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REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF DRUGS AND RELATED COMPOUNDS USING UNDERIVATIZED SILICA AS THE STATIONARY PHASE

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SUMMARY

The retention of organic compounds on silica in chomatographic systems with aqueous mobile phases has been investigated. The effects on retention of the type of silica and the pH of the mobile phase in the acidic range have been studied. The sample behaviour is similar to that observed in reversed-phase systems with an alkyl-bonded solid phase. The influence of the nature and the concentration of the mobile phase components on the retention of ionic samples has been examined. A retention model based on the distribution of ion pairs to the solid stationary phase is proposed. Different methods for the regulation of retention in these systems are given. Numerous examples of separations are presented.

INTRODUCTION

Silica gel is widely used as a solid phase in liquid chromatography with eluents of low polarity for the separation of various kinds of organic compounds but its adsorption properties towards organic samples in systems with aqueous mobile phases have been much less studied.

Iler[1] has shown in static experiments that hydrogen-bonding agents, such as alcohols, ketones and amides, and also ammonium ions of different kinds can be adsorbed on silica from aqueous solutions at pH 2.5. Some batch experiments have also been made by Crommen *et al.*[2] which indicate a significant adsorption of quaternary ammoniums to silica at low pH. Deviations between found and calculated retentions in chromatographic systems with an aqueous stationary phase coated on silica[2,3] give other indications of adsorption of ammonium ions at the silica–water interface.

Analogous systems with silica immersed in aqueous solutions have been studied more extensively with inorganic substances as samples and specially metal cations. A survey of this particular use of silica as inorganic ion exchanger is given in ref. 10.

This paper describes studies of chromatographic systems with silica as a solid stationary phase and buffered aqueous solutions as mobile phases. The influence of the mobile phase composition on the retention of organic samples in ionized form has been specially investigated and a model based on ion-pair distribution to the

solid phase, similar to that given by Schill and co-workers[4,5], is proposed. Reversed-phase systems suitable for various kinds of drugs and related compounds are presented.

EXPERIMENTAL

Apparatus

The pump was a Model 6000A solvent delivery system and the detector was a Model 440 absorbance detector measuring at a wavelength of 254 nm, both from Waters Assoc. The injector was a high-pressure valve from Valco Instruments, with a sample loop of 10 μl.

The columns were made of 316 stainless steel and had a length of 200 mm and I.D. 4.0 mm.

A Heto Type 02 PT 923 water-bath (Birkerōd, Denmark) was used to thermostat the chromatographic equipment.

The pH measurements were made with a Corning-EEL Model 109 digital pH meter.

Chemicals and reagents

The chromatographic supports were Nucleosil 100 (10 μm) from Macherey, Nagel & Co. (Düren, G.F.R.) and LiChrospher SI 100 (10 μm) and SI 500 (10 μm) from E. Merck (Darmstadt, G.F.R.).

1-Pentanol and other organic solvents were of pro analysi quality from Merck.

Camphor-10-sulphonic acid was obtained from Merck-Schuchardt (Munich, G.F.R.). Tetramethylammonium hydroxide (10% solution) was obtained from Merck and tetraethylammonium hydroxide (25% solution) from BDH (Poole, Great Britain). Tetrabutylammonium hydrogen sulphate (puriss) from AB Labkemi (Göteborg, Sweden) was used after neutralization with sodium hydroxide.

Sodium naphthalene-2-sulphonate was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). Sodium anthracene-2-sulphonate was synthesized according to Liebermann[6]. N-Methylamitriptyline bromide was synthesized from amitriptyline chloride according to Borg[7].

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

Chromatographic technique

After packing, the columns were washed with *n*-hexane, acetone, ethanol and water, before equilibration with aqueous mobile phase.

The mobile phases were degassed in an ultrasonic bath and thermostated at the experimental temperature (25.0°) in an air-thermostated cabinet. The mobile phase reservoir, the injector and the column were thermostated in a water-bath at $25.0 \pm <0.1°$.

The equilibration of the column was usually obtained after passage of about 50 ml of mobile phase.

Column stability

No significant changes in sample retention and column efficiency were observed for a long period of time (several months) while using aqueous mobile phases with pH ranging from 2 to 5 on silica columns. Before storage, columns were always re-equilibrated with ethanol, acetone and *n*-hexane.

RESULTS AND DISCUSSION

Determination of the volume of mobile phase in the column

Different samples were tested for the determination of the volume of mobile phase, V_m, in silica columns, using phosphate buffers of pH 2–3 as mobile phases. Inorganic anions, such as nitrate and dichromate, had slightly larger retention volumes than water samples in which the mobile phase was replaced by water. The latter gave distinct negative peaks with the UV-detector in the high sensitivity range.

The retention volumes obtained with water samples are in good agreement with those obtained on the same silica columns by using *n*-hexane–1-butanol (199:1) as non-polar mobile phase and carbon tetrachloride as non-retained sample. Porosity values, calculated from these retention volumes, were in the range 0.83–0.88 for the different kinds of silica tested. They are in accordance with the expected values for total porosity[8] and therefore water samples were used for determination of V_m in systems with aqueous mobile phases.

A slight decrease in V_m was observed when increasing concentrations of organic compounds, *e.g.*, 1-pentanol, were added to the aqueous mobile phase, which might indicate significant adsorption of these compounds on the solid phase. However, no determinations of the amounts of mobile phase components adsorbed on silica were made in this investigation. The value of V_m was controlled by injections of water samples when the composition of the mobile phase was changed.

Retention behaviour

Various kinds of organic compounds were tested as samples in chromatographic systems with silica as solid stationary phase and acidic aqueous buffers as mobile phases. Most of them, cationic and anionic as well as uncharged substances, were retained in these systems.

The retention increases with increasing hydrophobic character of the sample: the addition of one alkyl carbon gives an increase in log k' of about 0.2 while the increment in log k' for one aryl carbon is about 0.1. Log k' decreases by 0.3–0.4 unit on addition of one carboxy or hydroxy group (*cf.*, Table VI).

These retention data indicate that the sample behaviour is similar to that observed in ordinary reversed-phase systems with an alkyl-bonded solid phase.

It is also interesting that the retention of ammonium ions increases significantly with increasing degree of substitution at the nitrogen atom, quaternary ammonium compounds being the most strongly retained. A methyl substitution on a primary, secondary or tertiary ammonium group gives an increase in log k' of about 0.4.

Type of silica used as stationary phase

Different kinds of spherical silica microparticles ($d_p = 10\,\mu m$) were tested as solid stationary phases. Some retention data are presented in Table I, which indicate that the sample retention is related to the specific surface area of the silica.

The separation selectivity does not seem to be affected, however, the retention behaviour of widely different kinds of ionic and uncharged species being the same with the three solid phases tested.

A good batch-to-batch reproducibility of capacity ratios was obtained in these systems with aqueous buffers as mobile phases.

TABLE I

INFLUENCE OF THE TYPE OF SILICA USED AS STATIONARY PHASE

Mobile phase: phosphate buffer, pH 2.2. Stationary phase: spherically shaped silica (10 μm). S = specific surface area.

Sample	*log k'*		
	Nucleosil 100 (S = 300 m²/g)	*LiChrospher SI 100 (S = 250 m²/g)*	*LiChrospher SI 500 (S = 50 m²/g)*
Benzylamine	−0.14	−0.23	−0.70
Ephedrine	0.18	0.14	−0.32
Desipramine	0.85	0.76	0.49
Imipramine	1.25	1.10	0.83
Naphthalene-2-sulphonate	−0.39	−0.43	−0.80
Toluene	0.19	0.13	−0.43

pH of the mobile phase

The influence of the pH of the mobile phase on the adsorption properties of the solid phase was studied. Phosphate and citrate buffers with sodium as the only cation and an ionic strength of 0.1 were used as mobile phases.

As illustrated in Fig. 1, a change in pH from 2 to 5 has little influence on sample retention. The decrease in the retention of hydrophobic ammonium ions, such as desipramine, at about pH 2.8 seems to be related to the change from phosphate to citrate buffer. This might indicate that usual buffer components can also be adsorbed on silica and have an effect on retention. No significant differences in log k' were observed, however, with less retained compounds.

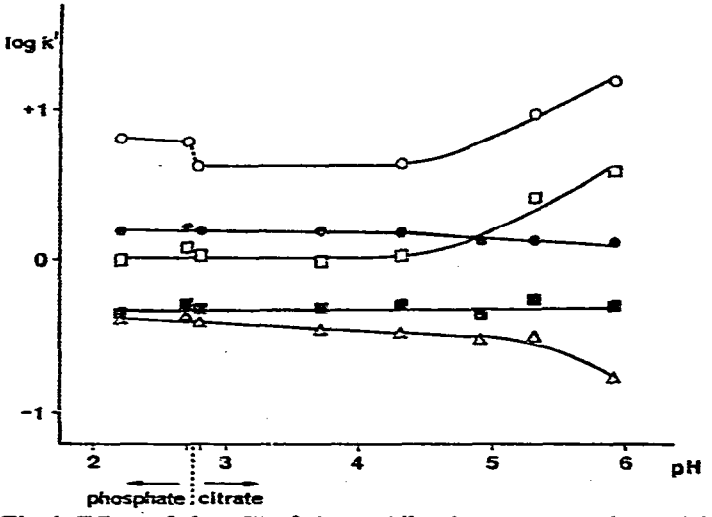

Fig. 1. Effect of the pH of the mobile phase on retention. Mobile phase: sodium phosphate or citrate buffer; ionic strength 0.1. Stationary phase: Nucleosil 100 (10 μm). Samples: ○, desipramine; □, phenethylamine; ●, toluene; ■, phenylalanine; △, naphthalene-2-sulphonate.

At pH above 5, an increase in pH gives rise to a drastic increase in the retention of cationic compounds, probably owing to increasing ionization of the silanol groups at the silica surface. Similar observations have been made previously in batch

experiments[1,2]. In the same pH range, the retention of anionic samples, such as naphthalene-2-sulphonate, decreases with increasing pH. The same tendency, although less marked, has been observed with uncharged molecules, such as toluene. Such a behaviour may also be explained by modification of the adsorption properties of silica owing to the dissociation of the silanol groups. The retention of the amino acid phenylalanine, which migrates as a zwitterion in that pH range, does not seem to be affected by pH changes in the mobile phase, probably by internal compensation of opposing effects.

Nature of the ionic components of the mobile phase

The effect on retention that can be obtained by changing the cationic component of the buffer used as the mobile phase is demonstrated in Table II. A change from sodium to different kinds of ammonium ions causes an increase in the retention of anionic samples, while the retention of cationic and uncharged compounds decreases. The concentrations of citrate, taken as buffer anion, and of the accompanying cation were kept constant.

Table II shows that the change in log k' increases with increasing hydrophobic character and degree of substitution at the nitrogen atom, which indicates that the magnitude of the effect is related to the tendency of the cation present in the mobile phase to be adsorbed on silica. The behaviour of sodium seems to be similar to that of hydrophilic primary amines, such as tris(hydroxymethyl)aminomethane. The most pronounced effect on retention was obtained with hydrophobic quaternary ammonium ions.

TABLE II

EFFECT OF THE NATURE OF THE CATIONIC COMPONENT PRESENT IN THE MOBILE PHASE

Mobile phase: citrate buffer, pH 3.6–3.8. Cations (C^+): 1, sodium; 2, tris(hydroxymethyl)aminomethane; 3, triethanolamine; 4, hexamethylenetetramine; 5, choline; 6, tetramethylammonium; 7, tetraethylammonium. $[C^+]_m = 0.1$ *M*. Stationary phase: Nucleosil 100 (10 μm).

Sample	*log k'*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Naphthalene-2-sulphonate	−0.49	−0.46	−0.24	−0.21	−0.14	−0.11	0.13
Amphetamine	0.21	0.18	−0.15	−0.26	−0.51	−0.55	−1.30
Atropine	0.78	0.70	0.40	0.20	0.04	−0.04	−0.60
Methylatropine	—	—	0.73	0.49	0.36	0.20	−0.31
Naphthalene	0.30	0.23	0.14	0.08	−0.07	−0.08	−0.22

Similar experiments were carried out with different anions present as acids in the mobile phase. As indicated by pH measurements, the acids tested were almost completely dissociated at the concentration used. Table III shows that the substitution of inorganic anions for the most hydrophobic anion camphorsulphonate gives rise to an increase in the retention of cationic compounds and a decrease in the retention of anionic and uncharged substances.

It can be seen from Tables II and III that the retention of ionic samples can be increased by ions of the opposite charge, counter ions, ("ion-pairing" effect) and

TABLE III

EFFECT OF THE NATURE OF THE ANIONIC COMPONENT PRESENT IN THE MOBILE PHASE

Mobile phase: 0.01 *M* solution of acid, pH 2.0–2.1. Stationary phase: Nucleosil 100 (10 μm).

Sample	*log k'*			
	Perchloric acid	*Nitric acid*	*Trichloroacetic acid*	*Camphor-10-sulphonic acid*
Phenylpropanolamine	−0.22	−0.21	−0.17	0.16
Benzylamine	−0.09	−0.09	−0.05	0.26
Amphetamine	0.25	0.27	0.33	0.58
Anthracene-2-sulphonate	−0.02	−0.03	−0.13	−0.40
Naphthalene	0.48	0.47	0.41	0.26

decreased by ions of the same charge ("competition" effect). The retention of uncharged samples can be reduced by both cationic and anionic components of the mobile phase, which means that ions and uncharged molecules can compete with each other for adsorption at the silica surface.

Ion-pair distribution

The investigation was focused mainly on the retention behaviour of compounds that migrate as ions in acidic mobile phases. The influence of counter ions on the retention of ionic samples, demonstrated in Tables II and III, might indicate that ionic compounds are distributed to silica as ion pairs. The distribution of ammonium ions to silica as ion pairs, in acidic media, has also been suggested by Iler[1].

If the sample ion, Q^+, is assumed to be distributed as an ion pair with the counter ion, Y^-, to the adsorbing surface, the distribution process can be described as follows[5]:

$$Q_m^+ + Y_m^- + A_s = QY \cdot A_s \quad (1)$$

The symbols m and s refer to mobile and stationary phases, respectively. $QY \cdot A_s$ represents the ion pair QY adsorbed on the silica surface, where it occupies an area A_s, usually called an adsorption site. The equilibrium constant, K_{QY}, for the distribution of the ion pair QY can be expressed by

$$K_{QY} = \frac{[QY \cdot A]_s}{[Q^+]_m \cdot [Y^-]_m \cdot [A]_s} \quad (2)$$

$[A]_s$ represents the number of moles of available adsorption sites per gram of solid phase. In accordance with the Langmuir model for monolayer adsorption, all of the adsorption sites are assumed to be equivalent.

Other components of the mobile phase can also be adsorbed at the silica surface, as ion pairs or in uncharged form, and will compete with the sample Q^+ for the available adsorption sites. The cation C^+, present in the mobile phase, can be distributed to the solid phase as an ion pair CY, which is assumed to occupy an adsorption site A_s. If QY and CY are the only adsorbed species, the maximum adsorption capacity of the retaining surface, K_0 (in moles per gram), can be expressed by the equation

$$K_0 = [A]_s + [QY \cdot A]_s + [CY \cdot A]_s \quad (3)$$

An expression for the capacity ratio of Q^+ can be obtained from eqns.2 and 3:

$$k'_Q = q \cdot \frac{[QY \cdot A]_s}{[Q^+]_m} = \frac{qK_0K_{QY}[Y^-]_m}{1 + K_{QY}[Q^+]_m \cdot [Y^-]_m + K_{CY}[C^+]_m \cdot [Y^-]_m} \quad (4)$$

where q is the ratio of solid phase to mobile phase (grams per litre) in the column and K_{CY} is the equilibrium constant for the ion-pair distribution of C^+ with Y^- (*cf.*, eqn. 2). Good peak symmetry was obtained in the sample concentration range studied. In this instance, the capacity ratio can be considered as independent of sample concentration and the term $K_{QY}[Q^+]_m \cdot [Y^-]_m$ in eqn. 4 can be neglected[5], which gives

$$k'_Q = \frac{qK_0K_{QY}[Y^-]_m}{1 + K_{CY}[C^+]_m \cdot [Y^-]_m} \quad (5)$$

A similar expression can be given for the capacity ratio of an anionic sample X^-:

$$k'_X = \frac{qK_0K_{CX}[C^+]_m}{1 + K_{CY}[C^+]_m \cdot [Y^-]_m} \quad (6)$$

where C^+ and Y^- are ionic components of the mobile phase and K_{CX} is the equilibrium constant for the ion-pair distribution of X^- with C^+.

The influence on k' of the nature of the ions present in the mobile phase (*cf.*, Tables II and III) is reflected in eqns. 5 and 6 by the magnitude of the constants for ion-pair adsorption QY, CX and CY.

Ionic strength of the buffer

The effect on retention obtained by using as mobile phases different dilutions of a phosphate buffer of pH 2.2 is shown in Table IV. The cationic component of the buffer, C^+, was sodium and the anion, Y^-, was dihydrogen phosphate at that pH.

The differences in $\log k'$ do not seem to be due to the change in pH, as no significant influence of pH on retention was observed in that pH range (*cf.*, Fig. 1). Eqns. 5 and 6 show that different retention effects can be obtained by the simultaneous change of $[C^+]_m$ and $[Y^-]_m$.

TABLE IV

INFLUENCE OF THE CONCENTRATION OF BUFFER COMPONENTS

Mobile phases: 1, phosphate buffer, pH 2.2, ionic strength 0.1; 2, 200 ml of mobile phase 1 diluted to 1000 ml with water, pH 2.35; 3, 50 ml of mobile phase 1 diluted to 1000 ml with water, pH 2.7. Stationary phase: Nucleosil 100 (10 μm).

Sample	*log k'*		
	1 ($[Na^+]_m = 0.1$ *M*)	*2* ($[Na^+]_m = 0.02$ *M*)	*3* ($[Na^+]_m = 0.005$ *M*)
Benzylamine	−0.14	−0.06	−0.02
Phenethylamine	0.00	0.12	0.15
Amphetamine	0.20	0.31	0.34
Toluene	0.19	0.31	0.32
Naphthalene-2-sulphonate	−0.39	−0.44	−0.54

The $\log k'$ of anionic samples, such as naphthalene-2-sulphonate, increases with increasing ionic strength of the buffer, which seems to indicate that the role of the "ion-pairing" effect with sodium is predominant in this instance. Cationic samples do not have exactly the same retention behaviour, as a decrease in $\log k'$ with increasing ionic strength has been obtained, which indicates a dominating "competition" effect of sodium. $[Na^+]_m$ seems to have a predominant influence on retention in both instances.

The change in the $\log k'$ of the uncharged compound toluene with buffer concentration indicates that the influence of the adsorption of inorganic ions on the retention of this compound is not negligible.

Concentration of counter ion

The variation of the capacity ratios of ionic samples with the concentration of the counter ion present in the mobile phase was studied.

With anionic samples (X^-), the quaternary ammonium compound choline was used as counter ion (C^+). Choline was added to the mobile phase as the dihydrogen citrate salt. The concentration of the anion (Y^-) was kept constant by addition of sodium dihydrogen citrate. By inversion of eqn. 6, the relationship between k'_X and $[C^+]_m$ becomes linear:

$$\frac{1}{k'_X} = \frac{1}{qK_0K_{CX}[C^+]_m} + \frac{K_{CY}[Y^-]_m}{qK_0K_{CX}} \tag{7}$$

The influence of sodium on the retention of anions cannot be considered as negligible, especially at low choline concentrations (*cf.*, Table II). If k'_0 represents the capacity ratio of the anionic sample in absence of choline, a correction to the found capacity ratio, k'_f, can be made by the following equation[5]:

$$k' = k'_f - \frac{k'_0 x}{1 + K_{CY}[C^+]_m \cdot [Y^-]_m} \tag{8}$$

where x is the ratio of $[Na^+]_m$ at a given $[C^+]_m$ to $[Na^+]_m$ at $[C^+]_m = 0$.

A first estimation of $K_{CY}[Y^-]_m$ was obtained from plots of $1/k'$ *versus* $1/[C^+]_m$ (*cf.*, eqn. 7). Only k'_f values obtained at high choline concentrations ($[C^+]_m = 0.05$–$0.1\ M$) were used in this instance. Fairly straight lines were obtained and an approximate value of $K_{CY}[Y^-]_m$ was given by the intercept/slope quotient. This value was used to correct k'_f according to eqn. 8. A better estimation of $K_{CY}[Y^-]_m$ could then be obtained by using the corrected capacity ratios in the plots. The same operation was repeated until a fairly constant value of $K_{CY}[Y^-]_m$ was obtained. The corrections were less than 10% of k'_f at $[C^+]_m \geq 0.05\ M$ but reached 30% at $[C^+]_m = 0.02\ M$.

Some examples of plots of $1/k'_X$ *versus* $1/[C^+]_m$ using corrected capacity ratios are given in Fig. 2. Fairly constant $K_{CY}[Y^-]_m$ values (22–27) were obtained for choline dihydrogen citrate with different anionic samples.

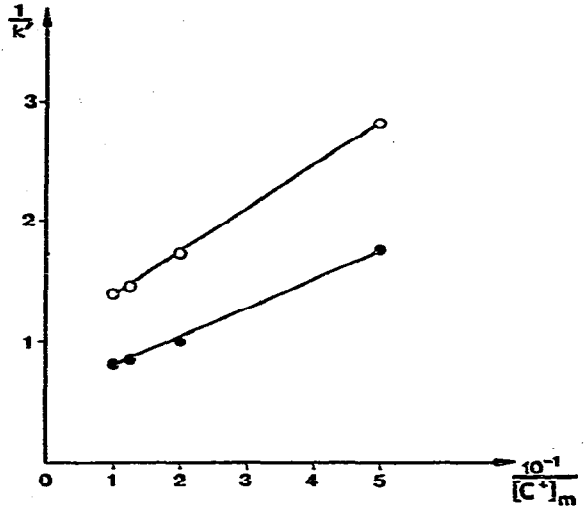

Fig. 2. Effect of counter ion concentration on retention of anions. Mobile phase: 0.1 *M* citrate buffer pH 3.7; cations, choline (C^+) and sodium. Stationary phase: Nucleosil 100 (10 μm). Samples: ○, naphthalene-2-sulphonate; ●, anthracene-2-sulphonate.

Similar experiments were carried out with cationic samples (Q^+), using camphorsulphonate as counter ion (Y^-). H^+ was the only cation (C^+) present in the mobile phase, and its concentration was kept constant by use of nitric acid. Strong acids can be considered to be distributed to the solid phase as ion pairs with H^+ and eqn. 5 can be used to express the relationship between k'_Q and $[Y^-]_m$. Linearization of this relationship is obtained by inversion of eqn. 5:

$$\frac{1}{k'_Q} = \frac{1}{qK_0K_{QY}[Y^-]_m} + \frac{K_{CY}[C^+]_m}{qK_0K_{QY}} \quad (9)$$

Some examples of plots of $1/k'_Q$ *versus* $1/[Y^-]_m$ are given in Fig. 3. The effect of the anion nitrate on the retention of the cations had also to be taken into account and the found capacity ratios (k'_f) were corrected according to eqn. 8. In this instance, the factor x in eqn. 8 represents the ratio of $[NO_3^-]_m$ at a given $[Y^-]_m$ to $[NO_3^-]_m$ at $[Y^-]_m = 0$. The product $K_{CY}[C^+]_m$ was estimated by successive approximations in the same way as for choline dihydrogen citrate. Larger corrections to k'_f were needed in this instance. $K_{CY}[C^+]_m$ values of 30–50 were found for camphorsulphonic acid with different cations as samples.

The linearity of the plots shown in Figs. 2 and 3 is fairly good and gives support to the assumption of ion-pair distribution in these systems. It is obvious, however, that further experimental studies should be made to confirm the validity of the retention model.

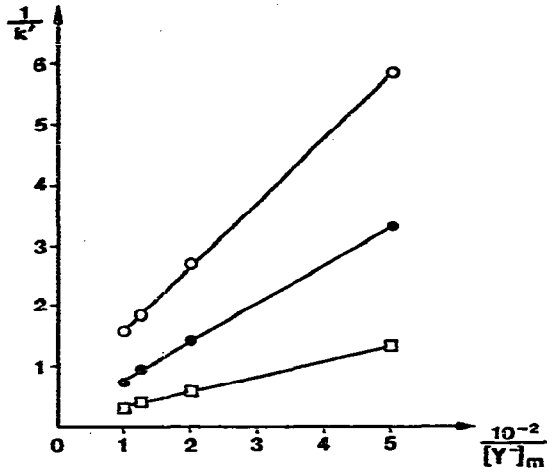

Fig. 3. Effect of counter ion concentration on retention of cations. Mobile phase: camphor-10-sulphonic acid (Y^-) + nitric acid, pH 2.1. Stationary phase: Nucleosil 100 (10 μm). Samples: ○, tyramine; ●, phenylpropanolamine; □, amphetamine.

Concentration of competing ion

Eqns. 7 and 9 indicate that it might also be possible to regulate the capacity ratio of ionic samples by changing the concentration of the ion of the same charge present in the mobile phase. This is demonstrated in Figs. 4 and 5.

Fig. 4 shows the influence of choline concentration ($[C^+]_m$) on the retention of cations and Fig. 5 shows the effect of camphorsulphonate concentration ($[Y^-]_m$) on the retention of anions. In both instances, the concentration of the counter ions was kept constant by addition of sodium dihydrogen citrate.

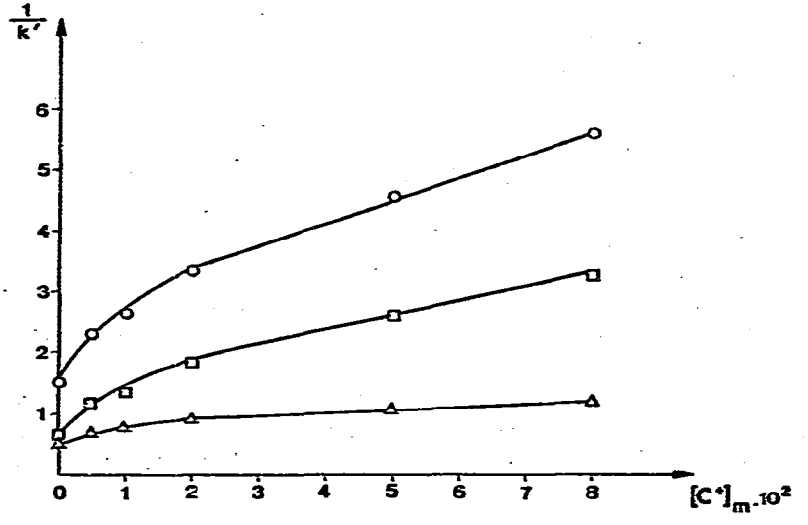

Fig. 4. Effect of concentration of competing cation on retention. Conditions as in Fig. 2. Samples: ○, benzylamine; □, amphetamine; △, naphthalene.

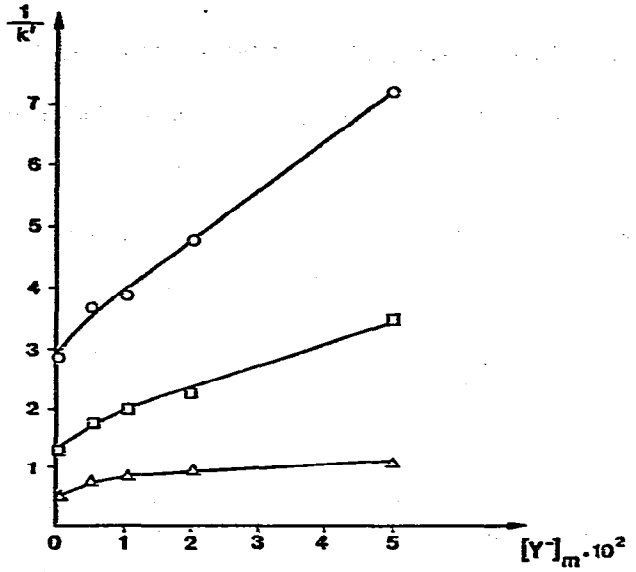

Fig. 5. Effect of concentration of competing anion on retention. Mobile phase: sodium camphor-10-sulphonate in citrate buffer, pH 3.7; $[Na^+] = 0.1$ *M*. Stationary phase: Nucleosil 100 (10 μm). Samples: ○, naphthalene-2-sulphonate; □, anthracene-2-sulphonate; △, naphthalene.

The curves in Figs. 4 and 5 show the same tendency, *i.e.*, a decrease in the slope with increasing concentration of competing ion. This tendency is especially pronounced at low concentrations, the curves approaching linearity at higher concentrations. A possible explanation is the presence of a limited number of stronger adsorption sites at the silica surface. When strongly adsorbed compounds, such as choline and camphorsulphonate, are present in the mobile phase in sufficiently high concentrations, these stronger sites will be completely covered, and the surface will acquire a more homogeneous character.

Fig. 4 shows that the curves are fairly linear in the choline concentration range 0.02–0.08 *M*. Estimations of $K_{CY}[Y^-]_m$ from the slope/intercept quotient within that concentration range gave values of 15–19 for different cationic samples. The agreement with the $K_{CY}[Y^-]_m$ values of 22–27 obtained with anonic samples (*cf.*, Fig. 2) in the same choline concentration range is acceptable.

$K_{CY}[C^+]_m$ for sodium camphorsulphonate was estimated from the slope/intercept quotient in the concentration range 0.01–0.05 *M*. As can be seen from Fig. 5, the plots are close to linearity in that concentration range. $K_{CY}[C^+]_m$ values of 25–30 were obtained.

Fig. 4 and 5 show that the retention of the uncharged compound naphthalene is also influenced by the concentration of the ionic components of the mobile phase.

Addition of lipophilic alcohols

The effects on retention of the nature and the concentration of the lipophilic alcohol added to the aqueous mobile phase are demonstrated in Table V and Fig. 6, respectively. Lipophilic alcohols are strongly adsorbed on silica and they reduce the retention of various kinds of samples.

TABLE V

EFFECT OF THE NATURE OF THE LIPOPHILIC ALCOHOL ADDED TO THE MOBILE PHASE

Mobile phase: phosphate buffer, pH 2.2, containing 1.0% (v/v) of lipophilic alcohol. Stationary phase: Nucleosil 100 (10 μm).

Sample	*log k'*			
	1-Pentanol	*1-Butanol*	*Isobutanol*	*tert.-Butanol*
Amphetamine	−0.38	−0.31	−0.31	−0.26
Desipramine	0.20	0.26	0.28	0.32
Atropine	0.23	0.30	0.30	0.34
Imipramine	0.49	0.59	0.62	0.70
Naphthalene-2-sulphonate	−0.68	−0.59	−0.61	−0.55
Toluene	−0.36	−0.29	−0.30	−0.22

Table IV shows that the "competition" effect of the alcohol increases with increasing carbon content and decreases slightly with increasing chain branching.

The influence of the concentration of the alcohol, P, can be expressed quantitatively by equations analogous to eqns. 7 and 9. The relationship between the capacity ratio of, *e.g.*, a cationic sample and the alcohol concentration can be given by

$$\frac{1}{k'_Q} = \frac{1}{qK_0K_{QY}[Y^-]_m} + \frac{K_p[P]_m}{qK_0K_{QY}[Y^-]_m} \quad (10)$$

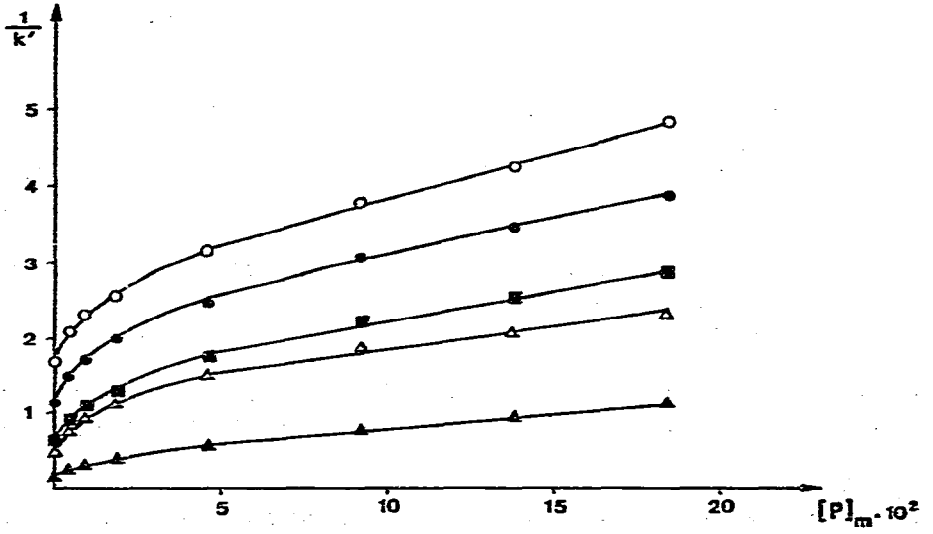

Fig. 6. Effect of concentration of lipophilic alcohol on retention. Mobile phase: sodium phosphate buffer, pH 2.2; ionic strength 0.1. Stationary phase: Nucleosil 100 (10 μm). Samples: ○, phenylpropanolamine; ●, anthracene-2-sulphonate; ■, amphetamine; △, naphthalene; ▲, desipramine.

TABLE VI

EFFECT OF THE 1-PENTANOL CONTENT IN THE MOBILE PHASE ON SEPARATION SELECTIVITY

Values given are $\log \alpha$ ($= \log k_2' - \log k_1'$).

Sample 1	*Sample 2*	*1-Pentanol concentration (%, v/v)*							
		0	*0.05*	*0.1*	*0.2*	*0.5*	*1.0*	*1.5*	*2.0*
Phenethylamine	Amphetamine	0.20	0.19	0.18	0.17	0.14	0.12	0.10	0.11
Phenylpropanolamine	Amphetamine	0.42	0.36	0.34	0.30	0.26	0.23	0.22	0.23
Phenylalanine	Phenethylamine	0.35	0.27	0.25	0.24	0.22	0.21	0.21	0.18
Phenylpropanolamine	Ephedrine	0.45	0.43	0.40	0.38	0.34	0.33	0.32	0.31
Naphthalene-2-sulphonate	Anthracene-2-sulphonate	0.33	0.29	0.27	0.25	0.24	0.22	0.20	0.18

TABLE VII

EFFECT OF SAMPLE CONCENTRATION ON CHROMATOGRAPHIC PROPERTIES

Experiment A: mobile phase, phosphate buffer, pH 2.2, 1.6 mm/sec, 7.5 MPa; stationary phase, Nucleosil 100 (10 μm); sample volume, 10 μl. Experiment B: mobile phase, phosphate buffer, pH 2.2, containing 1.5% (v/v) of 1-pentanol, 1.6 mm/sec, 8.3 MPa; other conditions as in experiment A.

Experiment	*Sample*	*Sample concentration (mole/l × 10^5)*	*k'*	*Asymmetry factor**	*H (mm)*
A	Phenylpropanolamine	1	0.64	1.2	0.08
		5	0.64	1.2	0.08
		20	0.62	1.7	0.08
	Phenethylamine	1	1.11	1.7	0.11
		5	1.08	2.2	0.11
		20	1.04	2.5	0.11
	Ephedrine	1	1.84	1.9	0.11
		5	1.78	2.3	0.12
		20	1.68	2.9	—
	Desipramine	1	6.6	4.9	—
		5	5.7	7.3	—
		20	4.9	10.7	—
B	Amphetamine	1	0.40	1.2	0.06
		5	0.40	1.1	0.07
		20	0.40	1.2	0.07
	Desipramine	1	1.09	1.3	0.09
		5	1.06	1.6	0.09
		20	1.04	1.7	0.09
	Imipramine	1	2.50	1.8	0.13
		5	2.39	2.2	0.13
		20	2.30	2.7	—
	Oxyphenonium	5	7.2	2.3	0.11
		20	6.8	4.2	—

* Back part of peak base/front part of peak base.

where K_p is the constant for the distribution of the alcohol to the solid phase. Fig. 6 shows plots of $1/k'$ *versus* $[P]_m$, P being 1-pentanol in this instance. The curves have the same shape as those shown in Figs. 4 and 5.

Estimations of K_p from the slope/intercept quotient were made in the 1-pentanol concentration range 0.05–0.15 *M*. Fairly constant K_p values (4–6) were obtained with both ionic and uncharged samples. This is a further indication that ions and uncharged compounds compete for the same adsorption sites.

Effect of mobile phase composition on chromatographic properties

Table VI shows that the separation selectivity decreases with increasing concentration of 1-pentanol in the mobile phase. The same effect was obtained by the addition of other strongly adsorbed ionic or uncharged compounds. The higher selectivity in the absence of a strongly competing agent might again be due to a significant contribution of stronger sites to retention under these conditions[9].

A comparison of the influence of sample concentration on k' and peak symmetry, with or without 1-pentanol present in the mobile phase, is given in Table VII. In both systems, the concentration effect was very limited at low k' values. With a simple buffer as the mobile phase, however, the tendency for changes in k' to occur with changes in concentration increases rapidly at $k' > 5$. In the presence of 1-pentanol, this tendency is much less pronounced. This favourable effect of 1-pentanol might be explained by an improvement in surface homogeneity, due to the complete coverage of the strongest adsorption sites by 1-pentanol molecules[9].

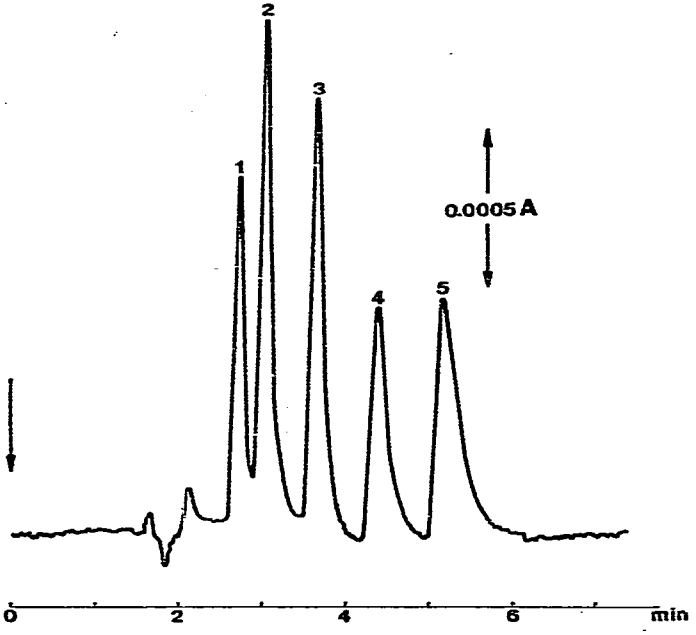

Fig. 7. Separation of adrenergics. Mobile phase: camphor-10-sulphonic acid (0.01 *M*), pH 2.1; 1.7 mm/sec; 7.7 MPa. Stationary phase: Nucleosil 100 (10 μm). Samples: 1 = norepinephrine bitartrate; 2 = epinephrine bitartrate; 3 = phenylephrine hydrochloride; 4 = isoproterenol sulphate; 5 = phenylpropanolamine hydrochloride.

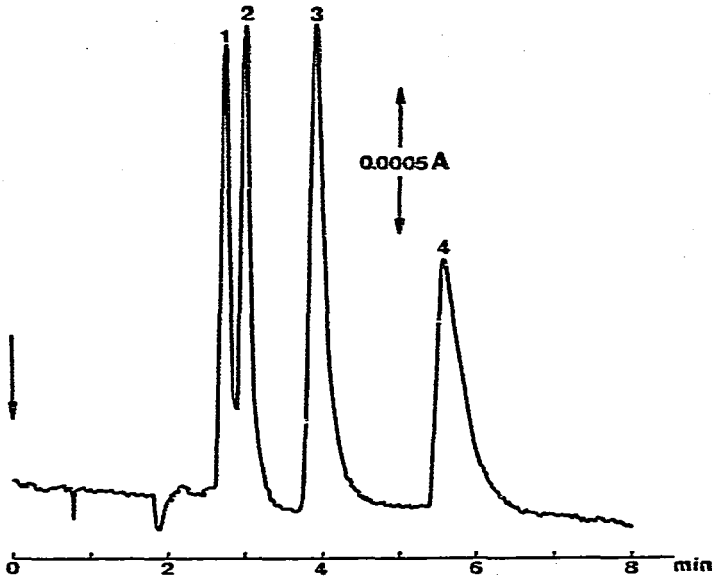

Fig. 8. Separation of biogenic amines. Conditions as in Fig. 7. Samples: 1 = dopamine hydrochloride; 2 = tyramine hydrochloride; 3 = tryptamine hydrochloride; 4 = phenethylamine hydrochloride.

The role of 1-pentanol or other strongly adsorbed compounds present in the aqueous mobile phase could be compared to that of, *e.g.*, water in systems with non-polar mobile phases.

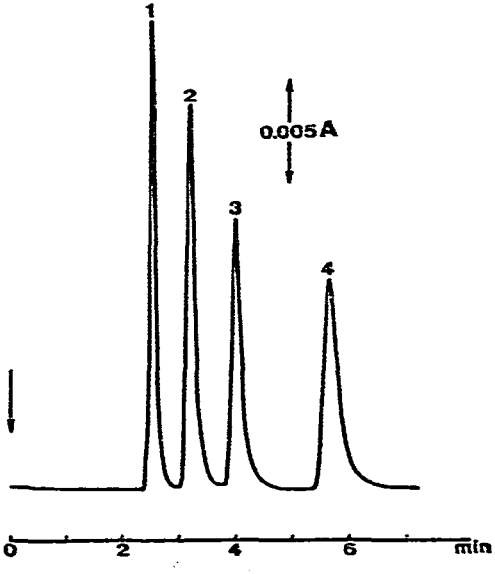

Fig. 9. Separation of sulphonamides. Mobile phase: camphor-10-sulphonic acid (0.02 *M*), pH 1.8; 1.7 mm/sec; 8.4 MPa. Stationary phase: Nucleosil 100 (10 μm). Samples: 1 = sulphanilamide; 2 = sulphadiazine; 3 = sulphamerazine; 4 = sulphamethazine.

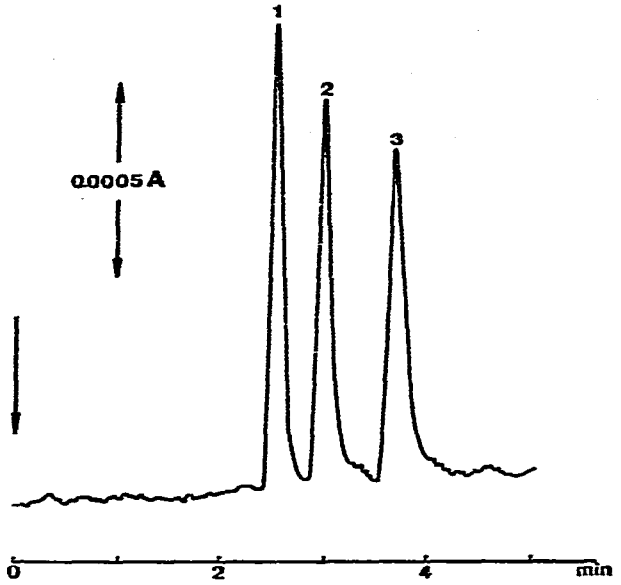

Fig. 10. Separation of amino acids. Conditions as in Fig. 9. Samples: 1 = tyrosine; 2 = tryptophan; 3 = phenylalanine.

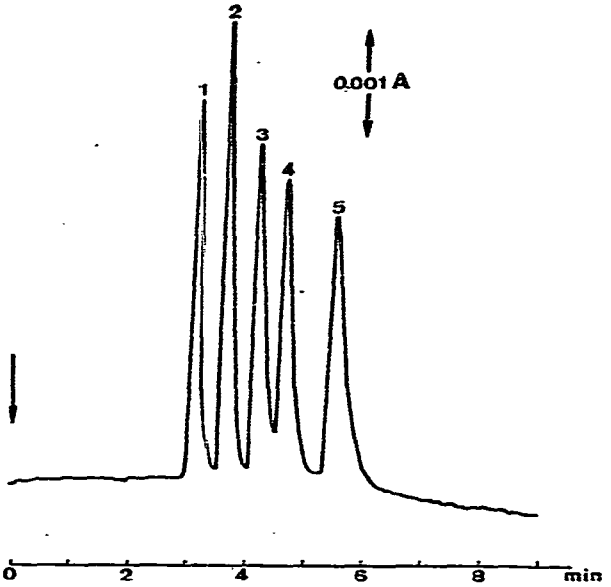

Fig. 11. Separation of carboxylic acids. Mobile phase: tetraethylammonium hydroxide (0.01 *M*)–citric acid (0.007 *M*), pH 4.9; 1.6 mm/sec; 8.4 MPa. Stationary phase: Nucleosil 100 (10 μm). Samples: 1 = gentisic acid; 2 = salicylic acid; 3 = benzoic acid; 4 = acetylsalicylic acid; 5 = hippuric acid.

As shown in Table VII, the plate height, H, in these systems corresponds to 6–13 d_p at a mobile phase speed of 1.6 mm/sec.

Applications to pharmaceutical analysis

Ion-pair reversed-phase systems suitable for various kinds of drugs and other compounds of biological interest have been constructed. Samples of widely different hydrophobic character could be chromatographed with suitable retention on the same silica columns by changing the composition of the aqueous mobile phase.

It has been shown that the retention of ionic samples can be increased by addition to the mobile phase of hydrophobic counter ions. This "ion-pairing" effect was used to regulate the retention of the most hydrophilic samples. Examples of chromatographic separations of hydrophilic ammonium ions with camphorsulphonate as counter ion are shown in Figs. 7–10. At a pH as low as 1.8, the sulphonamides and the amino acids are mainly present as cations in the mobile phase. The influence on retention of the introduction of phenolic groups is shown in Figs. 7, 8 and 10, while the effect of the addition of one methyl group is demonstrated in Fig. 9, by the separation of homologous sulphapyrimidines. Fig. 11 shows the separation of carboxylates as ion pairs with tetraethylammonium. At about pH 5, however, these samples might also be partly adsorbed as acids. The elution order in Fig. 11 is not exactly the same as that obtained in other ion-pair reversed-phase systems[5].

The retention of hydrophobic samples is more conveniently regulated by the addition of a competing ion or uncharged compound to the mobile phase. This is demonstrated in Figs. 12–16. As shown in Fig. 12, tetrabutylammonium can be used

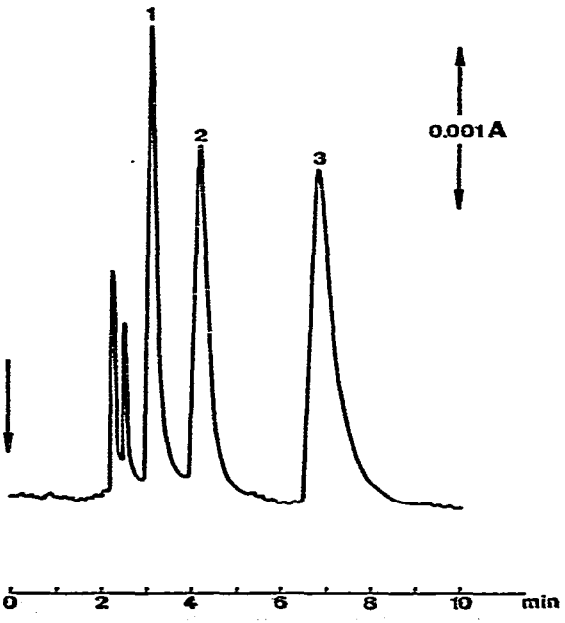

Fig. 12. Separation of tricyclic antidepressants. Mobile phase: tetrabutylammonium hydrogen sulphate (0.02 M) in sodium citrate buffer, pH 3.7; 1.6 mm/sec; 8.7 MPa. Stationary phase: Nucleosil 100 (10 μm). Samples: 1 = nortriptyline hydrochloride; 2 = amitriptyline hydrochloride; 3 = N-methylamitriptyline bromide.

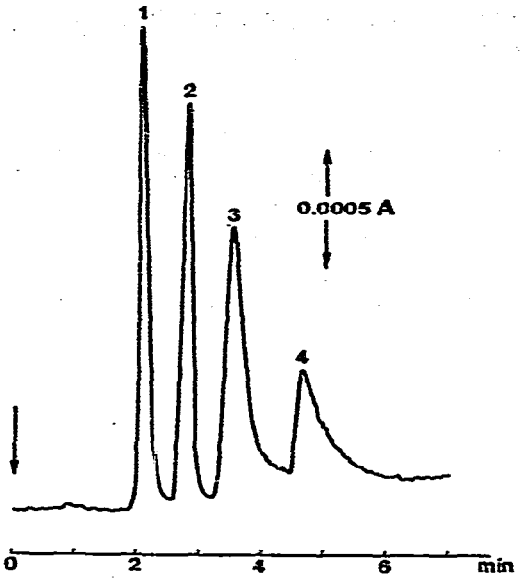

Fig. 13. Separation of sulphonates. Mobile phase: sodium butanesulphonate (0.01 *M*) in sodium citrate buffer, pH 3.7; 1.6 mm/sec; 7.7 MPa. Stationary phase: Nucleosil 100 (10 μm). Samples: 1 = sodium thymolsulphonate; 2 = sodium naphthalene-2-sulphonate; 3 = sodium anthracene-2-sulphonate; 4 = sodium camphorsulphonate.

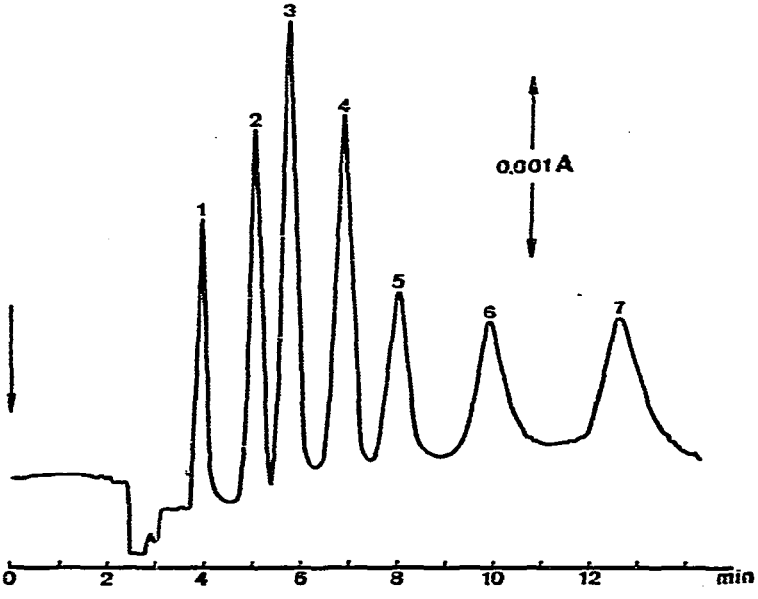

Fig. 14. Separation of anticholinergics. Mobile phase: sodium phosphate buffer, pH 2.2, ionic strength 0.1, +1.9% (v/v) 1-pentanol; 1.6 mm/sec; 7.7 MPa. Stationary phase: LiChrospher SI 100 (10 μm). Samples: 1 = scopolamine hydrobromide; 2 = atropine sulphate; 3 = benactyzine hydrochloride; 4 = adiphenine hydrochloride; 5 = methylatropine nitrate; 6 = oxypyrronium bromide; 7 = oxyphenonium bromide.

as competing cation to confer a suitable retention on the very hydrophobic ammonium ions. Fig. 12 demonstrates the effect on retention of methyl substitution at the nitrogen atom. Fig. 13 shows the separation of hydrophobic sulphonates in a system with butanesulphonate as competitor. In Figs. 14–16, the retention of cationic samples is regulated by the concentration of 1-pentanol in the mobile phase. Peaks 6 and 7 in Fig. 14 are closely related quaternary ammoniums with the same number of carbon atoms. The presence of two ammonium groups in prochlorperazine (Fig. 15) and in procaine (Fig. 16) decreases the hydrophobic character of these compounds.

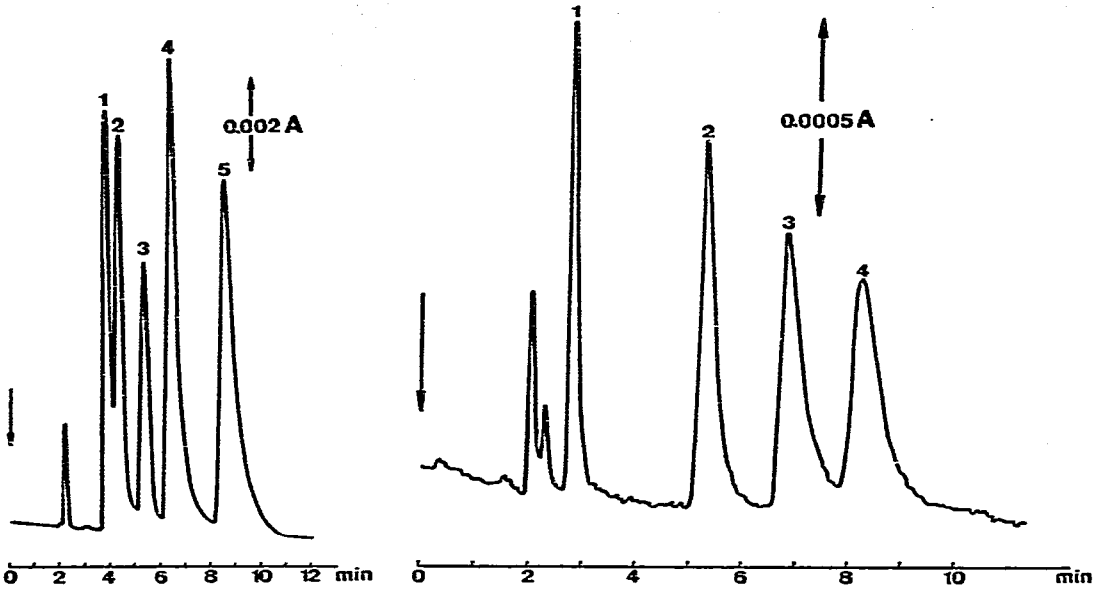

Fig. 15. Separation of phenothiazines. Mobile phase: sodium phosphate buffer, pH 2.2, ionic strength 0.1, +2.0% (v/v) 1-pentanol; 1.6 mm/sec; 8.4 MPa. Stationary phase: Nucleosil 100 (10 μm). Samples: 1 = prochlorperazine dimaleate; 2 = desmethylchlorpromazine hydrochloride; 3 = metopimazine; 4 = chlorpromazine hydrochloride; 5 = levomepromazine maleate.

Fig. 16. Separation of local anaesthesics. Mobile phase: sodium phosphate buffer, pH 2.2, ionic strength 0.1, +1.0% (v/v) 1-pentanol; 1.6 mm/sec; 7.7 MPa. Stationary phase: Nucleosil 100 (10 μm). Samples: 1 = benzocaine; 2 = procaine hydrochloride; 3 = lidocaine hydrochloride; 4 = amylocaine hydrochloride.

Amounts of sample in the nanogram range were usually used in the chromatograms presented in Figs. 7–16.

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