

Contributions to the calculation of retention data in ion-pair chromatography

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SUMMARY

The retention behaviour of organic bases (*m*- and *o*-aminophenol, dopamine) was investigated in the pH range 2,5–6,8 using aqueous phosphate buffer and constant ionic strength. The capacity factors were determined on two ODS-silica columns in the absence and presence of sodium octylsulphonate. Using the calculated values of the hydrophobic capacities of the columns, a logarithmic relationship is proposed between the capacity factor of the non-protonated base in the absence and presence of the ion-pairing reagent and the actual hydrophobic capacity of the column. For the retention ratios of the protonated base species, the electrostatic theory introduced by Stahlberg proved to be acceptable. At low pH the protonation of the octylsulphonate ion is also considered. The calculated and experimentally obtained capacity factors show acceptable agreement with each other.

INTRODUCTION

With the introduction of modern high-performance liquid column chromatography and the use of chemically bonded alkyl groups on silica as (reverse) stationary phases, an immense development of liquid chromatographic procedures began. During the development of the stationary phases, many efforts were made by the producers to develop products with guaranteed quality for long-term use. The most important point was reproducibility of the products, *i.e.*, the columns filled with the phases should have the same properties, and the second was the preservation of the original properties during the long-term use of the columns.

In spite of many efforts, if one wishes to change the column in a given separation, one has to consider the properties of the columns, even when the particle size, surface area, and nature of the alkyl groups are identical in both columns.

Several papers have been published on the theory of ion-pair chromatography in the last decade. Many models were introduced, but among them three main features can be distinguished. One is based on the formal description of the ion-pair formation reaction taking place between the ion-pairing reagent adsorbed on the stationary phase and the solute ion in the solution. This was treated in detail by Melander *et al.*¹

The second type is concerned with the more rigorous description of the equilibria taking place between the two phases, and considering also the limited amounts and the different binding abilities of the (polar and non-polar) binding sites of the stationary phase²⁻⁶. The third type model is different to the first two, and was introduced by Stahlberg and co-workers^{7,8}. In this model the electrostatic equation of Gouy and Chapman was used to describe the interaction between the ions present in the two phases.

In this paper we describe the calculations and considerations made in order to come closer to the solution of the problems that arise on changing columns in a given chromatographic separation.

EXPERIMENTAL

The experiments were carried out with a chromatographic system consisting of eluent reservoirs, Model 6000 A pumps (Waters Assoc.), Model 7010 six-port injection valves (Rheodyne), thermostated columns⁹ and a Type LC-55 UV-VIS spectrophotometric detector (Perkin-Elmer).

Two octadecylsilica columns were used: ODS-Hypersil (Shandon-Southern), 150 mm × 4.6 mm I.D., film thickness 5 μm; 1.52 g; C = 8.8%; $S_{\text{BET}} = 173 \text{ m}^2/\text{g}$; $V_0 = 1.8 \text{ ml}$; density $\rho = 0.817$; and ODS-Supelcosil (Supelco), 120 mm × 4.6 mm I.D., film thickness 5 μm; 1.3 g; C = 9.97%; $S_{\text{BET}} = 155 \text{ m}^2/\text{g}$; $V_0 = 1.5 \text{ ml}$; density $\rho = 0.838$. The Supelcosil column was new whereas the Hypersil column was well used.

The aqueous mobile phase contained phosphate buffer ($C_{\text{PO}_4} = 0.02 \text{ M}$) of various pH (2.5–6.6) and sodium bromide to keep the sodium ion concentration at $C_{\text{Na}} = 0.08 \text{ M}$. Octylsulphonic acid was used as the ion-pair-forming agent.

To ensure similar conditions for the stationary phases [similar coverage by the ion-pairing reagent (IP)], the concentrations in the mobile (C_{IP}) and stationary phases (\bar{C}_{IP}) were as follows: for the Hypersil column $C_{\text{IP}} = 0.005 \text{ M}$ and $\bar{C}_{\text{IP}} = 0.078 \text{ mmol/g}$ and for the Supelcosil column $C_{\text{IP}} = 0.0025 \text{ M}$ and $\bar{C}_{\text{IP}} = 0.088 \text{ mmol/g}$. Changes in these concentrations in the pH range investigated were not significant.

The solutes investigated and the logarithm of the protonation constants were as follows: *m*-aminophenol (MAP), $\log K = 4.25$; *o*-aminophenol (OAP), $\log K = 4.8$; and dopamine [DOP; 2-(3,4-dihydroxyphenyl)ethylamine], $\log K = 8.9$.

The capacity factors of the solutes were determined on both columns at different pH values, in the absence and presence of the ion-pairing reagent. The values obtained are presented in Figs. 1 and 2. The estimated capacity factors for the unprotonated and protonated bases are given in Table I.

The non-polar, hydrophobic capacity (q^0) was calculated from the carbon contents and surface areas of the stationary phases using the method of Berendsen *et al.*¹⁰. The polar capacity (q^x) was determined by breakthrough experiments, using 10^{-3} M hydroxide solution after acid treatment (with nitric acid) and washing of the column. The values found were as follows: Hypersil, $q^0 = 360$ and $q^x = 350 \text{ μmol/g}$, and Supelcosil, $q^0 = 510$ and $q^x = 190 \text{ μmol/g}$. Because the Hypersil column was well used, 300 μmol/g was used instead of the calculated 360 μmol/g in further calculations.

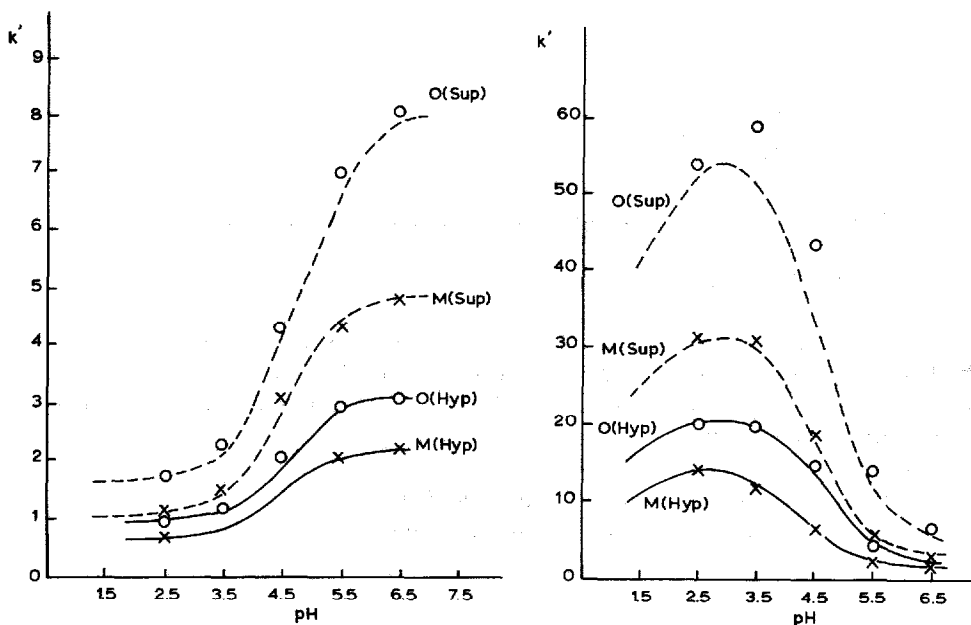


Fig. 1. Capacity factors of *o*-aminophenol (O) and *m*-aminophenol (M) at various pH values of the mobile phase using Hypersil and Supelcosil columns. The symbols (O and X) denote the values found experimentally and the full and dashed lines are the calculated values. $\log K_M = 4.5$; $\log K_O = 4.7$; $\log K_{IP} = 1.0$; $C_{IP} = 0.0\text{ M}$.

Fig. 2. Capacity factors of *o*-aminophenol (O) and *m*-aminophenol (M) at various pH values in the presence of octylsulphonate ion-pair forming ion, using Hypersil and Supelcosil columns. Symbols and lines as in Fig. 1. $C_{IP} = 0.005\text{ M}$ for the Hypersil column and 0.0025 M for the Supelcosil column.

The phase ratios used in the calculations were

$$\beta_{Hyp}^0 = \frac{0.300}{1.8/1.52} = 0.25$$

$$\beta_{Hyp}^x = \frac{0.350}{1.8/1.52} = 0.30$$

TABLE I
CAPACITY FACTORS OF UNPROTONATED AND PROTONATED BASES

Capacity factor	In absence of IP		In presence of IP	
	Hypersil	Supelcosil	Hypersil	Supelcosil
$k'_{B(MAP)}$	2.14	4.68	1.58	2.82
$k'_{BH(MAP)}$	0.65	1.0	14.0	36.0
$k'_{B(OAP)}$	3.1	8.0	2.0	5.0
$k'_{BH(OAP)}$	0.9	1.5	21	66
$k'_{B(DOP)}$	1.5	4.6	19	62

$$\beta_{\text{Sup}}^0 = \frac{0.510}{1.5/1.3} = 0.43$$

$$\beta_{\text{Sup}}^x = \frac{0.190}{1.5/1.3} = 0.17$$

where 0 and x refer to the hydrophobic and polar phase ratio, respectively.

RESULTS AND DISCUSSION

As can be seen from the data in Table I, the capacity factors for the all species are higher for the Supelcosil than for the Hypersil column. The difference is greater for the more hydrophobic species (MAP < OAP < DOP) both in the absence and presence of the ion-pairing agent.

Adsorption of the solutes in the absence of the ion-pairing reagent

The distribution ratios were calculated using the equation

$$d = k'/\beta \quad (1)$$

where k' is the capacity factor. For the non-protonated bases, $d_{\text{B(MAP)}} = 8.56$ for Hypersil and 10.88 for Supelcosil and $d_{\text{B(OAP)}} = 12.4$ for Hypersil and 18.60 for Supelcosil.

Assuming the following reaction^{3,6}:



where A is the adsorption site on the stationary phase

$$K_{\text{B}} = \frac{(\text{B})}{[\text{B}]q^0} \quad (2b)$$

where parentheses represent the concentration in the stationary phase and square brackets that in the mobile phase. We did not obtain identical K_{B} values for the two columns: $K_{\text{B(MAP)}} = 28.5$ for Hypersil and 21.8 for Supelcosil and $K_{\text{B(OAP)}} = 41.3$ for Hypersil and 37.2 for Supelcosil. Hence the adsorption strength is not linearly dependent on the hydrophobic capacity.

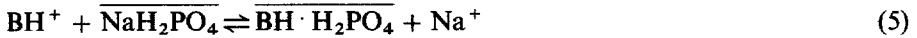
To establish whether the protonated base (cations) are bound by the polar or by the hydrophobic area of the column, indirect calculations were used to obtain the polar and non-polar contributions:

$$k'_{\text{BH(Hyp)}} = a_{\text{BH}}^x \beta_{\text{Hyp}}^x + a_{\text{BH}}^0 \beta_{\text{Hyp}}^0 \quad (3)$$

$$k'_{\text{BH(Sup)}} = a_{\text{BH}}^x \beta_{\text{Sup}}^x + a_{\text{BH}}^0 \beta_{\text{Sup}}^0 \quad (4)$$

As $\beta_{\text{Hyp}}^x > \beta_{\text{Sup}}^x$ and $\beta_{\text{Hyp}}^0 < \beta_{\text{Sup}}^0$, it was expected that the d_{BH}^x and d_{BH}^0 values found would give an indication of the sorption mechanism.

It was found in all instances that the polar contribution was almost zero compared with the hydrophobic contribution. Hence we suggest that the absorption of the protonated base takes place in the form of the ion pair formed with phosphate ions, $(\text{BH} \cdot \text{H}_2\text{PO}_4)$, *i.e.*,



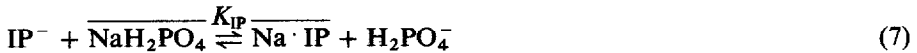
where the bars indicate compounds present in the stationary phase.

$$K_{\text{BH}} = \frac{(\text{BH})[\text{Na}^+]}{[\text{BH}^+]q^0} = d_{\text{BH}} \cdot \frac{[\text{Na}^+]}{q^0} \quad (6)$$

The calculated K_{BH} values for the two bases and for the two columns were as follows: $K_{\text{BH(MAP)}} = 0.69$ for Hypersil and 0.37 for Supelcosil and $K_{\text{BH(OAP)}} = 0.96$ for Hypersil and 0.55 for Supelcosil. The K_{BH} values obtained were different for the different phases. Hence we conclude that the adsorption strength does not depend linearly on q^0 .

Adsorption of the solutes in the presence of the ion-pairing reagent

The adsorption of the ion-pairing reagent may take place according to the following equation:



$$K_{\text{IP}} = \frac{(\text{IP})[\text{H}_2\text{PO}_4^-]}{[\text{IP}^-]q^0} \quad (8)$$

for the two columns the calculated values were found to be

$$K_{\text{IP(H)}} = (0.078 \cdot 0.02)/(0.005 \cdot 0.3) = 1.04 \text{ and } K_{\text{IP(S)}} = (0.088 \cdot 0.02)/(0.0025 \cdot 0.5) = 1.41. \text{ Hence the extent of adsorption cannot be described simply by eqn. 7.}$$

The adsorption of the non-protonated base in the presence of ion-pair forming species was always lower than that in its absence. It is true that the hydrophobic surface is partly occupied by the ion-pairing reagent, but a considerable part of the surface is still free. Therefore, the assumption of the displacement of ion-pairing ions by the solutes is not necessary.

However, we may attribute this lowering effect of the adsorbed ion-pairing reagent to the decreased hydrophobic capacity if we assume that the adsorption strength of the base depends on the accessible surface area not linearly but logarithmically, *i.e.*,

$$\log k'_B = \gamma q^0 + \log k_0 \quad (9)$$

As we know the k'_B values of the compounds in the absence of the ion-pairing reagent for Hypersil ($q = 0.3$) and for Supelcosil ($q = 0.51$), γ and k_0 can be calculated and also the $\log k'_B$ values corresponding to the actual capacities, which were left free in presence of the ion-pairing reagent (in this instance it was assumed that with the ion-pairing reagent one alkyl group was occupied). γ is characteristic for a given compound.

Calculated γ and k'_B values in the presence of the ion-pairing reagent were found as follows: for MAP, $\gamma_M = 1.7$ and $\log k_0 = -0.18$, for $q^0 = 0.3-0.078$ $k'_{B(IP)} = 1.57$ (Hypersil) and for $q^0 = 0.5-0.088$, $k'_{B(IP)} = 3.3$ (Supelcosil); and for OAP, $\gamma_0 = 2.05$ and $\log k_0 = -0.125$, for $q^0 = 0.3-0.078$, $k'_{B(IP)} = 2.13$ (Hypersil) and for $q^0 = 0.5-0.088$, $k'_{B(IP)} = 5.2$ (Supelcosil). The experimental values for k'_B are 1.58 and 2.82, respectively, for MAP, and 2.0 and 5.0, respectively, for OAP (see Table I).

The protonated base, having a positive loading, *i.e.*, being a cation, forms a hydrophobic ion-pair compound with the octylsulphonate ion (ion-pairing reagent) and therefore the capacity factor will be high.

According to the classical theories, used widely in the literature¹, an ion-exchange process takes place between the cations (sodium) of the ion-pairing reagent ion adsorbed on the stationary phase and the protonated base cation. Thus,



At equilibrium:

$$K_x = \frac{(\text{BH})[\text{Na}^+]}{[\text{BH}^+](\text{IP})} = d_{\text{BH}} \frac{[\text{Na}^+]}{(\text{IP})} \quad (11)$$

As the total amount of the ion-pair forming reagent is much higher than that of the protonated base cation, the ion-exchange equilibrium ratio, denoted by K_x , may be assumed to be constant. If we take the known concentrations in the experiments carried out with the two different columns, the calculated K_x values, however, were not found to be identical: MAP, 57.4 and 76.1; OAP, 86.1 and 139.5; and DOP, 78 and 131 for Hypersil and Supelcosil, respectively. If the ion-exchange mechanism were to be valid, the value of K_x would have been independent of the column used.

Reasonable values for the description of the retention of the protonated base cations can be obtained if the electrostatic theory and the equations introduced by Stahlberg and coworkers^{7,8} is used. According to the theory, the capacity factor is expressed by the following equation:

$$k'_{\text{BH}} = \beta^0 \exp\left(-\frac{\Delta G_{\text{AH}}^0 - z_{\text{BH}} F \Delta \psi}{RT}\right) \quad (12)$$

where ΔG_{AH}^0 is the chemical energy term, $-z_{\text{BH}} F \Delta \psi$ is the electrostatic energy term, z is the charge of the ion adsorbed (+1 for BH^+), F is the Faraday constant, R the gas constant, T temperature and ψ the electrostatic potential.

The ratio of the capacity factors in the presence and absence of the ion-pairing reagent (which is responsible for the electrostatic potential at the stationary phase) is simple:

$$\frac{k'_{\text{BH(IP)}}}{k'_{\text{BH(O)}}} = \exp\left(-z_{\text{BH}}\Delta\psi \cdot \frac{F}{RT}\right) \quad (13)$$

Taking the capacity factors found experimentally and using eqn. 13, the following values were found for $-z_{\text{BH}}\Delta\psi_0 F/RT (= X)$: for Hypersil [(IP) = 0.078], MAP 3.07, OAP 3.15 and DOP 2.54; and for Supelcosil [(IP) = 0.088], MAP 3.58, OAP 3.78 and DOP 2.60. The values obtained for MAP and OAP are similar, but those for DOP are different, as the structure and size of the ion are different. The ratios of the X values found for the two columns, $3.58/3.07 = 1.17$, $3.78/3.15 = 1.20$ and $2.60/2.54 = 1.02$, nearly correspond to the ratio of the concentrations of the ion-pair forming reagent (octylsulphonate) on the two stationary phases: $0.088/0.078 = 1.13$. The concentration of the ion-pairing reagent on the stationary phase under the given conditions is directly responsible for the electrostatic interaction.

CONCLUSIONS

In the absence of the ion-pair forming reagent the retention of the non-protonated and protonated base depends on the hydrophobic capacity of the column. The relationship between k' and q^0 is not linear but logarithmic.

The retention of the non-protonated base is lower in the presence of the ion-pair forming reagent than in its absence, and the difference ($\Delta \log k'$) corresponds to the surface capacity occupied by the ion-pairing reagent. No competition between the base and the ion-pairing reagent is considered.

The increased retention of the protonated base cations caused by ion-pair formation cannot be described with ion exchange, but can be described using the electrostatic theory, considering the concentration of the ion-pairing reagent on the stationary phase.

For the calculation of capacity factors from one column to another, if the experimental conditions are the same, the following equations can be proposed:

For the unprotonated base:

$$\log k'_2 - \log k'_1 = \gamma(q_2^0 - q_1^0) \quad (14)$$

$$\log k'_{(O)} - \log k'_{(IP)} = \gamma(\text{IP}) \quad (15)$$

where γ is characteristic of the solute and k_2 , q_2^0 and k_1 , q_1 refer to two columns of similar size and stationary phase;

For the protonated base:

$$\log k'_{\text{BH(IP)}} - \log k'_{\text{BH(O)}} = X' \quad (16)$$

$$\log k'_2 - \log k'_1 = X' \cdot \frac{(\text{IP})_2}{(\text{IP})_1} \quad (17)$$

where $X' = 0.43 X$

If we know k'_B and k'_{BH} , the pH dependence of the k' values in absence of the ion-pairing reagent can be calculated in the usual way¹:

$$k'_{B/BH} = k'_B \varphi_B + k'_{BH} \varphi_{BH} \quad (18)$$

In the presence of the ion-pairing reagent, the equation is similar but the protonation of the ion-pairing reagent is also considered, *i.e.*,

$$k'_{B/BH(IP)} = k_B \varphi_B + k_{BH} \varphi_{BH} \varphi_{IP} \quad (19)$$

The φ values are the molar fractions of the corresponding species:

$$\varphi_B = \frac{1}{1 + [H^+]K_B}; \quad \varphi_{BH} = \frac{[H^+]K_B}{1 + [H^+]K_B} \quad (20)$$

$$\varphi_{IP} = \frac{1}{1 + [H^+]K_{IP}} \quad (21)$$

where K_B and K_{IP} are the protonation constants of the base and of the ion-pairing ion, respectively.

The calculated and experimentally found values are shown in Figs. 1 and 2. As can be seen, the values are in good agreement with each other.

It must be stressed that the all above considerations and conclusions are valid only for the simple systems investigated and one must be very cautious in generalizing the results obtained in the calculation of capacity factors of solutes that have larger molecules or more complicated structures (with more polar groups, etc.).

SYMBOLS

A	adsorption site on the stationary phase
B, BH ⁺	non-protonated and protonated base species, respectively
[B], [BH ⁺], [IP]	concentrations of the species B, BH ⁺ and IP in the mobile phase
(B), (BH), (IP)	concentrations of the species B, BH ⁺ and IP in the stationary phase
C	carbon content of the column packing material (%)
C _{IP}	concentration of the ion-pair forming reagent in the eluent (mol/l)
\bar{C}_{IP}	concentration of the ion-pair forming reagent in the stationary phase (mmol/g)
<i>d</i>	distribution ratio (the subscript refers to the species)
DAP	dopamine
<i>F</i>	Faraday constant
ΔG_{BH}^0	free energy change of the adsorption of the species BH ⁺
Hyp	Hypersil
IP	ion-pair forming reagent
k'	capacity factor (the subscript refers to the species, the number to the columns)
k_0	parameter of the logarithmic eqn. 9
<i>K</i>	concentration equilibrium "constant" (the subscript refers to the reaction)

MAP	<i>m</i> -aminophenol
OAP	<i>o</i> -aminophenol
q^0	hydrophobic capacity of the stationary phase ($\mu\text{mol/g}$)
q^*	polar capacity of the stationary phase ($\mu\text{mol/g}$)
R	gas constant
S_{BET}	specific surface area of the column packing material obtained by the BET method (m^2/g)
Sup	Supelcosil
T	temperature (K)
V_0	dead volume of the column (ml)
z_{BH}	charge number of the species BH^+
X	exponent term of eqn. 13
β^0	phase ratio (hydrophobic)
β^*	phase ratio (polar)
γ	parameter of the logarithmic eqn. 9 (the subscript refers to the species)
ρ	density of the column packing (g/ml)
φ	molar fraction of a species; the subscript refers to the species
$\Delta\psi$	electrostatic surface potential change at the adsorption

REFERENCES

- 1 W. R. Melander, Cs. Horváth and I. Molnár, *Anal. Chem.*, 49 (1977) 42, 2295.
- 2 A. Tilly-Melin, Y. Askemark, K. G. Wahlund and G. Schill, *Anal. Chem.*, 59 (1979) 976.
- 3 A. Tilly-Melin, M. Ljungcrantz and G. Schill, *J. Chromatogr.*, 185 (1979) 225.
- 4 F. Riedo and E. Kováts, *J. Chromatogr.*, 239 (1982) 1.
- 5 J. Crommen, G. Schill, L. Hackzell and D. Westerlund, *Chromatographia*, 24 (1987) 252.
- 6 E. Arvidson, J. Crommen, G. Schill and D. Westerlund, *Chromatographia*, 24 (1987) 460.
- 7 J. Stahlberg, *J. Chromatogr.*, 356 (1986) 231.
- 8 J. Stahlberg and A. Furangen, *Chromatographia*, 24 (1987) 783.
- 9 A. Bartha and Gy. Vigh, *J. Chromatogr.*, 260 (1983) 337.
- 10 G. E. Berendsen, K. A. Pickaart and L. De Galan, *J. Liq. Chromatogr.*, 3 (1980) 1437.