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# STUDIES IN REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

# IV. THE RÔLE OF THE CHAIN LENGTH OF THE PAIRING ION

Á. BARTHA and Gy. VIGH
*Institute for Analytical Chemistry, University of Chemical Engineering, Veszprém (Hungary)*
and
H. A. H. BILLIET* and L. DE GALAN
*Laboratory for Analytical Chemistry, Technical University of Delft, Delft (The Netherlands)*
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## SUMMARY

Adsorption isotherms of straight chain sodium alkanesulphonates (from butane to octane) were determined by the breakthrough method in aqueous phosphate buffer on ODS-Hypersil, along with retention data of similarly and oppositely charged ionic and non-ionic solutes. The effects on the solute retention of the chain length of the pairing ion were examined both at varying and constant counter ion (sodium) concentrations, in terms of both the mobile phase and stationary phase pairing ion concentrations. A practical consequence of the use of systems with constant counter ion concentration is that pairing ions with different chain lengths (members of a homologous series) are interchangeable in terms of their surface concentrations.

## INTRODUCTION

The nature (type and chain length of the hydrophobic group) of the pairing ion is generally considered an important parameter in the retention of ionic solutes in reversed-phase ion-pair chromatography (RP-IPC). The retention of oppositely charged ions usually increases with increasing chain length of the pairing ion, when the mobile phase concentration, $P_m$, of the respective pairing ions is identical[1–5]. This effect was used by Bidlingmeyer[1] and Lurie and Demchuk[2,3] to improve the separation selectivity for mixtures of ionic and non-ionic solutes.

However, there are contradicting views on the origin of these selectivity changes, *i.e.*, different retention mechanisms have been invoked to explain the rôle of the chain length of the pairing ion[1–12]. Relevant experimental data are also contradictory.

Horváth *et al.*[4] determined the retention of catecholamines at varying $P_m$ of straight chain sodium alkyl sulphates ($C_4$, $C_6$, $C_8$, $C_{10}$). When used in identical mobile phase concentrations, the longer pairing ions caused greater retention. The re-

tention curves also exhibited maxima, at lower $P_m$ for the longer sulphates. The increased retention was explained by stronger ion-pair formation with the hydrophobic pairing ions. This ion-pair adsorption model seemed also to explain other experimental data[2–6]. On the other hand, van de Venne, Deelder and co-workers[7–9] correlated solute retention data with measured surface concentrations of pairing ions and proposed a dynamic ion-exchange model. They calculated the ion-exchange equilibrium constants, which were found to increase by a factor of 2 while going from hexane- to octanesulphonate and also from octane- to dodecanesulphonate, respectively.

Hung and Taylor[10,11] reported solute retention data measured with pairing ions of different structures, charges and chain lengths. They suggested an ion-exchange desolvation model to explain solute retention maxima and the effect of the type of pairing ion. Differences in solute retention were accounted for by the different hydrophobic surface areas of the pairing ions.

In these studies the counter ion, *e.g.*, sodium for alkyl sulphates, concentration, $C_m$, also increased as the pairing ion concentration increased.

There is another strategy, in which the counter ion concentration is kept constant while the mobile phase concentration of the pairing ion, $P_m$, is increased. Knox and Hartwick[12] used this technique. Along with the retention of catecholamines in the presence of octyl, decyl and lauryl sulphates, the adsorption isotherms of pairing ions were also measured in methanol–aqueous phosphate buffer (pH = 6) (20:80) eluents, at constant counter ion (sodium) concentration on Hypersil-ODS. The solute capacity ratios, $k$, when plotted against the surface concentrations, $P_s$, of pairing ions of different chain lengths almost coincided, indicating that electrostatic interaction in the stationary phase must be the major retention-controlling factor. However, these results contrast with those cited previously.

In Part III[13] we have demonstrated that both pairing ion adsorption and solute retention change with increasing $P_m$ depending on the counter ion concentration of the eluent. It was also suggested that the apparent contradictions in the literature were due to the different experimental conditions established by these two strategies. Our intention here is to examine the effect of pairing ion chain length, both at varying and constant counter ion concentration, upon the retention of ionic and non-ionic solutes. Solute retention data and adsorption isotherms of straight chain alkanesulphonates were determined simultaneously.

## EXPERIMENTAL

The drug samples and sodium butane- ($BuSO_3Na$), hexane- ($HexSO_3Na$) and octanesulphonates ($OctSO_3Na$) were from Janssen Chim. (Beerse, Belgium) and Merck (Darmstadt, F.R.G.), respectively.

The analytical column was packed with 5-$\mu$m ODS-Hypersil (Shandon Southern Products). The nitrogen BET surface area and the carbon content were 173 $m^2$ $g^{-1}$ and 8.8% (w/w), respectively, according to the manufacturer (Batch No. 8/1017).

The eluents were aqueous buffers containing 0–40% (v/v) methanol (MeOH), 25 m*M* $H_3PO_4$, 25 m*M* $NaH_2PO_4$ (pH = 2.1), different amounts (0–150 m*M*) of sodium bromide (Baker, Deventer, The Netherlands) and sodium alkanesulphonates.

The chromatographic equipment, eluent preparation, column regeneration and the experimental technique were as described[13].

## RESULTS AND DISCUSSION

The adsorption isotherms of straight chain sodium butane-, hexane- and octanesulphonates were determined on ODS-Hypersil in aqueous buffers by the breakthrough method[14]. For each ion-pairing reagent two series of measurements were carried out: (i) in eluents which contained only the phosphate buffer and increasing amounts of sodium alkanesulphonates (changing counter ion concentration, $[Na^+]$, and ionic strength); (ii) in eluents where the molar ratio of NaBr (initially 150 m*M*) to the surfactant was varied (keeping constant the counter ion concentration and ionic strength). The results are shown in Fig. 1: (a) the adsorption increases with the chain length; (b) the adsorption isotherms measured in sodium bromide-containing eluents (solid lines) are always higher than in the absence of added salt (dashed lines).

These results can be explained by the increasing hydrophobicity of the pairing ion and by salting-out effects, respectively (see also ref. 13). Again, the isotherms cannot be described by Langmuir or Freundlich type equations[13,14].

The effects of the mobile phase concentration, $P_m$, of sodium alkanesulphonates on the retention of positively charged adrenaline are shown in Fig. 2. The small difference in $k$ at $P_m = 0$ in the absence and presence of NaBr is caused by the salting-out effect. On the other hand, at $P_m = 150$ m*M*, the retention (in the $BuSO_3Na$ system) is the same in both cases because all NaBr is replaced by the surfactant[13]. At identical $P_m$, the capacity ratio, $k$, increases with the pairing ion chain length in both series of measurements.

Retention curves measured in eluents with changing counter ion concentration (dashed lines) show maxima at mobile phase concentrations of about 70 m*M* for

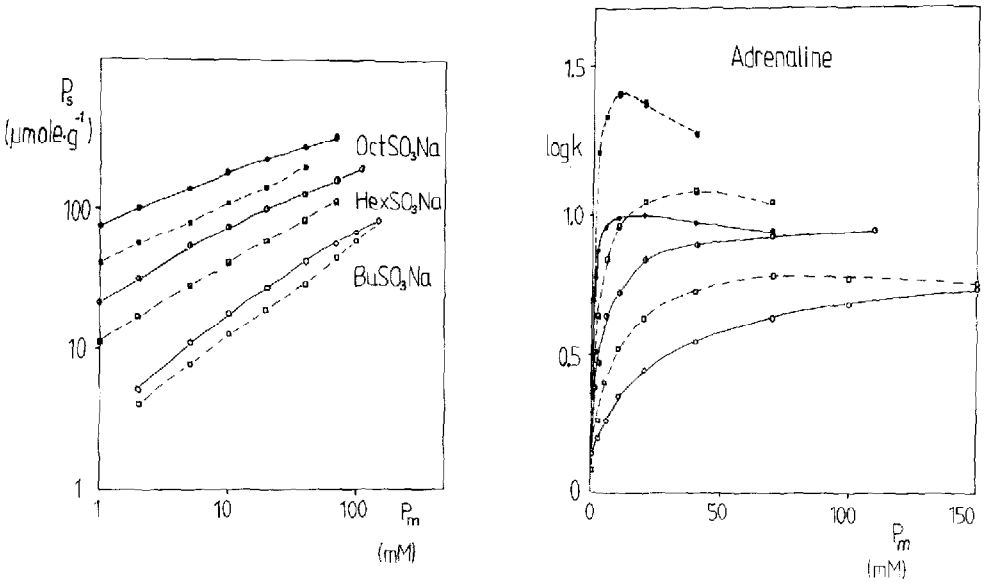



Fig. 1. Adsorption isotherms of $BuSO_3Na$ (○, □), $HexSO_3Na$ (◑, ◪) and $OctSO_3Na$ (●, ■) from aqueous 25 m*M* $H_3PO_4$–25 m*M* $NaH_2PO_4$ buffer (pH = 2.1), with changing (- - -) and constant (175 m*M* ) sodium concentration (——), on 5-μm ODS-Hypersil at 25°C.

Fig. 2. log $k$ data of adrenaline *vs.* the mobile phase concentration, $P_m$, of $BuSO_3Na$ (○, □), $HexSO_3Na$ (◑, ◪) and $OctSO_3Na$ (●, ■) in eluents with changing (- - -) and constant (——) sodium concentration.

$BuSO_3Na$, about 40 m*M* for $HexSO_3Na$ and 10 m*M* for $OctSO_3Na$ respectively. Similar behaviour has been observed by others[4,9,11]. These maxima cannot be explained by the formation of micelles in the eluent, as the critical micelle concentrations (CMCs) are in each case about ten times higher[15]. It should be noted that at constant counter ion concentration a retention maximum occurs only for $OctSO_3Na$ (at about $P_m$ = 20 m*M*), not for $BuSO_3Na$ or $HexSO_3Na$. In addition, higher retention is established by $HexSO_3Na$ in eluents without salt control, in comparison to $OctSO_3Na$ with salt control. Obviously, these observations are difficult to explain in terms of the mobile phase concentrations of pairing ions.

The same retention data for adrenaline are replotted in Fig. 3 against the measured surface concentrations, $P_s$, of the pairing ions. Two cases can be clearly distinguished: (i) a system with changing counter ion concentration; (ii) a system with counter ion control (at a high $C_m$ = 175 m*M* value). When salt control is used, pairing ions having different chain lengths result in identical solute retention (solid line) at a given $P_s$. In this system, solute retention also exhibits a maximum, although less pronounced. Similar maxima and little dependence on pairing ion chain length were observed by Knox and Hartwick[12] in salt-controlled, methanol–aqueous buffer (20:80) eluents.

We previously reported[16], retention maxima for negatively charged solutes when using positively charged tetrabutylammonium as pairing ion at constant counter ion (bromide) concentration. In salt-controlled eluents these maxima occur only at higher $P_s$[12,16] which, in our case, can be achieved only with $OctSO_3Na$. This explains the lack of maxima (in Fig. 2) for $BuSO_3Na$ and $HexSO_3Na$ with shorter alkyl chains, *i.e.*, lower adsorption (see also Fig. 1). On the other hand, when the counter ion concentration varies with increasing $P_m$, longer pairing ions yield greater retention at a given $P_s$ (dashed lines). Retention maxima still result with all pairing ions, but now occur at higher $P_s$ for the longer sulphonates, which means a reversed order compared to the $P_m$ scale in Fig. 2. In the absence of salt control, maxima occur at lower $P_s$ than in salt-controlled systems.

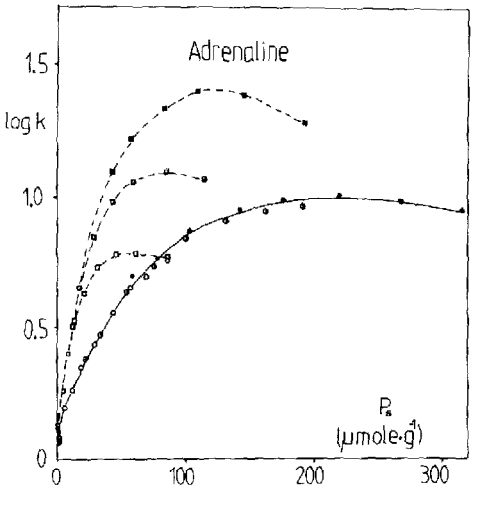



Fig. 3. log *k* data of adrenaline *vs.* the stationary phase concentration, $P_s$, of $BuSO_3Na$, $HexSO_3Na$ and $OctSO_3Na$. Symbols and other conditions as in Fig. 2.

We have previously found[13] that the maximum ratio of the surface concentration of the adsorbed pairing ion ($BuSO_3Na$) to the mobile phase concentration of the counter ion, $P_s/C_m$, coincided with the solute retention maxima. The adsorption isotherm data shown in Fig. 1 are replotted as $P_s/C_m$ *vs.* $P_s$ in Fig. 4 (points for $BuSO_3Na$ as in ref. 13). When the counter ion concentration in the eluent is kept constant ($C_m$ = 175 m*M*), then a straight (solid) line is obtained for $P_s/C_m$, irrespective of the chain length of the pairing ion. With changing counter ion concentration, characteristic maxima in the curves are observed for $P_s/C_m$ (dashed curves). These maxima can be explained by the levelling off of the adsorption isotherm ($P_s$), while $C_m$ (the sum of the concentrations of the counter ions from the initial buffer and from added pairing ion) steadily increases. Consequently, the maximum value and shape of $P_s/C_m$ curves for different pairing ions will depend both on their hydrophobicities and the initial counter ion concentration of the eluent.

In the absence of salt control, retention maxima and $P_s/C_m$ maxima occur at the same $P_s$ (see Figs. 3 and 4).

The area between the dashed and solid lines can be explored simply by changing the counter ion concentration of the eluent (see ref. 13). In practice, the slope of the $P_s/C_m$ *vs.* $P_s$ line ($C_m$ = constant) is determined by $C_m$. When pairing ions of different chain lengths are used at identical $P_m$, *e.g.*, as in refs. 1–5, the respective $P_s/C_m$ values fall on straight lines (dotted line in Fig. 4). Essentially, in these systems, identical ionic strengths are established by the pairing ion itself, rendering "semi-salt-controlled" systems.

It can also be said that the apparent selectivity effects of chain length observed in the absence of salt control are due to the different mobile phase conditions.

Although $P_s/C_m$ is considered to be a general correlating parameter[9,13], it fails to give a quantitative description of this chromatographic system. In Fig. 5 the re-

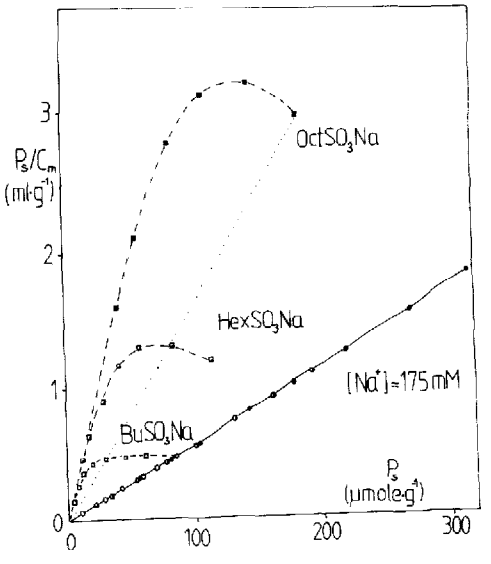



Fig. 4. The ratio of the concentrations of the adsorbed pairing ion ($RSO_3Na$) and the eluent counter ion ($Na^+$), $P_s/C_m$, as a function of the pairing ion concentration in the stationary phase, $P_s$. Conditions and symbols as in Fig. 1.

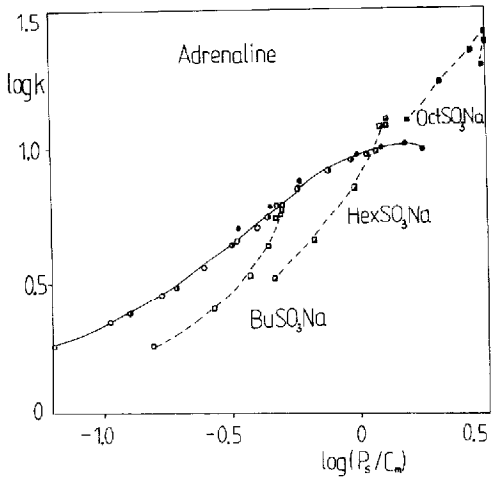



Fig. 5. log $k$ data of adrenaline *vs.* log $P_s/C_m$. Symbols and conditions as in Fig. 2.

tention data of adrenaline are correlated with $P_s/C_m$ values on the logarithmic scale. According to the dynamic ion-exchange model[7–9], this should yield a straight line. Indeed, in salt-controlled systems, at low $P_s/C_m$ (and $P_s$), a more or less linear behaviour is obtained which levels off at higher $P_s/C_m$ (with longer sulphonates). Retention data measured in the absence of salt control lie on other curves. They also exhibit an upper non-linear part, with a fold-over corresponding to the maxima in retention and $P_s/C_m$, as observed by Deelder and van den Berg[9,17].

The linear parts of the log $k$ *vs.* $P_s/C_m$ curves indicate to what extent the dynamic ion-exchange model can be applied. Clearly, the effects of simultaneous variation of $P_s$ and $C_m$ on solute retention cannot be accounted for only by $P_s/C_m$ over the whole range of experimental conditions.

Figs. 3 and 4 also provide an explanation for the contradicting experimental results and retention models cited in the Introduction. The apparent effects of pairing ion chain length originating from the changes of $P_s/C_m$ in the uncontrolled systems[4,5,10,11] led to suggestions of ion-pair adsorption or ion-exchange desolvation mechanisms.

When a constant counter ion concentration is maintained in the eluent, and the stationary phase pairing ion concentration, $P_s$, is the same, chain length has hardly any effect on the retention curve. This indicates that the primary retention-controlling interaction is electrostatic interaction between the solute ions and the adsorbed pairing ions. Its magnitude depends neither on the mobile phase concentration, $P_m$, nor on the chain length of the pairing ion. In other words, both parameters influence only the rate of adsorption of the pairing ion, but the hydrophobic chains are not involved directly in the primary solute–reagent interaction. This is supported by the retention changes of charged ionic solutes. As a typical example, the decreasing retention of *p*-toluenesulphonic acid (PTSA) is shown as a function of $P_s$ in Fig. 6. Again, when salt control is used, retention data obtained with pairing ions of different chain lengths hardly differ (solid line). With changing counter ion concentration (dashed curves), the retention of PTSA is decreased more and the extent is different for different chain lengths. The picture is very similar to that of

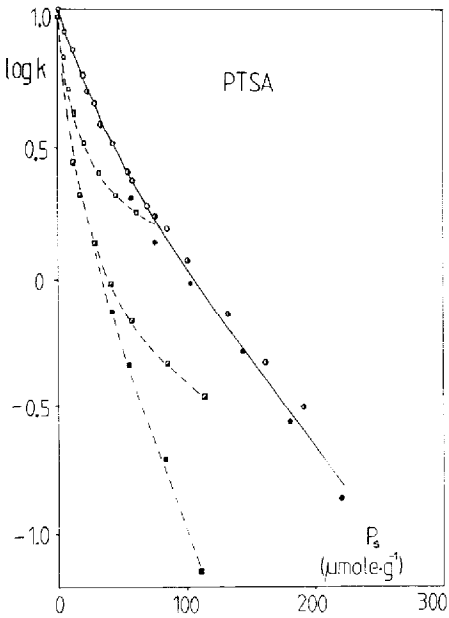



Fig. 6. log $k$ data of $p$-toluenesulphonic acid (PTSA) *vs.* the surface concentration, $P_s$, of $BuSO_3Na$, $HexSO_3Na$ and $OctSO_3Na$, in eluents with changing (---) and constant (175 m$M$) sodium concentration (——). Symbols and conditions as in Fig. 2.

oppositely charged solutes: ionic repulsion of charged solutes by the adsorbed pairing ions is less effective in eluents of higher counter ion concentration. When the mobile phase conditions are controlled, no chain-length selectivity differences can be discerned.

The validity of this conclusion has also been examined for eluents containing an organic modifier. The log $k$ data of adrenaline are plotted *vs.* $P_s$ of straight chain alkane sulphonates (from butane to dodecane) measured in 0, 10, 25 and 40% (v/v) methanol eluents, at constant counter ion (sodium) concentration (175 m$M$), in Fig. 7. As the methanol concentration increases, higher $P_m$ and long chain sulphonates are required to obtain sufficiently high $P_s$ values, *e.g.*, octane to dodecane at 40% (v/v) methanol. The retention behaviour is similar to that observed in the pure aqueous buffer. We have already reported[16] similar results for negatively charged solutes and tetrabutylammonium as pairing ion.

The capacity factors of phenol are plotted against the surface concentrations of pairing ions of different chain lengths in Fig. 8 (conditions as in Fig. 7). It is seen that solute retention decreases with $P_s$, and the decrease is largest in aqueous buffer. This behaviour cannot be explained by changes of $P_s/C_m$ and indicates that other retention-influencing parameters[18] should also be considered[19]. In fact, the retention of uncharged phenol is as insensitive towards the chain length of the adsorbed pairing ion as is that of adrenaline or PTSA

It should be noted that, in the separation of mixtures of different charged solutes, large selectivity changes can be achieved by varying the pairing ion concentration (see Fig. 9) or by changing the amount of the compensating salt (the slope of the log $k$ *vs.* $P_s$ curves of ionic solutes is altered).

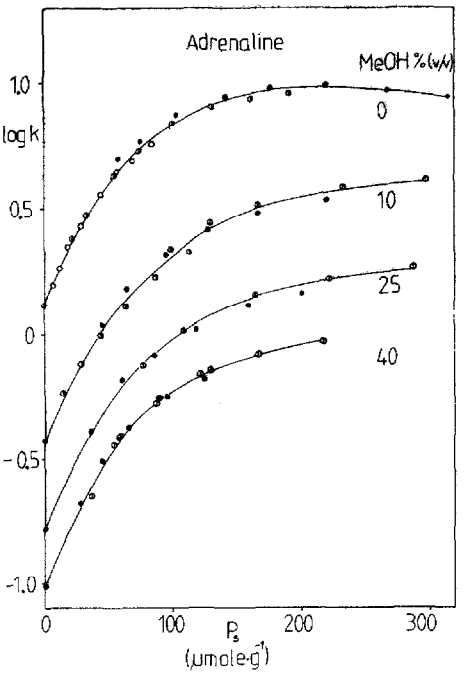
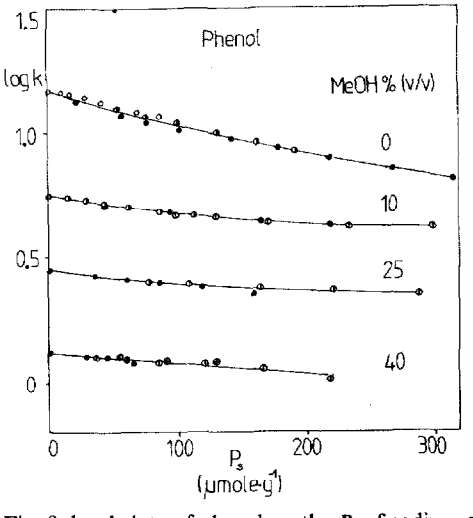



Fig. 7. log $k$ data of adrenaline *vs.* the surface concentration of $BuSO_3Na$ (○), $HexSO_3Na$(◑), $OctSO_3Na$ (●), $DecSO_3Na$ (Φ) and $DodecSO_3Na$ (⊕) pairing ions in eluents with 0, 10, 25 and 40% (v/v) methanol and constant (175 m$M$) sodium concentration.



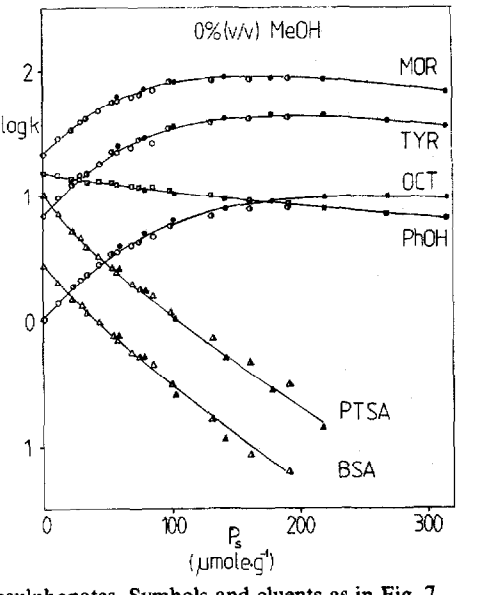




Fig. 8. log $k$ data of phenol *vs.* the $P_s$ of sodium alkanesulphonates. Symbols and eluents as in Fig. 7.

Fig. 9. log $k$ data of positively charged solutes (circles); octopamine (OCT), tyrosine (TYR) and morphine (MOR); negatively charged solutes (triangles), *p*-toluenesulphonic acid (PTSA) and benzenesulphonic acids (BSA) and uncharged solutes (squares); phenol (PhOH), *vs.* the surface concentration, $P_s$, of $BuSO_3Na$ (open symbols), $HexSO_3Na$ (half-filled symbols ) and $OctSO_3Na$ (filled symbols). Eluent: 25 m$M$ $H_3PO_4$–25 m$M$ $NaH_2PO_4$, aqueous buffer (pH = 2.1) with constant (175 m$M$) sodium concentration (controlled by the addition of 150–0 m$M$ NaBr).

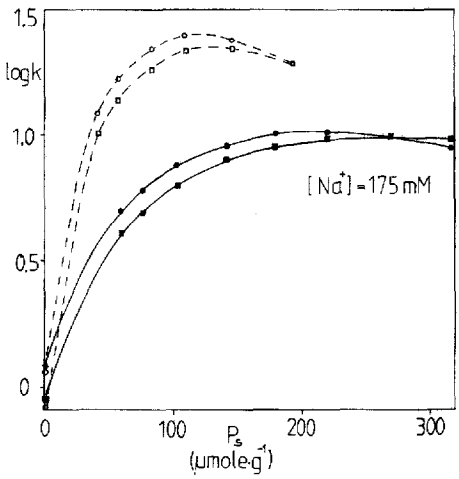



Fig. 10. log $k$ data of adrenaline (circles) and octopamine (squares) *vs.* the surface concentration, $P_s$, of $OctSO_3Na$ in aqueous buffer with changing (---) and constant (175 m$M$) sodium concentration (——).

When closely related solutes, with similar charges and structures, are to be separated, neither changes in $P_s$ nor $C_m$ (and especially not in the pairing ion chain length) will help (see Fig. 10). In this case, special selectivity effects should be invoked by changing the type of the pairing ion (charge, structure, optical activity, etc.) and/or the concentration and type of the organic modifier.

## CONCLUSIONS

When the counter ion concentration changes different retention (log $k$) *vs.* surface concentration ($P_s$) curves are obtained for pairing ions of different chain lengths. This behaviour, as well as the retention maxima observed in eluents without salt control (and $P_m \ll$ CMC) can be correlated by similar changes in the $P_s/C_m$ ratio. Although this parameter fails to provide a full description of the system (indicating also the limitations of the dynamic ion-exchange models), it points to the importance of controlling the counter ion concentration in eluents used in RP-IPC separations.

When the counter ion concentration is kept constant while $P_m$ (and $P_s$) is changed, the apparent selectivity of pairing ion chain length —arising from different $P_s/C_m$ values— disappears. When the mobile phase conditions ($C_m$, ionic strength) are strictly controlled, pairing ions of different chain lengths —at identical surface concentrations— will result in identical solute retentions. This holds also in eluents containing 0–40% (v/v) methanol as organic modifier and sodium alkanesulphonate pairing ions with chain lengths from $C_4$ to $C_{12}$.

Retention of ionic solutes is governed mainly by ionic interactions with the adsorbed pairing ion, but other (non-ionic) interactions should also be considered. The existence of such effects is indicated by the retention decrease of uncharged solutes and the retention maxima for oppositely charged ionic solutes occurring at high surface concentrations of pairing ions, in salt-controlled systems.

The use of constant counter ion concentration in the eluent means that the effects of individual changes in $P_s/C_m$ (depending on both the hydrophobicity of the pairing ion and the counter ion concentration of the initial buffer) can be excluded. In such a system, separations become more predictable and the effect of the different types (structure, charge, etc.) of pairing ions can be examined in terms of surface concentrations.

A practical consequence of the use of salt-controlled eluents is that pairing ions of different chain lengths (with identical structures) are interchangeable in terms of $P_s$. The chain length of the pairing ion can be chosen —without altering the separation— according to economic or other considerations (equilibration time, amount of reagent, price, etc.).

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