Journal of Chromatography, 185 (1979) 225–239

CHROM. 12,001

REVERSED-PHASE ION-PAIR CHROMATOGRAPHY WITH AN ADSORBING STATIONARY PHASE AND A HYDROPHOBIC QUATERNARY AMMONIUM ION IN THE MOBILE PHASE

I. RETENTION STUDIES WITH TETRABUTYLAMMONIUM AS CATIONIC COMPONENT

A. TILLY MELIN and M. LJUNGCRANTZ

Analytical Control, Astra Pharmaceuticals AB, S-151 85 Södertälje (Sweden)

and

G. SCHILL

Department of Analytical Pharmaceutical Chemistry, Biomedical Centre, University of Uppsala, Box 574, S-751 23 Uppsala (Sweden)

SUMMARY

The retention behaviour of some acids (acetylsalicylic acid, naphthalene-2-sulphonic acid, pentobarbital and vinbarbital) and bases (antipyrine, caffeine, codeine, dextropropoxyphene, hydroxyzine and Transergan) have been investigated using μBondapak C_{18} as stationary phase and a solution of tetrabutylammonium in acetonitrile + aqueous phosphate buffer of pH 3 and 6 (25:75) as the mobile phase.

The results indicate that the stationary phase contains sites with different adsorption abilities for hydrophobic ammonium ion pairs. Models for the retention have been developed and equilibrium constants for the distribution of the ion pairs to the hydrophobic stationary phase are presented.

INTRODUCTION

Pharmaceutical preparations often contain compounds with highly different properties, from more or less hydrophobic amines to aprotic compounds and acids. Reversed-phase chromatographic systems are often very suitable for the separation of such complex samples. The systems usually have an alkyl-bonded microporous silica as stationary phase and an aqueous buffer mixed with an organic modifier such as methanol or acetonitrile as the mobile phase. The pH of the mobile phase can be varied within the range 2–8, which permits the regulation of the retention of protolytes of widely different strengths.

In the pH range where the samples are present mainly in ionized form, they are retained as ion pairs and regulation of the retention can be effected by changing the nature and concentration of a counter ion[1]. The amines are often so strongly hydro-

phobic that they are highly retained even as ion pairs with very hydrophilic counter ions. A decrease in the retention can be achieved by an increase in the content of the organic modifier, but this will often make the systems less suitable for the separation of complex samples, as less hydrophobic sample components may become completely unretained. A decrease in the retention of the hydrophobic amines can also be obtained, however, by adding to the mobile phase another hydrophobic ammonium ion, which competes for the available adsorption sites[1]. The retention of anionic samples as ion pairs will, on the other hand, increase on addition of the hydrophobic ammonium ion and it is possible to regulate the capacity ratio of both cations and anions by changing the concentration and nature of the ammonium ion. Chromatographic systems constructed according to these principles will often enable highly selective isolation to be effected rapidly.

Numerous reversed-phase separations of acidic and basic drugs have been described, *e.g.*, acetylsalicylic acid[2,3], antipyrine[4], barbiturates[5–7], caffeine[3,8–10] and codeine[3,11,12]. The solid phase is often μBondapak C_{18}[2,4,6,8–13] but LiChrosorb RP-2[5], Nucleosil C_8[3], Spherisorb-10 ODS[14] and Partisil-10 ODS[15] are also used. The mobile phases are aqueous buffers of different pH with addition of, *e.g.*, methanol[4,7–9,11,12,14,16], acetonitrile[2,3,6,10,13,15] or butyronitrile[5]. Addition of ion-pair-forming agents such as 1-heptanesulphonic acid[7,11] and tetrabutylammonium[5] has been reported.

The aim of this investigation was to study the retention behaviour of cationic, anionic and uncharged samples in a reversed-phase system with an alkyl-bonded stationary phase and a mobile phase consisting of tetrabutylammonium dissolved in a mixture of phosphate buffer and acetonitrile. Acetylsalicylic acid, naphthalene-2-sulphonic acid, pentobarbital, vinbarbital, antipyrine, caffeine, codeine, dextropropoxyphene, hydroxyzine and Transergan were used as model compounds and the retention was regulated by changing the tetrabutylammonium concentration and the pH of the mobile phase. Models for the retention of ammonium ion pairs by sites of different character have been developed and used in the estimation of equilibrium constants for the distribution of the ion pairs to the stationary phase.

EXPERIMENTAL

Apparatus

The pump was an LDC 711-47 solvent delivery pump (Milton Roy Mini-Pump with a pulse dampener). The detector was a Vari-Chrom Liquid Chromatography Detector. The measurements were usually performed at a wavelength of 254 nm with the time constant set to 1 sec. The injector was a Valco valve injector with a sample loop of 25 μl.

The pH measurements were performed with a Radiometer PHM 64 Research pH meter, equipped with a Radiometer combined electrode.

All experiments were performed at ambient temperature (23°).

Chemicals and reagents

Tetrabutylammonium (TBA) iodide was obtained from AB Hässle (Mölndal, Sweden) and was converted into the hydroxide by shaking with silver oxide[17] and then neutralized with orthophosphoric acid. Acetonitrile was of Uvasol quality (E. Merck, Darmstadt, G.F.R.).

TABLE I

SAMPLES EXAMINED

Type	*Compound*	*Systematic name*	*pK_a*	*Ref.*
Acids	Acetylsalicylic acid	2-(Acetyloxy)benzoic acid	3.5	18
	Naphthalene-2-sulphonic acid	Naphthalene-2-sulphonic acid	<2	—
	Pentobarbital	5-Ethyl-5-(1-methylbutyl)barbituric acid	8.0; 12.6	19
	Vinbarbital	5-Ethyl-5-(1-methyl-1-butenyl)-barbituric acid	7.5; 12.3	19
Bases	Antipyrine	1,2-Dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one	1.5	20
	Caffeine	3,7-Dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione	<1	20
	Codeine	7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol	8.2	20
	Dextropropoxyphene	(*S*)-α-[2-(Dimethylamino)-1-methylethyl]-α-phenylbenzeneethanol propanoate	≈9*	21
	Hydroxyzine	2-(2-{4-[(4-Chlorophenyl)phenylmethyl]-1-piperazinyl}ethoxy)ethanol	2.1; ≈10*	22
	Transergan®	10*H*-Phenothiazine-10-carboxylic acid 2-(diethylamino)ethyl ester	≈9*	—

* Estimated.

The compounds used as samples are listed in Table I. They were of pharmacopoeial grade and were supplied by Astra Pharmaceuticals (Södertälje, Sweden).

The phosphate buffers were prepared with sodium as the cation and had an ionic strength of 0.05.

All other chemicals were of analytical-reagent grade and were used without further purification.

Column preparation

The column (200 × 3.2 mm I.D.) was made of 316 stainless steel with a polished inner surface, equipped with modified Swagelok connections and Altex stainless-steel frits (2 μm). μBondapak C_{18} of mean particle diameter 10 μm and with a specific area of 150 m^2/g according to the manufacturer (Waters Assoc., Milford, Mass., U.S.A.) was used as the chromatographic support.

The column was packed by the balanced-density slurry technique[23] with tetrachloroethylene as the suspending liquid. The packing procedure was performed at a pressure of 40 MPa. After packing, the column was washed with *n*-hexane, dichloromethane, ethanol and methanol before equilibration with the mobile phase.

Chromatographic technique

The mobile phases were prepared by mixing 25 volumes of acetonitrile and 75 volumes of phosphate buffer of pH 3.0 or 6.0. The pH values of the mobile phases mentioned below refer in all instances to the pH of the buffer component. Tetrabutylammonium dihydrogen orthophosphate was added to the buffers before mixing with the organic modifier. The mobile phases were degassed in an ultrasonic bath and thermostatted to ambient temperature (23°) before use.

The columns were equilibrated with mobile phase before use. A constant retention volume was usually obtained after pumping 50 column volumes. On changing the mobile phase the columns were washed with at least 50 ml of methanol to remove adsorbed ions[24].

The volume of mobile phase in the column, V_m, was determined by injecting a sample of potassium dichromate using acetonitrile–aqueous phosphate buffer (pH 8.0) (25:75) as the mobile phase[24].

The samples were dissolved in acetonitrile–phosphate buffer (25:75).

All results were obtained on the same column filling, which maintained constant properties for the whole of the experimental period.

The chromatographic results reported are the means from duplicate injections.

Determination of tetrabutylammonium in the column

The column was washed with 100 column volumes of methanol and the amount of tetrabutylammonium was determined by the picrate method[25].

RESULTS AND DISCUSSION

Ion-pair distribution to an adsorbing lipophilic stationary phase can be expressed by

$$Q^+_m + Z^-_m + A_s = QZ \cdot A_s$$

where Q^+_m and Z^-_m represent species in the hydrophilic mobile phase, A_s the available adsorption sites and $QZ \cdot A_s$ the adsorbed ion pair. A quantitative expression for the distribution process is given by the equilibrium constant, K_{QZ}, which is defined by

$$K_{QZ} = \frac{[QZ \cdot A]_s}{[Q^+]_m [Z^-]_m [A]_s} \quad (1)$$

From eqn. 1 it follows that it should be possible to regulate the distribution of one of the ionic species by changing the concentration of the counter ion in mobile phase. Several studies on reversed-phase ion-pair chromatography, however, have given results that indicate a more complex relationship between the distribution and the counter ion concentration[26,27]. A recent study has shown that the limited adsorption capacity of the stationary phase must be taken into consideration and a retention model is presented that well illustrates the chromatographic behaviour of carboxylic acids as ion pairs with quaternary alkylammonium ions[24].

For further development of the retention model it is necessary to study the retention behaviour of other kinds of samples, particularly ammonium ions of different structures. μBondapak C_{18} was preferred as the stationary phase as it gave good peak symmetry with the ammonium ion samples in contrasts to, *e.g.*, LiChrosorb RP-8 which was used in the previous studies.

Adsorption of tetrabutylammonium on the solid phase

The capacity of the stationary phase can be estimated from measurements of

the adsorption of tetrabutylammonium, Q^+. It is adsorbed as an ion pair with an anion from the mobile phase, Z^-, and the following expression is obtained:

$$K_o = [A]_s + [QZ \cdot A]_s \tag{2}$$

where K_o is the capacity of the stationary phase, *i.e.*, the maximum number of moles of QZ that can be adsorbed per gram of solid phase, $[A]_s$ is the number of available adsorption sites for QZ and $[QZ \cdot A]_s$ is the concentration of the adsorbed ion pair, the last two terms being expressed in moles per gram of solid phase.

Eqns. 1 and 2 can be combined to give

$$[QZ \cdot A]_s = \frac{K_o K_{QZ}[Q^+]_m[Z^-]_m}{1 + K_{QZ}[Q^+]_m[Z^-]_m} \tag{3}$$

Inversion gives

$$\frac{1}{[QZ \cdot A]_s} = \frac{1}{K_o} + \frac{1}{K_o K_{QZ}[Q^+]_m[Z^-]_m} \tag{4}$$

which shows that there should be a linear relationship between $1/[QZ \cdot A]_s$ and $1/[Q^+]_m$ if $[Z^-]_m$ is constant.

The adsorption of tetrabutylammonium ions was studied with mobile phases containing phosphate buffers of pH 3.0 and 6.0. Plots in accordance with eqn. 4 are given in Fig. 1 and the values of K_o and $K_{QZ}[Z^-]_m$, obtained from the intercept and the slope of the straight lines, are given in Table II. The capacity, K_o, of the μBondapak C_{18} phase used is slightly lower than that obtained for a LiChrosorb RP-8 phase with a mobile phase of very similar composition, while $K_{QZ}[Z^-]_m$ is about five times higher on μBondapak C_{18} than on LiChrosorb RP-8[24]. Tetrabutylammonium is obviously very strongly adsorbed to μBondapak C_{18} and even such a low mobile phase concentration of tetrabutylammonium as 0.007 *M* gives a site coverage of about 75%.

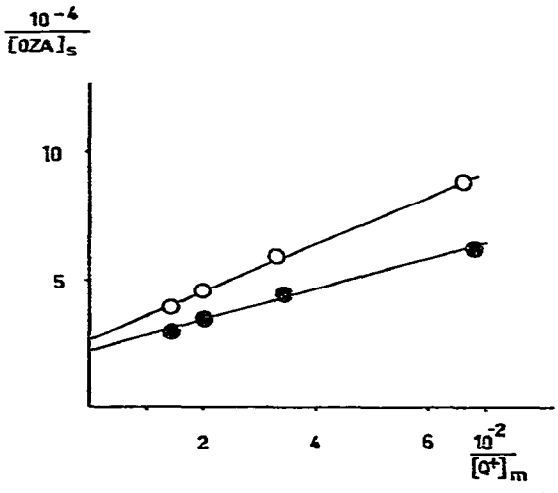

Fig. 1. Adsorption of tetrabutylammonium on the solid phase. Mobile phase: tetrabutylammonium, Q^+, in acetonitrile + aqueous phosphate buffer (25:75). ○, pH 3.0; ●, pH 6.0.

TABLE II

ADSORPTION OF TETRABUTYLAMMONIUM IONS

TBA concentration in the mobile phase: $1.5 \cdot 10^{-3}$–$7.0 \cdot 10^{-3}$ *M*.

pH of buffer	*Phosphate concentration (M)*	$K_0 \cdot 10^4$ *(mole/g)*	$K_{QZ}[Z^-]_m$	*Site coverage (%)*
3.0	0.04	0.34	330	31–67
6.0	0.03	0.38	500	37–77

Retention of acidic compounds as ion pairs

Acidic compounds can be retained in the system used by adsorption to the stationary phase both in acidic form and as ion pairs with tetrabutylammonium. When the pH of the mobile phase is much higher than the pK_a of the acid, HX, retention as an ion pair, QX, will dominate. As tetrabutylammonium also is adsorbed as an ion pair with a buffer anion, Z^-, the capacity can be expressed by

$$K_0 = [A]_s + [QX \cdot A]_s + [QZ \cdot A]_s \tag{5}$$

Eqn. 5 is based on the assumption that the ion pairs compete for the same adsorption sites and take up the same area of the retaining phase. The distribution of QX to the solid phase can be expressed by an equilibrium constant, K_{QX}, defined analogously to K_{QZ} (eqn. 1). Introduction of the equations for K_{QX} and K_{QZ} into eqn. 5 gives

$$[QX \cdot A]_s = \frac{K_0 K_{QX}[Q^+]_m[X^-]_m}{1 + K_{QZ}[Q^+]_m[Z^-]_m + K_{QX}[Q^+]_m[X^-]_m} \tag{6}$$

Assuming $K_{QX}[Q^+]_m[X^-]_m \ll (1 + K_{QZ}[Q^+]_m[Z^+]_m)$, which is a prerequisite for a concentration-independent retention of the sample and good peak symmetry, the capactity ratio, k'_X, is given by

$$k'_X = \frac{q[QX \cdot A]_s}{[X^-]_m} = \frac{qK_0K_{QX}[Q^+]_m}{1 + K_{QZ}[Q^+]_m[Z^-]_m} \tag{7}$$

where $q = W_s/V_m$ is the ratio of solid phase to mobile phase (g/l) in the column.

The anions of the acids are retained to some extent even when tetrabutylammonium is absent from the mobile phase. A compensation can be made according to eqn. 8, which is based on the assumption that the anions are retained as ion pairs with another cation of the mobile phase and compete for the same adsorption sites as the tetrabutylammonium ion pairs[24]:

$$k'_X = k' - \frac{k'_0}{1 + K_{QZ}[Q^+]_m[Z^-]_m} \tag{8}$$

where k'_0 is the capacity ratio in the absence of $[Q^+]_m$.

Transformation of eqn. 7 to a linear form gives

$$\frac{1}{k'_X} = \frac{1}{qK_0K_{QX}[Q^+]_m} + \frac{K_{QZ}[Z^-]_m}{qK_0K_{QX}} \tag{9}$$

which shows that a plot of $1/k'_X$ *versus* $1/[Q^+]_m$ should give a straight line if $[Z^-]_m$ is constant.

Chromatographic studies were performed with one strong acid (naphthalene-2-sulphonic acid), one acid of medium strength (acetylsalicylic acid with $pK_a = 3.5$) and two weak acids (pentobarbital and vinbarbital, with $pK_a > 7$). The separating efficiency was good, H being about 7 particle diameters at $k' \geqslant 5$ and a flow rate of 2.5 mm/sec. The changes in k'_X for acetylsalicylic acid with the concentration of tetrabutylammonium at pH 3 and 6 are shown in Fig. 2.

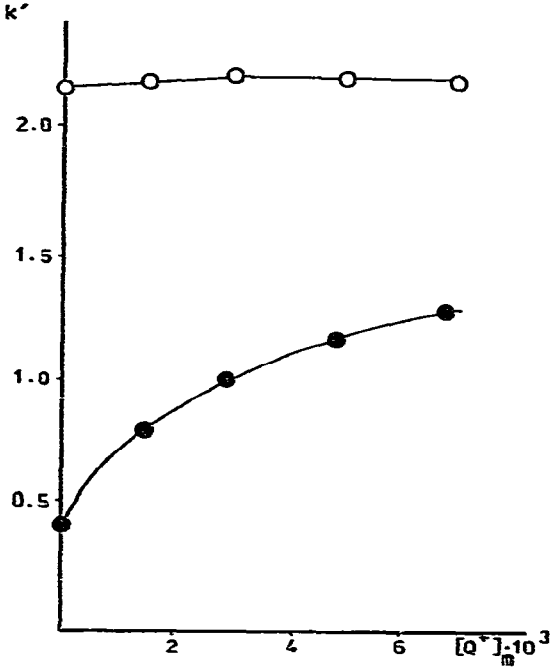

Fig. 2. Retention of acetylsalicylic acid at different concentrations of tetrabutylammonium, Q^+, in the mobile phase. Mobile phase: see Fig. 1. ○, pH 3.0; ●, pH 6.0.

At pH 6, acetylsalicylic acid is completely protolysed and mainly retained as an ion pair with tetrabutylammonium, and the increase in k'_X with increasing $[Q^+]_m$ can be assumed to follow eqn. 7. Naphthalene-2-sulphonic acid, which is completely protolysed at pH > 2, gave curves of this kind at both pH 3 and 6. Plots in accordance with eqn. 9 gave straight lines in all three instances, which proves the validity of the assumptions made. The constants for the distribution equilibria are given in Table III. They have been estimated from the intercept and the slope of the curves, measured values of $q = W_s/V_m$ and the values of the capacity, K_o, given in Table II.

The relation between the equilibrium constants of the samples, K_{QX}, is in accordance with expectation, naphthalene-2-sulphonic acid, which is the most hydrophobic of the acids owing to a higher carbon content, giving a significantly higher constant than acetylsalicylcic acid. The K_{QX} value for acetylsalicyclic acid obtained on the μBondapak C_{18} is significantly higher than that found with LiChrosorb RP-8 as solid phase[24]. The found values of $K_{QZ}[Z^-]_m$ are lower than the values obtained in the

TABLE III

RETENTION OF ACIDS AS ION PAIRS WITH TETRABUTYLAMMONIUM

TBA concentration: see Table II.

pH of buffer	*Phosphate concentration (M)*	*Sample*	k'_X *range*	$K_{QX} \cdot 10^{-3}$	$K_{QZ}[Z^-]_m$	$K_{QZ}[Z^-]_m$ *from Table II*
3.0	0.04	Naphthalene-2-sulphonic acid	1.18–5.28	48	170	330
6.0	0.03	Naphthalene-2-sulphonic acid	0.83–4.01	39	250	500
6.0	0.03	Acetylsalicylic acid	0.42–1.26	15	320	

adsorption studies. This might indicate that the retention model includes some too simplified assumptions, *e.g.*, the same adsorption area for different kinds of TBA ion pairs as well as the existence of only one kind of adsorption site.

Retention of uncharged acidic and basic compounds

In a mobile phase of pH 3, acetylsalicylic acid is mainly present in unprotolysed form. Changes in the tetrabutylammonium concentration of the mobile phase has an insignificant influence on the retention (see Fig. 2), which indicates that the sample is mainly retained in acidic form on a site on the stationary phase, where the influence of adsorption of tetrabutylammonium has little or no influence.

The influence of tetrabutylammonium on the retention of pentobarbital and vinbarbital is demonstrated in Fig. 3. Both compounds are very weak acids with $pK_a > 7$ and there are no indications that they are retained as ion pairs at pH 3 and 6. The retention decreases slightly, however, with increasing $[Q^+]_m$. The same observations were made with two very weak bases, antipyrine and caffeine, which are unprotolysed at pH $\geqslant$ 3. The slight decrease in the retention with increasing concentration of tetrabutylammonium might indicate that the uncharged samples are retained at a kind of adsorption site on the stationary phase, A^x, where tetrabutyl-

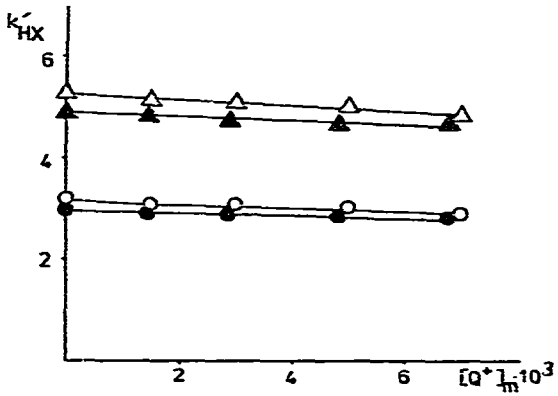

Fig. 3. Retention of barbituric acids. Mobile phase: see Fig. 1. Samples: pentobarbital, pH 3.0 (△) and 6.0 (▲); vinbarbital, pH 3.0 (○) and 6.0 (●).

ammonium will compete to a small extent. If the capacity of these sites of the stationary phase is K_0^x mole/g, the following expression is obtained:

$$K_0^x = [A^x]_s + [HX \cdot A^x]_s + [QZ \cdot A^x]_s \quad (10)$$

where $[A^x]_s$ is the number of available sites and $[HX \cdot A^x]_s$ and $[QZ \cdot A^x]_s$ the concentrations of the adsorbed species, all in moles per gram of solid phase. It must be emphasized that eqn. 10 is based on the approximation that HX and QZ take up the same area of the retaining phase.

An expression for the capacity ratio of HX, k'_{HX}, can be obtained by introducing an equilibrium constant for the sample, K_{HX}^x, defined by

$$K_{HX}^x = \frac{[HX \cdot A^x]_s}{[HX]_m [A^x]_s} \quad (11)$$

as well as a constant for the adsorbed ion pair, K_{QZ}^x, defined by analogy with eqn. 1. As the peak symmetry was good and the sample concentration was without influence on k'_{HX}, it is assumed that $(HX \cdot A^x]_s$ is negligible in eqn. 10. The equation for k'_{HX} will then take the form

$$k'_{HX} = \frac{q[HX \cdot A^x]_s}{[HX]_m} = \frac{qK_0^x K_{HX}^x}{1 + K_{QZ}^x [Q^+]_m [Z^-]_m} \quad (12)$$

The equation can be linearized by inverting it, which gives

$$\frac{1}{k'_{HX}} = \frac{1}{qK_0^x K_{HX}^x} + \frac{K_{QZ}^x [Q^+]_m [Z^-]_m}{qK_0^x K_{HX}^x} \quad (13)$$

The retention data for the uncharged compounds were plotted in accordance with eqn. 13. Good linearity was obtained in all instances, indicating that the model is an acceptable expression for the retention of uncharged acids and bases.

Values of $K_{QZ}^x [Z^-]_m$, estimated from the quotient between the slope and the intercept of the lines, are given in Table IV. A calculation based on the expression

$$K_0^x = [A^x]_s + [QZ \cdot A^x]_s = [A^x]_s (1 + K_{QZ}^x [Q^+]_m [Z^-]_m) \quad (14)$$

shows that less than 8% of the site is covered by QZ in the $[Q^+]_m$ range studied. The binding of QZ to this adsorption site is obviously weak, and the found constants are more than 30 times lower than the constant for the binding to the ion-pair adsorption site A (Tables II and III).

Retention of tertiary amines as ion pairs

The retention of organic ammonium ions can be decreased by adding a hydrophobic ammonium ion such as N,N-dimethyloctylammonium (DMOA) or N,N,N-trimethylnonylammonium to the mobile phase, as demonstrated by Wahlund and co-workers[28,29]. The effect is assumed to be due to competition for the available ad-

TABLE IV

RETENTION OF UNCHARGED COMPOUNDS IN THE PRESENCE OF TETRABUTYLAMMONIUM

TBA concentration: see Table II.

pH of buffer	*Phosphate concentration (M)*	*Sample*	*k' range*	$K^x_{QZ}[Z^-]_m$
3.0	0.04	Antipyrine	1.62–1.85	12
		Caffeine	0.97–1.05	8
		Pentobarbital	4.86–5.32	11
		Vinbarbital	2.89–3.18	13
6.0	0.03	Pentobarbital	4.69–4.93	7
		Vinbarbital	2.82–2.97	6

sorption sites in the stationary phase, and it has been shown that the retention can be regulated by change of the concentration of DMOA in the mobile phase[1].

The retention of hydrophobic tertiary ammonium ions can be regulated to a certain extent by the concentration of tetrabutylammonium in the mobile phase, as demonstrated in Fig. 4. The curves, showing the relationship between k' and $[Q^+]_m$, seem to flatten out at higher $[Q^+]_m$. Such effects can be obtained when the sample ions are retained by more than one kind of site and the binding abilities of the sites are affected differently by TBA. The previous studies (Table II and IV) have indicated that the stationary phase has two kinds of sites with different tendencies to bind tetrabutylammonium. If both sites are assumed to bind the hydrophobic sample ions, HB^+, as ion pairs with a buffer anion, Z^-, an expression for the capacity ratio, k'_{HB}, can be obtained by analogy with eqns. 7 and 12:

$$k'_{HB} = \frac{q[HBZ \cdot A]_s}{[HB^+]_m} + \frac{q[HBZ \cdot A^x]_s}{[HB^+]_m}$$

$$= \frac{qK_0K_{HBZ}[Z^-]_m}{1 + K_{QZ}[Q^+]_m[Z^-]_m} + \frac{qK^x_0K^x_{HBZ}[Z^-]_m}{1 + K^x_{QZ}[Q^+]_m[Z^-]_m} \quad (15)$$

The equilibrium constants for the sample, K_{HBZ} and K^x_{HBZ}, are defined by analogy with eqn. 1.

The hypothesis of two binding sites was tested by a graphical estimation of some of the equilibrium constants. This was performed graphically after linearization of eqn. 15 by substituting the expressions

$$\Delta k'_{HB} = qK^x_0K^x_{HBZ}[Z^-]_m \quad (16)$$

$$a = 1 + K^x_{QZ}[Q^+]_m[Z^-]_m \quad (17)$$

rearranging and inverting:

$$\frac{1}{k'_{HB} - \frac{\Delta k'_{HB}}{a}} = \frac{1}{qK_0K_{HBZ}[Z^-]_m} + \frac{K_{QZ}[Q^+]_m}{qK_0K_{HBZ}} \quad (18)$$

a was calculated by use of a mean value of $K^x_{QZ}[Z^-]_m$ obtained from Table IV.

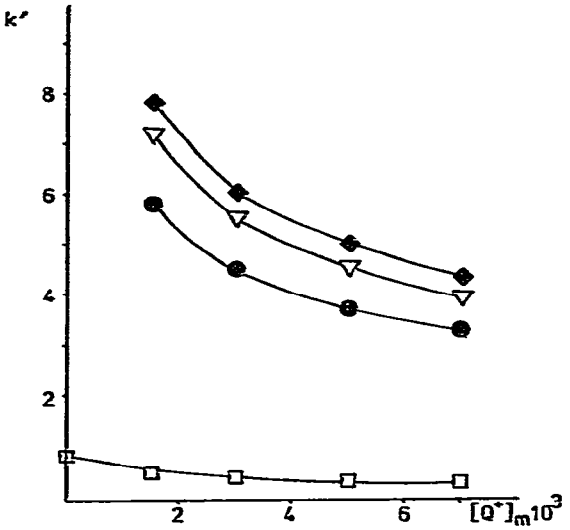

Fig. 4. Retention of tertiary amines. Mobile phase: tetrabutylammonium in acetonitrile + aqueous phosphate buffer (pH 3.0) (25:75). Samples: □, codeine; ▽, dextropropoxyphene; ◆, hydroxyzine; ●, Transergan.

$\Delta k'_{HB}$ is assumed to express the retention at site A^x, when the competition by tetrabutylammonium is negligible. It was estimated by testing in plots of $1/(k'_{HB} - \Delta k'_{HB}/a)$ *versus* $[Q^+]_m$ in accordance with eqn. 18. Plots were made for all of the tertiary ammonium ions and it was possible to find $\Delta k'_{HB}$ values that gave very good linearity in almost all instances. Examples are given in Fig. 5.

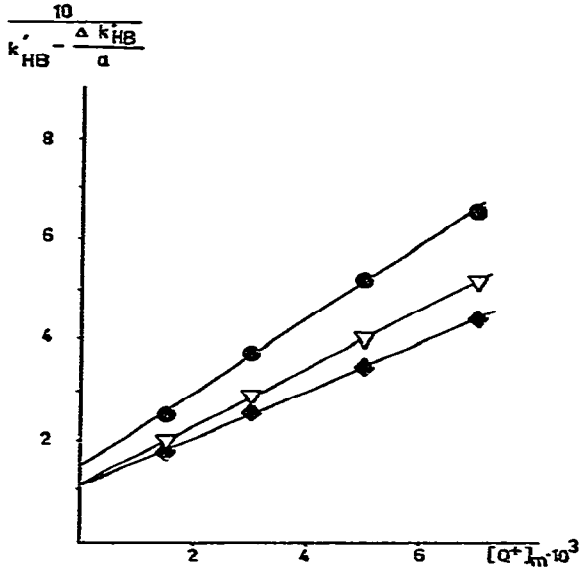

Fig. 5. Retention of tertiary amines compensated for the influence of a second adsorption site. Mobile phase: see Fig. 4. Samples: ▽, dextropropoxyphene; ◆, hydroxyzine; ●, Transergan.

TABLE V

RETENTION OF TERTIARY AMMONIUM IONS IN THE PRESENCE OF TETRABUTYLAMMONIUM

TBA concentration: see Table II.

pH of buffer	*Phosphate concentration (M)*	*Sample*	k'_{HD} *range*	$K_{HBZ}[Z^+]_m$	$\Delta k'_{HD}$	$K_{QZ}[Z^-]_m$	$K_{QZ}[Z^-]_m$ *from Table II*
3.0	0.04	Codeine	0.35– 0.50	12	0.276	442	330
		Dextropropoxyphene	3.94– 7.19	283	2.17	517	
		Hydroxyzine	4.35– 7.84	291	2.25	436	
		Transergan	3.28– 5.81	213	1.90	491	
6.0	0.03	Dextropropoxyphene	7.57–12.47	373	5.09	589	500
		Hydroxyzine	11.98–17.37	393	9.22	522	
		Transergan	8.29–12.48	319	6.40	642	

The found $\Delta k'_{HB}$ values are given in Table V. They constitute 30–80% of the total capacity ratio, k'_{HB}, and were determined with good precision, deviations from the given values of $\geqslant 1$% having a significant influence on the linearity of the plot.

Acceptable linearity could not be obtained for codeine at pH 6. The reason for this deviation has not been elucidated but it might be due to the fact that codeine is a considerably weaker base than the others and might be retained both as an ion pair and as a base at pH 6.

Constants estimated from the curves are given in Table V. $K_{HBZ}[Z^-]_m$ was obtained from the intercept, substituting a measured value of q and K_o from Table II. The relation between the values of $K_{HBZ}[Z^-]_m$ is in good agreement with predictions from liquid–liquid distribution data. Codeine, which is much less hydrophobic than the other amines, gives a considerably lower constant. The other three amines are highly hydrophobic and published distribution constants do not indicate any larger differences in hydrophobicity[21,22,30].

$K_{QZ}[Z^-]_m$ was obtained from the quotient slope/intercept. The found values are in acceptable agreement with those obtained by the adsorption measurements. The constants obtained from these studies are, however, estimated with the assumption of one adsorption site for tetrabutylammonium. If there are two sites with different adsorption properties, the total concentration of tetrabutylammonium in the stationary phase, C_{QZs}, is given by

$$C_{QZs} = [QZ \cdot A]_s + [QZ \cdot A^x]_s$$
$$= \frac{K_0 K_{QZ}[Q^+]_m[Z^-]_m}{1 + K_{QZ}[Q^+]_m[Z^-]_m} + \frac{K_0^x K_{QZ}^x[Q^+]_m[Z^-]_m}{1 + K_{QZ}^x[Q^+]_m[Z^-]_m} \quad (19)$$

The estimation of $K_{QZ}[Z^-]_m$ and K_o will require compensation for the adsorption of Q^+ to the second site, *i.e.*, $[QZ \cdot A^x]_s$. This is possible if the necessary constants are known. K_0^x is not known but an approximate calculation based on a mean value of $K_{QZ}^x[Z^-]_m$ from Table IV and the assumption of $K_0^x = K_o$ gave corrected values of K_o and $K_{QZ}[Z^-]_m$ deviating only about 5% from those given in Table II.

Influence of pH on retention

The data presented in the tables above show clearly that a change in pH of the mobile phase from 3 to 6 influences the capacity ratios and equilibrium constants. The results are summarized in Table VI.

TABLE VI

EFFECT OF pH INCREASE

Site	*Cation*	*Anion*	*Parameter*	R^*
A	*tert.*-Ammonium	$H_2PO_4^-$	$K_{HBZ}[Z^-]_m$	1.3–1.5
	TBA	$H_2PO_4^-$	$K_{QZ}[Z^-]_m$	1.5
	TBA	Naphthalene-2-sulphonate	K_{QX}	0.8
A^x	*tert.*-Ammonium	$H_2PO_4^-$	$\Delta k'_{HB}$	2.3–4.1
	H^+	Barbiturate	k'_{HX}	0.95
	TBA	$H_2PO_4^-$	$K_{QZ}^x[Z^-]_m$	0.6

* R = value at pH 6.0/value at pH 3.0.

The binding to site A is affected to only a moderate extent. At site A^x, on the other hand, a substantial increase in $\Delta k'_{HB}$ is obtained on increasing the pH, indicating a strong increase in the binding of the tertiary ammonium ions. The binding of the TBA ion pair decreases to some extent, while the weak acids are almost unaffected.

The fact that a considerable increase in the binding ability for ammonium ions with small substituents is observed when the pH is changed from 3 to 6 indicates that the site has weakly acidic properties and that it has such a position that it is not easily accessible to cations with bulky substituents such as tetrabutylammonium. It seems likely that the adsorption, at least in part, is due to non-derivatized silanol groups on the solid phase.

Applications

The easy regulation of the retention of both cationic and anionic compounds by the concentration of tetrabutylammonium in the mobile phase have made these chromatographic systems highly suitable for the separation of samples containing components with widely different chemical characters *e.g.*, pharmaceutical products.

A suitable tetrabutylammonium concentration is easily found by use of k' *versus* $[Q^+]_m$ curves of the kind demonstrated in Figs. 2–4. A typical chromatogram is shown in Fig. 6. Applications of the system will be discussed more extensively in a subsequent paper.

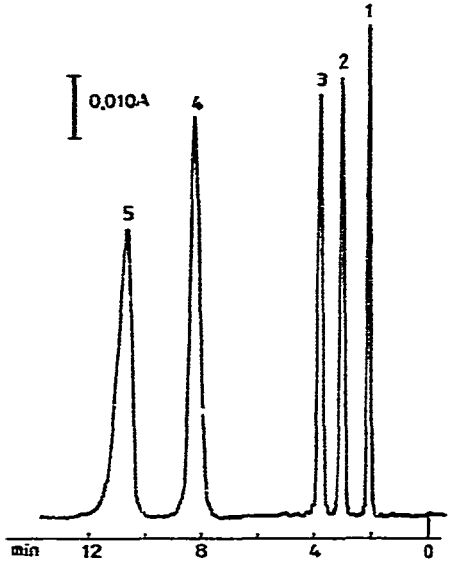

Fig. 6. Separation of acidic and basic compounds. Mobile phase: 10^{-3} *M* tetrabutylammonium in acetonitrile + aqueous phosphate buffer (pH 3.0) (25:75). Flow-rate: 3.2 mm/sec. Detection wavelength: 264 nm. Samples: 1 = caffeine; 2 = antipyrine; 3 = acetylsalicylic acid; 4 = Transergan; 5 = dextropropoxyphene.

REFERENCES

1 G. Schill and K.-G. Wahlund, in H. S. Herz and S. N. Chesler ((Editor), *National Bureau of Standards Special Publications 519, Proceedings of the 9th Materials Research Symposium 1978*, 1979, p. 509.

2 G. W. Peng, M. A. F. Gadalla, V. Smith, A. Peng and W. L. Chiou, *J. Pharm. Sci.*, 67 (1978) 710.
3 P. Helboe, A. K. Kristensen and M. Thomsen, *Arch. Pharm. Chem. Sci. Ed.*, 6 (1978) 141.
4 V. das Gupta and S. Sachanandani, *J. Pharm. Sci.*, 66 (1977) 897.
5 I. M. Johansson and K.-G. Wahlund, *Acta Pharm. Suecica*, 14 (1977) 459.
6 P. M. Kabra, B. E. Stafford and L. J. Marton, *Clin. Chem.*, 23 (1977) 1284.
7 I. Lurie, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 1035.
8 R. E. Hill, *J. Chromatogr.*, 135 (1977) 419.
9 F. Nielsen-Kudsk and A. K. Pedersen, *Acta Pharmacol. Toxicol.*, 42 (1978) 298.
10 J. J. Orcutt, P. P. Kozak, Jr., S. A. Gillman and L. H. Cummins, *Clin. Chem.*, 23 (1977) 599.
11 C. Olieman, L. Maat, K. Waliszewski and H. C. Beyerman, *J. Chromatogr.*, 133 (1977) 382.
12 V. das Gupta and A. G. Ghanekar, *J. Pharm. Sci.*, 66 (1977) 895.
13 G. R. Gotelli, P. M. Kabra and L. J. Marton, *Clin. Chem.*, 23 (1977) 957.
14 D. Howie, P. I. Adriaenssens and L. F. Prescott, *J. Pharm. Pharmacol.*, 29 (1977) 235.
15 R. A. Horvitz and P. I. Jatlow, *Clin. Chem.*, 23 (1977) 1596.
16 J. H. Knox and J. Jurand, *J. Chromatogr.*, 142 (1977) 651.
17 K. Gustavii and G. Schill, *Acta Pharm. Suecica*, 3 (1966) 259.
18 L. J. Edwards, *Trans. Faraday Soc.*, 46 (1950) 723.
19 D. A. Doornbos and R. A. de Zeeuw, *Pharm. Weekbl.*, 104 (1969) 233.
20 D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965.
21 K. O. Borg, H. Holgersson and P.-O. Lagerström, *J. Pharm. Pharmacol.*, 22 (1970) 507.
22 B.-A. Persson, *Acta Pharm. Suecica*, 5 (1968) 335.
23 R. E. Majors, *Anal. Chem.*, 44 (1972) 1722.
24 A. Tilly Melin, Y. Askemark, K.-G. Wahlund and G. Schill, *Anal. Chem.*, 51 (1979) 976.
25 K. Gustavii and G. Schill, *Acta Pharm. Suecica*, 3 (1966) 241.
26 D. Westerlund and A. Theodorsen, *J. Chromatogr.*, 144 (1977) 27.
27 C. Horváth, W. Melander, I. Molnár and P. Molnár, *Anal. Chem.*, 49 (1977) 2295.
28 K.-G. Wahlund and A. Sokolowski, *J. Chromatogr.*, 151 (1978) 299.
29 I. M. Johansson, K.-G. Wahlund and G. Schill, *J. Chromatogr.*, 149 (1978) 281.
30 G. Schill, R. Modin and B.-A. Persson, *Acta Pharm. Suecica*, 2 (1965) 119.