REVIEW ARTICLE

ION EXCHANGE RESINS IN ORGANIC ANALYSIS

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THE possible applications of ion exchange resins to organic and pharmaceutical analysis are now being actively explored by a number of workers. Further developments, particularly in the study of the physical chemistry of exchanges with organic ions should lead to notable simplifications of some of the older analytical processes.

The development of synthetic ion exchange resins dates from the paper of Adams and Holmes[1] describing the properties of comparatively unstable, weak cation and anion exchangers, prepared from phenol-formaldehyde and phenylenediamine polymers respectively. These authors were able to forecast some of the future applications of the resins in chemistry. During the last decade, much effort has been spent on the design and manufacture of stable, insoluble exchange resins having only one functional group each. Accounts of the preparations and properties of these new resins are given in books by Nachod[2] and Kunin and Myers[3], and reviews dealing with their applications have been written by Schubert[4] and Kressman[5].

A number of unifunctional resins are now available commercially which provide the analyst with a new group of reagents with consistent properties. Their particular advantage in analysis is that they can be set up in columns through which flows the solution with which they react. Considerable evidence exists to show that column reactions between resin and solution can be made quantitative.

The modern resins consist of a stable hydrocarbon matrix formed by co-polymerising unsaturated materials such as styrene and divinylbenzene. The reactive, functional groups may be introduced either before or after polymerisation. The weak cation exchangers can be prepared by the former method, for example, methacrylic acid and divinylbenzene are co-polymerised to give a resin containing carboxyl groups throughout its mass[6]. The strong cation exchangers are made by the latter method, spherical beads of the styrene, divinylbenzene co-polymer are sulphonated[7]. After sulphonation the resin beads retain their insolubility but they are able to swell up in water.

If the sulphonated resin is washed with water until the washings are neutral and is then put into a sodium chloride solution, the latter rapidly becomes acid owing to an interchange of cations between the solutions and the resin. The sulphonate ions are fixed onto the resin matrix but the hydrogen ions associated with them are free to move out into the solution, if an equivalent number of sodium ions diffuse into the resin to replace them. Eventually an equilibrium is set up:—

$(RSO_3^- + H^+)$	$+ (Na^+ + Cl^-) \rightleftharpoons$	$(RSO_3^- + Na^+)$	$+ (H^+ + Cl^-)$
resin, completely ionised	solution	sodium form of resin	solution

The increase of acidity of the sodium chloride solution gives a simple method for determining the extent of exchange[8]. The exchange occurs throughout the resin particle and not simply at the resin/solution boundary.

Ion exchange equilibria of this type can be interpreted qualitatively by the law of mass action. More exact interpretation involves consideration of the non-ideal behaviour of the ions, particularly in the resin phase where they are concentrated in a "solution" of the order 5N. Also, the volume changes of the resin on going from one ionic form to another involve energy changes due to the elastic forces of the resin matrix, which can effect the equilibria, e.g., these forces will tend to oppose conversion to a form which has a large specific volume. It is probably in consequence of this swelling effect that the strong exchangers show a marked preference for divalent ions.

The reversible ion exchange between a solution and a resin can be driven to completion in one direction by using a column technique. An excess of one of the reactants, the resin (usually a 20:1 or greater excess) is put into the column and the solution is allowed to flow through it, in this way one of the reaction products is continuously removed from the resin. For example, if a potassium chloride solution flows over an excess of strong anion exchanger in the hydroxyl form the effluent from the column consists of a potassium hydroxide solution. Under suitable conditions the exchange of chloride for hydroxide ions in the solution is quantitative.

The four types of unifunctional ion exchange resins of principal analytical interest are shown in Table I.

TABLE I

RESIN TYPES

Type	Functional group	Manufacturers		
		Permutit (G.B.)	Rohm and Haas (U.S.A.)	Dow Chemical Co. (U.S.A.)
A. Strong cation exchanger ..	Sulphonic acid	Zeo Karb 225	Amberlite IR–120	Dowex 50
B. Weak cation exchanger ..	Carboxylic acid	Zeo Karb 226	Amberlite IRC–50	
C. Strong anion exchanger ..	Quaternary ammonium	De Acidite FF	Amberlite IRA–400	Dowex 1 and 2
D. Weak anion exchanger ..	Primary amine	De Acidite E	Amberlite IR–45	

Some of these resins can be obtained in the convenient form of small spherical beads. In addition to their water insolubility, they are stable in organic solvents such as ethanol and acetone and they retain an appreciable exchange capacity in these solvents though rates of exchange are usually slower than in water.

Resins of type A and B are activated by conversion to the hydrogen form and this is achieved by passing an excess of 2N hydrochloric acid over the exchanger (resin) contained in a column. The resin is then washed with water until the washings are neutral. Types C and D are

similarly activated by conversion to the hydroxyl form with 2N sodium hydroxide solution.

In addition to the nature of the functional group of the exchanger, three other properties determine its characteristics. These are (1) the capacity, i.e., the concentration of exchangeable group in the resin, usually expressed in milli-equivalents/g. of dry resin, (2) the amount of cross-linking in the resin matrix and (3) the particle size of the resin. The degree of cross-linking is determined by the proportion of divinyl-benzene used in the co-polymerisation and this can have an appreciable effect on the chemical behaviour of the exchanger. For example, Partridge, Brimley and Pepper[9] report that variations in the degree of cross-linking of a sulphonated polystyrene resin give rise to important differences in its behaviour towards organic bases, and Saunders and Srivastava[10] state that a decrease in the degree of cross-linking of a carboxylic acid resin increases its equilibrium capacity for a large organic cation such as quinine. Variation of resin particle size has a very marked effect on rates of exchange, particularly if large ions are involved. Srivastava[11] found that an 80/120 B.S.S. sieved fraction of a carboxylic acid resin absorbed more quinine in 5 minutes from solution in ethanol (50 per cent.) than did a 20/40 B.S.S. fraction in 1 hour. The equilibrium capacities for quinine were however found to be the same in both cases.

In choosing or designing a resin for a particular application all these factors have to be taken into account. A low degree of cross-linking of the resin will facilitate exchange of large ions, but it will also cause large volume changes of the resin on conversion from one form to another. Similarly, the use of a strong exchanger will give rapid rates of exchange, but it may cause hydrolysis of labile organic materials since the strong exchangers are effective acid-base catalysts. Also it is often difficult to secure quantitative elution from the strong exchangers with a reasonably small volume of liquid. The weak cation exchangers have the disadvantage for column work, that they undergo a large increase in volume on conversion in water from the relatively unionised hydrogen form to the almost completely ionised salt form. This swelling is mainly an electrostatic effect arising from the mutual repulsions of ionised carboxyl groups held close to one another by the resin matrix. It can be very much reduced by using a solvent of low dielectric constant such as ethanol.

Physico-Chemical Studies with Exchange Resins

Most of the work so far published in this field has been concerned with the exchange of inorganic ions. Detailed studies of exchange equilibria have revealed the important part which volume changes and resin cross-linking play in determining positions of equilibrium[12,13]. The kinetics of exchange of inorganic ions have been examined by a number of workers[14,15,16], a recent paper being that of Reichenberg[17].

Saunders and Srivastava[10] have studied both equilibrium and kinetics of the absorption of quinine by a carboxylic acid resin from aqueous ethanol solutions. A period of several days was required for equilibrium

to be reached. The rate of absorption, being governed by the rate of diffusion of quinine through the resin particles, was greatly increased by reducing the particle size of the resin. They also examined in detail the absorption of a number of organic bases on to the resin[18]. Both rates of absorption and equilibrium capacities were found to be governed by the dissociation constants and molecular sizes of the absorbates. Kressman and Kitchener[19] have studied ion exchange equilibria with large organic cations and a phenolsulphonate cation exchanger. They found that the affinities of some of these ions for the resin increased with increasing ionic size. Kressman[20] has also studied separations of materials on the resins based on differences of ionic size.

Hale, Packham and Pepper[21] have observed the exchange of quaternary ammonium ions on sulphonated polystyrene resins and have reported an irreversible change in the swelling properties of the resin after such exchanges.

As has been pointed out by Tompkins[22], there is a paucity of background data concerning the constants of exchange reactions which makes difficulties in the development of new procedures in which the exchangers are used. There is no doubt that the full value of these resins in organic analysis will not be realised until more physico-chemical data concerning their interactions with organic compounds have been accumulated.

Resin columns. In analytical work the quantity of resin used should be as small an amount as will give a quantitative exchange. A tube containing 5 to 10 g. of resin in a column 20 cm. high is very suitable. There should be at least a 20:1 excess of resin. The resin should be slurried into a column already filled with solvent, in order to avoid formation of air bubbles. It is useful to have a capillary siphon at the outlet of the column to avoid accidental drying out. Solutions used should be roughly of 1 per cent. concentration. To achieve quantitative exchanges operating conditions such as flow rates, resin/solution ratio and volumes of eluting and washing liquids must be carefully evaluated, after elution and washing the column should be drained of liquid and washed again.

Applications of Ion Exchange Resins to Organic Analysis

These can be classified according to the function which the resin performs with respect to the solution.

(i) Complete removal of small ions from a solution.
(ii) Replacement of either the cations or the anions in the solution by hydrogen or hydroxyl ions respectively.
(iii) Removal of a material to be estimated from the solution onto the resin, followed by washing on the column and quantitative displacement from it.

Group I

Deionisation of water. This is an application of interest to all analysts. The process of removing dissolved salts from water by allowing it to flow successively over an anion and then a cation exchange resin was

first described by Adams and Holmes[1], using weak exchangers. The quality of the water obtained has been very much improved since then by using a column containing a mixture of strong anion and strong cation exchanger. This removes all small ions, including silicate and carbonate, from the water, giving a product containing only 1 part of solids per 25 million with a specific conductivity of 10^{-7} mhos./cm. at 20° C., comparable with the best conductivity water obtained by distillation in quartz apparatus.

Originally, the mixed resin column could not be easily regenerated and had to be discarded when the resins were spent, i.e., when the product no longer had an acceptable specific conductivity. However, a method has been developed[23], suitable for large-scale operation by which the two exchangers can be separated on the column, regenerated separately with acid and alkali, washed and then mixed together again, so that the column can be used for an almost continuous supply of deionised water. The deionised water so obtained easily fulfils the specification for distilled water of the British Pharmacopœia, the main contamination to be expected, organic matter, is well within the prescribed limits.

If the product is stored under suitable conditions it provides a supply of carbonate-free water, avoiding the necessity for boiling large volumes of ordinary distilled water.

Deionisation of colloids. Colloidal ions are only slowly taken up by ion exchange resins. Janus, Kenchington and Ward[24] have used this effect to produce gelatin sols of very low salt content. A 2 per cent. gelatin sol was passed through a column containing mixed strong anion and strong cation exchangers, at 40° C. so as to lower the viscosity of the sol. The exchangers removed all small ions from the liquid leaving a sol of specific resistivity, greater than 50,000 ohm/cm. and having an ash content, based on the weight of dry gelatin in the sol, of 0·003 per cent. The *p*H of the effluent was that of the isoelectric point of the gelatin.

Thompson[25] mentions the use of a strong cation exchanger for removing amino-acids from protein sols and Partridge[26] has discussed the use of ion exchange resins as molecular sieves for separating small and large ions.

The reviewer has found that it is possible to develop a quantitative assay of glycine in a gelatin sol, using a mixed resin column. The flow rate of the solution of glycine plus gelatin can be adjusted so that a negligible amount of gelatin is absorbed while the glycine is completely removed from the solution. The concentration of glycine in the sol is measured by the difference of the titres with caustic soda in the presence of formaldehyde of the solution before and after passage through the column.

Group II

Estimation of total ionic concentrations in water or colloid sols. In this type of estimation, the water or sol is allowed to flow over a strong exchanger to remove either the small cations or the small anions. The

effluent is then titrated with alkali or acid. Egner, Erikssen and Emanuelson[27] have used this method of determining the salt content of rain-water. Polis and Reinhold[28] have employed it to determine the total concentration of cations in serum; the serum is allowed to flow over a cation exchanger in the hydrogen form to give an acidic effluent, containing the anions of the serum, chloride, bicarbonate, phosphate, proteinate in the form of their acids. The effluent is aerated with carbon dioxide-free air to remove any free carbon dioxide present and is then titrated with alkali. The titre gives an estimate of the number of equivalents of total cation present in the original serum.

Preparation of carbonate-free alkali. It has always been a difficult problem to prepare solutions of sodium and potassium hydroxide completely free from carbonate. The standard method of preparation consists of making a saturated solution of the alkali in water and allowing it to stand for some time, the carbonate which is sparingly soluble is allowed to settle out. A much simpler procedure has been devised by Davies and Nancollas[29]. This is based on the preferential uptake of carbonate ions by a strong anion exchanger. The alkali solution is made up without any particular precautions and is allowed to flow through a column of strong anion exchanger, the effluent is almost free from carbonate. This technique has been developed further by Grunbaum, Schöniger and Kirk[30]. They required a very dilute (0·001N) carbonate-free caustic potash solution with a factor which would remain constant over a considerable period. They were able to achieve this by attaching a column containing a strong anion exchanger to the filling tube of an automatic burette. When the alkali solution was required a standard 0·001N solution of potassium chloride was run through the column and the effluent consisting of 0·001N potassium hydroxide was used for the titration. Quantitative conversion of chloride to hydroxide was effected by the column and since the standard solution was stored as inert potassium chloride, the hydroxide only being prepared immediately before titration, a solution was obtained whose factor remained constant over a period of several months.

Estimation of base in alkaloidal salts. This assay has been devised by Jindra and Pohorsky[31]. The alkaloidal salt solution is run through a column of preferably strong anion exchanger, though Jindra's earlier work was carried out with weak exchangers. Under suitable conditions a quantitative exchange of hydroxyl ions for the salt anions occurs and the liquid coming out of the columns consists of a solution of the alkaloid. This can be estimated by direct titration with standard acid. Fleming and the reviewer have found this method to be very suitable for the estimation of ephedrine hydrochloride and nicotine sulphate. In the latter case the exchanger not only removes the sulphate anions, but also takes out coloured components from impure solutions giving a colourless eluate which can be estimated polarimetrically. Jindra and Rentz[32] have further extended the method for the assay of local anæsthetics. One disadvantage of this method when the final estimation is carried out by acid titration, is that any inorganic salts present in the original material

will be converted to their hydroxides and so will add to the titre. This difficulty can perhaps be overcome by ashing or otherwise oxidising the sample and if appreciable amounts of inorganic matter are present, they can be dissolved in water and run through the column, the titre for this liquid being subtracted from that obtained for the original salt. This method could also be applied to the estimation of acid in salts of organic acids, by using a strong cation exchanger. The column effluent would then consist of a solution of the organic acid which could be estimated by titration.

Group III

This type of application of ion exchange resins provides an analytical tool by means of which substances in complex mixtures such as biological fluids can be removed and purified for estimation. In some cases it may give better results than older processes, such as extractions with immiscible solvents.

Separation of plant and animal tissue extractives. Partridge[33] has outlined a systematic procedure by which the water-soluble extractives of tissues can be separated into groups, using ion exchange columns. Individual members of groups were then separated by fractional displacement from the resins on a scale large enough to permit characterisation of new components. He describes a model experiment in which 16 amino-acids were isolated in a crystalline condition from the hydrolysis product of a protein. The hydrolysate was first treated to remove unchanged protein, either by dialysis or by precipitation with ethanol, coloured material was then removed by treatment with charcoal which also removed the aromatic amino-acids. The resulting solution was passed through a cation exchange resin which took out the positively charged amino-acids. A sample of the acid effluent was titrated with alkali to give an estimate of total anions present, the remainder was passed through a weak anion exchanger which removed the negatively charged amino-acids from the solution giving a final product which contained sugars and other neutral substances. Weakly cationic amino-acids such as histidine and lysine could be displaced from the cation exchanger by ammonia while arginine was displaced with caustic soda. Negatively charged amino-acids were removed from the anion exchanger with dilute hydrochloric acid. This technique has been applied successfully to the preparation of isotope-labelled amino-acids from proteins biosynthesised by organisms fed with labelled compounds.

Analysis of nucleic acid hydrolysates. Cohn[34] has used an exchange resin column to separate the amphoteric degradation products of nucleic acid hydrolysis. These mono ribonucleotides contain both phosphate and amino-groups and Cohn worked out the *p*H at which an optimum separation of components on an anion exchanger could be expected. From this he deduced that the mixture should be run through the exchanger (finely powdered strong anion exchanger) at *p*H 6, at which all the components carry a net negative charge. By lowering the *p*H of the eluting liquid in a stepwise fashion, the nucleotides were removed one after

another. Ultra-violet spectrophotometry and paper chromatography were used to analyse each fraction. By this method he was able to achieve a quantitative analysis of the acid hydrolysates and to identify previously unknown isomers of adenylic and geranylic acids.

Estimation of streptomycin in fermentation broth. Doery, Mason and Weiss[35] have developed an ion exchange method for the quantitative assay of streptomycin in broth. The broth was diluted with phosphate buffer to *p*H 9 and then clarified by centrifugation. A 5-ml. sample was then run through a column 4 mm. in diameter and 12 mm. high, of a weak cation exchanger which had been equilibrated with sodium bicarbonate. The resin retained the streptomycin cations quantitatively. It was washed with water and the streptomycin was displaced from it with 25 ml. of 0·2N hydrochloric acid. The effluent was then analysed by the standard maltol assay.

Estimation of sugars. The separation and assay of mixtures of sugars developed by Khym and Zill[36] is an example of the use of the resins with compounds which are normally non-ionic. The sugars were treated with borate solutions, with which they react to give complex negatively charged ions. The column used was charged with 200–400 mesh, strong anion exchanger, which was converted to the borate form with potassium tetraborate solution and then washed with water. The test solution, consisting of a mixture of sugars in dilute potassium borate solution, was put onto the column and elution was carried out with boric acid–borate buffer solutions. The eluate fractions were analysed for the various sugars by standard colorimetric methods and by paper chromatography. Sharp separations of fructose, galactose and glucose; mannose and fructose; ribose, arabinose and xylose; sucrose, fructose and glucose; sucrose and maltose were obtained in these borate columns.

Chambers, Zill and Noggle[37] have used similar borate columns to separate mixtures of glycosides, though in this case the separation appeared to be due to variations in the aglycone part of the glycoside molecules rather than to differences in the borate complex anion formed.

Analysis of aldehydes and ketones. Samuelson and his co-workers have also developed a specialised technique for using ion exchange resins with non-ionic organic compounds. Gabrielson and Samuelson[38] report that both aldehydes and ketones were taken out of solution by a strong anion exchanger column converted to the bisulphite form by pre-treating it with an excess of sodium bisulphite solution. By elution with water at 75° C., the ketone was removed from the column, but no aldehyde was detectable in the eluate. The aldehyde could be eluted quantitatively with an excess of sodium chloride solution.

Samuelson and Sjostrom[39] have extended this method to the estimation of sugar solutions. They found that sugars were quantitatively removed from aqueous ethanol solutions by the bisulphite form of a strong anion exchanger. They could then be fractionally eluted from the column.

Alkaloidal assay. For assaying alkaloids, particularly in coloured or otherwise impure solutions, the weak cation exchangers should be useful. To obtain fairly rapid results the exchanger should be in the form of a

fine powder and the alkaloid solution should be slightly alkaline. After absorption onto the exchanger, it is washed with solvent to remove non-basic impurities such as tannins, and then displaced with ammonia in the case of weak bases such as quinine or with hydrochloric acid if the base is stronger, for example, ephedrine. The purified alkaloid solution can then be assayed by titration, by evaporation to dryness or by polarimetry. Huyck[40] has shown the practicability of this method for ephedrine while Saunders and Srivastava have shown that quinine can be quantitatively removed from aqueous ethanolic solution by a weak cation exchanger and can be quantitatively displaced from the resin by ammonia solution.

Bjorling and Berggren[41] have used an inorganic silicate exchanger in the analysis of preparations containing tropa alkaloids. The exchanger was activated with a mixture of potassium chloride and acetic acid solutions, the solution of alkaloidal salt was flowed through the column when the alkaloid was transferred to the exchanger. It was then eluted with 0·2N hydrochloric acid and estimated spectrophotometrically.

Riboflavine determination. Fujiwara and Schimizu[42] have used a cation exchanger in the estimation of riboflavine in biological extracts, elution being carried out with pyridine–acetic acid solutions.

Estimation of methonium ions in serum. Child, working at the School of Pharmacy, has found that methonium ions can be quantitatively removed from aqueous solution or from serum by a weak cation exchange resin. In the latter case, the resin can be washed free of proteins and the methonium ion can then be displaced from the resin with hydrochloric acid. Results based on biological tests indicate that the displacement is quantitative.

Miscellaneous developments. Several groups of workers have prepared resins containing groupings other than the normal acidic or basic groups. Cassidy[43] has prepared so-called electron exchange resins containing reversible oxidation-reduction groups, by polymerising vinyl hydroquinone. Gregor, Taifer, Citarel and Becker[44] have prepared resins containing chelating groups, e.g., from *m*-phenylene diglycine, which give better possibilities of separating metal cations than the conventional resins.

Manecke[45] has suggested the use of an applied electric field across a horizontal resin exchanger column, in order to improve the sharpness of separation of ions on the column.

Application of Ion Exchange Resins in Pharmaceutical Analysis

Now that these resins are available commercially as standardised materials, comparable with other analytical reagents, it is worth considering whether they can be applied to simplify some of the standard pharmaceutical assays.

The most direct field of application would be in the estimation of salts. Alkaloidal and other organic base salts could be determined by the Jindra method and this is very much more simple and convenient than,

say, the steam distillation method recognised for estimating ephedrine in its salts[46]. Similarly, treatment of salts of organic acids with a strong cation exchanger followed either by direct titration or by extraction with organic solvent and subsequent titration would be more straightforward than the two-stage titration and extraction method used in some cases[47].

In the determination of small quantities of material in complex mixtures such as biological fluids, there is little doubt that ion exchange methods can offer a simpler and in some cases more reliable method of separation for assay than the older methods, such as solvent extraction.

Further work is required not only on direct empirical studies of optimum conditions for various assays, but also on the physico-chemical principles governing the uptake of organic materials by the resins and their subsequent displacement from them.

The resin manufacturers could assist further development of the applications of their products to organic analysis if they would supply them in the form of fine powders of mean particle diameter about 70 μ. Exchanges and absorptions with organic ions are often slow and they can be speeded considerably by reducing the particle size of the resin.

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