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STATIONARY PHASE EFFECTS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY OF ACIDS AND ION PAIRS

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SUMMARY

Reversed-phase partition chromatography has been performed with 1-pentanol as the stationary phase, applied on to the hydrophobic support by adsorption from an aqueous mobile phase containing a low concentration of 1-pentanol (1.9–2.5%).

By ion-pair chromatography of carboxylates with tetrapropylammonium and tetrabutylammonium as counter ions, ion pairs are retained by partition to the adsorbed layer of liquid stationary phase. Also for hydrophilic carboxylic acids, which are retained in uncharged form as the acids, the dominant retention mechanism is partition to the liquid stationary phase, while hydrophobic carboxylic acids are retained by an interaction with the hydrophobic support.

The interaction of the hydrophobic acids with the support can be regulated by varying the amount of 1-pentanol stationary phase, which is effected by changing the concentration of 1-pentanol in the mobile aqueous phase.

INTRODUCTION

Reversed-phase partition chromatographic systems have recently been introduced for the separation of hydrophilic organic acids as ion pairs with quaternary ammonium ions using aqueous solutions as mobile phases[1–3]. The principles of ion-pair distribution[4] and ion-pair chromatography[5] were applied to chromatographic systems with butyronitrile or 1-pentanol as the stationary phase coated on to hydrophobic supports by adsorption from the aqueous mobile phase.

This paper reports on a more detailed study of the procedure for coating different supports with the organic stationary phase. The aim of the study was also to investigate the influence of the support on the retention of acids and ion pairs. Acids of different hydrophobicity were used, and it was possible to distinguish between different retention mechanisms by changing the degree of coating with the stationary phase.

EXPERIMENTAL

Apparatus

The pump was an LDC Model 711-47 solvent delivery system (Milton-Roy

Minipump with pulse damper). The UV detector (LDC Model 1205 UV Monitor used at a wavelength of 254 nm) was thermostated by water (22.0°) circulating through a home-made cuvette cell holder with built-in channels.

Chemicals and reagents

1-Pentanol was of Fisher Scientific (Pittsburgh, Pa., U.S.A.) A.C.S. quality. Tetrabutylammonium (TBA) hydrogen sulphate from AB Labkemi (Göteborg, Sweden) was neutralized with sodium hydroxide prior to use. Tetrapropylammonium bromide (TPrABr) was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.).

Other substances were of analytical or reagent grade and were used without further purification.

Buffers were prepared with an ionic strength of 0.1.

Determination of batch distribution ratio

The experiments were performed according to Modin and Tilly[6] by using centrifuge tubes with equal phase volumes. Mechanical shaking for 30 min in a bath thermostated at 25.0° was used to equilibrate the phases.

The concentration of carboxylic acid was determined in both phases, or in the aqueous phase only, by UV photometry.

Chromatographic supports

The surface-modified chromatographic supports used were LiChrosorb RP-2, RP-8 and RP-18 of mean particle diameter 10 μm, obtained from E. Merck (Darmstadt, G.F.R.). According to the manufacturer, these supports are prepared from irregularly shaped, totally porous silica gels (LiChrosorb SI 60, specific pore volume 0.75 ml/g, specific surface area 500 m^2/g, for RP-2 and LiChrosorb SI 100, specific pore volume 1.0 ml/g, specific surface area 300 m^2/g, for RP-8 and RP-18) by reaction with dimethyldichlorosilane (RP-2), an octylchlorosilane (RP-8) or an octadecyldichlorosilane (RP-18). According to the manufacturer, the surface concentrations of the bonded silane, for the support batches used in this study, are 3.6, 4.75 and 5.0 μmole/m^2 for RP-2, RP-8 and RP-18, respectively, corresponding to degrees of derivatization of 43, 57 and 60%, respectively.

Columns

The column tubes (150 mm × 3.2 mm I.D.) were made of 316 stainless steel (Altex Scientific, Berkeley, Calif., U.S.A.). The volume of the empty column, both calculated and measured volumetrically, was 1.21 ml. The support was packed by a balanced density slurry technique as described previously[3].

Chromatographic technique

The chromatograph was thermostated either in an air-thermostated cabinet[3] at 24.0 ± 0.2° or by circulating water at 25.0 ± 0.1°. In the latter instance, the mobile phase reservoir was kept in a thermostated water-bath (HETO Type 02 PT 923 TC, Birkeröd, Denmark), which also thermostated the separation column by pumping the bath liquid through a jacket around the column.

Tubings that were not thermostated, and also the injector and the pump head,

were carefully insulated. The temperature of the pump head must not be above the chromatographic temperature. The ambient temperature was about 23°.

The mobile phase reservoir (mostly 200 ml in volume) should be well filled and carefully tightened if no free stationary liquid phase is present as an upper layer. When free stationary phase liquid was present in the reservoir, a tendency for the column to become overloaded with stationary phase was sometimes observed.

Preparation of the mobile phase

The mobile phase is prepared by dissolving appropriate concentrations of the buffer and the counter ion in de-ionized water. The whole, or part, of the solution is saturated with the stationary phase liquid (1-pentanol) in a separating funnel placed in the water-bath (or the cabinet). After phase separation, the solution is filtered through glass-wool (to avoid droplets of 1-pentanol) into a reservoir flask. Free 1-pentanol can be placed as an upper layer in the reservoir, but this was not done in most instances. If undersaturated mobile phases are to be used, the saturated solution is mixed with a suitable volume of non-saturated solution.

Mobile phase saturated with 1-pentanol will contain 2.5% (v/v) of dissolved 1-pentanol.

Components of the aqueous solution intended to constitute the mobile phase may be extracted into the organic stationary phase liquid when the phases are being saturated. This occurs, for example, with tetrabutylammonium, which can be extracted as an ion pair with dihydrogen phosphate, hydrogen sulphate and sulphate.

In order to avoid a decrease in the counter-ion concentration in the aqueous phase exceeding 1%, the phase volume ratio used when saturating the phases should be

$$V_{aq}/V_{org} \geq 99\, D_Q \qquad (1)$$

where D_Q is the distribution ratio, $C_{Q,org}/C_{Q,aq}$, between the organic and aqueous phase of the counter ion, Q, originally present in the aqueous phase.

The concentrations of tetrabutylammonium and tetrapropylammonium in the mobile phase are easily tested by the picrate extraction method[7].

Coating procedure

The column, filled with methanol or a methanol–water mixture, is eluted with the 1-pentanol-containing mobile phase until the retention volumes of unretarded and retarded samples become constant. When equilibrium is attained, the eluate can be set on recycling.

During the storage, the columns should be filled with 1-pentanol-saturated water.

RESULTS AND DISCUSSION

Coating with stationary phase

As demonstrated earlier[2], hydrophobic supports can be coated with an organic stationary phase by equilibration with an aqueous mobile phase containing a suitable concentration of the organic liquid.

The volume of mobile phase needed to reach equilibrium depends on the

TABLE I

COATING OF SURFACE-MODIFIED SUPPORTS WITH 1-PENTANOL

Mobile phase: aqueous solution saturated with 1-pentanol (2.5%, v/v). Temperature: 24° (air thermostat).

Support	*Flow-rate (ml/min)*	*Volume of mobile phase needed for saturation (ml)*	*1-Pentanol adsorbed (ml)*
RP-2	1.0	17–20	0.29
RP-8	0.8	130	0.35
RP-18	0.8	100	0.28

properties of the support. Results obtained on surface-modified supports of different hydrophobic character (LiChrosorb RP-2, RP-8 and RP-18) are given in Table I. The packings were freshly prepared and the mobile phase was saturated with 1-pentanol.

The speed of coating was 5–6 times higher on RP-2 than on the other supports, owing to a more efficient uptake of 1-pentanol. RP-2 takes up 60–70% of the amount of 1-pentanol passed through the column until saturation, while RP-8 and RP-18 only take up about 10%.

In all instances saturation is obtained within 2 h at a flow-rate of 1 ml/min.

Amount of stationary phase

The volume of 1-pentanol in the stationary phase on the column, V_{PeOH}, was determined by gas chromatographic (GC) analysis[2]. The content of water in the stationary phase was not determined, but it was assumed that the concentration of water in the stationary 1-pentanol phase is equal to the solubility of water in 1-pentanol, *i.e.*, 6.2% (v/v)[8]. The volume of stationary phase, V_s, was thus obtained by multiplying the volume of stationary 1-pentanol by 1.07.

The volume of the mobile phase, V_m, was obtained by injecting dichromate with $k' = 0$.

The volumes of stationary and mobile phase found on columns of the three different supports are given in Table II, which also gives porosity values, ε_m, calculated by

$$\varepsilon_m = V_m/V_0 \tag{2}$$

where V_0 is the volume of the empty column. Another porosity value, ε, which is constant for a given column packing, is obtained from the measured V_s and V_m by

$$\varepsilon = (V_s + V_m)/V_0 = \varepsilon_s + \varepsilon_m \tag{3}$$

ε_s will be the fraction of the column volume that is occupied by the stationary phase liquid, which fills the pores of the packing material.

When ε is known, the phase volume ratio, V_s/V_m, can be calculated for any value of V_m according to

$$V_s/V_m = (\varepsilon/\varepsilon_m) - 1 \tag{4}$$

TABLE II

COLUMNS COATED WITH 1-PENTANOL
Conditions: see Table I.

Support		V_{PeOH} *(ml)*	V_s *per gram of support** *(ml/g)*	V_m *(ml)*	V_s/V_m*	ε_m	ε
Type	*Amount*** *(g)*						
RP-2	0.56	0.30	0.57	0.60	0.53	0.50	0.76
RP-2	0.56	0.27	0.52	0.66	0.44	0.55	0.78
RP-2	0.56	0.29	0.55	0.67	0.46	0.55	0.81
RP-2	0.56	0.29	0.55	0.61	0.51	0.50	0.76
RP-8	0.55	0.35	0.67	0.58	0.65	0.48	0.79
RP-8	0.55	0.35	0.67	0.59	0.63	0.49	0.80
RP-18	—	0.28	—	0.56	0.54	0.46	0.71

* $V_s = V_{PeOH} \cdot 1.07$.
** Mean values.

ε_m will be dependent on the amount of stationary phase (see eqn. 3) and is thought to approach a minimum value of *ca.* 0.4 (equal to the inter-particle porosity) when the pores are completely filled with stationary liquid phase[9,10], *i.e.*, a maximum value of ε_s is reached.

Table II shows that the highest loading with stationary phase and the highest phase volume ratio were obtained on the RP-8 columns.

Further illustrations of the result of coating with a stationary phase can be obtained by calculations of the degree of filling of the pores of the support with the stationary 1-pentanol phase.

The specific pore volume and surface area of the modified supports are lower than on the non-modified silica[11]. However, for the modified supports these data are lacking, although some have been published for similar materials[11], and therefore an approximate calculation of the degree of filling of the pores was made by comparing ε_s values calculated from the data in Table II by eqn. 3 with maximum ε_s values obtained from eqn. 3 by use of $\varepsilon_m = 0.4$ (the inter-particle porosity, see above). These estimations showed that the pores of the RP-2 support were filled to *ca.* 70% while those of the RP-8 and RP-18 supports were filled to *ca.* 80%.

These results were obtained by using the air thermostat. Improved thermostating by using circulating water gave slightly lower ε_m values on all supports, indicating that better filling of the pores is obtained.

It is also possible to deduce that on the RP-8 columns the stationary phase amounts to 4 moles of 1-pentanol per mole of octylsilane on the support surface. The calculation was based on an octylsilane surface concentration of 4.75 μmole/m^2.

Retention during the coating

The properties of the stationary phase during the course of the coating was studied by chromatographing retarded and unretarded samples. The retention volume, V_R, of a sample with a distribution ratio D between the stationary and the mobile phase is given by

$$V_R = V_m + V_s \cdot D \tag{5}$$

$V_m + V_s$ is constant and an increase of the volume of the stationary phase, ΔV_s, gives rise to a corresponding decrease in V_m. This gives the following expression for V_R during the coating process:

$$V_R = V_m^0 + V_s^0 \cdot D + (D - 1) \cdot \Delta V_s \quad (6)$$

where V_m^0 and V_s^0 are the values of V_m and V_s when $\Delta V_s = 0$. The change in retention volume during the coating process will depend not only on the increase in the volume of the stationary phase, but also on the magnitude of the distribution ratio.

Some examples of retention changes in a system based on ion-pair distribution are shown in Fig. 1. A methanol-filled packing was eluted with 1-pentanol-saturated mobile phase. The effect of the adsorption of 1-pentanol on the support was followed by repeated determinations of V_R of four compounds with different k' values with about a 10-min interval between sample injections. V_m was obtained on each injection by the front peak which coincided with a sample with $k' = 0$, *e.g.*, dichromate.

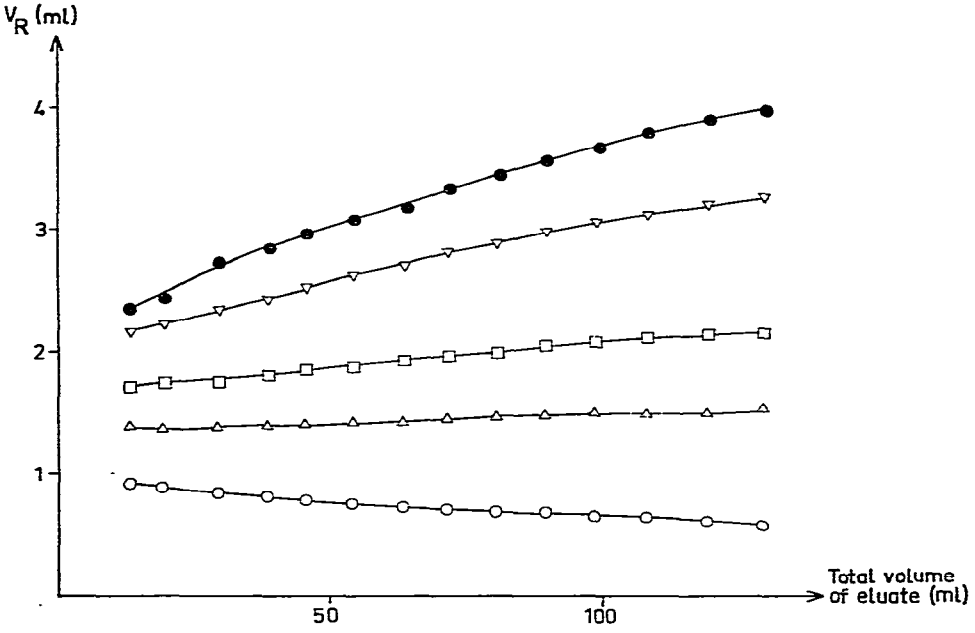

Fig. 1. Retention volume during the coating procedure. Mobile phase: tetrabutylammonium (0.03 *M*, pH 7.4) saturated with 1-pentanol (0.8 ml/min). Support: LiChrosorb RP-8. $V_s + V_m = 0.96$ ml. Samples: ○, front peak, △, 4-hydroxybenzoic acid; □, 3-hydroxybenzoic acid; ▽, benzenesulphonic acid; ●, benzoic acid.

The increase in V_R of all of the sample components indicate that their distribution ratios were larger than unity (eqn. 6).

Further insight into the coating procedure is given by Fig. 2, which shows the change in the phase volume ratio on the column as a function of the volume of the eluate. V_s/V_m was calculated from ε_m, measured during the coating, and ε, which was determined after completion of the coating, according to eqn. 4 (see Table II).

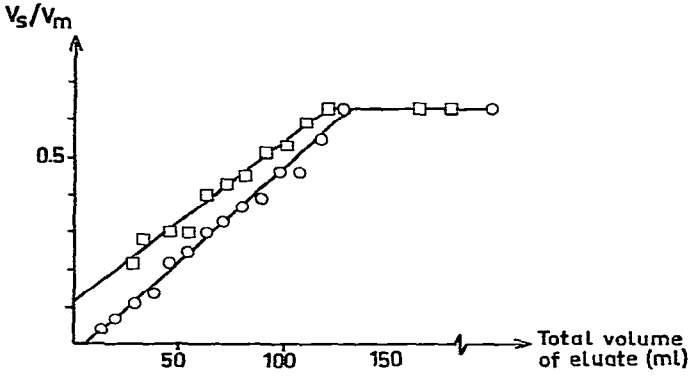

Fig. 2. Phase volume ratio during the coating. Conditions as in Fig. 1. ○, First coating; □, second coating.

Fig. 2 demonstrates the coating of a newly packed column as well as re-coating of the same support after removal of the first coating by injection of methanol.

In both instances V_s/V_m increases linearly, approaching a constant level. The second coating gives a higher intercept, probably due to incomplete removal of the stationary phase from the first coating. The final phase volume ratio is the same in both instances, which demonstrates the reproducibility of the technique.

Stability

The stability of the coating was tested by measuring V_R for three carboxylic acids and V_m during the coating procedure and the following 7 days (Fig. 3). The system was run in the recycling mode after the completion of the coating (136 ml of eluate). No significant changes in V_R and only a very slight increase in V_m were observed.

Change of 1-pentanol concentration in the mobile phase

The amount of stationary phase can be regulated by changing the concentration of 1-pentanol in the mobile phase. Table III gives the characteristics of coated columns obtained by equilibration with mobile phases with 1-pentanol concentrations corresponding to 75, 95 and 100% relative saturation of 1-pentanol. V_s was calculated from the determined ε_m and the known ε according to eqn. 3.

The coating procedure was not followed in detail, but it was observed that the final V_s/V_m was attained very rapidly at the lowest 1-pentanol concentration (75% saturation) and within 150 ml of eluate at 95% saturation.

The amount of stationary phase increases drastically with increasing 1-pentanol concentration in the mobile phase. It is obvious that the distribution ratio of 1-pentanol, D_a, between the adsorbent and the mobile phase

$$D_a = \frac{\text{moles of 1-pentanol per g of adsorbent}}{\text{moles of 1-pentanol per ml of mobile phase}} \quad (7)$$

increases with increasing 1-pentanol concentration in the mobile phase. This change in the adsorptive properties of the solid phase with increasing coating indicates that

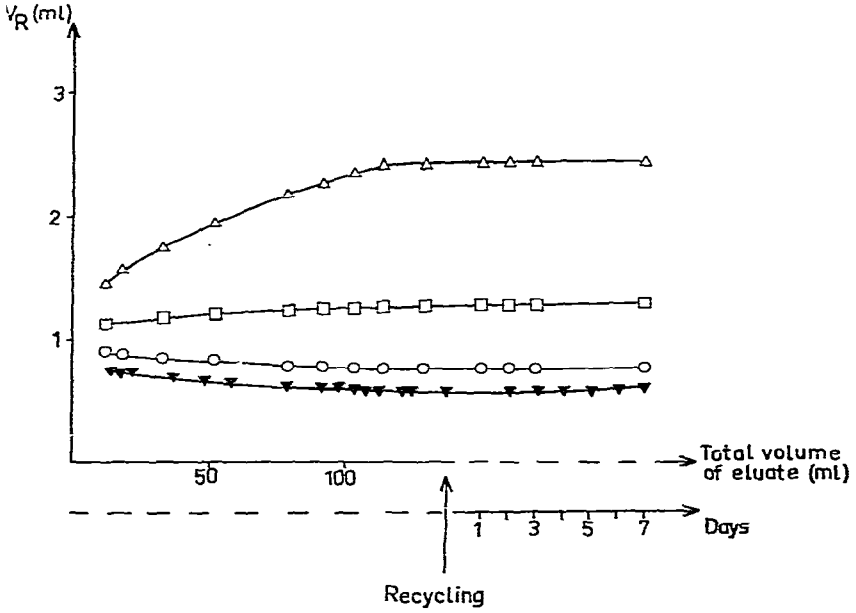

Fig. 3. Stability of the column coating. Mobile phase: phosphate buffer (pH 5.4) saturated with 1-pentanol (0.36 ml/min, no free 1-pentanol in the reservoir). Support: LiChrosorb RP-8. Samples: △, 4-hydroxybenzoic acid; □, 3-hydroxybenzoic acid; ○, 3-aminobenzoic acid; ▼, front peak.

TABLE III

STATIONARY PHASE VOLUME AT DIFFERENT 1-PENTANOL CONCENTRATIONS IN THE MOBILE PHASE

Mobile phase: phosphate buffer (pH 8.0) containing 1-pentanol. Support: LiChrosorb RP-8 (10-μm). $V_s + V_m = 0.96$ ml.

1-Pentanol in mobile phase		ε_m	V_s *(ml)*	V_s/V_m
%	*Rel. saturation (%)*			
1.9	75	0.62	0.21	0.27
2.4	95	0.55	0.30	0.46
2.5	100	0.47	0.40	0.71

its surface is completely covered and a multi-molecular layer of 1-pentanol is formed, which of course is in accordance with the results on pore filling above.

Relationship between k' and V_s/V_m

In reversed-phase partition chromatography, the capacity ratio, k', depends on the phase volume ratio according to

$$k' = (V_s/V_m)\, D \tag{8}$$

where D is the distribution ratio between the stationary and mobile liquid phases. Accordingly, the capacity ratio should increase linearly with increasing V_s/V_m.

If the retention is due to mechanisms other than distribution to the liquid stationary phase, different relationships between k' and V_s/V_m can be obtained. Approaches to the study of such effects have been discussed[12]. For a solute that also interacts with the surface of the solid support by an adsorption mechanism, the capacity ratio can be expressed by

$$k' = (V_s/V_m)\, D + (A_s/V_m)\, K_s \tag{9}$$

where A_s is the surface area available for interaction and K_s is an adsorption coefficient.

An increase in V_s can decrease the influence from the adsorption by a decrease in A_s and if the adsorption retention mechanism dominates the resulting effect will be a decrease in k' with increasing V_s/V_m.

By studying the change in the capacity ratio as a function of V_s/V_m, indications of retention mechanisms other than liquid–liquid distribution can be obtained. These data can be obtained by using different 1-pentanol concentrations in the mobile phase, which will affect V_s/V_m as discussed above (see Table III). The measurement of the capacity ratio during the continuous change in V_s/V_m which occurs in a coating procedure can also give this information.

Approximate capacity ratios can be calculated from measurements during the coating procedure, by use of the relationship

$$k'_{appr.} = (V_R - V_m)/V_m \tag{10}$$

where V_m and V_R are determined at each injection of sample. This experimental approach will not give true capacity ratios, as V_s/V_m changes during the chromatographic run. From Fig. 2 it follows that the increase in V_s/V_m is about 0.0045 per millilitre of eluate and by avoiding phase volume ratios below 0.2 and D values above 23 the error in $k'_{appr.}$ is always kept below 10% (relative). This makes it possible to distinguish between different retention mechanisms if either of them dominates in the studied range of V_s/V_m.

Hydrophilic acids, retained as acids and ion pairs. Relationships between the capacity ratio ($k'_{appr.}$) of hydrophilic acids and the phase volume ratio, measured during the coating procedure, are shown in Figs. 4 and 5.

Fig. 4 gives results obtained when the solutes were chromatographed in acidic form with phosphate buffer of suitable pH as the mobile phase. The linear relationships with intercepts close to the origin indicate that eqn. 8 is valid and that the distribution to the adsorbed 1-pentanol layer is the dominating retention mechanism.

Fig. 5 gives results from a study with the solutes retained as ion pairs with tetrabutylammonium. At the pH used, the solutes are completely unretained in acidic form. The increase in k' with increasing phase volume ratio indicates that the 1-pentanol layer serves as the stationary phase for retention of the ion pairs. No explanation was found for the positives intercepts.

The semi-logarithmic presentation of the results in Fig. 6 shows that the selectivity (*i.e.*, $\Delta \log k'_{appr.}$) is unaffected by the change in V_s, which is a further indication of the validity of eqn. 8 in retention of the ion pairs.

Hydrophobic acids, retained as acids and ion pairs. Carboxylic acids containing

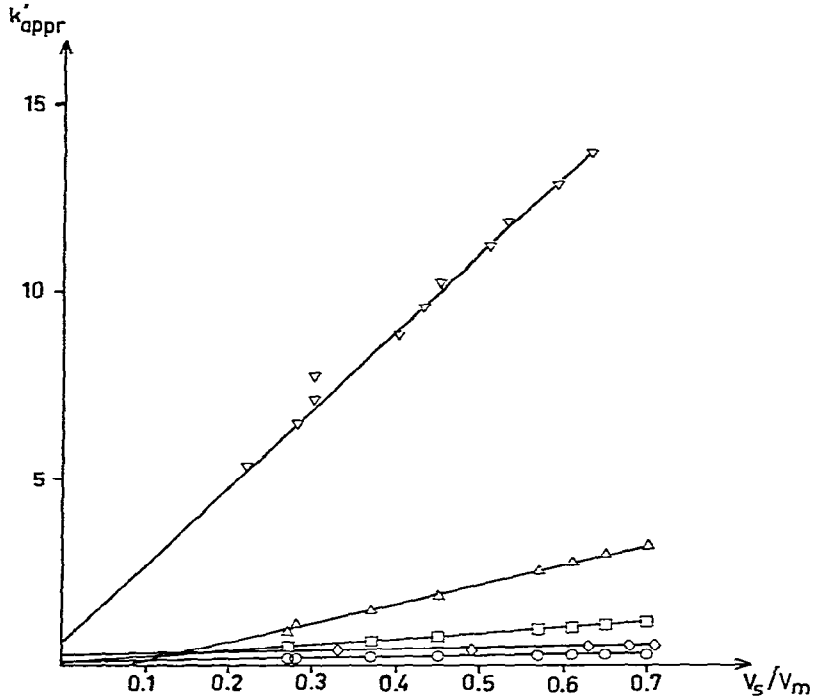

Fig. 4. Capacity ratios of hydrophilic acids during the coating procedure. Mobile phase: phosphate buffer (pH 5.44) saturated with 1-pentanol (0.36 ml/min, pH 7.44 for phenobarbital). Support: LiChrosorb RP-8. Samples: ▽, phenobarbital; △, 4-hydroxybenzoic acid; □, 3-hydroxybenzoic acid; ◇, 4-hydroxyphenylacetic acid; ○, 3-aminobenzoic acid.

naphthyl or diphenyl groups showed a decreasing retention during the course of the coating. A closer study of this effect was made by varying the amount of stationary phase using different concentrations of 1-pentanol in the mobile phase, as discussed above.

Results obtained in the acid distribution mode, using mobile phases of pH 8, are given by the symbols connected by continuous lines in Fig. 7, while the symbols connected by broken lines give results obtained with tetrapropylammonium (0.03 *M*) present in the mobile phase, in order to retain the substances as ion pairs. The capacity ratios decreased in both instances with increasing V_s/V_m, but the decrease was smaller in the presence of tetrapropylammonium.

The decrease in the capacity ratios in the acid distribution mode can be explained by the acids being adsorbed to the hydrophobic surface of the support (*cf.*, eqn. 9 and the discussion above).

The net capacity ratio of the ion pair distribution, k'_{QX}, was calculated from

$$k'_{QX} = k'_X - k'_{HX} \tag{11}$$

where k'_{HX} is the capacity ratio by retention as acid, *i.e.*, when buffer of pH 8 constitutes the mobile phase, and k'_X is the capacity ratio when tetrapropylammonium

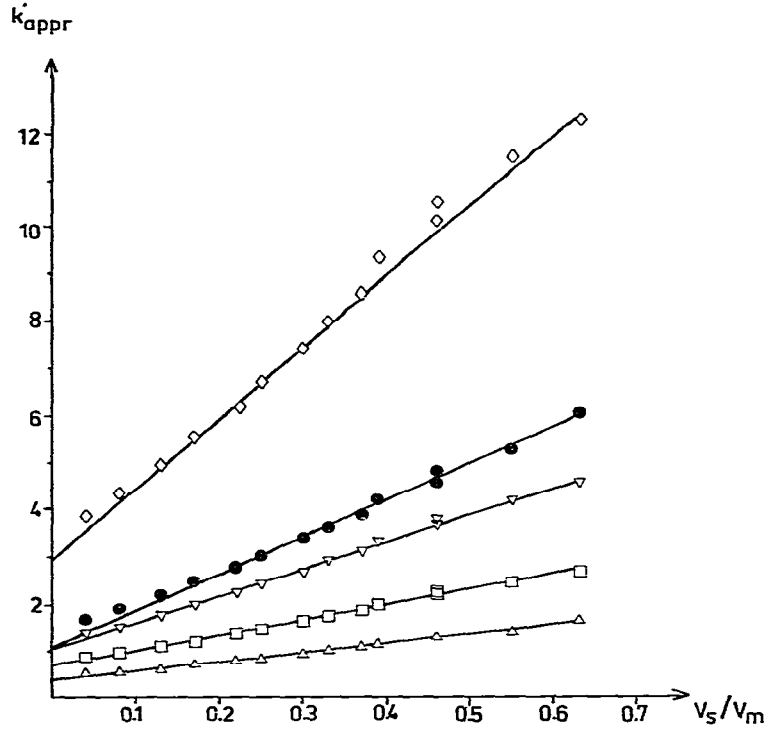

Fig. 5. Capacity ratios of ion pairs of hydrophilic acids during the coating procedure. Mobile phase: tetrabutylammonium (0.03 M, pH 7.4) saturated with 1-pentanol (0.8 ml/min). Support: LiChrosorb RP-8. Samples: ◊, toluene-4-sulphonic acid; ●, benzoic acid; ▽, benzenesulphonic acid; □, 3-hydroxybenzoic acid; △, 4-hydroxybenzoic acid.

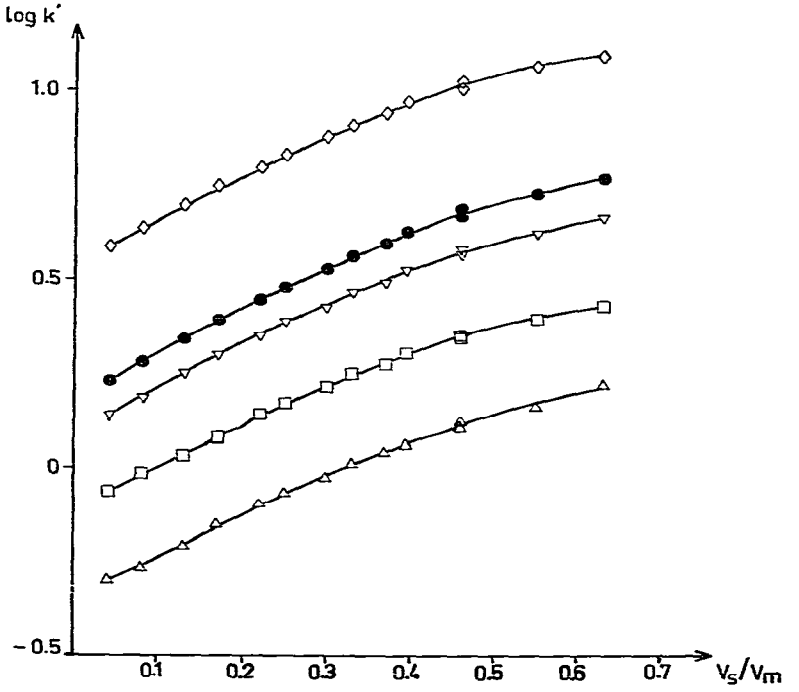

Fig. 6. Selectivity of ion pairs of hydrophilic acids during the coating procedure. Conditions and samples as in Fig. 5.

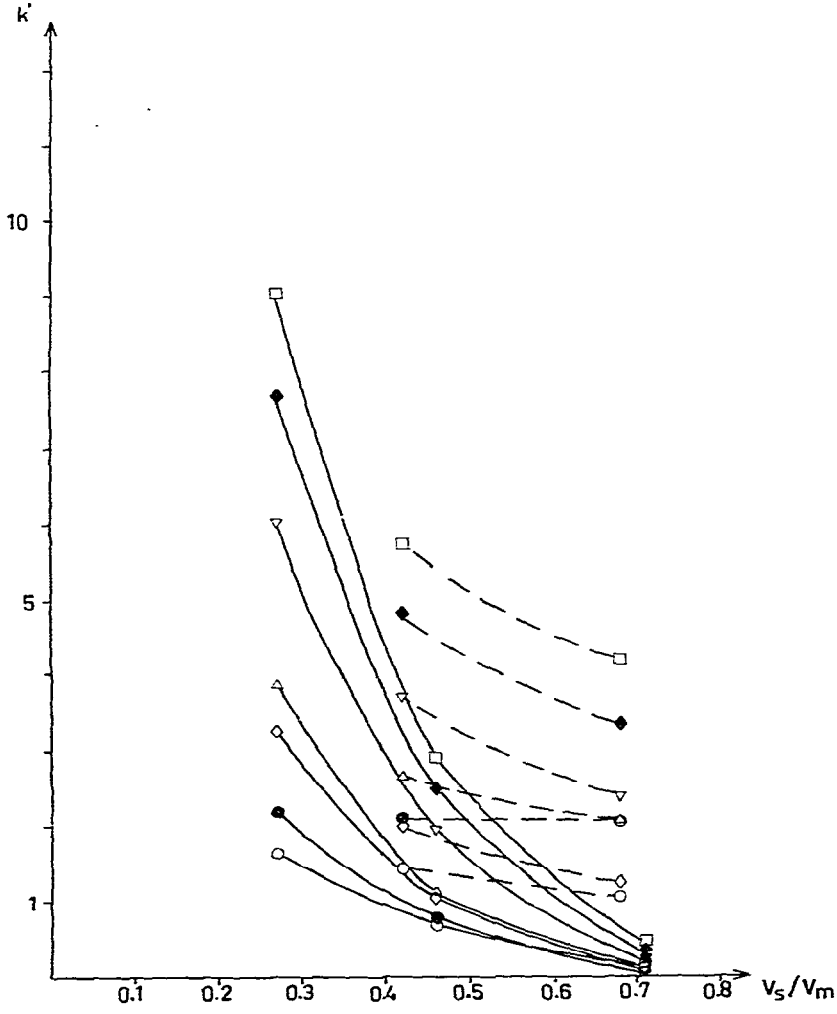

Fig. 7. Capacity ratios of hydrophobic acids in the acidic and ion-pair mode at different phase volume ratios. Mobile phase: ——— (acidic mode), pH 7.96 ($V_s/V_m = 0.27$), pH 8.00 ($V_s/V_m = 0.46$), pH 7.98 ($V_s/V_m = 0.71$); – – – (acidic and ion-pair mode), tetrapropylammonium (0.03 M), pH 7.99 ($V_s/V_m = 0.42$), pH 7.94 ($V_s/V_m = 0.68$). Stationary phase: 1-pentanol. Support: LiChrosorb RP-8. Samples: ○, 6-hydroxy-α-methyl-2-naphthylacetic acid; ●, benzilic acid; ◇, 1-naphtylacetic acid; △, diphenylacetic acid; ▽, 6-methoxy-2-naphthylacetic acid; ◆, 2-naphthoic acid; □, 6-methoxy-α-methyl-2-naphthylacetic acid.

(0.03 *M*) is present in the mobile phase at the same pH, resulting in retention of both the acid and the ion pair. As addition of tetrapropylammonium to the mobile phase caused a slight change in V_s/V_m and pH, k'_{HX} had to be corrected to the value valid when ion-pair distribution occurred. Correction for the difference in V_s/V_m was made by interpolation in Fig. 7 (solid lines). The correction for the difference in pH was made in one instance (pH 7.94) and was based on the expression for the capacity ratio of a compound that migrates as acid at pH $> pK'_{HX} + 2$ (*cf.*, ref. 13):

$$k'_{HX} = \frac{a_{H+}\, k'_K}{K'_{HX}} \tag{12}$$

where k'_K is the capacity ratio of HX when it migrates as acid at pH $<$ pK'_{HX} $-$ 2 and K'_{HX} is the apparent acid dissociation constant defined by

$$K'_{HX} = \frac{a_{H+}\,[X^-]}{[HX]} \tag{13}$$

The change in the magnitude of the capacity ratio, $\Delta \log k'_{HX}$, for a pH change, ΔpH, is then

$$\Delta \log k'_{HX} = -\Delta \mathrm{pH} \tag{14}$$

Plots of the found values for k'_{QX} *versus* the phase volume ratio are given in Fig. 8. The corrections for k'_{HX} made in the calculation of k'_{QX} amount of 25–50% and 2–10% of the final value of k'_{QX} given in Fig. 8 for the lower and the higher phase volume ratios, respectively. The increase in k'_{QX} with increasing V_s/V_m clearly indicates that the ion-pair retention is effected by the stationary 1-pentanol phase (*cf.*, eqn. 8).

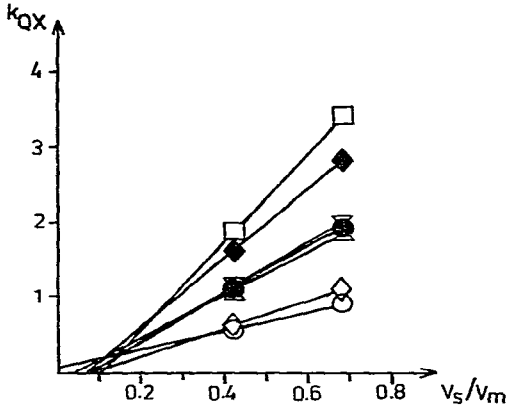

Fig. 8. Capacity ratios of hydrophobic acids in the ion-pair mode at different phase volume ratios. Conditions and samples as in Fig. 7. k'_{QX} calculated according to eqn. 6 from data in Fig. 7.

Conclusion. It can be concluded that the retention is due to two mechanisms, one involving distribution to the stationary 1-pentanol phase, which dominates for hydrophilic acids both by distribution as acids and ion pairs, and the other an interaction with the support, which is apparent for the more hydrophobic acids in acidic form but not as ion pairs.

The interaction of the support with the acidic form of the compounds decreases with increasing amount of stationary 1-pentanol phase, *i.e.*, when the thickness of the layer of stationary phase increases.

The ion pairs of the acids are not adsorbed to the support, probably owing to their polar nature, but they are partitioned to the more polar stationary phase, 1-pentanol.

Influence of the support on the extraction constant

As the stationary liquid phase is adsorbed on to a hydrophobic surface, it would be expected to have different properties from a free liquid phase and consequently different properties when adsorbed on different supports.

However, only small such effects were observed in ion-pair chromatography of hydrophilic carboxylic and sulphonic acids as seen from determinations of the conditional extraction constants, E^*_{QX}, from the column results. E^*_{QX} was determined from the measured capacity ratios by the relationship[1]

$$k'_{QX} = V_s V_m^{-1} E^*_{QX} C_Q \tag{15}$$

where C_Q is the total concentration of the counter ion, Q, in the mobile phase.

The found conditional extraction constants for RP-2, RP-8 and RP-18 as supports are plotted in Fig. 9 and a difference can be seen between those obtained on the moderately hydrophobic RP-2 (not completely derivatized with silane compound) and those on the more hydrophobic RP-8 and RP-18 supports. The lower values on the

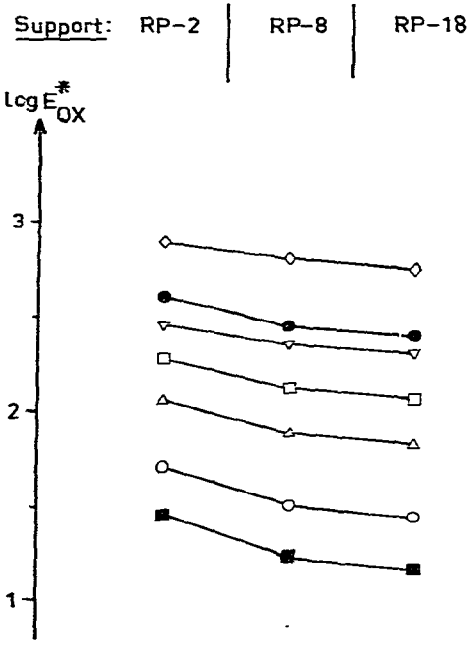

Fig. 9. Influence of the support on the extraction constant determined on the column. Stationary phase: 1-pentanol. Counter ion (Q): tetrabutylammonium. RP-2 column: $V_m = 0.61$ ml; $V_s/V_m = 0.50$; $C_Q = 0.0299$ *M* (pH 7.9). RP-8 column: $V_m = 0.56$; $V_s/V_m = 0.65$; $C_Q = 0.0289$ *M* (pH 7.4). RP-18 column: $V_m = 0.56$ ml; $V_s/V_m = 0.54$; $C_Q = 0.0346$ *M* (pH 7.4). Samples: ◇, toluene-4-sulphonic acid; ●, benzoic acid; ▽, benzenesulphonic acid; □, 3-hydroxybenzoic acid; △, 4-hydroxybenzoic acid; ○, 3-aminobenzoic acid; ■, 4-aminobenzoic acid.

more hydrophobic supports might be caused by a lower water content in the adsorbed 1-pentanol phase, as the water probably solvates ion pairs[14,15].

Distribution data in batch extractions and chromatography

A comparison of column data with batch distribution data (with the free liquid phases) obtained with 1-pentanol as organic phase is given in Table IV for 4-hydroxybenzoic acid distributed as acid. The capacity ratio calculated from the batch distribution ratio agrees well with the found capacity ratio obtained with LiChrosorb RP-2 as support. This shows that also on this support the retention of hydrophilic acids is caused only by the stationary liquid phase.

TABLE IV

COMPARISON OF COLUMN AND BATCH DISTRIBUTION OF 4-HYDROXYBENZOIC ACID IN THE ACIDIC MODE

Aqueous phase (batch and column): citrate buffer, pH 4.03. Organic phase (batch and column): 1-pentanol. Support: LiChrosorb RP-2. $V_s/V_m = 0.45$. $k'_{calc} = V_s \cdot V_m^{-1} \cdot D_{HX}$; $D_{HX} = C_{HX,org}/C_{HX,aq}$.

Method	D_{HX}	k'_{calc}	k'_{found}	k'_{found}/k'_{calc}
Batch distribution	44			
Column chromatography		20	15.3	0.8

Determination of distribution constants for drugs and other biologically active compounds between octanol and water has attracted much interest and such data have been collected by Leo *et al.*[16]. They also give regression equations by which distribution constants to many organic solvents can be calculated from the octanol data.

Table V gives calculated values of the distribution constants

$$K_{d(HX)} = \frac{[HX]_{org}}{[HX]_{aq}} \tag{16}$$

for a number of hydrophilic carboxylic acids between primary pentanols and water, obtained from distribution constants in the system octanol–water by use of the regression equation[16].

The distribution constants of a number of hydrophilic carboxylic acids obtained in the phase system 1-pentanol–water by the present reversed-phase chromatographic method are also given in Table V. They were obtained from the capacity ratios, k'_{HX}, by the expression[13]

$$\log K_{d(HX)} = \log k'_{HX} + pH + \log (a_{H^+} + K'_{HX}) - \log \frac{V_s}{V_m} \tag{17}$$

In the calculations, acid dissociation constants (thermodynamic) taken from ref. 17 were used, if necessary.

The agreement between calculated and experimentally determined constants is very good. A large deviation is obtained in only one instance, where data for the octanol–water system were lacking and the calculation had to be based on $K_{d(HX)}$ determined with another organic solvent.

TABLE V

EXPERIMENTAL AND CALCULATED DISTRIBUTION CONSTANTS

Chromatographic conditions: Mobile phase, phosphate buffer (pH 2.08) (pH 5.44 for toluic acids); stationary phase, 1-pentanol; support, LiChrosorb RP-8; phase volume ratios (V_s/V_m), 0.61 for 2-hydroxy benzoic acid, 0.60 for phenylacetic acids and 0.68 for the others. Calculated constants: valid for primary pentanols–water, obtained by a regression equation[16] from experimental values in octanol–water.

Sample	*Log* $K_{d(HX)}$		*Δlog* $K_{d(HX)}$
	By chromatography	*Calculated*	
Benzoic acid	1.96	1.78	0.18
2-Hydroxybenzoic acid	2.26	2.10	0.16
3-Hydroxybenzoic acid	1.66	1.48	0.18
4-Hydroxybenzoic acid	1.69	1.55	0.14
Phenylacetic acid	1.60	1.48*	0.12
2-Hydroxyphenylacetic acid	1.09	0.96	0.13
3-Hydroxyphenylacetic acid	1.13	0.96	0.17
4-Hydroxyphenylacetic acid	1.06	0.41**	0.65
o-Toluic acid	2.29	2.32**	−0.03
m-Toluic acid	2.39	2.19	0.20
p-Toluic acid	2.29	2.11	0.18
5-Hydroxyindole-3-acetic acid (5-HIAA)	0.87	0.87	0

* Experimental value in primary pentanols-water (batch extraction)[16].

** From experimental values in solvents other than alcohols.

The results show that the octanol–water distribution constants available can be used for the estimation of capacity ratios in the present chromatographic system, which is based on the phase system 1-pentanol–water. In most instances it will not be necessary to re-calculate the data from octanol–water to primary pentanols–water, as it involves a correction by only about 0.1–0.2 log unit. Further, distribution constants determined chromatographically in the pentanol–water system can be used to predict distribution constants in octanol–water systems.

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REFERENCES

1 K.-G. Wahlund, *J. Chromatogr.*, 115 (1975) 411.
2 K.-G. Wahlund and U. Lund, *J. Chromatogr.*, 122 (1976) 269.
3 B. Fransson, K.-G. Wahlund, I. M. Johansson and G. Schill, *J. Chromatogr.*, 125 (1976) 327.
4 G. Schill, in J. A. Marinsky and Y. Marcus (Editors), *Ion Exchange and Solvent Extraction*, Vol. 6, Marcel Dekker, New York, 1974, p. 1.
5 S. Eksborg and G. Schill, *Anal. Chem.*, 45 (1973) 2092.
6 R. Modin and A. Tilly, *Acta Pharm. Suecica*, 5 (1968) 311.

7 K. Gustavii and G. Schill, *Acta Pharm. Suecica*, 3 (1966) 241.
8 J. A. Riddick and W. B. Bunger, in A. Weissberger (Editor), *Techniques of Chemistry, Vol. II, Organic Solvents*, Wiley-Interscience, New York, 3rd ed., 1970, p. 157.
9 J. C. Giddings, *Dynamics of Chromatography*, Part I, Marcel Dekker, New York, 1965, p. 199.
10 H. Engelhardt, J. Asshauer, U. Neue and N. Weigand, *Anal. Chem.*, 46 (1974) 336.
11 K. K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
12 J. R. Conder, D. C. Locke and J. H. Purnell, *J. Phys. Chem.*, 73 (1969) 700.
13 I. M. Johansson and K.-G. Wahlund, *Acta Pharm. Suecica*, 14 (1977) 459.
14 P.-O. Lagerström, *Acta Pharm. Suecica*, 12 (1975) 215.
15 B.-A. Persson, *Acta Pharm. Suecica*, 5 (1968) 343.
16 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
17 G. Kortüm, W. Vogel and K. Andrussow, *Dissociation Constants of Organic Acids in Aqueous Solution*, Butterworths, London, 1961.