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# STUDIES IN REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

## V. SIMULTANEOUS EFFECTS OF THE ELUENT CONCENTRATION OF THE INORGANIC COUNTER ION AND THE SURFACE CONCENTRATION OF THE PAIRING ION

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SUMMARY

Retention data of the positively charged adrenaline ion, when plotted against the surface concentration of the adsorbed alkylsulphonate pairing ion and the mobile-phase concentration of the inorganic counter ion (sodium), fall on a common retention surface, irrespective of the chain length of the pairing ion used. This common retention surface can be used to reconcile the apparently conflicting reports on the effects of the concentration and chain length of the alkylsulphonate pairing ion and the concentration of the inorganic counter ion. It can also be used for a better understanding and control of both retention and separation selectivity in reversed-phase ion-pair chromatography.

## INTRODUCTION

In reversed-phase ion-pair liquid chromatography, oppositely charged solutes experience a characteristic retention maximum as the mobile-phase concentration of the pairing ion is increased[1–16]. Equilibrium effects[1,2,17–19], micelle formation at high pairing ion concentrations[6], ion-pair formation and decreasing adsorption capacity of the stationary phase[5], a decrease in the available hydrophobic surface area caused by the adsorption of the pairing ion[8,9], changes in the surface potential in the adsorbed layer of the pairing ion[11,20,21], ion-pair formation coupled with increasing inorganic counter ion concentration[22] and a decrease in the interfacial surface tension brought about by the adsorbed pairing ion[23] have been invoked to explain the occurrence, shape and position of the retention maxima. Solute retention was reported to vary with the chain length of the pairing ion[6,8,9,24] and also to be independent of the chain length of the pairing ion[10,15]. Further, apparently conflicting results were reported concerning the effects of the concentration of the counter ion: both linear[3] and non-linear[11,25] relationships were observed between the retention and the ratio

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of the surface concentration of the pairing ion and the mobile-phase concentration of the inorganic counter ion. These observation prompted us to re-examine the simultaneous effects of the concentration and chain length of the pairing ion and the concentration of the inorganic counter ion. We have found that the retention of positively charged solutes is determined simultaneously by the mobile phase concentration of the inorganic counter ion ($C_m$) and the surface concentration of the adsorbed alkylsulphonate pairing ion ($P_s$), and is independent of the chain length of the pairing ion.

## EXPERIMENTAL

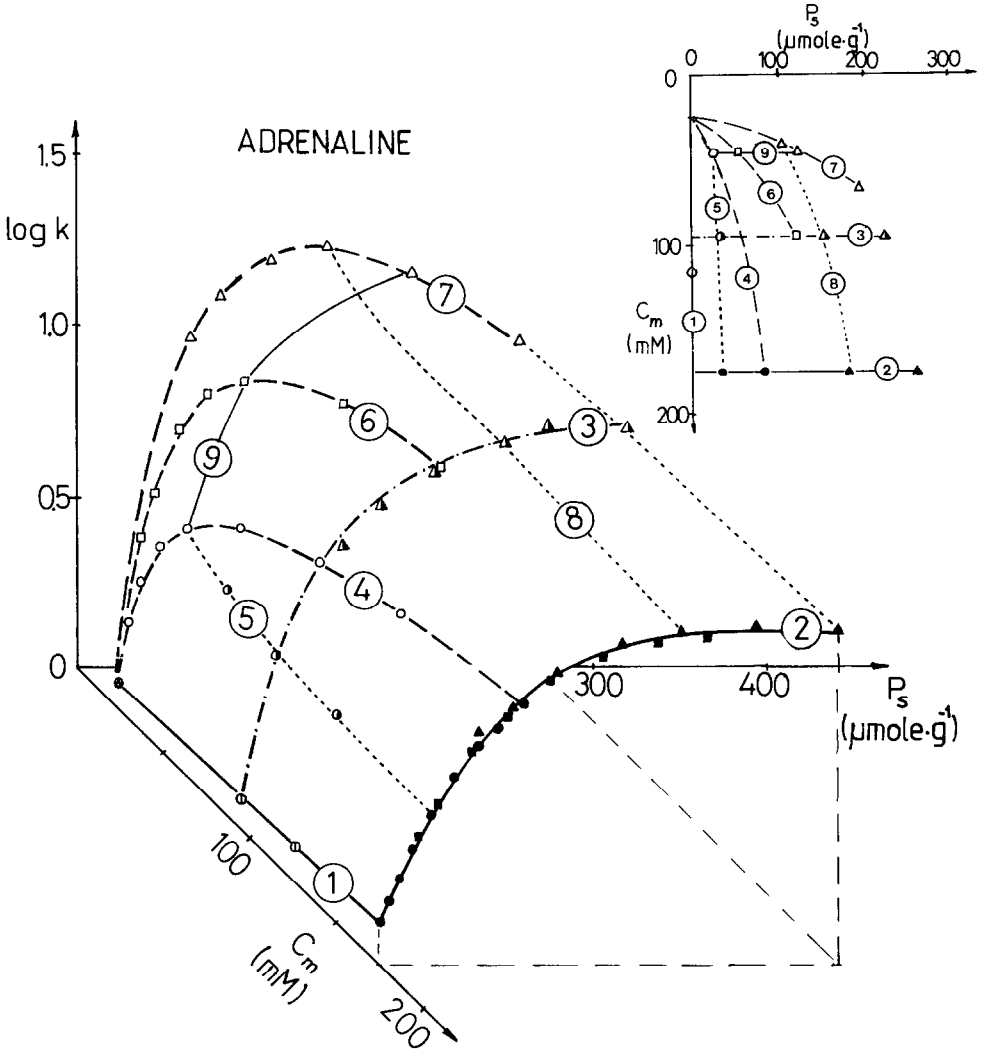
The solutes were obtained from Janssen (Beerse, Belgium) and the ion-pairing reagents [sodium 1-butanesulphonate ($BuSO_3Na$), sodium 1-hexanesulphonate ($HexSO_3Na$) and sodium 1-octanesulphonate ($OctSO_3Na$)] and the buffer components from Merck (Darmstadt, F.R.G.). Mobile phases were prepared from deionized water, and contained 25 m*M* $H_3PO_4$, 25 m*M* $NaH_2PO_4$ (pH 2.1) and various amounts of NaBr and/or the pairing ions.

Hypersil ODS (Shandon, London, U.K.) (5 $\mu$m) with a nominal carbon content of 8.8% (w/w) and a BET surface area of 173 $m^2/g$ (according to the manufacturer) was used as the stationary phase. The equipment, chromatographic conditions and the experimental technique used were as described previously[13,26]. They permit the simultaneous determination of both the adsorption isotherms and the retention data of the solutes[15,25]. All measurements were made at 25 ± 0.1°C.

## RESULTS AND DISCUSSION

In Part IV[15], we showed that both the retention (log $k$, where $k$ is the capacity ratio) and the position of the retention maxima of the oppositely charged solutes varied with both the concentration and the chain length of the pairing ion when the mobile-phase concentrations of both the buffer and the inert salt (NaBr) were kept constant and the concentration of the pairing ion was increased (*i.e.*, when the ionic strength of the eluent was different from point to point). However, when the ionic strength of the eluent was kept constant by decreasing the concentration of the inert salt (NaBr), as the concentration of the pairing ion increased (*i.e.*, a constant Na concentration was maintained), identical solute retention was observed at identical surface concentrations of the pairing ion, irrespective of the chain length of the latter. The maxima in log $k$ also occurred at the same surface concentration, irrespective of the chain length of the pairing ion (*cf.*, Figs. 2 and 3 in ref. 15). These observations indicated that the effects of the counter ion concentration and the surface concentration of the pairing ion should be evaluated simultaneously.

The retention of adrenaline is shown as log $k$ in Fig. 1, plotted against both the measured surface concentration ($P_s$) of the various alkanesulphonate pairing ions and the mobile-phase concentration of the inorganic counter ion, sodium ($C_m$). The inset (top right) shows the respective $P_s$ and $C_m$ points obtained with pairing ions of different chain lengths in eluents of different ionic strength. Lines 4, 6 and 7 in the inset represent the surface concentrations that are obtained when the concentration of sodium alkanesulphonate in the eluent is increased ($BuSO_3$, $HexSO_3$ and $OctSO_3$,




Φ $C_m$=25-175 mM, (0-150 mM NaBr) ①

● $BuSO_3Na$
■ $HexSO_3Na$
▲ $OctSO_3Na$
$C_m$=175 mM ②

Δ $OctSO_3Na$, $C_m$=95 mM ③

○ $BuSO_3Na$, no salt ④

◑ $P_m$=20 mM $BuSO_3Na$, $C_m$=45-175 mM ⑤

□ $HexSO_3Na$, no salt ⑥

△ $OctSO_3Na$, no salt ⑦

$P_m$=10 mM $OctSO_3Na$, $C_m$=35-175 mM ⑧

$BuSO_3Na$
$HexSO_3Na$
$OctSO_3Na$
$P_m$=20 mM
$C_m$=45 mM
⑨

Fig. 1. Retention (log $k$) of adrenaline as a function of the stationary phase concentration ($P_s$) of alkanesulphonate pairing ions and eluent concentration ($C_m$) of the inorganic counter ion (sodium). Mobile phase, 25 m$M$ $H_3PO_4$–25 m$M$ $NaH_2PO_4$ buffer (pH 2.1); stationary phase, 5 μm Hypersil ODS; temperature, 25°C.

respectively) and the eluent contains no added NaBr. Lines 5 and 8 represent the surface concentrations that are obtained when the concentration of the pairing ion ($BuSO_3$ and $OctSO_3$, respectively) in the eluent is kept at a constant, low value, but the concentration of sodium bromide is increased. Lines 9, 3 and 2 show the surface concentrations that can be established at constant ionic strength (salt-controlled system) with increasing concentrations in the mobile phase of $BuSO_3$, $HexSO_3$ and $OctSO_3$.

The retention of adrenaline above the plane of $P_s$ and $C_m$ is shown in the main part of Fig. 1. Line 1 represents the slight increase in retention that occurs when the ionic strength of the eluent that contains no pairing ion is increased by the addition of NaBr. This behaviour is in agreement with the prediction of the solvophobic retention theory of reversed-phase liquid chromatography[27].

As shown by lines 2 and 3, all log $k$ values fall on the same line when the ionic strength of the eluent is kept constant (*i.e.*, the retention surface is intersected by a plane parallel to that of log $k$ and $P_s$), irrespective of the chain length of the pairing ion used. A single retention maximum is observed at a fairly high $P_s$ value (*ca.* 250 $\mu$mol/g). This means that, in a salt-controlled system, it is the surface concentration of the pairing ion that determines the value of log $k$, not its chain length.

Curves 4, 6 and 7 represent the log $k$ values that were obtained when the concentration of $BuSO_3$, $HexSO_3$ and $OctSO_3$, respectively, was increased in an eluent that contained no other inorganic salt. Thus, as the mobile-phase concentration of the various pairing ions is increased, both the eluent concentration of the counter ion (Na) and the surface concentration of the respective pairing ion is increased. As the extent of adsorption depends on both the hydrophobicity and mobile-phase concentration of the pairing ion, this type of eluent preparation is not represented by a straight line in the plane of $P_s$ and $C_m$, but rather by curves 4, 6 and 7 in the inset. Hence the observed retention data lie on non-linear intersections of the curved retention surface. Lines 5 and 8 represent another non-linear intersection of the retention surface. In this instance, the mobile-phase concentration of the pairing ion is kept constant, while both the sodium concentration of the eluent and the pairing ion concentration on the surface are increased by the addition of the inert salt (NaBr). Meanwhile, the retention varies from a point on curve 7 through curve 3 to curve 2.

Hence, in reversed-phase ion-pair chromatography the retention is controlled, simultaneously, by both the mobile-phase concentration of the counterion and the surface concentration of the pairing ion and a single parameter, for example the $P_s/C_m$ ratio proposed in the dynamic ion-exchange model[11] cannot fully describe the chromatographic system[25].

The changing shape of the retention curve and the changing position of its maximum are demonstrated in Fig. 2. Here, the retention of adrenaline is shown in three different eluent systems, one with no salt control, one controlled at a medium counter ion concentration and the third controlled at a high counter ion concentration. These retention curves, in fact, represent different intersections of the retention surface shown in Fig. 1. The maximum that is such a prominent feature when the ionic strength of the eluent varies is barely noticeable in the salt-controlled systems. Its prominence depends only on the manner in which the common, doubly curved retention surface is intersected.

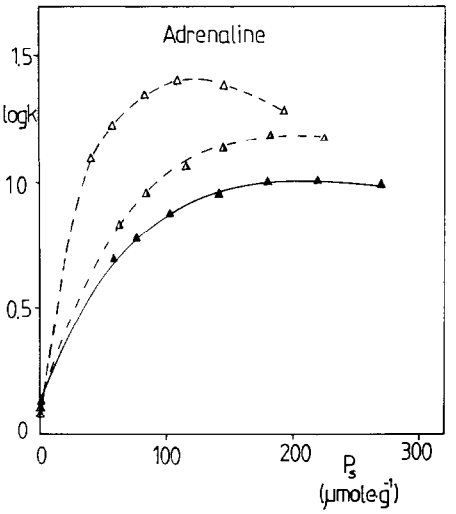



Fig. 2. Retention of adrenaline as a function of the surface concentration of octanesulphonate at different counter ion concentrations. Conditions as in Fig. 1. △---△, No salt; △-.-.-△, $[Na^+]$ = 95 m*M*; ▲—▲, $[Na^+]$ = 175 m*M*.

There is another consequence of the observation that in a salt-controlled system all retention data fall on the same line as $P_s$ increases, irrespective of the chain length of the pairing ion. In the four-parameter thermodynamic retention model of ion-pair chromatography the retention maximum was attributed to the pairing ion-related decrease of the interfacial surface tension[23]. The decrease in retention depends only on the product of the adsorption surface area requirement of the solute and the change in the interfacial surface tension. Numerical fitting of the model to a few sets of retention data, taken from the literature, produced a good fit, indicating that the model was applicable. However, alkanesulphonates of different chain length present at identical surface concentrations lead to different interfacial surface tension values. Therefore, an individual retention maximum should be seen for each pairing ion at a different, characteristic surface concentration. This is clearly not the case, indicating that the existence of retention maxima cannot be explained by the decreased surface tension alone.

The apparent chain length dependence of the position of the retention maximum in terms of either the mobile-phase concentration[5–9,11,12] or the stationary-phase concentration of the pairing ion[8,9–16] can also be explained by Fig. 1. With an eluent of varying ionic strength, the corresponding $P_s$ and $C_m$ data pairs follow a different curve for each pairing ion (lines 4, 6 and 7 in the inset for $BuSO_3$, $HexSO_3$ and $OctSO_3$ respectively) as the concentration of the pairing ion is increased. Consequently, the observed retention curves represent different intersections of the same common retention surface along different curves in the plane of $P_s$ and $C_m$, and not genuinely different interactions. As long as the surface concentrations and mobile phase counter ion concentrations are identical, the chain length of the pairing ion plays no role in the retention.

Fig. 1 can be used to illustrate another observation in reversed-phase ion-pair

chromatography, *viz.*, at identical mobile phase concentrations the longer chain pairing ions lead to larger solute retention[6–11,14–16,18,24,28–30]. This case is represented by curve 9 in Fig. 1. At identical $C_m$, the longer the chain the more the pairing ion is adsorbed. Hencer the retention surface is intersected by a plane parallel to that of log $k$ and $P_s$. It was suggested that the separation selectivity in reversed-phase ion-pair chromatography can be improved by mixing eluents that contain pairing ions of different chain lengths at the same mobile-phase concentration[28–30]. As shown in Fig. 1 this leads to different $P_s$ and $C_m$ values when the ionic strength in not controlled, or to different $P_s$ values when the ionic strength is controlled. The same retention could be established by using only a single pairing ion of the appropriate mobile-phase concentration.

The existence of the common retention surface rationalizes the practical rule of pairing ion selection[15,19,31]: the pairing ion that covers the broadest $P_s$ range without presenting problems with solubility or micelle formation is to be preferred. Any point on the retention surface can be realized by varying the mobile-phase concentration of both the pairing ion and the counter ion. At constant ionic strength, one moves in a plane parallel to the plane of log $k$ and $P_s$. When the mobile phase concentration of the pairing ion is varied and the ionic strength is not controlled, one moves along both the $C_m$ and $P_s$ axis and, consequently, the retention changes in a manner that is difficult to control and predict.

Separation selectivity can be varied differently by moving parallel to either the $C_m$ or the $P_s$ axis. For a non-charged solute and oppositely charged solute pair that are eluted at the same time, an increase in $C_m$ at constants $P_s$ will hardly influence (slightly increase) the retention of the non-charged solute, whereas it will significantly decrease the retention of the charged solute[15,32,33]. On the other hand, as shown in Fig. 3 for adrenaline and octopamine, the separation selectivity for two closely related, similarly charged solutes is almost independent of the salt concentration (inorganic ionic environment in the mobile phase), but changes somewhat with the surface concentration of the pairing ion (which modifies the selective interactions between the solutes and the stationary phase). Both $C_m$ and $P_s$ can be used to control the retention of charged solutes but only the latter can be used to alter the separation selectivity for charged solutes.

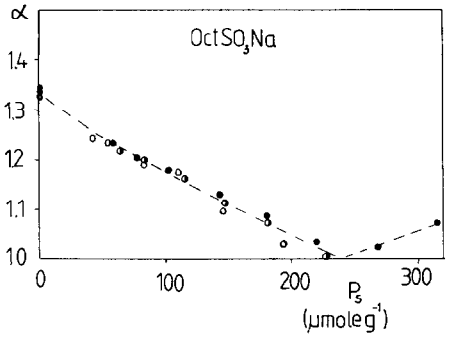



Fig. 3. Relative retention of adrenaline and octopamine as a function of the surface concentration of octanesulphonate at different counter ion concentrations. Conditions as in Fig. 1. ●, $[Na^+]$ = 175 m$M$; ◑, $[Na^+]$ = 95 m$M$; ○, no salt.

## CONCLUSIONS

A common retention surface is obtained when the retention of positively charged adrenaline is plotted against the eluent concentration of the inorganic counter ion (sodium) and the surface concentration of the adsorbed alkanesulphonate pairing ion of different chain length. At constant surface concentration, the retention of the oppositely charged solute will decrease monotonously with the concentration of the counter ion. At constant eluent counter ion concentration (constant ionic strength), the retention of the oppositely charged solute will increase with increasing surface concentration of the pairing ion and pass through a maximum. The resulting retention surface is a doubly curved surface. The existence of the common retention surface permits the interchangeable use of alkanesulphonates of different chain length without influencing the separation. By judicious choice of both ionic strength and surface concentration, both retention and separation selectivity can be controlled.

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