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SOLUTE–SOLVENT INTERACTIONS IN ION-PAIR LIQUID CHROMATOGRAPHY OF AMINES ON NON-POLAR BONDED PHASES USING 1-PENTANOL AND N,N-DIMETHYLOCTYLAMINE AS ORGANIC MODIFIERS

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SUMMARY

Adsorption isotherms were determined for the partition of 1-pentanol and N,N-dimethyloctylamine (DMOA) between mobile aqueous phases and a chemically bonded silica (LiChrosorb RP-8). The adsorption isotherms were combined with capacity factors from the retention of alprenolol and some related secondary amines (solutes) in a model for the calculation of the influence of the modifiers on the retention of the solutes. The retention model includes the distribution of DMOA and the solutes as ion pairs to two different kinds of adsorption sites on the solid phase with different affinities and capacities for the adsorbed compounds.

INTRODUCTION

Liquid chromatography on chemically bonded non-polar stationary phases has become a widely used technique for the separation of organic compounds with various physico-chemical properties. For charged eluites, ion-pairing agents have been used as additives to the mobile aqueous phase to regulate retention. Reversed-phase ion-pair chromatography has been extensively investigated by Wahlund and co-workers[1–3], who used dynamic coating of the hydrophobized particles with 1-pentanol. The retention mechanism in systems with a non-polar bonded phase and a counter ion in the aqueous mobile phase has been widely discussed in the last few years. According to Terweij-Groen *et al.*[4] retention proceeds via dynamic ion exchange, and Scott and Kucera[5] presented some evidence that ion exchange was the major retention mechanism. As pointed out by Horváth *et al.*[6], the different conclusions about the retention mechanism may be due to the experimental conditions used in the different studies, *e.g.*, the hydrophobicity of the counter ion, the concentration range investigated and the content of organic solvent in the mobile phase.

In recent papers Schill and co-workers[7,8] have been treating the retention of the solutes as ion-pair adsorption to sites with different properties on the bonded phase. A similar approach has also been used by Crommen[9]. This means of evaluating which parameters are decisive for the retention of the ion pairs is in accordance with what was suggested by Snyder[10] for liquid–solid chromatography. In all of these considerations about the retention mechanism in liquid chromato-

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graphy on bonded phases, the heterogeneous nature of the solid phase in conjunction with the difficulties involved in ensuring reproducibility from one batch to another seems to be the obstacle to obtaining deeper knowledge of this subject. This was emphasized by Unger[11] in his book on porous silica and by Colin and Guiochon[12] in a review. Several parameters are not completely understood, *e.g.*, the role of unreacted silanol groups on the retention on non-polar bonded phases[13]. Horváth and Melander[14] first assumed that accessible silanol groups played only a minor role in the retention, but later found it necessary to mask such functions by an amine component in the mobile phase in the separation of different ionizable organic substances[15]. However, as established by Unger[11], thorough discrimination between different kinds of silanol groups is highly speculative and standardization should preferably be performed by chromatographic means, *i.e.*, by adjusting the eluent composition to maintain the chromatographic performance as regards efficiency and sequence of elution.

Wahlund and Sokolowski[16] found that ion-pair chromatography of tricyclic amines on bonded phases gave strongly tailing peaks, which could be overcome by the addition of a long-chain ammonium compound to the mobile phase. In later work[17] alkylammonium ions with different structures were tested for their ability to improve the chromatographic performance of the ion pairs. A retention model consisting of adsorption sites of different nature was used to evaluate the results. Adsorption isotherms for the organic modifiers were not determined, however.

The aim of this investigation was firstly to develop a separation system for hydrophobic amines as ion pairs with a satisfactory and reproducible chromatographic performance irrespective of different batches of bonded packing material Secondly, it was of interest to elucidate the retention behaviour of the solutes and the interaction between the modifiers, 1-pentanol and N,N-dimethyloctylamine (DMOA), and the amine solutes. Adsorption isotherms were determined by variation of the concentration of the modifiers in the aqueous mobile phase. The experimental data were evaluated by a similar approach to that used by Tilly-Melin *et al.*[8] and Sokolowski and Wahlund[17], indicating that a two-site model for both the two modifiers and the solutes was most likely. From the elucidation of the isotherms it has been possible to calculate and predict capacity factors and to determine the accumulation of modifiers on the bonded phase.

THEORETICAL

A retention model according to principles established by Schill and coworkers[7,8] were used and the different modifiers involved were assumed to compete with each other and the sample components for the available adsorption sites on the solid phase.

Adsorption of neutral modifiers

The monolayer adsorption of a neutral modifier, *e.g.*, 1-pentanol, is expressed by the equation

$$Pe_m + A_s = PeA_s \qquad (1)$$

If $(Pe)_m$ is the concentration of 1-pentanol in the mobile phase, $(A)_s$ is the number of available adsorption sites and $(PeA)_s$ is the number of moles of adsorbed 1-pentanol per gram of solid phase, the equilibrium constant, K_{Pe}, is given by

$$K_{Pe} = \frac{(PeA)_s}{(Pe)_m (A)_s} \tag{2}$$

The total concentration of adsorbed 1-pentanol constituting a monolayer is K_0, which is defined as

$$K_0 = (A)_s + (PeA)_s \tag{3}$$

Adsorption of modifiers as ion pairs

Ammonium compounds, *e.g.*, DMOA, added to the mobile phase can be adsorbed as ion pairs with a counter ion, X_m^-, according to the equation

$$D_m^+ + X_m^- + A_s = DXA_s \tag{4}$$

with the corresponding equilibrium constant, K_{DX}, defined as

$$K_{DX} = \frac{(DXA)_s}{(D^+)_m (X^-)_m (A)_s} \tag{5}$$

If one adsorbed ion pair is assumed to cover a surface area equivalent to one molecule of adsorbed 1-pentanol, the total amount of adsorbed modifiers is given by

$$K_0 = (A)_s + (PeA)_s + (DXA)_s \tag{6}$$

The influence of 1-pentanol on the adsorption of DMOA can be calculated by combination of eqns. 2, 5 and 6 which gives

$$(DXA)_s = \frac{K_0 K_{DX} (D^+)_m (X^-)_m}{1 + K_{DX} (D^+)_m (X^-)_m + K_{Pe} (Pe)_m} \tag{7}$$

Retention of amines

A sample amine, HB^+, is assumed to be adsorbed as an ion pair according to the same principle as discussed for DMOA. If the equilibrium constant of the sample, K_{HBX}, is defined as for DMOA (eqn. 5) and the term $(HBXA)_s$ is included in eqn. 6, an expression for the amount of adsorbed sample can be derived by combination of eqns. 2, 5 and 6:

$$(HBXA)_s = \frac{K_0 K_{HBX} (HB^+)_m (X^-)_m}{1 + K_{HBX} (HB^+)_m (X^-)_m + K_{DX} (D^+)_m (X^-)_m + K_{Pe} (Pe)_m} \tag{8}$$

The capacity factor of the sample, k'_{HB}, is defined as

$$k'_{HB} = \frac{W_s (HBXA)_s}{V_m (HB^+)_m} \tag{9}$$

where W_s is the weight of the solid phase in grams and V_m is the volume of the mobile phase on the column in litres. Combination of eqns. 8 and 9 gives

$$k'_{HB}V_m = \frac{W_s K_0 K_{HBX} (X^-)_m}{1 + K_{HBX} (HB^+)_m (X^-)_m + K_{DX} (D^+)_m (X^-)_m + K_{Pe} (Pe)_m} \quad (10)$$

EXPERIMENTAL

Chemicals and reagents

N,N-Dimethyloctylamine (DMOA) was obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.) and distilled before use. The amines used as solutes (Fig. 1) were of pharmacopoeial grade and supplied as chlorides by the Department of Organic Chemistry, AB Hässle (Mölndal, Sweden). 1-Pentanol was of Fisher Scientific A.C.S. quality and all other chemicals were of analytical-reagent grade and used without further purification.

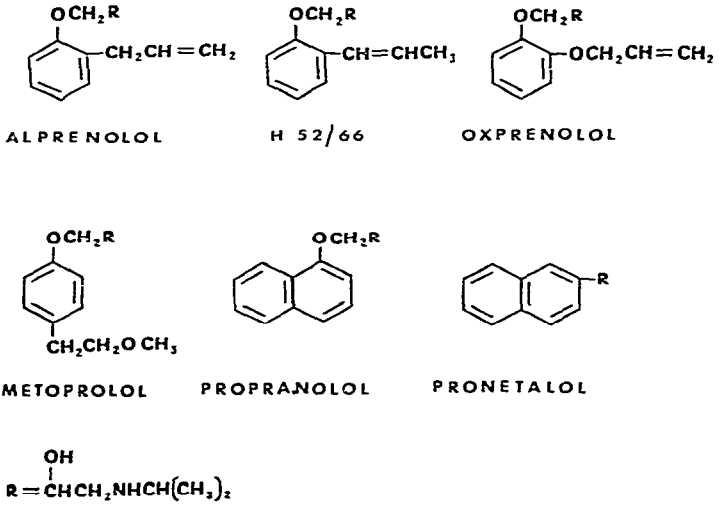

Fig. 1. Structures of the compounds studied.

Liquid chromatographic (LC) system

The liquid chromatograph was assembled from an LDC 711-47 pump with a pulse damper, a Rheodyne sampling valve with a sample loop of 20 μl and a Cecil 212 spectrophotometer operated at 270 nm. The stainless-steel columns (150 × 4.0 mm I.D.) were equipped with modified Swagelok connections and were packed with LiChrosorb RP-8, 5 μm (E. Merck, Darmstadt, G.F.R.). A Thermomix 1441 waterbath (B. Braun, Melsungen G.F.R.) was used to thermostat the mobile phase and the column at 23.0 ± 0.1°C.

The mobile phase consisted of 0.1 *M* phosphoric acid and a total of 0.05 *M* DMOA + sodium hydroxide in which an appropriate amount of 1-pentanol was dissolved. Before equilibration of the mobile phase, 100 ml of ethanol were passed through the column. The volume of the mobile phase in the column was determined by injecting either mobile phase of slightly changed composition or potassium nitrate

dissolved in the mobile phase. When the mobile phase contained DMOA, potassium nitrate was retained on the column and could not be used.

Gas chromatographic assay of adsorbed DMOA and 1-pentanol

The amount of 1-pentanol and DMOA adsorbed on to the bonded phase was determined by gas chromatography after elution from the LC column with 100 ml of ethanol. A Perkin-Elmer 3920B gas chromatograph equipped with a flame-ionization detector and stainless-steel columns (1.8 m × 2 mm I.D.) was used with nitrogen as the carrier gas at a flow-rate of 25 ml/min.

1-Pentanol was assayed on a column containing 5% of Carbowax 20M on Chromosorb G, DMCS. The column and injector temperatures were 90 and 230°C, respectively. 2-Pentanol was used as an internal standard.

DMOA was determined on a column containing 16% of Apiezon L and 4% of Carbowax 20M on Gas-Chrom P coated with 5% of potassium hydroxide. The column and injector temperatures were 165 and 230°C, respectively. Tributylamine was used as an internal standard. The sample was alkalified with 5 *M* sodium hydroxide solution before injection on to the column.

The results were compensated for the content of 1-pentanol or DMOA in the volume of mobile phase within the column.

RESULTS AND DISCUSSION

Adsorption of 1-pentanol

The adsorption isotherm of 1-pentanol illustrated in Fig. 2 has an upper concentration limit owing to the solubility in the aqueous mobile phase. In the high

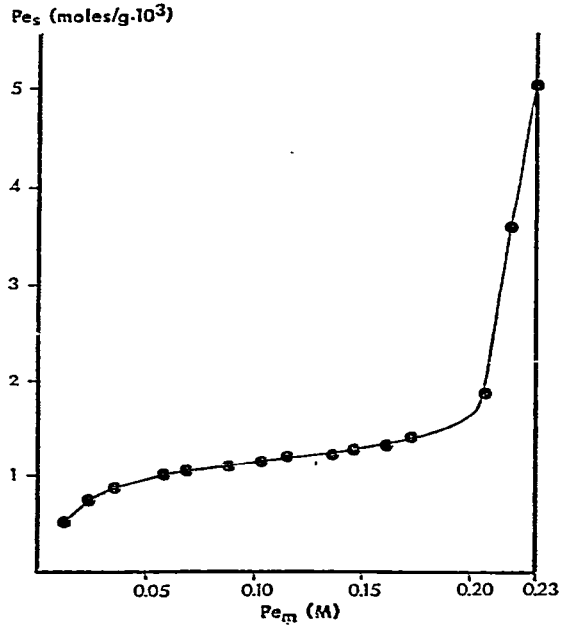

Fig. 2. Adsorption isotherm for 1-pentanol. Mobile phase: 1-pentanol in aqueous phosphate buffer (pH 2.2). Column: LiChrosorb RP-8, 5 μm.

concentration range a bulk phase is formed on the solid phase, which is indicated by a rapid increase in the amount of adsorbed 1-pentanol. In this study the concentrations of 1-pentanol in the mobile phase were below 0.12 mole/l and the alcohol is adsorbed according to a Langmuir isotherm for monolayer adsorption. The equilibrium constant of 1-pentanol and the monolayer capacity of the solid phase as defined in eqns. 2 and 3 were determined according to the usual principles for the calculation of monolayer adsorption[10], which gave $K_{Pe} = 55$ mole/l and $K_0 = 1.3 \cdot 10^{-3}$ mole/g.

Adsorption of DMOA and interaction with 1-pentanol

Isotherms for the adsorption of DMOA as ion pairs with bromide–phosphate are presented in Fig. 3. A decrease in the amount of adsorbed DMOA with increasing

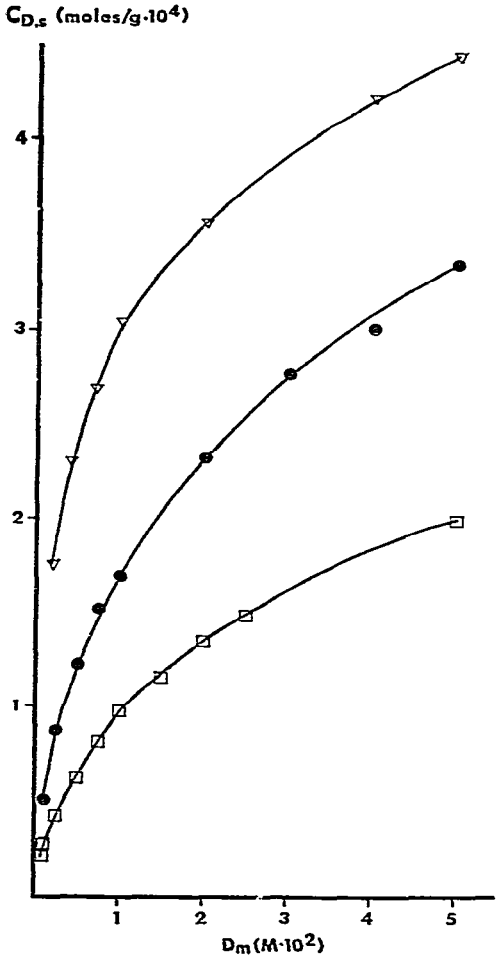

Fig. 3. Effect of 1-pentanol on the adsorption isotherm of DMOA. $C_{D,s}$ = total concentration of adsorbed DMOA. Mobile phase: DMOA and 0.05 *M* KBr in aqueous phosphate buffer (pH 2.2). ▽, $(Pe)_m = 0$; ●, $(Pe)_m = 0.0345$ *M*; □, $(Pe)_m = 0.115$ *M*.

concentration of 1-pentanol in the mobile phase is in accordance with the theory of competitive adsorption of the different modifiers as given in eqns. 6 and 7.

The experimental data presented in Fig. 3 could not be fitted to the Langmuir isotherm as the adsorption of DMOA was comparatively high at low mobile phase concentrations. This indicates saturation of the surface at high concentrations of DMOA or non- equivalent surface sites on the solid phase[10]. The hypothesis of two different types of binding sites was tested by the introduction of a second adsorption site A^x in eqn. 7 with the corresponding equilibrium constant K^x_{DX} and the monolayer capacity K^x_0 defined analogously to eqns. 5 and 6. An expression for the total amount of adsorbed DMOA on the two sites, $C_{D,s}$, can be derived in accordance with eqn. 7, which gives

$$C_{D,s} = \frac{K_0 K_{DX} (D^+)_m (X^-)_m}{1 + K_{DX} (D^+)_m (X^-)_m + K_{Pe} (Pe)_m} + \frac{K^x_0 K^x_{DX} (D^+)_m (X^-)_m}{1 + K^x_{DX} (D^+)_m (X^-)_m + K^x_{Pe} (Pe)_m} \quad (11)$$

It was assumed that DMOA is strongly adsorbed on one of the adsorption sites, A, and almost completely covers all of these sites when present at a high concentration in the mobile phase:

$$K_{DX} (D^+)_m (X^-)_m \gg 1 + K_{Pe} (Pe)_m \quad (12)$$

Eqn. 11 can then be transformed into

$$\frac{1}{C_{D,s} - K_0} = \frac{1 + K^x_{Pe} (Pe)_m}{K^x_0 K^x_{DX} (D^+)_m (X^-)_m} + \frac{1}{K^x_0} \quad (13)$$

By estimating a value of K_0 a linear adsorption isotherm was obtained [$(D^+)_m > 0.01$] from which the monolayer capacity and the equilibrium constants for adsorption of DMOA and pentanol on site A^x could be calculated (eqn. 13). The results are presented in Fig. 4 and Table I.

TABLE I

EQUILIBRIUM CONSTANTS FOR DMOA, 1-PENTANOL AND ALPRENOLOL

Solid phase, LiChrosorb RP-8; aqueous phase, 0.05 *M* potassium bromide in 0.1 *M* phosphate buffer (pH 2.2).

Site	$(Pe)_m$	$(D^+)_m$	$K_0 \cdot 10^5$	$K_{DX} (X^-)_m$	$K_{Pe} (Pe)^n_m$	$K_{HBX} (X^-)_m$
A	0	0.001–0.005	7.9	$1.0 \cdot 10^4$		
	0.0345	0.001–0.005	10.4		13.4	$1.34 \cdot 10^4$
	0.115	0.001–0.01	8.6		34.6	$0.65 \cdot 10^4$
A^x	0	0.007–0.05	38	50		
	0.0345	0.02 –0.05	50		1.77	76
	0.115	0.025–0.05	58		9.71	35

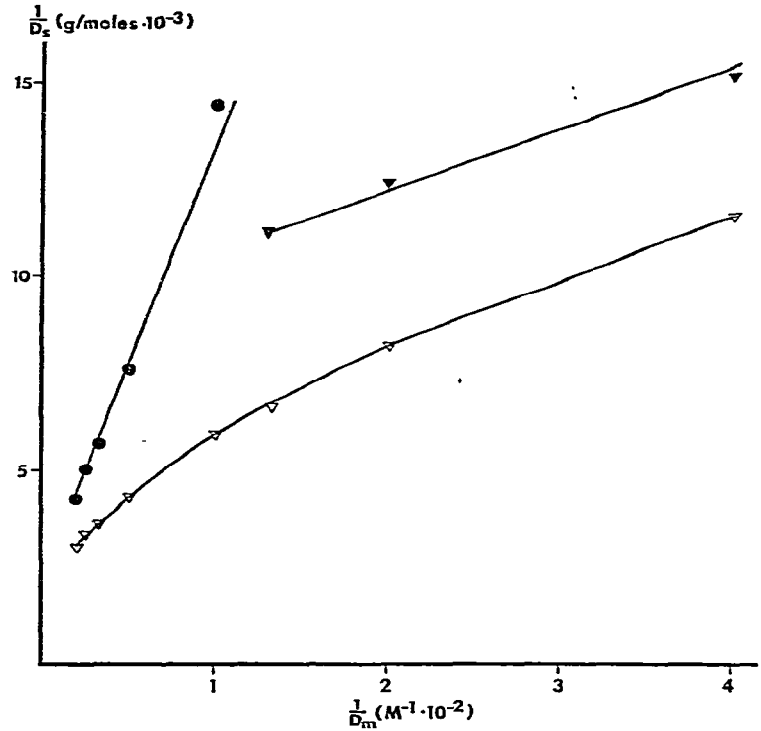

Fig. 4. Adsorption of DMOA according to a two-site adsorption model. Mobile phase: DMOA, 0.0345 *M* 1-pentanol and 0.05 *M* KBr in aqueous phosphate buffer (pH 2.2). ▽, Total adsorption of DMOA on the solid phase; ●, adsorption of DMOA on site A^x; ▼, adsorption of DMOA on site A.

Considering the magnitude of K^x_{DX} and K^x_{Pe} the following expression is valid at low concentrations of DMOA in the mobile phase:

$$K^x_{DX}\,(D^+)_m\,(X^-)_m \ll 1 + K^x_{Pe}\,(Pe)_m \tag{14}$$

Eqn. 11 can then be transformed into

$$\frac{1}{C_{D,s} - (D^+)_m\,a} = \frac{1 + K_{Pe}\,(Pe)_m}{K_0 K_{DX}\,(D^+)_m\,(X^-)_m} + \frac{1}{K_0} \tag{15}$$

in which

$$a = \frac{K^x_0 K^x_{DX}\,(X^-)_m}{1 + K^x_{Pe}\,(Pe)_m} \tag{16}$$

Eqn. 15 was used to evaluate the adsorption of DMOA to site A. The inverted value of the slope from eqn. 13 was inserted as the term *a* in eqn. 15. This compensation for adsorption of DMOA to site A^x gave a good linearity at low concentrations of DMOA in the mobile phase, as illustrated in Fig. 4. The equilibrium constants calculated from eqn. 15 are given in Table I.

The monolayer capacity of site A, K_0, is about five times lower than the capacity of site A^x, K_0^x, and about 15 times lower than the monolayer capacity for one-site adsorption of 1-pentanol. The last result indicates that the area required by an adsorbed 1-pentanol molecule is smaller than that for an adsorbed ion pair. This has not been considered in eqns. 11–16 but is taken into account in Table I by the introduction of an exponent n for $(Pe)_m$.

The relationship between the equilibrium constants for adsorption of DMOA to the two different sites is in accordance with the assumption of a significantly higher affinity of DMOA to site A. This is illustrated in Fig. 5, which shows the adsorption isotherms calculated from the equilibrium constants and monolayer capacities.

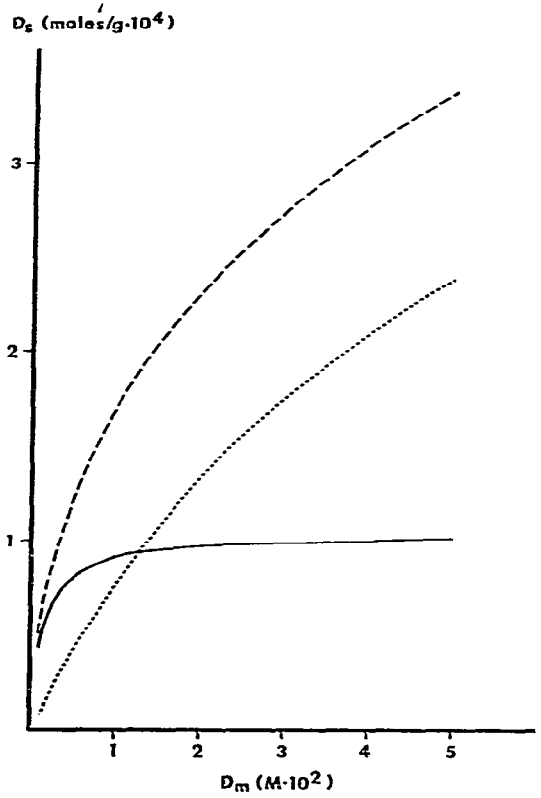

Fig. 5. Adsorption isotherms for DMOA calculated from the constants in Table I. Mobile phase as in Fig. 4. — — —, Total adsorption of DMOA on the solid phase; - - - -, adsorption of DMOA on site A^x; ———, adsorption of DMOA on site A.

Retention of sample amines

The influence of DMOA on the retention of some structurally related secondary amines is illustrated in Fig. 6. The retention is decreased significantly by even very low concentrations of DMOA in the mobile phase. At higher concentrations the effect levels off, owing to the limited capacity of site A, which then will be completely covered by DMOA.

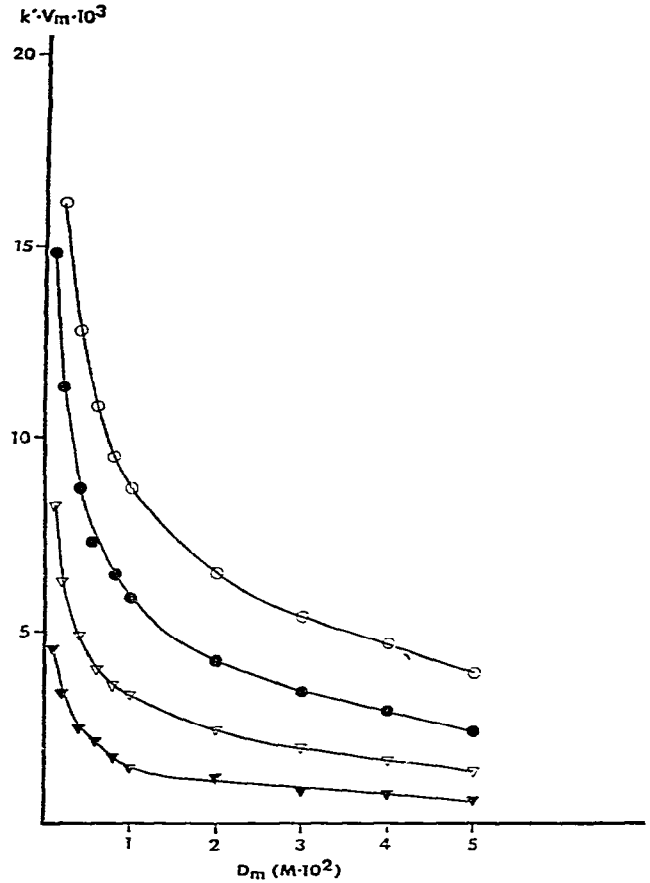

Fig. 6. Retention of some related hydrophobic amines at different concentrations of DMOA in the mobile phase. Mobile phase: DMOA and 0.115 *M* 1-pentanol in aqueous phosphate buffer (pH 2.2). ○, Propranolol; ●, alprenolol; ▽, pronetalol; ▼, oxprenolol.

Application of the two-site adsorption model to the expression for the capacity factor presented in eqn. 10, assuming that $K_{DX}(D^+)_m \gg K_{HBX}(HB^+)_m$, will give the following equation:

$$k'_{HB}V_m = \frac{W_s K_0 K_{HBX}(X^-)_m}{1 + K_{DX}(D^+)_m(X^-)_m + K_{Pe}(Pe)_m} + \frac{W_s K_0^x K_{HBX}^x (X^-)_m}{1 + K_{DX}^x(D^+)_m(X^-)_m + K_{Pe}^x(Pe)_m} \quad (17)$$

The adsorption process at site A was estimated from capacity factors of alprenolol at low concentrations of DMOA in the mobile phase, when eqn. 14 is assumed to be valid. Eqn. 17 can then be transformed into

$$\frac{1}{k'_{HB}V_m - a} = \frac{1 + K_{Pe}(Pe)_m}{W_s K_0 K_{HBX}(X^-)_m} + \frac{K_{DX}(D^+)_m}{W_s K_0 K_{HBX}} \quad (18)$$

in which

$$a = W_s K_0^x K_{HBX}^x (X^-)_m / [1 + K_{Pe}^x (Pe)_m] \quad (19)$$

At site A^x the capacity factors of alprenolol at high concentrations of DMOA in the mobile phase were utilized, eqn. 12 being valid. Eqn. 17 is then rearranged to

$$\frac{1}{k'_{HB} V_m - \frac{b}{(D^+)_m}} = \frac{1 + K_{Pe}^x (Pe)_m}{W_s K_0^x K_{HBX}^x (X^-)_m} + \frac{K_{DX}^x (D^+)_m}{W_s K_0^x K_{HBX}^x} \quad (20)$$

in which

$$b = W_s K_0 K_{HBX} / K_{DX} \quad (21)$$

By estimation of values for the constants a and b linear plots of $1/k'_{HB} \cdot V_m$ against $(D^+)_m$ were obtained. The value of a found was in good agreement with the inverted value of the intercept calculated from eqn. 20, and the value of b found was in good agreement with the value calculated from the slope in eqn. 18.

Equilibrium constants for alprenolol calculated from eqns. 18 and 20 are presented in Table I. A chromatogram for the separation of alprenolol and some structurally related amines as ion pairs with dihydrogen phosphate is shown in Fig. 7.

Influence of the anion

Bromide and dihydrogen phosphate were used as counter ions in the ion pairing of DMOA and the sample amines. There was a linear relationship between the capacity factor of alprenolol and the concentration of bromide in the mobile phase. An intercept was obtained which was due to concominant adsorption as phosphate ion pair, as shown in a separate study. Whether DMOA is adsorbed solely as an ion pair to both sites was not clear from our studies, as no separate quantitation of adsorbed anions was made. Such studies were performed by Bijsterbosch[18], who found that cationic surfactants were adsorbed according to a two-step isotherm, the counter ion (bromide) being involved only in the second step.

Two-site adsorption and chromatographic performance

The ability of DMOA to improve the chromatographic behaviour of hydrophobic amines was reported by Wahlund and Sokolowski[16,17]. They examined from a structural point of view different alkylammonium compounds as modifiers in the mobile phase and found that the kind of substituent on the amino group was of critical importance for the chromatographic performance. Melander *et al.*[15] found similar effects of amines as additives to the mobile phase and suggested that they exerted their influence by interaction with and masking of free silanol groups on the surface of the chemically-bonded packing. If so, the combined adsorption to the bonded hydrophobic moiety and to unreacted silanol groups might explain the structural effects observed for amines as modifiers and as solutes.

In the evaluation of the results of this study according to the two-site adsorption model, a significant difference between the adsorption of the ion pairs and of

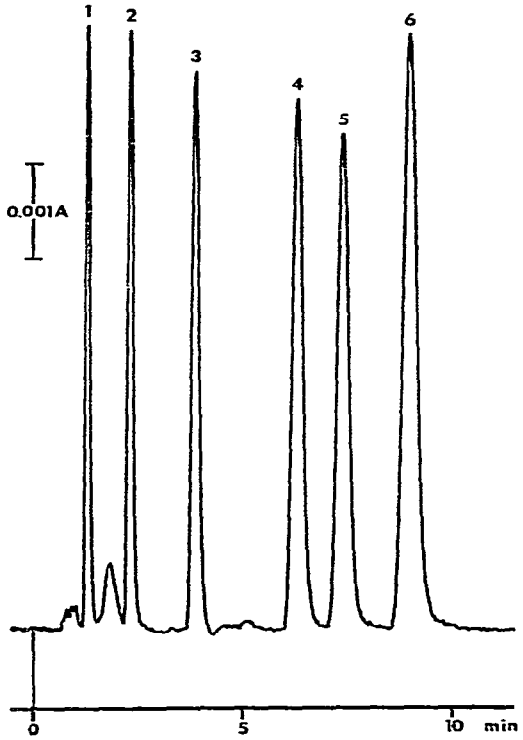

Fig. 7. Separation of alprenolol and some structurally related amines. Mobile phase: 0.01 *M* DMOA and 0.115 *M* 1-pentanol in aqueous phosphate buffer (pH 2.2). Flow-rate: 1.0 ml/min. Detection wavelength: 270 nm. Samples: 1, metoprolol; 2, oxprenolol; 3, pronetalol; 4, alprenolol; 5, H 52/66; 6, propranolol.

1-pentanol was observed (Table I). While the adsorption to site A^x is of the same magnitude for the studied components, there is a much higher affinity of the ion pairs of DMOA and alprenolol to site A than is the case for 1-pentanol. The selective adsorption of these ion pairs to site A may indirectly be the reason for the poor chromatographic behaviour of alprenolol and related amines in the absence of suitable modifiers such as DMOA. The strong binding constants of site A compared with site A^x indicate that parts of the surface of the solid phase other than the bonded material is involved in the adsorption process, *e.g.*, free silanol groups.

The two-site adsorption model proved to be useful in the evaluation of equilibrium constants from the adsorption isotherm of the modifiers. For a deeper understanding of the structural effects, modifiers and solutes with properties different to those of DMOA and the sample amines studied here should be examined with respect to adsorption and chromatographic behaviour. The outcome of such studies will contribute to chromatographic separations with maintained performance and efficiency irrespective of variations in the properties of the chemically bonded nonpolar packing and independent of the nature of the sample amines.

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