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REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF DRUGS AND RELATED ORGANIC COMPOUNDS

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SUMMARY

Procedures are given for high-performance ion-pair chromatography of organic ammonium compounds (*e.g.*, noradrenaline, dopamine, synephrine, ephedrine, zimelidine, imipramine, desipramine, amitriptyline and nortriptyline) with dihydrogen phosphate, bromide, cyclohexylsulphamate, dicyclohexylsulphamate or octylsulphate as counter ions and 1-pentanol as the liquid stationary phase on LiChrosorb-RP. With methylene chloride as the stationary phase, the highly hydrophilic noradrenaline and adrenaline can also be separated.

A long-chain alkylammonium compound must be added to the system in the chromatography of hydrophobic amines and all types of quaternary ammonium compounds to prevent peak deformation.

Both the liquid stationary phase and the support affect the selectivity.

INTRODUCTION

The determination of drugs, their metabolites and endogenous compounds in biological samples from humans and animals is an essential part of current drug research and of the development of new drug therapy. In recent years ion-pair chromatography has become an important technique in this field as it opens up possibilities for the systematic control of the separation of ionic compounds of widely different types. The use of the technique in drug research has been summarized in some recent reviews[1–3].

The demand for an intimate knowledge of the composition of biological samples has increased the interest for mild analytical techniques that give rise to a minimum of changes in the composition of the sample. One means of achieving this is to use chromatographic systems that make direct injection of the biological fluid possible. A first step in that direction was the development of ion-pair chromatographic systems with an aqueous mobile phase (reversed-phase systems) by Wahlund[4].

The fairly simple technique that can be used for the isolation of anionic compounds has been demonstrated in several papers[5–7], while recently a solution has been found to the problems that arise with hydrophobic cationic samples[8].

This paper presents a survey of systems for the separation of hydrophilic and

hydrophobic organic ammonium compounds. The control of retention and separation selectivity by the choice of type and concentration of counter ion as well as the type and amount of liquid stationary phase is demonstrated, and methods are given for the selection of phase systems. The elimination of side-effects, that give rise to peak deformation and low separating efficiency, by addition of long-chain ammonium compounds to the system is also demonstrated.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of an LDC Model 711-26 or 711-47 solvent delivery system (Milton-Roy minipump with pulse damper), an LDC Model 1205 UV monitor with an 8-μl cell and a measuring wavelength of 254 nm and a Valco high-pressure valve injector with 8- or 30-μl loops.

The column tubes were made of 316 stainless steel with a polished surface (Altex Scientific, Berkeley, Calif., U.S.A.). They were used with dimensions of 150 × 4.5 mm, 100 × 4.5 mm and 150 × 3.2 mm and were equipped with modified Swagelok connectors and Altex stainless-steel frits (2 μm). The water-bath for thermostating was an HETO 02 PT 923 TC (Birkeröd, Denmark).

Chemicals and reagents

1-Pentanol was of A.C.S. quality from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Methylene chloride, a "zur Analyse" product from E. Merck (Darmstadt, G.F.R.), was extracted with water to remove ethanol.

Sodium cyclohexylsulphamate (zur Synthese) was obtained from Merck-Schuchardt (Munich, G.F.R.) and sodium *n*-octylsulphate (zur Tensidanalyse) from Merck. Potassium dicyclohexylsulphamate was kindly supplied by AB Hässle (Mölndal, Sweden).

Tetrapropylammonium iodide (TPrAI), tetrabutylammonium iodide (TBAI) and N,N,N-trimethylnonylammonium bromide (TMNABr), from Eastman-Kodak (Rochester, N.Y., U.S.A.) were usually converted into hydroxides by shaking their aqueous solutions with silver oxide. The hydroxides were converted into phosphates by adding orthophosphoric acid and diluted to an ionic strength of 0.1. N,N-Dimethyloctylamine (DMOA), obtained from ICN-K & K Labs. (Plainview, N.Y., U.S.A.), was distilled.

All amines and quaternary ammonium compounds used as chromatographic samples were of pharmacopoeial or equivalent grade. Their structures are given in Tables I and II.

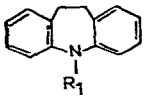
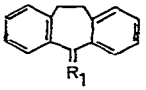
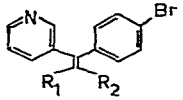
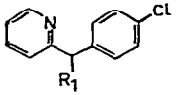
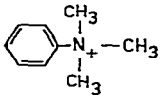
Zimelidine and derivatives were kindly supplied by AB Astra Läkemedel (Södertälje, Sweden) and the amphetamine homologues by Dr. Gösta Hallström. N-Methylimipramine was kindly supplied by Dr. P.-O. Lagerström. It can be synthesized from imipramine chloride and obtained as the phosphate[9]. N-Methylamitriptyline bromide was synthesized from amitriptyline chloride according to Borg[10].

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

The buffers were prepared at an ionic strength of 0.1 from orthophosphoric acid and sodium hydroxide.

TABLE I

STRUCTURES OF SOME OF THE SAMPLES

Formula	*Name*	R_1	R_2
	Desipramine	$(CH_2)_3NHCH_3$	—
	Imipramine	$(CH_2)_3N(CH_3)_2$	—
	N-Methylimipramine	$(CH_2)_3N^+(CH_3)_3$	—
	Trimipramine	$CH_2CH(CH_3)CH_2N(CH_3)_2$	—
	Nortriptyline	$CH(CH_2)_2NHCH_3$	—
	Amitriptyline	$CH(CH_2)_2N(CH_3)_2$	—
	N-Methylamitriptyline	$CH(CH_2)_2N^+(CH_3)_3$	—
	Zimelidine (Z-isomer)	$CH_2N(CH_3)_2$	—
	Zimelidine (E-isomer)	H	$CH_2N(CH_3)_2$
	N-Demethylzimelidine (E-isomer)	H	CH_2NHCH_3
	Chlorpheniramine	$CH_2CH_2N(CH_3)_2$	—
	N,N,N-Trimethylaniline	—	—

Chromatographic supports

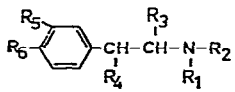
LiChrosorb RP-18 (10 μm), RP-8 (10 μm) and RP-8 (5 μm) were obtained from Merck. Spherisorb ODS (5 μm) was obtained from Phase Separations (Queensferry, Great Britain).

Column preparations and testing

Packing. The columns were packed by a balanced-density slurry technique[11]

TABLE II

STRUCTURES OF SOME OF THE SAMPLES

Formula	*Name*	R_1	R_2	R_3	R_4	R_5	R_6
	Phenylethylamine	H	H	H	H	H	H
	Amphetamine	H	H	CH_3	H	H	H
	α-Ethylphenylethylamine	H	H	C_2H_5	H	H	H
	α-Isopropylphenylethylamine	H	H	$CH(CH_3)_2$	H	H	H
	N-ethylephedrine chloride	C_2H_5	CH_3	CH_3	CH	H	H
	Ephedrine	H	CH_3	CH_3	OH	H	H
	Norephedrine	H	H	CH_3	OH	H	H
	Pholedrine	H	CH_3	CH_3	H	H	OH
	Tyramine	H	H	H	H	H	OH
	p-Hydroxyephedrine	H	CH_3	CH_3	OH	H	OH
	Synephrine	H	CH_3	H	OH	H	OH
	Dopamine	H	H	H	H	OH	OH
	Adrenaline	H	CH_3	H	OH	OH	OH
	Noradrenaline	H	H	H	OH	OH	OH

using tetrachloroethane as the suspending liquid. The initial packing pressure was 40,000 kPa. After packing, the fillings were washed with 300 ml of *n*-hexane and 120 ml of acetone.

The quality of the columns was tested with methanol–water (6:4) as the mobile phase, injecting 2-phenylethanol, 2,6-dimethylphenol and 2,3,5-trimethylphenol. The accepted columns gave a plate height of less than six times the support particle diameter for capacity ratios above 2, at a flow-rate of 0.6 mm/sec.

Coating. The columns were coated with liquid stationary phase by passing mobile phase, containing the organic liquid, through the column. Equilibrium was usually reached after the passage of 100–150 column volumes of mobile phase[12].

The total volume of liquid phase in each column was obtained by separate measurements of the volume of mobile phase, V_m, and stationary phase, V_s. V_m was determined by injecting mobile phase with a slightly different content of counter ion. V_s was determined on the 1-pentanol-coated column by stripping off the stationary liquid with ethanol–water and determining the amount of 1-pentanol by gas chromatography on an OV-225 column. In all further experiments on that column, V_s was obtained as the difference between the total volume of liquid phase in the column and V_m, also for methylene chloride-coated columns assuming the same total volume.

Mobile phase. The mobile phases were prepared from aqueous buffers. The cationic components (TBA, TMNA, TPrA and DMOA) were added and the solutions were normally saturated with the stationary organic liquid at 25.0 ± 0.1° using only a slight excess of organic liquid[12]. When a lower content of organic liquid was required, saturated and unsaturated solutions were mixed. The counter ion (anion) was finally dissolved in the solution.

The solubility of 1-pentanol in water is 2.5 vol.-% and that of methylene chloride 1.0 vol.-% (ref. 13).

Chromatographic technique

The reservoir, the injector and the column were maintained thermostattically at 25.0 ± 0.1°, in most instances by immersion in a water-bath. The detector cell was maintained thermostattically at 22° by circulating water[12] when the mobile phase was saturated with the stationary liquid phase.

All injected samples were dissolved in the mobile phase.

RESULTS AND DISCUSSION

Principle

Ion-pair chromatography is based on a liquid–liquid distribution technique for ionic compounds usually called ion-pair extraction. This process can be illustrated by the following example.

A cation, Q^+, can be transferred from an aqueous into an organic phase, if it is accompanied by an equivalent amount of an ion of the opposite charge, X^- (the counter ion), as illustrated by the process

$$Q^+_{aq} + X^-_{aq} = Q^+_{org} + X^-_{org}$$

with the equilibrium constant, K_{ie}, given by

$$K_{ie} = [Q^+]_{org} [X^-]_{org} [Q^+]^{-1} [X^-]^{-1} \quad (1)$$

In an organic phase of low polarity, Q^+ and X^- will form an ion pair, QX:

$$Q^+_{org} + X^-_{org} = QX_{org}$$

The equilibrium constant for this association process, K_{ia}, is defined as

$$K_{ia} = [QX]_{org} [Q^+]^{-1}_{org} [X^-]^{-1}_{org} \quad (2)$$

The lower the polarity of the organic phase, the higher is K_{ia} (ref. 14).

As most studies have been performed with organic phases of low polarity and in such concentration ranges that the ion pair dominates and the concentration of the ions is negligibly small, the process has usually been described by a gross reaction that consists of the above two equilibria:

$$Q^+_{aq} + X^-_{aq} = QX_{org}$$

The equilibrium constant for the gross reaction, usually called the extraction constant, E_{QX}, is defined as

$$E_{QX} = [QX]_{org} [Q^+]^{-1} [X^-]^{-1} \quad (3)$$

An expression for the distribution ratio of Q^+, D_Q, can be based on eqn. 3, which gives the equation

$$D_Q = [QX]_{org} [Q^+]^{-1} = E_{QX} [X^-] \quad (4)$$

From this expression, it follows that the distribution of Q^+ can be regulated by the concentration of the counter ion, X^-, and by the nature of X^- and the properties of the organic phase which will affect the extraction constant, E_{QX} (*cf.*, ref. 14).

It must be emphasized that eqn. 4 is based on the assumption that $[Q^+]_{org}$ is much smaller that $[QX]_{org}$. This is not so when the total concentration of Q^+ is low and an organic phase of medium polarity, *e.g.*, a higher alcohol, is used. A combination of eqns. 1 and 3 will give the following expression for the distribution ratio:

$$D_Q = \frac{[QX]_{org} + [Q^+]_{org}}{[Q^+]} = \left(E_{QX} + \frac{K_{ie}}{[X^-]_{org}}\right) [X^-] \quad (5)$$

$[X^-]_{org}$ and D_Q change with the total concentration of Q^+, but this can be prevented by the addition of a foreign cation, which is extracted to the organic phase with X^-. This will stabilize $[X^-]_{org}$ and consequently also D_Q (refs. 8 and 15).

Control of the capacity ratio

An expression for the capacity ratio in a reversed-phase system for ion-pair chromatography can be based on eqn. 4 when the ion-pair formation dominates:

$$k'_Q = (V_s/V_m) E_{QX} [X^-] \quad (6)$$

The means for regulation of k' are the phase volume ratio, V_s/V_m, and, in accordance with the principles of ion-pair extraction, the concentration and the nature of the counter ion and the nature of the stationary phase.

The basis for eqn. 6 is the assumption that the sample is retained by the liquid stationary phase. It has been shown by Wahlund and Sokolowski[8] that ion pairs also can be retained by adsorption on the solid phase, and that the influence of the adsorption increases with decreasing volume of stationary phase. This means that the influence of a change of V_s/V_m can be rather complex and deviate considerably from the simple relationship given in eqn. 6.

Choice of counter ion

The capacity ratio can be varied within wide limits by the choice of the counter ion. For hydrophobic amines, small hydrophilic counter ions such as dihydrogen phosphate or bromide can be used[8], while more hydrophilic compounds require hydrophobic counter ions in order to obtain a suitable retention.

An estimation of the extraction constant for an ion pair of the sample can often be made from published data[1,14] and a suitable counter ion then selected. The final choice of counter ion and its concentration can be made after some chromatographic test runs. The results of such a test series are given in Fig. 1.

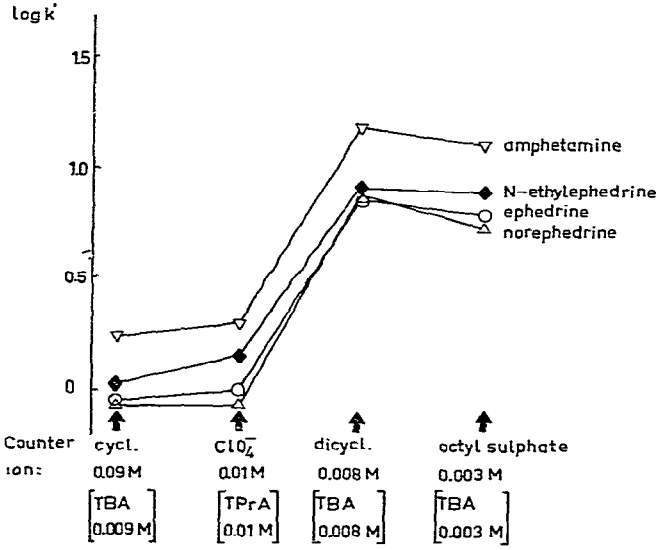


Fig. 1. Effects of counter ion on retention. Mobile phase: counter ion (cycl. = cyclohexylsulphamate, dicycl. = dicyclohexylsulphamate) + quaternary ammonium ion in phosphate buffer (pH 4.2–4.6), saturated with 1-pentanol. Stationary phase: 1-pentanol. Support: LiChrosorb RP-18 (10 μm).

The samples are four rather hydrophilic phenylethylamine derivatives, and four anions of highly different hydrophobic character were used as counter ions with 1-pentanol as the liquid stationary phase. Cyclohexylsulphamate gives a rather low retention. An increase in the size of the counter ion by another six carbon atoms to dicyclohexylsulphamate would, according to normal rules of liquid–liquid distribution[16,17], increase the extraction constant and the capacity ratio by about 2.5

logarithmic units. This will give too high a retention, and it is therefore necessary to decrease the counter ion concentration about 10-fold (*cf.*, eqn. 6). The increase in k' obtained in the chromatographic run with dicyclohexylsulphamate as counter ion agrees fairly well with these approximate calculations.

Octylsulphate would give an even higher extraction constant as it is known that organic sulphates give considerably higher extraction constants than other anions with the same number of alkyl carbon atoms[5,14]. A further decrease in the counter ion concentration is then necessary in order to keep k' at a suitable level.

Counter ion concentration

The final regulation of the retention can be made by changing the concentration of the counter ion in the mobile phase. A demonstration with octylsulphate as counter ion is given in Fig. 2.

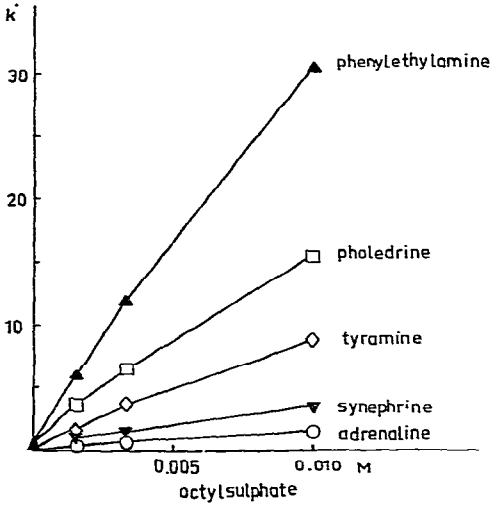


Fig. 2. Capacity ratio and counter ion concentration. Mobile phase: octylsulphate in phosphate buffer (pH 3.0), saturated with 1-pentanol. Stationary phase: 1-pentanol. Support: LiChrosorb RP-18 (10 μm).

The relationships between k' and the counter ion concentration are qualitatively in accordance with eqn. 6, which indicates that the samples are retained mainly as ion pairs with octylsulphate while the retention with the buffer component dihydrogen phosphate is almost negligible. As the slopes of the lines increase with increasing extraction constant, it is obvious that the possibilities of regulating the capacity ratio are rather limited for highly hydrophilic compounds with small extraction constants such as adrenaline and synephrine. It is also inconvenient when the extraction constant is very high, as for phenylethylamine, as the counter ion concentration must then be so low that the chromatographic system might be unstable.

A separation in this chromatographic system is demonstrated in Fig. 3. Differences in the degree of substitution of the amino group and in the number and positions of the hydroxyl groups give rather high separation factors and a rapid and efficient separation is possible.

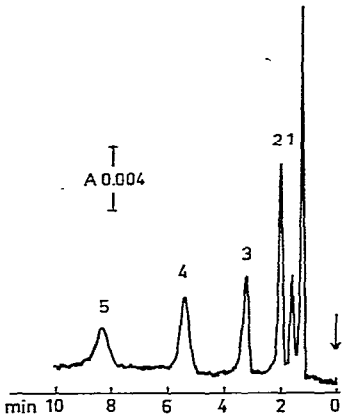


Fig. 3. Separation of phenylethylamine derivatives. Mobile phase: octylsulphate (0.0014 *M*) in phosphate buffer (pH 3.0), saturated with 1-pentanol (2.1 mm/sec, 2100 kPa, V_m = 1.01 ml). Stationary phase: 1-pentanol (V_s/V_m = 0.55). Support: LiChrosorb RP-18 (10 μm). Peaks: 1 = adrenaline (0.36 μg); 2 = dopamine (0.87 μg); 3 = tyramine (0.66 μg); 4 = pholedrine (0.49 μg); 5 = phenylethylamine (0.52 μg).

Liquid stationary phase

It is known from liquid–liquid distribution studies that the solvating properties of the organic phase have a very large influence on the separation selectivity of the system[18]. Hydrogen bonding between solute and solvent is of particular significance in this respect, and it is important to have systems where the hydrogen-bonding ability of the organic phase can be varied.

Application of a liquid stationary phase can be made very easily by using its ability to be adsorbed on a hydrophobic chromatographic support from an aqueous solution (the mobile phase). This has been achieved successfully with 1-pentanol and butyronitrile[5]. Attempts to extend this technique to less polar organic solvents such as methylene chloride are reported in Fig. 4.

The secondary ammonium compound adrenaline and its N-demythylation product noradrenaline cannot be separated as ion pairs with octylsulphate when the strongly hydrogen bonding 1-pentanol is used as the stationary phase. A good separation can be obtained, however, if 1-pentanol is replaced with the weakly hydrogen donating methylene chloride. The aminophenols have different retention in this system, even when octylsulphate is absent and phosphate can be assumed to act as the counter ion, but the addition of octylsulphate is necessary to obtain a suitable retention.

N-Methylation of noradrenaline (to adrenaline) increases log k' by about 0.4 unit, while removal of the hydroxyl group in the side-chain (to dopamine) gives an increase of about 0.8 log unit.

Retention mechanism

Studies of the influence of the amount of liquid stationary phase can give important information about the retention mechanism in reversed-phase chromatography of ion pairs as well as uncharged compounds[12]. The amount of liquid phase can easily be changed by varying the concentration of the organic liquid in the mobile phase.

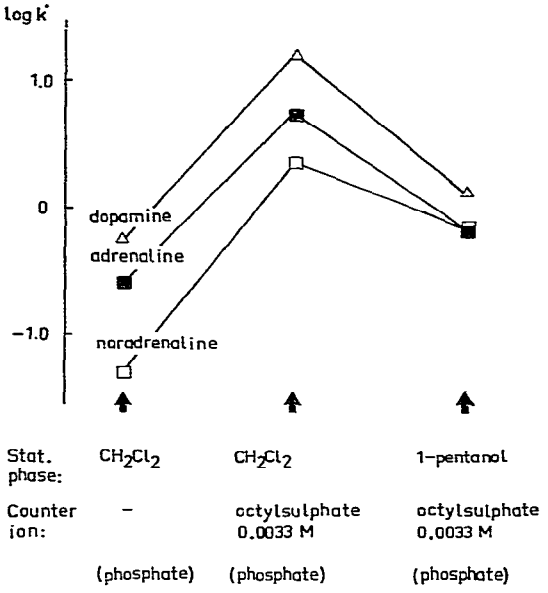


Fig. 4. Stationary phase effects. Mobile phase: counter ion in phosphate buffer (pH 3.0), saturated with stationary liquid phase. Stationary phase: methylene chloride ($V_s/V_m = 0.54$); 1-pentanol ($V_s/V_m = 0.55$). Support: LiChrosorb RP-18 (10 μm).

The variation of k' for a series of octylsulphate ion pairs on changing V_s/V_m is demonstrated in Fig. 5. A decrease in the volume of 1-pentanol that is applied as the stationary phase on the support not only decreases the retention but also changes the difference between log k' of the samples, *i.e.*, the separation selectivity of the system. The reason for this effect must be differences in the retention mechanisms for the four amines. If the capacity ratio had been in accordance with eqn. 6, all four lines would be parallel with slopes of unity and intercepts equal to log E_{QX} [X^-]. The results show that none of the ion pairs is retained exactly in accordance with eqn. 6 but liquid–liquid distribution of the ion pairs dominates in all instances.

The influence of methylene chloride as liquid stationary phase on the retention of the octylsulphate ion pairs of adrenaline and noradrenaline is demonstrated in Fig. 6. A decrease in the volume of stationary phase increases k', which shows that adsorption of the ion pair on the solid phase causes the retention in this system. This should also hold for the results in Fig. 4 (*cf.* ref. 8).

It should be noted that the systems with 1-pentanol and butyronitrile as stationary liquid phases have almost unlimited stability under carefully thermostatted conditions, while methylene chloride is slowly stripped off the support, probably owing to the difficulties in keeping the concentration of methylene chloride in the mobile phase constant, owing to its high vapour pressure (b.p. 39.8°)[13].

Anionic samples

Both hydrophilic and hydrophobic anions can usually be separated with high efficiency in ion-pair chromatographic systems. Quaternary alkylammonium ions have so far mainly been used as counter ions.

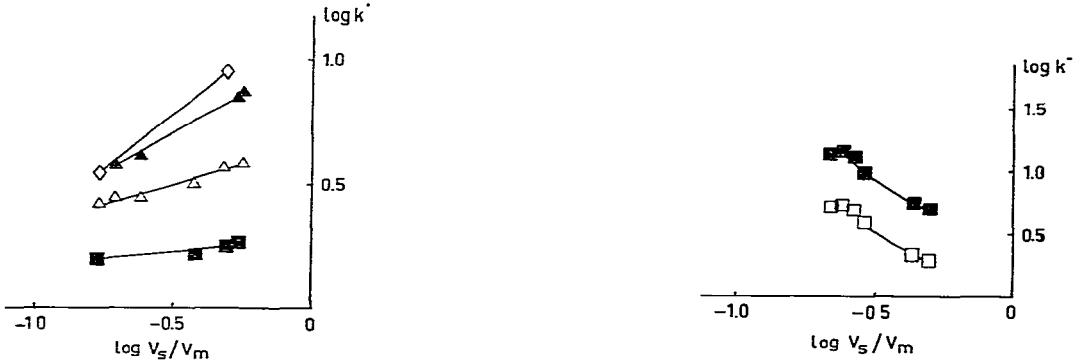


Fig. 5. Retention and selectivity at different stationary phase loading of 1-pentanol. Mobile phase: octylsulphate (0.01 *M*) in phosphate buffer (pH 3.0), containing 1-pentanol to 60–100% of its solubility. Stationary phase: 1-pentanol. Support: LiChrosorb RP-18 (10 μm). Samples: ◇, tyramine; ▲, *p*-hydroxyephedrine; △, dopamine; ■, adrenaline.

Fig. 6. Retention and selectivity at different stationary phase loading of methylene chloride. Mobile phase: octylsulphate (0.0033 *M*) in phosphate buffer (pH 3.0), containing methylene chloride in different concentrations. Stationary phase: methylene chloride. Support: LiChrosorb RP-18 (10 μm). Samples: ■, adrenaline; □, noradrenaline.

The good properties of these systems have been demonstrated in separations of naphthylacetates[12] and sulphonamides, barbiturates and their metabolites[5,6]. Hydrophobic, weak acids can be retained in these systems both as ion pairs and acids, which opens up possibilities of regulating the retention and the selectivity by varying the pH[6].

Hydrophilic cationic samples

The separation of cationic compounds by ion-pair chromatography can be performed with counter ions with highly different structures. A suitable retention can be obtained by a hydrophobic counter ion in low concentration as well as by a higher concentration of a more hydrophilic counter ion, as was demonstrated in Fig. 1.

The choice of counter ion can also have a considerable influence on the separating efficiency. The studies on phenylethylamine derivatives showed that the fairly hydrophilic counter ion cyclohexylsulphamate gives rise to chromatograms with rather tailing peaks, while a much better peak symmetry is obtained with the hydrophobic octylsulphate.

Previous studies have shown that such tailing can be due to ion-pair dissociation in the organic stationary phase[8,15], and addition of a hydrophobic cation such as tetrabutylammonium has been used to suppress or control the disturbing dissociation process (*cf.*, eqn. 5). Such an addition also gave in this instance a considerable improvement in the peak symmetry. A demonstration of the separating efficiency that can be obtained with cyclohexylsulphamate in the presence of tetrabutylammonium is given in Fig. 7, which shows the separation of some homologous phenylethylamines. Each addition of one methylene group increases log k' by 0.2–0.3 unit.

The selectivity of systems with octylsulphate as counter ion is demonstrated in a separation of seven phenylethylamine derivatives shown in Fig. 8. Introduction of

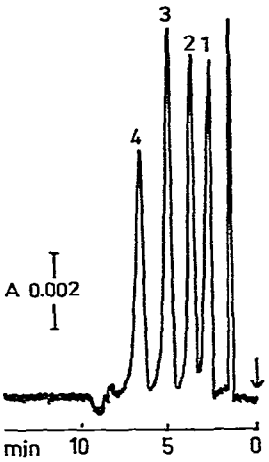


Fig. 7. Separation of phenylethylamine homologues. Mobile phase: cyclohexylsulphamate (0.08 *M*) + tetrabutylammonium (0.008 *M*) in phosphate buffer (pH 2.2), saturated with 1-pentanol (1.7 mm/sec, 1350 kPa, V_m = 0.99 ml). Stationary phase: 1-pentanol (V_s/V_m = 0.59). Support: LiChrosorb RP-18 (10 μm). Peaks (1.7–3.1 μg of each amine injected): 1 = phenylethylamine; 2 = amphetamine; 3 = α-ethylphenylethylamine; 4 = α-isopropylphenylethylamine.

one hydroxyl group decreases log k' by about 0.3 unit while lengthening of the alkyl chains gives an increase of 0.2 unit.

The separating efficiency is, however, even after the addition of tetrabutylammonium, better with the most hydrophobic counter ion, octylsulphate, than with cyclohexylsulphamate and dicyclohexylsulphamate, particularly in the lower k' range,

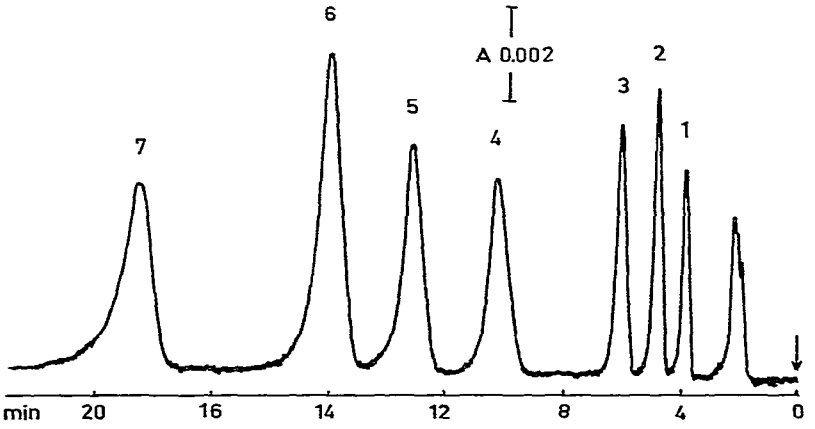


Fig. 8. Separation of phenylethylamine derivatives after addition of tetrabutylammonium to the mobile phase. Mobile phase: octylsulphate (0.003 *M*) + tetrabutylammonium (0.003 *M*) in phosphate buffer (pH 4.2), saturated with 1-pentanol (1.7 mm/sec, 1700 kPa, V_m = 1.12 ml). Stationary phase: 1-pentanol (V_s/V_m = 0.40). Support: LiChrosorb RP-18, 10 μm. Peaks: 1 = adrenaline (0.35 μg); 2 = synephrine (0.48 μg); 3 = *p*-hydroxyephedrine (1.0 μg); 4 = pholedrine (0.88 μg); 5 = ephedrine (4.7 μg); 6 = N-ethylephedrine (8.8 μg); 7 = amphetamine (2.8 μg).

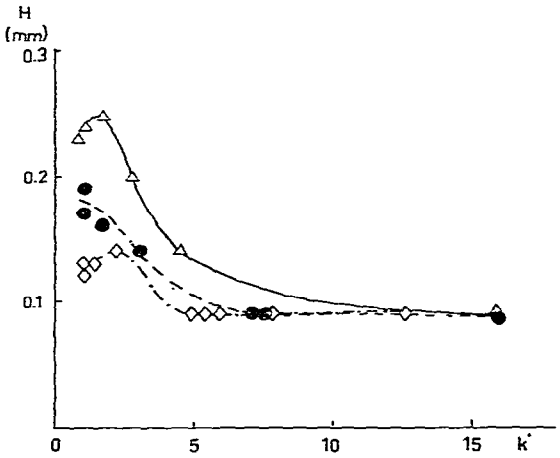


Fig. 9. Effect of counter ion on column efficiency. Mobile phase: △, cyclohexylsulphamate (0.09 *M*) + TBA (0.009 *M*); ●, dicyclohexylsulphamate (0.008 *M*) + TBA (0.008 *M*); □, octylsulphate (0.003 *M*) + TBA (0.003 *M*); in phosphate buffer (pH 4.2–4.6), saturated with 1-pentanol. Stationary phase: 1-pentanol. *Support:* LiChrosorb RP-18 (10 μm). Samples: hydrophilic amines (see Table II).

as demonstrated in Fig. 9. This indicates that the peak broadening is also dependent on the structure of the ion pair.

Hydrophobic cationic samples

A further illustration of the complex retention behaviour of cationic compounds in reversed-phase systems is given by the hydrophobic ammonium ions. They

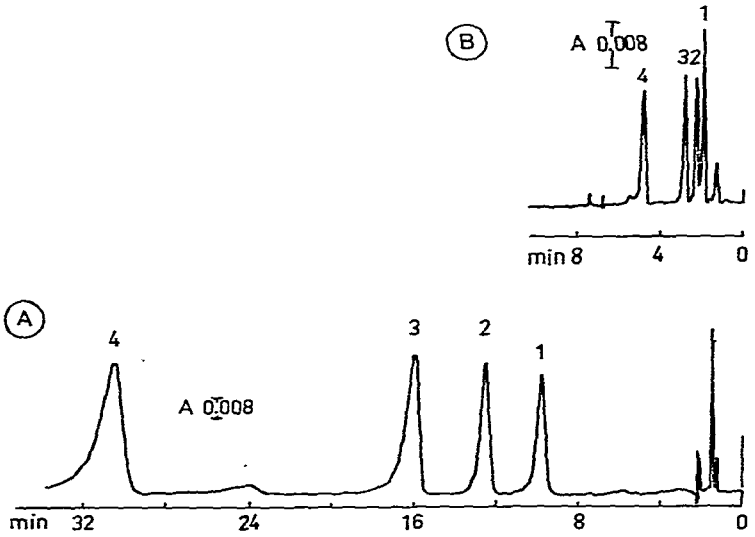


Fig. 10. Separation of hydrophobic amines with and without addition of dimethyloctylammonium. Mobile phase: (A) cyclohexylsulphamate (0.05 *M*); (B) cyclohexylsulphamate (0.05 *M*) + DMOA (0.01 *M*); in phosphate buffer (pH 2.2), containing 1-pentanol to 50% of its solubility. Stationary phase: 1-pentanol. Support: LiChrosorb RP-8 (5 μm). Peaks: 1 = zimelidine (E-isomer); 2 = N-demethylzimelidine (E-isomer); 3 = chlorpheniramine; 4 = zimelidine (Z-isomer).

give, in simple ion-pair chromatographic systems, highly tailing peaks and a retention considerably higher than expected[8]. The abnormal behaviour of these ion pairs cannot be corrected by addition of tetrabutylammonium, and mechanistic studies have shown that the retention is dominated by adsorption of the ion pairs on the surface of the solid support. Addition of a long-chain, tertiary or quaternary alkylammonium ion to the system gives a large decrease in k' and a considerable improvement in the peak symmetry. A demonstration of the effect of the addition of dimethyloctylammonium to a system with cyclohexylsulphamate as the counter ion is given in Fig. 10.

A survey of the retention behaviour of some hydrophobic amines in different systems is given in Fig. 11. In systems where the mobile phase contains only buffer and counter ion, the retention increases with increasing hydrophobic character of the counter ion, as expected. The addition of dimethyloctylammonium (DMOA) reduces the binding ability of the support surface considerably. The cyclohexylsulphamate *i.e.*, the most hydrophobic ion pairs, have a lower retention than the phosphate, *i.e.*, the most hydrophilic ion pairs in the absence of dimethyloctylammonium.

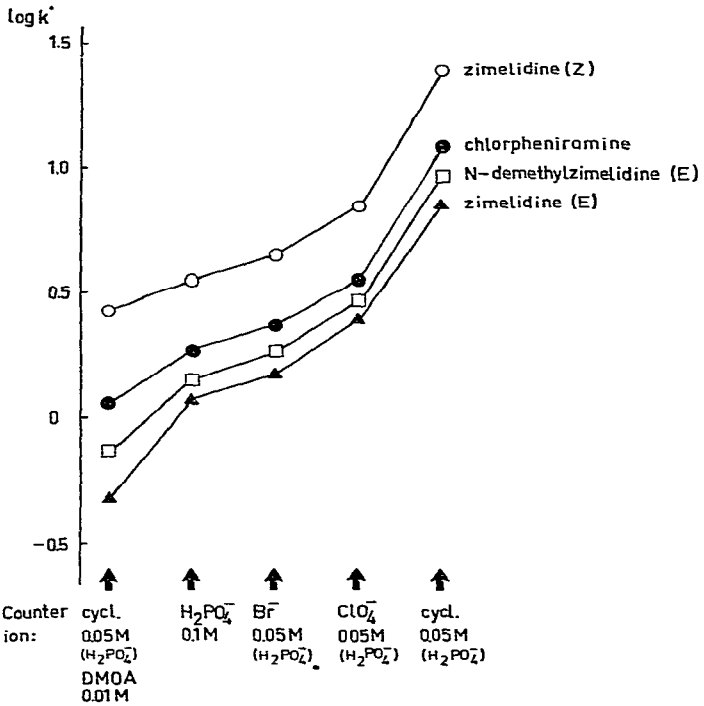


Fig. 11. Effect of counter ion on retention. Mobile phase: counter ion (cycl. = cyclohexylsulphamate) in phosphate buffer (pH 2.0–2.2), containing 1-pentanol to 50% of its solubility. Stationary phase: 1-pentanol. Support: LiChrosorb RP-8 (5 μm).

Both hydrophilic and hydrophobic quaternary ammonium ions have shown even greater tendencies than the hydrophobic amines for deviating chromatographic behaviour, giving highly deformed peaks in simple ion-pair chromatographic systems. Addition of a long-chain ammonium ion to the mobile phase eliminates these

disturbances and the quaternary ammonium ions give symmetrical peaks and high separating efficiencies comparable with those of the amines. A demonstration is given in Fig. 12, which shows the separation of a quaternary, a secondary and a primary ammonium ion as ion pairs with octylsulphate and with trimethylnonylammonium present in the mobile phase.

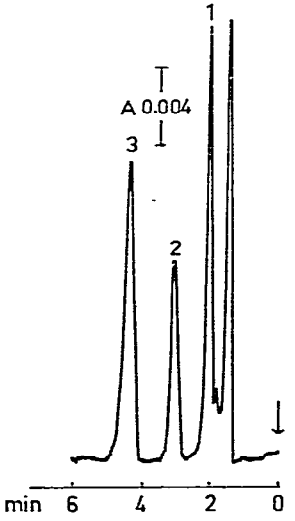


Fig. 12. Separation of quaternary, secondary and primary ammonium ions. Mobile phase: octylsulphate (0.0017 *M*) + TMNA (0.0013 *M*) in phosphate buffer (pH 3.0), saturated with 1-pentanol (3.7 mm/sec, 2100 kPa, V_m = 1.23 ml). Stationary phase: 1-pentanol (V_s/V_m = 0.28). Support: LiChrosorb RP-18 (10 μm). Peaks: 1 = trimethylaniline; 2 = pholedrine; 3 = phenylethylamine.

Support effects

The character of the adsorption forces, which have such an important influence on the retention and the general chromatographic behaviour, have so far not been clarified. The solid phase (support) can have a considerable influence in some instances. A demonstration is given in Fig. 13, which shows the retention on three different supports: LiChrosorb RP-8 (5- and 10-μm particles) and Spherisorb ODS (5-μm particles)[19]. The samples were hydrophobic ammonium compounds containing tricyclic ring systems. Dihydrogen phosphate was used as the counter ion and 1-pentanol as the stationary liquid phase. Trimethylnonylammonium was added to the mobile phase in order to improve the separating efficiency of the chromatographic system.

The retention order on RP-8 is in accordance with the normal behaviour for ion-pair distribution between 1-pentanol and an aqueous phase[20]. On ODS, the secondary ammonium ions are much less retained and the retention order is the same as for ion pairs with chloride when methylene chloride is used as the organic phase[18]. This indicates that the retaining phase has hydrocarbon-like properties, in contrast to the situation on RP-8. It should also be noted that the separating efficiency is much lower on ODS than on RP-8, even if the peak symmetry in all instances is good. The separation of some imipramine derivatives on RP-8 is demonstrated in Fig. 14. The demethylation of the quaternary ammonium compound (N-methylimipramine) in-

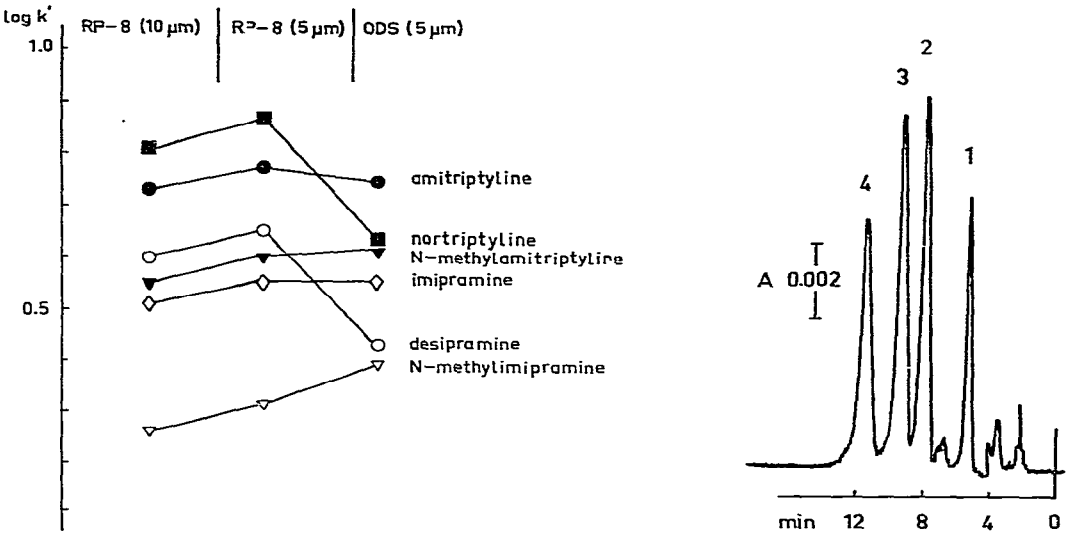


Fig. 13. Support effects. Mobile phase: TMNA (0.03 *M*) in phosphate buffer (pH 2.0), saturated with 1-pentanol. Stationary phase: 1-pentanol.

Fig. 14. Separation of tricyclic ammonium compounds. Mobile phase: TMNA (0.036 *M*) in phosphate buffer (pH 2.0), saturated with 1-pentanol (1.4 mm/sec, 5000 kPa, V_m = 0.66 ml). Stationary phase: 1-pentanol (V_s/V_m = 0.45). Support: LiChrosorb RP-8 (10 µm). Peaks: 1 = N-methylimipramine; 2 = imipramine; 3 = desipramine; 4 = trimipramine.

creases log k' by about 0.25 unit, while the demethylation of the tertiary amine gives an increase of only 0.1 unit.

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