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MECHANISM OF RETENTION OF ACIDIC SOLUTES BY OCTADECYL SILICA USING QUATERNARY AMMONIUM PAIRING IONS AS ION EXCHANGERS

C. T. HUNG and R. B. TAYLOR*

School of Pharmacy, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen AB9 1FR (Great Britain)

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SUMMARY

The adsorption isotherms of four commonly used quaternary ammonium pairing ions in aqueous solution on ODS Hypersil are reported. The capacity factors of four substituted benzoic acids as test solutes are measured as a function of pairing ion concentration under conditions of complete solute ionisation. The chromatographic behaviour of an undissociated solute, 5-hydroxymethylfurfuraldehyde, is also examined under these conditions. Over the wide range of pairing-ion concentrations used, maximum values of capacity factors are observed.

A mechanism of retention, combining desolvation and ion exchange, which considers the effect of adsorbed pairing ion on surface available for desolvation is proposed. On the basis of this mechanism an equation relating capacity factor and adsorbed pairing-ion concentration is derived. The data obtained in this investigation are fitted to the equation and the desolvation and ion-exchange constants are estimated. The behaviour of the neutral solute 5-hydroxymethylfurfuraldehyde is examined in the light of the derived equation.

INTRODUCTION

Although ionised solute species may be chromatographed using alkyl-modified silica with aqueous solvents[1], the addition of low concentrations of quaternary amines to the mobile phase has been shown to improve separation of acidic solutes[2,3]. Corresponding improvement in the chromatography of basic solutes has been obtained by the addition of alkyl sulphonates[4].

The mechanism of retention of such ionised solutes has been interpreted[5–9] on the basis of ion pairs, formed between the ionised solute and the added pairing ion, being adsorbed by the hydrophobic stationary phase. Following evidence that the pairing ion, originally in the aqueous mobile phase, is adsorbed by the stationary phase, an alternative mechanism based on the *in situ* formation of an ion exchange matrix was proposed[10]. Several workers have demonstrated the phenomenon of

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pairing-ion adsorption on a quantitative basis, fitting results obtained to various isotherm types[2,8,11,12].

Quantitative evidence for the ion-pairing mechanism has been based largely on the linear dependence of solute capacity factor with mobile phase pairing-ion concentration[6,8,13]. Recent work taking into account competitive adsorption of the pairing ion, although producing an alternative dependence, has supported this mechanism[14].

That adsorption of pairing ion occurs on chemically bonded reversed phases has been taken as evidence of an ion-exchange mechanism being operative. Quantitative relationships between solute capacity factors or distribution coefficients and mobile phase pairing-ion concentration have been established[2,11] using an appropriate isotherm. In addition the inverse dependence of capacity factors on counter-ion concentration[15] has further supported the ion exchange hypothesis. Only recently, however, has capacity factor dependence on experimentally determined adsorbed pairing-ion and mobile phase counter-ion concentrations been demonstrated[16].

The ion-pairing concept also differs from that of ion exchange in that it indicates that desolvation of the ion pair is more readily achieved than that of the ionised solute alone. The ion-exchange mechanism takes no account of the hydrophobicity of the solute but implies that separation is based on differences in the ion-exchange constants of various solutes.

It has been shown by several workers[2,5,9,17,18] that, over wide ranges in mobile phase pairing-ion concentration, the dependence of capacity factors on pairing-ion concentration is complex. It may reach a plateau or indeed pass through a maximum. This would indicate that the processes occurring during such chromatographic separations are considerably more complex than those described by either the ion-pairing or ion-exchange approaches.

It is the purpose of the present work to determine the adsorption behaviour of several of the more commonly used quaternary ammonium alkyl pairing ions on ODS Hypersil as a typical capped C_{18} stationary phase. Using various benzoic acids as test solutes, it is intended to examine the relationships between capacity factors and pairing-ion concentration both in the mobile and stationary phases. It is intended to use sufficiently wide concentration ranges to observe the reported decrease of capacity factor and to relate this to the coverage of the adsorbent surface by pairing ion.

EXPERIMENTAL

Apparatus

Chromatographic column loading and column desorption were carried out using an Applied Chromatography Systems 750/3 dual piston pump and a Pye Unicam LC-3 variable wavelength UV detector. Injection for chromatography was via a Rheodyne 7125 valve. Chromatographic columns were usually 50 × 5 mm I.D. slurry packed although, for tetrabutylammonium sulphate, a very short 4 × 5 mm I.D. column was made. Columns for isotherm measurements were 250 × 5 mm I.D. All measurements were made at ambient temperature.

Materials

Tetramethylammonium sulphate (TMA), tetraethylammonium bromide (TEA) tetrabutylammonium sulphate (TBA), 2,4-dimethylbenzoic acid (2,4DMBA), naphthoic acid (NA) and 4-ethylbenzoic acid (4EBA) were obtained from Aldrich (Milwaukee, WI, U.S.A.). Cetrimide (CTAB) was obtained from I.C.I. Pharmaceuticals (Macclesfield, Great Britain) and benzoic acid (BA) from Koch-Light Labs. (Colnbrook, Great Britain). 5-Hydroxymethylfurfuraldehyde (5HMF) was obtained from Sigma (St. Louis, MO, U.S.A.). Water was double-distilled in glass and all other reagents were of Analar or comparable quality. ODS Hypersil was purchased from Shandon (London, Great Britain).

Procedures

The adsorption isotherms for TMA, TEA, TBA and CTAB were determined by pumping the appropriate concentrations of pairing ion in water through the column until the absorbance, measured at a suitable low wavelength, was constant with time. Although the absorbance was not linear with concentration, the equilibrium state could be estimated by this procedure. Ethanol was used to desorb the pairing ion until the absorbance of the eluent was again constant, and the total eluent was collected. The mass of the pairing salt deloaded was determined after evaporating the solvent under reduced pressure and was corrected for the dead volume of the system. The deloading process usually required some 200 column volumes and twice this volume was passed. At the lowest concentrations of TMA employed, the coefficient of variation measured on four replicates was 16%.

Chromatographic measurements

A standard eluent of 0.002 *M* disodium hydrogen phosphate was employed at pH 7.4 containing the appropriate concentration of pairing salt. The column was equilibrated to this concentration before chromatographic results were recorded. The four benzoic acids used as solutes were chromatographed at a concentration of 0.01 *M* and a detection wavelength of 240 nm. 5HMF was injected at a concentration of 0.005 *M* and monitored at the same wavelength.

RESULTS AND DISCUSSION

Isotherms

The isotherms obtained for the various pairing ions are shown in Fig. 1. The curves obtained for the symmetrical tetraalkyl pairing ions resemble Type L according to the Giles classification[19]. This form is taken to indicate gradual surface coverage of an adsorbent, terminating in a plateau region where the amount adsorbed does not vary with the solution concentration. At low concentrations this isotherm will approximate to a Type C or linear isotherm, with the amount adsorbed directly proportional to the solution concentration. The surfactant pairing ion (CTAB) appears quite different and is markedly H Type. This indicates high adsorption even at very low solution concentration with no proportional region present. This is consistent with previously reported work where the time required for equilibration of a C_{18} phase with low concentrations of cetrimide has been emphasised[18]. It would

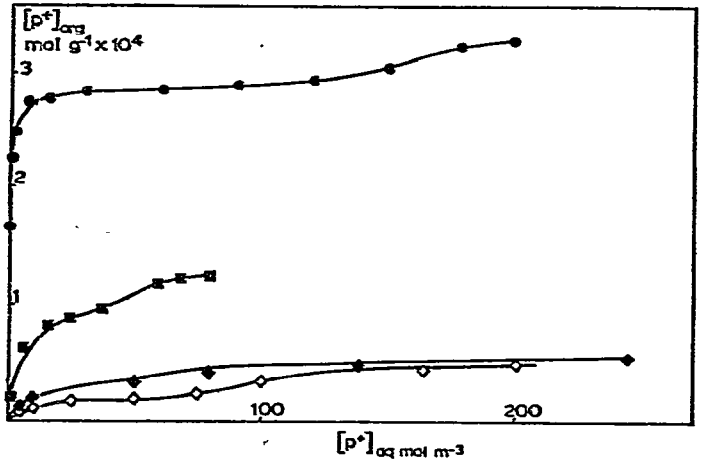

Fig. 1. Adsorption isotherms for various quaternary ammonium pairing ions for the ODS Hypersil–water system. ◇, TMA; ◆, TEA; ■, TBA; ●, CTAB.

appear from the present results that regulation of the surface concentration of very hydrophobic pairing ions would be very critical in aqueous solutions.

Although the results in Fig. 1 are represented as isotherms, it is obvious that each pairing ion used has a different plateau in terms of amount adsorbed. Indeed the larger the pairing ion, the more surface there appears to be available. This limits the usefulness of the isotherm as a general method of describing the adsorption behaviour of different pairing ions on the C_{18} surface. No attempt therefore is made to fit the curves to Freundlich or Langmuir equations. The adsorption data are used directly in subsequent sections to estimate the area of the C_{18} surface covered by a given pairing ion at appropriate aqueous concentrations. A relevant property of the pairing ion with respect to its adsorption would appear to be the hydrophobicity as measured by some characteristic parameter of that part of the pairing ion in contact with the C_{18} surface. A reasonable choice is the hydrophobicity of the longest hydrocarbon chain of the pairing ion. Correlation of the surface concentration at 0.05 *M* aqueous phase concentration with the Hansch π parameter[20] produces a regression line

$$\text{Amount adsorbed } (\mu\text{mol g}^{-1}) = 46.5\pi - 18 \qquad R^2 = 0.991$$

which indicates a good proportional relationship at a plateau region on the isotherms.

These results in aqueous solution show that the commonly used quaternary ammonium ions are indeed adsorbed to varying extents on the C_{18} silica and that the extent depends more upon the nature of the pairing ion than upon the aqueous-phase concentration. It is a consequence of this that such adsorbed species may act as *in situ* ion exchangers for other solutes and also that, depending on the nature and concentration of adsorbed pairing ion, some of the C_{18} surface will be occupied and thus be unavailable for the desolvation of solutes during chromatography. This latter aspect is considered further below, in the interpretation of the capacity factor variations for charged and uncharged solutes with pairing ion type and concentration.

Theoretical

The retention of an ionised solute in the presence of pairing ion on a C_{18} reversed-phase system may be considered as occurring by two separate mechanisms. One is the desolvation of the solute on the C_{18} surface. This may be minimal in the case of a highly ionised solute resulting in short retention times but may be appreciable if the solute is hydrophobic even when ionised.

The nett retention volume is given by

$$V_{r1} - V_m = K_1 A'_s \tag{1}$$

where V_{r1} is the measured retention volume, V_m the volume of mobile phase in the column, A'_s the surface area of the stationary phase available for desolvation and K_1 the desolvation constant or partition coefficient.

The other mechanism suggested is by an ion-exchange equilibrium of the form

$$(P_n^+C^{n-})_{org} + nA^-_{aq} \rightleftharpoons n(P^+A^-)_{org} + C^{n-}_{aq}$$

$(P_n^+C^{n-})_{org}$ refers to a monovalent pairing ion together with the possibly multivalent (n) counter ion adsorbed to the C_{18} surface, and A^- a monovalent anion as solute.

An ion-exchange constant can be written as

$$K_{IE} = \frac{[P^+A^-]^n_{org}\,[C^{n-}]_{aq}}{[P_n^+C^{n-}]_{org}\,[A^-]^n_{aq}} \tag{2}$$

From which, if the distribution for A^- between organic and aqueous phases is written as

$$D_{A^-} = \frac{[P^+A^-]_{org}}{[A^-]_{aq}} \tag{3}$$

by substitution for $[P^+A^-]_{org}$, we have

$$D_{A^-} = \left(\frac{K_{IE}\,[P_n^+C^{n-}]_{org}}{[C^{n-}]_{aq}}\right)^{1/n} \tag{4}$$

Such a relationship alone does not allow for any desolvation of the ion pair formed at the C_{18} surface due to the hydrophobicity of the solute. It does not account for differences in distribution, and thus retention, among different solutes other than on purely electrostatic terms. To explain the variation in retention, it is suggested that an ion-exchange reaction occurs between the ionised solute and the adsorbed pairing ion followed by desolvation of the neutralised solute on to the C_{18} surface. This desolvation will be proportional to the hydrophobicity of the solute as described by a desolvation constant K_2, which will act to increase the electrostatic effect of ion exchange. K_2, although analogous to K_1, may be of a different magnitude since it refers to a solute with no nett ionic charge. The retention of the bound species in on exchange will also be proportional to the area of the stationary phase available for desolvation, so that the nett retention volume for a solute retained by an ion-exchange–desolvation mechanism is given by

$$V_{r2} - V_m = K_2 A'_s \left(\frac{K_{IE}\,[P_n^+C^{n-}]_{org}}{[C^{n-}]_{aq}}\right)^{1/n} \tag{5}$$

The total nett retention volume ($V_{rT} - V_m$) is given by

$$V_{rT} - V_m = K_1 A_s' + K_2 A_s' \left(\frac{K_{IE}\,[P_n^+ C^{n-}]_{org}}{[C^{n-}]_{aq}}\right)^{1/n} \tag{6}$$

and the column capacity factor k' by

$$k' = \frac{1}{V_m}\left\{K_1 A_s' + K_2 A_s' \left(\frac{K_{IE}\,[P_n^+ C^{n-}]_{org}}{[C^{n-}]_{aq}}\right)^{1/n}\right\} \tag{7}$$

A'_s is related to the total area of the C_{18} stationary phase, A_s, by the relationship

$$A_s' = A_s - [P_n^+ C^{n-}]_{org}\, A_P \tag{8}$$

where A_P is the area per mole occupied by a particular adsorbed pairing ion. A_s' is difficult to define but can be thought of as the effective area available for adsorption of any species in terms of C_{18} surface.

Substituting A_s' in the expression for k' we obtain

$$k' = \frac{1}{V_m}(A_s - [P_n^+ C^{n-}]_{org}\, A_P)\left\{K_1 + K_2 \left(\frac{K_{IE}\,[P_n^+ C^{n-}]_{org}}{[C^{n-}]_{aq}}\right)^{1/n}\right\} \tag{9}$$

This equation relates the capacity factor of a given solute to the adsorbed pairing-ion concentration and mobile phase counter-ion concentration, taking into account the effective area of C_{18} available for desolvation. It shows a much more complex dependence of k' on adsorbed pairing ion concentration than has hitherto been suggested.

The form of the dependence of k' on adsorbed pairing-ion concentration can be seen more readily if the situation of monovalent counter ion only is considered. The equation can be rearranged after setting n to unity as

$$k' = \frac{1}{V_m}\left(A_s K_1 - K_1\,[P^+C^-]_{org}\, A_P + K_2 K_{IE} A_s \frac{[P^+C^-]_{org}}{[C^-]_{aq}} - K_2 K_{IE} A_P \frac{[P^+C^-]_{org}^2}{[C^-]_{aq}}\right) \tag{10}$$

k' is thus expressed as a quadratic in $[P^+C^-]_{org}$ which exhibits a maximum value. This is in agreement with the results obtained by several workers when considering the capacity factor dependence on aqueous pairing-ion concentration. Following the schematic representation of the mechanism of retention in solvophobic chromatography[21] and ionic interaction chromatography[22], the two processes treated above are diagrammatically represented in Fig. 2.

Chromatographic results

The dotted curves in Figs. 3(a–d) represent the variation in k' for the four acidic solutes with aqueous or mobile phase concentrations of the various pairing ions. For the tetramethyl, tetraethyl and tetrabutyl compounds, clearly observed maxima are evident. The maxima are more pronounced the greater the hydrophobicity

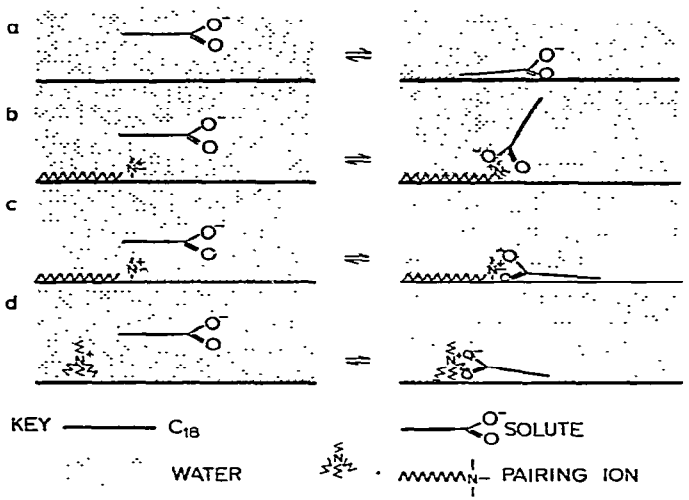

Fig. 2. Schematic representation of the postulated retention mechanisms discussed in the text. (a) Desolvation of an ionised solute; (b) ion exchange involving an adsorbed pairing ion without subsequent desolvation of solute; (c) and (d) ion exchange reinforcing desolvation of solute for adsorbed surfactant (c) and non surfactant (d) pairing ions.

of the solute and the pairing ion. The k' values become very large, reflecting the marked ion-exchange–solvophobic retention effect in wholly aqueous eluent. In the case of cetrimide, the only experimentally accessible k' values occur on the decreasing portion of the curve. This is interpreted as a consequence of the H-type isotherm obtained for this compound whereby, even at extremely low concentrations, appreciable concentrations of adsorbed pairing ion exist.

The increase of k' with $[P_n^+C^{n-}]_{aq}$ has previously been taken to support both the ion exchange[23] and the ion-pairing[24] interpretation of retention. Neither of these mechanisms, however, can account for the general decrease of k' observed in this and previous investigations where high pairing-ion concentrations were used. The decrease in k' has been attributed to micelle formation in the case of cetrimide by previous workers. In the present investigation, however, the dercease is seen to be general even for the non-surface-active pairing ions. The theoretical treatment of the model described above predicts this decrease when taken in conjunction with the experimentally measured adsorbed pairing ion concentrations, $[P_n^+C^{n-}]_{org}$. In eqns. 9 and 10, k' may decrease owing to two quite dissimilar effects. The C_{18} surface area available for desolvation subsequent to ion exchange will decrease according to the term $A_s - [P_n^+C^{n-}]_{org}A_P$. Also k' will decrease as $[C^{n-}]$ increases as a result of added pairing salt. Under the experimental conditions chosen, using low ionic strength buffer to minimise purely hydrophobic chromatography, it is not possible to separate these two effects, *i.e.* increasing $[P^+C^{n-}]_{aq}$ will tend to increase $[P_n^+C^{n-}]_{org}$ according to the isotherm, thus decreasing available surface, and will also increase $[C^{n-}]_{aq}$ directly.

In order to test quantitatively the fit of the derived equations (9 and 10) with the experimental points, the following procedure was adopted. The value of A_s for

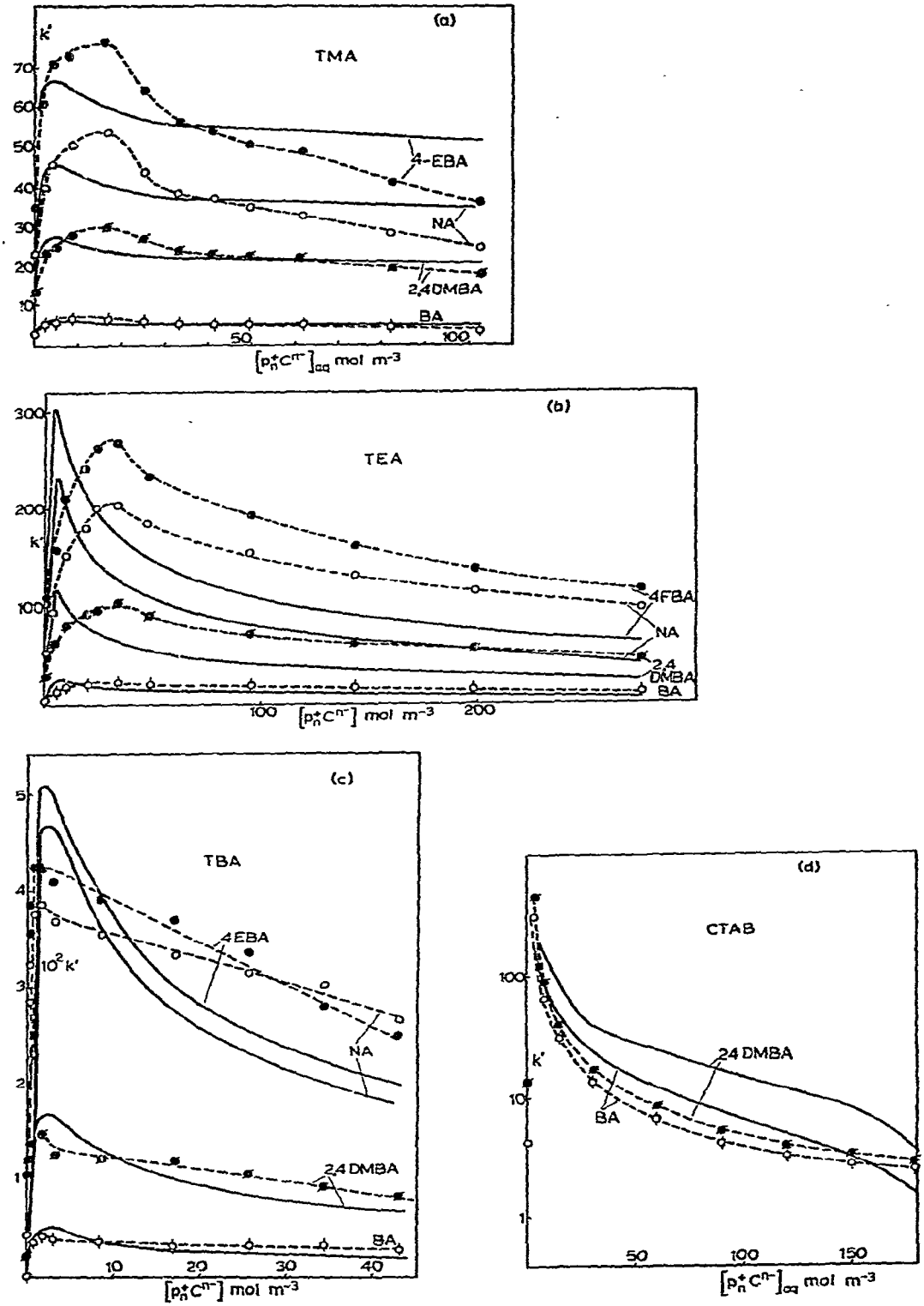

Fig. 3. Plots showing the variation of capacity factor (k') with aqueous pairing-ion concentration for the four acidic solutes and various pairing ions (a–d). Dashed lines represent experimental results. Full lines represent calculated curves.

the 5-μm silica ODS was taken[25] as 142 $m^2\ cm^{-3}$. The factor A_P was calculated on the basis of accepted atomic dimensions, bond angles and lengths for each of the pairing ions in the fully extended conformation using data and calculations contained in the CAMSEQ software system[26]. The areas of the symmetrical pairing ions were calculated as circles with alkyl chain lengths as radius, and that of cetrimide as the molecule in a flat position on the adsorbent surface. For various aqueous concentrations of pairing ion, the corresponding $[P_n^+C^{n-}]_{org}$ value was obtained from the appropriate isotherm. The value of $[C^{n-}]_{aq}$ was calculated to include the contribution from the buffer. The constant K_1 was estimated from the chromatographic results obtained in the absence of pairing ion for each solute. Using an iterative computer program (HOOKE) to produce the best least-squares fit of the experimental data k' *vs.* $[P_n^+C^{n-}]_{org}$, the composite constant $K_2K_{IE}^{1/n}$ was evaluated for each solute and pairing ion. The corresponding graphs of k' *vs.* $[P_n^+C^{n-}]_{aq}$ using those derived constants are shown in Fig. 3 as full lines.

To demonstrate further the fit of the proposed model, Figs. 4(a–d) show experimental (dotted lines) and calculated (full lines) values of k' as a function of adsorbed pairing ion concentration $[P_n^+C^{n-}]_{org}$. The experimental curves in these plots indicate more forcefully that k' continues to decrease while $[P_n^+C^{n-}]_{org}$ is essentially constant.

The calculated lines in Figs. 3 and 4 show good agreement with the experimental values in terms of the general shape of the curve produced, especially in the region of decreasing k'. The position of the predicted maxima on the pairing-ion concentration scale do not consistently lie above or below the experimental values, indicating some error in the values allocated to the variables in eqn. 9. The calculated curve was found to be insensitive to the value of A_s but was highly sensitive to $[P_n^+C^{n-}]_{org}$ obtained from the isotherms. The experimental data points for the more hydrophobic naphthoic and 4-ethylbenzoic acids were not sufficiently representative of the parabolic shape of the curve to enable values of K_2K_{IE} to be obtained for the cetrimide pairing ion.

That the magnitudes of the experimental and calculated k' values are in agreement over such widely different values based on the best value of one adjustable constant is taken as evidence that the proposed ion-exchange–desolvation process is of major significance in the retention of the ionised acids in C_{18}–aqueous pairing-ion systems. The optimum values of the composite $K_2K_{IE}^{1/n}$ constant are shown in Table I, together with the average value of K_1 obtained for a particular solute in the absence of pairing ion. The K_2K_{IE} values represent the ion-exchange–desolvation process and are seen to increase with the hydrophobicity of solute for a given pairing ion, as does

TABLE I

K_1 AND $K_2K_{IE}^{1/n}$ VALUES CALCULATED TO PRODUCE THE BEST FIT OF EXPERIMENTAL DATA

Solute	$K_1 \cdot 10^3$	$K_2K_{IE}^{1/2}$ *(TMA)* $\cdot 10^5$	K_2K_{IE} *(TEA)*	$K_2K_{IE}^{1/2}$ *(TBA)* $\cdot 10^4$	K_2K_{IE} *(CTAB)*
BA	2.28	2.18	0.103	1.69	0.104
2,4 DMBA	9.52	11.48	0.519	5.76	0.208
NA	15.7	18.76	1.05	16.5	
4 EBA	24.4	26.23	1.36	17.9	

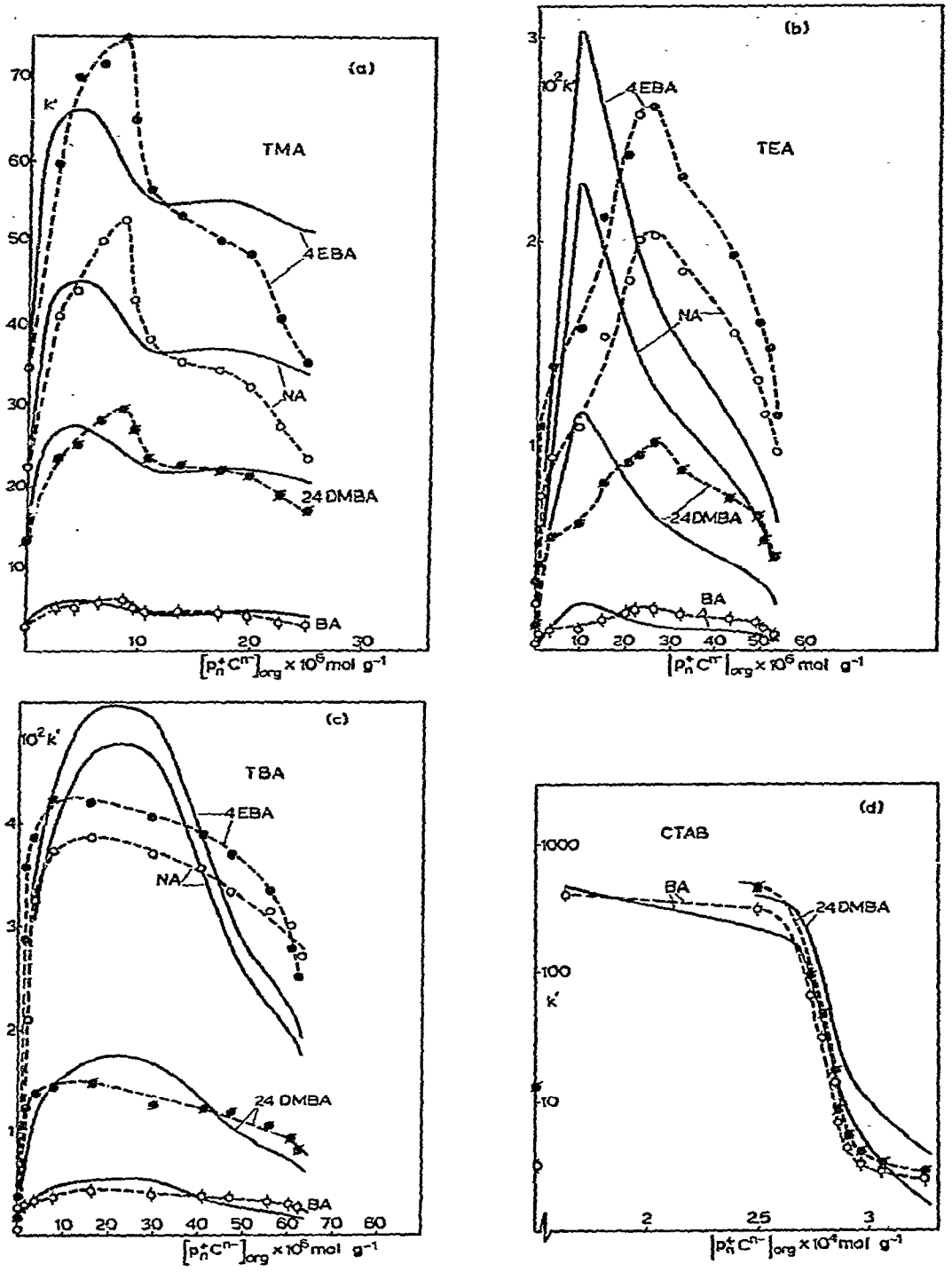

Fig. 4. Plots showing the variation of capacity factors (k') with adsorbed pairing-ion concentration for the four acidic solutes and various pairing ions (a–d). Dashed lines and full lines as in Fig. 3.

K_1. Considerable fluctuation, however, is apparent for the same solute as a function of pairing ion. There is no regular increase in $K_2K_{IE}^{1/n}$ with hydrophobicity of pairing ion as would be expected in an ion-pairing phenomenon where the K_2 representing desolvation would refer to the ion pair. In order to separate the components of desolvation and ion exchange, the approximation is made that $K_1 = K_2$, *i.e.* that the desolvation energy of a solute is the same under ion-exchange as under non-ion-exchange conditions. This enables the K_2 term, which in any case should be constant on this mechanism between pairing ions for any solute, to be eliminated and the K_{IE} constant to be evaluated taking into account the particular value of (n) for the pairing salt used. These results are shown in Table II. The K_{IE} values are substantially constant for all solutes within a given pairing ion, and even for different pairing ions the values are of comparable magnitude. This is taken to indicate that the electrostatic binding thouhgt to be operative is the same for all pairing ions and solutes, with the $K_2K_{IE}^{1/n}$ term dictating retention and resolution since in all cases this term is much larger than K_1. It must be stated that the $K_2K_{IE}^{1/n}$ values obtained for cetrimide, even for those solutes where an acceptable fit was obtained, are likely to be low owing to the lack of data at very high k' values.

TABLE II

K_{IE} VALUES CALCULATED FOR SOLUTE-PAIRING ION SYSTEMS ASSUMING $K_1 = K_2$

Solute	$K_{IE} \cdot 10^{-6}$			
	TMA	*TEA*	*TBA*	*CTAB*
BA	0.922	4.54	55.4	4.50
2,4 DMBA	1.45	5.45	36.3	2.60
NA	1.41	6.67	100.9	
4 EBA	1.15	5.58	53.8	

The behaviour of 5HMF in the presence of pairing ion yields further evidence of the surface coverage aspect of the proposed mechanism. In the absence of an ion-exchange mechanism, eqns. 9 and 10 reduce to

$$k' = \frac{K_1}{V_m}(A_s - [P_n^+C^{n-}]_{org}\, A_P) \qquad (11)$$

The plot of k' against $[P_n^+C^{n-}]_{org}\, A_P$ of all pairing ions for 5HMF is shown in Fig. 5. While eqn. 11 predicts a constant gradient of K_1/V_m, the data points for three of the four pairing ions used lie on a curve of constantly decreasing slope. This may indicate a changing desolvation constant owing to a different alkyl surface being formed progressively owing to adsorption of pairing ion. The tetramethylammonium pairing ion appears not to conform to this pattern and no explanation can be given. The results of 5HMF indicate one possible oversimplification in the proposed mechanism, in that only the C_{18} surface is considered with regard to solute desolvation.

In the light of these results, the proposed ion-exchange–desolvation mechanism appears to account for the experimentally observed variation of k' with pairing-ion concentration. It is evident that both the concentration and type of pairing ion have

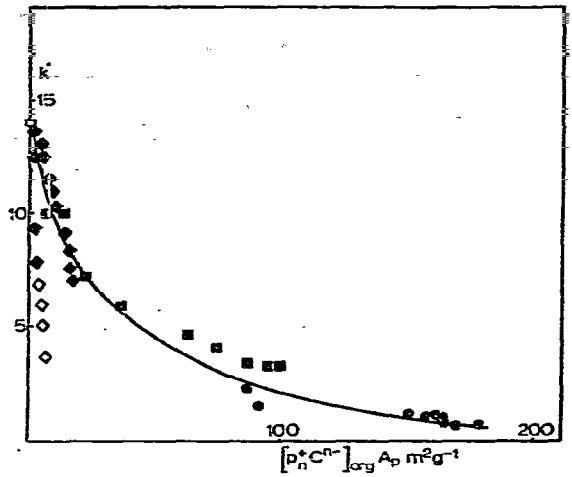

Fig. 5. Plot showing the variation of capacity factor for 5-hydroxymethylfurfuraldehyde as a function of the area of C_{18} surface calculated as being covered by various pairing ions at the concentrations studied. ◊, TMA; ◆, TEA; ■, TBA; ●, CTAB.

a marked effect and that prediction of retention is difficult without detailed knowledge of the isotherm characteristics and the operative ion-exchange constants. There appears no difference in the mode of action of the various pairing ions other than in their adsorption characteristics. It is likely that the addition of an organic modifier to bring the k' values to reasonable magnitudes will alter the isotherm shape[27] as well as reducing the very powerful hydrophobic effect exhibited in the present work. Further investigation is required to verify this quantitatively using the proposed mechanism and derived equation, especially in the case of very highly adsorbed pairing ions such as cetrimide. It is possible that the change in isotherm with organic modifier concentration will enable rationalisation of the very variable chromatographic ion-pairing results obtained by different investigators.

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