ION EXCHANGERS IN ANALYTICAL CHEMISTRY: APPLICATIONS AND PROBLEMS

By Professor Otto-Erich Schultz
Pharmazeutisches Institut der Universität, Kiel

At the International Congress for Applied Chemistry in London in 1909, Siedler advanced the idea that artificial zeolites could be applied to quantitative analysis. Siedler was right although permutites were at first only applied in a limited way to analytical methods. Thus a synthetic zeolite was used for the separation of ammonia from urine for colorimetric determination[1], and a permutite was used to exchange the alkaline earth metals for the alkali metals in a volumetric method for sulphuric acid in tap water[2,3]. The general applicability of permutites in analytical chemistry remained limited, however, owing to their instability towards acids and bases. It was not until the discovery and development of ion exchangers on a resin base that the way was opened for their many possible applications to analysis.

Individual Applications

Quantitative inorganic analysis

In qualitative analysis ion exchangers serve for the removal of interfering anions such as phosphate, oxalate, or tartrate[4,5,6]. After the precipitation of the sulphide group and the removal of hydrogen sulphide from the weak acid solution, this latter is slowly passed through a cation exchange column in the H-form. The cations are bound to the exchanger and the anions remain in solution and appear in the effluent. The cations are then eluted with 4N hydrochloric acid and estimated. In this procedure a green chromium (III) salt may pass through the column in small amounts since Cr^{+++} can form anionic or neutral complexes. Complex cyanides can be separated in a similar way[7].

Separations of metals can be carried out in various ways with ion exchangers. The amphoteric metals zinc, aluminium, molybdenum, tungsten and antimony can be separated from the non-amphoteric metals after exchange on a cation exchanger, by solution with alkali as complex anions; molybdenum and tungsten with a 2 per cent. caustic soda solution, zinc and aluminium with a 5 per cent. and antimony with a 10 per cent. solution[8–10].

The separation of molybdenum in qualitative analysis can be achieved by a method developed by Klement, wherein the molybdate is bound as a complex with organic acids[11,12]. A weak mineral acid solution containing the molybdate together with other cations is treated with excess citric acid and passed through a cation exchanger in the H-form. The molybdenum passes into the effluent as a complex and can be recovered quantitatively.

Bismuth can be eluted with a 1 per cent. potassium iodide solution from a cation exchange column laden with bismuth, copper and lead. Traces

of copper are also eluted, but not in sufficient quantity to interfere with the direct colorimetric determination of bismuth in the eluate.

From a solution of arsenic, antimony and tin, a cation exchanger of the sulphonic acid type holds back the antimony and tin, the arsenic appearing in the effluent[8].

Arsenic and antimony can be separated by means of a strong basic anion exchanger in the sulphate form which quantitatively holds back the arsenic but not the antimony[13].

QUANTITATIVE INORGANIC ANALYSIS

Determination of Cations

In quantitative analysis, many processes can be much simplified in that the cations can be exchanged for hydrogen ions on a cation exchanger in the H-form, the liberated acid determined with standard alkali and thus the equivalent amount of original cation can be calculated. This method can also be used for salts which are soluble only in acids, for example, calcium phosphate. In this case the increase in hydrogen ions is found and from this the content of cations can be calculated. Samuelson and colleagues and Klement and others have used this method in many cases with success. The determination of the anions in the solution is equally possible. Table I gives a series of possible separations.

For solutions containing several cations and anions, this method is however only applicable in rare cases. Here a simplification of the methods previously applied is possible in that the cations, exchanged in the usual way on a column, are dissolved with hydrochloric acid and determined in the acid solution.

TABLE I

THE USE OF EXCHANGERS IN THE DETERMINATION OF IONS

Cation	Anion	Exchanger	Reference
K	SO_4		(without separation of anions from cations)
Alkalis	PO_4		
Al, Fe	PO_4		
Ca	SO_3 (Sulphite-hydrochloric acid)		91
Na, K	$Fe(CN)_6'''$, $Fe(CN)_6''''$	H-exchanger	7
Na, K	$Co(CN)_6'''$, $Fe(CN)_5NO''$ $Cr(CN)_6$, $Mo(CN)_8''''$ $Cr(CN)_8''''$	H-exchanger	7
Na, K, Mg, NH_4, Cu, Ca	Cl	Wolfatit K	92
Sr, Ba	Cl	"	92
Cd	Acetate	"	92
Co	NO_3	"	92

In certain instances, owing to the instability of the anions in acid solution, an NH_4-exchanger must be employed instead of an H-exchanger. For example, the separation of the alkali metals from chromate, molybdate, tungstate, phosphomolybdate, phosphotungstate and silicotungstate and the determination of sodium and potassium in the presence of vanadate can be thus effected (Table II). Owing to the oxidising action of chromates in an acid medium and the instability of the other anions in acid solution, these cannot be treated with an exchanger in the H-form[7,10].

TABLE II

SEPARATION OF ALKALI METALS

Cations	Anions	Exchanger	Reference
Na, K	CrO_4''; MoO_4'', WO_4'', $P(Mo_9O_{10})_4'''$, $P(W_3O_{10})_4'''$	NH_4-Exchanger	7
Na, K	VO_3'	NH_4-Exchanger	10

A further possibility in quantitative determination of cations is to elute them with hydrochloric acid after they have been bound to a column as described above, and then to assay them in the hydrochloric acid solution. This procedure is especially useful when interfering anions must be removed. In the methods heretofore adopted an interfering anion had to be converted into an insoluble compound and removed by filtration. With exchangers this removal of interfering anions is accomplished very simply. Treatment of the solution with a cation exchanger binds the cations; and the anions which remain in solution, appear in the effluent. The cations are then eluted with hydrochloric acid and are determined without interference. Moreover the free acid in the effluent can be titrated and related to the cation content.

There are examples of this method; potassium in the presence of sulphuric acid, alkalis in the presence of phosphoric acid[5,6,14,15] and the estimation of aluminium and iron in the presence of phosphoric acid[16].

By complex formation of cations further possibilities may be envisaged, particularly the separation of cations from one another[7]. Thus the alkali metals can be separated from iron and cobalt. The mixture containing the alkali metals together with iron and cobalt is added to a solution of hydrocyanic acid which has been neutralised with ammonia. The heavy metals thus form complex cyanides, and, after passage through a column of exchange resin, appear in the effluent. The alkali metals are exchanged, eluted with hydrochloric acid and determined in the acid solution.

Finally, the different affinities of cations for the exchange resin can be used for their quantitative separation, for example, the separation of lithium, sodium and potassium and also cadmium and zinc by elution of a Wofatit KS column loaded with a mixture of the ions. Separation is brought about by the passage of the eluted cations through the unloaded part of the column which must therefore be sufficiently large. By means of a suitable apparatus for measuring conductivity, curves can be obtained showing when each cation appears in the effluent. By previous calibration with known solutions, the method can be made quantitative[17,18].

Determination of Anions

According to Samuelson, the anions which are principally determined are those which are bound as neutral salts, the solutions of which are passed through a cation exchanger in the H-form, and the equivalent amount of acid thus liberated is titrated with standard alkali. Table III gives possible separations and these proceed smoothly. Weak acids are adsorbed as non-polar molecules by the exchanger. For them, the

TABLE III

THE USE OF EXCHANGERS IN THE DETERMINATION OF IONS

Cations	Anions	Exchanger	Reference
Na, K	SO_4, NO_3	Wofatit KS	19
Li, Na, K, NH_4, Mg, Ca, Sr, Ba, Co, Ni, Zn, Mn, Al, Fe, Cr	NO_3, ClO_4, PO_4	Wofatit	7
Na, Cu, Fe^{++}, Cr Va	Cl, SO_4, NO_3, PO_4	Wofatit Sulphonic acid organolites	4, 7
K, Na, NH_4, Mg, Ca	SO_4	Wolfatit K Wofatit KS	21, 22, 93, 94
Al, Zn, Co, Fe, Cr (violet)			
Li, Na, K, NH_4, Ca, Sr, Ba, Co	Cl		95
K (black powder)	NO_3		7
Alkalimetal, NH_4, Cr, Fe, Al	PO_4	Wofatit K and KS	92, 96, 97
Li, Na, K, NH_4, Mg, Ca, Sr, Ba, Zn, Mn, Co, Ni, Al, Cr (green and violet)	Br, I, ClO_3		98
Fe	Br, ClO_3		98
Fe, Al, Co, Mn, Zn	SeO_3		98
Cd	Cl, Br, I, SO_4, NO_3, ClO_4, ClO_3, PO_4, Acetate, Oxalate		
Na	$H_4P_2O_7$	Wofatit K and KS	92
Alkalimetal	HPO_3		92
Ca, Fe	SiO_3		99
Na	Oxalate		92
Li, Na, K, NH_4, Mg, Ca, Sr, Ba, Zn, Mn, Co, Ni, Al, Fe, Cd, Cu, Pb	Acetate	Wofatit KS	6, 92, 24
Li, Na, K, NH_4, Mg, Ca, Sr, Ba, Zn, Mn, Co, Ni, Cd, Cu	Oxalate		6, 92, 24
Na	Tartaric acid	Wofatit K	92
K	$H_4Fe(CN)_6$	Wofatit K	92
K	HF	Wofatit K	92
K	CNS	Wofatit K	92
Mg	ClO_4	Wofatit KS	92
Na	$C_6H_5SO_3'$	Wofatit K	92
K	S_2O_6''	Wofatit KS	92

separation is quantitative only when large quantities of wash-water are used[19,20].

Bromate and iodate are reduced in contact with cation exchangers in the H-form and cannot therefore be separated.

For the determination of phosphoric acid, freshly precipitated alkaline earth metal phosphates are shaken with excess cation exchanger in the H-form. The precipitates dissolve completely in two minutes; the cations are bound on the exchanger and free phosphoric acid remains in the solution. Phosphoric acid can thus be separated from Li, Na, K, NH_4, Mg, Al, Fe^{+++}[7]. With Cr^{+++} separation can be effected in mixtures of chrome alum and chromium nitrate. On the contrary however separation is not possible with green Cr(III) salt solutions or solutions containing Cr^{+++} which have been boiled before treatment with the ion exchanger owing to the formation of complex cations[4].

Ion exchangers can also be used to advantage to remove ions which interfere with the usual quantitative procedures.

The precipitation of sulphate with barium chloride for gravimetric determination of sulphate suffers considerable interference in the presence of cations such as Al^{+++}, Cr^{+++}, Ca^{++}, whose removal with cation exchangers can be effected without difficulty. A pure sulphate solution

free from interfering ions is obtained in the effluent[21,22]. Any nitric acid present simultaneously can be removed by evaporation of the effluent. The presence of phosphoric acid requires the usual procedure when precipitating sulphate with barium chloride. In the presence of green Cr(III)-salt solutions this method fails since, owing to the formation of complex sulphates of chromium, a part of the chromium appears in the effluent and a part of the sulphuric acid is bound as a complex with the chromium and retained on the exchanger. With violet chromium solutions correct values are obtained by working at low temperatures[23]. Complications can likewise arise with neutral solutions of iron ammonium alum since sulphate can be bound as cationic complexes[7]. On the other hand quantitative ion exchange is not affected by the hydrolysis of beryllium sulphate[24]. Other possible ways of removing interfering ions are given in Table IV.

TABLE IV

REMOVAL OF INTERFERING IONS

Method	Interfering Ions	Removal
Gravimetric determination of sulphate	Fe^{+++}, Al^{+++}, Cr^{+++}, Ca^{++}	H-Cation exchanger: Recovery of pure sulphuric acid.
Polarographic determination of selenide	Fe^{+++}, Al^{+++}, Co^{++}, Mn^{++}, Zn^{++}	H-Cation exchanger: separation of interfering ions.

The different affinities of chloride and bromide for a strongly basic anion exchange resin can be used for their separation in mixtures. On elution with a sodium nitrate solution at a controlled efflux rate, the first fraction contains no halide, the second fraction chloride and the third bromide[25].

Separation of the Rare Earth Metals

The separation of the rare earth metals or their compounds, which is very difficult without the use of ion exchangers, can be performed elegantly with their aid. The method utilises the ability of the rare earth metals to form complexes with citric acid the stability of which depends on the pH, and which exhibit very different degrees of adsorbability by the exchanger.

Analysis of Complexes

With the aid of resin exchangers, metal complexes may be separated into cationic, anionic and neutral complexes. The solution containing the complexes is passed successively over a cation- and then over an anion exchanger when the cationic- and then the anionic complexes are respectively exchanged. The neutral complexes appear in the effluent in both cases. Quantitative determinations are carried out on the eluates and on the effluent and the results give the composition of the complex types.

MICROCHEMICAL DETERMINATIONS

Inorganic Determinations

Wiesenberger was the first to use resin exchangers for microchemical estimations. He carried out neutral salt splitting with Wofatit-K and

estimated the acid alkalimetrically. After dissolving the neutral salts in water and treatment with the H-cation exchanger, he titrated the free acid in the effluent with 0·01N sodium hydroxide solution[26].

In human serum the calcium fraction can be determined easily with cation exchangers[27]. The calcium is exchanged by contact of the serum with Na-Wofatit-F, eluted with hydrochloric acid and determined in the eluate in quantities of the order of 0·1 to 1·0 mg.

In the determination of rubidium- and caesium salts in quantities of 0·15 milliequivalents, small-scale columns have been used for the microanalysis with good results[28].

Acetyl-group Determination

This determination is one of the most difficult organic group analyses. The micromethod of Freudenberg and Weber[29–31], which, according to figures reported in the literature gives excellent results, is by no means easy to carry out, but has been simplified by Wiesenberger[32] by the use of ion exchangers. He saponifies the ethyl acetate, obtained with *p*-toluene-sulphonic acid in the presence of ethanol, with strong caustic soda (instead of the 0·02N sodium hydroxide previously used) and treats the sodium acetate solution containing excess alkali with an H-cation exchanger so that all the Na^+ is exchanged with H^+. The liberated acetic acid is then titrated with 0·01N sodium hydroxide solution.

Exchange of Aldehydes and Ketones

The exchange of aldehydes and ketones can be achieved on an anion exchange column laden with bisulphite due to the well-known formation of hydroxy sulphonic acids thus:[33–37]

$$RCHO + HSO_3' \rightleftharpoons RCH(OH)SO_3'$$
$$R_2CO + HSO_3' \rightleftharpoons R_2C(OH)SO_3'$$

The reaction is reversible; elution is therefore possible with either alkalis or acids.

USE OF ION EXCHANGERS IN PLANT ANALYSIS

Alkaloids

The problems and possibilities of the quantitative estimation of alkaloids has been examined in detail by Büchi and Furrer[38]. Alkaloidal salts form large cations in aqueous solution:

$$Alk.HCl \rightleftharpoons [Alk.H]^+ Cl'.$$

These undergo exchange with cation and anion exchangers.

Use of Cation Exchangers

$$R^-\cdot H^+ + [Alk.H]^+Cl' \rightarrow R^-\cdot[Alk.H]^+ + HCl$$

In these exchanges (R = exchanger) the cation forms a salt-like compound with the ion exchanger. The alkaloid may then be recovered with alkali or ammonia according to the following equation:

$$R^-\cdot[Alk.H]^+ + NH_4OH \rightarrow R^-\cdot NH_4^+ + Alk. + H_2O.$$

Sparingly soluble alkaloidal bases are precipitated on the column and are extracted with an organic solvent[39,40]. When ethanolic ammonia is used, the liberated free bases are simultaneously dissolved by the ethanol.

Use of Anion Exchangers

$$R^{+}\cdot OH' + [Alk.H]^{+}\cdot Cl' \rightarrow R^{+}\cdot Cl' + Alk. + H_2O.$$

During the exchange of alkaloidal salts with anion exchangers in the OH-form, the salt-forming ion is exchanged with OH' and the liberated alkaloidal base precipitated in the column. It is then eluted with an organic solvent.

Anion exchangers for the exchange of alkaloids must possess groups of a certain basic strength, otherwise selective exchange is not possible. An anion exchanger of the quaternary ammonium type converts not only alkaloids but alkali, ammonium and amine salts quantitatively into their bases or carbonates. Anion exchangers of the weak basic type do not completely convert strongly basic alkaloids to the free bases, for example, ephedrine and cotarnine. In both cases incorrect results are obtained; in the first case values are too high and in the second case too low[41].

For the analyses only ion exchangers with a loose network can be employed[42–45]. The natural and synthetic silicate exchangers have an average pore diameter of 3 to 5 Å[46]. The greatest diameter of the quinine ion is 15 Å[38]. Such ion exchangers are thus ill-adapted for the ion exchange adsorption of the large alkaloid cations. The earliest investigations with organic ion exchangers showed that even with these the total exchange capacity was not nearly reached[47].

Of the many cation resin exchangers examined, Duolite C-10 proved best for exchange with cinchona alkaloids[38]. Even here the use of strong ethanolic solutions somewhat lowered the exchange performance, but with a 46 per cent. ethanolic solution complete exchange was effected.

The elution of alkaloids from cation exchange resin in quantitative estimations is possible principally in two ways.

The alkaloid bound on the resin in the H-form, is exchanged with H^{+}-ions from an acid. The initial amount of acid is known and the excess acid which is not exchanged with alkaloid is back-titrated; or, the alkaloid is exchanged with other cations and is then transformed with alkali into the free base which separates on the column. To determine the alkaloid titrimetrically, two conditions must be fulfilled. Firstly, an easily volatile base must be employed, which can be driven off before the titration, and secondly the alkaloidal base must be dissolved with an organic solvent.

For the first of these two processes a considerable excess of acid is essential[38] both for sulphonic acid and carboxylic acid resins for the quantitative recovery of the alkaloid. With the second process of alkaline regeneration, very rapid quantitative recovery is achieved with an ethanolic ammonia solution which acts both as alkali and solvent[35].

In the determination of the total alkaloid content of Extractum Cinchonæ Pharm. Helv. V and Cortex Cinchonæ Pharm. Helv. V by exchange

on Duolite C-10, both with the H^+– and NH_4^+ forms, coloured compounds and mineral cations can be separated from the alkaloids using an aqueous ammonia solution containing a small amount of ammonium chloride[38]. For the determination of the total alkaloids of cinchona bark, both the filtration method and the contact exchange method are suitable. In this latter the exchanger is mixed directly with the bark from which the resin is easily separated by decantation with distilled water. For both methods, a water-formic acid mixture serves as solvent[38].

Ion exchangers can also be used for the separation of substances which interfere with the quantitative estimation of alkaloids.

After making alkaline with aqueous ammonia solution, ipecacuanha root is extracted with ether, the ether solution separated, and after the addition of sulphuric acid, the ether is removed. The sulphuric acid—emetine solution is passed through a column of synthetic alumino-silicate exchanger in the ammonium form, which effects separation of accompanying coloured materials. The exchanged alkaloid is eluted with a 10 per cent. ethanolic ammonia solution, and the alkaloid estimated titrimetrically after evaporation of the solvent. The values obtained were very good. The organic exchangers Amberlite IR-100 and Zeo-Karb were both unsuited to this method[48].

In the colorimetric estimation of morphine in pathological urine co-extracted materials interfere (extraction method of Pierce and Plant). These can be separated using synthetic zeolite (Permutit) since the morphine is exchanged but not the interfering substances. The morphine is eluted with saturated sodium carbonate solution and estimated colorimetrically by the addition of Folin-Denis phenol reagent[49].

The differing basic properties of alkaloids can be used for their separation. Cation exchangers of the carboxylic type enable weakly basic alkaloids (e.g., strychnine and caffeine) to be separated from strongly basic ones (e.g., quinine, brucine and nicotine)[43].

Glycosides

In the glycoside sphere, ion exchangers have been little used up till now. Anionotropic aluminium oxide[50] was used in the isolation of digicornin. By chromatography of sugars and some glycosides on borate-buffered exchange columns, separation can be effected owing to the formation of different borate complexes conditioned by the spacial configuration of the OH-groups[51].

Glycosides of Mustard Oil. The anionic character of mustard oil glycosides, shown by their ability to migrate in electrophoresis experiments[52], enables the exchange of the glycoside anions on an anion exchanger to take place with ions originally present.

Strongly basic ion exchangers (Lewatit MI and Amberlite IR-400) in the OH-form exchange accompanying materials besides the glycoside, so that the eluate residue contains 60 to 70 per cent. of glycoside. The weakly basic ion exchangers Amberlite IR-45 and Amberlite IR-4B yield an eluate whose residue consists of up to 90 per cent. of glycoside.

Anionotropic aluminium oxide-Woelm can also be used in the same way as the weakly basic anion exchangers.

Anthraquinone Glycosides[53]. For the analysis of drug extracts containing anthraquinone glycosides strongly basic anion exchangers have been shown to be the best (Lewatit MI and Amberlite IR-400). On elution with glacial acetic acid, the materials retained on the column pass gradually into the eluate without separation. Flavone derivatives as well as anthraquinone glycosides and anthraquinone aglycones can be identified in the eluate.

Determination of Reducing Sugars. The estimation of reducing sugars in plant extracts is frequently inaccurate owing to the presence of other reducing substances. These interfering substances may be removed, however, with ion exchangers. After passage through a two-stage column or treatment of the solution with mixed resins, it is freed from interfering substances. The ability of monosaccharides to form sugar-borate complexes with borate ions, and to react with bisulphite enables an exchange to be effected. Fructose, glucose, mannose and galactose in dilute sodium borate solution are quantitatively retained by strongly basic ion exchangers in the OH-form[54]. Xylose and mannose are quantitatively held back from an aqueous solution by an ion exchanger in the bisulphite form[55]. Fructose does not react with such an exchanger and can thus be separated from xylose and mannose (Fig. 1). By stepwise elution with ethanol solutions of decreasing concentration a separation is effected since the stability of the addition compounds differs[55].

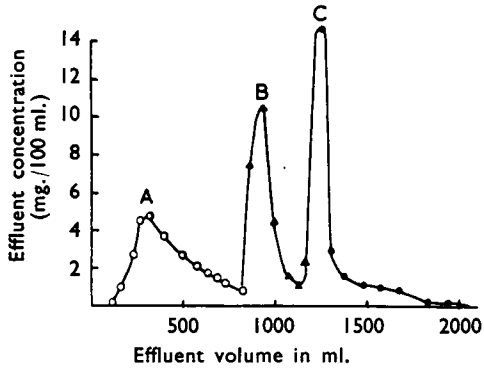

FIG. 1. Chromatographic separation of monosaccharides by stepwise elution.
Column: 9 × 150 mm.; Amberlite IRA-400 [HSO_3^-; <0·12 mm.].
Flow rate: 0·7 ml./min.
Elutriants: × 99·5 per cent. ethanol (by weight).
▲ 95·0 per cent. ethanol.
● water.
A. Fructose. B. Glucose. C. Mannose.

The carbonyl content (end-groups) of polysaccharides are estimated by transformation to carboxyl groups by the cyanhydrin method with $Na^{14}CN$. The radioactive carboxylic acid derivative is exchanged on an anion exchange resin and thus separated from other polysaccharides. The radioactivity after elution gives the carbonyl content (end-groups)[56].

APPLICATION OF ION EXCHANGERS IN THE ANALYSIS OF DRUGS

Local Anæsthetics

Of the local anæsthetics, larocaine, tutocaine, percaine, procaine, amylocaine, amethocaine, can be exchanged with Amberlite IRA-400 and

quantitatively determined[57]. Local anæsthetics in tablets and solutions for injection could not be determined since these preparations contained too great a quantity of electrolytes[58], but a procaine determination in an ointment was successful[57].

Sympathomimetic Drugs

Of sympathomimetics, methylamphetamine, ephetonal, ephedrine, naphazoline and amphetamine were estimated using without difficulty Amberlite IRA-400[59]. Compounds with one or more phenolic hydroxyl groups attached to the benzene ring were, however, not determinable, e.g., adrenaline, sympatol. They are bound only partly by the exchanger. The authors explain this as due to the weakening of the basic properties of these compounds by the phenolic hydroxyl groups which is in agreement with the work of Kunin and McGarvey[60], who found that phenols could be bound with a strongly basic exchanger[58].

Determination of Sympathomimetic Amines and Antihistamines in Tablets

The quantitative analysis of sympathomimetic amines[59] (ephedrine, methylamphetamine, ephetonal, naphazoline) and antihistamines[61] (antazoline, mepyramine, promethazine) was carried out in the following way[58]:

An extract with 10 ml. of 50 per cent. ethanol of tablets or dragees containing 20 to 100 mg. of active constituent is filtered through cotton wool. The diluents retained on the filter paper are extracted several times with hot 95 per cent. ethanol to make a total quantity of 50 ml. of extraction liquid. The combined filtrates are passed through a column containing 8 to 10 g. of Amberlite IRA-400 in the carbonate form at a rate of 7 ml. per minute. For washing through, 40 to 80 ml. of hot 95 per cent. ethanol is sufficient. After dilution with 40 ml. of hot distilled water, the solution is then titrated potentiometrically with 0·1N hydrochloric acid, or a suitable indicator (methyl red) may be used.

Regeneration of the column is effected with 10 ml. of 4 per cent. caustic soda solution, followed by washing with water until the effluent is no longer alkaline to phenolphthalein. The column cannot be used indefinitely since it is gradually rendered unusable by "filling" materials.

Determination of Spasmolytic and Cough-sedative Materials in Tablets

Substances such as acedicon, caramiphen, amprotropine and adiphenine can be assayed by the above method for the determination of the content in tablets of sympathomimetic amines and antihistamines[58].

Amino-acids

Ion exchange chromatography is of significance for the analytical and preparative separation of amino-acids. Different exchange potentials of different ions with similar properties enable separation to be effected using ion exchangers in principle exactly as in ordinary chromatographic analysis. Work on amino-acids and nucleotides as well as on the separation of the rare earth metals and isotopes in the inorganic realm have contributed much to the development of ion exchange chromatography.

Amino-acids are firstly separated into groups and then these are separated into their individual components.

In group-separations, exchangers with a functional group are selected, which selectively exchange only a few acids with isoelectric points in a definite pH-range from a series of amphoteric amino-acids with isoelectric points extending over a wide pH-interval. Thus the groups of aliphatic neutral, aliphatic basic and aliphatic amino-acids with two carboxyl groups can be separated (Table V).

TABLE V

SEPARATION OF AMINO-ACIDS

Cation exchanger	Exchange	References
Sulphonic acid type in H-form	Total amino-acids	62, 100, 104,
Sulphonic acid type in salt form	From neutral solution only basic amino-acids	105
	From acid solution also neutral amino-acids	
Carboxylic acid type: at pH 4·7	Total basic amino-acids	106
at pH 7·0	Lysine and arginine	106
Anion exchanger:		
Strong basic type in OH-form	Total amino-acids except arginine	
Strong basic type	Dibasic amino-acids	101, 107
Wofatit M, previously treated with 0·2N acetic acid	Tryptophane	63, 108

Aliphatic and aromatic amino-acids are separated from one another with activated charcoal. The aromatic amino-acids (phenylalanine, tyrosine, tryptophane) are selectively adsorbed by activated charcoal[62,63].

Kunin and Winters[64] combined anion- and cation exchangers and separated acid, neutral and basic groups, and the latter into arginine, lysine and histidine as shown in Figure 2.

The chromatographic separation[65–68] of the amino-acids depends upon the degree of ionisation of individual acids, the van der Waals forces between the ion exchanger and the acids and lastly the charge on the ions[69]. When there are little or no van der Waals forces, the distribution of the amino-acids must take place according to their dissociation constants. With methionine, van der Waals forces operate so that it does not appear in the series of the other amino-acids in accord with their dissociation constants during separation, but is more strongly adsorbed. When the van der Waals forces are reduced by raising the temperature, it then appears in approximately the position in the series of amino-acids, conditioned by its dissociation constant[67].

Experimentally a cation exchanger is used and the amino-acids taken up by the resin are displaced with bases which have a higher affinity for it (e.g., NH_3 or NaOH). The eluate is analysed by means of its conductivity or pH or better, collected with a fraction-collector and analysed by paper chromatography.

By such a procedure, however, many amino-acids are not separable, but groups are obtained. In order to effect a separation, further methods have been developed. After a further adsorption, the fraction can be treated with another eluant[70] or it can be eluted step-wise by successive

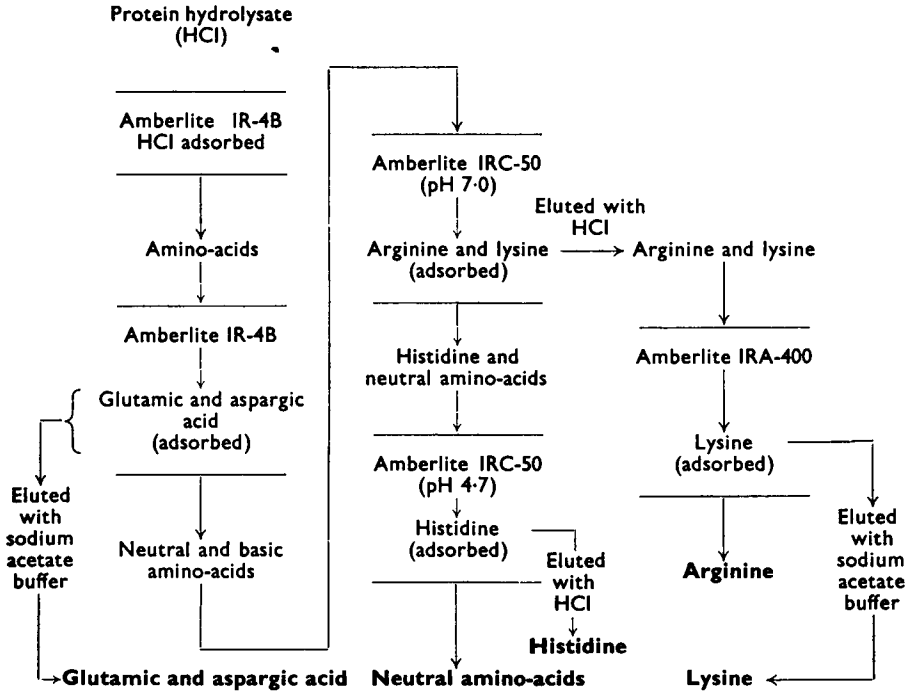

FIG. 2. Scheme for the separation of amino-acids into three groups by means of synthetic ion exchange resins.

solvents with increasing acid concentration. Also, using a resin in the Na-form buffer solutions of increasing pH may be employed[71,72].

In the separation of an amino-acid mixture containing 3 to 6 mg. the following acids were quantitatively obtained by using a buffer of pH 3·4, and were divided off according to their maxima: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine and valine; at a pH of 4·25 the following acids were obtained: methionine, *iso*leucine, leucine, tyrosine and phenylalanine; at pH 8·3, histidine; at pH 9·2, lysine and at pH 11, arginine[72].

Nucleotides are separated on the same principle as amino-acids[73].

Vitamins

Ion exchangers in determination of vitamins serve not only to remove interfering substances but also for the separation of the vitamins themselves.

Vitamin B_1. Vitamin B_1 can be quantitatively determined in ultra-violet light after oxidation to thiochrome in an alkaline medium. When vitamin B_1 is to be detected in biological material, substances may be co-extracted which interfere with the determination. Their removal can be achieved with ion exchangers, since they are retained. Synthetic zeolites[74,75] or Amberlite IR-100 in the free acid- or in the Na-form[76] or cation exchangers of the carboxylic acid type in the Na-form may be used[64].

For details of the method *cf.* ref. [77]. By this method aneurine was detected in urine[78–81], blood[82] and cereals[81–83]. The determination of aneurine after separation with an ion exchanger can be done colorimetrically[84].

Vitamin B_2. Vitamin B_2 can be separated from vitamin B_1 with ion exchangers and then quantitatively determined.

Vitamin B_6. Vitamin B_6 is exchanged on a cation exchanger of the carboxylic acid type[85] and thus freed from interfering substances which are co-extracted from yeast and which render accurate determination impossible.

Nicotinamide. For the removal of substances which interfere with the exact quantitative fluorimetric estimation of nicotinamide, the extract is adjusted to pH 5·0 and treated with a cation exchanger of the carboxylic acid type in the Na-form. At this pH no nicotinamide is exchanged. Then the anionic impurities are removed with a strong basic anion exchanger in the OH-form[86].

Panthenol can be separated by means of a column of Amberlite IRA-400 in the OH-form from ascorbic acid and vitamins of the B-complex-aneurine, riboflavine, pyridoxine, nicotinic acid and panthotenic acid, and can be quantitatively determined colorimetrically in the effluent[87].

Antibiotics

Penicillin. With penicillin it is evident that the degree of cross-linking of the resin has a decisive influence on the exchange capacity for such a large molecule as penicillin[88].

Streptomycin. A streptomycin broth can be purified to such an extent by the use of ion exchangers, that a sufficiently pure streptomycin solution is obtained which gives reliable and accurate results on determination.

The broth is diluted with a 0·2M disodium phosphate solution to a strength of 20 to 50 units per ml., adjusted to pH 8·5 to 9·0 with 0·2N sodium hydroxide solution and centrifuged. The prepared solution (5 ml.) is passed through a cation exchange column in the Na-form followed by 0·5 ml. of distilled water, and then followed by wash-water at an efflux rate of 0·3 ml./minute. The streptomycin cations are adsorbed quantitatively at this pH. Immediately after the wash-water has passed through the column, 25 ml. of 0·2N hydrochloric acid is passed at 0·5 ml./minute. The first 20 ml., which contains the eluated streptomycin free from interfering substances, is used for the determination.

Streptomycin is hydrolysed to maltol by the addition of 0·2 ml. of 4N sodium hydroxide solution to 4 ml. of the corresponding acid eluate; it is heated for 6 minutes in a boiling water bath and immediately cooled to room temperature in ice. Ultra-violet absorption at 322 mμ is measured before and after heating in a 1 cm. cell of a Beckman-Spectrophotometer[89].

Aureomycin. For the quantitative determination of aureomycin in blood and urine a synthetic zeolite (Decalso) may be used, which retains it, and from which it can be dissolved at an elevated temperature with 5 per cent. sodium carbonate solution. The quantitative determination is carried out fluorimetrically on the eluate[90].

Very recently an attempt has been made to combine paper chromatography and ion exchangers by impregnating the paper with ion exchangers.

This short review shows how varied are the possible applications in analytical chemistry opened up by the use of ion exchangers. Their further development too, will take a long time to explore fully.

REFERENCES

1. Folin and Bell, *J. biol. Chem.*, 1917, **29**, 329, through Kolb, *Chem. Ztg.*, 1924, **48**, 557.
2. Bahrdt, *Z. anal. Chem.*, 1927, **70**, 109.
3. Kehren and Stommel, *Chem. Ztg.*, 1927, **51**, 913.
4. Samuelson, *Z. anal. Chem.*, 1939, **116**, 328.
5. Klement, *ibid.*, 1944, **127**, 2.
6. Klement and Dmytruk, *ibid.*, 1948, **128**, 109.
7. Samuelson, *Svensk kem. Tidskr.*, 1945, **57**, 158.
8. Lur'e Yu. Yu. and Filippova, *Zavodskaya Lab.*, 1948, **14**, 159, through ref. 41, p. 159.
9. Lur'e Yu. Yu. and Filippova, *ibid.*, 1947, **13**, 539, through ref. 41, p. 159.
10. Runeberg and Samuelson, *Svensk kem. Tidskr.*, 1945, **57**, 250.
11. Klement, *Z. anal. Chem.*, 1952, **136**, 17.
12. Klement, *Kemisk Maanedsblad, Kbn.*, 1952, **33**, 37.
13. Klement, through ref. 41, p. 159.
14. Runeberg, *Svensk kem. Tidskr.*, 1945, **57**, 114.
15. Runeberg and Samuelson, *ibid.*, 1945, **57**, 91.
16. Samuelson, *ibid.*, 1940, **52**, 241.
17. Wickbold, *Z. anal. Chem.*, 1951, **132**, 401.
18. Kressmann and Kitchener, *J. chem. Soc.*, 1949, 1190.
19. Erler, *Z. anal. Chem.*, 1949, **129**, 209.
20. Erler, *ibid.*, 1950, **131**, 106.
21. Samuelson, *Svensk kem. Tidskr.*, 1940, **52**, 115.
22. Goehring and Darge, *Z. anal. Chem.*, 1943, **125**, 180.
23. Klement, *ibid.*, 1951, **133**, 205.
24. Djurfeldt, Hansen and Samuelson, *Svensk kem. Tidskr.*, 1947, **57**, 14.
25. Rieman and Lindenbaum, *Analyt. Chem.*, 1952, **24**, 1199.
26. Wiesenberger, *Mikrochemie*, 1942, **30**, 176.
27. Klement, *Naturwissenschaften*, 1950, **37**, 211.
28. Samuelson, *Technisk Tidskr.*, 1946, No. 23.
29. Freudenberg and Weber, *Z. angew. Chem.*, 1925, **38**, 28.
30. Rappaport, *Biochem. Z.*, 1934, **251**, 432.
31. Viditz, *Mikrochim. Acta*, 1937, **1**, 326.
32. Wiesenberger, *Mikrochemie*, 1942, **30**, 241.
33. Samuelson, *Swed. Pat.*, 1946, 125, 443.
34. Gabrielson and Samuelson, *Svensk kem. Tidskr.*, 1950, **62**, 214.
35. Gabrielson and Samuelson, *Acta chem. scand.*, 1952, **6**, 738.
36. Gabrielson and Samuelson, *Svensk kem. Tidskr.*, 1952, **64**, 150.
37. Samuelson, *Elektrochem.*, 1953, **57**, 207.
38. Büchi and Furrer, *Arzneimitt.-forsch.*, 1953, **3**, 1.
39. Jindra and Pohorsky, *J. Pharm. Pharmacol.*, 1950, **2**, 361.
40. Jindra and Pohorsky, *ibid.*, 1951, **3**, 344.
41. Samuelson, *Ion Exchangers in Analytical Chemistry*, Wiley, N.Y., 1952, p. 208.
42. Cernescu, Diss., Eidg. Techn. Hochschule, Zurich, 1933.
43. Kunin, *Analyt. Chem.*, 1949, **21**, 87.
44. Mukherjee and Gupta, *J. Proc. Inst. Chemists (India)*, 1949, **21**, 83.
45. Mukherjee, Gupta and Bhattacharyya, *J. Indian chem. Soc.*, 1950, **27**, 156.
46. Wiegner and Russel, *J. Soc. chem. Ind.*, 1931, **50**, 65 T.
47. Applezweig, *J. Amer. chem. Soc.*, 1944, **66**, 1990.
48. Lassło and Webster, *J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 193.
49. Oberst, *J. Lab. Clin. Med.*, 1938, **24**, 318.
50. Tannira, Kobayashi and Tokita, *Jap. Med.*, 1948, **1**, 206.
51. Chambers, Zill and Noggle, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 461.
52. Schultz and Barthold, *Arzneimitt.-forsch.*, 1952, **2**, 532.
53. Schultz and Mayer, *ibid.*, in the press.
54. Khym and Zill, *J. Amer. chem. Soc.*, 1951, **73**, 2399.
55. Samuelson and Sjöström, through ref. 41, p. 198.
56. Isbell, *Science*, 1951, **133**, 532.
57. Jindra and Rentz, *J. Pharm. Pharmacol.*, 1952, **4**, 645.

58. Jindra and Motl, *Pharmazie*, 1953, **8**, 547.
59. Jindra and Rentz, *Československ. farm.*, 1952, **1**, 625.
60. Kunin and McGarvey, *Industr. Engng Chem.*, 1949, **41**, 1265.
61. Jindra and Motl, *Československ. farm.*, 1952, **1**, 632.
62. Schramm and Primisigh, *Z. physiol. Chem.*, 1947, **282**, 271.
63. Tiselius, Drake and Hagdahl, *Experientia*, 1947, **3**, 21.
64. Kunin and Winters, *Industr. Engng Chem.*, 1949, **41**, 460.
65. Partridge and Brimley, *Biochem. J.*, 1949, **44**, 513.
66. Partridge, Brimley and Pepper, *ibid.*, 1950, **46**, 334.
67. Partridge and Brimley, *ibid.*, 1951, **48**, 313.
68. Partridge and Brimley, *ibid.*, 1951, **49**, 153.
69. Davies, *ibid.*, 1949, **45**, 38.
70. Partridge, *ibid.*, 1949, **45**, 459.
71. Stein and Moore, *Cold Spring Harbor Symposia Quant. Biol.*, 1949, **14**, 179.
72. Stein and Moore, Abstr. of Papers, *Amer. chem. Soc.*, 118th Meeting, 1950, p. 10M.; *J. biol. Chem.*, 1951, **192**, 663.
73. Cohn and Carter, *J. Amer. chem. Soc.*, 1950, **72**, 2606, 4273.
74. Hennessy, *Industr. Engng Chem.* (Anal.), 1941, **13**, 216.
75. Hennessy and Cerecedo, *J. Amer. chem. Soc.*, 1939, **61**, 179.
76. Myers, *Industr. Engng Chem.*, 1943, **35**, 858.
77. *Methods of Vitamin Assay*, New York, 1951, p. 111.
78. Egana and Meiklejohn, *J. biol. Chem.*, 1941, **141**, 859.
79. Mason and Williams, *ibid.*, 1942, **146**, 589.
80. Papageorge and Lamar, *Arch. Biochem.*, 1947, **14**, 310.
81. Nose and Tashiro, *J. Japan. biochem. Soc.*, 1949, **21**, 130.
82. Fujita and Yamadori, *Arch. Biochem.*, 1950, **28**, 94.
83. Dawbarn, *Australian J. exp. Biol., med. Sci.*, 1949, **27**, 207.
84. Hochberg and Melnick, *J. biol. Chem.*, 1944, **156**, 53.
85. Shimizu and Shiba, *J. chem. Soc. Japan* (*pure Chem. Sect.*), 72, 442; *J. Japan Chem.*, 1951, **5**, 55.
86. Kato and Shimazu, *Science*, 1951, **144**, 12.
87. Wollish and Schmall, *Analyt. Chem.*, 1950, **22**, 1033.
88. Kunin and Myers, *Diss. Faraday Soc.*, 1949, No. 7, 114; through ref. 41, p. 234.
89. Doery and Mason, *Analyt. Chem.*, 1950, **22**, 1038.
90. Saltzman, *J. Lab. clin. Med.*, 1950, **35**, 123.
91. Samuelson, *Svensk. Papp.-Tidn.*, No. 3, 1945.
92. Wickbold, *Z. anal. Chem.*, 1951, **132**, 241.
93. Samuelson, *Svensk kem. Tidskr.*, 1939, **51**, 195.
94. Samuelson, *ibid.*, 1942, **54**, 124.
95. Samuelson and Darge, Diss. Techn. Hochschule, Stockholm, 1944.
96. Helrich and Rieman, *Analyt. Chem.*, 1947, **19**, 651.
97. Kortüm, Kotzüm-Seiler and Finkh, *Chemie*, 1945, **58**, 37.
98. Samuelson, *IVA*, 1946, **17**, 5.
99. Lagerstrom, Samuelson and Scholander, *Svensk Papp-Tidn*, 1949, **52**, 113.
100. Hems, Page and Waller, *J. Soc. chem. Ind.* (*Lond.*), 1948, **67**, 77.
101. Cannan, *J. biol. Chem.*, 1944, **152**, 401.
102. Cannan, *Ann. N.Y. Acad. Sci.*, 1946, **47**, 135.
103. Freudenberg, Walch and Molter, *Naturwissenschaften*, 1942, **30**, 87.
104. Levy and Coon, *J. biol. Chem.*, 1951, **192**, 807.
105. Cleaver, Hardy and Cassidy, *J. Amer. Chem. Soc.*, 1945, **67**, 1343.
106. Suzuki, Hagiwara and Kubota, *Japan J. pharm. Chem.*, 1951, **23**, 191.
107. Kibrick, *J. biol. Chem.*, 1944, **152**, 411.
108. Rauen, *Angew. Chem.*, 1948, **60** Q, 250.