

CHROM. 16,553

STUDIES IN REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

III. THE EFFECT OF COUNTER ION CONCENTRATION

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(Received December 23rd, 1983)

SUMMARY

Adsorption isotherms of sodium 1-butanedisulphonate and retention data of both positively and negatively charged ions have been determined in the presence and absence of sodium bromide, in aqueous phosphate buffer, on ODS-Hypersil. Large differences are found in the retention of ionic solutes when the concentration of the pairing ion in the mobile phase is increased over the same range with constant or changing counter ion concentration. Retention maxima are observed for positively charged ions when the pairing ion and the counter ion concentrations increase simultaneously, but not when the counter ion concentration in the eluent is kept constant at a high value. A unifying picture is given for the retention of oppositely charged solutes in terms of the ratio of the concentration of the adsorbed pairing ion and the mobile phase concentration of the counter ion, P_s/C_m .

INTRODUCTION

In previous publications from this laboratory^{1,2} we have described a rapid procedure for the optimization of the mobile phase composition in high-performance liquid chromatography (HPLC). Initially, the procedure was applied to the organic modifier content in binary and ternary solvent systems. In later studies, it was used for optimization of the eluent pH in silica-based columns³ and of the organic modifier content in ion-exchange chromatography on alumina⁴.

An important area for mobile phase optimization is ion-pair chromatography (IPC). Indeed, the retention in reversed-phase (RP) IPC depends on various parameters, such as the type and concentration of the ion-pairing reagent, the organic modifier content, the eluent pH and the concentration of inorganic counter ions.

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However, before optimization can be contemplated, it is necessary to acquire a better insight into the mutual influence of these chromatographic parameters and upon solute retention.

In previous studies on IPC in mixed aqueous-organic mobile phases we have shown that solute retention is primarily governed by the surface concentration of the ion-pairing reagent⁵⁻⁷. This was demonstrated for tetrabutylammonium as the pairing cation and methanol as organic modifier. The concentration of the counter anion (bromide) was kept strictly constant in these studies. In the present investigation we use an anionic pairing ion (butanesulphonate) and study the influence of the corresponding counter cation (sodium).

When the concentration of a pairing ion is varied, the concentration of the counter ion, *i.e.*, sodium for negatively charged sulphonates, can vary correspondingly⁸⁻¹⁴ or it can be kept constant by the addition of compensating amounts of another—generally non-ion-pairing—sodium salt^{6,7,15,16}. A comparative study to reveal the difference between these two strategies has not yet been reported. On the other hand, several examples are given in the literature of the effect of added inorganic salts at constant concentration of pairing ion in the mobile phase^{8,10,11,15-21}. An increased counter ion concentration enhances the adsorption of pairing ions to the stationary phase^{10,11,19,20} and generally decreases the retention of oppositely charged solute ions^{8,10,11,15-21}.

The observations have been discussed qualitatively in terms of competitive ion-pair adsorption models^{9,15,17}, quantitatively by dynamic ion-exchange models^{10,11,14,19} and recently by the ion-interaction model²⁰. Of these theories, only the dynamic ion-exchange model^{10,11} provides a quantitative description of the simultaneous change of the counter ion concentration, C_m , and the surface concentration of the pairing ion, P_s , when salt is added to the eluent. It predicts a linear relationship between the capacity factor, k , and the ratio of these two parameters, regardless of the effect employed to change P_s/C_m . Besides studies reporting good linearity for this relationship^{8,11,16,19}, non-linearity^{12,17,18} and other discrepancies have also been reported^{10,12,16,21}.

The present study is intended to clarify the rôle of the counter ion under rigidly controlled conditions, by simultaneous measurement of pairing ion adsorption isotherms and solute retention data.

EXPERIMENTAL

All chemicals and salts used were of analytical reagent grade. Drugs and sodium alkyl sulphonates were purchased from Janssen Chim. (Beerse, Belgium) and Merck (Darmstadt, F.R.G.), respectively. Sodium bromide, NaH_2PO_4 and H_3PO_4 were from Baker (Deventer, The Netherlands). Distilled and ion-exchanged water was used for the preparation of buffer solutions and eluents.

The chromatographic system comprised two Model 6000 A pumps, a Model 440 absorbance detector (254 nm), a R 401 differential refractometer (Waters Assoc., Milford, MA, U.S.A.), thermostatted columns (150 mm \times 4.6 mm I.D.; Chrompack, The Netherlands), two six-port injection valves (Model 7120 Rheodyne, 10- μl loop; Valco N60) and a dual-channel recorder (BD 41, Kipp en Zonen, The Netherlands). The analytical column was slurry packed with 5- μm ODS Hypersil (Shandon South-

ern Products), with a nitrogen BET surface area of $173 \text{ m}^2 \text{ g}^{-1}$ and a carbon content of 8.8% (w/w) according to the manufacturer (Batch No. 8/1017). The set-up of the equipment, the chromatographic conditions and eluent preparation were as described previously^{5,6}. The eluents were aqueous buffers containing 25 mM H_3PO_4 and 25 mM NaH_2PO_4 (pH = 2.1) and different amounts of sodium bromide (NaBr) and/or sodium 1-butanedisulphonate (BuSO_3Na). In one series of measurements the concentration of the phosphate buffer was also changed.

After a series of chromatographic measurements, the column was flushed with 50 ml aqueous buffer containing bromide or phosphate ions in the original concentration. The adsorbed pairing ion was washed from the column with successive 50-ml portions of phosphate buffer (pH = 2.1) containing 0, 25, 50 and 75% (v/v) methanol, respectively. Finally the column was equilibrated again by pumping through 0.5–1 l of the respective aqueous buffer.

In spite of the careful treatment, a slow degradation of the column was observed during the washing procedure, which resulted in a 1–2% decrease in retention of all solutes. For the conclusions of the present study, this degradation was considered to be acceptable.

RESULTS AND DISCUSSION

In this study we chose a common ion-pairing system, where the pairing ion (BuSO_3Na) concentration was varied up to 150 mM. According to an earlier study by Horváth *et al.*⁹, this is well beyond the concentration where all positively charged test solutes show retention maxima.

First the effect of the added sodium bromide was studied in the absence of the pairing ion (Fig. 1). In accordance with solvophobic theory²², the $\log k$ values of all test solutes increase linearly with the salt content irrespective of their charge. The retention increase is largest for positively charged solutes, lowest for neutrals and intermediate for negatively charged ions, but in all cases $\log k$ varies by less than 0.1 unit, when the concentration of NaBr is increased from zero to 150 mM.

In the presence of ion-pairing reagent the effect of added NaBr is much larger. This is clear from Fig. 2 which presents the retention of various charged solutes as a function of the mobile phase concentration of the pairing ion, P_m , under two different conditions. The dashed curves represent the situation where the sodium concentration increases from 0 to 150 mM as a result of the addition of pairing ion (BuSO_3Na). The solid curves were measured at constant sodium concentration (175 mM) by proportionally decreasing the amount of added NaBr from 150 mM to zero. In either case, the retention data at zero and 150 mM NaBr in Fig. 1 provide the starting points for the retention curves of the charged solutes in Fig. 2. When the sodium concentration increases (dashed curves) the retention of negatively charged solutes decreases steadily, whereas the positively charged solutes show characteristic retention maxima, at a P_m value of about 80 mM. It should be noted that this concentration is about ten times lower than the estimated critical micelle concentration of BuSO_3Na ²³. When the counter ion concentration (sodium) is kept constant (solid curves) no maxima are observed. Indeed, the addition of NaBr results in a significant decrease in the retention of the positively charged solutes and an increase in the retention of the negatively charged solutes. On the other hand, the retention data

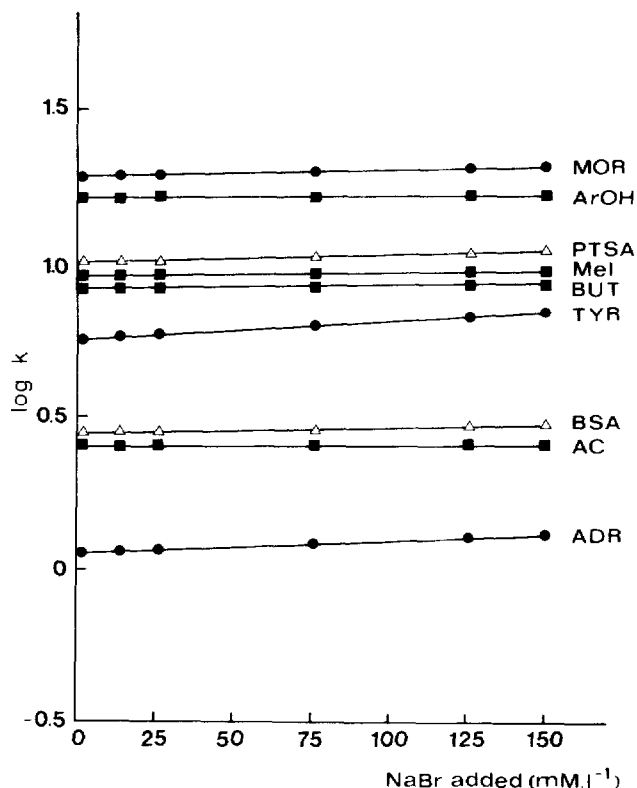


Fig. 1. The effect of added sodium bromide (NaBr) on the retention of positively charged (●), negatively charged (△) and neutral (■) solutes. Mobile phase: aqueous buffer, 25 mM H₃PO₄ + 25 mM NaH₂PO₄ (pH = 2.1). Stationary phase: 5- μ m ODS-Hypersil. Temperature: 25°C. Solutes: ADR = adrenaline; AC = acetone; BSA = benzenesulphonic acid; TYR = tyrosine; BUT = 2-butanone; MeI = methyl iodide; PTSA = *p*-toluenesulphonic acid; ArOH = phenol; MOR = morphine.

coincide within experimental error at the final data point ($P_m = 150$ mM) where the eluent composition is the same.

The corresponding adsorption isotherms of the pairing ion are shown in Fig. 3. In accordance with other studies^{10,11,19,20}, in the presence of extra salt, a higher adsorption of the pairing ion is observed at the same P_m . Again, the two curves converge to the same final point. Both adsorption isotherms fail to satisfy the Langmuir equation over the entire concentration range. The highest adsorption value ($85.6 \mu\text{M g}^{-1} = 0.5 \mu\text{M m}^{-2}$) measured at 150 mM is rather low in comparison to adsorption data of longer alkyl sulphonates¹⁴ and sulphates¹⁶ measured on ODS-Hypersil, *e.g.*, $2 \mu\text{M m}^{-2}$ for octanesulphonate.

The differences between the dashed and the solid curves in Figs. 2 and 3 may be attributed to an effect of the counter ion or of the ionic strength. To distinguish between these, two other experiments were carried out. In both, the mobile phase concentration of BuSO₃Na was maintained constant at $P_m = 20$ mM and the sodium concentration was varied from 45 to 175 mM by addition of NaBr or by increasing

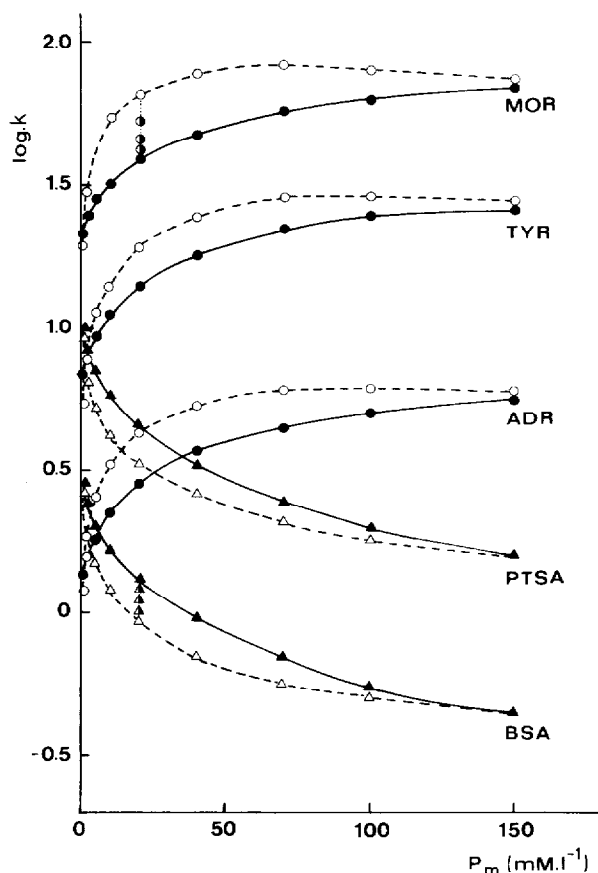


Fig. 2. The capacity factor of ionized solutes vs. the mobile phase concentration, P_m , of sodium 1-butanedisulphonate (BuSO_3Na) with changing (dashed line) and constant (solid line) sodium concentration. Eluent: phosphate buffer as in Fig. 1 with BuSO_3Na (0–150 mM) or phosphate buffer (25 mM sodium) with BuSO_3Na (0–150 mM) and NaBr (150–0 mM). The dotted line for morphine and benzenesulphonic acid illustrates the effect of added NaBr at a constant P_m of 20 mM.

the concentration of the $\text{H}_3\text{PO}_4\text{--NaH}_2\text{PO}_4$ (1:1) phosphate buffer. As a result, different ionic strength values can be established at the same concentrations of pairing ion, P_m , and counter ion, C_m . Fig. 4 shows that the increase in P_s (observed in Fig. 3) is almost linearly related to the sodium concentration and virtually independent of the ionic strength under the given conditions.

Similar effects were reported for the adsorption of alkyl sulphonates at fluid–fluid interfaces. The “salting out” effect in these systems primarily depends on the mean ionic activity of the sulphonate and the sodium, while the non-surface-active anion, accompanied by the counter ion (in added salt), influenced the ionic strength as well as the mean ionic activity coefficient in the solution^{24,25}. In our case the latter effect has less influence on the adsorption in comparison to the changes in the counter ion concentration. Recently, Bidlingmeyer and Warren²⁰ reported S-shaped curves for pairing ion adsorption data as a function of the logarithmic salt concentration,

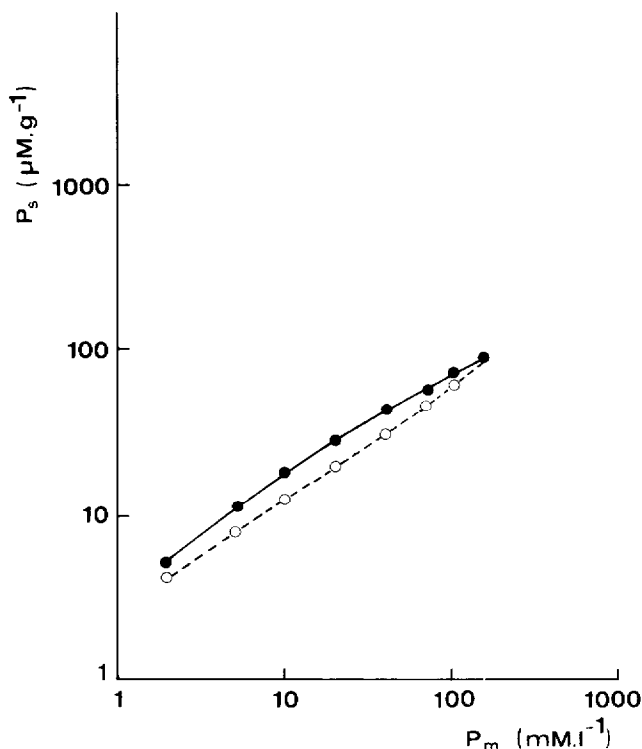


Fig. 3. Adsorption isotherms of sodium 1-butan-1-ylsulfonate from an aqueous 25 mM H_3PO_4 + 25 mM NaH_2PO_4 buffer (pH = 2.1) with changing (dashed curve) and constant (175 mM) sodium concentration (solid curve), on 5- μm ODS-Hypersil at 25°C.

over a much wider concentration range (10^{-4} – 10^{-1} M). When plotted logarithmically, our data of Fig. 4 also show a slight S-shape.

Fig. 5 presents the corresponding retention behaviour of neutral and charged solutes at constant pairing ion concentration ($P_m = 20$ mM) as a function of the counter ion concentration varied through addition of NaBr (dashed curves) or phosphate buffer (solid curves). The log k of neutral methyl iodide increases slightly and linearly with the sodium concentration. Somewhat higher retention values are observed with the phosphate series. This suggests that the retention of neutral solutes is modified by the entire salt content, *i.e.*, the ionic strength, rather than only by the counter ion concentration. The changes are comparable with those given in Fig. 1 and can be attributed to the increase of the hydrophobic contribution of solute retention. The log k of similarly charged aromatic sulphonic acids increases non-linearly by almost 0.2 units, when the sodium concentration is varied from 45 to 175 mM. The data for the phosphate series systematically exceed those for the bromide series, by an amount comparable with that observed for methyl iodide. Again the effect of the ionic strength may be responsible through the non-electrostatic contribution to the retention of the negatively charged solutes. Alternatively, the increase of the pK_a values with ionic strength may decrease the ionization of the acids and

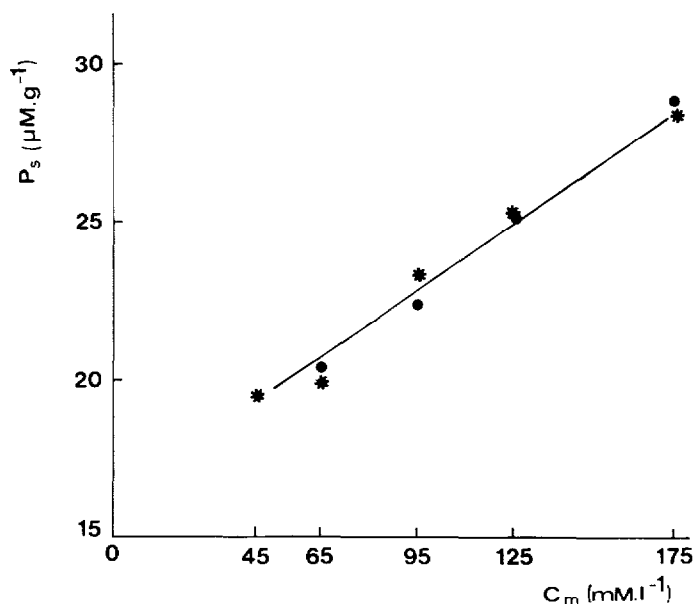


Fig. 4. The effect of added sodium on the adsorption of sodium 1-butanedisulphonate at constant mobile phase concentration of the pairing ion, $P_m = 20$ mM. The sodium concentration, C_m , was varied by adding sodium bromide (●), or by increasing the H_3PO_4 - NaH_2PO_4 (1:1) buffer concentration (*).

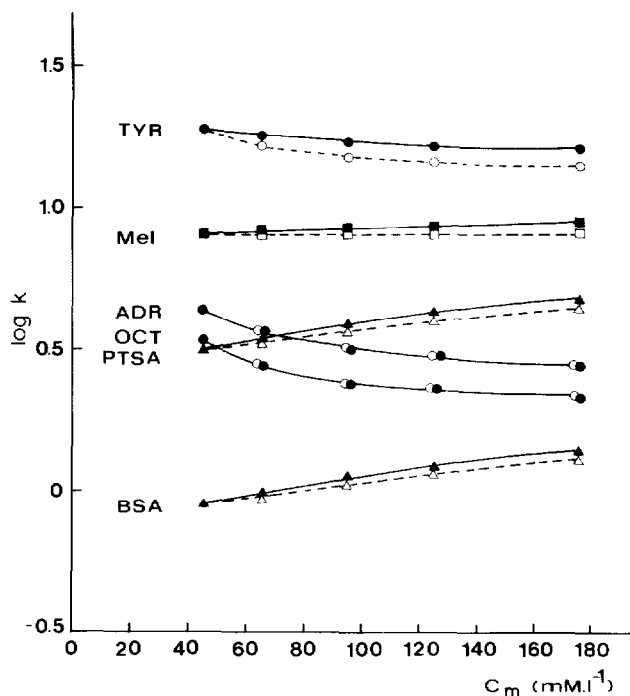


Fig. 5. Log k data for different solutes at constant pairing ion concentration, $P_m = 20$ mM, as a function of the sodium concentration in the mobile phase varied by the addition of NaBr (---) or phosphate buffer (—). OCT = octopamine.

enhance their retention. All changes caused by the differences in ionic strength are small in comparison to the counter ion effect. In contrast to the above two cases, the retention of positively charged solutes decreases non-linearly (by $0.2 \log k$ units) with the sodium concentration, and the retention data measured for either the bromide or the phosphate series practically coincide. Only tyrosine shows a different behaviour: the decrease is less and there is a significant effect of ionic strength. The possibility of tyrosine occurring as a zwitterion, with an acidic pK_a of 2.6^{26} close to the pH of the buffer, would lead us to expect a strong influence of the ionic strength for this solute.

The absence of an additional effect of ionic strength in both the pairing ion adsorption isotherm (Fig. 4) and the retention of positively charged solutes (Fig. 5) also suggests a strong correlation between these two parameters. To examine this, the retention data of Fig. 2 have been replotted as a function of the surface concentration of the pairing ion by means of the adsorption isotherms in Fig. 3. The results are presented in Fig. 6. However, whereas the adsorption of the pairing ion increases

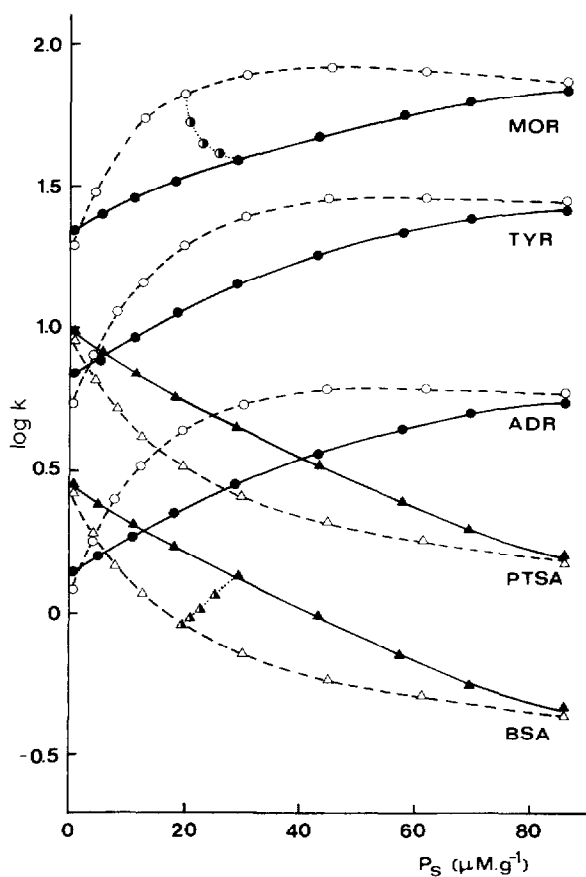


Fig. 6. $\log k$ data for ionized solutes vs. the stationary phase concentration of BuSO_3Na , P_s . Symbols and other conditions as in Fig. 2. The data for morphine and benzenesulphonic acid at constant P_m (20 mM) are taken from the bromide series (dotted line).

with increasing salt content (Fig. 2), the retention of oppositely charged solutes decreases (Fig. 5). As a result, the difference between the dashed and solid curves in Fig. 6 is even more striking than in Fig. 2. Without the addition of NaBr, the retention curves are again decidedly non-linear (dashed line) and maxima are observed in agreement with other studies^{9,14}. On the other hand, the retention increases almost linearly when the counter ion is controlled in the eluent (solid line), for both oppositely and similarly charged solutes.

When the retention data of oppositely charged solutes are replotted on a linear scale, the curves at constant C_m are virtually linear. This is in accord with the theoretical expression provided by the dynamic ion-exchange model¹⁰⁻¹²

$$k = K_{ie} P_s / C_m \quad (1)$$

where P_s/C_m is the phase ratio and K_{ie} is the ion-exchange equilibrium constant. Now, if P_s/C_m is plotted as a function of P_m or P_s for our two sets of experimental conditions, we obtain the curves in Fig. 7. There is a striking resemblance of these

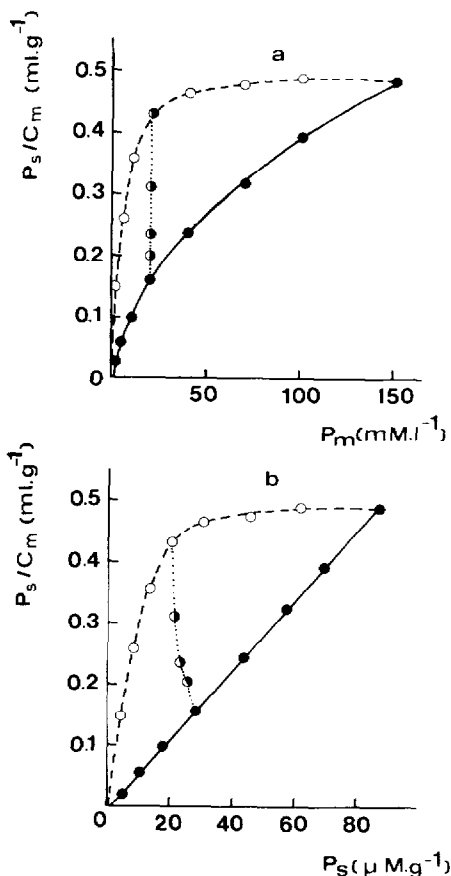


Fig. 7. The ratio of the adsorbed pairing ion (BuSO_3Na) and eluent counter ion (sodium) concentration, P_s/C_m , as a function of the pairing ion concentration in the mobile phase (a), P_m , and in the stationary phase (b), P_s . Conditions as in Figs. 3 and 4. Dotted lines refer to added NaBr at constant P_m .

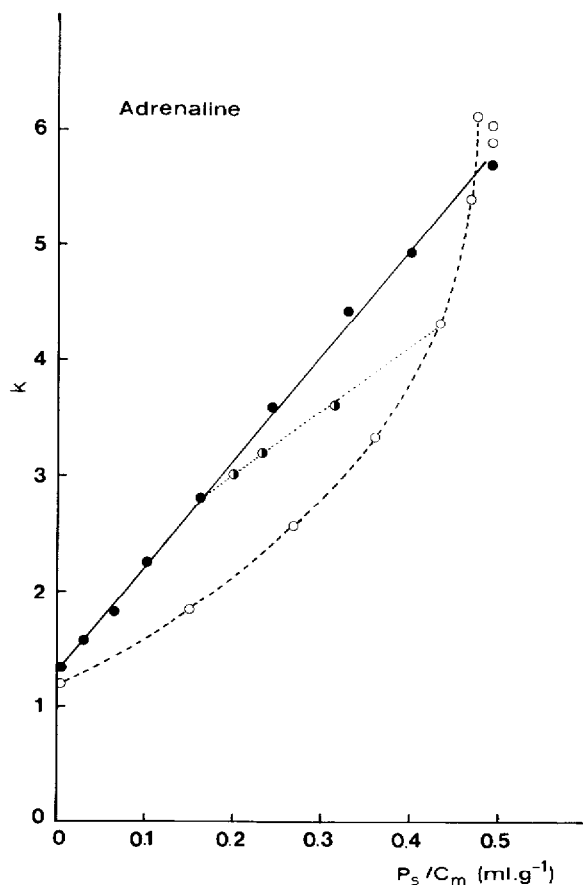


Fig. 8. The capacity factor of adrenaline, k , against the P_s/C_m ratio, at changing counter ion, C_m , and pairing ion, P_m , mobile phase concentration (dashed line); at constant P_m (20 mM) and changing C_m (dotted line); and at constant C_m (175 mM) and changing P_m (solid line). Conditions as in Figs. 2-6.

curves to the retention curves of oppositely charged solutes in Figs. 2 and 6. When plotted *versus* P_m , both P_s/C_m curves are non-linear, whereas at constant C_m (solid curves), P_s/C_m is, of course, proportional to P_s . Also, when the counter ion concentration varies with the pairing ion (dashed curves), maxima are observed for P_s/C_m just as for the retention curves in Figs. 2 and 6 at a similar P_m value of 100 mM. It might, therefore, be hypothesized that the parameter P_s/C_m includes the effects of both the pairing ion and the counter ion upon the retention of oppositely charged solutes.

As a typical example, Fig. 8 presents the retention of positively charged adrenaline as a function of P_s/C_m . At a constant counter ion concentration, C_m , the capacity factor is linearly related to P_s/C_m . However, when the counter ion concentration is varied (dashed curve) a different, concave curve is obtained. This difference indicates that the effect of the counter ion is not completely described by the parameter P_s/C_m in the dynamic ion-exchange model and that there is a second effect. A

similar behaviour has been reported by Deelder *et al.*¹² with octanesulphonate as the pairing ion and sodium as the counter ion. These authors ascribed the non-linear behaviour to an increase of K_{ic} with P_s . However, this hypothesis is in contrast with the linear variation of k with P_s at constant C_m , as demonstrated by the solid line in Fig. 8.

When P_m is kept constant and C_m is varied, k increases with P_s/C_m (dotted line). Straight lines were observed also in similar studies by Van de Venne¹⁰ and Knox and Hartwick¹⁶. Their retention data presented as k against P_s/C_m give a smaller slope when C_m was varied at constant P_m , in comparison to those measured at changing P_m and constant C_m . Fig. 8 explains this apparently contradictory behaviour, showing that the same P_s/C_m ratio can result in different solute retentions when the eluent composition (ionic strength) is different.

When a more hydrophobic pairing ion is used, P_m varies over a more restricted concentration range. When in this case C_m is not kept constant, its variation is also smaller and the increase of P_s/C_m is of primary importance. With reference to Figs. 6 and 7 (dashed lines) the levelling off of P_s/C_m with P_m or P_s remains responsible for the maxima in the solute retention curves. This provides an explanation for the retention maxima, reported in other studies, when the counter ion concentration was changed upon pairing ion addition and micelle formation could be excluded^{9,12,13}.

However, retention maxima were also observed when using more hydrophobic pairing ions at constant counter ion concentration^{6,16}. Micelle formation in the eluent^{16,19} or other retention decreasing effects—originating from high pairing ion adsorption²⁷—will ultimately level off or maximize k or $\log k$. Although these effects have no significant influence in the case of BuSO_3Na , the linearity of k vs. P_s/C_m (at constant C_m) is limited to lower values of the surface concentration. When sodium sulphonate pairing ions with longer alkyl chains are used, this relationship has been shown to level off at higher surface concentrations²⁸. This leads to the conclusion that, when using a pairing ion with higher hydrophobicity, without controlling the counter ion concentration in the eluent, solute retention maxima can arise from the combined effects of P_s/C_m changes and other retention decreasing effects (accompanied with high P_s).

CONCLUSIONS

We have shown in this study that the counter ion concentration can have a significant effect on both pairing ion adsorption and solute retention.

Different retention is observed for ionic solutes when the concentration of the pairing ion in the mobile phase is increased with or without compensating for the changes in the counter ion concentration in the eluent. The retention is decreased for positively charged ions, increased for negatively charged ones, but hardly affected for neutral solutes, when salt is added.

Retention maxima are observed for positively charged solutes when the counter ion concentration increases with addition of pairing ion. When the counter ion concentration and ionic strength in the eluent are constant, a simple linear relationship is observed between k and P_s/C_m . Both these observations can be explained by similar changes of the P_s/C_m ratio. The decrease in retention of these solutes, when the counter ion concentration is increased at constant P_m , can also be rationalized

by the decrease of P_s/C_m . However, in spite of its primary importance, P_s/C_m has been shown not to be the only parameter to describe solute retention, when large changes occur in the eluent composition (ionic strength).

We also note that there are no significant differences in log (selectivity) for positively charged solutes, regardless of whether C_m is kept constant or changed (the log k vs. P_m or P_s curves are nearly parallel).

In view of the more complex retention behaviour when C_m is allowed to vary and the relative simplicity, *i.e.*, better predictability, when C_m is kept constant, the latter system is preferred for further investigation including optimization of ion-pairing systems. It has the further advantage that the effect of other parameters (chain length, type of pairing ion) can be examined in terms of the surface concentration, P_s and C_m , under constant mobile phase conditions, C_m and ionic strength.

ACKNOWLEDGEMENT

A grant from SEA (Scientific Exchange Agreement) Foundation to Á. Bartha is appreciated.

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