Journal of Chromatography, 189 (1980) 299–316

CHROM. 12,468

PEAK TAILING AND RETENTION BEHAVIOUR OF TRICYCLIC ANTIDEPRESSANT AMINES AND RELATED HYDROPHOBIC AMMONIUM COMPOUNDS IN REVERSED-PHASE ION-PAIR LIQUID CHROMATOGRAPHY ON ALKYL-BONDED PHASES

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(First received July 10th, 1979; revised manuscript received September 11th, 1979)

SUMMARY

Peak tailing of tricyclic antidepressant amines was studied on a range of alkyl-bonded silicas in reversed-phase ion-pair chromatographic systems. Mobile phases were mixtures of methanol and buffers containing phosphate or bromide as counter-ions.

Alkylammonium ions added to the eluent eliminated peak tailing and decreased the retention of the amines.

A retention model for ion-pair adsorption is used for evaluation of the results. It is found that the stationary phase contains adsorption sites with different ability to retain ammonium compounds and uncharged compounds.

INTRODUCTION

Liquid chromatography with photometric detection is well suited to the separation and determination of tricyclic antidepressant amines, *e.g.* in monitoring the parent drug and its metabolites in human serum. In reversed-phase liquid chromatography, where a mobile phase of high aqueous content is used, ion-pair chromatography[1] of amines can be performed in the acidic and neutral pH range where the available alkyl-bonded microparticulate silica materials used as solid phases are stable.

We have previously found that the reversed-phase ion-pair systems often result in the strong tailing of peaks of hydrophobic ammonium compounds and that this behaviour can be corrected by adding to the eluent organic ammonium compounds that block the disturbing adsorption sites[2,3]. Similar observations were made by Van der Maeden *et al.*[4] in reversed-phase chromatography on μBondapak C_{18} of some quaternary ammonium compounds, and they claim that residual silanol groups in the alkyl-bonded solid phase are responsible for the disturbing adsorption. Kraak and Bijster[5] obtained asymmetric peak shapes of tricyclic antidepressants with acidic mobile phases on Nucleosil C_{18} and LiChrosorb RP-8, but improvement was observed

when the samples were retained as bases at high pH in the presence of an amine in the eluent. Knox and Pryde[6] also observed good peak symmetry on SAS silica for similar compounds when an eluent containing ammonia and lauryl sulphate was used. Proelss *et al.*[7] obtained improved peak symmetry after addition either of an amine or of an organic anionic counter-ion (pentane sulphonate) on μBondapak C_{18} as solid phase. Twitchett and Moffat[8] also obtained badly tailing peaks on this solid phase for organic ammonium compounds in mobile phases of methanol and buffer. On the other hand, Brodie *et al.*[9] seem to obtain good peak symmetry in a similar system using Partisil ODS.

We have previously observed[3] that the retention of organic ammonium ions in ion-pair adsorption systems can be decreased by introducing in the mobile aqueous phase an alkylammonium ion, which will compete with the sample ammonium ions for the adsorption sites[1]. This will improve the chances of regulating retention in ion-pair chromatography.

This paper examines the role of alkyl-bonded silicas for the peak symmetry of hydrophobic ammonium compounds retained as ion-pairs with phosphate and bromide in reversed-phase systems using methanol and buffer in the mobile phase. The effect of the addition of alkylammonium ions as tail reducers is discussed, and the regulation of the retention of cationic solutes by the anionic counter-ion and by an alkylammonium ion is evaluated .

EXPERIMENTAL

Apparatus

The pump was an LDC Model 711-47 solvent delivery system (Milton-Roy Minipump with pulse dampener) and the detector was an LDC Model 1205 UV Monitor used at 254 nm wavelength. A Valco CV-6-HPax sample injection valve (for 3000 p.s.i.) with a 10.1-μl loop was used.

The columns were of stainless steel with a polished inner surface, equipped with modified Swagelok connectors and Altex stainless-steel frits (2 μm) and were 10 cm × 4.5 mm I.D., unless otherwise stated.

A water-bath, HETO type 02 PT 923 TC (Birkerød, Denmark), was used to thermostat the chromatograph, and the pH was measured with an Orion Research Model 801 A/digital pH meter equipped with an Ingold combined electrode type 401.

Chemicals and reagents

Methanol was of Merck (Darmstadt, G.F.R.) p.a. quality.

Tetramethylammonium bromide (TMABr) from Merck, N,N,N-trimethylnonylammonium bromide (TMNABr) and tetrabutylammoniumiodide (TBAI) from Eastman-Kodak (Rochester, N.Y., U.S.A.) were converted into the hydroxides by shaking their aqueous solutions with silver oxide.

N,N-Dimethyloctylamine (DMOA) was either obtained from K & K Labs. (Plainview, N.Y., U.S.A.) and distilled, or synthesized by a modified Eschwailer–Clarke reaction[10] and distilled. N,N-Dimethylethylamine (DMEA) was synthesized by the same method as DMOA. No impurities could be found in the DMOA or the DMEA when examined by IR and NMR spectroscopy.

N,N-Dimethylcyclohexylamine (DMCHA) puriss, was from Fluka (Buchs,

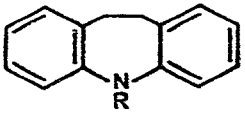
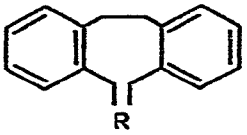
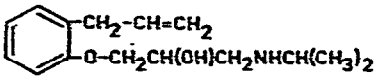
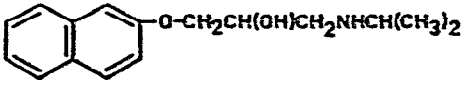
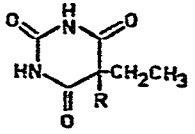
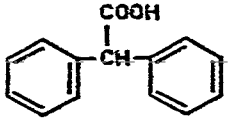
Switzerland). Triethylamine (TrEA) zur Synthese and tripropylamine (TrPrA) zur Synthese were from Merck-Schuchardt (München, G.F.R.).

Amines, quaternary ammonium compounds and acids used as chromatographic samples are listed in Table I. They were of Pharmacopoeial grade. Alprenolol and imipramine were supplied by Hässle (Mölndal, Sweden), desipramine by Ciba-Geigy (Basel, Switzerland), trimipramine by Leo (Helsingborg, Sweden), nortriptyline by Pharmacia (Uppsala, Sweden), amitriptyline by Merck Sharp & Dohme (Rahway, N.J., U.S.A.) and propranolol by I.C.I. (Macclesfield, Great Britain). N-Methylimipramine was kindly supplied by Dr P.-O. Lagerström. It can be synthesized from imipramine chloride and obtained as the phosphate[11]. N-Methylamitriptyline bromide was synthesized from amitriptyline chloride as described by Borg[12].

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

TABLE I

LIST OF SAMPLES

Formula	*Name*	*R*
	desipramine	$-(CH_2)_3NHCH_3$
	imipramine	$-(CH_2)_3N(CH_3)_2$
	trimipramine	$-CH_2CH(CH_3)CH_2N(CH_3)_2$
	N-methylimipramine	$-(CH_2)_3\overset{\ominus}{N}(CH_3)_3$
	nortriptyline	$=CH(CH_2)_2NHCH_3$
	amitriptyline	$=CH(CH_2)_2N(CH_3)_2$
	N-methylamitriptyline	$=CH(CH_2)_2\overset{\ominus}{N}(CH_3)_3$
	alprenolol	
	propranolol	
	barbital	$-CH_2CH_3$
	amobarbital	$-(CH_2)_2CH(CH_3)_2$
	diphenylacetic acid	

Column preparation and use

The solid phases used are listed in Table II. They were packed in the column by the balance-density slurry packing technique described by Majors[13]. LiChrosorb RP-8 and Spherisorb ODS were suspended in tetrachloroethane and after packing the columns were washed with hexane and acetone. μBondapak C_{18} and ODS-Hypersil were suspended in chloroform and the columns were washed with methanol. Nucleosil C_8 and Nucleosil C_{18} were suspended in chloroform but washed with hexane and acetone. LiChrosorb RP-18 was both packed and washed with ethanol.

TABLE II

SOLID PHASES USED

Name	*Manufacturer*	*Batch no.*	*Nominal particle diameter (μm)*
LiChrosorb RP-8	Merck (Darmstadt, G.F.R.)	VV 1535	5
LiChrosorb RP-18		VV 1524	5
Nucleosil C_8	Macherey-Nagel (Düren, G.F.R.)	8031	5
Nucleosil C_{18}		8051	5
ODS-Hypersil	Shandon Southern Products (Cheshire, Great Britain)	6 A 512	5
μBondapak C_{18}	Waters Assoc. (Milford, Mass., U.S.A.)	36 C 3 R	10
Spherisorb ODS	Phase Separations (Queensferry, Clwyd, Great Britain)	MH 14/141	5

After washing, an eluent containing methanol and water (6:4) was pumped through the column. About 30 column volumes were needed to get a stable baseline for all packings, with the exception of Nucleosil C_{18} which needed about 200 column volumes.

All columns were tested with the methanol–water eluent before use. The solutes used for the testing were phenol, 2-phenylethanol, 2,6-dimethylphenol and 2,3,5-trimethylphenol. Columns having a reduced plate height, $h = H/d_p$, less than 10 at a flow-rate of 1 mm/sec and asymmetry factors less than 2 were accepted for this study. To measure the asymmetry factor (ASF), a perpendicular was drawn from the vertex, formed by the two peak tangent lines, to the base-line. The back part of the peak base-line divided by the front part gives the ASF.

All columns were checked for constant sample retention throughout a series of experiments. In quantitative evaluations of the retention on μBondapak C_{18}, data were only included which were obtained at relatively low concentrations of the ammonium additive, DMOA. Higher concentrations brought about a change of the bonded material, which was revealed by an increase of retention and decrease of separation selectivity of cationic samples.

The presence of some alkylammonium additives in the eluent caused a slow increase in the pressure drop when LiChrosorb RP-8 was used as solid phase. Also voids occurred in the top of the column packing, which had to be filled up. After a while the pressure drop reached such a level that the column had to be discarded. Using a buffer of pH 2, the column life-time was *ca.* 10 days when eluents containing the quaternary ammonium compounds TMNA and TMA were used. In the presence of the tertiary ammonium compound DMEA the column lasted 5 weeks. When the

tertiary ammonium additive DMOA was used with a buffer of pH 3 the column lifetime was much longer: 2.5 months.

Chromatographic technique

The chromatograph was thermostated by circulating water taken from a water-bath kept at 25.0 ± 0.1°. The eluent reservoir was kept in the thermostated water-bath, which also thermostated the separation column by pumping the water through a glass-jacket mounted on the column. The pump was thermostated by pumping the water through a pump-head with built-in channels. Also the tubings from the reservoir to the pump and from the pump to the injector valve were thermostated by circulating water through jackets made of PVC-tubing mounted with Swagelok heat-exchanger tees.

The columns were equilibrated with 65 ml of eluent. After that, the eluent was recycled in a volume of *ca.* 250 ml.

The samples were dissolved in 1:1 methanol–phosphate buffer (pH 2 or 3) and the injected volume was 10.1 μl.

The hold-up volume of the column, V_m, used in measurements of capacity ratios was determined from the peak obtained when phosphate buffer (pH 2 or 3), methanol or sodium nitrate were injected. Sodium nitrate is preferred, but in cases when the eluent contained alkylammonium ions, a sample of methanol or phosphate buffer with the same pH as the eluent was used.

The capacity ratio, k', was measured from the retention volume, V_R, at the peak maximum by $k' = (V_R - V_m)/V_m$.

Preparation of the eluent

The eluent was prepared by mixing equal volumes of methanol and phosphate buffer (pH 2 or 3) of ionic strenght 0.10 and $H_2PO_4^-$ concentration of 0.10 M, if not otherwise stated. The phosphate buffers were prepared by mixing phosphoric acid with either sodium hydroxide or sodium dihydrogen phosphate. When the eluent contained alkylammonium ions an equivalent amount of the sodium hydroxide was exchanged for tetraalkylammonium hydroxide or amine in order to keep the ionic strength constant.

Mixing the buffers with methanol results in a volume decrease of 3.3%, for which the actual concentration of species in the eluent was corrected. The actual concentration of $H_2PO_4^-$ in the eluent was not exactly known because the dissociation constant of phosphoric acid in a medium of 50% methanol is not available.

RESULTS AND DISCUSSION

Peak tailing on different solid phases

In a previous study[2] we showed that when tricyclic antidepressant drugs were chromatographed as phosphate ion-pairs in a liquid–liquid system with pentanol as the stationary phase, strong tailing of peaks was observed. This was ascribed to an interaction with the support material, which was LiChrosorb RP-8. When this material was used as stationary phase in the present liquid–solid phase system with methanol–phosphate buffer as eluent similar results were obtained. Seven commercially available hydrophobic solid phases were thus tested, including two octyl-derivatized and five octadecyl-derivatized silicas (Table III). The solutes chromato-

TABLE III

RETENTION AND ASYMMETRY ON DIFFERENT SOLID PHASES

Eluent: 1:1 methanol–phosphate buffer (pH 3). C^0 = Sample concentration.

Solutes	$C^0 \cdot 10^4$	*Nucleosil* C_8, *5 μm*		*LiChrosorb RP-8, 5 μm*		*μBondapak* C_{18}, *10 μm*		*Nucleosil* C_{18}, *5 μm*		*ODS-Hypersil, 5 μm*		*LiChrosorb RP-18, 5 μm*		*Spherisorb ODS, 5 μm**	
		k'	*ASF*	*k'*	*ASF*	*k'*	*ASF*	*k'*	*ASF*	*k'*	*ASF*	*k'*	*ASF*	*k'*	*ASF*
Desipramine	3.9–4.1	3.79	2.8	8.24	3.6	3.81	1.5	12.2**	3.1	12.5	5.93	17.3	4.4	39.9	3.3
Imipramine	3.6–3.7	3.60	2.2	8.00	5.6	3.43	1.5	11.4	3.6	13.6	5.9	20.8	3.6	79.6	2.7
Trimipramine	3.3–3.9	4.54	1.9	10.3	4.4	4.51	1.4	15.1	3.3	17.3	3.7	29.5			
N-Methylimipramine	3.4	9.16	2.9	6.69	6.1	3.05	1.6	9.56	6.0	12.5	5.0	18.4	5.4	>150	
Propranolol	16–17	1.61	3.0	2.75***	2.9	1.54	1.5	3.98	3.3	3.23	4.7	3.54	4.2	9.64	3.5
Amobarbital	39	3.06	1.4	4.21	1.3	2.70	1.3	6.94	1.2	4.23	2.0	4.72	1.7	3.29	1.2
Diphenylacetic acid	20–23	5.74	1.4	8.38†	1.3	4.82	1.4	15.7	1.4	8.72	1.9	10.3	1.6	8.11	1.2

* Column 150 × 4.5 mm I.D.

** $C^0 = 8.4 \cdot 10^{-4}$.

*** $C^0 = 7.1 \cdot 10^{-4}$.

† $C^0 = 9.4 \cdot 10^{-4}$.

graphed were five ammonium compounds (secondary, tertiary and quaternary) and, for comparison, two weak acids, amobarbital and diphenylacetic acid. The ammonium compounds are retained in cationic form whereas the acids are predominantly retained in uncharged form at the prevailing pH.

Good peak symmetry of the acids is obtained on all solid phases whereas the ammonium compounds have acceptable symmetry (ASF < 2) only on one of them (μBondapak C_{18}). This solid phase also gives the lowest retention of the cationic solutes. The other solid phases cause strong tailing and high retention of the ammonium compounds. It is clear that the tailing effect is selective for the cationic compounds as the uncharged solutes give symmetrical peaks even though the samples are about 10 times more concentrated than most of the ammonium compounds.

Addition of alkylammonium ions to reduce tailing

In the previous study[2] peak tailing was eliminated by adding to the eluent a long-chain ammonium compound such as DMOA or TMNA. In the present case, with methanol–phosphate buffer as the eluent, peak symmetry was greatly improved when 0.05 *M* DMOA was added (compare Tables III and IV). Chromatograms in Fig. 1 illustrate the gain in resolution obtained after addition of DMOA to the eluent when using ODS-Hypersil as solid phase.

TABLE IV

RETENTION AND SYMMETRY AFTER ADDITION OF DMOA TO THE ELUENT

Eluent: 0.050 *M* DMOA in 1:1 methanol–phosphate buffer (pH 3).

Solute	$C^{\circ} \cdot 10^4$	*LiChrosorb RP-8, 5 µm*		*µBondapak C_{18}, 10 µm*		*ODS-Hypersil, 5 µm*		*LiChrosorb, RP-18, 5 µm*		*Spherisorb* ODS, 5 µm*	
		k'	*ASF*	*k'*	*ASF*	*k'*	*ASF*	*k'*	*ASF*	*k'*	*ASF*
Desipramine	3.9	2.04	1.3	1.46	1.4	2.53	1.8	2.54	1.7	2.68	1.3
Imipramine	3.6	1.76	1.2	1.26	1.5	2.17	1.7	2.14	1.5	3.43	1.1
Trimipramine	3.5–3.9	2.28	1.1	1.57	1.3	2.82	1.6	2.78	1.6	4.15	1.1
N-Methylimipramine	3.4	1.00	1.3	0.90	1.5	1.29	2.0	1.23	1.8	3.70	1.5
Propranolol	16–17	0.69	1.3	0.49	1.5	0.79	2.8	0.77	2.0	0.93	1.4
Amobarbital	39	3.30	1.2	2.15	1.5	3.41	1.9	3.56	1.6	2.36	1.6
Diphenylacetic acid	9.4–20	9.91	1.1	5.02	1.4	11.7	1.9	12.1	1.7	10.9	1.5

* Column 150 × 4.5 mm. I.D.

We investigated the effect of a range of tertiary and quaternary ammonium additives having different types of alkyl groups, including different alkyl chain lengths. The study was made on LiChrosorb RP-8 and the results are summarized in Fig. 2, where the ASFs, obtained after addition of the different ammonium compounds to the eluents, are plotted.

Bulky additives with several carbons in each alkyl group, such as tetrabutyl- and tripropylammonium, gave little or no improvement of the symmetry, but a clear improvement is seen for the others which are characterized by two or more short alkyl substituents. N,N-Dimethyl substituents in the tertiary ammonium compounds and N,N,N-trimethyl substituents in the quaternary ammonium compounds seem to be especially effective for reducing tailing. This is obvious when comparing the

Fig. 1. Comparison of peak shapes in the absence and presence of DMOA in the eluent. Solid phase: ODS-Hypersil, 5 μm. Peaks: 1 = N-methylimipramine ($3.37 \cdot 10^{-4}$ *M*); 2 = imipramine ($3.28 \cdot 10^{-4}$ *M*); 3 = desipramine ($3.32 \cdot 10^{-4}$ *M*); 4 = trimipramine ($3.34 \cdot 10^{-4}$ *M*). (a) Eluent: 1:1 methanol–phosphate buffer (pH 2.84); 1.11 mm/sec; 34 bars. (b) Eluent: DMOA 0.05 *M* in 1:1 methanol–phosphate buffer (pH 2.84); 1.11 mm/sec; 38 bars.

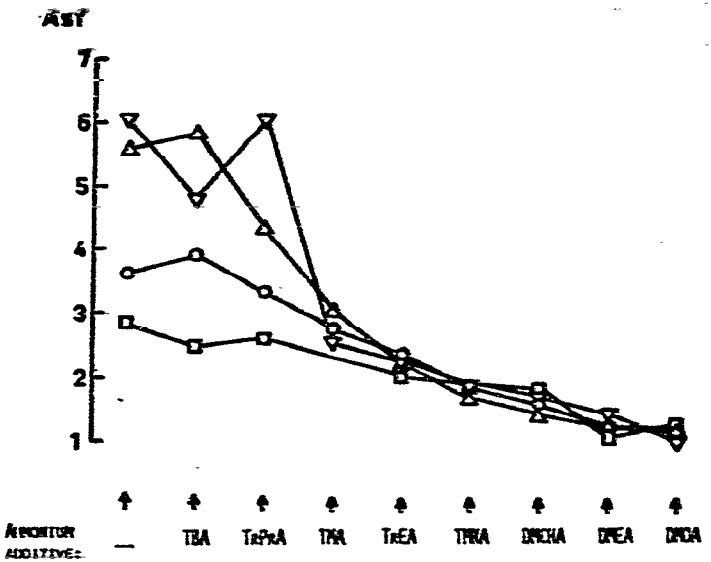


Fig. 2. Peak asymmetry of ammonium compounds in the presence of different ammonium additives in the eluent. Additives: TBA = tetrabutylammonium; TrPrA = tripropylammonium; TMA = tetramethylammonium; TrEA = triethylammonium; TMNA = trimethylnonylammonium; DMCHA = dimethylcyclohexylammonium; DMEA = dimethylethylammonium; DMOA = dimethyloctylammonium. Eluent: 0.05 *M* additive in 1:1 methanol–phosphate buffer pH 2.0–3.3; (exceptions: TMA and TMNA 0.03 *M*). Solid phase: LiChrosorb RP-8, 5 μm. Samples: ▽, N-methylimipramine ($3.37 \cdot 10^{-4}$ *M*); △, imipramine (($3.64 - 3.80) \cdot 10^{-4}$ *M*); ○, desipramine (($3.51 - 4.05) \cdot 10^{-4}$ *M*); □, propranolol ($15.8 \cdot 10^{-4}$ *M* but $7.09 \cdot 10^{-4}$ *M* for DMOA, DMEA and no additive).

effects of DMOA and DMCHA with tripropylammonium, which only slightly improves symmetry. This structure-dependent effect is also indicated in the chromatographed samples: propranolol, which is a secondary ammonium compound with two bulky substituents, in contrast to the other cationic samples, tails only moderately even though it is applied in four times higher concentration.

The total number of methylene groups in the ammonium additives is of little importance for the tail-reducing effect. This is obvious when comparing the effect of DMEA with that of DMOA or TMA with TMNA. The differences are small.

Only the broad trends in Fig. 2 have been discussed as the concentrations of additives and samples are slightly different.

Influence of the concentration of ammonium additives on tailing

Increasing the concentration of an additive is found to decrease the ASF, as illustrated in Fig. 3 for addition of DMEA. Three cationic samples, desipramine, imipramine and N-methylimipramine, and one uncharged compound are included. Acceptable peak symmetry (ASF < 2) was obtained using 0.05 *M* DMEA in the eluent. Addition of DMOA gave ASFs < 2 for the same substances already at a concentration of 0.03 *M* (lower concentrations were not investigated), which shows that DMOA is a more effective tail-reducing additive than DMEA.

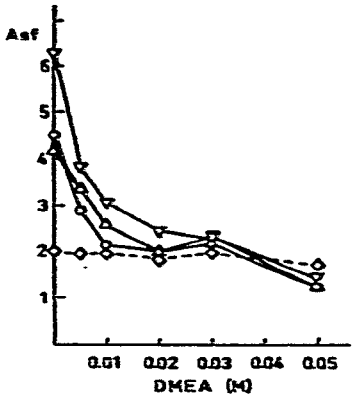


Fig. 3. Effect of the concentration of DMEA on peak asymmetry. Eluent: DMEA in 1:1 methanol–phosphate buffer (pH 2.0). Solid phase: LiChrosorb RP-8, 5 μm. Samples: ∇, N-methylimipramine ($3.37 \cdot 10^{-4}$ *M*); $\triangle$, imipramine (($3.64–3.80) \cdot 10^{-4}$ *M*); $\bigcirc$, desipramine (($3.51–3.98) \cdot 10^{-4}$ *M*); $\Diamond$, barbital ($1.57 \cdot 10^{-2}$ *M*).

Sample concentration effects

The influence of the sample concentration of the cationic solutes on their peak symmetry is illustrated in Fig. 4 with imipramine as the sample. The role of the solid phase (LiChrosorb RP-8 and μBondapak C_{18}) and of the ammonium compounds added to the eluent can also be studied in the figure. When no additive is present the symmetry is acceptable on μBondapak C_{18}, but only up to a sample concentration of 1×10^{-3} *M*. At higher concentrations peaks start to tail considerably even on this solid phase. The presence of 0.03 *M* DMOA in the eluent results in symmetrical peaks on both solid phases even at the highest sample concentration. It is seen, as already noted above, that DMOA is more effective in reducing tailing than DMEA.

Further illustration of the sample concentration effect is given in Fig. 5 where the capacity ratios (measured on the peak maximum) are plotted as a function of the

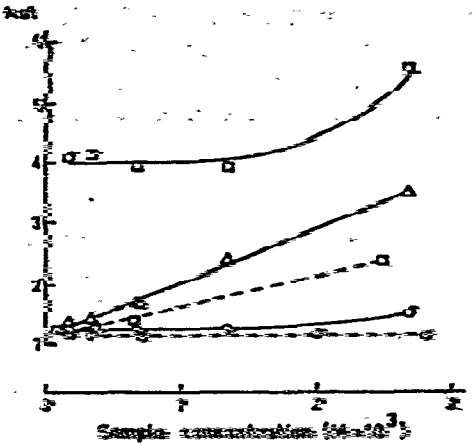


Fig. 4. Peak asymmetry factor of imipramine at different sample concentration. Eluent: 1:1 methanol–phosphate buffer (pH 2 or 3). Solid phases: ———, LiChrosorb RP-8, 5 μm (column 150 × 4.5 mm I.D.); - - -, μBondapak C_{18}, 10 μm. Ammonium additives: □, no additive; ○, DMOA 0.03 *M*; △, DMEA 0.03 *M*.

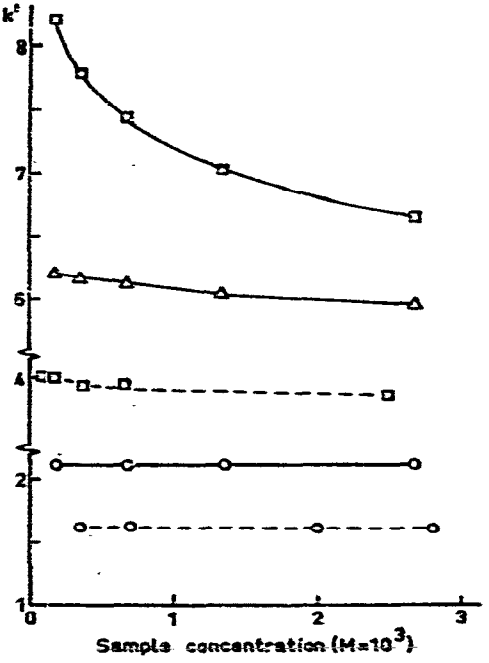


Fig. 5. Retention of imipramine at different sample concentrations. Conditions as in Fig. 4.

initial sample concentration. As expected, capacity ratios strongly depend on the sample concentration on LiChrosorb RP-8, whereas on μBondapak C_{18} retention is only slightly influenced. Addition of DMOA in the eluent gives constant retention on both solid phases.

Ion-pair retention

Retention model. In the chromatographic systems where alkyl-bonded silicas are used in combination with eluents composed of water and a water-miscible solvent such as acetonitrile or methanol, it may be appropriate to regard the stationary phase as an adsorbing surface. This approach has given a useful retention model for chromatography of carboxylate anions as ion-pairs with quaternary alkylammonium ions in acetonitrile systems[14].

In the present study, where cationic samples are chromatographed, the adsorption equilibrium is expressed by

$$Q_m^+ + Z_m^- + A_s \rightleftharpoons QZA_s$$

where Q_m^+ is a cationic sample present in the mobile phase and Z_m^- is a counter-ion present in the buffer of the mobile phase, in the present case phosphate ion. The species in the mobile phase are distributed to the stationary phase where they, as an ion-pair QZA_s, occupy an area called an adsorption site, A_s. The equilibrium constant for the process is given by

$$K_{QZ} = \frac{[QZA]_s}{[Q^+]_m\,[Z^-]_m\,[A]_s} \tag{1}$$

where concentrations in the mobile phase are given in moles/l and concentrations in the stationary phase are expressed in moles/g of solid phase. $[A]_s$ is the concentration of free adsorption sites in moles/g of solid phase at equilibrium.

The capacity ratio of Q^+ depends on the phase-ratio, W_s/V_m, and the distribution ratio, $[QZA]_s/[Q^+]_m$, according to

$$k'_Q = \frac{W_s}{V_m} \cdot \frac{[QZA]_s}{[Q^+]_m} \tag{2}$$

where the phase-ratio is the ratio of solid phase to mobile phase in the column expressed in g/l. Combination with eqn. 1 gives

$$k'_Q = \frac{W_s}{V_m} \cdot K_{QZ}[Z^-]_m\,[A]_s \tag{3}$$

The stationary phase has a limited adsorption capacity which is characterized by K_0 according to

$$K_0 = [A]_s + [QZA]_s \tag{4}$$

A combination of eqns. 1–4 gives

$$k'_Q = \frac{W_s\,K_0\,K_{QZ}\,[Z^-]_m}{V_m\,(1 + K_{QZ}\,[Q^+]_m\,[Z^-]_m)} \tag{5}$$

At sufficiently high sample concentrations, $[Q^+]_m$, the capacity ratio will decrease with increasing sample concentration resulting in tailing peaks (the adsorption isotherm becomes non-linear)[14].

If a cationic species B^+, *e.g.* one of the alkylammonium additives, is present in the eluent it may also be distributed to the stationary phase as ion-pair with Z^-:

$$B_m^+ + Z_m^- + A_s \rightleftharpoons BZA_s$$

The adsorption capacity will now be given by

$$K_0 = [A]_s + [QZA]_s + [BZA]_s \quad (6)$$

Then the capacity ratio of Q^+ will be given by

$$k'_Q = \frac{W_s K_0 K_{QZ} [Z^-]_m}{V_m (1 + K_{QZ} [Q^+]_m [Z^-]_m + K_{BZ} [B^+]_m [Z^-]_m)} \quad (7)$$

which indicates how the capacity ratio is influenced by the presence in the mobile phase of B^+, which competes with the sample for the adsorption sites.

Peak tailing effects. The competing effect of B^+ on the distribution of the sample to the adsorption sites can be used to decrease sample peak tailing because it decreases the influence of the sample concentration in the denominator of eqn. 7. This effect was demonstrated on the different solid phases (see Fig. 3 and Table IV). However, there are several indications that the tailing observed is not due to retention on a homogeneous stationary phase with one single type of adsorption sites, as is assumed in eqn. 7. Fig. 6 shows the influence on the retention (on LiChrosorb RP-8) of increasing the concentration of DMEA in the mobile phase. In the region where the cationic solutes tail (*cf.* Fig. 3) their retention decreases rapidly with increasing concentration of DMEA, but at the higher concentrations the retention levels off and does not seem to approach zero as would be the case if eqn. 7 were valid.

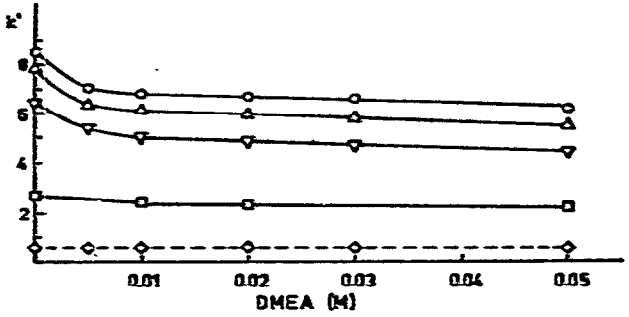


Fig. 6. Effect of the concentration of DMEA on retention. Conditions as in Fig. 3. Samples: □, propranolol (7.09×10^{-4} *M*); otherwise as in Fig. 3.

Further, it can be shown that the sample concentration applied to the LiChrosorb RP-8 column (Table III) is probably too low to cause sample overloading of a homogeneous stationary phase. This argument is based on the following discussion. The sample concentration in the mobile phase, $[Q^+]'_m$, that causes noticable peak asymmetry may be defined as that which gives a decrease of the capacity ratio of 10% from its value, k'^0_Q, at infinitely low sample concentration (*cf.* eqn. 5), *i.e.* a decrease of the distribution ratio by the same extent (see eqn. 2). By combination of eqns. 2, 3 and 4 the corresponding sample concentration in the stationary phase, $[QZA]'_s$, will be given by

$$[QZA]'_s = 0.1 K_0 \quad (8)$$

while the sample concentration in the mobile phase is given by

$$[Q^+]'_m = \frac{W_s}{V_m} \cdot \frac{0.1\,K_0}{k'_Q} \qquad (9)$$

where $k'_Q = 0.9 \cdot k'^0_Q$.

A calculation of $[Q^+]'_m$ on the trimipramine peak obtained on LiChrosorb RP-8 (Table III) was made using a K_0 value of $6 \cdot 10^{-5}$ moles/g, which was found for the adsorption of tetrabutylammonium on LiChrosorb RP-8[14]. The observed peak maximum k' value of 10 was used, as k'^0_Q could not be measured, and thus only an approximate value for $[Q^+]'_m$ of $4 \cdot 10^{-4}$ M was obtained. The initial sample concentration is even lower than this, which means that peak tailing should not occur. Therefore, the drastic peak tailing yet observed is probably due to a heterogeneous composition of the stationary phase with more than one type of adsorption sites where the disturbing sites give relatively high equilibrium constants.

Two-site adsorption model. Even the result obtained on μBondapak C_{18}, on which the cationic samples gave symmetrical peaks (Table III), indicates that the stationary phase has a heterogeneous composition. The retention of ammonium compounds and an uncharged acid was studied as a function of the concentration of an alkylammonium additive (DMOA) in the mobile phase. These results are presented in Fig. 7. As in Fig. 6 the retention of the cationic solutes decreases with increasing concentration of DMOA and seems to level off at the higher concentrations, whereas the weak acid amobarbital ($pK_a \approx 8$) shows a very small decrease. This indicates that there may be two kinds of retaining phases (adsorption sites), one on which amobarbital is predominantly retained and on which DMOA has an almost insignificant competing effect, and a second on which DMOA competes with the cationic samples. These may be retained on the first type of site as well.

In an attempt to explain the retention of the cations we assume that they are retained on two types of site according to the following equation:

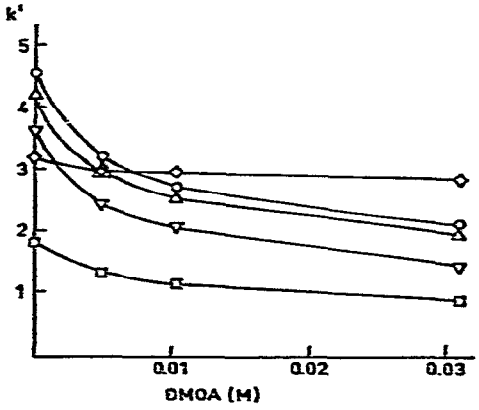


Fig. 7. Regulation of retention by DMOA on μBondapak C_{18}. Eluent: DMOA in 1:1 methanol–phosphate buffer (pH 3.0). Solid phase: μBondapak C_{18}, 10 μm (column 150 × 4.5 mm I.D.). Samples: ○, desipramine; △, imipramine; ▽, N-methylimipramine; □, propranolol; ◇, amobarbital.

$$k'_Q \cdot V_m = W_s K_0 K_{QZ}[Z^-]_m + \frac{W_s K_0^* K_{QZ}^* [Z^-]_m}{1 + K_{BZ}^* [B^+]_m [Z^-]_m} \quad (10)$$

The first term comes from the retention ($k'_{Q,(A)}$) on the sites, A, that are unaffected by DMOA and the second term from the retention ($k'_{Q,(A^*)}$) on the DMOA-influenced sites, A*. The equation is derived analogously to eqns. 5 and 7, omitting the term containing the sample concentration because peaks are symmetrical. K_0, K_{QZ}, K_0^*, K_{QZ}^* and K_{BZ}^* are the respective adsorption capacities and equilibrium constants on the two sites. V_m was incorporated in the dependent variable because it decreased slightly (from 1.58 to 1.48 ml) with increasing DMOA concentration ($[B^+]_m$). As the experiments were performed at constant phosphate concentration the equation can be simplified to

$$k'_Q \cdot V_m = a + \frac{b}{1 + K_{BZ}^* [B^+]_m [Z^-]_m} \quad (11)$$

where $a = W_s K_0 K_{QZ}[Z^-]_m$ and $b = W_s K_0^* K_{QZ}^* [Z^-]_m$ and then further rearranged and inverted to

$$\frac{1}{k'_Q \cdot V_m - a} = \frac{1}{b} + \frac{K_{BZ}^* [Z^-]_m}{b} \cdot [B^+]_m \quad (12)$$

For each cationic solute the value of a was fitted to give a straight line for a plot of the left-hand side *versus* $[B^+]$. After an initial visual test of linearity, the fit that gave the smallest residual deviation in a least squares regression was chosen, and Fig. 8 shows some examples of such plots. As can be seen the linearity was good. From each line the values of $K_0 K_{QZ}[Z^-]_m$, $k'_{Q,(A)}$, $K_0^* K_{QZ}^* [Z^-]_m$ and $K_{BZ}^* [Z^-]_m$ were calculated from

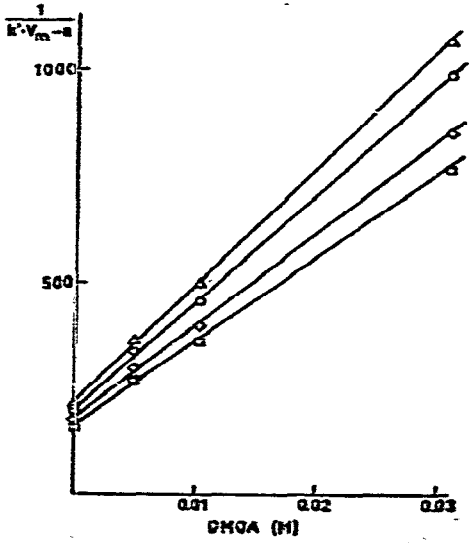


Fig. 8. Examples of plots of eqn. 12. Conditions as in Fig. 7. Samples: △, imipramine; ○, desipramine; ◇, N-methylamitriptyline; □, trimipramine.

the slope and the intercept and the results are summarized in Table V. The value of $K^*_{BZ}[Z^-]_m$, which contains the equilibrium constant for the adsorption of DMOA, and thus is obtained from the chromatographic retention of different samples, shows excellent constancy which supports the validity of the retention model (eqn. 10). Preliminary results which indicate a substantial adsorption of DMOA on the solid phases used in this study are in line with this model, but should be extended to the independent determination of $K^*_{BZ}[Z^-]_m$ from the adsorption isotherm of DMOA to confirm the validity of the model.

TABLE V

EVALUATION OF RETENTION OF AMMONIUM COMPOUNDS

Conditions as in Fig. 7*.

Solute	$K_o K_{QZ}[Z^-]_m \cdot 10^3$	$k_{Q,(A)}$**	$K^*_o K^*_{QZ}[Z^-]_m \cdot 10^3$	$K^*_{BZ}[Z^-]_m$
Desipramine	2.07	1.40	4.98	127
Imipramine	1.91	1.29	4.59	126
N-Methylimipramine	1.15	0.78	4.38	118
Trimipramine	2.09	1.41	6.16	121
Nortriptyline	2.41	1.63	6.42	129
Amitriptyline	2.17	1.47	5.92	126
N-Methylamitriptyline	1.41	0.95	5.60	122
Propranolol	0.89	0.60	1.94	131

* $W_s = 1.0$ g.

** Calculated for $V_m = 1.48 \cdot 10^{-3}$ l.

On both types of site the selectivity for separation of a tertiary ammonium compound from its N-demethylation product (secondary ammonium compound) is rather low and of the same magnitude. This is illustrated in Fig. 9, where the calculated capacity ratios on the two sites are plotted for some of the compounds studied. From the logarithmic representation the differences in selectivity between the two sites can be easily observed. The most remarkable difference is seen for the quaternary ammonium compound relative to the secondary and tertiary ammonium compounds. This might indicate that one of the sites has hydrogen-accepting properties as the possibility of hydrogen bonding is the main difference between the differently substituted ammonium compounds.

The reason for the heterogeneity of the stationary phase may be the presence of residual silanol groups. It is well known that different ways of preparing alkyl-bonded phases, *e.g.* by using mono-, di- or trichlorosilanes and having different silica gels as starting material, can lead to unequal degrees of coating of the silanol groups and that there will always be a certain amount of residual silanol groups[15]. Depending on the silanization reagent and the reaction conditions they will be more or less accessible for solute interaction[16–18]. The tail-reducing effect of the different alkylammonium additives discussed above, among which it was found that bulky derivatives were less effective, indicate that steric effects are important for the interaction with the disturbing sites.

Regulation of the retention by the counter-ion (bromide). The ion-pair retention model assumes that the retention of the cationic solutes can be regulated by con-

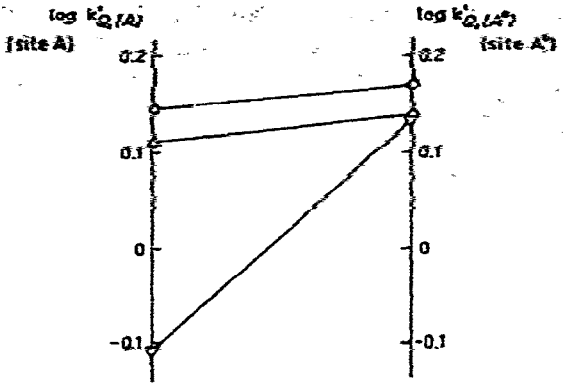


Fig. 9. Comparison of selectivity on the two sites. Data taken from Table V. Samples: ○, desipramine; △, imipramine; ▽, N-methylimipramine.

trolling the concentration of the counter-ion in the mobile phase. In the previous discussion this variable was not studied because the counter-ion, dihydrogen phosphate, was held at constant concentration. The influence of the counter-ion was, however, briefly studied using bromide (added as sodium bromide to the phosphate buffer) and with LiChrosorb RP-8 as the solid phase. The results are shown in Fig. 10. DMOA was added to reduce tailing.

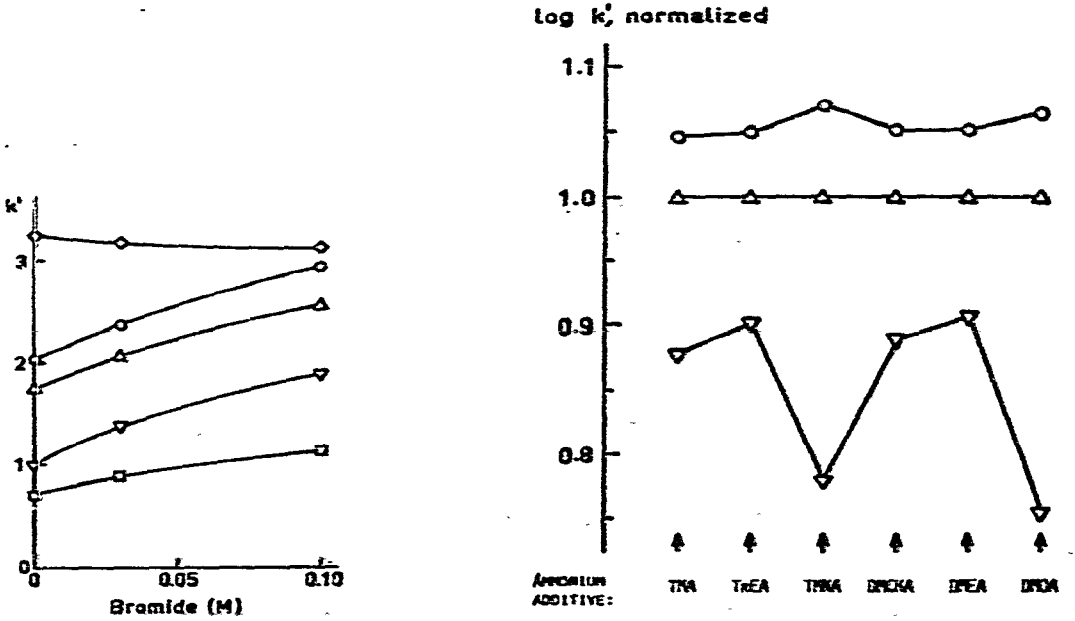


Fig. 10. Regulation of the retention of ammonium compounds and an uncharged compound by bromide counter-ion. Eluent: NaBr in 1:1 methanol–phosphate buffer (pH 2.9) containing 0.05 *M* DMOA. Solid phase: LiChrosorb RP-8, 5 μm. Samples: ◇, amobarbital; ○, desipramine; △, imipramine; ▽, N-methylimipramine; □, propranolol.

Fig. 11. Separation selectivity with different ammonium additives in the eluent. Conditions as in Fig. 7. Samples: ○, desipramine; △, imipramine; ▽, N-methylimipramine.

In line with the model, there is a steady increase of the retention of the cations, although with a slightly decrasing slope. The uncharged compound is almost unaffected. This indicates that the phase on which the sample bromide ion-pairs are retained may be only slightly influenced by a competing adsorption of DMOA–Br ion-pairs (*cf.* eqn. 10).

The practical value of using bromide to regulate the capacity ratio of the cations is limited owing to the low slopes of the curves. This is in contrast to a partition chromatographic system using a bulk liquid, pentanol, as the stationary phase[2] where a much higher change in retention was obtained for the same substances and with bromide as counter-ion. Use of more hydrophobic, organic, anions as counter-ions in the present system will probably be more useful.

Separation selectivity; effect of the type of ammonium additive and solid phase

The separation selectivity on LiChrosorb RP-8, obtained after addition of the different alkylammonium compounds to the eluent (to avoid tailing), was studied for the substances desipramine, imipramine and N-methylimipramine, which have different degrees of substitution on the ammonium group. The conditions were the same as in Fig. 2, but only phase systems giving ASFs less than 3 were included. The results are summarized in Fig. 11, which gives the logarithm of the capacity ratios normalized to log $k' = 1$ for imipramine. Normalization rendered the comparison between the different phase systems easier, as the degree of retention was very different.

Only minor differences in separation factor occur between the secondary and the tertiary ammonium compounds, but the separation factor for the quaternary relative to the others changes notably. The highest separation factor is obtained in those phase systems that contain the long-chain TMNA and DMOA in the mobile phase. The logarithm of the separation factor between imipramine and N-methylimipramine is found to be 0.25 in the DMOA system. A very similar value, 0.26, was

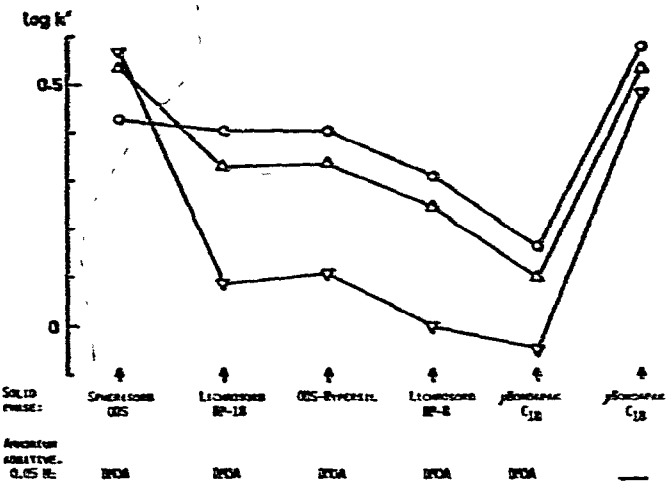


Fig. 12. Separation selectivity on different solid phases. Data taken from Tables III and IV. Samples as in Fig. 11.

obtained when these substances were retained on the DMOA-independent sites on μBondapak C_{18} (see the log $k'_{Q.(A)}$ values in Fig. 9) with the same mobile phase. This indicates that the stationary phase that retains the samples on LiChrosorb RP-8 after the addition of DMOA may have the same properties as the DMOA-independent sites on μBondapak C_{18}.

The separation selectivity on some different solid phases for the same substances as in Fig. 11 is presented in Fig. 12 in the form of log k' values. 0.05 *M* DMOA was present in the eluent to ensure symmetrical peaks. On all solid phases except Spherisorb ODS the selectivities are very similar. The latter solid phase obviously has very different properties from the others, as the retention order is completely reversed.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Natural Science Research Council. We are grateful to Professor Göran Schill for critical reading of the manuscript, and to Kristrún Davídsdottír for technical assistance.

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