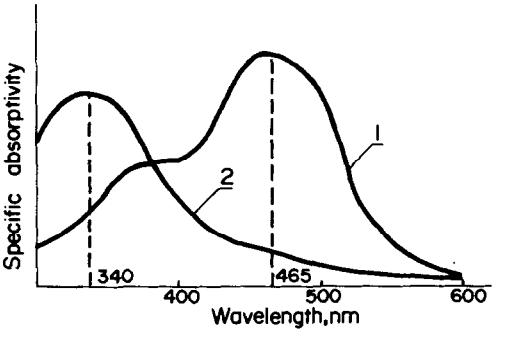

Fig. 1. Absorption spectra of ruthenate (curve 1) and osmate (curve 2) in 2*M* NaOH medium.

Fusion with sodium hydroxide alone is unsatisfactory. It was found that at a 5:1 mass ratio of sodium hydroxide and peroxide ruthenium is completely oxidized to ruthenate at dull-red heat. A larger fraction of sodium peroxide in the flux is unfavourable because it strongly attacks the silver crucible. Up to about 30 mg of ruthenium can be fused with a mixture of 1 g of sodium hydroxide and 0.2 g of the peroxide. Any silver removed from the crucible during the fusion does not interfere in the determination.

The molar absorptivity of ruthenate (RuO_4^{2-}) at 465 nm in 2*M* sodium hydroxide is 1740 ± 16 l. mole^{-1}.cm^{-1} ($n = 5$, significance level $\alpha = 0.05$). When the absorbance is measured immediately after preparation of the solutions it is not affected by the alkali concentration in the range 0.1–2*M*, but higher concentrations decrease the absorbance (*e.g.*, by 1.7% with 4*M* alkali). Alkaline ruthenate solutions obey Beer's law up to a ruthenium concentration of 50 μg/ml.

The stability of the absorbance of the ruthenate solutions was found to depend on the concentration of ruthenium as well as on the amount and quality of the sodium hydroxide used for the fusion. The stability increases with increasing concentration of ruthenium, the colour at a ruthenium concentration > 0.6 mg/ml being stable for about a month (at the optimum alkali concentration). When Merck *p.a.* sodium hydroxide was used the absorbance of 50-μg/ml ruthenium solutions in 2–4*M* alkali was constant for 24 hr. When other kinds of sodium hydroxide were used, the absorbance was constant for shorter periods (4–10 hr).

The duration and temperature of heating of the alkaline ruthenate solution before the absorbance measurement have no effect on the stability of the solution.

Elements that give coloured ions in alkaline medium interfere, and include the platinum metals, the error caused being + 4% by Pd, + 5% by Ir, + 7% by Pt and + 8% by Rh when these metals are present in 5:1 w/w ratio to Ru. Osmium causes + 10% error when present in 1:1 w/w ratio to Ru.

Determination of ruthenium in some ruthenium(IV) compounds

Ruthenium dioxide is easily fused with sodium hydroxide (without addition of sodium peroxide), the ruthenium being converted into the sexivalent state. The absorption spectrum of the ruthenate solution obtained is identical with that of the ruthenate obtained from ruthenium metal. Analysis of a sample of ruthenium dioxide gave 75.95% ruthenium (standard deviation 0.12%, $n = 6$). The theoretical content is 75.94%.

Fusion of lead ruthenite ($PbRuO_3$) with sodium hydroxide gives a product which dissolves in water to give an alkaline solution of ruthenate and plumbite (which does not interfere in the determination). To prevent the attack of lead on the silver crucible and possible losses of ruthenium, the crucible is covered with a protective layer of sodium hydroxide before the sample is added. Formation of a clear solution is accelerated by heating for 15 min on a steam-bath. Analysis of a sample of pure lead ruthenite gave 28.2$_9$% ruthenium (standard deviation 0.09%, $n = 6$). The theoretical content is 28.35%.

Fusion of bismuth pyroruthenate ($Bi_2Ru_2O_7$) with sodium hydroxide gives orange ruthenate, but during dissolution of the melt in water a brown-black precipitate, insoluble in 2*M* sodium hydroxide, is formed, which retains small amounts of ruthenium. However, if the suspension is heated with 4*M* sodium hydroxide at 50° for about 20 min the ruthenium quantitatively passes into solution as RuO_4^{2-}. Analysis of pure bismuth pyroruthenate gave 27.1$_4$% ruthenium (standard deviation 0.13%, $n = 6$). The theoretical content is 27.60%.

Determination of osmium as osmate

If osmium metal powder is fused at dull-red heat with sodium hydroxide (with or without sodium peroxide) and the cooled melt dissolved in water, a solution containing osmium in the form of pale yellow osmate is obtained. The absorption spectrum of osmate in 2*M* sodium hydroxide is shown in Fig. 1 (curve 2). The absorption maximum appears at 340 nm. The alkaline osmate solutions are less stable with time than the corresponding ruthenate solutions, a 50-μg/ml osmium solution in 2*M* sodium hydroxide retaining constant absorbance for only 30 min, and fading because of aerial oxidation. The decomposition is slower in closed vessels, but practically unaffected by light. The absorbance increases with increasing sodium hydroxide concentration. In 2*M* sodium hydroxide the molar absorptivity at 340 nm is 2745 ± 21 l.mole^{-1}.cm^{-1} ($n = 5$). Beer's law is obeyed up to an osmium concentration of 80 μg/ml.

Ruthenium and osmium mutally interfere because of the overlap of their absorption spectra, but this can be turned to advantage by measuring the absorbance at the two wavelengths of maximal absorption and

Table 1. Results of osmium and ruthenium determination in mixtures of both metals (by fusion as for ruthenium metal and measurement at 340 and 465 nm)

Taken, *mg*		Found, *mg*	
Os	Ru	Os	Ru
2.78	2.79	2.67	2.77
2.34	2.37	2.35	2.42
2.17	3.35	2.15	3.32
3.75	3.80	3.62	3.83
3.98	3.75	3.86	3.73
4.67	3.69	4.59	3.62
4.00	20.54	3.86	21.0$_3$
4.65	19.87	4.52	19.2$_5$
19.93	3.94	19.3$_5$	3.78
20.84	4.87	21.1$_2$	4.76

use of simultaneous equations. As usual in such cases, the precision is impaired, and the errors may be appreciable (Table 1).

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ENTHALPIMETRY WITH CATALYTIC REACTIONS—I DETERMINATION OF SULPHIDE SOLUTION BY THE IODINE–AZIDE REACTION

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Summary—Sulphide in the range 0.02–0.5 μmole is determined by direct injection enthalpimetry with relative errors and coefficient of variation within 3%.

Direct injection enthalpimetry (DIE) is one of the main types of enthalpimetric analysis and the procedure is very simple. Rapid DIE methods have been proposed for the quality control analysis of various products.[1,2] One drawback of the technique is the lack of sensitivity. Smith *et al.*[3] improved the sensitivity by using a function generator and lock-in amplifier. Hansen *et al.*[4] proposed a method for determining nitrite at the nmole level, based on the reaction with sulphamic acid. The method is too time-consuming for routine analysis because the titrant and titrand have to be brought to the same initial temperature. High sensitivity can be obtained by the use of catalytic reactions, although these are not fundamental solutions to the difficulty. There have been many enthalpimetric–kinetic methods based on catalytic reactions.[5–9] However, they are also time-consuming and extraneous heat effects brought about by stirring and Joule heat changes during measurements lead to serious errors.

Iodometric procedures are most generally used for determination of inorganic sulphide. Iodine, iodate, hypohalite, permanganate *etc.* have been used as the oxidant and instrumental methods for detecting the end-point have been investigated.[10] Thermometric and enthalpimetric determinations of sulphide have been described by Sajó *et al.*[11] and Marik-Korda *et al.*[12] However, the titration procedures are not sensitive enough to measure trace amounts of sulphides with good precision.

It is well known that the iodine–azide reaction is catalysed by metallic sulphides, such as ZnS and CdS, and by sulphide ions in solution, and this has been used as a basis for qualitative and quantitative analysis for traces of sulphides.[13–15] Sensitive titrimetric methods based on the reaction have been described by several workers.[16–18]

The present paper gives a direct-injection enthalpimetry method for determining trace amounts of sulphide by use of the iodine–azide reaction. The sample solution is injected into the iodine–azide mixture. The reaction takes place instantly on injection of the sample and terminates within 2 min. The rise in temperature is monitored with a thermistor in a Wheatstone bridge circuit, and is proportional to the amount of sulphide.

EXPERIMENTAL

Apparatus

A thermometric titrator (TOA electric TMT-3A) with an electronic recorder was used. The injector was a 1-ml microsyringe. The reaction vessel was a 30-ml Dewar flask in a thermostat at 25 ± 0.1°.

Reagents

All reagents used were of analytical reagent grade. A 0.2*M* iodine (I_2) solution in 1.0*M* potassium iodide was prepared and standardized by titration with thiosulphate. The sodium azide was checked iodometrically by the method of Feigl *et al.*[19] Buffer solutions (0.1*M*) of potassium phthalate–sodium hydroxide (pH 5.5), potassium dihydrogen phosphate–sodium hydroxide (pH 6–8) and ammonium chloride–ammonia (pH 9 and 10) were used. A stock sulphide solution (0.05*M*) was made by dissolving sodium sulphide enneahydrate crystals in 0.1*M* sodium hydroxide and standardized with standard iodine and thiosulphate solutions. Dilute standard sulphide solutions were prepared by diluting the freshly standardized stock solution with freshly boiled and cooled distilled water for each measurement. The iodine–azide solution was prepared by dissolving 45.5 g of sodium azide in 250 ml of 0.08*M* iodine (I_2) solution, adjusting to pH 7.0 with hydrochloric acid and making up to 1 litre with phosphate buffer (pH 7.0). The solution was allowed to stand for 3 hr to stabilize. The solution could be used for a month if stored at 4° in a refrigerator.

Procedure

A 10-ml portion of the iodine–azide solution was pipetted into the reaction vessel and a 500-μl portion of sample taken in the syringe. The loaded syringe was placed in position, stirring started, and thermal equilibrium reached. A sufficient length of base-line was recorded, the sample

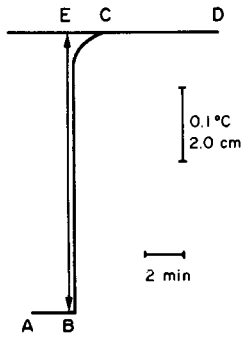

Fig. 1. Enthalpogram for sulphide. The solution: 10 ml of 0.02*M* iodine, 0.7*M* sodium azide at pH 7.0. Sulphide: 1.0×10^{-7} mole in 500 μl. Line AB is the initial isothermal base-line, line BC is the reaction curve and line CD is the post-reaction region. Point B is the injection point. Pulse height is BE.

solution injected quickly, and the temperature change recorded; the sensitivity was chosen by trial and error. A determination took 10 min.

A calibration curve was prepared in the usual way with standard sulphide solutions.

RESULTS AND DISCUSSION

The important variables in the reaction are the pH and the concentrations of azide, iodide, iodine and sulphide.[16–18] Their influence was investigated by injection of (*a*) sulphide solution into iodine–azide solution, (*b*) iodine solution into azide–sulphide solution and (*c*) azide solution into iodine–sulphide solution. The catalysis by sulphide occurred in (*a*) and (*b*).

Sulphide solutions buffered at various pH values were injected into solutions that were 0.02*M* in iodine (I_2), 0.1*M* in potassium iodide, 0.7*M* in sodium azide and at various pH values. Figure 1 shows an enthalpogram obtained at pH 7.0. Enthalpograms for other solutions were similar in shape. The reaction took place rapidly immediately after injection of the sulphide solution and terminated within 2 min. If buffer alone was injected, no temperature pulses were observed. Figure 2 illustrates the influence of pH on the temperature change, *i.e.*, the extent of the catalysed reaction. The extent of reaction was greater in acidic solutions than in basic solutions. The pH-dependence was least over the pH range 6.5–7.5.

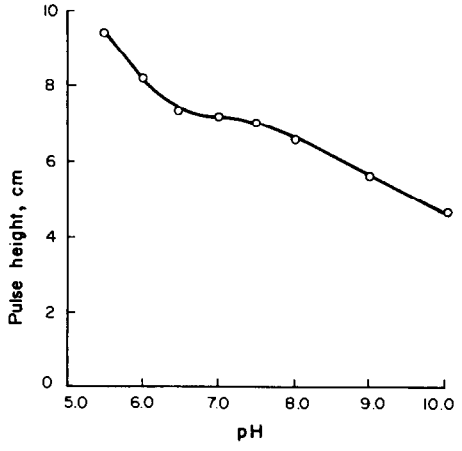

Fig. 2. Influence of pH on the pulse height. Other conditions as for Fig. 1.

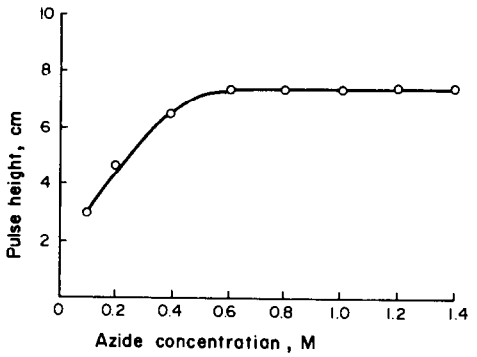

Fig. 3. Influence of azide concentration on the pulse height. Conditions as for Fig. 1.

When iodine solution was injected into sulphide-free azide solution, exothermic pulses 1–2 cm in height on the recorder chart were observed at various pH values and may be attributed to the reaction of iodine with azide. Because of volatilization of hydrogen sulphide from the azide–sulphide solutions, the pulse height obtained on injection of iodine decreased with increase in the lapse of time between preparation of the reaction medium and injection. At pH 5.5, 6.0, 7.0, 8.0 the pulse heights measured 4 min after preparing the solutions were 17.7, 13.1, 10.8, 7.6% less, respectively, than those measured 1 min after the preparation. It was concluded that injection of the iodine solution into the azide–sulphide solution is an impractical procedure.

The influence of the concentrations of sodium azide on the pulse height at pH 7.0 is shown in Figs. 3 and 4. The extent of reaction was independent of the azide concentration over the range 0.6–1.4*M*. The extent of reaction increased linearly with iodide concentration over the range 0.05–2.0*M*, but the presence of large quantities of iodide slowed down the velocity of the reaction. The times taken to complete the reaction at 1.0 and 2.0*M* iodide concentrations were 4 and 7 min, respectively.

Changing the iodine concentration from 0.01 to 0.1*M* had little effect on the extent of the reaction.

A calibration curve giving the amount of sulphide ion in the aliquot injected (500 μl) was prepared covering the range 2×10^{-8}–5×10^{-7} mole by use of an iodine–azide solution (0.02*M* iodine, 0.1*M* potassium iodide and 0.7*M* sodium azide) at pH 7.0. The plot of pulse height against amount of sulphide was linear and passed through the origin.

Any initial temperature difference of less than ±0.3° between the solution and sample solution did

Table 1. Results of the determination of known amounts of sodium sulphide in the injected sample

Sulphide taken, μmole	Sulphide found, μmole	Deviation, %	C.V.* (n = 7), %
0.0201	0.0206	+2.5	1.8
0.0402	0.0408	+1.5	1.3
0.0804	0.0813	+1.1	1.4
0.161	0.163	+1.2	1.3
0.322	0.325	+0.9	1.0
0.502	0.507	+1.0	1.1

* Coefficient of variation.

Table 2. Results obtained for copper metal samples (1 g)

Sample	Sulphur, μg/g			Certificate value, μg/g
	n	$\bar{x}$	C.V, %	
Crude copper	4	131	2.5	130
Copper LC	5	32.8	4.1	32
Copper HC	5	15.5	4.8	15

Mean values ($\bar{x}$), number of determinations (n) and coefficients of variation (C.V) are given.

not affect the catalytic reaction pulse-height since the smallest detectable change in temperature was 0.04°.

Metal ions which precipitate as sulphides in acidic or basic solutions interfered. The reaction was slightly catalysed by Ag_2S and HgS. Catalysis by CuS, PbS, CdS, NiS, CoS, MnS, FeS, SnS and ZnS was less than that by sulphide ion. For example, when a suspension of zinc sulphide (4×10^{-8} mole) was injected into the solution, the time taken for termination of the reaction was about 20 min and the extent of the reaction was about half that with sulphide ion. Interference occurred with sulphur compounds which catalyse the reaction, such as thiocyanates, thiosulphates, and thioketones. Catalysis by potassium thiocyanate, sodium thiosulphate and thiourea was about 2–3 times that by sulphide ion and the reactions stopped within 1.5 min. Sodium sulphite or sodium nitrite up to 0.01M in the sample solution did not interfere by their reaction with iodine or azide because the resultant temperature change was far less than the lower limit of temperature detection.

The results for solutions of known concentrations (Table 1) show that sulphide in the range 2×10^{-8}–5×10^{-7} mole can be determined with relative errors and coefficients of variation not exceeding 3%. The method was applied to the determination of sulphur in copper metal. The sample was dissolved in a mixture of hydriodic acid, hypophosphorous acid and hydrochloric acid,[20] and the evolved hydrogen sulphide swept with nitrogen into an absorption vessel. The apparatus was similar to that used by Watson *et al.*[21] The hydrogen sulphide was absorbed from the gas stream in 10 ml of 0.01M sodium hydroxide. An aliquot (500 μl) of this solution was taken in the syringe and the subsequent operations were as described in the procedure. The results (Table 2) are in agreement with the certificate values and the method gave coefficients of variation of less than 5%.

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SIMULTANEOUS MICRODETERMINATION OF CARBON, HYDROGEN AND CHLORINE OR BROMINE IN ORGANIC COMPOUNDS BY VARIOUS RAPID EMPTY-TUBE COMBUSTION METHODS

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Summary—A standard Pregl absorption tube packed with polyurethane foam mixed with a cation-exchanger in the silver form, backed with a layer of "Anhydrone", and connected between the water and carbon dioxide absorption tubes and kept at room temperature, has been employed successfully for the simultaneous determination of chlorine or bromine together with carbon and hydrogen. This foam tube is suitable for use with the Belcher-Ingram, rapid straight empty-tube and rapid flash-combustion methods.

Various methods[1–5] have been used for the retention of halogen-containing combustion products in microdetermination of carbon and hydrogen in organic compounds by combustion in oxygen,[1–5] and for the simultaneous determination of carbon, hydrogen and halogen. Metallic silver in various forms,[6–10] at temperatures between 400 and 600°, is most commonly used for halogen retention. Copper,[11,12] antimony,[13] bismuth,[14] manganese dioxide,[15–17] lead dioxide,[18] silica gel,[19] strontium silicate[20] and the products from thermal decomposition of potassium permanganate[21] have also been used.

Recent work[22] has demonstrated that open-cell polyurethane foam can be used for the quantitative retention of iodine produced during the combustion of iodine-containing compounds.

In the present work, the possibility of using the foam for the retention of chlorine and bromine and for the simultaneous determination of carbon, hydrogen and either of these halogens, has been investigated. Three rapid empty-tube combustion procedures[23–25] were used.

EXPERIMENTAL

Reagents and materials

All reagents were of microanalytical-reagent grade unless otherwise specified. Flexible polyurethane foam, a polyether of open-cell type, was supplied by Greiner K. G. Schaumstoffwerk-Kremsumunster, Austria. Polyurethane–Varion KS heterogeneous ion-exchange foam[26] was kindly provided by Professor Dr. T. Braun, Institute of Inorganic and Analytical Chemistry, L. Eötvös University, Budapest, and contained 40% w/w of Varion KS sulphonated polystyrene cation-exchanger, 0.3–0.5 mm particle size (Nitrokemia, Fuzfogyartslep, Fuzfo, Hungary).

The unloaded polyurethane foam (in cubes of about 5 mm edge) was washed three times with acetone and then dried at 80°. Silver foam was prepared by shaking 5 g of heterogeneous cation-exchange foam with 50 ml of 0.5*M* silver nitrate in a stoppered flask for 1 hr on a mechanical shaker, decanting the solution, and repeating the shaking with fresh silver nitrate solution. The "silver-foam", *i.e.*, ion-exchange foam in the silver-form, was dried at room temperature in a vacuum desiccator for 6 hr.

Preparation of the silver-foam tube

A standard Pregl absorption tube was two-thirds filled with 1 g of the dried silver-foam followed by a thin quartz-wool plug, the rest being filled with "Anhydrone" (14–22 mesh).

Procedures

The procedures used with the Belcher–Ingram empty-tube method,[23] the straight empty-tube method[24] and the flash-combustion method[25] were essentially those in the literature cited, but with the modifications suggested by Gawargious and Farag[9] for the quantitative decomposition of highly halogenated compounds. The halogen absorption tube was connected between the water and carbon-dioxide absorption tubes and kept at room temperature. After the combustion, the combustion train was flushed with oxygen for 15 min. The absorption tubes were detached and wiped as usual. The weights of the absorption tubes were recorded on the 3rd minute for water, the 6th for carbon dioxide and the 10th for halogen.

RESULTS AND DISCUSSION

Simultaneous determination of carbon, hydrogen and chlorine or bromine, has gained little popularity, because the halogen absorption vessel must generally be heated. Polyurethane foam, however, can retain relatively large quantities of iodine at room temperature.[22] Unfortunately, it proves not to be successful for chlorine or bromine.

Table 1. Simultaneous microdetermination of carbon, hydrogen and chlorine or bromine in halogenated organic compounds with silver-foam as external absorbent for halogen (mean of 4 determinations)

No.	Compound		Theoretical, %	Belcher–Ingram method		Straight empty-tube method		Flash combustion method	
				Found, %	Std. devn., %	Found, %	Std. devn., %	Found, %	Std. devn., %
1.	*p*-Chlorobenzoic acid	C	53.70	53.74	0.38	53.89	0.20	53.77	0.20
		H	3.22	3.34	0.28	3.03	0.11	3.25	0.20
		Cl	22.64	22.67	0.41	22.77	0.32	22.80	0.20
2.	Arginine. HCl	C	34.21	34.23	0.37	34.43	0.22	34.27	0.24
		H	7.18	7.23	0.32	6.99	0.06	7.15	0.28
		Cl	16.83	16.92	0.26	16.97	0.17	16.85	0.34
3.	Benzidine. HCl	C	56.04	56.15	0.24	56.29	0.13	56.02	0.30
		H	5.49	5.56	0.19	5.10	0.09	5.53	0.20
		Cl	27.57	27.40	0.13	27.68	0.31	27.45	0.22
4.	Chloroisatin	C	52.91	52.92	0.22	52.78	0.19		
		H	2.22	2.31	0.34	2.12	0.05		
		Cl	19.53	19.58	0.29	19.71	0.25		
5.	Phenylsemi-carbazide. HCl	C	44.80			44.74	0.17		
		H	5.37			5.10	0.06		
		Cl	18.90			18.62	0.25		
6.	*p*-Chloroaniline	C	56.49			56.65	0.26		
		H	4.74			4.41	0.11		
		Cl	27.79			27.73	0.30		
7.	Chloranil	C	29.23	29.11	0.26	29.34	0.24	29.16	0.14
		Cl	57.77	57.61	0.23	57.47	0.16	55.55	0.33
8.	Hexachloroethane	C	10.15	10.12	0.09	10.36	0.24	10.09	0.22
		Cl	89.95	89.92	0.10	89.95	0.39	86.49	1.24
9.	*p*-Bromobenzoic acid	C	41.82	41.75	0.13	42.07	0.05	41.79	0.18
		H	2.51	2.51	0.13	2.65	0.03	2.59	0.13
		Br	39.75	39.67	0.22	39.68	0.22	39.66	0.19
10.	*p*-Dibromobenzene	C	30.55	30.42	0.18	30.66	0.13	30.56	0.24
		H	1.71	1.70	0.27	1.78	0.11	1.87	0.15
		Br	67.75	67.59	0.28	67.85	0.13	67.53	0.05
11.	*p*-Bromophenol	C	41.65	41.52	0.27	41.87	0.10	41.64	0.26
		H	2.91	2.87	0.20	3.04	0.15	2.95	0.24
		Br	46.19	46.13	0.25	46.39	0.03	46.09	0.25
12.	9-Bromophenanthrene	C	65.39	65.47	0.34	65.48	0.15	65.49	0.29
		H	3.53	3.62	0.11	3.75	0.06	3.51	0.07
		Br	31.08	31.01	0.22	31.22	0.06	31.07	0.26
13.	Bromanil	C	17.01	17.21	0.27	17.01	0.21	17.11	0.23
		Br	75.44	75.29	0.06	75.14	0.11	73.28	0.82
14.	Bromoaniline	C	41.89			41.80	0.24		
		H	3.52			3.46	0.12		
		Br	46.45			46.56	0.29		
15.	5,7-Dibromo-8-hydroxyquinoline	C	35.68			35.67	0.31		
		H	1.66			1.64	0.20		
		Br	52.75			52.50	0.28		

Braun *et al.*[26,27] have prepared a heterogeneous ion-exchange foam in which a finely ground commercial ion-exchange resin (*e.g.*, Varion KS) is built into a polyurethane foam matrix of the open-cell, polyether type. The mechanical properties of this ion-exchange foam were the same as those of the foam containing no ion-exchange resin, and the distribution of the ion-exchange resin grains was uniform.

We thought that this ion-exchange foam in the silver form might have a higher efficiency than polyurethane foam for the absorption of chlorine and bromine from the combustion products.

The silver-foam was packed in a Pregl absorption tube and backed with anhydrone to retain any moisture removed from the foam layer by the fast flow of oxygen, and used at room temperature between the water and the carbon dioxide absorption tubes.

The results obtained by all three methods are summarized in Table 1. No problems were encountered in the analysis of halogenated organic compounds by the Belcher–Ingram and rapid straight empty-tube methods but with the flash combustion method low halogen figures were obtained for chloranil, bromanil and hexachloroethane, probably because of incomplete decomposition of these thermally stable organic compounds by this combustion method.

Using the silver-foam material at room temperature is more convenient than using a silica absorption tube (packed with silver gauze) at 500°.[9]

The method was then examined for the analysis of

organic compounds containing nitrogen together with carbon, hydrogen, halogen and oxygen, and found suitable (cf. Table 1) if a manganese dioxide tube is connected between the silver-foam tube and the carbon dioxide tube, for the removal of acidic nitrogen oxides from the combustion products. The method cannot be used for compounds containing sulphur, however.

Although the results show no overall bias, the precision is poorer than that obtainable when the same combustion methods are used for the determination of only carbon and hydrogen, and the halogen is determined separately by methods such as oxygen-flask combustion and titration, but may be adequate for certain control-analysis purposes.

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TURBIDIMETRIC DETERMINATION OF SOME SULPHUR COMPOUNDS IN ETHANOL FROM FERMENTATION

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Summary—A simple method is described which can be used for the determination of certain sulphur compounds found in industrial ethanol obtained from fermentation of molasses. The method is based on the turbidimetric determination of sulphate after the sample has been treated with dilute hydrogen peroxide solution, by precipitation of the sulphate with barium chloride under appropriate conditions. Several samples of fermentation ethanol have been analysed by this method and the sulphur contents found compared with the total acidity.

The recent trend towards the use of biomass material for the production of fuel and chemicals is growing as oil prices increase. Brazil is already blending *ca.* 20% of fermentation ethanol (produced from sugar cane and to a lesser degree from cassava) in gasoline and is planning to switch completely to ethanol in the near future. There are also projects to use ethanol as a chemical feedstock.

Other countries, such as the Federal Republic of Germany and the U.S.A. are studying the use of methanol from coal or gas-derived synthetic gas, as a motor fuel.[1] However, there are disadvantages in the use of methanol owing to its toxicity and corrosiveness, which suggested the development of a plant where methanol could be converted into gasoline, by a company in the U.S.A.[2]

Corrosiveness due to the use of ethanol as motor fuel was reported as early as 1907[3] when fermentation ethanol was used as a motor fuel. According to McIntosh[3] this corrosiveness was due to impurities in the denaturing agent (usually pyridine) employed.

However, in 1941, Martraire[4] suggested that the corrosiveness of fermentation ethanol was due to the presence of sulphur compounds such as SO_2, H_2S (produced from SO_2 by reduction, when other secondary fermentation processes occurred, owing to contamination of the must[4]) and possibly mercaptans and thio-ethers.

Martraire[4] also called attention to the fact that if ethanol containing SO_2 is used mixed with gasoline as motor fuel it will produce a fuel with corrosive properties.

Later, in 1944, after reporting that the main objection of motorists at that time to the replacement of gasoline by a fuel containing a high proportion of ethanol was corrosion, Staub[5] established that the corrosiveness was due to the presence of SO_2 and that the amount present was in most cases higher when the ethanol was obtained from molasses produced by a process where the clarification used treatment with sulphite in excess. This also resulted in an ethanol with high acidity, with values as high as 200 μg/ml (expressed as acetic acid), compared with 4 μg/ml when the usual clarification process was employed.[5] Other sources of sulphur compounds could be the sulphuric acid added during the fermentation process to decrease the pH, and the ammonium sulphate used as a source of nitrogen, although these chemicals are used in only small quantities.

Besides being corrosive, sulphur compounds are also objectionable when ethanol is used as raw material for the catalytic synthesis of acetaldehyde and butadiene.[6]

Although turbidimetry has been widely suggested or employed for the determination of sulphate in water,[7] soil, plants,[8] urine, cement,[9] the semi-quantitative (comparative) determination of sulphate in wine and vinegar[10] and for the determination of the sulphur content of pure organic compounds[11] there is no reference in the literature examined to the application of this technique for the determination of traces of sulphur compounds in fermentation ethanol.

The present work describes a simple method for the

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determination of SO_2, SO_3, SO_3^{2-}, S^{2-} and SO_4^{2-} in ethanol by turbidimetry, adapting the technique described by Sheen *et al.*[7] After the sample has been refluxed with dilute hydrogen peroxide solution in alkaline medium, the sulphate produced is precipitated with barium chloride.

EXPERIMENTAL

Apparatus

A Klett-Summerson photoelectric colorimeter with a filter having its maximum transmission at 420 nm was used. Any nephelometer or spectrophotometer could be used.

Chemicals

The reagents used were of analytical grade. Clear colourless glycerol was used to prepare the conditioning solution,[12] made by dissolving 120 g of sodium chloride in *ca.* 400 ml of water, adding 15 ml of concentrated hydrochloric acid, and 500 ml of glycerol, then diluting to 1 litre with distilled water. Other conditioning solutions[8,13] which have been proposed for the turbidimetric determination of sulphate can also be used.

Calibration curve

A calibration curve is prepared with a range from 0 to 15 μg of sulphur per ml, by use of fractions from a standard solution of sulphate (45 μg of sulphate per ml, equivalent to 15 μg/ml as sulphur) made by dissolving 0.0916 g of potassium sulphate in 1 litre of distilled water. Portions of this solution are taken to prepare appropriate dilutions of 2.5, 5.0, 10 and 12.5 μg/ml (as sulphur). Each solution is then treated as in the procedure, the oxidation step being omitted. The calibration curve is linear.

Determination of sulphur

Pipette 50 ml of ethanol (or a suitable sample containing 0.6–0.15 mg of sulphur) into a 250-ml Erlenmeyer flask (B 24/29 ground joint). Add 15 ml of freshly prepared 5% hydrogen peroxide solution and 5 ml of 0.15*M* sodium hydroxide. Fit a 300-mm long Liebig condenser. Reflux on a hot-plate for 1 hr, remove the condenser and evaporate the solution to *ca.* 40 ml. After cooling, transfer to a 50-ml standard flask, and make up to volume with distilled water.

Transfer 25 ml to a 100-ml beaker, add 5 ml of the conditioning solution, stir, and measure any turbidity, which must be subtracted from the total turbidity determined as follows.

Add 0.3 g of barium chloride crystals, stir until dissolution is complete, let stand for 2 min, stir for another 15 sec and immediately determine the total turbidity.

The remainder of the 50 ml can be used to repeat the determination, after appropriate dilution if the amount of sulphate present is higher than the limit of the calibration curve.

Reagent blanks must be run frequently, especially when new reagents are employed or when the aged conditioning solution becomes slightly yellow, which sometimes happens.

Determination of total acidity

Total acidity, expressed as acetic acid, is determined by titration of 25 ml of sample with 0.01*M* sodium hydroxide, with phenolphthalein as indicator.

RESULTS AND DISCUSSION

Several samples of industrial ethanol (not fuel grade) ranging from 92 to 96% v/v alcohol content were analysed by this method, which has been used at COPERBO as a routine method.[14]

Table 1 shows some of the results obtained, mainly with samples with high total acidity and high sulphur content (which ranged from nil to *ca.* 80 μg/ml). As observed by Staub,[5] when the ethanol sample has a high sulphur content it also shows high acidity.

Although the method has not been compared with any other classical method, such as the flame-combustion method usually employed for control of oil-refinery products, it can be recommended as a guide to the amount of sulphur compounds present. It will probably not detect mercaptans, if present, as these compounds will most likely be oxidized to the corresponding disulphides.[15] Sulphide in alkaline medium can however, be oxidized with hydrogen peroxide to sulphate.[9] The bisulphite addition product formed between acetaldehyde and SO_2, which accounts for 75–100% of the "bound SO_2" in wine, is hydrolysed in alkaline medium, freeing the SO_2.[16]

Advantages and problems encountered when the turbidimetric method is employed have already been discussed by Sheen *et al.*,[7] according to whom, in the typical range of the turbidimetric method (0–100

Table 1. Determination of sulphur and total acidity

Sample	Ethanol, % *v/v**	Sulphur, *μg/ml*	Total acidity, *μg/ml*†
1	96	Nil	37
2	96	Nil	31
3	96	0.2§	43
4	96	2.9	45
5	96	8.3	35
6	96	54‡	480
7	92	65‡	449
8	96	67‡	464
9	96	79‡	482

* According to the manufacturer.

† As acetic acid.

§ 100 ml of sample concentrated to 50 ml.

‡ 25 ml of sample and appropriate dilution before the precipitation step.

μg/ml as sulphate; 0–33 μg/ml as sulphur) the results agree within the tolerance expected when compared with a gravimetric method.

Recently, Lemoch,[13] after comparing the turbidimetric method for the determination of sulphate in plants (with an acacia solution as conditioner) with a gravimetric and a colorimetric method, concluded that the turbidimetric method was the most convenient and rapid procedure.

Although the specification limit established for total acidity in ethanol as a motor fuel in Brazil is low (30 μg/ml, as acetic acid), the corrosive properties will presumably be different if this acidity is due to mineral acids rather than organic acids.

When fermentation ethanol was used in the past as raw material for industrial organic synthesis (acetaldehyde and butadiene) by heterogeneous catalysis, a limit of 6 μg/ml was specified for the level of sulphur present.[6]

If larger sample volumes are taken and the volume after the oxidation step is reduced, 0.2 μg of sulphur per ml can be determined, although in certain cases the presence of impurities in the sample introduces some turbidity, which appears during the concentration step, and this must be subtracted as described in the procedure.

This method, owing to its simplicity and the low price of the equipment involved, could be conveniently adopted, even by the small alcohol fermentation plants planned for installation in Brazil in the near future.

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ANNOTATION

THE USE OF APPROXIMATION FORMULAE IN CALCULATIONS OF ACID–BASE EQUILIBRIA

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Summary—Details of diagrams recently presented for the regions where approximation formulae give the pH of mono- and diprotic acids with an error of less than 0.02 pH unit are discussed.

Narasaki[1] has recently reported calculations to identify the regions of concentration and dissociation constants where approximation formulae for calculation of $[H^+]$ and pH in acid–base equilibria give the pH with an error < 0.02. Similar calculations were done by me some time ago not only for pure solutions of single acids[2] but also for more complex systems.[3] A first comparison of Narasaki's diagrams with mine showed broad agreement of the results, but suggested some differences in the shape of the curves. Full comparison was impossible because the units on the axes were different. In order to investigate the differences in the diagrams I recalculated some of my results, and found a very definite difference in some of the details of the diagrams. In this note, the differences are reported and discussed.

CALCULATIONS

The exact pH was calculated by computer to a precision of better than ± 0.001 (the results were the same if the pH was calculated to ± 0.01) from the exact polynomial equations [Narasaki's[1] equations (10) and (18)] by using a general subroutine previously described[4] for the calculation of pH. The only formula of those considered by Narasaki[1] which had been included in my previous analysis of pure solutions of single acids[2] was the simplest of all, *i.e.*,

$$[H^+] = \sqrt{K_A C_A} \qquad (1)$$

$$pH = \tfrac{1}{2}pK_A - \tfrac{1}{2}\log C_A \qquad (1')$$

where K_A is the dissociation constant of a monoprotic acid or the first dissociation constant of a diprotic acid, K_{A_1}. None of the second-degree approximations included in my previous study[3] was considered by Narasaki.[1] The difference between the pH value given by formula (1') and the exact pH was calculated for successive sets of values of the variables (C_A and K_A for monoprotic acids, C_A, K_{A_1} and K_{A_2} for diprotic acids) by a program which provided results as tables of calculation errors. The diagrams (Figs. 1 and 2) were drawn from these tables.

RESULTS AND DISCUSSION

The results obtained for a monoprotic acid are presented in Fig. 1, which is a corrected version of Fig. 2 of my original work,[2] turned upside down to allow it to be compared with Narasaki's[1] Fig. 1, case C. Inside the area limited by curves $+1$ and -1 the calculation error which resulted from replacement of the exact equation by formula (1') is less than 0.01 pH unit. Similarly, inside the area limited by curves -5 and $+5$ the calculation error is smaller than 0.05 pH unit. On the central line or in the area marked zero the error is zero (when rounded off to two places of decimals); to the left it is negative and to the right it is positive. Comparison of Fig. 1 with Narasaki's Fig. 1 case C, shows general agreement in the area where formula (1') give results with a small error. However the area obtained by Narasaki[1] for an error of less than ± 0.02 pH units is slightly smaller than that in the present diagram for an error of less than ± 0.01 pH unit, and not slightly larger as expected. Moreover, the curves obtained in my work are continuous, and extended into the region of low concentration and pK_A (top left corner of the figure). This detail was not shown in Narasaki's diagram, probably because it was drawn from a rather restricted set of calculated values or because the calculations were not precise enough.

For comparing the results for diprotic acids, my previous plots (Figs. 3 and 4)[2] were unsuitable because values of pK_{A_2} instead of pK_{A_1} (which was used in Narasaki's Figs. 2–4) were plotted on the x-axis, so the results had to be recalculated and replotted. When the ratio K_{A_2}/K_{A_1} was used as a variable, as in Narasaki's Fig. 2, it was found that the agreement was not complete. The differences found were of the same type as above. The results are shown in Fig. 2, where the value of pK_{A_2} (not the ratio

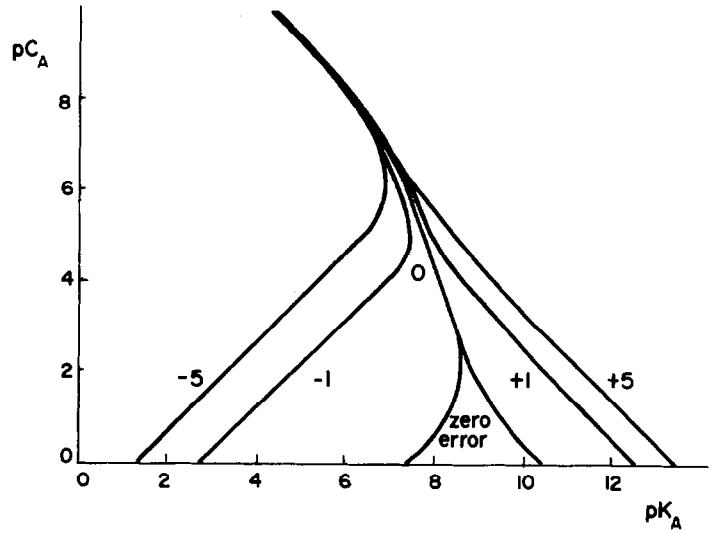

Fig. 1. Range of applicability of equation (1′) for monoprotic acids. The number near each line gives the calculation error multiplied by 100.

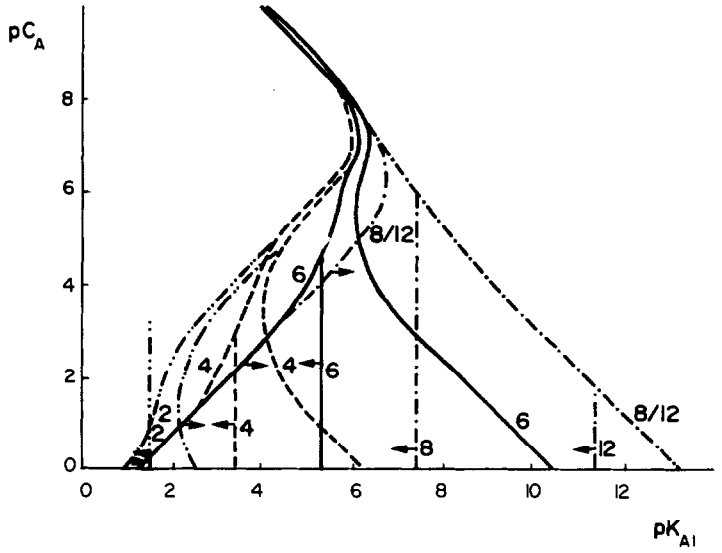

Fig. 2. Range of applicability of equation (1′) for diprotic acids. Lines are labelled with the appropriate values of pK_{A2}. Areas of applicability are indicated by arrows, and vertical lines refer to the condition $pK_{A1} \leqslant pK_{A2} - 0.60$.

K_{A2}/K_{A1}) is used as the variable, for the sake of convenience (tables of acid dissociation constants list values of successive constants and not ratios). For each value of pK_{A2} the calculation error introduced by the use of formula (1′) is smaller than 0.05 pH units inside the area limited by the two lines marked with the value of pK_{A2} (the line on the left corresponds to negative errors, that on the right to positive errors). The vertical lines refer to the condition $pK_{A1} \leqslant pK_{A2} - 0.60$, which is true for all known real dibasic acids. As with monoprotic acids, the areas extend into the region of low concentration and pK_A, which again was not found by Narasaki.[1] It is interesting to note that when the value of pK_{A2} is increased to *ca.* 8, there is enlargement of the area where formula (1′) can be used without introducing appreciable error. This is expected because, when the second dissociation becomes weaker, its contribution to the production of protons in solution decreases. Thus, the first dissociation dominates protolytic equilibria and the use of formula (1), where only the first dissociation is considered, leads to a smaller error. For values of pK_{A2} larger than 8 the second dissociation is completely negligible so the area stops widening. For such large values of pK_{A2} the diprotic acid behaves like a monoprotic acid and the lines in Fig. 2 coincide with those of Fig. 1.

CONCLUSIONS

Diagrams like those presented by Narasaki[1] should be used with caution, because the lines which limit the

areas seem to be very sensitive to calculation procedures: precise diagrams seem to be obtained only when the calculated values are sufficiently precise and numerous.

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LETTER TO THE EDITOR

COMMENT ON "MACHINE COMPUTATION OF EQUILIBRIUM CONCENTRATIONS - SOME PRACTICAL CONSIDERATIONS" BY D.J. LEGGETT

SIR,

The original program MINIQUAD[1] was adapted for concentration calculation by Leggett.[2] In the original MINIQUAD the initial guess for all free component concentrations is set at 10^{-7}M. Leggett added a section by which "free metal-ion concentrations are set equal to total metal-ion concentrations and free ligand concentrations are computed on the premise that no complexation has occurred",[2] and used the following code to achieve this goal.

```
 1          IF (IP .GT. 1) GO TO 2000
 2          DO 147 I = 1, NC
 3          SUM = 1.0
 4          IF (LIGAND (I) .EQ. 0) GO TO 147
 5          DO 146 K = 1, NK
 6          IF (JQR (I,K) .LT. 1) GO TO 146
 7          W = BABS (K)
 8          SUM = SUM + W * CX (NCPL) ** JQR (NCPL,K)
 9      146 CONTINUE
10          CX (I) = TOTC (I)/SUM
11      147 CONTINUE
12          DO 148 I = 1, NC
13          IF (LIGAND (I) .GT. 0) GO TO 148
14          CX (I) = TOTC (I)
15      148 CONTINUE
16      2000 CONTINUE
```

Lines 12 - 15 set free metal-ion concentration equal to total metal concentration, whereas lines 2 - 11 attempt to calculate free ligand concentration by correcting for protonation of ligands by means of

$$[L] = T_L / (1 + \beta_1[H] + \beta_2[H]^2 + \beta_3[H]^3 + \ldots)$$

where T_L is total ligand concentration and charges are omitted. However, there is an error in this coding. Line 6 checks whether the ith ligand is present in the kth complex, and if so proceeds with updating SUM. The error is introduced by the fact that no attempt is made to update SUM for only protonated ligands and to discard complexes of the type $M_iL_jH_k$. As a result of this, the computed free ligand concentrations are incorrect.

The modification below rectifies this situation and performs the task desired by Leggett. However, even this modified code handles only H_iL-type protonated ligands, and will not correctly treat H_iL_j-type complexes without further modifications. It is presented here only to permit correct implementation of Leggett's MINIQUAD B;[2] caution must be exercised in its use as its underlying assumptions are valid (but not necessarily!) only when the calculations are started at low pH.

```
      IF (IP .GT. 1) GO TO 2000
      DO 147 I = 1, NC
      SUM = 1.0
      IF (LIGAND (I) .EQ. 0) GO TO 147
      DO 146 K = 1, NK
      IF (JQR (I,K) .LT. 1) GO TO 146
C.....IF A COMPLEX CONTAINS ANOTHER METAL OR LIGAND, DISCARD IT
      DO 5000 LCHECK = 1, NC
         IF (LCHECK .NE. I .AND. JQR (LCHECK,K) .NE. 0) GO TO 5001
 5000    CONTINUE
      W = BABS (K)
      SUM = SUM + W * CX (NCPL) ** JQR (NCPL,K)
 5001 CONTINUE
  146 CONTINUE
      CX (I) = TOTC (I)/SUM
  147 CONTINUE
      DO 148 I = 1, NC
      IF (LIGAND (I) .GT. 0) GO TO 148
      CX (I) = TOTC (I)
  148 CONTINUE
 2000 CONTINUE
```

10 September 1980

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The Editorial Board and the Publisher of *Talanta* have great pleasure in announcing that the Louis Gordon Memorial Award for 1979, for the paper judged the best written of those published in *Talanta* during the year, has been made to Saad S. M. Hassan and M. H. Eldesouki, Department of Chemistry, Ain Shams University, Cairo, Egypt, for their paper "Determination of chloramphenicol in pharmaceutical preparations by the cadmium ion-selective electrode, spectrophotometry and atomic-absorption spectrometry", *Talanta*, 1979, **26**, 531.

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New atomic-absorption spectrometric, potentiometric and polarographic methods for the determination of nitrates: SAAD S. M. HASSAN. (10 April 1980)
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Detection of iodine and thiocarbonyl sulphur in organic compounds: KRISHNA K. VERMA and SUNIL K. DUBEY. (11 April 1980)
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Pyrithioxine dihydrochloride monohydrate estimation: N. M. SANGHAVI and KOYNA JOSHI. (15 April 1980)
Detection and semiquantitative determination of metal ions by capillary solid-state spot-tests: M. QURESHI, ALI MOHAMMAD and G. G. RAJU. (16 April 1980)
Electrothermal atomization of calcium and strontium in a molybdenum microtube: MASAMI SUZUKI and KIYOHISA OHTA. (17 April 1980)
Atomic-absorption spectrometric determination of metals and silicon in tar-sands fly-ash: C. O. GOMEZ-BUENO, G. L. REMPEL and D. R. SPINK. (9 April 1980)
Stability constants of chloro-complexes of cadmium(II) in sea-water conditions: MARIA de LURDES, S. SIMÕES, MARIA CÂNDIDA, T. A. VAZ, and J. J. R. FRAÚSTO DA SILVA. (22 April 1980)
Determination of a trace amount of bromine in rocks by ion-exchange chromatography and direct potentiometry with an ion-selective electrode: H. AKAIWA, H. KAWAMOTO and K. HASEGAWA. (22 April 1980)
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Electrothermal atomic-absorption spectrophotometry of barium: Assessment of the method for its determination in brines and caustic soda solutions: FADHIL JASIM and MAHMOOD M. BARBOOTI. (24 April 1980)
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Solution studies on chelates of nickel(II), zinc(II) and cadmium(II) with 2-thiopicolinanilide: M. MOLINA, C. MELIOS and N. BARELLI. (10 April 1980)
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PMR assay of natural products in pharmaceuticals—V: Assay of ephedrine hydrochloride in tablets: M. M. A. HASSAN, M. UPPAL ZUBAIR and J. S. MOSSA. (25 April 1980)
Stability constants for the complexation of transition-metal ions with fulvic and humic acids in sediments, measured by gel filtration: SHIZUKO HIRATA. (29 April 1980)
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Volumetric determination of uranium in low-grade uranium ores by the ferrous ion–phosphoric acid reduction method: A. HITCHEN and G. ZECHANOWITSCH. (31 August 1979)

Solvent extraction of molybdenum(VI) as thioglycollate complex with *N*-benzylaniline into chloroform: S. P. RAO, R. NANDINI BHARGAVA and B. SITARAM. (25 October 1979)

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Diphenyl and dipyridylglyoxal bis(benzoylhydrazone) as analytical reagents: M. SILVA, M. GALLEGO and M. VALCÁRCEL. (7 November 1979)

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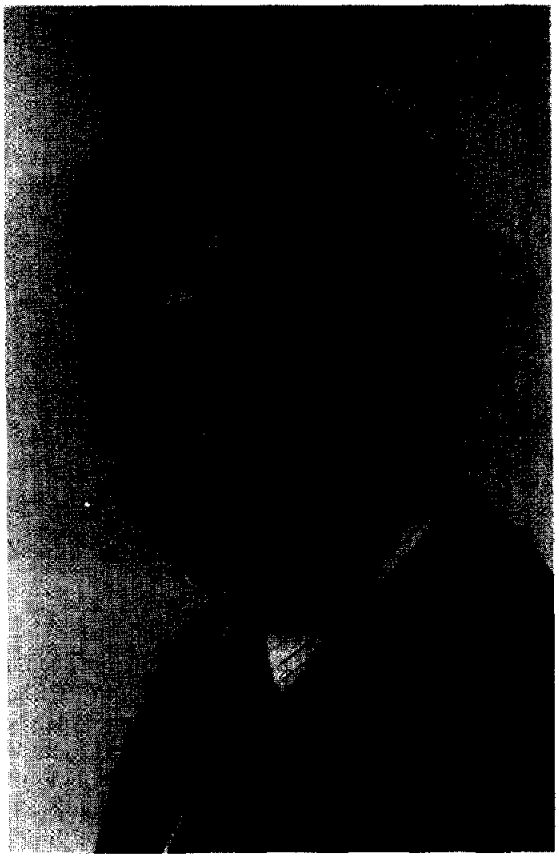

L. J. KRICKA

Dr L. J. KRICKA was born in 1947 in Karlovy Vary, Czechoslovakia, and obtained a B.A. (Hons.) in Chemistry from York University, and later a D.Phil. in the same university in 1971. After a Research Fellowship at the University of Liverpool he joined the Medical School at the University of Birmingham in 1973 as Lecturer in Clinical Chemistry, and has been there since. His research interests are broad, encompassing non-isotopic immunoassays (e.g. enzyme and luminescence immunoassays), two-dimensional and thin-film electrophoresis, analytical applications of chemi- and bio-luminescence analysis, the application of isotachophoresis to clinical problems, and computer-assisted learning in clinical chemistry. He is co-author with Dr P. M. S. Clark of a book "Biochemistry of Alcohol and Alcoholism" and editor of a companion volume (due out at the end of the year) on "Medical Consequences of Alcohol Abuse". He is at present co-editing a book on luminescence, "Chemical and Biological Luminescence".

P. R. BONTCHEV

PROFESSOR P. R. BONTCHEV was born in 1933 in Bourgas, Bulgaria. He obtained his B.Sc. in Chemistry at the University of Sofia in 1956 and Ph.D. from the same university in 1965. In 1975 he obtained the Dr. Sc. degree from the Bulgarian Academy of Sciences. From 1956 he has been in the Analytical Department of the Sofia University, where in 1969 he was promoted to Associate Professor. He was Vice-Dean of the Faculty of Chemistry, Sofia University, in the period 1976–1979. His works are mainly in the fields of catalytic analysis, co-ordination chemistry, and analysis of biological materials and physiologically active substances. He has published over 80 papers in these fields and two books—"Mechanisms of Inorganic Reactions in Solution" (together with G. Nikolov) and "Complexation and Catalytic Activity" (in Bulgarian, and translated into Russian). He is author of the text-book "Introduction to Analytical Chemistry" (two editions in Bulgarian and one in Russian).

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Substoichiometric isotope-dilution analysis for tin with salicylideneamino-2-thiophenol, using complexation in non-aqueous medium: Application to the analysis of coal fly-ash: HISANORI IMURA and NOBUO SUZUKI. (27 March 1980)
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Use of tervalent and tetravalent manganese for the determination of arsenic(III), uranium(IV), molybdenum(V), tin(II) and titanium(III): N. RUKMINI, V. S. N. P. KAVITHA and K. RAMA RAO. (9 April 1980)
Improved method for titrimetric determination of sulphide, using the iodine–azide reaction: NOBUTOSHI KIBA and MOTOHISA FURUSAWA. (9 April 1980)

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Séparation chromatographique des azépines: Nanochromatographie sur couche mince et chromatographie en phase liquide sous pression: A. VILLET, J. ALARY et A. COEUR. (27 November 1979)
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Contributions to the study of azo-derivatives of heterocyclic compounds as analytical reagents—I: 1,2,4-(3-Triazolylazo)-2-naphthol as a new analytical reagent: J. CACHO and C. NERIN. (16 January 1980)
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Contribution to the study of azo-derivatives of heterocyclic compounds as analytical reagents—II: Spectrophotometric determination of nickel with 1,2,4-(3-triazolylazo)-2-naphthol. Application to the analysis of steel: J. CACHO and C. NERIN. (22 January 1980)
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Indirect polarographic microdetermination of organic sulphur compounds: MOUAYED Q. AL-ABACHI, FAWZI H. AL-DABBAGH and S. T. SULAIMAN. (5 February 1980)

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TALANTA ADVISORY BOARD

The Editorial Board and Publishers of *Talanta* take pleasure in welcoming the following new members of the Advisory Board of the journal.

H. SANKE GOWDA
PASCHOAL E. A. SENISE

They also wish to record their sincere thanks for the help given by

G. JOHANSSON
N. JORDANOV
A. P. KRESHKOV
R. PERRY

who retire from the Advisory Board.

PROFESSOR H. SANKE GOWDA received his B.Sc.(Hons.) degree from the University of Mysore and M.Sc. and D.Sc. degrees from Andhra University. He worked in the research school of Professor R. Belcher and received the Ph.D degree from the University of Birmingham, U.K., in 1959. He joined the Faculty of Chemistry, Central College, Bangalore as Lecturer in 1948, becoming Assistant Professor in 1956 and Professor and Head of the Department of Postgraduate Studies and Research in Chemistry, Manasa Gangotri, University of Mysore, Mysore in 1966. He was the Director of the Summer Institute in Chemistry for College Teachers for three years and organized the National Symposium on Microchemical Techniques in Mysore in 1978. He has delivered lectures at Romanian Universities under the Indo-Romanian Cultural Exchange Programme in 1970 and at Research Institutes in the USSR under the Indo-Soviet Cultural Exchange Programme in 1979. He is a Recorder of the Chemistry Section of the Indian Science Congress Association, Fellow of the Indian Chemical Society and member of many academic bodies of the Mysore University.

His research interests are in analytical chemistry with special interest in molecular absorption spectroscopy, redox indicators, oxidimetry and co-ordination chemistry. He has founded a large research school of 20 candidates, many of whom have taken the Ph.D. degree. He has published about 100 original research papers in national and international journals.

PROFESSOR PASCHOAL E. A. SENISE was born 19 August 1917 at São Paulo, Brazil; he obtained his B.S. in Chemistry, University of São Paulo (USP), 1937; D.Sc., USP, 1942; was Research Fellow, Louisiana State University, 1950–52; appointed "Livre-docente" of Inorganic and Analytical Chemistry, USP, 1956; Professor in-charge, USP, 1958; Full Professor, USP, 1965–; Director of the "Instituto de Química" (Chemistry Department) USP, 1970–74; 1978–; Co-ordinator General of Post-graduate studies, USP, 1970–; Member of the University Council, USP, 1968–; Member (1960) and Vice-President of the Brazilian Academy of Sciences, 1965–; Member of the Scientific Board of the Brazilian Research Council, 1968–; Member of the Sigma-Xi Society, International Chapter, 1958; Fellow of the American Association for the Advancement of Science, 1963; ordinary member of the following scientific societies: Chemical Society, Analytical Division; American Chemical Society; American Association for the Advancement of Science; Brazilian Chemical Society; Brazilian Society for the Advancement of Science. His main fields of research are co-ordination compounds in solution, solvent extraction and electroanalytical studies.

SUBJECT INDEX

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PUBLICATIONS RECEIVED

Ion-Selective Electrode Reviews, Vol. 1, No. 1: J. D. R. THOMAS (ed.), Pergamon, Oxford, 1979.

On the basis of the three reviews in the first issue, those working with ion-selective electrodes will welcome this new twice-yearly journal. Although prepared from camera-ready typescript, the text is clear and free of typographical errors. The editor, Dr J. D. R. Thomas, has struck a nice balance in this issue, covering an applied topic (industrial analysis), a research topic which is generally familiar but all too extensive and diverse (calcium ISEs) and one of which few have direct experience but many wish to know more (ISFETs).

"Designing Calcium Electrodes" by Moody and Thomas (26 pages, 80 references) concentrates on the choice of solvent mediator and ion-exchanger/sensor for optimizing calcium selectivity relative to common interferents, although some attention is paid to mechanical design. This review covers the literature to the end of 1978 very thoroughly and is undeniably useful to those wishing to make calcium electrodes.

"Ion-Selective Field Effect Transistors" by Janata and Huber (48 pages, 65 references) is the most timely of the three reviews. It covers theoretical and practical aspects of ISFETs up to October 1978 and I found it most instructive.

"Industrial Applications" by P. L. Bailey (50 pages, 167 references) first clearly sets out the requirements, advantages and disadvantages of ISEs for those in charge of analytical laboratories and then describes, determinand by determinand, the industrial uses of ISEs. The main issues are brought out for each electrode, although the discussion of the calcium electrode could have been fuller and the water hardness electrode is almost overlooked. A statement of why lead and cadmium electrodes are not used would have been better than neglecting them altogether and residual chlorine is too important a subject to be ignored, despite the paucity of sources. Despite these minor omissions, the review as a whole is balanced and thorough.

DEREK MIDGLEY

Statistical Theory and Methodology of Trace Analysis: C. LITEANU and I. RÎCĂ, Ellis Horwood., Chichester, England, 1980. pp. 446. $61.90/£25.00.

Specialization parallels scientific advance. Over the years the Reviewer has seen the monograph treatment of *applied* statistics "zoom in" first on science and technology, then on chemistry, and more recently on chemical analysis. Now even higher power is used by Doctors Liteanu and Rîcă to focus on trace analysis! Their monograph is probably the first emphasizing the treatment of data for detection and determination at trace levels. The book should be of benefit to all trace analysts, whatever their scientific discipline, concerned with the reliability of results and should be added to libraries having significant holdings in analytical chemistry.

With a work on applied statistics, authors must sail between Scylla and Charybdis: they must not founder on the rocks of so many examples that insufficient room is left for advanced topics, yet not be sucked into a whirlpool of mathematics that can drown many readers. Liteanu and Rîcă have steered well and achieve an elegant balance between statistical theory and applications.

The mathematical treatment has been kept relatively condensed and a notation of modest complexity is adopted and persisted in. Analysts with a time-eroded knowledge of advanced mathematics will have to face only a few matrices, vectors, integrals, and the like; and they can use the final equations even if the derivation is only imperfectly appreciated. For many topics, practical examples having the trappings of major instrumental methods for trace analysis (e.g., emission spectrography and spectrometry, X-ray fluorescence, spectrophotometry) are presented in summary form. The Reviewer would have welcomed more examples even at the cost of redundancy (*repetitio est mater studiorum*).

The authors demonstrate well the vitality of their subject and include some topics that are still undergoing research and final formulation. Many chapters cite from 50 to over 100 papers that can be consulted by interested readers. Some of the "newer" topics covered will delineate the merit of the work: testing hypotheses, information processing of results, stability of analytical systems, relation between signal and concentration, analytical signal, detection and resolution, detection and determination limits, increasing signal-to-noise ratio in analytical chemistry, pattern recognition, analysis of non-homogeneous materials, to name a few.

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NOTICES

MINERALOGICAL ASSOCIATION OF CANADA

SHORT COURSE:

NEUTRON ACTIVATION ANALYSIS IN THE GEOSCIENCES

A Short Course on Neutron Activation Analysis (NAA) in the Geosciences, sponsored by the Mineralogical Association of Canada, will be held at Halifax, N.S., during 16–18 May 1980. It immediately precedes the annual meetings of the Geological and Mineralogical Associations of Canada in Halifax during 19–21 May 1980.

Intensive lecture sessions, demonstrations, discussions, and exhibits are planned on: development and present status of NAA; general principles; neutron sources; γ-ray detectors; data acquisition and reduction; instrumental and radiochemical methods; applications to mineralogy, petrology, subsurface mineral exploration, and environmental studies. The course is designed to provide an overview for those who are not experts in the methods.

Further information and registration forms may be obtained from:

Gunter K. Muecke
Department of Geology
Dalhousie University
Halifax, Nova Scotia B3H 3J5
Canada

AMERICAN VACUUM SOCIETY

27th NATIONAL SYMPOSIUM

CALL FOR PAPERS

The 27th National Symposium of the American Vacuum Society will be held 13–17 October 1980, at the Detroit Plaza Hotel. Topics include surface science, thin films, electronic materials and processes, vacuum technology, vacuum metallurgy, and education training. The deadline for submission of abstracts is 6 June 1980. For information, contact J. A. Thornton, Program Chairman, Telic Corporation, Santa Monica, California, or Nancy Hammond, American Vacuum Society, New York.

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PUBLICATIONS RECEIVED

Medical and Biological Applications of Electrochemical Devices: J. KORYTA (ed.), Wiley, Chichester, 1980. pp. 331 + ix. £27.00.

The stated aim of this book is to treat comprehensively those electroanalytical devices which have been introduced into the laboratory or clinic during the preceding one or two decades, and one must state at the outset that this aim is by and large achieved. Following an introductory chapter, there are four chapters on the use of ion-selective electrodes (ISEs), including a very incisive and comprehensive one on liquid-membrane ion-selective electrodes and one on the use of solid-state ISEs in clinical chemistry. The other two chapters on ISEs cover measurements using these devices in single cells and in excitable tissues. The following three chapters deal with, respectively, polarographic oxygen determination in biological systems, voltammetric approaches to the study of whole-organ physiology, and the determination of 1,4-benzodiazepine derivatives in biological materials. None of these techniques can be regarded at present as readily usable in clinical situations, although the chapter on polarographic oxygen determination is superbly comprehensive. The book concludes with a fairly thorough chapter on the applicability of enzyme electrodes, both potentiometric and polarographic, in biomedical investigations.

Whilst the very great current interest in the use of electroanalytical devices in the Life Sciences makes this book very timely, it would have benefited from a clearer separation between what one might call the Art of the Possible in the research laboratory and the Art of the Soluble in the clinic. On this basis, therefore, one is left with the impression that the book is aiming at two audiences who really have very little in common. The prospective buyer should hope, in a book of this price, to be able to benefit from reading the whole of it. I fear that this hope may be unfulfilled for many, and, whilst it will be of use in the library, the high price of this work may put off the individual purchaser.

DOUGLAS KELL

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MARY MASSON

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Microweighing in Vacuum and Controlled Environments: A. W. CZANDERNA and S. P. WOLSKY, Elsevier, Amsterdam, 1980. pp. 418. $78.00; Dfl 160.00.

In analytical chemistry all quantitative statements are based on absolute mass determinations. Thus weighing is not only one of the most important basic analytical operations, but also among the most precise and accurate methods of determination, being surpassed or equalled by few other measurement techniques. In the present climate of instrumental analysis the analyst frequently forgets the balance and its recent development, regarding it merely as a commercially available tool for routine analysis. The balance is likewise less thoroughly dealt with in modern textbooks on analysis, since gravimetric methods are now more seldom used, and the balance is used mainly for weighing samples and standard substances. Even in organic elemental microanalysis, which in its classical period gave great impetus to balance development, speed and electronic digital presentation are more esteemed than extremely high absolute sensitivity in the lower ng range. Most analysts, therefore, are unaware of the intensive development of the microbalance during the last 20 years, not only with respect to automation but also in finding better solutions to specific tasks, especially in connection with the study of adsorption, desorption, decomposition and depletion processes on matrix surfaces, or changes in stoichiometry of thin layers in vacuum or a controlled atmosphere, as a function of temperature.

In surface investigation by means of vacuum microbalances, over 400 papers have been published in the last 10 years, but most of them did not appear in the analytical literature. In connection with surface analysis (by AES, ESCA, SIMS and other instrumental methods) in particular, the absolute determination of very small changes in the mass of the surface layers, by microweighing in vacuum or defined gas phases, has undergone a renaissance as a reference and/or calibration

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FOREWORD

The Editorial Board and Publisher of *Talanta* take great pleasure in presenting this special issue in honour of Professor Harvey Diehl, on the occasion of his 70th birthday anniversary. Professor Diehl has made many highly significant contributions in various fields of analytical chemistry, and his work on the value of the Faraday must rank, in attention to detail and achievement of extremely high precision, with the best of the work done on atomic weight determinations by the giants of the past. It was felt that this Honour Issue would not be complete without an example of Professor Diehl's own work, so (without his consent being sought) his most recent paper is included; it typifies his thoroughness and originality in research. The photograph on the cover is of Professor Diehl's office blackboard during the work on the structure of fluorescein.

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NOTICES

INTERNATIONAL SYMPOSIUM ON ELECTROANALYSIS IN CLINICAL, ENVIRONMENTAL AND PHARMACEUTICAL CHEMISTRY 13–16 APRIL 1981

The following plenary lectures will be presented at this Symposium to be held at UWIST, Cardiff and organized by the Electroanalytical Group of the Analytical Division of the Royal Society of Chemistry:

Opening Lecture: J. D. R. Thomas, UWIST, Cardiff, Wales.

Electrochemical Polarization Phenomena of Simple Models of Biological Membranes: J. Koryta, J. Heyrovský Institute of Physical Chemistry and Electrochemistry, Prague, Czechoslovakia.

Ion-selective Field Effect Transistors: Principles and Applications in Clinical Chemistry and Biology: J. Janata, University of Utah, Salt Lake City, U.S.A.

Analytical Uses of Piezoelectric Crystals for Air Pollution Monitoring: G. G. Guilbault, University of New Orleans, U.S.A.

Applications of Electrochemical Methods in the Analysis of Pharmaceutical Preparations: E. Pungor, The Technical University, Budapest, Hungary.

Applications of Electroanalytical Instrumentation to Control Monitoring Work in Water and Water Purification: R. Briggs, Water Research Centre, Stevenage Laboratory, England.

Details from:

Short Courses Section (Electroanalysis Symposium)
UWIST
Cardiff, CF1 3NU
Wales, U.K.

32nd PITTSBURGH CONFERENCE AND EXPOSITION ON ANALYTICAL CHEMISTRY AND APPLIED SPECTROSCOPY ATLANTIC CITY, NEW JERSEY, U.S.A., 9–13 MARCH 1981

The following symposia are being organized.

1. Studies of Catalysts by Raman, Photoacoustic and/or IR Spectroscopy
2. Analytical Chemistry—New Directions for this Decade
3. Analytical Chemistry and Process Stream Analysis
4. Analysis of High Purity Water
5. Surface Analysis of Materials
6. Industrial Hygiene and the Analytical Chemist
7. Scaled Down HPLC and Practical Micro-scale LC Systems
8. The Future of Capillary Column Gas Chromatography
9. Analytical Laboratory Computer Networking
10. Synfuels from Coal
11. Recent Developments in the Electrochemical Determination of Pollutants
12. Sampling and Analysis of Airborne Contaminants from Fossil Fuel—Mobile and Stationary Sources
13. Consumer Products and the Analytical Chemist
14. Environmental Pollution—Problems, Measurements and Standards
15. Tracking Drugs as They Travel Through the Body
16. Clinical Toxicology
17. Dal Nogare Award and Symposium
18. Coblentz Society Award and Symposium
19. Fourier Transform Spectroscopy (FTS)/Computer Assisted Dispersive Spectroscopy (CADS)
20. Spectroscopy Society of Pittsburgh Award
21. Society for Analytical Chemists of Pittsburgh Award
22. Hasler Award

A short course on Approaches to Laboratory Computerization will be held on Friday and Saturday, 13 and 14 March, 1981.

General papers *are not* restricted to the symposia topics. Papers may be contributed in all areas of the disciplines of Analytical Chemistry and Applied Spectroscopy. In 1980, over 760 papers were submitted for the Technical Program.

Those authors wishing to present papers in the 1981 Pittsburgh Conference Technical Program should submit four copies of a 300-word abstract to:

Mrs Linda Briggs, Program Secretary
Pittsburgh Conference
437 Donald Road
Pittsburgh, PA 15235, U.S.A.

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Short Courses Section (Electroanalysis Symposium),
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The programme of the Symposium will emphasise methodology and applications of electroanalytical methods, especially regarding ion-selective electrodes, gas sensers and polarography. Aspects of development, operation and mechanisms as well as new areas, such as use of piezoelectric crystals will be included where these are likely to have relevance to progress in electroanalysis in the biomedical and environmental fields.

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method, and from this point of view alone it was a useful task by the editors to outline in this monograph the state of the art of microweighing. In addition, 11 authors with international reputations in the field have surveyed special areas of development or application.

Czanderna and Wolsky survey the history, definition and classification of weighing principles, calibration techniques, auxiliary equipment for vacuum microbalances, interferences, and applications. The microanalyst will miss applications to micro and ultramicro elemental analysis, however, and thermogravimetry and differential thermal analysis have been deliberately omitted.

Schwoebel deals with the theory, construction and handling of beam microbalances, Massen and Poulis treat the sources of error in microweighing in controlled environments, and Robens reviews physical adsorption studies. Czanderna covers chemisorption studies, and Kollen and Vasofsky write about simultaneous microgravimetric and residual gas analyser measurements. The last four chapters are on mass change and infrared spectra (Angell), catalysts and catalytic processes (Fuller), high-temperature reactions (Gulbransen and Brassart) and unusual applications of the microbalance (Gast).

The book has more than 1000 references, carefully chosen with regard to interdisciplinary aspects, and covers practically all aspects of microweighing. It should be useful to a wide range of readers, such as chemists, physicists, materials scientists, biologists, engineers and geologists. For the analyst it is practically a point of honour not to lose sight of modern developments in microweighing, which was formerly the most important fundamental technique at his disposal, even if he uses the technique only for solving special problems. The book therefore, which has a price corresponding to its contents and get-up, ought to be available in any analyst's library.

G. TÖLG

Solution Equilibria: F. R. HARTLEY, C. BURGESS and R. ALCOCK, Horwood, Chichester, 1980. pp. 361. £26.00 (hard cover), £6.90 (paper).

This book gives an up-to-date account of the many methods that have been used for the determination and evaluation of stability constants, and discusses their interpretation and application in a range of sciences. After an introduction to the basic concepts, the authors consider the problem of determining the number and nature of species in solution. They then discuss the various methods available for treatment of experimental data. Methods utilizing secondary concentration variables and other "pre-computer" methods are compared and contrasted with linear and non-linear least-squares methods. The effects of systematic and random errors are described, and the statistical treatment of errors, including the necessity for weighting when the variances of residuals are not equal, is discussed. The experimental methods described include potentiometry, ultraviolet and visible spectrophotometry, spectroscopy (infrared, Raman, *etc.*), distribution methods, polarography and related techniques, and calorimetric methods. Special attention is given to the problems of studying very weak, very strong and polynuclear complexes and the interaction of metal ions with polyelectrolytes. The four "Case Studies" are perhaps the best part of the book. Each study describes how, for the particular system, data were obtained. Then, step by step, the methods used to achieve complete quantitative characterization of the system are explained. The appendices give some notes on matrix algebra and two computer programs written by the authors. A useful innovation is the bookmark listing the principal symbols used and their meanings.

There are a number of typographical errors, such as M instead of ML in equation 3.2, and α instead of θ in equation 15.29. This second error is related to the use in the literature of both α_c, the degree of formation, as defined in this book, and $\alpha_{M(L)}$ as used by Ringbom, called θ here, which is equal to $1/\alpha_c$ when α_c refers to free ligand or metal. The authors could usefully have mentioned the possible confusion, but they themselves are also a little confused since they state that Kragten's *Atlas of Metal–Ligand Equilibria in Aqueous Solution* gives plots of α_c whereas it actually gives plots of Ringbom's α_M function. The computer programs have been printed from photographs of the computer print-out, and the reproduction is not wholly satisfactory. Also, the instructions for use could have been rather more helpful, and a sample set of data for use in debugging the program would have been a useful addition.

MARY MASSON

Gel Chromatography: Theory, Methodology, Applications: TIBOR KREMMER and LÁSZLÓ BOROSS, Wiley, Chichester, 1979. pp. 299. £16.50.

This is a revised version of the Hungarian book "Gélkromatográfia". The translator, Mrs. M. Gábor, and all those concerned with the production of this book in Hungary, have produced a text which reads well and is essentially free from trivial errors. It is, however, difficult to avoid placing it in the *déjà vu* category: there is little in this text that is an advance over the longer established books on this aspect of chromatography. A rapid scan of the bibliography presented reveals that, of the *ca.* 900 references presented, only 2 refer to work published in 1977, some 20 to 1976 with around a further 50 references to publications dated 1975 and 1974. We have therefore a text that leans heavily on the sixties and early seventies; although it is stolid, thorough and reliable, it is not a source of inspiration nor of new ideas to any significant extent.

D. M. W. ANDERSON

Talanta Vol. 27, pp. iii to iv
Pergamon Press Ltd 1980. Printed in Great Britain

NOTICES

INTERNATIONAL SYMPOSIUM ON ELECTROANALYSIS IN CLINICAL, ENVIRONMENTAL AND PHARMACEUTICAL CHEMISTRY 13–16 APRIL 1981

The following plenary lectures will be presented at this Symposium to be held at UWIST, Cardiff and organized by the Electroanalytical Group of the Analytical Division of the Royal Society of Chemistry:

Opening Lecture: J. D. R. Thomas, UWIST, Cardiff, Wales.

Electrochemical Polarization Phenomena of Simple Models of Biological Membranes: J. Koryta, J. Heyrovský Institute of Physical Chemistry and Electrochemistry, Prague, Czechoslovakia.

Ion-selective Field Effect Transistors: Principles and Applications in Clinical Chemistry and Biology: J. Janata, University of Utah, Salt Lake City, U.S.A.

Analytical Uses of Piezoelectric Crystals for Air Pollution Monitoring: G. G. Guilbault, University of New Orleans, U.S.A.

Applications of Electrochemical Methods in the Analysis of Pharmaceutical Preparations: E. Pungor, The Technical University, Budapest, Hungary.

Applications of Electroanalytical Instrumentation to Control Monitoring Work in Water and Water Purification: R. Briggs, Water Research Centre, Stevenage Laboratory, England.

Details from:

Short Courses Section (Electroanalysis Symposium)
UWIST
Cardiff, CF1 3NU
Wales, U.K.

32nd PITTSBURGH CONFERENCE AND EXPOSITION ON ANALYTICAL CHEMISTRY AND APPLIED SPECTROSCOPY ATLANTIC CITY, NEW JERSEY, U.S.A., 9–13 MARCH 1981

The following symposia are being organized.

1. Studies of Catalysts by Raman, Photoacoustic and/or IR Spectroscopy
2. Analytical Chemistry—New Directions for this Decade
3. Analytical Chemistry and Process Stream Analysis
4. Analysis of High Purity Water
5. Surface Analysis of Materials
6. Industrial Hygiene and the Analytical Chemist
7. Scaled Down HPLC and Practical Micro-scale LC Systems
8. The Future of Capillary Column Gas Chromatography
9. Analytical Laboratory Computer Networking
10. Synfuels from Coal
11. Recent Developments in the Electrochemical Determination of Pollutants
12. Sampling and Analysis of Airborne Contaminants from Fossil Fuel—Mobile and Stationary Sources
13. Consumer Products and the Analytical Chemist
14. Environmental Pollution—Problems, Measurements and Standards
15. Tracking Drugs as They Travel Through the Body
16. Clinical Toxicology
17. Dal Nogare Award and Symposium
18. Coblentz Society Award and Symposium
19. Fourier Transform Spectroscopy (FTS)/Computer Assisted Dispersive Spectroscopy (CADS)
20. Spectroscopy Society of Pittsburgh Award
21. Society for Analytical Chemists of Pittsburgh Award
22. Hasler Award

A short course on Approaches to Laboratory Computerization will be held on Friday and Saturday, 13 and 14 March, 1981.

General papers *are not* restricted to the symposia topics. Papers may be contributed in all areas of the disciplines of Analytical Chemistry and Applied Spectroscopy. In 1980, over 760 papers were submitted for the Technical Program.

Those authors wishing to present papers in the 1981 Pittsburgh Conference Technical Program should submit four copies of a 300-word abstract to:

Mrs Linda Briggs, Program Secretary
Pittsburgh Conference
437 Donald Road
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 *August 29, 1980.* Abstracts received after this date cannot be guaranteed consideration for inclusion in the 1981 Technical Program.

Exposition of Modern Laboratory Equipment

In 1980, the Modern Laboratory Equipment presented at the Conference totalled 458 exhibitors occupying 1056 booths and 32 seminar rooms in which was displayed the latest equipment available for Analytical Chemistry and Spectroscopic work. Anyone desiring to reserve exhibit space or to obtain additional information regarding the 1981 Exposition should contact:

Dr Richard Obrycki, Exposition Chairman
Pittsburgh Conference
437 Donald Road
Pittsburgh, PA 15235, U.S.A.

SIXTH AUSTRALIAN SYMPOSIUM ON ANALYTICAL CHEMISTRY
The Australian National University, Canberra, 23–28 August, 1981
ANALYTICAL CHEMISTRY DIVISION
THE ROYAL AUSTRALIAN CHEMICAL INSTITUTE

Research papers and poster presentations are invited for this Symposium in all areas of analytical chemistry but especially in geochemical and mineral analytical chemistry, food and drug analysis, new developments in analytical instrumentation and the education of analytical chemists.

Further information is available from the Honorary Secretary:

B. J. Stevenson
P.O. Box 1397
Canberra City, A.C.T. 2601
Australia

Talanta, Vol 27, pp iii–iv
Pergamon Press Ltd 1980 Printed in Great Britain

PUBLICATIONS RECEIVED

Coulometric Analysis: E. PUNGOR (Ed.), Akadémiai Kiadó, Budapest, 1979. Pp. 302 + x. $27.00.

This is a collection of plenary and discussion lectures presented at a conference in Matrafüred in 1978. Eight plenary lectures review a range of aspects of coulometric analysis, some being more readable and helpful than others. I found Prof. Hulanicki's survey of coulometric titrations in non-aqueous solvents particularly interesting, but such a choice is inevitably a personal one.

The fourteen discussion papers covered a variety of topics, including the determination of sulphide, thiols, acids and bases, metals, chloride, platinum, organic acids, dithiocarbamates, and hydrazines.

The many central European authors have done well to present their papers in English, but I feel the editor would have been well advised to omit most of the discussion questions, particularly following the plenary lectures, where the questioners seem to suggest that they had either been asleep during the lecture or missed the points which the lecturer was trying to make.

To conclude, I found here some interesting papers which other workers in the field would want to read, though there is probably not much which could not be found elsewhere. Indeed, one might well ask whether a broad choice of topics from several different authors is the best way of presenting an up to date picture of such a specialized field.

IAIN L. MARR

Handbuch der analytischen Chemie, Quantitative Analyse, Band 4aγ, **Zinn (Tin):** J. W. PRICE and R. SMITH. Springer Verlag, Berlin, 1978. Pp. 262 + xv. DM 146.00.

Contrary to what the title might lead one to expect, this book is in English throughout, which will certainly open the wide market which this book deserves. The authors make it clear that they do not quote every possible reference connected with the analysis and determination of tin, but have selected carefully to provide significant and important background material to all the points they wish to make. This helps greatly towards making this such an eminently readable book from which one forms the impression that here are two people who not only have experience in the field, but also are enthusiastic to pass on their experience to the newcomer. Background information of other kinds slip in—on ores, alloys, tinplate, plating solutions, organotin compounds and various industrial processes—to fill out the bare bones of selected methods, showing why these are needed and where the problems lie. I have enjoyed reading this book and have learned much from it. If the expense can be justified, then this book should be bought by all those working in tin chemistry.

IAIN L. MARR

GLC and HPLC Determination of Therapeutic Agents. Parts 2 and 3: K. TSUJI (Ed.), Dekker, New York, 1979. Pp. xiv + 520 and xiv + 548.

These two books constitute the second and third parts of Volume 9 of the Chromatographic Science Series, which is devoted to the determination of therapeutic agents by chromatographic methods. Part 2, which contains Chapters 14–24, deals with the analysis by GC and HPLC of narcotics, barbiturates, antipsychotic drugs, antihypertensive agents and vasodilators, together with some antibiotics and steroids. Part 3, which contains Chapters 25–40, is concerned with anti-inflammatory agents, prostaglandins, glycosides, anticoagulants, diuretics, X-ray contrast agents, alkaloids, vitamins and sugars. A critical approach is maintained in the review of literature methods and possible new methods for the determination of some drugs are suggested. Numerous examples of gas chromatograms and high pressure liquid chromatograms illustrate the text.

J. R. MAJER

Analysis of Drugs and Metabolites by Gas Chromatography–Mass Spectrometry, Vol. 6: B. J. GUDZINOWICZ and M. J. GUDZINOWICZ, Dekker, New York, 1979. Pp. x + 446.

This is the latest addition to the multi-volume comprehensive treatise on the analysis of drugs by GC/MS. The treatment in the present volume is, as with previous volumes, precise and detailed. Extensive accounts of analytical determinations of specific drugs are given, so that the procedures may readily be reproduced. Data on the accuracy and limits of detection for individual methods are collected and compared in Tables, together with the retention volumes of the drugs. Many chromatograms and pharmacokinetic graphs are reproduced from original papers to aid in the interpretation of the text. Two main classes of drugs are dealt with in this book. They are the cardiovascular group, which includes glycosides, vasodilators, anticoagulants, diuretics and antisclerosis agents and the antihypertensive group, which includes hypoglycemic agents and thyroidal hormones.

J. R. MAJER

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EDITORIAL REVIEW

Just as a camel is defined as a horse designed by a committee, a committee might be defined as a body that makes a simple working rule almost unintelligible. A committee does not deliberately set out to do this, of course, but if it consists of more than about one person, the appeasement of its more bigoted members by inclusion of their notions, amendments, improvements (?) and other quirks will almost certainly ensure that the committee fails to keep to its intentions. These thoughts originate from contemplation of some of the products of the IUPAC commissions on nomenclature, and their consequences for practical scientists. Now nobody would deny that definitions are necessary in order to avoid mutual misunderstanding, or that the various commissions concerned have worked long and hard to arrive at such definitions, but the wisdom of some of the resulting dictates may certainly be questioned. Why shorten mole to mol, for example, and then insist on using dm^3 instead of l for litre? Why say the word litre is regarded as a special name for the cubic decimetre (and hence imply that it is strictly synonymous with it) and then say that it must not be used to express results of high precision? Either the terms are interchangeable in all respects or they must mean two different things. One example given (by IUPAC) of the meaning of the mole is an absurdity: we are asked to consider the entity $\frac{1}{2}Ca^{2+}$, and this can only mean that calcium ions are exactly divisible into two equal fragments, and that these are capable of existence. If they are not in fact thus divisible, there is no physical meaning to the concept, and this example should be withdrawn. It is, of course, obvious that it arose from an attempt to use the advantages of the concept of chemical equivalents, while denying that any exist.

A good example of the benefits and irritations (in this case remarkably many and agreeably few, respectively) to be derived from committee work is provided by the IUPAC Compendium of Analytical Nomenclature.* This work, compiled by three analysts with world-wide reputations, and is a painstaking attempt to provide universally acceptable definitions of the vast number of technical terms and concepts used in modern analytical chemistry. That they succeed in their aim to such a large extent as they do is a tribute to their patience and devotion. The book itself is almost completely free from printer's errors, and those noticed are readily corrected (the subscript 2 misssing in SO_2 on page 58, and the misalignment of Table 2.2.15 on page 13); on line 2 of page 180 it may be that "mole concept" was intended instead of "whole concept".

That having been said, and the authors duly thanked and congratulated, certain questions must be asked, if only in the hope of arousing further discussion of certain controversial points. Thus on page 9 it is stated that use of range-tables to estimate standard deviations from small numbers of results is not recommended, but most statistical texts state that the range method is more efficient (in the mathematical sense) than the usual calculation method when the number of variates is small. On page 10 the term "relative error" is also disapproved of, although it is probably less likely to be misinterpreted than "percentage error". On page 11, it is stated that a normal distribution is to be expected for analytical results, but there are many practising analysts, especially those interested in trace and environmental analysis, who would dispute this. On page 39 the totally unnecessary term "formality" is defined; it is a redundancy for molarity, itself a term that IUPAC would like to suppress on grounds of possible confusion with molality. IUPAC might ponder on the morality of this in view of the fact that molarity is used far more extensively than molality as a concentration unit, and is far more sensible in terms of practical use. The notion on p. 178 that molality is useful for titration purposes is ludicrous—with weight burettes it is the solution that is weighed, so mole/kg of solution is more logical as the concentration unit (and is used as such by analysts). On pages 59 and 61 there is an unfortunate example of right and left hands not working in unison, as the same symbol (K_D) is used for two different concepts, and two different symbols (K_D and K_{ex}) for the same constant.

The section that may cause most concern, however, is the Appendix on the use of equivalents and normalities. In 1969 IUPAC Commission V.3 appointed a working party to consider this question of whether normality should be abandoned in favour of molarity, and the "normal" members of that party produced arguments sufficiently cogent, and definitions sufficiently clear, to convince even the most committed "molar" members that *both* systems had their place and uses and that both should be retained. It is doubtful whether members of that working party would recognize from the Appendix the outcome of their deliberations. What the working party proposed was that the mole and equivalent should be related by a very simple definition, and that normality should also be related to the mole in a simple way. In essence, the equivalent was to be defined as that amount of a substance (of specified formula) which in a given acid-base reaction would react with or release

* **Compendium of Analytical Nomenclature:** H. M. N. H. IRVING, H. FREISER and T. S. WEST, IUPAC/Pergamon, Oxford, 1978. Pp. viii + 223. $25.00.

1 mole of hydrogen ions or react with (or be equivalent to) 1 equivalent of another substance, and in a redox reaction would release or accept 1 mole of electrons or react with (or be equivalent to) 1 equivalent of another substance. A normal solution would contain 1 equivalent per litre of solution. These definitions are indeed in the Appendix, but are buried in a welter of what seems irrelevant, unnecessary and unnecessarily complicated detail. The numerical value of the equivalent is readily seen from the reaction equation, and there is no need whatever for the invention of an "equivalence factor", which seeems a typical committee artefact. The discussion of the apparent anomaly (on pp. 182–183) of the iodide/iodate reaction is not as helpful as it might be and is indeed a little misleading because it does not state the facts fully enough. If the reaction

$$KIO_3 + 5KI + 6HCl = 3I_2 + 6KCl + 3H_2O$$

is being used to determine the *iodide*, then the equivalent of the iodate *is* $\frac{1}{5}$ mole, no matter what subsequently happens to the iodine. If the iodine is then titrated with thiosulphate, then only $\frac{5}{6}$ of the thiosulphate used corresponds to the iodide being determined. However, this particular reaction is practically unusable for this purpose because of the difficulty in detecting the end-point. Instead, the reaction is used as a *source of iodine*, either for convenience in iodine titrations or for determining *iodate* (by reaction with *excess* of iodide, and titration of the iodine with thiosulphate). In either case 1 mole of iodate gives rise to 3 moles (6 equivalents) of I_2, which will then be reduced to I^- either by the reductant to be determined by titration with the iodine, or by the thiosulphate used in the iodate determination, so the equivalent of the iodate will be $\frac{1}{6}$ mole, whichever way we look at the reaction (so the oxidation state method still gives the correct answer). The only valid objection to the use of normality is the fact that the equivalent depends on the reaction used, and that the same substance may have more than one equivalent and hence a mistake might be made if a solution of such a substance were labelled only in terms of its normality, without specification of the reaction type it was to be used for. Even this objection will not stand examination in the light of common sense. Even in a microprocessor age people still have tongues in their heads, or can read and write (after a fashion) if they are scientists, and even in the present state of education might be expected to realize that running a standard takes only a few minutes longer, and might be a good idea anyway, as a check on the value given on the label. Moreover, the person using the solution is the one most likely to have prepared it (or had it prepared) and so ought to know what the label means. Much more insidious is the risk of error when molarity is used without specification. It is not so long ago that the Royal Institute of Chemistry examiners were asked to re-mark some examination answers because the question referred to 0.1*M* ferric alum and the examiners used the old "double formula" whereas many candidates used the 12-hydrate formula. What would the authors of the Compendium understand by 0.1*M* iodine? Does iodine mean I or I_2? On the face of it the risk is the same as for normality, but is really more subtle because of the very insistence that molarity is invariable (whereas normality is not) and the consequent belief that errors cannot be made, with the danger that the need to state the species referred to will be forgotten. This risk is not great in the laboratory (for the same reasons as for normality) but it *is* in publication of methods unless the species concerned is stated.

In general, normality is far easier to use than molarity for acid-base and redox reactions: the stoichiometric factor from the reaction equation is already incorporated in arriving at the equivalent and so is not necessary in the calculations. On the other hand, normality is not meaningful in complexation and precipitation reactions, where molarity *must* be used. In polymer chemistry and biochemistry where the molecular weight (or relative molecular mass as IUPAC would say) may not be knowable or known, then calculations must be done in terms of equivalents (or moles) of a determinable group per unit weight (or mass). A by-product of the use of equivalents is that Faraday's laws and the principles of coulometry and electrochemistry become much simpler to comprehend. The Appendix to the Compendium does not really clarify the concept of normality. What is IUPAC going to do if molarity is also abandoned (as ISO appears to desire)? Perhaps it should ask itself whether such moves are a result of lack of thought and presentation (perhaps the term iupacity might be coined to describe such obfuscation!)

To sum up—beg, buy, borrow or steal the book, read it, and use it as far as the dictates of commonsense and editors will allow.

R. A. CHALMERS

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Pergamon Press Ltd 1980. Printed in Great Britain

PUBLICATIONS RECEIVED

Probing Polymer Structures (Advances in Chemistry Series No. 174): J. L. KOENIG (editor), American Chemical Society, Washington, D.C., 1979, $33.00 pp. 277.

This book is based on a 1977 symposium sponsored by the Divisions of Polymer Chemistry and Analytical Chemistry of the American Chemical Society. It comprises thirteen contributions dealing with current developments in instrumental methods of characterizing commercial polymers. The title word "Structure" is used in a very broad sense, since many of the techniques deal with aspects of dynamic mechanical and tensile testing. Indeed, classical analysis methods, such as vibrational spectroscopy, are omitted except where they provide information on transitions and relaxation processes. Subjects included are: electrical noise associated with thermal transitions and flow, acoustic emission under tensile load, inelastic electron tunnelling spectroscopy, quasi-elastic laser-light scattering, the nanotensilometer, Brillouin scattering, multipass Fabry-Perot spectroscopy, measurement of non-linear viscoelastic properties.

G. GORDON CAMERON

Foundations of Chemical Analysis: O. BUDEVSKY, Ellis Horwood, Chichester, 1979. Pp. 372. £18.50 (Student edition, limp cover, £7.50).

In this book Budevsky aims to introduce the student to what is often considered to be a confusing branch of chemistry. He does so in a clear methodical manner, first introducing the basic concepts of thermodynamics, structure of solvents, and acid–base equilibria, with many worked examples. From these fundamental considerations he develops theoretical and practical approaches to titrimetry, complexometry, gravimetry and redox equilibria, again with worked examples and clear diagrams throughout the text.

The construction of logarithmic concentration diagrams and of titration curves based on these is particularly well explained and emphasis is repeatedly placed on graphical methods for representing titrations. Acid–base titration in non-aqueous solvents is very fully dealt with, and the section on complex-formation equilibria introduces the Ringbom α-coefficient and conditional stability constant simply and clearly. The use of EDTA as a complexing agent is discussed in detail. Precipitation equilibria and titrations are dealt with at length, with emphasis again placed on titration curves and their construction. Consideration of redox reactions leads to the introduction of redox titrations and potentiometry, sections on indicator and reference electrodes.

This textbook is to be recommended to the student approaching analytical chemistry for the first time, presenting as it does the basic principles of classical analysis in such a lucid fashion, aided by its many diagrams and examples.

BRENDA J. ADAM

Organic Reagents for Copper: F. J. WELCHER and E. BOSCHMANN, Krieger, Huntington, New York, 1979. Pp. xv + 614. $34.50.

With 5291 references to over 1500 organic reagents for the determination of copper, this book ought to be the last word on the subject. Surely editors must call a moratorium and refuse all further papers on reagents for copper, unless they are clear improvements on the 3 or 4 standards. The book is well produced, carefully compiled and contains a lot of practical information. Unfortunately, like many of the papers in the field, it is so uncritical that it cannot be recommended, although referees and editors should find it useful.

D. BETTERIDGE

75 Years of Chromatography—a Historical Dialogue: L. S. ETTRE and A. ZLATKIS (Eds.), Elsevier, Amsterdam, 1979. Pp. XIV + 502. $49.75.

It was a happy idea to ask 59 pioneers of chromatography to contribute a short account of their own contributions and to give their impressions of the early days. Each account is preceded by a brief biography provided by the editors. Their personal accounts have received minimal editing, and this makes the book a most interesting cross section of the scientific community. Hindsight may be 20/20, but there are fascinating and diverse reasons given for initiating crucial experiments.

A few criticisms can be made. The articles are given in alphabetical order, so there is no sense of the general order of progression and this is not rectified by a chronological table; the editors have not made any critical comments on the accounts of the contributors, which is friendly, but not conducive to good history; some of the early stars are missing.

It is however a most readable book, pre-eminently one to be dipped into and savoured over a long period. It is strongly recommended to all those, young or old, for whom science is an adventure.

D. BETTERIDGE

Mass Spectrometry, Part A: CHARLES MERRITT, JR. and CHARLES N. MCEWEN (Editors), Dekker, New York, 1979. Pp. 304. $35.00.

Although in no sense intended as an introductory treatment, this volume, number 3 in the Practical Spectroscopy series, begins with an excellent review of the development of mass spectrometry as an integral part of chemistry, achieving this in

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DECEMBER

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The book has more than 1000 references, carefully chosen with regard to interdisciplinary aspects, and covers practically all aspects of microweighing. It should be useful to a wide range of readers, such as chemists, physicists, materials scientists, biologists, engineers and geologists. For the analyst it is practically a point of honour not to lose sight of modern developments in microweighing, which was formerly the most important fundamental technique at his disposal, even if he uses the technique only for solving special problems. The book therefore, which has a price corresponding to its contents and get-up, ought to be available in any analyst's library.

G. TÖLG

Solution Equilibria: F. R. HARTLEY, C. BURGESS and R. ALCOCK, Horwood, Chichester, 1980. pp. 361. £26.00 (hard cover), £6.90 (paper).

This book gives an up-to-date account of the many methods that have been used for the determination and evaluation of stability constants, and discusses their interpretation and application in a range of sciences. After an introduction to the basic concepts, the authors consider the problem of determining the number and nature of species in solution. They then discuss the various methods available for treatment of experimental data. Methods utilizing secondary concentration variables and other "pre-computer" methods are compared and contrasted with linear and non-linear least-squares methods. The effects of systematic and random errors are described, and the statistical treatment of errors, including the necessity for weighting when the variances of residuals are not equal, is discussed. The experimental methods described include potentiometry, ultraviolet and visible spectrophotometry, spectroscopy (infrared, Raman, *etc.*), distribution methods, polarography and related techniques, and calorimetric methods. Special attention is given to the problems of studying very weak, very strong and polynuclear complexes and the interaction of metal ions with polyelectrolytes. The four "Case Studies" are perhaps the best part of the book. Each study describes how, for the particular system, data were obtained. Then, step by step, the methods used to achieve complete quantitative characterization of the system are explained. The appendices give some notes on matrix algebra and two computer programs written by the authors. A useful innovation is the bookmark listing the principal symbols used and their meanings.

There are a number of typographical errors, such as M instead of ML in equation 3.2, and α instead of θ in equation 15.29. This second error is related to the use in the literature of both α_c, the degree of formation, as defined in this book, and $\alpha_{M(L)}$ as used by Ringbom, called θ here, which is equal to $1/\alpha_c$ when α_c refers to free ligand or metal. The authors could usefully have mentioned the possible confusion, but they themselves are also a little confused since they state that Kragten's *Atlas of Metal–Ligand Equilibria in Aqueous Solution* gives plots of α_c whereas it actually gives plots of Ringbom's α_M function. The computer programs have been printed from photographs of the computer print-out, and the reproduction is not wholly satisfactory. Also, the instructions for use could have been rather more helpful, and a sample set of data for use in debugging the program would have been a useful addition.

MARY MASSON

Gel Chromatography: Theory, Methodology, Applications: TIBOR KREMMER and LÁSZLÓ BOROSS, Wiley, Chichester, 1979. pp. 299. £16.50.

This is a revised version of the Hungarian book "Gélkromatográfia". The translator, Mrs. M. Gábor, and all those concerned with the production of this book in Hungary, have produced a text which reads well and is essentially free from trivial errors. It is, however, difficult to avoid placing it in the *déjà vu* category: there is little in this text that is an advance over the longer established books on this aspect of chromatography. A rapid scan of the bibliography presented reveals that, of the *ca.* 900 references presented, only 2 refer to work published in 1977, some 20 to 1976 with around a further 50 references to publications dated 1975 and 1974. We have therefore a text that leans heavily on the sixties and early seventies; although it is stolid, thorough and reliable, it is not a source of inspiration nor of new ideas to any significant extent.

D. M. W. ANDERSON

method, and from this point of view alone it was a useful task by the editors to outline in this monograph the state of the art of microweighing. In addition, 11 authors with international reputations in the field have surveyed special areas of development or application.

Czanderna and Wolsky survey the history, definition and classification of weighing principles, calibration techniques, auxiliary equipment for vacuum microbalances, interferences, and applications. The microanalyst will miss applications to micro and ultramicro elemental analysis, however, and thermogravimetry and differential thermal analysis have been deliberately omitted.

Schwoebel deals with the theory, construction and handling of beam microbalances, Massen and Poulis treat the sources of error in microweighing in controlled environments, and Robens reviews physical adsorption studies. Czanderna covers chemisorption studies, and Kollen and Vasofsky write about simultaneous microgravimetric and residual gas analyser measurements. The last four chapters are on mass change and infrared spectra (Angell), catalysts and catalytic processes (Fuller), high-temperature reactions (Gulbransen and Brassart) and unusual applications of the microbalance (Gast).

The book has more than 1000 references, carefully chosen with regard to interdisciplinary aspects, and covers practically all aspects of microweighing. It should be useful to a wide range of readers, such as chemists, physicists, materials scientists, biologists, engineers and geologists. For the analyst it is practically a point of honour not to lose sight of modern developments in microweighing, which was formerly the most important fundamental technique at his disposal, even if he uses the technique only for solving special problems. The book therefore, which has a price corresponding to its contents and get-up, ought to be available in any analyst's library.

G. TÖLG

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D. M. W. ANDERSON

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 *August 29, 1980.* Abstracts received after this date cannot be guaranteed consideration for inclusion in the 1981 Technical Program.

Exposition of Modern Laboratory Equipment

In 1980, the Modern Laboratory Equipment presented at the Conference totalled 458 exhibitors occupying 1056 booths and 32 seminar rooms in which was displayed the latest equipment available for Analytical Chemistry and Spectroscopic work. Anyone desiring to reserve exhibit space or to obtain additional information regarding the 1981 Exposition should contact:

Dr Richard Obrycki, Exposition Chairman
Pittsburgh Conference
437 Donald Road
Pittsburgh, PA 15235, U.S.A.

SIXTH AUSTRALIAN SYMPOSIUM ON ANALYTICAL CHEMISTRY
The Australian National University, Canberra, 23–28 August, 1981
ANALYTICAL CHEMISTRY DIVISION
THE ROYAL AUSTRALIAN CHEMICAL INSTITUTE

Research papers and poster presentations are invited for this Symposium in all areas of analytical chemistry but especially in geochemical and mineral analytical chemistry, food and drug analysis, new developments in analytical instrumentation and the education of analytical chemists.

Further information is available from the Honorary Secretary:

B. J. Stevenson
P.O. Box 1397
Canberra City, A.C.T. 2601
Australia

Talanta, Vol. 27, p. iv
Pergamon Press Ltd 1980. Printed in Great Britain

ERRATA

In the paper by G. Ackermann and J. Kothe in *Talanta* 1979 26, 696. On page 696 the sixth line from the foot of the left-hand column should read: (Nonova)[5] bzw. $8{,}3 \cdot 10^4$ (Kitano und Ueda)[4]].

In the book review of Foundation of Chemical Analysis by O. Budevski in the February issue 1980 the price of the students edition was given incorrectly, it should be £5.90/$14.80.

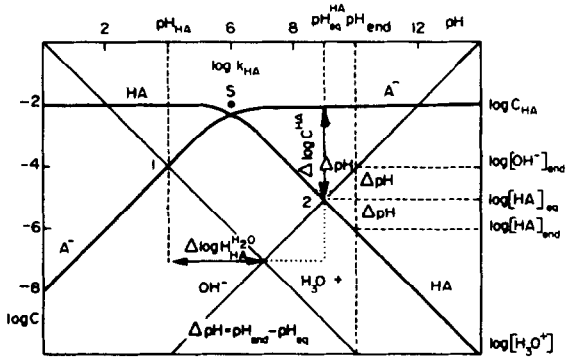
Talanta, Vol. 27, p. v
Pergamon Press Ltd 1980. Printed in Great Britain

ERRATA

In the paper by T. Nagai, *Talanta*, 1980, **27**, 25, on page 26, in Table 2, the last line should read:

0.180 0.155 ± 0.010 3.5.

In the paper by E. Wänninen, *Talanta*, 1980, **27**, 29, the following corrections are necessary. On page 29 in Fig. 1, arrow-heads are to be inserted at the ends of the vertical full line labelled ΔpH, as shown below:



On page 31 a solidus should be inserted in equation (18), as follows:

$$K_{HX} = [HX]/[H_3O^+][X^-]. \quad (18)$$

On page 32, the last equation in (*c*) in *Example 3* should read:

$$(\%e)_{HX} = \pm(1.25 \times 0.36 + 0.25 \times 18) = 5.0$$

and the corresponding equation in (*c*) of *Example 4* should read:

$$(\%e)_{HX} = \pm(1.25 \times 0.36 + 0.25 \times 1.8) = 0.9$$

In the paper by I. Tsukahara and M. Tanaka, *Talanta*, 1980, **27**, 655, on page 657, left-hand column, line 2, 150 μg of Au should read: 15 μg of Au.

Talanta, Vol 27, p v
Pergamon Press Ltd 1980 Printed in Great Britain

ERRATUM

In the paper by H. Narasaki, *Talanta*, 1980, **27**, 194, equation (21) should read:

$$[H^+] = \frac{-(K_1 + C_H) + \sqrt{(K_1 + C_H)^2 + 4K_1C_A}}{2} + nK_2 \qquad (21)$$

Talanta, Vol. 27, p. vi
Pergamon Press Ltd 1980. Printed in Great Britain

NOTICE

THE THIRD INTERNATIONAL CONFERENCE ON PHYSICOCHEMICAL METHODS FOR WATER AND WASTEWATER TREATMENT Lublin (Poland) 21–25 September 1981

The Conference is co-sponsored by Maria Curie-Sklodowska University, the Polish Chemical Society and the Chief Technical Organization of Poland.
Physicochemical methods for water and wastewater treatment will be the theme for the Conference. Papers are required on any subject related to the theme. However, special attention will be given to:

—application of ion-exchange for water and wastewater treatment,
—recovery of water and chemicals from wastewater,
—application of catalytic processes for wastewater treatment.

LECTURES, SUBMISSION OF PAPERS

The main lectures (45 min each) will be presented at the symposium. A limited number of papers covering original, unpublished work on the symposium subjects will be accepted for presentation. (Presentation time for these papers will be 15 min with 5 additional minutes for discussion). Anyone wishing to contribute a lecture or paper should submit a synopsis of 25–30 typewritten lines in English not later than 30 January 1981.

Full details and registration form available from

Dr Lucjan Pawlowski,
3rd International Conference,
Department of Chemical Technology,
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20-031 Lublin,
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AUTHOR INDEX