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REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF ANTIDEPRESSIVE AND NEUROLEPTIC AMINES AND RELATED QUATERNARY AMMONIUM COMPOUNDS

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SUMMARY

Reversed-phase ion-pair liquid chromatographic systems for amines and quaternary ammonium compounds have been studied with 1-pentanol as the organic liquid stationary phase and aqueous solutions containing dihydrogen phosphate, bromide and cyclohexylsulphamate as counter ions. Strong tailing was avoided and a good separation efficiency was obtained after addition of N,N,N-trimethylnonylammonium or N,N-dimethyloctylamine to the mobile phase.

It is shown that the favourable influence of the long-chain ammonium compounds was due to the elimination of retention mechanisms other than liquid–liquid distribution.

An ion-pair retention mechanism possibly involving the surface of the support is proposed and excellent separations of *cis–trans* isomers could be obtained.

INTRODUCTION

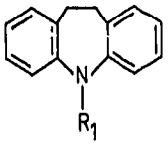
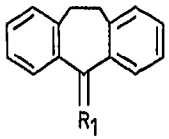
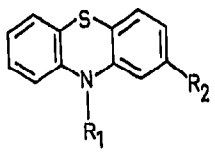
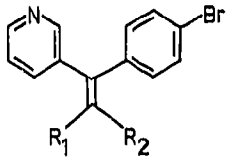
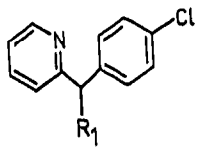
Ion-pair chromatographic separations have found wide application in the separation of amines and quaternary ammonium ions of pharmacological and biochemical importance[1]. These systems have an aqueous stationary phase, containing the counter ion, and an organic solvent as the mobile phase.

The construction of liquid chromatographic systems with an organic liquid as the stationary phase and an aqueous mobile phase has made ion-pair chromatographic separations possible with the counter ion dissolved in the mobile phase[2,3]. These systems have the advantage that the choice of a suitable counter ion and its concentration are easily made by test runs directly on the chromatographic column[4]. They are particularly suitable for hydrophilic and aqueous samples (*e.g.*, biological fluids) which can be dissolved directly in the mobile phase[5].

Systems with butyronitrile and 1-pentanol as stationary phases have been used for separation of acidic drugs and their metabolites[4–6]. Stationary phases with a high extraction ability, such as 1-pentanol, make these systems suitable for the separation

TABLE I

SAMPLES USED

Formula	*Name*	R_1	R_2
	Desipramine·HCl	$-(CH_2)_3NHCH_3$	–
	Imipramine·HCl	$-(CH_2)_3N(CH_3)_2$	–
	N-Methylimipramine	$-(CH_2)_3\overset{\oplus}{N}(CH_3)_3$	–
	Nortriptyline	$=CH(CH_2)_2NHCH_3$	–
	Amitriptyline·HCl	$=CH(CH_2)_2N(CH_3)_2$	–
	N-Methylamitriptyline·Br	$=CH(CH_2)_2\overset{\oplus}{N}(CH_3)_3$	–
	Dixyrazine	$-CH_2CH(CH_3)CH_2-N\,\square\,N-(CH_2)_2-O-(CH_2)_2OH$	–H
	Perphenazine	$-(CH_2)_3-N\,\square\,N-(CH_2)_2OH$	–Cl
	Fluphenazine·HCl	$-(CH_2)_3-N\,\square\,N-(CH_2)_2OH$	$-CF_3$
	Zimelidine·2HCl·H_2O (Z-isomer)	$-CH_2N(CH_3)_2$	–H
	(E-isomer, oxalate)	–H	$-CH_2N(CH_3)_2$
	(E-isomer, oxalate)	–H	$-CH_2NHCH_3$
	Chlorpheniramine maleate	$-CH_2CH_2N(CH_3)_2$	–

of hydrophilic samples, but it is shown in this paper that they are equally useful for hydrophobic compounds if the mobile phase has a suitable composition.

This paper describes an investigation of the chromatographic behavior of hydrophobic amines in reversed-phase systems with 1-pentanol as the stationary phase and inorganic and organic anions of different hydrophobicity as counter ions in the aqueous mobile phase.

The study was concentrated on some closely related hydrophobic ammonium compounds: the tertiary amines imipramine and amitriptyline, their N-demethylation products desipramine and nortriptyline and the quaternary ammonium ions N-methylimipramine and N-methylamitriptyline. Runs were also made with other hydrophobic tertiary amines with related structures.

EXPERIMENTAL

Apparatus

Ordinary high-performance liquid chromatographic equipment was used, as described earlier[5]. The column was of 150 mm length and 3.2 mm I.D.

A water-bath, HETO Type 02 PT 923 TC (Birkeröd, Denmark), was used to thermostat the chromatograph.

The pH was measured with an Orion Research Model 801 A digital pH meter equipped with an Ingold Type 401 combined electrode.

In the batch experiments, a Zeiss PMQ II spectrophotometer with 10-mm cells was used.

Chemicals and reagents

1-pentanol was of Fisher Scientific (Pittsburgh, Pa., U.S.A.) A.C.S. quality. Tetrabutylammonium iodide (TBAI), tetrapentylammonium iodide (TPeAI) and N,N,N-trimethylnonylammonium bromide (TMNABr) (Eastman-Kodak, Rochester, N.Y., U.S.A.) were converted into hydroxides by shaking their aqueous solutions with silver oxide and then further converted into phosphates by adding orthophosphoric acid. Tetrabutylammonium hydrogen sulphate ($TBAHSO_4$) (puriss., AB Labkemi, Göteborg, Sweden) was used after adjustment of pH with appropriate buffer components.

N,N-dimethyloctylamine, DMOA (ICN–K & K Labs., Plainview, N.Y., U.S.A.) and N-methyloctylamine (Eastman-Kodak) were distilled. Octylamine (puriss.) was obtained from Fluka (Buchs, Switzerland) and dihexylamine (zur Synthese) from Merck-Schuchardt (Munich, G.F.R.). Sodium cyclohexylsulphamate (sodium cyclamate) (zur Synthese) was obtained from Merck-Schuchardt.

The amines and quaternary ammonium compounds (pharmacopoeial grade) used as chromatographic samples are listed in Table I. Zimelidine and derivatives were kindly supplied by AB Astra Läkemedel (Södertälje, Sweden). N-Methylimipramine was kindly supplied by Dr. P.-O. Lagerström; it can be synthesized from imipramine chloride and obtained as the phosphate[7]. N-Methylamitriptyline bromide was synthesized from amitriptyline chloride according to Borg[8].

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

Column preparation

The support used was LiChrosorb RP-8 of mean particle diameter 10 μm (E. Merck, Darmstadt, G.F.R.). It was packed in the column by a balanced-density slurry packing technique[9] using tetrabromoethane + tetrachloroethylene (1:5) as the suspending liquid. After packing, the filling was washed with *n*-hexane and acetone.

The support was coated with 1-pentanol as stationary liquid phase *in situ* by a dynamic coating method described elsewhere[10].

All results were obtained on a single column filling, mostly completely coated with stationary phase, and used for a period of 9 months.

Chromatographic technique

The basic technique has been described elsewhere[5,10].

The chromatograph was thermostated at 25.0 $\pm$ 0.1°. No pre-column had to be used. The sample volumes were 1–20 μl and the samples were dissolved in the mobile phase.

The mobile phase was prepared by dissolving appropriate concentrations of the counter ions and other components in an aqueous phosphate buffer. The concentration of the buffer was adjusted to give $[H_2PO_4^-] = 0.10$ in the mobile phase, unless otherwise stated. The counter ions (dihydrogen phosphate, bromide and cyclohexylsulphamate) were used as their sodium salts.

When saturating the mobile phase with the stationary phase liquid (1-pentanol), care was taken to avoid losses of components of the aqueous phase by using a minimum excess volume of 1-pentanol[10].

The mobile phase reservoir contained about 200 ml of mobile phase. A small excess (about 0.5 ml) of 1-pentanol was present in the reservoir, in order to maintain saturation of the mobile phase.

The volume of mobile phase within the column (V_m) was determined from the retention volume of synephrine, a hydrophilic secondary amine, which is unretained.

Determination of batch distribution, extraction and dissociation constants

The experiments were performed in centrifuge tubes with equal phase volumes of 1-pentanol and the aqueous phase, which were pre-saturated with each other. Equilibrium was attained by mechanical shaking for 30 min in a thermostated bath at 25.0°.

The ionic strength of the aqueous phase was 0.1. The ammonium salts were initially dissolved in the aqueous phase and, after equilibration of the phases, the total concentration of the cationic species was determined, in either one or both phases, by photometry in the UV region for imipramine and by the picrate extraction method[11] for TBA.

The constants were evaluated by slope analysis. For imipramine, the distribution constant was calculated according to Persson[12] by using a constant sample concentration in the organic phase with a phosphate buffer of pH 4–5 as the aqueous phase. Ion-pair extraction and dissociation constants were evaluated according to Persson and Schill[13] and Gustavii and Schill[11] by varying the sample concentration (see also Schill[14] for the principles of ion-pair extraction).

RESULTS AND DISCUSSION

Symbols

$$K_{d(A)} = \frac{[A]_{org}}{[A]} = \text{distribution constant of the base A.}$$

$$K'_{HA^+} = \frac{a_{H^+}\,[A]}{[HA^+]} = \text{apparent acid dissociation constant of } HA^+.$$

$$E_{HAX} = \frac{[HAX]_{org}}{[HA^+]\,[X^-]} = \text{extraction constant of the ion pair HAX.}$$

$$E^*_{HAX} = \frac{C_{HAX,org}}{C_{HA}\,C_X} = \text{conditional extraction constant of the ion pair HAX.}$$

$$k_{diss(HAX)} = \frac{[HA^+]_{org}\,[X^-]_{org}}{[HAX]_{org}} = \text{dissociation constant of the ion pair HAX.}$$

Concentrations without subscripts refer to the aqueous phase.

Ion-pair dissociation

Preliminary determinations of extraction constants indicated that dihydrogen phosphate and bromide would be suitable as counter ions with the actual cationic samples. However, it is known that the ion-pair dissociation constants in 1-pentanol are rather high[15] (see Table II). The ion-pair dissociation might influence the symmetry of the chromatographic peaks[3,16] through the effect of the degree of ion-pair dissociation on the conditional extraction constant, E^*_{HAX}, of the sample ion pair. The conditional extraction constant increases with decreasing sample concentration[3,14] and the capacity ratio of the sample will change concurrently with E^*_{HAX} according to

$$k'_{HA} = (V_s/V_m)\,E^*_{HAX}C_X \qquad (1)$$

Symmetrical chromatographic peaks are obtained if the degree of ion-pair dissociation is kept constant. This is achieved if a high and constant concentration of the counter ion in the organic phase, $[X^-]_{org}$, is maintained. This situation can be obtained by adding to the aqueous mobile phase a cation, Q^+, which brings about a suitable ion-pair extraction of the counter ion into the stationary phase[3,16]. The required concentration of Q^+ in the aqueous mobile phase can be calculated by the expression for the conditional extraction constant of the sample ion pair (HAX)[17,18]:

$$E^*_{HAX} = E_{HAX}\left(1 + \frac{k_{diss(HAX)}}{[X^-]^{\pm}\cdot(E_{HAX}\,k_{diss(HAX)}\,[HA^+] + E_{QX}\,k_{diss(QX)}\,[Q^+])^{\pm}}\right) \qquad (2)$$

The required concentration of Q^+ depends on the concentration of the sample on the column, but also on the dissociation constant and the concentration of the counter

ion. However, the conditional extraction constant will always be independent of the concentration of the sample, $[HA^+]$, in the mobile phase if

$$E_{HAX}k_{diss(HAX)}[HA^+] \ll E_{QX}k_{diss(QX)}[Q^+] \quad (3)$$

Table II presents some extraction and dissociation constants of imipramine, HA^+, and tetrabutylammonium (TBA), Q^+, determined by batch distribution experiments.

TABLE II

ION-PAIR EXTRACTION AND DISSOCIATION CONSTANTS

Organic phase: 1-pentanol. Base distribution of imipramine: log $(K_{d(A)} \cdot K_{HA^+}) = -4.91$.

Ion pair	*Aqueous phase*	*Log E*	*Log k_{diss}*	*Log ($E k_{diss}$)*
TBA–H_2PO_4	NaH_2PO_4 (0.10 *M*)			−3.17
TBA–Br	NaBr (0.10 *M*)	1.58	−3.15	
Imipramine–H_2PO_4	Phosphate buffer (pH 3.00); $[H_2PO_4^-] = 0.10$ *M*	1.1	−3.8	

Substitution of the constants of the ion pairs of $H_2PO_4^-$ in eqn. 3 indicates that a TBA concentration of 0.03 *M* in the mobile phase will suffice to prevent changes in E^*_{HAX} for sample concentrations up to 10^{-3} *M*, and thus give symmetrical chromatographical peaks.

However, in chromatographic experiments imipramine gave too retarted and strongly tailing peaks with a mobile phase of 0.028 *M* TBA phosphate in phosphate buffer of pH 2.00 and 1-pentanol as the stationary phase. Some improvement was obtained by using the more hydrophobic tetrapentylammonium, but strong tailing remained. TPeA–H_2PO_4 can be expected to have about the same ion-pair dissociation constant as TBA–H_2PO_4 (ref. 14) but the extraction constant is about 10 times higher owing to the addition of four CH_2 groups in TPeA. Each methylene group is expected to give a contribution of 0.23 log-unit to the extraction constant, as seen from results on imipramine and trimipramine with 1-pentanol as the organic phase[2]. Trimipramine is a derivative of imipramine in which a methyl group is present in the 2-position in the carbon side-chain. Consequently, the use of TPeA will further improve the possibilities of keeping the ion-pair dissociation at a constant degree according to eqns. 2 and 3.

The batch distribution experiments indicated that ion-pair dissociation in the organic phase was the only side-reaction within the phase system that can give a change in E^*_{HAX} with the concentration of the sample. As E^*_{HAX} was kept constant by the addition of the quaternary ammonium compounds and strong tailing still remained, it is obvious that the poor chromatographic behaviour cannot be caused by solution equilibria in either of the two liquid phases. Some additional retention mechanism, probably an interaction with the hydrophobic support surface, obviously causes the tailing of the peaks.

Addition of long-chain amines and quaternary ammonium ions to the mobile phase

Dihydrogen phosphate as counter ion. A considerable improvement in chromatographic performance was obtained when a long-chain amine, N,N-dimethyl-

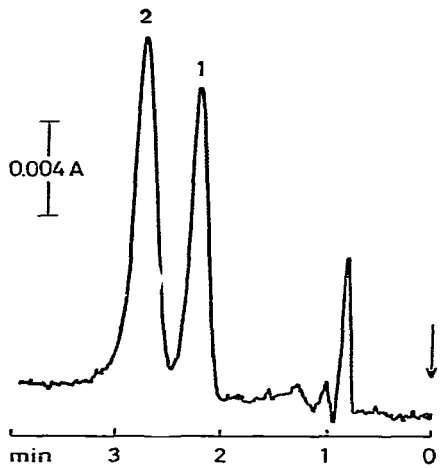


Fig. 1. Separation of imipramine and desipramine with DMOA in the mobile phase. Mobile phase: phosphate buffer (pH 2.00), DMOA 0.028 *M*, saturated with 1-pentanol; 3.1 mm/sec; 31 bar; V_m = 0.54 ml. Stationary phase: 1-pentanol, V_s/V_m = 0.78. Support: LiChrosorb RP-8, 10 μm. Peaks: 1 = imipramine (1.0 nmole, 330 ng); 2 = desipramine (1.1 nmole, 330 ng).

octylamine (DMOA), was added to the mobile phase. A chromatogram demonstrating the separation of imipramine and desipramine is shown in Fig. 1.

It is likely that the samples are retained as ion pairs with dihydrogen phosphate. Evidence for this is obtained by a calculation of the capacity ratio based on eqn. 1, data for the chromatographic system from Fig. 1 and the extraction constant of imipramine in Table II. Assuming that $E^*_{HAX} = E_{HAX}$, the calculation gave k'_{HA} = 1.0, which is in fairly good agreement with the found value of 1.72. The calculation of E^*_{HAX} by means of eqn. 2 is impossible owing to the lack of constants for DMOA, but a computation by use of the constant for TBA–H_2PO_4 (Table II) gave $E^*_{HAX} = E_{HAX} \cdot 1.1$. This indicates that the difference between the found and the calculated k' values can, at least in part, be due to the fact that the imipramine ion pair is retained partly dissociated in the stationary 1-pentanol phase.

A further indication of the ion-pair retardation of the samples is given by the agreement in the observed selectivities for desipramine and imipramine in chromatographic and batch extraction experiments. In Fig. 1 a separation factor (α) of 1.37 was found, while the batch extraction[2] gave a separation factor of 1.48.

Tests were also made with mobile phases containing primary and secondary amines such as *n*-octylamine, N-methyloctylamine and N,N-dihexylamine, but no satisfactory chromatograms were obtained with the exception of N-methyloctylamine, which gave acceptable peak symmetry for amines but not for quaternary ammonium compounds. It was also noticed that the tertiary amine imipramine always showed stronger tailing than desipramine (for the same amount of sample), which shows that the structure of the sample also is of importance.

A long alkyl chain seems to be a necessary structural element in the amines added to the mobile phase. This is supported by the observation that N,N,N-tripropylamine added to the mobile phase gave strongly tailing peaks while, as discussed above, the long-chain unsymmetrical DMOA gave good peak symmetry.

Addition of an unsymmetrical, long-chain quaternary ammonium ion, N,N,N-trimethylnonylammonium (TMNA), gave results as good as those obtained with DMOA. A separation of a secondary, a tertiary and a quaternary ammonium ion is demonstrated in Fig. 2. It is important to note that the retention is higher in the presence of TMNA than in the presence of DMOA. Imipramine has $k' = 3.43$ in the TMNA system and $k' = 1.72$ in the DMOA system, in spite of the fact that V_s/V_m was considerably lower in the TMNA system. The separation factor of desipramine and imipramine is slightly lower with TMNA in the mobile phase ($\alpha = 1.23$) than with DMOA (see above, Fig. 1).

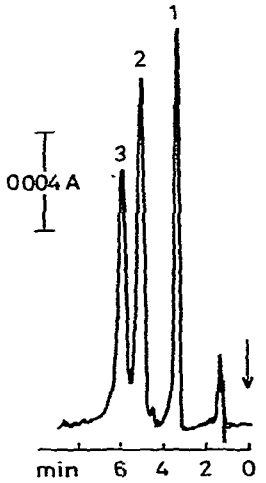


Fig. 2. Separation of imipramine and derivatives with TMNA in the mobile phase. Mobile phase: phosphate buffer (pH 1.95), TMNA 0.036 *M*, saturated with 1-pentanol; 2.9 mm/sec; 100 bar; $V_m = 0.67$ ml. Stationary phase: 1-pentanol, $V_s/V_m = 0.43$. Support: LiChrosorb RP-8, 10 μm. Peaks: 1 = N-methylimipramine (0.67 nmole, 200 ng); 2 = imipramine (1.2 nmole, 390 ng); 3 = desipramine (1.4 nmole, 440 ng).

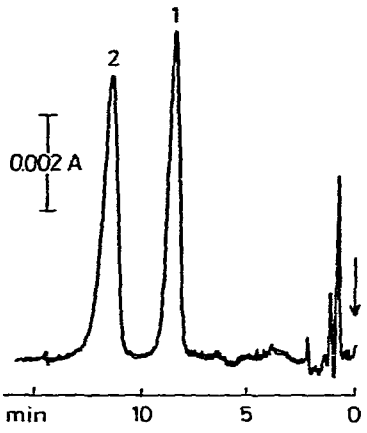
The positive results with TMNA indicates that the effect of the added ammonium ion is highly dependent on structure as the same concentration of TBA gave strong tailing and too high a retention (see above).

Bromide as counter ion. Bromide gives higher extraction constants than dihydrogen phosphate[8] and a mobile phase of 0.043 *M* sodium bromide in 0.028 *M* DMOA in phosphate buffer of pH 2.00 gave increased retentions of both imipramine and desipramine, as shown in Fig. 3. The same system was also used for some hydrophobic phenothiazine derivatives with a piperazine group in the side-chain. The separation of dixyrazine, perphenazine and fluphenazine is shown in Fig. 4. The good peak symmetry is obvious from Figs. 3 and 4.

The retention data of some ammonium compounds are given in Table III for the ion pairs with both dihydrogen phosphate and bromide.

Supports effects

The interaction of the samples with the support was briefly investigated by



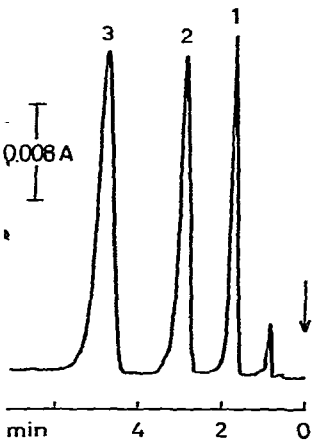


Fig. 3. Separation of imipramine and desipramine with bromide in the mobile phase. Mobile phase: NaBr 0.043 *M*, phosphate buffer (pH 2.00), DMOA 0.028 *M*, saturated with 1-pentanol; 3.0 mm/sec; 28 bar; $V_m = 0.55$ ml. Stationary phase: 1-pentanol, $V_s/V_m = 0.75$. Support: LiChrosorb RP-8, 10 μm. Peaks: 1 = imipramine (0.87 nmole 280 ng); 2 = desipramine (0.90 nmole, 280 ng).

Fig. 4. Separation of neuroleptics. Mobile phase: NaBr 0.043 *M*, phosphate buffer (pH 2.00), DMOA 0.028 *M*, saturated with 1-pentanol; 3.0 mm/sec; 64 bar; $V_m = 0.55$ ml. Stationary phase: 1-pentanol, $V_s/V_m = 0.75$. Support: LiChrosorb RP-8, 10 μm. Peaks: 1 = dixyrazine (0.47 nmole, 200 ng); 2 = perphenazine (0.48 nmole, 190 ng); 3 = fluphenazine (0.88 nmole, 420 ng).

TABLE III

RETENTION DATA (CAPACITY RATIOS) OF AMMONIUM COMPOUNDS

Mobile phase: phosphate buffer (pH 2.00); DMOA 0.028 *M*; counter ion. Stationary phase: 1-pentanol. Support: LiChrosorb RP-8. Phase volume ratio: 0.77.

Sample	*Counter ion*	
	$[H_2PO_4^-] = 0.10$ *M*	$[H_2PO_4^-] = 0.10$ *M*; $[Br^-] = 0.043$ *M*
Dixyrazine	0.19	1.05
Perphenazine	0 47	2.52
Fluphenazine	0.92	4.75
Imipramine	1.72	9.12
N-Methylamitriptyline	1.42	9.37
Desipramine	2.38	12.6
Amitriptyline	3.01	16.4
Nortriptyline	4.09	22.1

the method of changing the amount of stationary phase discussed previously[10]. Both an adjustment of V_s by using different concentrations of 1-pentanol in the mobile phase under equilibrium conditions, and a continuous change of V_s obtained during the coating procedure with 1-pentanol-saturated mobile phase, were used to evaluate the effects.

The samples were some closely related secondary and tertiary amines (see Table I), including a pair of *cis–trans* isomers. Cyclohexylsulphamate was used as the counter ion and the mobile phase also contained DMOA and TBA. The variation of the capacity ratios with the phase volume ratio on the column is shown in Fig. 5.

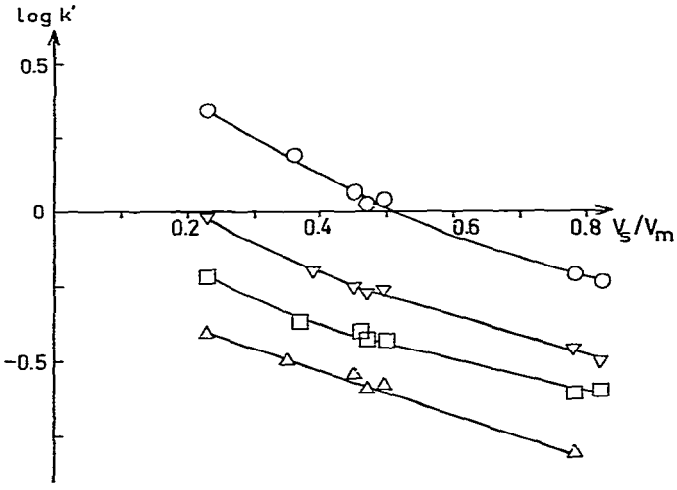


Fig. 5. Variation of the capacity ratios with the phase volume ratio on the column. Mobile phase: cyclohexylsulphamate 0.05 *M*, phosphate buffer (pH 2.21), $[H_2PO_4^-]$ 0.01 *M*, DMOA 0.01 *M*, $TBANaSO_4$ 0.03 *M*, saturated with 1-pentanol at phase volume ratios of 0.35–0.82, 50% saturated with 1-pentanol at a phase volume ratio of 0.23. Stationary phase: 1-pentanol. Support: LiChrosorb RP-8, 10 μm. Samples: ○, zimelidine (Z-isomer); ▽, chlorpheniramine; □, N-demethylated E-isomer of zimelidine; △, E-isomer of zimelidine.

The phase volume ratio, V_s/V_m, was calculated from the measured V_m and the known volume of liquid in the column ($V_s + V_m$) (ref. 10). Similar curves, but with lower capacity ratios, were obtained with bromide as the counter ion.

The capacity ratios decrease with increasing phase volume ratio, *i.e.*, increasing amount of stationary phase. This shows that the retention cannot follow the relationship

$$k' = (V_s/V_m)D \tag{4}$$

where D is the distribution ratio of the samples between the two liquid phases. Some retention mechanism other than distribution to 1-pentanol obviously dominates the retention of the amines. No studies have been made to test if both DMOA and TBA are necessary for the good chromatographic performance of the samples which was obtained (see Fig. 6).

With bromide and cyclohexylsulphamate as counter ions, different capacity ratios are obtained during the whole coating procedure. This indicates that an ion-pair retention, not due to a distribution to the stationary liquid phase, is taking place. Such effects may also be present in the systems with dihydrogen phosphate as the counter ion and mobile phases with added DMOA and TMNA, which were discussed above.

The selectivity seems to be independent of the content of stationary phase and peaks of excellent symmetry were obtained, as is obvious from Fig. 6. The *cis–trans* isomers are well resolved with a separation factor of 5.7. The secondary amine is more retarded than its N-methylated derivative, and the separation factor between

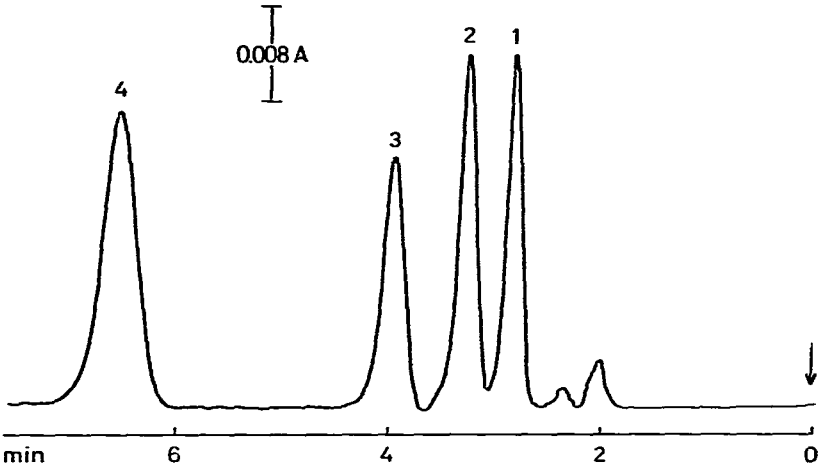


Fig. 6. Separation of cis–trans isomers. Mobile phase: cyclohexylsulphamate 0.05 *M*, phosphate buffer (pH 2.21), [$H_2PO_4^-$] 0.01 *M*, DMOA 0.01 *M*, $TBANaSO_4$ 0.03 *M*, 50% saturated with 1-pentanol, 1.2 mm/sec; 64 bar; V_m = 0.78 ml. Stationary phase: 1-pentanol, V_s/V_m = 0.23. Support: LiChrosorb RP-8, 10 μm. Peaks: 1 = E-isomer of zimelidine (0.29 nmole, 120 ng); 2 = N-demethylated E-isomer of zimelidine (0.26 nmole, 100 ng); 3 = chlorpheniramine (0.36 nmole, 140 ng); 4 = zimelidine (*Z*-isomer; 0.27 nmole, 110 ng).

them is 1.54. This is close to the values obtained for similar compounds such as desipramine and imipramine by distribution as chloride ion pairs with 1-pentanol as the stationary phase both in batch and on reversed-phase columns[2]. Organic phases of 1-pentanol and cyclohexane also give similar separation factors (1.5–1.8)[19,20].

The value of the separation factor for the secondary and tertiary ammonium ions indicates that the stationary phase, which in addition to 1-pentanol may retain the ion pairs, has properties similar to those of a 1-pentanol phase.

CONCLUSIONS

Hydrophobic ammonium ions behave anomalously in the reversed-phase ion-pair separation systems studied. It seems likely that this is associated with the hydrophobic and possibly also the surface-active nature of the samples and may be caused by interactions with the surface of the support.

The disturbances in the chromatographic behaviour can be eliminated by addition of long-chain tertiary or quaternary ammonium ions to the aqueous mobile phase.

Indications have been obtained of an ion-pair retention mechanism that does not involve the stationary liquid phase but possibly the hydrophobic surface of the support.

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