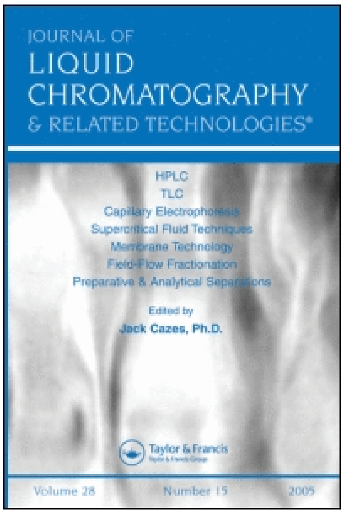

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EFFECTS OF COUNTER IONS IN ION-PAIR LIQUID CHROMATOGRAPHY OF HYDROPHOBIC AMINES ON NON-POLAR BONDED PHASES

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ABSTRACT

The retention behaviour of alprenolol and related hydrophobic amines in ion-pair adsorption systems has been examined with particular emphasis on the influence of different mono- and divalent counter ions (dihydrogenphosphate, bromide, perchlorate, dimethylcyclohexyl sulphate, sulphate and ethylenediaminetetraacetate). N,N-dimethyloctylamine (DMOA) and 1-pentanol were used as modifiers in the aqueous eluent and LiChrosorb RP-8 as stationary phase.

The retention is evaluated according to a two-site adsorption model and equilibrium constants are given for ion pair adsorption of DMOA. The retention of alprenolol has been evaluated in terms of ion exchange with DMOA and the ion-exchange constants are shown to be of the same magnitude and independent of the nature of the counter ion used. The ion-pair adsorption and the ion-exchange approaches are analogous expressions for the distribution process governing the retention.

INTRODUCTION

Hydrophobic amines can be separated on non-polar bonded silica phases by use of aqueous eluents containing suitable modifiers and ion-pairing agents (1-3). Recent studies (3-6) have shown that the retention of the solutes follows a model which comprises adsorption of modifiers and solutes to two different types of sites on the stationary phase. It has been postulated by others (7) that the chemically bonded phase constitutes one type of site with a high binding capacity, and that the other type of site is due to unsilanized hydroxyl groups on the surface of the silica. These silanol groups have a high affinity to hydrophobic amines but a low capacity, leading to overloading of solutes which, on the chromatograms, gives rise to tailing peaks and concentration-dependent capacity ratios.

The chromatographic behaviour can be improved by reducing the influence of the silanol groups by the presence of a suitable modifier in the eluent. The modifier competes with the solutes and reduces their adsorption on to the solid phase. Long chain aliphatic ammonium compounds, e.g. dimethyloctylamine (DMOA), have proved to be suitable as modifiers (1). Their effect may be due to interaction both with the carbon chains of the bonded phase and with unreacted silanol groups. Little attention has been paid to the possibilities of regulating the retention in such systems through the nature of the counter ion and its concentration in the eluent.

In the present paper mono- and divalent anions were examined as ion-pairing agents for hydrophobic amines and retention data were analysed according to the two-site adsorption model. It was found that the nature and concentration of the counter ions determine the extent of adsorption of DMOA on to the solid phase. The retention seems to be only indirectly dependent on the nature of the anion and can be treated either as ion-pair adsorption or as ion exchange between DMOA and the solutes, cf. (8-10). The ion-exchange approach seems to be particular suited for divalent anions as ion-paring agents in the eluent.

EXPERIMENTAL

Chemicals and Reagents

N,N-Dimethyloctylamine (DMOA) was purchased from ICN Pharmaceuticals (Plainview, NY, U.S.A.) and distilled before use.

Alprenolol and the other amines used as solutes (3) were supplied by the Department of Organic Chemistry, AB Hässle, and so was 3,5-dimethylcyclohexylsulphate (DMCHS) used as counter ion (potassium salt). 1-Pentanol was of Fisher Scientific A.C.S. quality and all other chemicals were of analytical-reagent grade and used without further purification.

Liquid Chromatographic System

The liquid chromatograph consisted of an Altex 110 A pump, a Rheodyne Model 70-10 sampling valve with a sample loop of 20 µl and an LDC spectroMonitor III spectrophotometer operated at 270 nm. Chromatographic columns (150 x 4.0 mm) were packed with LiChrosorb RP-8, 5 µm, from E. Merck and operated at 1.0 ml/min at 23 ± 1°C.

The eluents were aqueous buffers prepared from inorganic or organic acids, DMOA and sodium hydroxide, in which 1-pentanol, 0,115 moles/L, and appropriate amounts of other counter ions (DMCHS or KBr) were dissolved. The columns were conditioned with methanol before equilibriation with the eluent. As a rule, test solutions injected contained 3×10^{-4} moles/L of each of the amines.

RESULTS AND DISCUSSION

Symbols

A detailed symbol list and derivation of the equilibrium equations have been presented earlier (ref. (3)).

$[A]_s$	Available adsorption sites expressed in moles per gram of solid phase.
$[QXA]_s$, $[HBXA]_s$, $[Pe_nA]_s$	Adsorbed species in moles per gram of solid phase.
$K_{QX} = \frac{[QXA]_s}{[Q^+]_m [X^-]_m [A]_s}$	Equilibrium constant for adsorption of Q^+ as ion pair with X^- on site A.

$K_{HBX} = \dfrac{[HBXA]_s}{[HB^+]_m\ [X^-]_m\ [A]_s}$	Equilibrium constant for adsorption of HB^+ as ion pair with X^- on site A.
$K_{Pe} = \dfrac{[Pe_nA]_s}{[Pe]_m^n\ [A]_s}$	Equilibrium constant for adsorption of 1-pentanol on site A.
$K_{Q_2Y} = \dfrac{[Q_2YA]_s}{[Q^+]_m^2\ [Y^{-2}]_m\ [A]_s}$	Equilibrium constant for adsorption of Q^+ as ion pair with Y^{-2} on site A.
$K_o = [A]_s+[QXA]_s+[HBXA]_s+[Pe_nA]_s$	Adsorption capacity of site A.

Corresponding symbols are used for a second adsorption site A^x.

$q = W_s/V_m$	Ratio of amount of solid phase (g) to volume of eluent in the column (L).
$k'_{HB} = \dfrac{q\ [HBXA]_s}{[HB^+]_m}$	Capacity ratio of the amine HB^+ retained as ion pair with X^-.

Retention Principles

The retention of ionic solutes on a non-polar solid phase can be regulated by the kind and concentration of ionic modifiers present in the aqueous eluent. The modifiers are adsorbed as ion pairs on the stationary phase in an equilibrium which can be expressed quantitatively by combining the expressions for K_o and K_{QX} given above. Ionic and neutral modifiers will compete with the solute for the adsorption capacity of the solid phase. The effect of this competition is summarized in the following expression for the capacity ratio of a solute, HB^+, migrating in a system with Q^+, X^- and 1-pentanol in the eluent (cf. (3)).

$$k'_{HB} = \frac{q\ K_o\ K_{HBX}\ [X^-]_m}{1 + K_{Pe}\ [Pe]_m^n + K_{QX}\ [Q^+]_m\ [X^-]_m} \qquad (1)$$

The equation is valid under the assumption that the adsorbed solute is a negligible fraction of K_o. The retention can, under these conditions, just as well be considered as an ion exchange process.

$$QXA_s + HB^+_m = HBXA_s + Q^+_m \tag{2}$$

with the ion-exchange constant, K_{ie}, defined by

$$K_{ie} = \frac{[HBXA]_s\ [Q^+]_m}{[QXA]_s\ [HB^+]_m} \tag{3}$$

A substitution of the expressions for the equilibrium constants, K_{QX} and K_{HBX} into eq. (3) gives

$$K_{HBX} = K_{ie}\ K_{QX} \tag{4}$$

It should be emphasized that ion-pair adsorption and ion exchange are analogous expressions for the distribution processes behind the retention under the conditions given above.

Two-site Adsorption

The retention models used in earlier studies (3-6) are based on assumptions of adsorption to two kinds of adsorption sites with different binding ability. The retention can then be expressed by

$$k'_{HB} = k'_{HBA} + k'_{HBA^x} \tag{5}$$

where k'_{HBA} and k'_{HBA^x} express the retention on sites A and A^x, respectively.

The binding to A, which is the stronger of the sites, is determined by the constants K_o, K_{HBX}, K_{QX} and K_{Pe}, while the binding to the weaker site A^x can be expressed by K^x_o, K^x_{HBX}, K^x_{QX} and K^x_{Pe}. The constants for binding to A are determined by measurements of k'_{HB} at low concentrations of Q^+ and X^-, where the influence of site A^x is minor and can be compensated for (3). The estimate of the constants is made by slope analysis based on eq. (6) obtained by inversion of eq. (1) after substitution of eq. (4).

$$\frac{1}{k'_{HB}} = \frac{1 + K_{Pe}\,[Pe]^n_m}{q\,K_o\,K_{ie}\,K_{QX}\,[X^-]_m} + \frac{[Q^+]_m}{q\,K_o\,K_{ie}} \qquad (6)$$

The constants for binding to site A^x are estimated at such high concentrations of Q^+ and X^- that site A is completely covered. The retention on site A, k'_{HBA}, is then constant, which gives the expression

$$k'_{HB} - k'_{HBA} = \frac{q\,K^x_o\,K^x_{ie}\,K^x_{QX}\,[X^-]_m}{1 + K^x_{Pe}\,[Pe]^n_m + K^x_{QX}\,[Q^+]_m\,[X^-]_m} \qquad (7)$$

The constants are determined by slope analysis after estimation of k'_{HBA}, as shown in a previous paper (3).

Monovalent Counter Ions

Hydrophilic counter ions are often used to promote suitable retention by chromatography of hydrophobic amines. Tables 1 and 2 give constants for dihydrogenphosphate, with alprenolol as solute in a system with LiChrosorb RP-8 as adsorbent, and an eluent of dimethyloctylamine (DMOA) in a phosphate buffer pH 2.2 containing 1-pentanol 0.115 M. It has not been possible to perform the measurements at constant ionic strength, but the variations have in most cases not exceeded the range 0.06 - 0.12.

The constants obtained with variation of the concentration of dihydrogenphosphate show good agreement with those obtained with variation of DMOA (Q^+). The calculation of the constants in

TABLE 1

Equilibrium Constants from Retention of Alprenolol on Site A as Ion Pair with Monovalent Counter Ions

X^-	$[X^-]_m$	$[Q^+]_m$	$K_{QX}.10^{-6}$	$K_o.K_{ie}.10^5$
$H_2PO_4^-$	0.005-0.1	0.001	1.1	1.2
$H_2PO_4^-$	0.057	0.001-0.005	0.70	2.1
ClO_4^-	0.002-0.025	0.001	8.4	1.9
Br^-	0.1	0.001-0.005	0.21	4.7
DMCHS	0.0005	0.001-0.005	24	11

Q^+ = DMOA, DMCHS = dimethylcyclohexyl sulphate. The constants for Br^- and DMCHS determined with 0.0076 M dihydrogenphosphate in the eluent. Estimates made with $K_{Pe}.[Pe]_m^n = 34.6$ and $q = 760$.

TABLE 2

Equilibrium Constants from Retention of Alprenolol on Site A^x as Ion Pair with Monovalent Counter Ions

X^-	$[X^-]_m$	$[Q^+]_m$	$K^x_{QX}.10^{-3}$	$K^x_o.K^x_{ie}.10^4$
$H_2PO_4^-$	0.01-0.07	0.05	3.5	2.7
$H_2PO_4^-$	0.057	0.01-0.05	4.8	2.2
ClO_4^-	0.02-0.13	0.02	16	3.3
Br^-	0.1	0.01-0.05	0.78	4.5
DMCHS	0.002	0.01-0.04	260	3.1

The constants for Br^- and DMCHS determined with 0.057 M dihydrogenphosphate in the eluent. Estimates made with $K^x_{Pe}.[Pe]_m^n = 9.71$ and $q = 760$.

the Tables has been made by use of values for K_{Pe} and K^x_{Pe} given in ref. (3).

The values of $K_o.K_{ie}$ and $K^x_o.K^x_{ie}$ are almost constant and seem to be independent of the counter ion used. $K^x_o.K^x_{ie}/K_o.K_{ie}$ is about 15. Previous studies (3) have shown that K^x_o/K_o is about 7, which indicates that K^x_{ie}/K_{ie} is about 2, i.e. of about the same magnitude.

The effect of changing the concentration of the ions in the eluent on the capacity ratio can be illustrated graphically by a diagram constructed according to principles given in the Appendix. The influence of $[H_2PO_4^-]_m$ and $[DMOA]_m$ on the retention of alprenolol is illustrated in Fig. 1. At low concentration of DMOA (Fig. 1 a), the adsorption to the stronger site dominates and k'_{HB} can be varied within a much wider range than at the higher concentration of DMOA (Fig. 1 b), where the adsorption to the stronger site is almost constant.

Two or more counter ions are often present in the eluent and involved in the ion-pair distribution, one of them being added to regulate the retention and the others to provide pH buffering. The retention of a cationic solute, HB^+, in an eluent containing

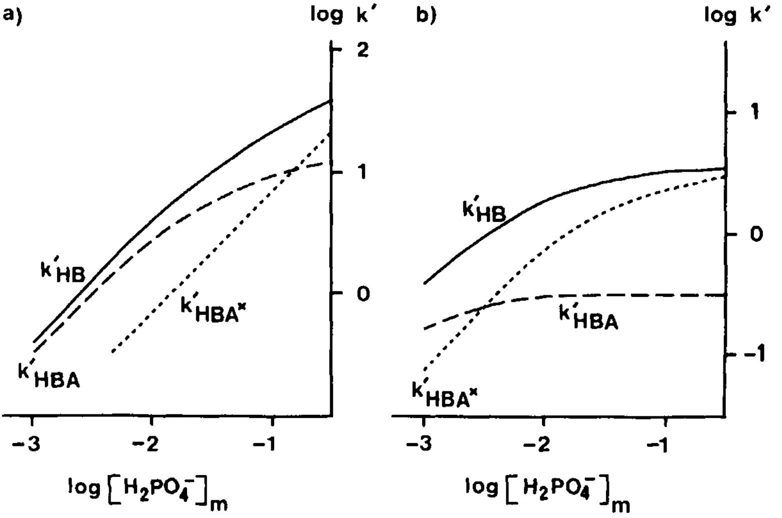

FIGURE 1. Retention of alprenolol as ion pair with dihydrogenphosphate. Eluent: DMOA and 0.115 M 1-pentanol in phosphate buffer pH 2.2. k'_{HB}, k'_{HBA} and k'_{HBA^x} are the overall capacity ratio and the capacity ratios for site A and A^x respectively. a) $[DMOA]_m$ = 0.001 M, b) $[DMOA]_m$ = 0.05 M.

Q^+, X^- and Z^-, can be derived analogously to eq. (1). If the magnitude of the ion-exchange constant is assumed to be independent of the anion, the following relationship is valid

$$K_{ie} = K_{HBX}/K_{QX} = K_{HBZ}/K_{QZ} \tag{8}$$

The expression for the capacity ratio of HB^+ then has the following form

$$k'_{HB} = \frac{q\ K_o\ K_{ie}\ (K_{QX}\ [X^-]_m + K_{QZ}\ [Z^-]_m)}{1 + K_{Pe}\ [Pe]^n_m + [Q^+]_m(K_{QX}\ [X^-]_m\ + K_{QZ}\ [Z^-]_m)} \tag{9}$$

The equilibrium constant of a counter ion, X^-, can be estimated in the presence of another anion, if the equilibrium constant of the latter, K_{QZ}, is known. The estimate can be made by slope analysis based on eq. (9) in inversed form.

$$\frac{1}{k'_{HB}} = \frac{1 + K_{Pe}\ [Pe]^n_m}{q\ K_o\ K_{ie}\ (K_{QX}\ [X^-]_m + K_{QZ}\ [Z^-]_m)} + \frac{[Q^+]_m}{q\ K_o\ K_{ie}} \tag{10}$$

In order to enable a good estimate, the condition used should give

$$K_{QX}\ [X^-]_m \gg K_{QZ}\ [Z^-]_m \tag{11}$$

This approach had to be adapted to the two-site theory in accordance with the principles given above (cf. eq. (7)).

The constants of bromide and dimethylcyclohexyl sulphate (DMCHS) are given in Tables 1 and 2. $H_2PO_4^-$ was present as buffering component in the mobile phase and the constants $K_{QZ} = 0.7 \times 10^6$ and $K^x_{QZ} = 4.8 \times 10^3$, taken from the Tables, were used to compensate for the adsorption of $H_2PO_4^-$ as ion pair. The K_{ie} found for DMCHS on site A deviates significantly from the values obtained with the inorganic counter ions. If this is due to the differences in size between the organic and the inorganic ions is at present not possible to elucidate.

The constants in Tables 1 and 2 show that the differences in extracting ability between the inorganic ions are rather small in the chromatographic system in comparison with liquid-liquid systems (11-13). $H_2PO_4^-$ shows a remarkably high constant compared to Br^- and ClO_4^-.

The regulation of the retention of a hydrophobic amine such as alprenolol can as a rule be made by changing the concentration of $H_2PO_4^-$, which then has the double function of counter ion and buffering component in the eluent. The influence of the counter ion on the retention of alprenolol when DMOA (Q^+) is present in the eluent is demonstrated in Fig. 2. The construction of the diagram is based on values of the ion-exchange constants from Tables 1 and 2. The Figure shows that a more hydrophilic counter ion such as $H_2PO_4^-$ gives a considerably larger range of variation of k'_{HB} than the more hydrophobic ions ClO_4^- and DMCHS.

A combination of two counter ions with different extracting ability can in certain cases have advantages. An example is given in Fig. 3, which shows a comparison of the retention when the

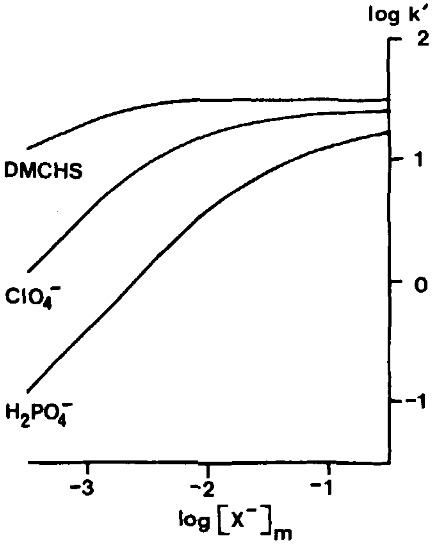

FIGURE 2. Regulation of the retention of alprenolol by the concentration of dihydrogenphosphate, perchlorate and dimethylcyclohexyl sulphate. Eluent: Counter ion, 0.01 M DMOA and 0.115 M 1-pentanol in aqueous solution of pH 2.

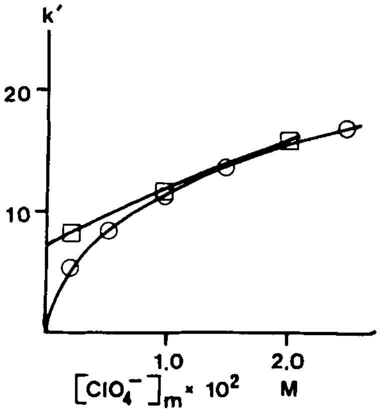

FIGURE 3. Regulation of the retention of alprenolol with perchlorate. Eluent: 0.001 M DMOA, 0.115 M 1-pentanol and sodium perchlorate with □ 0.01 M dihydrogenphosphate or ○ perchloric acid (pH ≃ 2.2) as buffering agent.

counter ions are perchlorate alone and a combination of perchlorate and dihydrogenphosphate. The relationship between k'_{HB} and $[ClO_4^-]_m$ is non-linear when perchlorate is the only counter ion, while an almost linear curve is obtained when $H_2PO_4^-$ is present in constant concentration in the eluent. An exact regulation of the retention is much easier to perform in the latter system.

Divalent Counter Ions

Sulphuric acid and ethylenediaminetetraacetic acid (EDTA) were tested as counter ions in pH ranges, where they are present mainly in divalent form. The chromatographic performance of the hydrophobic solutes in this system was as good as with monovalent counter ions. If the retention follows an ion-exchange model analogous to that applied to systems with monovalent counter ions, the following equilibrium should be valid

$$Q_2YA_s + HB_m^+ = QHBYA_s + Q_m^+ \qquad (12)$$

If the equilibrium constant of this process is K_{ie}, the capacity ratio of HB^+ can be expressed by the following equation derived in analogy with the corresponding equations for monovalent counter ions.

$$k'_{HB} = \frac{q\ K_o\ K_{ie}\ K_{Q_2Y}\ [Q^+]_m\ [Y^{-2}]_m}{1 + K_{Pe}\ [Pe]_m^n + K_{Q_2Y}\ [Q^+]_m^2\ [Y^{-2}]_m} \tag{13}$$

Estimates of constants according to a two-site model based on eq. (13) in inversed form gave the results presented in Tables 3 and 4.

The ion-exchange constant, K_{ie}, has the same value as obtained for monovalent counter ions (cf. Table 1), which confirms the validity of the ion-exchange model used.

TABLE 3

Equilibrium Constants from Retention of Alprenolol on site A as Ion Pair with Divalent Counter Ions

Y^{-2}	$[Y^{-2}]_m$	$[Q^+]_m$	$K_{Q_2Y}.10^{-10}$	$K_o.K_{ie}.10^5$
$EDTA^{-2}$	0.002-0.01	0.001	3.6	1.9
SO_4^{-2}	0.002-0.05	0.001	3.3	1.8

Q^+ = DMOA. Estimates made with $K_{Pe}.[Pe]_m^n = 34.6$ and $q = 760$.

TABLE 4

Equilibrium Constants from Retention of Alprenolol on site A^x as Ion Pair with Divalent Counter Ions

Y^{-2}	$[Y^{-2}]_m$	$[Q^+]_m$	$K^x_{Q_2Y}.10^{-6}$	$K^x_o.K^x_{ie}.10^4$
$EDTA^{-2}$	0.01-0.05	0.02	8.0	1.8
SO_4^{-2}	0.02-0.2	0.02	3.8	2.3

Estimates made with $K^x_{Pe}.[Pe]_m^n = 9.71$ and $q = 760$.

CONCLUSIONS

Ion-pair systems containing DMOA as modifier provide liquid chromatographic separations of hydrophobic amines with good performance. DMOA is also a major tool for regulating retention.

The nature and concentration of the counter ion are means to regulate retention. This effect levels off at high surface coverage of DMOA, i.e. at high content in the mobile phase or with hydrophobic counter ions.

The thermodynamics of the retention of the solute amines can be treated as ion-pair adsorption or as ion exchange, the two models being equivalent.

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APPENDIX

The change of the capacity ratio of a cationic solute, HB^+, with the concentration of the mobile phase components X^- and Q^+ can be illustrated by a diagram. It is based on expressions analogous to eq. (1) and separate constructions are made for each of the sites.

Eq. (1) can, after combination with eq. (4) be written in the following form:

$$k'_{HBA} = \frac{a\,[X^-]_m}{[Q^+]_m\,([X^-]_m + b\,K_{QX}^{-1}\,[Q^+]_m^{-1})} \qquad (14)$$

where $a = q\,K_o\,K_{ie}$ and $b = 1 + K_{Pe}\,[Pe]_m^n$.

The diagram gives the relationship between log k'_{HB} and log $[X^-]_m$. The construction is based on the following limiting cases

I. $[X^-]_m >> b\ K_{QX}^{-1}\ [Q^+]_m^{-1}$ (15)

gives

$$\log k'_{HBA} = \log a\ [Q^+]_m^{-1} \tag{16}$$

which is a horizontal line with the intercept $\log a\ [Q^+]_m^{-1}$.

II. $[X^-]_m << b\ K_{QX}^{-1}\ [Q^+]_m^{-1}$ (17)

gives

$$\log k'_{HBA} = \log a\ K_{QX}\ b^{-1} + \log [X^-]_m \tag{18}$$

which is a line with a slope of +1 going from the construction point ($\log k'_{HBA} = 0$; $\log [X^-]_m = - \log a\ K_{QX}\ b^{-1}$).

The final line will follow the lowest of the two lines in the diagram, passing 0.3 log units below the intersection point. The capacity ratio of HB^+, k'_{HB}, is the sum of k'_{HBA} and $k'_{HBA}x$.