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CHROMATOGRAPHY IN AQUEOUS SOLUTION ON STYRENE–DIVINYLBENZENE RESINS

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SUMMARY

The ability of porous styrene–divinylbenzene polymers to adsorb aromatic compounds from aqueous solutions decreases on prolonged use in aqueous media, the decrease being related to shrinkage of the resin. The capacity can be restored by treatment with aqueous ethanol. The resins are unsuitable for difficult separations in aqueous eluents but are useful for the concentration of solutes from aqueous solutions and for separations of organic solutes into groups. Aromatic acids, phenolic aldehydes and phenols are retained in 5 m*M* sulphuric acid while carbohydrates pass into the effluent. The aromatic compounds are separated in groups of increasing acid strength by displacement with Tris, sodium carbonate buffers and sodium hydroxide. Complications occur when the aromatic acids or aldehydes are strongly hydrophobic.

INTRODUCTION

Adsorption on non-polar cross-linked polymers such as macroporous styrene–divinylbenzene resins (*e.g.*, Amberlite XAD-2) has been employed for the concentration and group separation[1–4] of small amounts of organic solutes in water and aqueous solutions. The coarse resin particles are designed for industrial applications. Even with crushed particles, sorption equilibrium is established very slowly. The distribution coefficients are not reproducible and the resins are unsuitable for chromatographic separations of solutes with similar structures[5].

Resins of this type have now become available as fine beads, but their utilization in practical analyses has been little explored[6]. We report here on their application to the group separation of organic compounds in aqueous media.

EXPERIMENTAL

Jacketed columns were packed with Hitachi gel 3011 (12 ± 5 μm) soaked for 4 days in methanol, which was then replaced with ethanol (93%, w/w). The column was filled by slurry packing in 50% aqueous ethanol at 50 °C. Unless stated otherwise, this temperature was maintained throughout. The column was then conditioned by passing 5 m*M* sulphuric acid through the column at the flow-rate to be used in

subsequent experiments. Amberlite XAD-2 was crushed and sieved to obtain a particle size of 0.06–0.12 mm. The resin was then treated in the same manner as the Hitachi gel.

The aromatic solutes in the effluents were recorded by continuous measurement of the absorbance at 254 nm. The breakthrough capacity was arbitrarily chosen as the effluent volume at the moment when the effluent concentration, C, was equal to 1% of the influent concentration C_0. The effluent volume was calculated in bed volumes, the bed volume being determined after the first experiment in each series. No correction was applied for the compression of the resin bed after prolonged use of the column or for the swelling during treatment with aqueous ethanol.

In the group separations a piece of Teflon tubing which served as an injection loop was filled (0.35 ml) with the sample solution, which was then swept into the column with the eluent. Both added and separated amounts of aromatic compounds were determined spectrophotometrically at the wavelength of maximum absorption. The amounts added were determined in the eluents used in the group separation. The pH was increased to 7.5 in the solutions containing carboxylic acids, while the solutions containing phenolic compounds were acidified to pH 4.5 before analysis.

The Tris buffer of pH 6.5 used for elution of carboxylic acids contained 6.0 $g \cdot l^{-1}$ of 2-amino-2-hydroxymethyl-1,3-propanediol and 2.95 $g \cdot l^{-1}$ acetic acid. A carbonate buffer of pH 10.0 containing 1.85 $g \cdot l^{-1}$ of sodium hydrogen carbonate and 1.65 $g \cdot l^{-1}$ sodium carbonate was employed for the elution of phenolic aldehydes. In some experiments a carbonate buffer of pH 9.5 was used (3.0 $g \cdot l^{-1}$ of sodium hydrogen carbonate and 1.0 $g \cdot l^{-1}$ sodium carbonate). The eluents were boiled continuously before being introduced into the column.

RESULTS AND DISCUSSION

Shrinkage of the resin

A study of the elution curves for aromatic compounds in aqueous media on a column packed with the Hitachi gel showed that the results were irreproducible and that the efficiency decreased on prolonged use of the column. The column was therefore emptied and re-packed after conditioning the resin in 93% ethanol for 24 h. The re-packed column was reconditioned with 5 m*M* sulphuric acid (40 column volumes), which led to a 5% decrease in height.

Breakthrough curves for benzyl alcohol in 5 m*M* sulphuric acid were then recorded several times over a period of 19 days. The curves were asymmetric with a steep rise in concentration after the breakthrough point but with slow saturation of the resin (Fig. 1). This type of curve is typical for systems with convex sorption isotherms. Between the experiments 5 m*M* sulphuric acid was pumped continuously through the column. The width of the breakthrough curves increased with time and the breakthrough capacity for benzyl alcohol decreased by approximately 25% after 19 days. During the whole period (starting before the reconditioning with sulphuric acid) the height of the resin bed decreased by 11%.

The column was then emptied and the resin was slurried repeatedly in 93% ethanol for 24 h. The resin was filtered off, slurried in 50% ethanol and transferred quantitatively back into the column. The column was again conditioned with 40 column volumes of 5 m*M* sulphuric acid. The breakthrough curve for benzyl alcohol recorded immediately after conditioning with sulphuric acid showed that the capacity

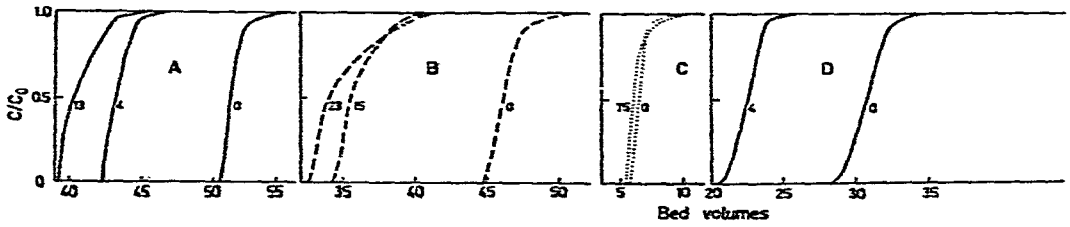

Fig. 1. Breakthrough curves at 50 °C for 1.5 m*M* benzyl alcohol in 5 m*M* sulphuric acid recorded on different days. Column dimensions: 160 × 2.5 mm. Nominal linear (empty tube) flow-rate: 10.5 cm·min^{-1}. A, Hitachi gel in aqueous solution (0.33 g of resin per 1 ml bed volume); B, reconditioned Hitachi gel in aqueous solution; C, reconditioned Hitachi gel in 5 m*M* sulphuric acid in 20% (w/w) ethanol; D, XAD-2 in aqueous solution (0.29 g of resin per 1 ml bed volume). The values on the curves refer to the number of days after conditioning with ethanol.

was restored to approximately 90% of that found in the first experiment. Sulphuric acid was again pumped continuously through the column.

Again, a broadening of the breakthrough curves and a decreased breakthrough capacity were observed with increasing time. The compression of the resin bed was virtually the same as that observed in the first series of experiments.

For comparison, experiments were carried out with Amberlite XAD-2 after conditioning in ethanol under the same conditions. The breakthrough curves were wider and of the same shape as observed for the Hitachi gel. The breakthrough capacity decreased significantly with increasing time of contact with the aqueous solution. During the whole period the values were lower than those obtained for the Hitachi gel. Almost identical results were obtained in analogous experiments with conditioning in methanol. The height of the resin bed decreased by 4% when the ethanol was displaced by 40 bed volumes of 5 m*M* sulphuric acid. An additional decrease of 8% was observed after the column had been used for 6 days.

The results show that the ability of the styrene–divinylbenzene resins to retain aromatic compounds from aqueous solution decreased with increasing time of contact with water and that the decrease is related to shrinkage of the resin particles. The results explain the observation that elution chromatography on this resin in purely aqueous eluents results in irreproducible positions of the elution peaks.

After these experiments the column with Hitachi gel was kept in 20% ethanol for almost 3 months. Breakthrough curves for benzyl alcohol in 5 m*M* sulphuric acid in 20% ethanol were then recorded (Fig. 1). Between these experiments the column was kept in 5 m*M* sulphuric acid in 20% ethanol. Before recording the breakthrough curves the solution in the column was replaced with freshly prepared solution. Fig. 1 shows that the breakthrough curves were sharper and less asymmetric than those recorded in aqueous solution. In nine determinations of the breakthrough capacity for 1.5 m*M* benzyl alcohol made within a period of 75 days the lowest value was 5.3 and the highest 5.6 column volumes. As expected, the retention of benzyl alcohol was suppressed markedly in the presence of ethanol. For comparison, experiments were carried out with 1.5 m*M* phenylacetic acid under identical conditions. For this acid the corresponding figures were 8.0 and 8.2 column volumes. No systematic change in swelling with time was observed. Evidently, no significant blockage of the adsorption sites occurred when the resin was kept in contact with 20% ethanol. The

results explain why aqueous alcoholic solutions are preferred to water as eluents in elution chromatography.

Application in group separations in aqueous media

The primary purpose of this investigation was to devise a method for group separations of (1) strongly polar compounds such as sugars and aliphatic hydroxy acids, (2) aromatic carboxylic acids, (3) phenolic aldehydes and (4) phenols. The main part of the investigation was directed towards low-molecular-mass compounds of the types present in spent cooking liquors from pulp mills and in bleach liquors after oxygen bleaching of wood pulp.

To achieve complete sorption of aromatic carboxylic acids the sample solutions were acidified so that the carboxylic acids were virtually non-ionized. Hydrochloric and sulphuric acids can be used with equally good results, but as hydrochloric acid is more corrosive and interferes with the determination of the strongly polar organic compounds by chromic acid oxidation, only experiments with solutions containing sulphuric acid (5 mmol·l^{-1}) are reported here. Experiments with 1–5 mg of glucose, arabinose, gluconic acid or galactaric acid in 5 m*M* sulphuric acid loaded on a column packed with the Hitachi gel showed that displacement with 5 m*M* sulfuric acid at 50 °C gave a sharp elution band which was recorded with a refractive index detector. The baseline was approached after approximately 1.5 bed volumes of the acid had been introduced into the column. Elution with pure water instead of dilute acid can result in the elution of carboxylic acids retained by the resin. Chromic acid oxidation of the collected eluate fraction showed that the relative error in the determination was less than ± 1.0%. Glucose and gluconic acid were also determined after separation from benzoic acid, vanillin and phenol, which were retained quantitatively by the resin. The differences between the amounts of glucose and gluconic acid added and found were 1.0% or less.

After displacement of the strongly polar non-electrolytes and acids it is convenient to elute the less polar acids as a group by increasing the pH so that the acids become ionized. In an application of Amberlite XAD-2 described by Burnham *et al.*[2] benzoic acid was eluted with 0.05 *M* sodium hydrogen carbonate solution while phenols that remained in the resin were subsequently displaced with sodium hydroxide. In this work sugars and strongly polar carboxylic acids, as already mentioned, were displaced with dilute mineral acid. To avoid disturbances due to evolution of carbon dioxide we applied a Tris buffer of pH 6.5 to elute the aromatic carboxylic acids. Another advantage is that most phenolic compounds are hardly affected in this medium while strongly acidic phenols are partially ionized in sodium hydrogen carbonate solution and move down the column at an appreciable rate. This is illustrated by the experiments with vanillin (4-hydroxy-3-methoxybenzaldehyde) referred to in Fig. 2. With Tris no traces of vanillin were found within 70 bed volumes.

Benzoic acid was eluted more rapidly with Tris buffer at 50 °C than at 30 °C (Fig. 3). The elution bands exhibited a sharp front and considerable tailing. The chromatogram indicated a recovery of 99.9% after approximately 8.5 bed volumes at 30 °C compared with 5 bed volumes at 50 °C.

Vanillic and 4-hydroxybenzoic acids, which are stronger acids, were displaced more rapidly than benzoic acid with the Tris buffer. In experiments at 50 °C with the

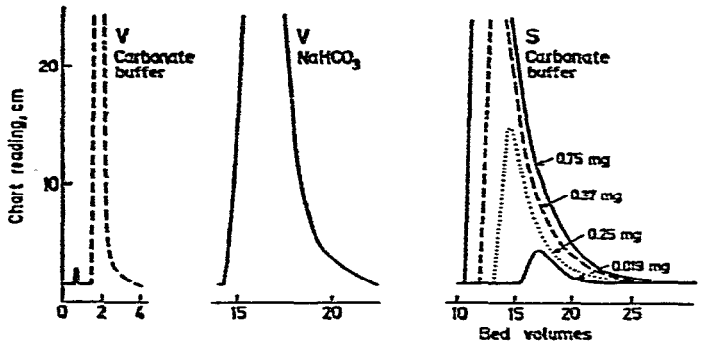

Fig. 2. Displacement of 0.35 mg of vanillin (V) with 0.05 *M* sodium hydrogen carbonate (full line) or with carbonate buffer at pH 10 (broken line) and displacement of various amounts of 2-hydroxybenzaldehyde (S) with the carbonate buffer. Column: 380 × 4.5 mm. Nominal linear flow-rate: 2.8 cm·min^{-1}. Temperature: 50 °C. The ghost peak with a maximum at 0.65 bed volume was virtually identical in both eluents.

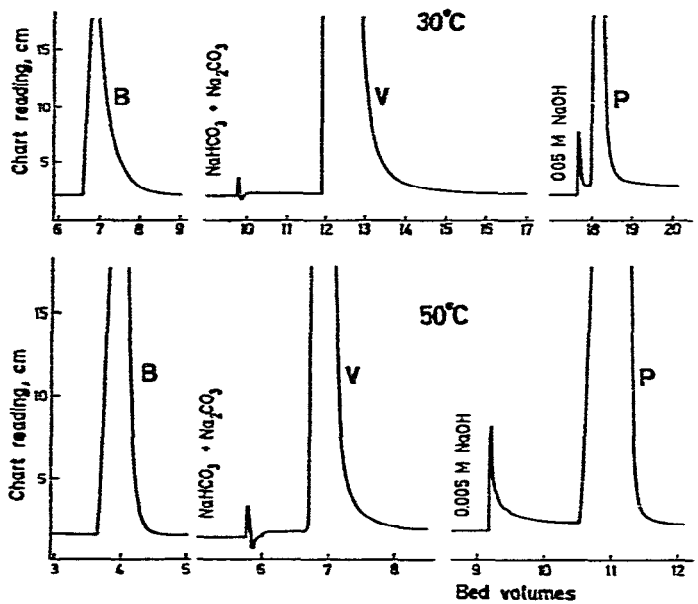

Fig. 3. Separation of 0.35 mg of benzoic acid (B) by elution with Tris, 0.35 mg of vanillin (V) by elution with carbonate buffer at pH 10 and 0.40 mg of phenol (P) by elution with sodium hydroxide. The compounds were dissolved in 0.35 ml of 5 m*M* sulphuric acid containing 4.5 mg of glucose which was washed out with 2 bed volumes of the acid before the elution with Tris. Column: 380 × 4.5 mm. Nominal linear flow-rate: 2.8 cm·min^{-1}.

addition of 0.66 mg of 4-hydroxybenzoic acid, a sharp front appeared at 0.9 bed volume while the elution was complete after approximately 3.5 bed volumes. Spectrophotometric determinations showed that the differences between the amounts added and found were less than 1.0%.

The complete elution of vanillin with a large volume of aqueous sodium hydrogen carbonate solution suggests that rapid elution of those phenolic compounds

which are fairly strong acids can be achieved in a carbonate buffer of higher pH. As shown in Fig. 2, a large amount of vanillin applied to the column was eluted completely with a carbonate buffer of pH 10 within less than 4 bed volumes. No interfering decomposition of the aldehyde was observed.

The results for 2-hydroxybenzaldehyde (salicylaldehyde) in Fig. 2 show that an increased addition led to a lower peak elution volume and that tailing occurred even when the amount added was lowered to 19 μg. Hence, the sorption isotherm is convex even at a low column loading.

A comparison between the displacement of various compounds with the aqueous carbonate buffer of pH 10 is shown in Table I. It can be seen that 4-hydroxybenzaldehyde was eluted much more rapidly than 2-hydroxybenzaldehyde. Intramolecular hydrogen bonding and the four carbon atoms in sequence lacking oxygen-containing substituents will lead to larger hydrophobic interactions for 2-hydroxybenzaldehyde than for its isomers. As expected, 3-hydroxybenzaldehyde appeared at a position between its isomers. Similarly, 3-hydroxy-4-methoxybenzaldehyde was held more strongly than vanillin. The slow elution of the hydrophobic species shows that hydrophobic interactions are important even when the phenolic groups are ionized. The elution curves indicated quantitative recovery of all aldehydes. A comparison between the amount of 3-hydroxybenzaldehyde added and that determined in the isolated fraction showed that the recovery was better than 99%.

TABLE I

POSITION OF THE BAND CONTAINING 0.70 mg OF VARIOUS AROMATIC COMPOUNDS DURING ELUTION AT 50 °C WITH AQUEOUS CARBONATE BUFFER AND WITH METHANOL MIXED WITH THE BUFFER (1:3)

Compound	*Position of elution band (bed volumes)*		
	Aqueous buffer	*Buffer mixed with methanol*	
		Compressed resin bed	*Stirred resin bed*
4-Hydroxy-3-methoxybenzaldehyde	1.2– 4.5	0.4– 3.5	0.4– 2.8
3-Hydroxy-4-methoxybenzaldehyde	9.5– 21	1.0– 6.0	1.1– 4.2
2-Hydroxybenzaldehyde	10 – 24	1.0– 6.5	1.3– 4.5
3-Hydroxybenzaldehyde	5.7– 13	0.9– 5.0	1.0– 3.6
4-Hydroxybenzaldehyde	0.9– 2.9	0.4– 2.5	0.4– 2.2
Phenol	17 – 31	3.0–10	3.5– 6.3
2-Methoxyphenol	75 –125	7.0–21	7.6–13

Experiments at 30 °C showed that the vanillin band appeared later and was much broader than at 50 °C (Fig. 3).

Phenol and 2-methoxyphenol were displaced slowly with the aqueous carbonate buffer of pH at 50 °C (Table I). At 30 °C phenol was retained much more strongly and appeared first between 56 and 71 bed volumes. Incomplete conversion to phenolate ions is the main explanation for the slow elution. It is therefore recommended that the pH of the eluent be increased further to achieve a rapid elution of phenols of these types. Aqueous sodium carbonate or sodium hydroxide solutions can be used to advantage. Fig. 3 shows that phenol is eluted as a fairly sharp band with 0.005 *M* sodium hydroxide at 50 °C. It is clear that reproducible

results can be obtained only if atmospheric carbon dioxide is excluded during the preparation and storage of the eluent. If excess of sodium hydroxide does not disturb the continued analysis of the eluted fraction it is advantageous to use stronger sodium hydroxide solution, *e.g.*, 0.05 *M*, which was the eluent used by Burnham *et al.*[2] for the elution of phenol and 2-methylphenol (*o*-cresol) from XAD-2. Fig. 3 shows that phenol was eluted as a sharp band at 30 °C. The tailing decreased when the temperature was increased to 50 °C. Spectrophotometric determinations of vanillin and phenol in the fractions eluted with the carbonate buffer and with sodium hydroxide, respectively, confirmed that the elution was quantitative. The differences between the amounts added and found were within 1.0%.

The effect of the flow-rate on the separation of benzoic acid, vanillin and 2-methoxyphenol is illustrated in Fig. 4. A decreased column efficiency with increasing flow-rate is reflected in increased tailing and decreased peak heights. The interstitial volume in columns with macroporous resins is not a well defined quantity[7] and the flow-rate in the packed part of the column therefore cannot be determined accurately. It is, evident however, that the highest nominal linear flow-rate (27.7 $cm \cdot min^{-1}$) corresponds to a flow-rate of at least 1 $cm \cdot sec^{-1}$ in the resin bed.

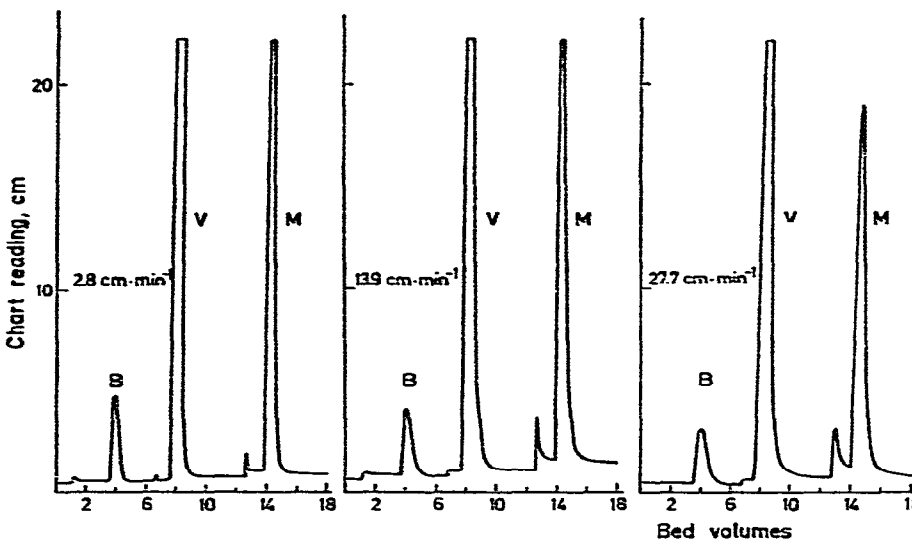

Fig. 4. Influence of the nominal linear flow-rate on the separation of 0.17 mg of benzoic acid (B) by elution with Tris (0–6 bed volumes), 0.17 mg of vanillin (V) by elution with carbonate buffer at pH 10 (6–12 bed volumes) and 0.18 mg of 2-methoxyphenol (M) by elution with 0.05 *M* sodium hydroxide. Temperature: 50 °C. Column: 380 × 4.5 mm.

At this flow-rate the pressure drop was 12 MPa compared with 1.2 MPa at the lowest flow-rate. In the experiment with a nominal linear flow-rate of 13.9 $cm \cdot min^{-1}$ the pressure drop was 4.8 MPa. The resin bed was compressed by 4.7% when the nominal linear flow-rate was increased from 2.8 to 27.7 $cm \cdot min^{-1}$.

As shown in Fig. 4, the volume of each eluent passed into the column before the solutes were first detected in the eluate was virtually unaffected by the flow-rate.

In group separations the first part of the eluate can be discarded provided that the absorbance is controlled or that only known solutes have to be determined. It is of interest that for the solutes studied the volume that can be discarded is not affected to any appreciable extent by the flow-rate. In analyses of unknown mixtures it is safest, however, to collect the whole eluate in order to prevent losses. With regard to the increased tailing, the volume that has to be collected must be increased when a high flow-rate is employed. The recorded curves indicate that at the highest flow-rate each group of compounds was recovered to the extent of 99.9% in an eluate volume of 2 bed volumes, which is within less than 3 min. At the lowest flow-rate the same recoveries were obtained within approximately 1 bed volume, which corresponds to a 5-fold increase in time. Evidently, a considerable shortening of the time for the group separation can be obtained at the expense of an increased dilution of the eluate fractions.

Difficulties in eluting some phenolic compounds with sodium hydroxide from a cross-linked non-polar resin have been reported by Junk *et al.*[3]. Hence, 18 ml of 0.05 *M* sodium hydroxide were required to elute, 3,5-dimethylphenol at room temperature from a column containing 0.2 g of Amberlite XAD-2, while, 2,3,6-trimethylphenol required approximately 25 ml. Our experiments with the Hitachi gel at 50 °C showed that no 2,3,6-trimethylphenol (0.49 mg added) appeared in the eluate when 64 bed volumes of the eluent had been introduced. Under the same conditions (Fig. 5) 3,5-dimethylphenol was displaced between 8.4 and 21 bed volumes. Hence, the position relative to that of 2,3,6-trimethylphenol differed markedly on the Hitachi gel compared with that reported for XAD-2. As expected, 2-methylphenol was displaced more rapidly than 3,5-dimethylphenol but less rapidly than phenol, while 2-methoxyphenol took a position between phenol and 2-methylphenol. The experiments confirm that in aqueous solution hydrophobic solutes can be retained very strongly by the resin even when the solutes are ionized.

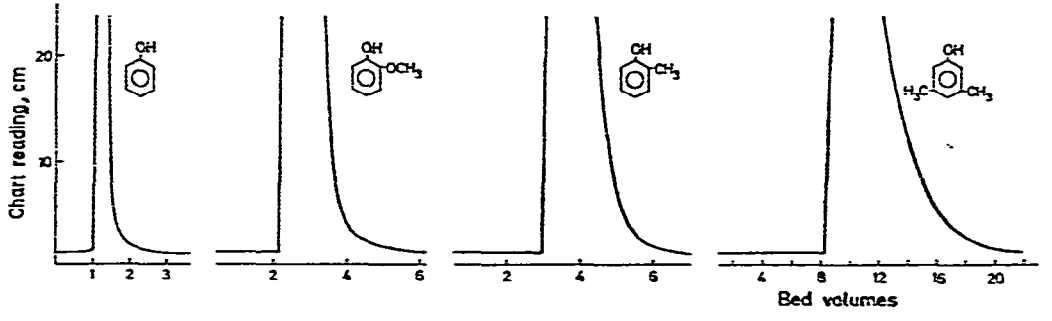

Fig. 5. Displacement of phenol, 2-methoxyphenol, 2-methylphenol and 3,5-dimethylphenol with 0.05 *M* sodium hydroxide at 50 °C. Separate experiments with 1.2 mg of each compound. Column 380 × 4.5 mm. Nominal linear flow-rate: 2.8 cm·min^{-1}.

Elution with aqueous methanol or ethanol

The results given above show that it is impractical to use aqueous sodium hydroxide for the elution of strongly hydrophobic phenols. Compounds of this type appeared much more rapidly in eluents containing methanol or ethanol. Hence, experiments with 0.05 *M* sodium hydroxide in 20% (w/w) ethanol showed that 0.49 mg of 2,3,6-trimethylphenol was eluted between 7.0 and 12 bed volumes, while the same amount of 3,5-dimethylphenol gave an elution band between 1.6 and 6 bed volumes.

When the sample to be analysed contains phenols both with and without hydrophobic substituents the compounds can be eluted together with sodium hydroxide in aqueous methanol or ethanol. In some types of analyses, *e.g.*, of samples containing large amounts of phenol and 2-methoxyphenol together with minor amounts of hydrophobic phenols, it can be advantageous to elute phenol and 2-methoxyphenol with aqueous sodium hydroxide and the more hydrophobic compounds with sodium hydroxide in either aqueous ethanol or aqueous methanol. In this way the sample solution is divided into five fractions instead of four as obtained when only aqueous eluents are employed.

When phenols that are easily oxidized by air in alkaline media are present in the solution to be analysed, the use of aqueous ethanol or methanol for the elution of the last fraction is also recommended. Most phenols are eluted with a reasonable volume of 50% ethanol even if no alkali is added. Hence, 3,5-dimethylphenol and 2,6-dimethylphenol were eluted between 2.2 and 6.8 bed volumes at 50 °C while 2,3,6-trimethylphenol appeared between 3.8 and 8.5 bed volumes.

The situation is more complex when compounds belonging to the other groups are strongly hydrophobic. Hence, aromatic carboxylic acids containing hydrophobic substituents are eluted very slowly with the aqueous Tris buffer even if the dissociation is almost complete. As an example, it can be mentioned that 35 μg of 4-chlorobenzoic acid was eluted between 19 and 27 bed volumes at 50 °C on the column referred to in Fig. 2. With a mixture of methanol and the Tris buffer in the ratio 1:3 (v/v) the same amount was eluted as a sharp band between 2.2 and 7 bed volumes. In this medium benzoic acid appeared as a sharp band between 0.8 and 4 bed volumes. As expected, the alcohol present in the eluent also suppresses the distribution coefficients of solutes which are undissociated in the Tris buffer. These solutes will therefore move down the column at a much higher rate than in the absence of alcohol. The application of organic solvents can therefore lead to complications in the analysis of the subsequent fractions. For example, vanillin (0.35 mg), which was held very strongly in the aqueous Tris buffer, was eluted between 13 and 27 bed volumes when methanol mixed with the buffer (1:3) was used as the eluent. With an increased proportion of methanol (1:2) in the eluent, vanillin appeared between 5 and 12 bed volumes. In this medium phenol added in the same amount appeared between 6 and 9 bed volumes while 2-hydroxybenzaldehyde was eluted between 32 and 50 bed volumes. Evidently, the possibilities of eluting aromatic compounds in order of decreasing acid strength disappear when a large proportion of alcohol is present in the eluent.

In agreement with the results obtained with the Tris buffer, phenolic aldehydes and phenols were eluted more rapidly with a mixture of methanol and the carbonate buffer of pH 10 than with the aqueous carbonate buffer. The results in Table I show that very large effects of methanol were obtained with the solutes that were held strongly in the aqueous buffer, while only a small effect was obtained with, for instance, vanillin. In the mixed solvent overlapping was obtained between phenol and the phenolic aldehydes under the conditions applied here. Theoretically, it should be possible to obtain a better separation by using a buffer solution of a lower pH. Experiments with 2-hydroxybenzaldehyde and phenol were therefore made with a buffer containing an increased proportion of sodium hydrogen carbonate (pH 9.5). In mixtures of methanol with this buffer (Fig. 6) 2-hydroxybenzaldehyde appeared as a band with a sharp front but some tailing. It can be seen that although large amounts were applied to the column a satisfactory separation of the compounds was achieved.

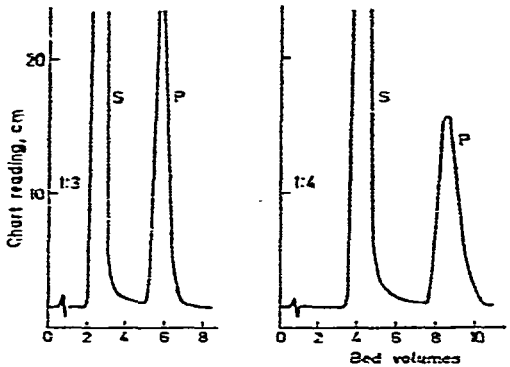

Fig. 6. Separation of 0.34 mg of 2-hydroxybenzaldehyde (S) from 0.32 mg of phenol (P) by elution at 50 °C with a mixture of methanol and a carbonate buffer (pH 9.5) in the proportions 1:3 and 1:4. Column: 380 × 4.5 mm. Nominal linear flow-rate: 7.3 cm·min^{-1}.

A decreased proportion of methanol from 1:3 to 1:4 led to a slower elution and to a broadening of the elution curves of both compounds.

As already mentioned, an increased pressure drop in the column can be observed when mixed solvents are employed. Hence, a displacement of an aqueous eluent with a mixture of water with methanol or ethanol leads to a higher counter pressure than that predicted from the change in viscosity. The observation is explained by a swelling of the resin particles. In experiments with resin beds compressed during elution with the aqueous eluents at high flow-rates it may be necessary to decrease the flow-rate when the mixed solvent is employed. Hence, when aqueous ethanol (20%) was pumped through the column referred to in Fig. 4 which had been used in aqueous media at a nominal linear flow of 27.7 cm·min^{-1} the counter pressure at a given flow-rate was much higher than was obtained in water. In this instance it was necessary to decrease the linear flow-rate to 10 cm·min^{-1} to maintain a pressure drop of 12 MPa. When switching over to aqueous eluents again the counter pressure decreased significantly although the bed volume decreased further. After the column had been used for about 1 month with aqueous eluents and eluents containing 20% methanol or 20% ethanol alternately, the pressure drop in the mixed eluents was 12 MPa at a linear flow-rate of 7 cm·min^{-1}, while in aqueous solution the linear flow-rate could be increased to about 12 cm·min^{-1} without this pressure being exceeded. To avoid an excessively high counter pressure when working alternately with different eluents, re-packing of the column might be necessary. In most group separations columns of moderate length are used. Stirring with a stainless-steel rod can therefore be used instead.

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