Displacement Chromatography on Synthetic Ion-exchange Resins

5. SEPARATION OF THE BASIC AMINO-ACIDS

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In Part 1 of this series of papers (Partridge & Westall, 1949) it was shown that, whilst the base-exchange resin Zeo Karb 215 may be used with success for the separation of the neutral and acidic amino-acids, certain difficulties arise when the separation of basic substances is attempted. These difficulties were traced to the phenolic hydroxyl groups of the resin. Resins of the sulphonated phenol-formaldehyde type are multifunctional when in equilibrium with solutions of high pH. Titration curves (Partridge & Westall, 1949, Fig. 4) show that over the range pH 1–7 the sulphonic acid groups only are dissociated, but when the pH rises from 7 to 12 the phenolic hydroxyl groups also dissociate and thus over this range the resin retains cations on both types of group.

Measurement of the boundary widths of fronts due to the retention of sodium ions from a range of buffered solutions of different pH showed that sharp boundaries were obtained over the range pH 1–7, but that under more alkaline conditions the boundaries became progressively wider (Partridge & Westall, 1949, fig. 7). This suggested that the rate of exchange between sodium and hydrogen ions was rapid under acid conditions when the sulphonic acid groups only were involved, but was much slower when phenolic hydroxyl groups also played a part.

Other experiments showed that the effect was also important when one base displaced another from the resin, and successful separations were obtained only with components of lower basic strength than ammonia; in the experiments on the fractionation of a protein hydrolysate, described in Part 3 of this series (Partridge, 1949*a*), ammonia was used as the displacement developer, in spite of the fact that this reagent was insufficiently basic to displace arginine. In practice it was found that the affinity of ammonia for the resin was not much greater than that of lysine and thus this amino-acid also was not readily displaced and could not be recovered quantitatively. The difficulty with which arginine and lysine are recovered from a column of Zeo Karb 215 either by elution with hydrochloric acid or by displacement with sodium hydroxide has been stressed by Hems, Page & Waller (1948), who were unable to obtain satisfactory recoveries of these two amino-acids in experiments with protein hydrolysates.

In order to overcome this difficulty experiments have now been carried out with samples of another type of resin which contained no phenolic hydroxyl groups. This was a resin of the sulphonated crosslinked polystyrene type. As will appear below, attempts to use a commercial sample of this resin resulted in failure, and for this reason variously modified samples of sulphonated crosslinked polystyrene resin were prepared in the laboratory.

It soon became apparent that the rate of exchange of large organic bases varied considerably with the degree of crosslinking of the resin, and sharp boundaries were only obtained on resin samples in which the structure was relatively open. However, resins of open structure suffered from the corresponding disadvantage of excessive shrinkage with increases in the ionic strength of the solution phase, and it was necessary to take special steps to minimize disturbance of the boundaries due to this cause.

EXPERIMENTAL

Preparation of sulphonated crosslinked polystyrene

The preparation of sulphonated crosslinked polystyrene (sulphonated PSX) involves two stages, first the copolymerization of styrene and divinylbenzene in bead form, and secondly sulphonation of the resulting copolymer (D'Alelio, 1942). Styrene and divinylbenzene, together with benzoyl peroxide (1% w/v) as polymerization catalyst, were shaken vigorously at room temperature with a 2% (w/v) aqueous dispersion of 'Promulsin' (a cellulosic derivative obtained from the Watford Chemical Co.). The hydrocarbon phase dispersed in the weak gel, forming spherical droplets which were sufficiently stable to allow polymerization to be effected at 80° for 18 hr. without further agitation. The product, after removal of 'Promulsin' by acid hydrolysis (2 N-H_2SO_4 at 100° for 4 hr.) followed by washing, consisted of regular spheres.

The copolymer was sulphonated by heating with excess conc. H_2SO_4 in presence of Ag_2SO_4 as catalyst at 100° for

8 hr. The product was poured into cold water and washed thoroughly by decantation. It was then treated alternately with 2 N-NaCl and 2 N-HCl (three cycles), washed with water, air-dried and sieved.

Determination of swelling and shrinkage

Sulphonated PSX is a hygroscopic swelling gel. The moisture regains of a typical sample at 32% and 75% R.H. and at saturation were 0·2, 0·6 and 1·5 g. of water per g. of dry hydrogen resin. The observed regain increases with decreasing degree of crosslinkage. The volume shrinkage observed with increase in concentration of ambient solution is equivalent to desorption and is also dependent on the degree of crosslinkage.

Regain at saturation was measured by weighing a saturated sample after removal of excess liquid water by centrifuging. A sample of the resin in the hydrogen form (0·5 g.) was placed in a weighed filter tube, and the whole immersed in water at 20° for 1 hr. (absorption of water is complete in a few min.). The filter tube was closed with a rubber cap and centrifuged at 2000 rev./min. for 24 hr. The temperature was maintained at 20° throughout by running the centrifuge in an air thermostat. After weighing, the saturated sample was dried at 105° *in vacuo* and reweighed.

Approximate values for shrinkage resulting from increase in ionic strength were obtained by a column method. Hydrogen resin (10 g.) were backwashed with distilled water in a graduated column. The volume of the column of resin was noted after allowing to settle for 1 hr. with tapping. Corresponding settled volumes were then obtained after backwashing with HCl solutions of different strengths.

Determination of titration curves

Sulphonated PSX was regenerated with 2 N-HCl and washed until the effluent was free from chloride and had a pH in the range 4–5. Samples of air-dry resin (0·5 g.) were weighed into a series of 100 ml. flasks and at the same time samples were taken for determination of moisture content. To each of the flasks were added different amounts of NaOH keeping the solid/liquid ratio approximately constant (75 ml. of solution/0·5 g. of solid). A parallel series of experiments was made in presence of NaCl as added neutral electrolyte. After shaking for 24 hr., the pH of the equilibrated solution was determined with a glass electrode, using a Cambridge Alki electrode for pH's >9. Finally, 25 ml. samples of solution were titrated for acid liberated or exess base.

The amounts of Na^+ ion retained (in mmol. per g. of dry hydrogen resin) are plotted against pH in Fig. 1.

Properties of resins used

In all, four samples of sulphonated PSX were examined, three prepared in the laboratory and one a commercial product.

The degree of crosslinkage of the samples prepared in the laboratory was systematically varied by varying the proportion of divinylbenzene copolymerized with styrene (Table 1).

The capacity of each of the three products was independent of particle size and corresponded with the value calculated for a monosulphonic acid of crosslinked polystyrene. Table 1 demonstrates the marked reduction in swelling and shrinkage which results from increased crosslinking.

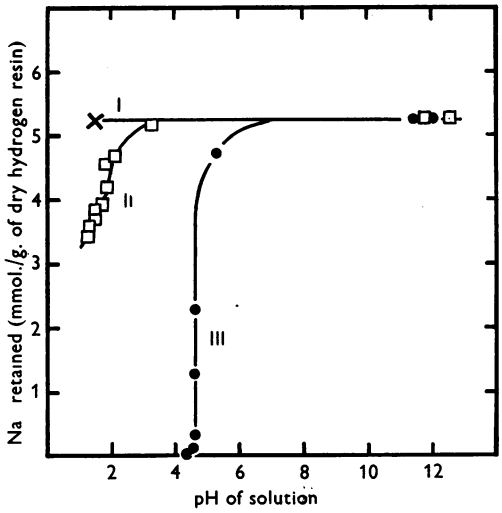

Fig. 1. The effect of pH on the Na^+ retained by sulphonated crosslinked polystyrene: I, in the presence of 5 M-NaCl; II, in the presence of 0·1 M-NaCl; III, in the absence of added salt.

The commercial sample (capacity = 5·0 mmol. per g. of dry H^+ resin) had a saturation regain of 0·75 g. of water per g. of dry resin and volume shrinkages slightly smaller than Chemical Research Laboratory sample no. 2169. It was apparently more highly crosslinked than any of the materials prepared in the laboratory.

Exploratory separation experiments suggested that the highly swelling samples offered the best prospect of success, hence sample no. 2088 was chosen for a more detailed investigation of physical properties and ion-exchange characteristics. The results obtained are summarized below.

Effect of pH *on the retention of sodium ions*

It is seen from Fig. 1 that the amount of Na^+ taken up by sulphonated PSX is independent of pH, at least for all pH values >1·5, provided there is a large excess of Na^+ in solution. The exchange reaction may be represented by

$$HR + Na^+_{solution} \rightleftharpoons NaR + H^+_{solution},$$

where HR denotes sulphonated PSX.

Table 1. *Properties of laboratory-prepared sulphonated crosslinked polystyrene*

Chemical Research Laboratory reference no.	Nominal content of divinylbenzene (%)	Capacity (mmol./g. of dry H^+ resin)	Saturation regain (g. of water/g. of dry H^+ resin)	Volume shrinkage of H^+ resin in 1 N-HCl (%)	2 N-HCl (%)	5 N-HCl (%)
2088	4	5·30	1·80	10	15	25
3168	5	5·30	1·47	5	12	20
2169	10	5·25	0·82	0	4	10

If the resin behaves as a strong acid and is fully ionized at all pH's, the amount of Na^+ taken up at equilibrium depends solely on the affinity constant and the ratio $[Na^+]/[H^+]$ in solution. Provided this latter ratio is large and no other ionizable group is present, the uptake of Na^+ must be independent of pH and equal to the total capacity. The lower values obtained at low pH's in absence of added salt or with 0·1 N-NaCl are due to the ratio $[Na^+]/[H^+]$ being < 100.

The behaviour of sulphonated PSX is entirely consistent with that expected of a unifunctional, strongly acidic, exchanger (Hale & Reichenberg, 1949).

The retention of amino-acids and bases by a sample of sulphonated polystyrene resin

The procedure adopted for the determination of ϵ (the amount of base retained by the hydrogen form of the resin expressed as mmol./g.) has already been described in detail for the absorption of bases and amino-acids by Zeo Karb 215 (Partridge & Westall, 1949) and, since the same procedure was followed in the present work, no further description need be given. The air-dried Na resin was graded by sieving in the dry state and the 80–120 mesh/in. fraction was used for packing the column.

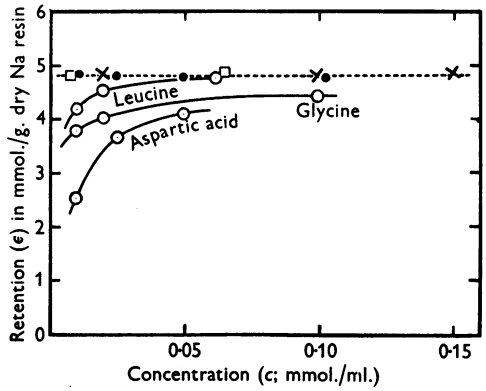

Fig. 2. The retention of a number of bases and amino-acids on sulphonated PSX resin no. 2088 (80–120 mesh/in.). Dotted line, arginine - - □ - -, ammonia - - × - -, NaOH - - ● - -.

Fig. 2 shows values of ϵ obtained for a range of amino-acids of varying isoelectric point from aspartic acid (pI, 2·77) to arginine (pI, 10·76). The curves are plotted in the conventional form as distribution isotherms and show the amount (mmol.) of solute taken up by 1 g. of resin from pure aqueous solutions of various concentrations. Since the resin was normally stored in the Na form the results are given in terms of the weight of the resin in its dry Na condition and, for convenience, this convention has been adopted throughout the remainder of the paper. The retention volumes in frontal analysis of the solutions of NH_3, NaOH and arginine were measured by titration of successive fractions of the effluent with standard acid. Aspartic acid was determined by titration with NaOH and leucine by formaldehyde titration. Electrical conductivity readings were used for the estimation of glycine.

It will be observed that the isotherms of NH_3 and NaOH are almost horizontal over the concentration range 0·01–0·15 M, showing that the retention over this range is stoicheiometric. The isotherm of arginine falls on the same straight line in the range 0·01–0·06 M. Thus, when the amino-acid is taken up from isoelectric solutions of this concentration it behaves stoicheiometrically as a univalent base. The neutral amino-acid, leucine, is taken up almost to the same extent as arginine from concentrated solution, but at dilutions below 0·05 M the retention falls off rapidly.

The shape of the titration curves of the resin (Fig. 1) suggests that aspartic acid (pI, 2·77) and glutamic acid (pI, 3·22) should be rather slightly retained from isoelectric solution, and the isotherm of aspartic acid (Fig. 2) shows this to be the case. Glycine (pI, 5·97) occupies an intermediate position between aspartic acid and leucine.

Boundary widths of fronts formed on sulphonated PSX resin by various bases

The method used for the determination of boundary widths was that given in Part 1 of this series (Partridge & Westall, 1949). Table 2 shows the results of a number of determinations with various bases and amino-acids. Resin sample no. 2088 was used for these experiments, and the determinations were carried out with a column of 9·0 mm. diameter and 92 mm. height, containing 1·7 g. (dry wt.) of the resin.

Table 2. *The width of various boundaries on sulphonated polystyrene resin columns*

(4% Divinylbenzene, 80–120 mesh/in.)

Boundary	Rate, S (cm./hr.)	Boundary width, λ (cm.)
NaOH	12·5	1·5
	3·9	0·5
Ammonia	5·3	1·0
	11·3	1·2
Arginine	1·5	1·8
	8·9	1·5
Leucine	3·2	1·5
	9·1	1·7
Glycine	9·8	0·7
	20	1·1
Aspartic acid	5·3	1·7
	10·6	1·9

The determinations refer to the widths of the boundaries of the fronts due to solutions of the bases on a column packed with the resin in its acid form, and thus the results give a qualitative indication of the readiness of displacement of H^+ by other cations. The widths measured were of the same order for all the bases and amino-acids and were generally rather lower than those observed with samples of Zeo Karb 215 of similar particle size. Since mechanical packing defects in the column frequently give rise to oblique fronts and these prevent the observation of boundaries narrower than 0·5–1·0 cm., the dependence of the boundary width (λ_{90}, as defined by Partridge & Westall, 1949) on the rate of progression of the front down the column (S) is not brought out clearly by the data in Table 1; but as in the case of Zeo Karb 215, it is clear that no real advantage is to be obtained by employing progression rates slower than 10–15 cm./hr. Variation of the concentration of the inflowing solutions over the range 0·01–0·1 M had no effect on the boundary widths observed.

Recovery of arginine and lysine from columns of sulphonated crosslinked polystyrene resin

Arginine was not readily eluted by dilute HCl and quantitative elution required large volumes of acid. When a column containing 1·7 g. of resin sample no. 2088 was saturated with arginine it was found that while 84% of the amino-acid was eluted by passage of 50 ml. of N-HCl, no less than 250 ml. was required to elute 98%. Higher concentrations of HCl were more effective and elution of 99% of the arginine held on the column was obtained by passage of 45 ml. of 3 N-HCl. However, the displacement of arginine by NaOH proceeded smoothly and quantitatively, sharp boundaries being formed between the arginine and NaOH bands. In one experiment an aqueous solution (75 ml.) of arginine monohydrochloride (1·58 g.) was allowed to pass through a column containing 1·7 g. (dry wt.) of the resin (sample no. 2088). The column was then rinsed with a small quantity of water and the arginine displaced by passage of 0·15 N-NaOH, the rate of flow being adjusted to establish the migration of the Na^+ boundary at about 10 cm./hr. The effluent was collected in 10 ml. fractions and the displacement followed by paper chromatography. The arginine in the effluent solution was then estimated by a modification of the Sakaguchi method (Devine, 1941) and showed a recovery of 99%.

No difficulty was encountered in the recovery of lysine from columns of sulphonated PSX by displacement with solutions of NaOH, provided that development was carried out without delay. However, in chromatograms carried out with the hydrolysis products of egg albumin the presence of a number of peptides of lysine has been observed, and it is not clear whether this was due to a synthetic reaction catalysed by the resin or to the presence in the hydrolysate of a small residue of resistant material. The presence of such peptides in similar circumstances was observed by Block (1947), and in this case the evidence appeared to point to resynthesis.

The effect, as we have observed it, may be illustrated by reference to a particular experiment in which the more basic amino-acids of a protein hydrolysate were allowed to remain on a column of sulphonated PSX at 0° for 10 days. The chromatogram obtained by displacing the mixture with 0·2 N-NaOH showed the presence of a small amount of peptide material. This was concentrated at the leading edge of the lysine band, and a filter-paper chromatogram (solvent: butanol-acetic acid mixture) showed it to consist of four distinct components (R_F: (*a*) 0·37, strong spot; (*b*) 0·33, weak spot; (*c*) 0·25, strong spot; (*d*) 0·04, weak spot). Further paper chromatograms were cut so as to isolate the spots due to the individual compounds; the cut pieces were then extracted with water and the eluates hydrolysed with 5·5 N-HCl at 100° for 30 hr. Further chromatography of the hydrolysis products showed that all the fractions contained lysine and that the two stronger spots *a* and *c* contained respectively leucine and valine in addition to lysine.

The presence of such peptides has not been observed in chromatograms of mixtures of amino-acids of known purity, and it cannot be excluded that they may have been present, as such, in the hydrolysate of the protein; it may be significant that the peptides appeared to be very resistant to hydrolysis and that digestion with 5·5 N-HCl at 100° for 18 hr. was insufficient to complete the reaction.

Packing disturbances due to the shrinkage of sulphonated PSX resin

Although the fronts formed by the passage of solutions of the amino-acids through columns packed with the resin in its acid form had sharp boundaries (cf. Table 2) it was found that when mixtures of the amino-acids were placed on the columns and subsequently displaced by NaOH, only the first amino-acid boundary was sharp and the bands formed by the succeeding components overlapped one another considerably. This behaviour could only be understood in terms of disturbing effects caused by the volume changes taking place in the resin. As the first front traverses the column the resin shrinks owing to the increase in the ionic strength of the solution phase. This does not disturb the first front, but destroys the uniformity of packing in the part of the column already traversed. Subsequent fronts are then distorted by the presence of channels and gaps between the resin and the walls of the filtration tube.

It is clear that such disturbances would be reduced by the choice of an operation cycle in which changes in ionic strength were minimized. In the separation of the more basic amino-acids it is permissible to saturate the column, before use, with a solution of a base weak enough to be displaced by the amino-acids, and a considerable diminution of changes in swelling can be obtained by preliminary saturation of the column with glycine hydrochloride. Accordingly, the following operation cycle was adopted for the separation experiments described below: (1) the column was regenerated with 2 N-HCl, (2) it was then washed with 0·1 N-HCl, (3) a solution of glycine hydrochloride (0·1 M), sufficient to saturate the column, was then applied, (4) the mixture of basic amino-acids was applied at 0·15–0·2 M-concentration, (5) displacement was carried out with 0·15–0·2 M-NaOH.

A further method by which an improvement in the separations was obtained was by dividing the column into two portions; a long upper part and a short lower part. The disturbance in a short column by changes in the volume of the resin particles is less serious than that in a long column, for in the latter case part of the weight of the column packing is supported by the sides of the filtration tube, and such a column does not resettle so readily under gravity. A short column, placed in position below the main long column, acts as a corrector and increases the sharpness of the boundaries as they pass through it.

Both these expedients appeared to improve the performance of narrow laboratory columns in separating mixtures of the more basic amino-acids and, in the next section, examples are given of separations carried out by these means.

Separation of mixtures of the more basic amino-acids

The object of these experiments was to make a direct comparison of the performance of the various resin samples under as nearly as possible identical conditions. Sample 2169 and the commercial sample did not swell excessively, but nevertheless the same precautions to reduce disturbances due to swelling were taken with these samples as with resins of lower degree of crosslinking. Therefore the columns were made in two sections, and were saturated with glycine hydrochloride as described above.

The construction of the columns was generally similar to that described by Partridge & Westall (1949). Since the

volume occupied by a given weight of resin when packed wet into a column varied to a marked degree with the cross-linking and was different for each of the four resin samples, it was not possible to fix both the dry weight of resin and the height occupied in filtration tubes of the same bore. For the purpose of these experiments the height of the packed column was fixed, and the amount of resin taken was chosen to fill the columns to a fixed height when in equilibrium with 0·1 N-HCl. The main column was in each case 27 cm. high, packed in a glass tube of 11 mm. diameter; and the height of the lower column was 2·5 cm. (diameter 13·5 mm.). Both columns were provided with glass floats to prevent disturbance to the top surface of the resin by the impact of the drops of solution flowing into the tubes. The weight of resin (dry wt., Na form) packed into the two filtration tubes in each case was as follows: sample no. 2088, 8·5 g.; sample no. 2168, 10·6 g.; sample no. 2169, 16·2 g.; commercial sample, 20·6 g.

The columns were regenerated and saturated with 0·1 M-glycine hydrochloride solution as described in the preceding section. The test solution contained L-leucine, 0·784 g.; L-histidine monohydrochloride, 1·148 g.; L-lysine dihydrochloride, 1·315 g.; L-arginine monohydrochloride, 1·263 g. dissolved in water (100 ml.). This was allowed to flow through the column at a rate of 40 ml./hr. Displacement development was carried out with 0·15 N-NaOH, the solution being applied at such a rate as to allow the complete development of the column in 6 hr. (rate of progression of the Na^+ boundary, 5 cm./hr.).

The effluent solution was collected in fractions of equal volume (10 ml.) and a drop from each fraction taken for the preparation of filter-paper chromatograms. The results of the fractionations are shown in Fig. 3.

Fig. 3 (*a*) shows the analyses of the effluent obtained with the most highly swelling resin (no. 2088). Calculation of the boundary widths between the various amino-acid bands show these to be approx. 1 cm. for the two boundaries of the lysine band and approx. 2 cm. for the boundaries of the leucine band. Thus the separation was as sharp as could be expected with a column of over 1 cm. in diameter and it is clear that, with boundaries as sharp as this, excellent recoveries could be obtained by increasing the length of the column and increasing the amount of the amino-acid mixture applied to it.

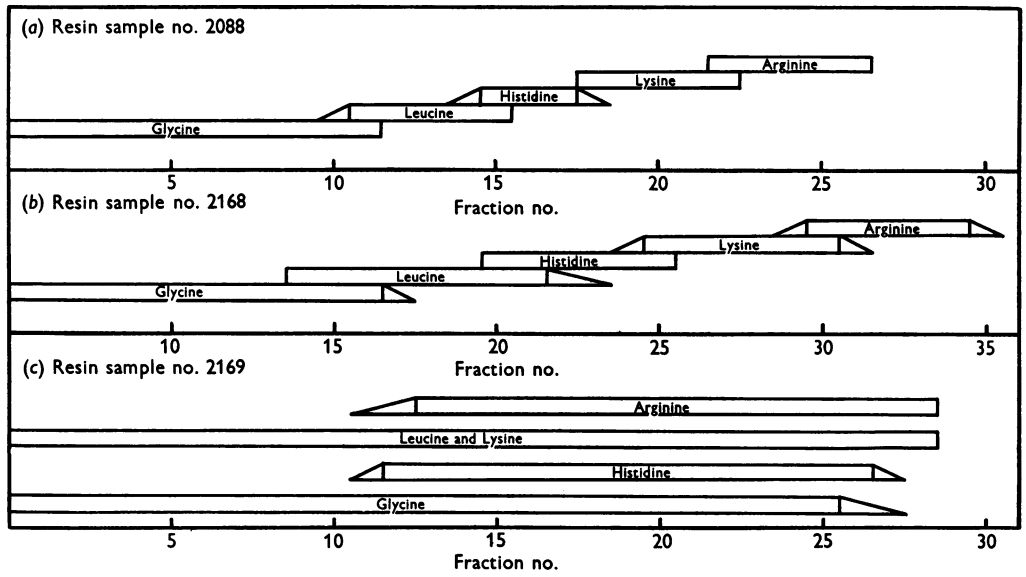

Fig. 3. Chromatogram prepared by displacing with NaOH mixtures of leucine, histidine, lysine, and arginine from columns packed with various samples of sulphonated crosslinked polystyrene resin (40–70 mesh/in.). The columns were treated with 0·1 M-glycine hydrochloride before application of the amino-acid mixture.

Fig. 3 (*b*) shows the chromatogram obtained with resin no. 2168. In this case the boundaries were not as sharp (boundary widths, 3·6–1·8 cm.), but a useful separation was obtained. Boundary widths of the order shown by this resin can well be tolerated with large columns (of 1 in. or more in diameter), and since the sample suffered less swelling than sample no. 2088 it is probable that its use would be preferable in work on a preparative scale.

The chromatogram obtained with sample no. 2169 is shown in Fig. 3 (*c*). Here, no useful separation was obtained, and similar results were given by experiments carried out with the commercial sample, which was even more crosslinked than no. 2169. Since the separations obtained in these and other experiments were so poor, no further work was carried out with either of these resins.

Use of sodium chloride solution as displacement developer

In Part 4 of this series (Partridge, 1949*b*) the effect of using NaCl as the displacement developer instead of a free base was described. Using Zeo Karb 215, a rather remarkable change in the order of displacement was observed, and use was made of the effect to secure the recovery of glucosamine and histidine in a pure condition. The following was the order of displacement of a series of bases from a Zeo

Karb column by dilute NH_3 solution: leucine, histidine, glucosamine, lysine, NH_3. With 0·05–0·10 M-NaCl as displacement developer the order was: glucosamine, Na^+, leucine, histidine, lysine. It was considered of interest to determine if the same reversal of order occurred with the sulphonated PSX resin, particularly as this resin is more generally suitable for use with the stronger bases.

The column was packed with 8·5 g. (dry wt.) of resin sample no. 2088 and was identical with that used in the experiment illustrated in Fig. 3 (*a*). The test solution (100 ml.) contained D-glucosamine hydrochloride, 0·634 g.; L-leucine, 0·392 g.; L-histidine monohydrochloride, 0·567 g.; L-lysine dihydrochloride, 0·637 g. After regeneration, the column was washed with 0·05 N-HCl. Preliminary saturation of the column with glycine hydrochloride was unnecessary since the ionic strength of the solution was maintained by the presence of HCl derived from the base hydrochlorides and from the NaCl solution used as displacement developer. After passage of the test solution, 0·05 M-NaCl (400 ml.) was applied at a rate of 200 ml./hr.; the solution was then replaced by a stronger solution of NaCl (0·10 M, 400 ml.) and the rate slowed to about 150 ml./hr. In order to displace histidine and lysine, the NaCl solution was then replaced by 0·15 N-NaOH which was applied at 120 ml./hr.

The resulting chromatogram is illustrated in Fig. 4 (*a*) which shows that the four components were well separated. The leading boundary of the histidine band was contaminated by a little leucine indicating that the volume of NaCl solution was not quite sufficient to complete the elution of leucine. The position of the Na^+ front is indicated by the curve in Fig. 4 (*b*) which shows that NaCl breaks through at fraction 25, immediately behind the glucosamine rear boundary. The curve shows the concentration of HCl in the effluent and was obtained by titrating successive fractions with standard NaOH solution to the end point of bromthymol blue.

DISCUSSION

The use of sulphonated crosslinked polystyrene instead of the sulphonated phenol-formaldehyde resin used in previous work has proved of particular value for the separation of the three basic amino-acids: histidine, lysine and arginine. This was to be expected since it was considered that the slow rate of exchange of strong bases by the latter type of resin was due to the intervention of phenolic hydroxyl radicals. However, not all samples of sulphonated crosslinked polystyrene resin were effective and a further point of interest has emerged from the study of resin samples prepared in the laboratory in which the degree of crosslinking varied. In exchange reactions involving organic bases of relatively high molecular volume the structure of the polymer network is an important consideration, and such reactions proved to occur more readily with the more highly swelling and less strongly crosslinked samples than with those of closer structure. From this it appears that the relationship between the molecular volume of the solute and the size of the apertures in the resin framework, through which the exchanging cations must pass in both directions, may be critical. A similar conclusion has been reached from studies of the rates of exchange of organic bases of different

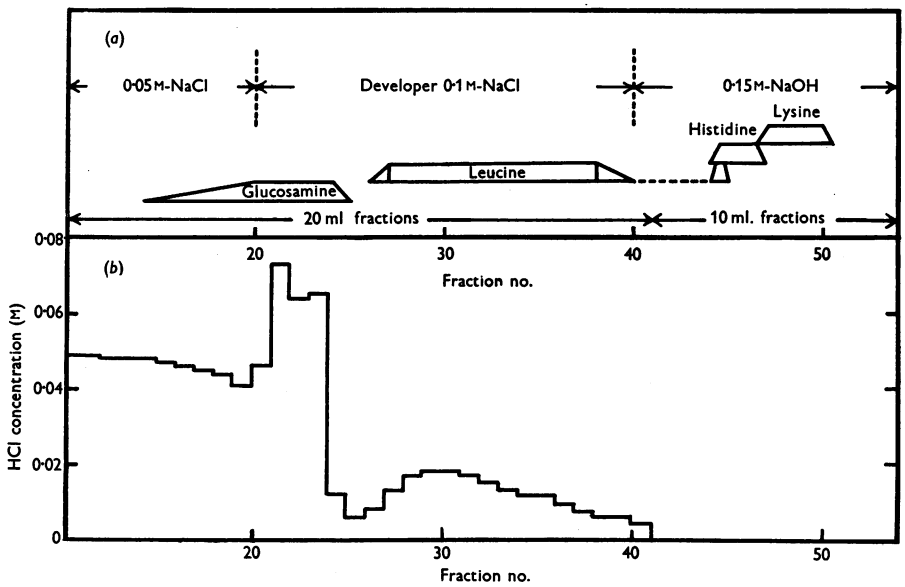

Fig. 4. (*a*) Chromatogram prepared by displacing a mixture of glucosamine, leucine, histidine and lysine from a column of sulphonated PSX resin, first with an increasing concentration of NaCl, followed by 0·15 M-NaOH. (*b*) Concentration of HCl in the effluent. Each fraction was titrated with standard NaOH to the end point of bromthymol blue.

molecular size on a single sample of resin (Kressman & Kitchener, 1949); the effect has not been described previously as a direct consequence of variations in the molecular structure of the resin.

Determination of the widths of boundaries of fronts due to various solutes in columns packed with the resins gives a qualitative picture of the overall rate of the exchange reaction involved, but accurate quantitative results cannot be obtained by this method where the exchange rates are fast and the boundaries correspondingly narrow. The main reason for this is that boundaries are invariably more or less oblique due to mechanical unevenness in the packing of the columns and thus boundary widths of less than 0·5–1·0 cm. are rarely observed in practice. Where the rate of an exchange reaction is slow, as for instance in the case of resins of large particle size, there exists the possibility of correlating the exchange rate and the boundary width more accurately.

Although the more highly swelling resin samples possess the advantage of rapid reaction, they suffer from the corresponding disadvantage of excessive shrinkage with changes in ionic strength. It was observed that disturbance due to this cause was more serious in long narrow columns than in shorter columns of wider bore, and consequently the choice of the most suitable type of resin must depend upon the scale of the experiments envisaged and upon the detailed design of the column. From experience gained in this work it appeared that resins made with 4–5 % of divinylbenzene were suitable for fractionation experiments on a small laboratory scale. However, on the basis of the data so far available, no decision can be made concerning the most suitable resin for preparative work on a larger scale. In order to place the design of larger-scale columns on a rational basis, detailed information on the swelling properties and rates of exchange for a range of resins is required. This information may be obtained more systematically and with greater certainty from the results of static experiments rather than from direct observation of columns. The detailed design of column assemblies of high efficiency must await the collection of such data.

SUMMARY

1. The use of sulphonated phenol-formaldehyde cation-exchange resins in the preparation of displacement chromatograms leads to difficulties in the separation of basic amino-acids. These difficulties are due to the phenolic hydroxyl groups of the resin and an attempt has been made to avoid them by use of a sulphonated crosslinked polystyrene resin containing no phenolic hydroxyl groups.

2. Three samples of polystyrene resin prepared in the laboratory and one commercial preparation were investigated. Variations in the degree of crosslinking produced by variations in the amount of divinylbenzene used in the preparation of the resin gave rise to important differences in the behaviour of the sulphonated product towards organic bases.

3. Two resin samples with a low degree of crosslinking gave sharp boundaries in displacement chromatograms using basic amino-acids, but suffered from the defect of excessive shrinkage with changes in the ionic strength of the solution phase. The most highly crosslinked laboratory sample and the commercial sample did not shrink excessively, but the boundaries observed in displacement chromatograms carried out under comparable conditions were very diffuse.

4. In order to minimize the disturbances of the column associated with excessive shrinkage, two expedients were adopted: (*a*) the operation cycle was adjusted to avoid large changes in the ionic strength of the solution phase; (*b*) the continuity of the column packing was broken near the base of the column.

5. Employing these expedients, mixtures of leucine, histidine, lysine and arginine were separated successfully.

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