

CHROMSYMP. 479

SELECTIVITY EFFECTS BETWEEN IONIC AND NEUTRAL SOLUTES USING HYDROPHOBIC PAIRING IONS

R. B. TAYLOR* and R. REID

School of Pharmacy, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen AB9 1FR (U.K.)
and

C. T. HUNG

School of Pharmacy, University of Otago, Dunedin (New Zealand)

SUMMARY

The equations of the ion-exchange desolvation mechanism of hydrophobic ion pairing are expressed in terms of mobile phase pairing-ion concentration, assuming a linear isotherm. The variation of capacity factors in a mixture of hydrophobic acids and neutral solutes with cetyltrimethylammonium pairing ion concentration at pH 7 is examined for several acetonitrile concentrations. The mixture comprises 9-anthropic, 2-naphthoic and benzoic acids, together with their corresponding hydrocarbons. The variation of capacity factors in a mixture of hydrophilic bases and acids with pairing ion concentration is examined for pentane and octane sulphonate and lauryl sulphate at pH 2 for a single acetonitrile concentration. This mixture consists of norepinephrine, epinephrine and dopamine, together with homovanillic acid, dihydroxyphenylacetic acid and vanillylmandelic acid. It is shown that marked differences in selectivity with alteration in elution order can be achieved between ionic and neutral solutes as a result of changes in pairing ion type and concentration and organic modifier concentration. The adequacy of the modified equations in accounting for the observed effects is discussed and an attempt is made to generalise how the chromatographic variables studied affect retention and thus resolution in mixtures of ionic and neutral solutes.

INTRODUCTION

Several different theories have appeared in the literature describing the processes which occur during ion pairing with hydrophobic pairing ions in reversed-phase systems^{1–5}. These theories, for anionic pairing ions, have been reviewed elsewhere⁶. Of these, only the ion exchange desolvation mechanism provides a single explanation for both the maximum in the curve of capacity factor against pairing ion for ionic solutes and the concomitant decrease in the capacity factor of a neutral solute. The ion-exchange desolvation approach considers that the decrease observed at high pairing-ion concentrations is a direct result of coverage of the alkylsilica surface with adsorbed pairing ion, causing a decrease in surface area to be available for desolvation of any solute.

The enhancement of retention of ionic solutes by addition of hydrophobic ion pairing agent is now used as a matter of routine. The decrease of retention of neutral species, on the other hand, has been regarded as slight compared to the enhancement effects^{7,8} and has only recently been applied practically⁹.

In many mixtures encountered in biomedical^{9,10} fields a general separation problem encountered is that of resolving a mixture containing acidic, neutral and basic organic solutes. If chromatography is carried out at pH 2, such a mixture will exist as cationic and neutral species if the pK_a values of the acids and bases present are greater than three. An anionic pairing ion will enhance the retention of the cationic solutes while decreasing the retention of neutral species. On the other hand, if a high pH mobile phase is used, such a mixture will exist as anionic and neutral species. While the upper limit of pH usable with alkylsilica stationary phases is relatively low (7–8), at this pH most organic acids are predominantly in the anionic form and many bases essentially undissociated. Cationic pairing ion will thus increase the retention of acids while decreasing that of neutral and basic compounds. In such circumstances, the opposite directions of variation of capacity factor with pairing-ion concentration can be used to produce changes in selectivity between the charged and neutral groups of compounds.

To date, few data have appeared in the literature to indicate the magnitude or the variables affecting the decrease of k' for a neutral solute with pairing-ion concentration, particularly relative to the behaviour of ionic solutes of similar hydrophobicity. The variation of capacity factor of an ionic solute $k'_{c^{\mp}}$ with adsorbed monovalent pairing ion concentration $[P^{\pm}]_{org}$ has been derived as⁵

$$k'_{c^{\mp}} = \frac{1}{V_m} \left(A_s K_{1c} - K_{1c} A_p [P^{\pm}]_{org} + K_{2c} K_{IE} A_s \frac{[P^{\pm}]_{org}}{[C^{\mp}]_{aq}} - K_{2c} K_{IE} A_p \frac{[P^{\pm}]_{org}^2}{[C^{\mp}]_{aq}} \right) \dots \quad (1)$$

where the symbols have their previous meaning⁵. For a neutral solute the capacity factor k'_n is given by

$$k'_n = \frac{1}{V_m} (K_{In} A_s - K_{In} A_p [P^{\pm}]_{org}) \dots \quad (2)$$

These equations are of little practical application unless the value of $[P^{\pm}]_{org}$ is known. $[P^{\pm}]_{org}$ is believed to be related to the experimentally controlled $[P^{\pm}]_{aq}$ or concentration of pairing ion in the mobile phase by some form of adsorption isotherm. Linear isotherms¹¹ and also those following Freundlich and Langmuir type equations have been used to fit the adsorption data with varying success^{12–14}. It has also been shown that no single isotherm type can fit such data over all concentration ranges, and that the shape of the isotherm alters with mobile phase organic modifier concentrations¹⁵.

In the present work the above equations are modified using a linear isotherm and used to predict the magnitude of the maximum in $k'_{c^{\mp}}$ and its position on the

$[P^\pm]_{\text{aq}}$ scale. These predictions are compared with the experimental results obtained in two model systems.

While the assumption of a linear isotherm will not be accurate for all pairing ions at all concentrations, it is likely that it will be adequate at low surface coverage, *i.e.* for weakly adsorbed pairing ions or at high organic modifier concentration.

THEORETICAL

Predictions from existing equations

Eqns. 1 and 2 can be expressed in terms of $[P^\pm]_{\text{aq}}$ using the linear isotherm

$$[P^\pm]_{\text{org}} = K_0[P^\pm]_{\text{aq}} \dots \quad (3)$$

where K_0 is the desolvation equilibrium constant of the hydrophobic pairing ion. The modified equations can be written as

$$k'_{c^\mp} = \frac{1}{V_m} \left(K_{1c}A_s - K_{1c}K_0A_p[P^\pm]_{\text{aq}} + K_{2c}K_{IE}K_0A_s \frac{[P^\pm]_{\text{aq}}}{[C^\mp]_{\text{aq}}} - K_{2c}K_{IE}K_0^2A_p \frac{[P^\pm]_{\text{aq}}^2}{[C^\mp]_{\text{aq}}} \right) \dots \quad (4)$$

and

$$k'_n = \frac{1}{V_m} (K_{1n}A_s - K_{1n}K_0A_p[P^\pm]_{\text{aq}}) \dots \quad (5)$$

respectively.

These modified equations allow the effect of organic modifier concentration, which will alter the value of K_0 for a given pairing ion and hydrophobicity of pairing ion which also determines K_0 in any given solvent to be examined more readily as a function of $[P^\pm]_{\text{aq}}$. For a neutral solute eqn. 5 predicts that k'_n should decrease linearly with increase in $[P^\pm]_{\text{aq}}$ and that the slope of this line should increase the more hydrophobic the pairing ion (K_0) and/or the solute (K_{1n}). For an ionic solute eqn. 4 predicts a characteristic maximum in the curve of k'_{c^\mp} against $[P^\pm]_{\text{aq}}$ and this has been experimentally observed for diverse solutes by various workers^{1,5,7,15}. After differentiation of eqn. 4 with respect of $[P^\pm]_{\text{aq}}$ and setting the gradient equal to zero, it can be shown that

$$\frac{2K_{2c}K_{IE}K^2A_p[P^\pm]_{\text{aq(max)}}}{V_m[C^\mp]_{\text{aq}}} = \frac{K_{2c}K_{IE}K_0A_s}{V_m[C^\mp]_{\text{aq}}} - \frac{K_{1c}K_0A_p}{V_m}$$

Since $A_p \ll A_s$ and $K_{1c} \ll K_{2c}K_{IE}$ ¹⁵ the term $\frac{K_{1c}K_0A_p}{V_m}$ is neglected and

$$[P^\pm]_{\text{aq(max)}} = \frac{A_s}{2A_pK_0} \dots \quad (6)$$

This shows that the maximum in the $k'_c{}^\pm$ against $[P^\pm]_{\text{aq}}$ curve will occur at smaller values of $[P^\pm]_{\text{aq}}$ the larger or more hydrophobic the pairing ion used. Also, the magnitude of $k'_c{}^\pm$ obtained at this maximum can be obtained by substituting for $[P^\pm]_{\text{aq}(\text{max})}$ in eqn. 4 to give

$$k'_c{}^\pm(\text{max}) = \frac{1}{V_m} \left(K_{1c}A_s - \frac{K_{1c}A_s}{2} + \frac{K_{2c}K_{IE}A_s^2}{2A_p[C^\mp]_{\text{aq}}} - \frac{K_{2c}K_{IE}A_s^2}{4A_p[C^\mp]_{\text{aq}}} \right)$$

Since $K_{1c} \ll K_{2c}K_{IE}$

$$k'_c{}^\mp(\text{max}) = \frac{K_{2c}K_{IE}A_s^2}{4V_m A_p [C^\mp]_{\text{aq}}}$$

Since $[C^\mp]_{\text{aq}}$ varies with pairing ion concentration, if buffer concentration is low

$$[C^\mp]_{\text{aq}(\text{max})} = [P^\pm]_{\text{aq}(\text{max})} = \frac{A_s}{2K_0 A_p}$$

and

$$k'_c{}^\mp(\text{max}) = \frac{K_{2c}K_{IE}K_0 A_s}{2V_m} \dots \quad (7)$$

Thus, the maximum value of $k'_c{}^\mp$ will increase as the size of the pairing ion used. This maximum value will also increase in any given solvent system as the hydrophobicity of the solute increases (K_{2c}).

The use of a non-linear isotherm in place of eqn. 3 does not alter the form of eqns. 6 and 7 but would affect the concentration dependence of k' in eqns. 4 and 5.

Choice of solute systems

A solute mixture (Set I) of benzene (B), naphthalene (N), and anthracene (A) together with the corresponding carboxylic acids benzoic acid (BA), 1-naphthoic acid (NA) and 9-anthroic acid (AA) is used to represent very hydrophobic compounds requiring an appreciable proportion of organic modifier to obtain reasonable k' values. This set of solutes provides large differences in hydrophobicity between different acids and neutral compounds while minimal hydrophobicity differences exist between each hydrocarbon and the corresponding undissociated acid. These compounds are used to examine the effect of organic modifier concentration for a single highly adsorbed pairing ion cetyltrimethylammonium (CTAB) at pH 7. To examine the effect of different hydrophobicity of pairing ion, a set of solutes (Set II) is used; consisting of compounds either cationic or neutral at pH 2. These compounds comprise norepinephrine (NE), epinephrine (E) and dopamine (DA), together with some of the accepted metabolites of these catecholamines which are acidic namely vanillylmandelic acid (VMA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). These compounds, all have octanol-water partition coefficients less than five,

represent a more hydrophilic group than the aromatic hydrocarbons and have reasonable capacity factors at low concentrations of organic modifier which allows demonstration of the effect of pairing ion hydrophobicity.

EXPERIMENTAL

A Waters Assoc. liquid chromatography system was employed consisting of M6000A pump, fixed-wavelength (254 nm) ultraviolet detector, system controller and data module. Injection was by the WISP^(TM) system and columns of various lengths between 15 and 200 mm, 5 mm in diameter were slurry-packed at high pressure with Hypersil ODS (Shandon Southern). Water was purified by a MilliQ^(TM) system and HPLC-grade acetonitrile was used as supplied (Rathburn Chemicals). Pairing ions pentane sulphonate and octane sulphonate (PSA) and (OSA) were used as the sodium salts as supplied (Aldrich Chemicals) and laurylsulphate (SLS) also as the sodium salt was obtained from Fisons Scientific. Cetrinide (CTAB) was obtained from Thornton and Ross. Benzene and naphthalene were of reagent quality and anthracene was purified by recrystallisation. Benzoic acid, 1-naphthoic acid and 9-anthroic acid were used as supplied (Aldrich Chemicals). The solute set representing catecholamines and metabolites were obtained as indicated previously⁹.

Measurement of k' was carried out by chromatographing ionic and neutral solutes sequentially in the same solvent. Retention times were recorded to 0.01 min and t_0 , was determined by injection of water. Where necessary small t_0 values for very short columns were calculated from data obtained on longer columns. A fixed volume of solvent was recycled, and pairing ion concentration was altered by adding weighed amounts of solid pairing ion directly to the solvent and equilibrating.

RESULTS AND DISCUSSION

The results of the chromatographic measurements on Set I using CTAB as pairing ion at four different acetonitrile concentrations are shown in Fig. 1a-d. The effects of increasing the proportion of organic modifier in an ion-pairing system is twofold. Using the nomenclature of eqns. 3-5; the effect of increase of acetonitrile is firstly to reduce the desolvation constants K_{1c} and K_{1n} . This is seen as the marked decrease in capacity factor for all solutes in absence of pairing ion as acetonitrile concentration is increased. Under these conditions the hydrophobicity of the neutral solute is in all cases greater than the corresponding acid anion and very large differences between k'_c - and k'_n are observed. The second effect of increasing acetonitrile concentration is to decrease the amount of pairing ion adsorbed at any $[P^+]_{aq}$, *i.e.* K_0 in eqn. 3 is reduced. In addition, K_{2c} the desolvation constant for the ionic solute after ion exchange is also reduced and both of these effects account for the decrease in the maximum value of k'_c - observed as acetonitrile concentration increases. The decrease in K_0 with increasing acetonitrile concentration is also verified by the observation that the maximum value of k'_c - occurs at smaller $[P^+]_{aq}$ the lower the acetonitrile concentration. This latter effect is so marked that maximum k'_c - values for the carboxylic acids could not be determined directly in 10% acetonitrile, but from the data obtained, occur at $[P^+]_{aq}$ values less than 1 mM. The $[P^+]_{aq}$ value at which the maximum k'_c - occurs is constant for all solutes in a given solvent system

which indicates that it is a surface phenomenon while the maximum value of k'_c varies strongly with hydrophobicity of the particular solute which is consistent with the dependence of $k'_{c-(max)}$ on K_{2c} in eqn. 7 for different solutes.

The effect of addition of pairing ion on the retention of the three neutral solutes tested is to decrease the capacity factors at all acetonitrile concentrations measured. The magnitude of this decrease, as measured by the initial gradient of the k'_n against $[P^+]_{aq}$ curve, increases as the acetonitrile concentration decreases. This is in agreement with the dependence of k'_n on K_0 in equation 5 and points to the decrease being

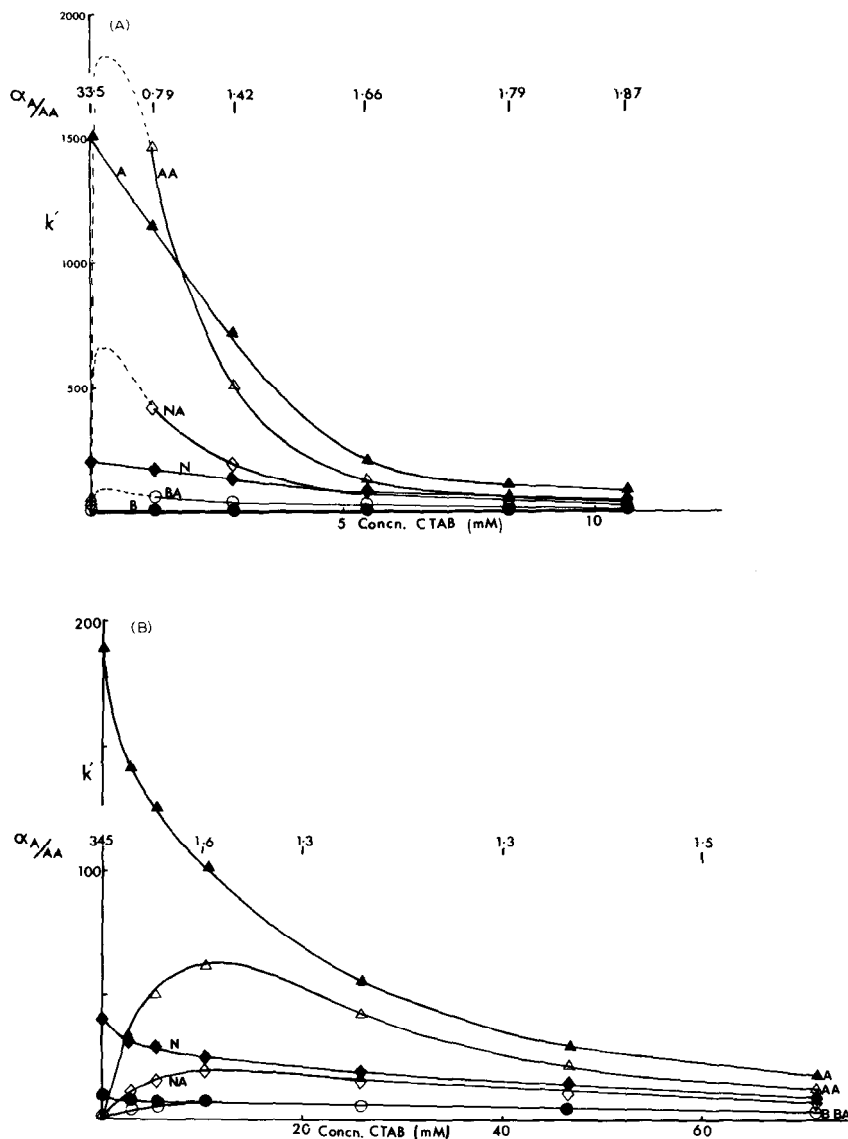


Fig. 1.

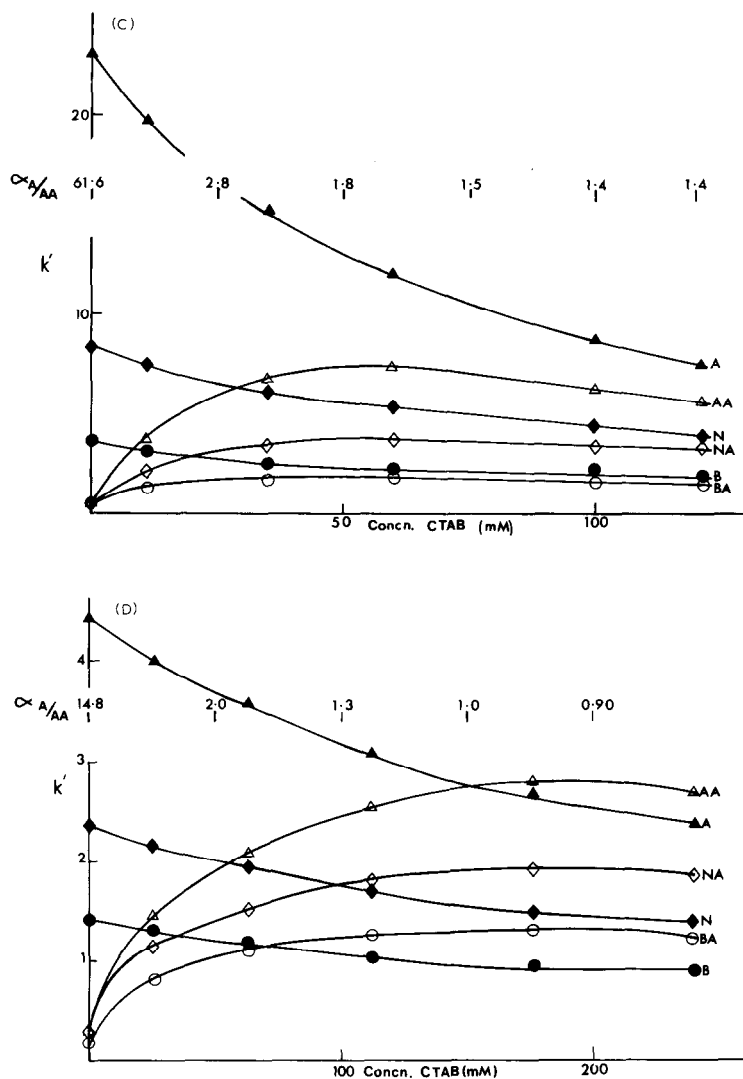


Fig. 1. Variation of capacity factor, k' , with CTAB concentration for different mobile phase proportions of acetonitrile, using solute Set I at pH 7 (a) 10%, (b) 30%, (c) 50%, (d) 70% (v/v) in acetonitrile and 10 mM buffer. Compound identification as in the text.

a result of decreased surface availability. The decrease of k'_n over the $[P^+]_{aq}$ range investigated, for all acetonitrile concentrations, is of the same order of magnitude as the increase in k'_c - observed under corresponding conditions for comparable solutes. This is evidence that the desolvation aspect of retention in such systems is of comparable importance to that of ion exchange or ion interaction. This suggestion is supported by the observation that the decrease of k'_n with $[P^+]_{aq}$ is greater the more hydrophobic the solute as described by the appearance of K_{1n} in eqn. 5.

Also shown in Fig. 1 are values of selectivity (α) between the A/AA pair as a

function of $[P^+]_{aq}$. At all acetonitrile concentrations these decrease from high values in the absence of pairing ion to values ranging between 1 and 2. In the situation of high adsorption of CTAB as a result of small acetonitrile concentration Fig. 1a the order of elution is reversed over certain $[P^+]_{aq}$ ranges. It is, however, of more interest to consider Set I as a whole and to observe that particularly at low loadings of pairing ion, Fig. 1c and d, that considerable elution order changes are apparent among different ionic and neutral solutes.

Fig. 2a-c shows the results obtained using solute Set II. These results confirm the observations made above for the situation where variable surface coverage of the

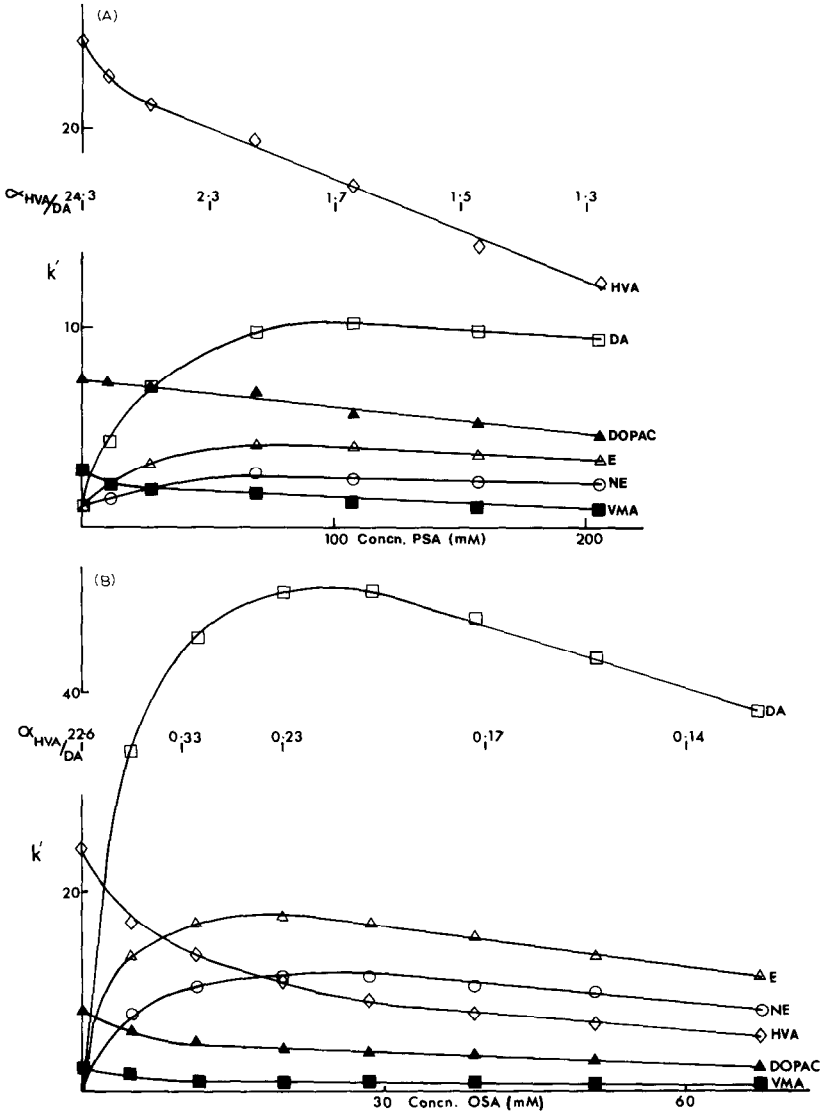


Fig. 2.

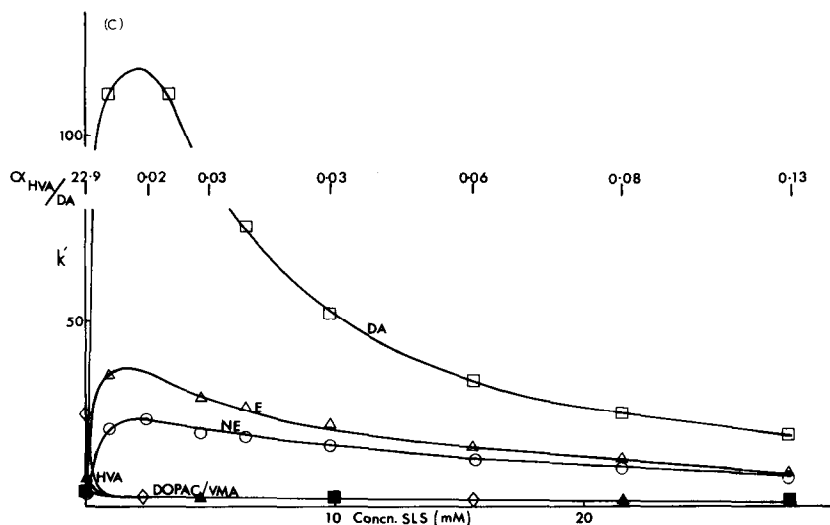


Fig. 2. Variation of capacity factor with pairing ion concentration, using solute Set II at pH 2 in 5% (v/v) acetonitrile and 10 mM buffer. (a) Pentanesulphonate, (b) octanesulphonate, (c) laurylsulphonate. Compound identification as in the text.

alkylsilica is produced by pairing ions of different hydrophobicity for a mixture of hydrophilic solutes in which the ionic species require anionic pairing ions for retention. It is seen that as the pairing ion hydrophobicity increases so k'_{o+} at the maximum becomes larger and occurs at smaller $[P^-]_{aq}$ values for the cationic solutes as predicted in eqns. 7 and 6, respectively. Also, for the undissociated acids behaving as neutral solutes the initial slope of the k'_n against $[P^-]_{aq}$ curve becomes steeper with increasing pairing ion hydrophobicity, as predicted in eqn. 5. The differences in solute hydrophobicity are not so marked as in Set I, but again qualitatively, it is seen that in Fig. 2a the slope of HVA ($P_{octanol/water} = 3.7$) is much greater than that of VMA ($P_{octanol/water} = 0.46$) verifying the dependence of this slope on K_{in} in eqn. 5. In Fig. 2 selectivity values are shown for the three pairing ions studied using the HVA/DA pair and selectivity is seen to change as a function of $[P^-]_{aq}$. The predicted linearity in the decrease of k'_n with $[P^\pm]_{aq}$ is not observed in Figs. 1 or 2. This may be an indication of the lack of linearity in the isotherm which would be consistent with the most linear relationships being obtained for Figs. 1d and 2a where surface coverage would be low. Alternatively the term $A_p[P^\pm]_{org}$ used to describe surface area occupied by pairing ion may be an oversimplification if, for example, micelle type aggregates are formed on the stationary phase surface.

It is apparent from Figs. 1 and 2 that similar changes in the shape of the k' against $[P^\pm]_{aq}$ curves can be obtained as a result of either decreasing acetonitrile concentration or of increasing the hydrophobicity of the pairing ion used and that both cause marked changes in selectivity between the classes of ionic and neutral solutes. This supports, at least qualitatively, the ideas contained in the ion-exchange desolvation mechanism of ion pairing outlined above. However, it has been shown that alterations of organic modifier concentration affects both ionic and neutral sol-

utes to a similar degree, affecting as it does both desolvation constants. Alteration of hydrophobicity of pairing ion at a fixed organic modifier concentration enhances the selectivity differences between these classes of solutes. This is shown by comparison of the selectivity values shown in Figs. 1 and 2. In Fig. 1, $\alpha_{A/AA}$ for all acetonitrile concentrations tends to approximately 1 while in Fig. 2 $\alpha_{HVA/DA}$ changes from 1 to 0.1 to 0.02 at certain $[P^-]_{aq}$ as the pairing ion used becomes larger.

CONCLUSIONS

In order to generalise the above findings Fig. 3 is drawn, summarising the effects of organic modifier concentration and pairing ion hydrophobicity for different solutes for the situations where pH is adjusted to either end of the chromatographic range and where buffer ionic concentration is constant. Such a generalised picture has been found to be of help in selecting chromatographic conditions to achieve desired analytical separations^{9,16}. If for example analysis of only ionic solutes is required a large pairing ion at low acetonitrile concentration will elute neutral compounds rapidly and retain ionic solutes (Fig. 2c). For retention of all components of such a mixture the acetonitrile concentration is adjusted to obtain suitable retention of neutral compounds according to their hydrophobicity and a suitable pairing ion is used at an appropriate concentration to enhance retention of the ionic solutes without drastically decreasing retention of neutral compounds. Such a situation is shown in Figs. 1c and 2a.

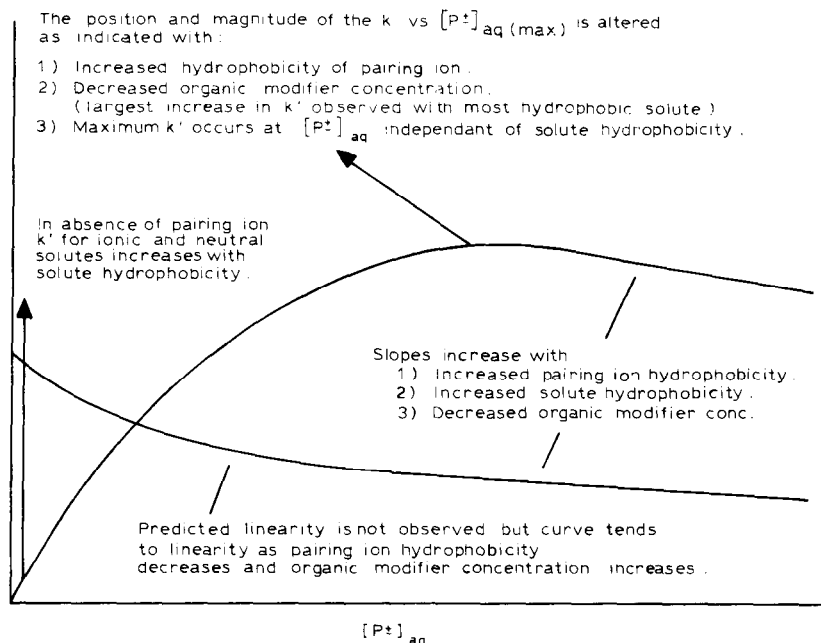


Fig. 3. General effects of some chromatographic variables on the characteristic k' against pairing ion concentration curves, obtained for ionic and neutral solutes in presence of oppositely charged pairing ion.

ACKNOWLEDGEMENT

One of us (R.B.T.) wishes to thank D. J. F. Ayim, University of Ibadan, for helpful discussion.

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