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Extension of the electrostatic retention model of reversed-phase ion-pair high-performance liquid chromatography to include the effect of the eluent pH

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ABSTRACT

The electrostatic retention model is extended to predict the retention changes of monoprotic weak acids and bases as the eluent pH and the mobile phase concentration of the ion-pairing reagent are varied simultaneously in reversed-phase high-performance liquid chromatography. At constant ionic strength and organic modifier concentration, the magnitude of solute retention shifts can be predicted from the model equations using a very limited set of experimental data. The effect of the eluent pH on the adsorption of the sodium octylsulphonate pairing ion and the surface potential was studied. Predictions from the model were compared with experimental retention data and good agreement was found for both oppositely and similarly charged solute ion – pairing ion combinations. The advantages and limitations of applying the electrostatic retention model in the optimization of ion-pair chromatographic separations are discussed in detail.

INTRODUCTION

In reversed-phase high-performance liquid chromatography (RP-HPLC) the variation of eluent pH and/or the concentration of the organic modifier often leads to satisfactory separations of mixtures of weak organic acids and/or bases. However, when these components are accompanied by solutes of other charge type (strong acids, bases or non-ionic components) and relative hydrophobicity, one may need to add ion-pairing reagents to the eluent in order to achieve the proper separation of all components of interest.

Owing to the increased number and interdependence of mobile phase variables, the selectivity optimization of such ion-pair chromatographic (IPC) separations can become a complex task. The successful optimization of the separations precludes the correct selection of the initial value(s) and range of eluent variables (*i.e.*, the optimization parameter space) [1–4] where the search for the optimum conditions will be performed. In contrast to regular RPC systems, this is not a trivial task in ion-pair

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chromatography and it often requires a large number of preliminary experiments. The number of the initial chromatographic runs could be reduced if the solute retention shifts, caused by the addition of pairing ions, could be predicted.

A number of retention models [5–8] have been suggested in RP-IPC to describe the capacity factor (k') of ionizable solutes when an ion-pairing reagent is added to the eluent at various pH values. However, in order to elucidate the retention-modifying effect of the pairing ion, the dynamic ion-exchange [5] constants, the ion-pair formation or adsorption constants [6] for each solute ion or the parameters of the assumed Freundlich-type pairing ion adsorption [7] must determined. As a result, these models require a large amount of experimental data determined under widely varying chromatographic conditions to predict solute retention changes.

Stahlberg and co-workers developed an electrostatic theory for reversed-phase ion-pair chromatography [9–11] which has been successfully used to account for the effect of the type and concentration of the inorganic counter ions [12] and the organic modifier(s) [13]. The electrostatic retention model gives a physically consistent description of the retention processes and it allows practical equations to be derived to determine the retentions of fully ionized solutes (*i.e.*, strong acids and bases) from very few experimental data [14].

In this study, the electrostatic model was used to describe the retention of monocharged weak acids and bases as the eluent pH and the concentration of the ion-pairing reagent are varied simultaneously. A set of equations was derived to predict the direction and magnitude of retention changes for both oppositely and similarly charged solute ion-pairing ion combinations. Predictions from the theory are compared with experimental retention data and it is shown that the equations can be used to predict the solute retention shifts (assuming that the charge type of the solutes is known) from a very limited set of experimental data.

THEORY

In reversed-phase chromatography, the capacity factor of partly ionized solutes (k'_c) can be expressed as the sum of the capacity factors for the charged (k'_i) and the uncharged species (k'_0), multiplied by the corresponding equilibrium fraction (f)

$$k'_c = (1 - f)k'_0 + fk'_i \tag{1}$$

The addition of an ion-pairing reagent to the eluent will primarily influence the retention of the ionized form of the solute. The basic assumption of the electrostatic retention model of RP-IPC [9–11] is that the adsorbing pairing ions and the counter ions form an electrical double layer at the stationary phase surface and create a difference in the electrostatic potential (ψ_0) between the stationary phase and the bulk mobile phase. According to the theory, the capacity factor of the fully ionized solute (k'_i) of charge z_i is given as

$$k'_i = k'_{0i} \exp(-z_i F \psi_0/RT) \tag{2}$$

where k'_{0i} is the capacity factor of the fully ionized form of the solute in the absence of pairing ion, F is the Faraday constant, ψ_0 is the difference in the electrostatic potential

between the surface of the stationary phase and the bulk mobile phase, R is the gas constant and T is the absolute temperature.

The fraction of ionized components (A^-) for monoprotic weak acids (HA) is defined as

$$f = (A^-)/\{(A^-) + (HA)\} \tag{3}$$

and it can be expressed using the acid dissociation constant (K_a) of the weak acid as

$$f = K_a/(K_a + [H^+]) \tag{4}$$

where $[H^+]$ is the hydrogen ion concentration. For monoprotic weak acids ($z_i = -1$), the substitution of eqns. 2 and 4 into eqn. 1 results in

$$k'_{acid} = \frac{k'_{HA} + K_a/[H^+]k'_{0A^-}\exp(F\psi_0/RT)}{1 + (K_a/[H^+])} \tag{5}$$

where k'_{HA} is the capacity factor of the non-ionized form of weak acid HA and k'_{0A^-} is the capacity factor of the ionized form in the absence of pairing ion.

Similar reasoning can be used to derive the corresponding equation for monoprotic ($z_i = +1$) weak bases (BH^+)

$$k'_{base} = \frac{k'_B + [H^+]/K_a k'_{0BH^+}\exp(-F\psi_0/RT)}{1 + ([H^+]/K_a)} \tag{6}$$

where k'_B is the capacity factor of the non-ionized form of weak base B and k'_{0BH^+} is the capacity factor of the ionized form in the absence of pairing ion.

In the absence of pairing ions, ψ_0 is defined to be zero, *i.e.*, the exponential term equals one. As a result, eqns. 5 and 6 reduce to the well known expressions developed for the regular reversed-phase chromatographic mode [15]. The above model assumes that the ion-pairing reagent is fully ionized throughout the pH range used (*i.e.*, $z_A = \pm 1$). The electrostatic potential has positive values ($\psi_0 > 0$) for positively charged and negative values ($\psi_0 < 0$) for negatively charged pairing ions. It is also assumed that the capacity factor of both the ionized (k'_{0i}) and the non-ionized form (k'_0) of the solute are constant and independent of the concentration of the pairing ion. The effect of the pairing ion on k'_0 is usually negligible compared with its influence on k'_{0i}, as a first approximation.

The organic modifier content and the ionic strength of the mobile phase influence the capacity factor and the protonation constant of the solutes, in addition to the electrostatic potential (if a pairing ion is present). To allow for a meaningful test of the model equations, the above two variables were kept constant throughout this study.

EXPERIMENTAL

All test compounds were of analytical-reagent grade and were obtained from different suppliers. Buffer components and sodium bromide were obtained from

Reanal (Budapest, Hungary) and sodium octylsulphonate from Merck (Darmstadt, Germany).

The eluents contained 10% (v/v) methanol, aqueous phosphate or citrate or acetate–malonate buffer and sodium bromide. Eluents with constant ionic strength (0.1 *M*) and varying pH values between 2.5 and 6.5 were prepared by titrating eluent A*i* (A1 = 20 m*M* H_3PO_4–80 m*M* NaBr; A2 = 20 m*M* citric acid–80 m*M* NaBr; A3 = 10 m*M* acetic acid–10 m*M* malonic acid–80 m*M* NaBr) with eluent B*i* (B1 = 10 m*M* NaH_2PO_4–10 m*M* Na_2HPO_4–50 m*M* NaBr; B2 = 20 m*M* trisodium citrate–20 m*M* NaBr; B3 = 10 m*M* sodium acetate–10 m*M* malonic acid–20 m*M* NaOH–50 m*M* NaBr). When 5 m*M* sodium octylsulphonate was used as ion-pairing reagent, the sodium bromide concentration was decreased by the same amount in each eluent.

An LC 5600 liquid chromatograph, UV (set to 254 nm) and RI detectors (all from Varian Aerograph, Walnut Creek, CA, U.S.A.) equipped with two Model 7010 injection valves (Rheodyne, Cotati, CA, U.S.A.) were used. The analytical column was packed with 5-μm Hypersil ODS (200 mm × 4.6 mm I.D.; Shandon, U.K.). The set-up of the equipment allowed for the simultaneous determination of solute capacity factors and pairing ion adsorption data [16]. The temperature was kept at 25°C. The eluent pH was measured with a glass electrode (Radelkis, Hungary) calibrated for aqueous standard solutions. Model calculations were performed on an IBM AT compatible computer (MS DOS 4.0, 1 MB RAM, 20 MB hard disk).

RESULTS AND DISCUSSION

Effect of eluent pH on surface potential

In order to test eqns. 5 and 6, one must determine not only solute capacity factors, but also the value of the difference in the electrical potential created by the presence of the pairing ion. In practice, eqn. 2 can be rearranged and used to calculate the actual ψ_0 values from the k' data for a fully ionized solute measured in the absence (k'_{0i}) and in the presence (k'_i) of the pairing ion of charge z_A

$$(z_iF)/(RT)\psi_0 = -\ln (k'_i/k'_{0i}) \quad (7)$$

The capacity factor data for the strong base dopamine ($z_i = +1$) were used for the calculation of ψ_0 in the pH range 2.5–6.5. The results are summarized in Table I. It can be seen that the resulting electrical potential is virtually independent of the variation of the eluent pH between 2.5 and 6.5.

The experimental adsorption data of the sodium octylsulphonate ion-pairing reagent are also included in Table I. The surface concentrations were determined from the breakthrough curves of the pairing ion, *i.e.*, from experiments independent of the capacity factor measurements used for the calculation of the ψ_0 data. The breakthrough curves were recorded at each pH value on the freshly regenerated column [16] prior to the measurements of k' data. The adsorption data show a slight decrease at higher pH values, which is just the opposite behaviour compared with the changes in k' values for the positively charged dopamine. This indicates a slightly increasing negative (repulsive) electrical charge on the surface of the silica-based stationary phase, which can be associated with dissociation of surface silanol groups. However, the relatively constant level of the pairing ion adsorption and the corresponding

TABLE I

ELUENT pH *VS.* CAPACITY FACTOR DATA FOR THE STRONG BASE DOPAMINE (pK = 8.80) IN THE ABSENCE (k'_{0i}) AND IN THE PRESENCE (k'_i) OF PAIRING ION, THE SURFACE POTENTIAL (ψ_0) CALCULATED FROM EQN. 7 AND THE ADSORPTION DATA (n_A) OF SODIUM OCTYLSULPHONATE CORRESPONDING TO 5 mM MOBILE PHASE CONCENTRATION

See Experimental for other conditions.

pH	k'_{0i}	k'_i	ψ_0 (mV)	n_A (μmol/g)
2.5	1.50	17.39	−62.8	78.6
3.5	1.56	18.45	−63.3	78.4
4.5	1.55	18.64	−63.8	78.5
5.5	1.65	19.34	−63.1	77.4
6.5	2.24	19.42	−55.4	73.1

surface potential suggests that the effect of the dissociation of the silanol groups is still negligible. At higher pH values the effect of dissociating silanol groups on the adsorption of positively charged ion-pairing reagents may become more significant [5,17,18].

The level of the surface concentration (78 μmol/g) of the sodium octylsulphonate and the resulting average surface potential (−62 mV) both fall in the region where the linearized retention equation of the electrostatic retention model (*i.e.*, eqn. 2) can be applied [19]. The capacity factor of the fully ionized dopamine increases by a factor of 11 when 5 mM pairing ion is added to the eluent. Practical chromatographic work is often performed under conditions similar to those used in this study, providing data for a realistic test of the suggested equations.

Comparison of experimental and theoretical retention behaviours of ionic solutes

The capacity factor (k') *vs.* eluent pH data for the positively charged ($z_i = +1$) octopamine and the negatively charged 6-hydroxynaphthalene-2-sulphonic acid ($z_i = -1$) are shown in Figs. 1 and 2. Data points measured in the absence and in the presence of 5 mM sodium octylsulphonate are represented by filled squares and circles, respectively.

The retention data of both solutes drift slightly with increasing eluent pH. However, as a first approximation the capacity factors in the absence of pairing ion (k'_{0i}) can be considered constant. Generally, with careful selection of the stationary phase and the buffer system, close to ideal retention behaviour can be obtained for strong acids and bases [3]. This idealized behaviour is represented by the dashed lines, which were placed as close as possible to the experimental points at an arbitrary level along the k' axis. For fully ionized solutes eqn. 2 is used (instead of eqn. 5 or 6) to calculate the change in capacity factors when a pairing ion is added to the eluent. The use of eqn. 2 requires a knowledge of k'_{0i} and the surface potential corresponding to the given pairing ion concentration. The results in Table I indicate that one can use an average value for ψ_0, independent of the eluent pH.

The solid lines in Figs. 1 and 2 represent k' data which were calculated from eqn. 2, using only the k'_{0i} value of the solutes (dashed line) and an average value

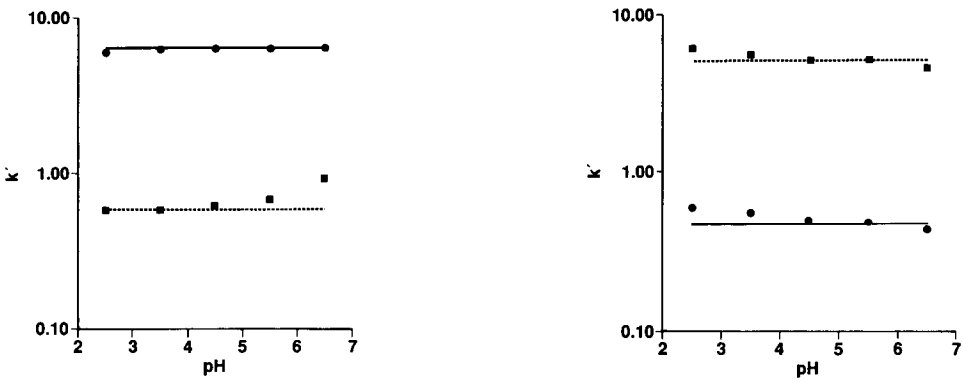

Fig. 1. k' *vs.* pH data for positively charged octopamine in (■) the absence and (●) the presence of sodium octylsulphonate (5 mM) pairing ion. The theoretical retention plot in the presence of the pairing ion (solid line) was calculated from eqn. 2 ($z_i = +1$, $F\psi_0/RT = -2.398$), assuming idealized retention behaviour (dashed line) in the absence of pairing ion, *i.e.*, constant $k'_{0i} = 0.59$. Column, Hypersil ODS; eluent 10% (v/v) methanol in 20 mM aqueous phosphate buffer containing sodium bromide (constant 0.1 M ionic strength); temperature, 25°C.

Fig. 2. k' *vs.* pH data for negatively charged 6-hydroxynaphthalene-2-sulphonic acid (6HN2SA) in (■) the absence and (●) the presence of sodium octylsulphonate (5 mM) pairing ion. The theoretical retention plot in the presence of the pairing ion (solid line) was calculated from eqn. 2 ($z_i = -1$, $F\psi_0/RT = -2.398$), assuming idealized retention behaviour (dashed line) in the absence of pairing ion, *i.e.*, constant $k'_{0i} = 5.2$. Other conditions as in Fig. 1.

(−62 mV) for the surface potential, determined from the k' data for dopamine (*cf.*, Table I). Compared with the size of the retention changes, remarkably good agreement is found between the experimental and calculated k' data both for increasing (Fig. 1) and decreasing (Fig. 2) solute retention.

The capacity factor *vs.* pH data for a weak base (adenine) and a weak acid (2,4-dihydroxybenzoic acid) are shown in Figs. 3 and 4. In the absence of a pairing ion, at pH 2.5, adenine is protonated (pK 4.12) and it has a lower retention (k'_{0i}) than its non-ionic form (k'_0) at pH 6.5. On the other hand, the retention of 2,4-dihydroxybenzoic acid is lower at high pH where the carboxyl group is fully dissociated (pK_a = 3.21). The experimental k' data for both solutes (filled squares) follow the S-shaped pattern well known in reversed-phase chromatography. The theoretical k' data (dotted lines) were calculated from eqns. 5 and 6 assuming a zero value for the surface potential. It is important to note that only two experimental k'_{0i}, k'_0 data measured at the two limiting pH values and the literature pK values of the solutes were used in the calculations, *i.e.*, no curve fitting was applied.

When the eluent contains 5 mM sodium octylsulphonate as a pairing ion, at low pH the k' (filled circles) of the positively charged adenine increases from 2.07 (k'_{0i}) to 20.4 (k'_{base}). This value is 2.5 times higher than the retention of the uncharged form ($k'_0 = 8.2$) of adenine. Owing to the increased negative surface potential in the presence of the pairing ion, the retention of 2,4-dihydroxybenzoic acid decreases dramatically and it elutes close to the column dead volume ($k'_{acid} = 0.05$) at high pH. The larger deviation between the predicted and experimental points at $k' < 0.5$ may be attributed to the error in measuring short retention times.

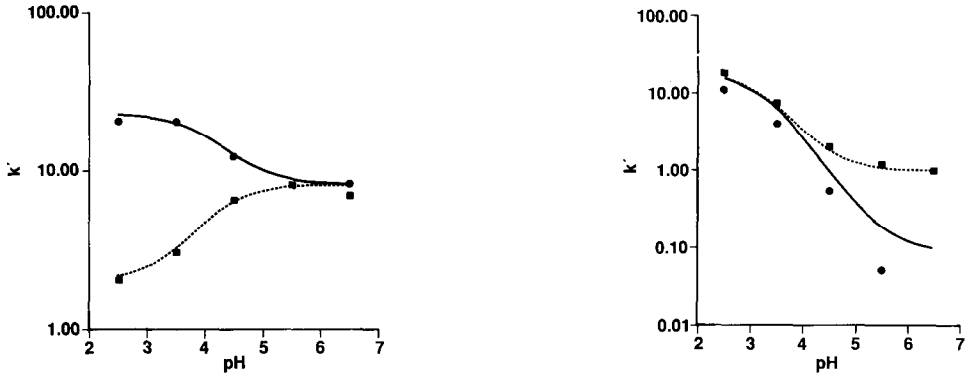

Fig. 3. k' *vs.* pH data for the weak base adenine ($z_i = +1$) in (■) the absence and (●) the presence of sodium octylsulphonate (5 mM) pairing ion. The theoretical retention plots in the absence (dashed line) of the pairing ion ($\psi_0 = 0$) and in the presence (solid line) of the pairing ion were calculated from eqn. 6 (pK = 4.12, k'_0 = 8.2, k'_{0i} = 2.07, $z_iF\psi_0/RT = -2.398$). Other conditions as in Fig. 1.

Fig. 4. k' *vs.* pH data for the weak acid 2,4-dihydroxybenzoic acid (2,4-DHBA) in (■) the absence and (●) the presence of sodium octylsulphonate (5 mM) pairing ion. The theoretical retention plots in the absence (dashed line) of the pairing ion ($\psi_0 = 0$) and in the presence (solid line) of the pairing ion were calculated from eqn. 5 ($z_i = -1$, pK = 3.2, k'_0 = 18.5, k'_{0i} = 6.85, $F\psi_0/RT = -2.398$). Other conditions as in Fig. 1.

The simultaneous effect of pairing ion addition and pH variation was calculated again from eqns. 5 and 6 (solid lines). The theoretical predictions use the pK_a, k'_0 and k'_{0i} values as described above; only the average value determined for the surface potential is introduced. None of the experimental k' data measured in the presence of the pairing ion (filled circles) were used for fitting the curves. It can be seen that the direction and magnitude of the retention changes caused by the pairing ion addition are predicted correctly by the theory.

In Fig. 5 the k' *vs.* pH data for *p*-aminophenol are shown in three different buffer

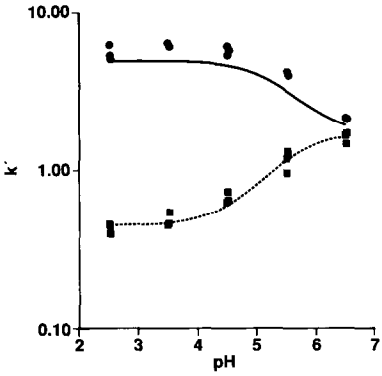

Fig. 5. k' *vs.* pH data for the weak base *p*-aminophenol ($z_i = +1$) in (■) the absence and (●) the presence of sodium octylsulphonate (5 mM) pairing ion. The theoretical retention plots in the absence (dashed line) of the pairing ion ($\psi_0 = 0$) and in the presence (solid line) of the pairing ion were calculated from eqn. 5 (pK = 5.38, k'_0 = 1.7, k'_{0i} = 0.45, $F\psi_0/RT = -2.398$). Eluent, 10% (v/v) methanol in aqueous 20 mM phosphate, citrate or acetate–malonate buffer, containing sodium bromide (constant 0.1 M ionic strength). Other conditions as in Fig. 1.

TABLE II

EXPERIMENTAL (k'_{exp}) AND THEORETICALLY PREDICTED (k'_{calc}) CAPACITY FACTORS OF FULLY IONIZED BASIC SOLUTES ($z_i = +1$) AT pH 2.5, IN THE PRESENCE OF 5 m*M* SODIUM OCTYLSULPHONATE ($F\psi_0/RT = -2.398$)

k'_{0i} is the capacity factor measured in the absence of pairing ion; basic pK values from ref. 21.

Solute	pK	k'_{0i}	k'_{exp}	k'_{calc}
Adenine	4.12	2.07	20.40	22.77
Cytidine	4.20	1.13	12.20	12.43
Creatinine	4.80	0.35	3.88	3.85
p-Aminophenol	5.38	0.45	6.14	4.95
Dopamine	8.80	1.61	17.38	17.71
Octopamine	9.60	0.58	5.89	6.49

systems (phosphate, citrate and acetate–malonate) with identical ionic strength (0.1 *M*). Again, theoretical k' data were calculated from eqn. 6, using the literature pK value, two experimental k'_{0i}, k'_0 data measured in the phosphate buffer system at pH 2.5 and 6.5 without pairing ion and assuming either zero (dotted line) or the average (−62 mV) value (solid line) for the electrical potential. The deviation between the predicted and the measured k' data is comparable to the relatively small differences caused by changing the type of buffer anions. This result supports the generality and practical applicability of the suggested model to other buffer systems.

The experimental and theoretically predicted k' data are compared for some other bases and acids in Tables II and III, respectively. The results are given for eluents with pH values where the compounds were ionized (see pK values) in order to test predictions for the possible largest retention changes with the addition of the pairing ion. In these calculations, only the charge type (z_i) and the capacity factor of the ionized solute (k'_{0i}) measured in the absence of a pairing ion and the average value of the surface potential were used (see eqn. 2). Again, there is generally an acceptable agreement between the experimental and the predicted k' data. When the initial (k'_{0i}) or the resulting (k'_{exp}) capacity factor values are lower than 1, the differences between the

TABLE III

EXPERIMENTAL (k'_{exp}) AND THEORETICALLY PREDICTED (k'_{calc}) CAPACITY FACTORS OF FULLY IONIZED ACIDIC SOLUTES ($z_i = -1$) AT pH 5.5, IN THE PRESENCE OF 5 m*M* SODIUM OCTYLSULPHONATE ($F\psi_0/RT = -2.398$)

k'_{0i} is the capacity factor measured in the absence of pairing ion; acidic pK_a values from ref. 22.

Solute	pK_a	k'_{0i}	k'_{exp}	k'_{calc}
6-Hydroxynaphthalene-2-sulphonic acid	<0.5[a]	5.19	0.49	0.47
p-Toluenesulphonic acid	0.67[a]	3.24	0.20	0.29
2,6-Dihydroxybenzoic acid	1.22	6.85	0.49	0.62
2,4-Dihydroxybenzoic acid	3.21	1.20	0.05	0.18
3,4-Dihydroxyphenylacetic acid	4.3	0.84	0.18	0.08

[a] Approximate values.

experimental and calculated values are large. For k' values larger than 1, the deviations are in the range 10–20% relative.

The above results indicate that the electrostatic retention model can be advantageously used to estimate the magnitude of retention shifts for ionic solutes, when a pairing ion is added to the eluent at various pH values, from a very limited set of experimental data. In order to approximate the k' *vs.* pH behaviour of monocharged (monoprotic) solutes in the absence of pairing ion (*cf.*, dashed lines in Figs. 1–5), the charge type of the components, pK_a, k'_0 and/or k'_{0i} values must be known. The addition of a pairing ion to the eluent often results in significantly enhanced or decreased analysis times. Therefore, the assessment of these retention shifts is of practical importance. In order to estimate the magnitude of k' changes, one must determine the value of the difference in the electrical potential. This requires only the measurement of k' of one or two fully ionized solutes in eluents of a given pH without and with ion-pairing reagent. In other words, the IPC system can be calibrated for surface potential from the retention data for a well known standard.

As a result, measurements in three eluents already provide starting point for the model to estimate the possible retention shifts. The advantage of the model is that after calibrating for the surface potential, new k' data of ionized solutes can be guessed before actually measuring the sample mixture in the presence of a pairing ion. This feature is very useful during the selection of the type and initial concentration of the pairing ions [20].

An important area of such applications is in the selection of initial mobile phase compositions prior to systematic selectivity optimization [2–4]. In this early stage of the chromatographic method development, the primary aim is to obtain chromatograms with reasonable analysis times and errors of 10–20% in predicted retention times are usually acceptable.

Retention models are often sought also in the course of the subsequent selectivity optimization. Some computer-aided procedures [8,23–26] fit multi-parameter equations through the experimental retention data points and calculate different optimization criteria as a function of the mobile phase variables. For the efficient operation of these methods the solute capacity factors must be estimated to within less than 1–2% for a broad range of experimental conditions [27]. It must be noted that neither the earlier retention models [5–8] nor the electrostatic model in its present form can comply with these requirements.

CONCLUSIONS

Based on the electrostatic theory of reversed-phase ion-pair chromatography, a set of equations have been developed to describe the retention changes of monoprotic weak acids and bases when an ion-pairing reagent is added to eluents with different pH values. According to the model, the retention of the ionized form of the solutes depends on the surface potential. Hence the surface potential can be determined from the capacity factor data for a fully ionized solute, measured in the absence and the presence of a pairing ion. Both the surface potential and the adsorption of the sodium octylsulphonate pairing ion were found to be constant when the eluent pH was varied between 2.5 and 6.5 at constant ionic strength and organic modifier concentration.

The retention equations have been tested by calculating the expected retention

behaviours of strong and weak acids and bases in the presence of a pairing ion, using only literature pK values, capacity factor data for the solutes (measured in the absence of the pairing ion) and the surface potential (determined from the retention shift of one fully ionized compound). The predictions from the model agreed well with experimental retention data for both oppositely and similarly charged solute ion–pairing ion combinations, and also for eluents with different buffer anions. The calculated and measured capacity factor data differed by 10–20% relative when the results were not affected by the experimental errors of measuring short retention times. The practical advantage of the proposed equations is that they allow one to estimate the magnitude of solute retention changes from a very limited amount of experimental data.

REFERENCES

1 P. J. Schoenmakers and M. Mulholland, *Chromatographia*, 25 (1988) 737.
2 Á. Bartha, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 464 (1989) 225.
3 G. K. C. Low, Á. Bartha, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 478 (1989) 21.
4 J. K. Strasters, F. Coolsaet, Á. Bartha, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 499 (1990) 523.
5 J. L. M. van de Venne, J. L. H. Hendrikx and R. S. Deelder, *J. Chromatogr.*, 167 (1978) 1.
6 A. Tilly-Melin, Y. Askemark, K.-G. Wahlund and G. Schill, *Anal. Chem.*, 51 (1979) 976.
7 R. C. Kong, B. Sachok and S. N. Deming, *J. Chromatogr.*, 199 (1980) 307.
8 A. P. Goldberg, E. Nowakowska, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 316 (1984) 241.
9 J. Ståhlberg, *J. Chromatogr.*, 356 (1986) 231.
10 J. Ståhlberg and A. Furangen, *Chromatographia*, 24 (1987) 783.
11 J. Ståhlberg, *Chromatographia*, 24 (1987) 820.
12 J. Ståhlberg and I. Hagglund, *Anal. Chem.*, 60 (1988) 1958.
13 Á. Bartha, Gy. Vigh and J. Ståhlberg, *J. Chromatogr.*, 506 (1990) 85.
14 Á. Bartha and J. Ståhlberg, *J. Chromatogr.*, 535 (1990) 181.
15 Cs. Horváth, W. Melander and I. Molnar, *Anal. Chem.*, 49 (1977) 142.
16 Á. Bartha and Gy. Vigh, *J. Chromatogr.*, 260 (1983) 337.
17 R. H. A. Sorel, A. Hulshoff and S. Wiersema, *J. Liq. Chromatogr.*, 4 (1981) 1961.
18 O. A. G. J. van der Houwen, R. H. Sorel, A. Hulshoff, J. Teeuwsen and A. W. M. Indemans, *J. Chromatogr.*, 393 (1981) 209.
19 J. Ståhlberg and Á. Bartha, *J. Chromatogr.*, 456 (1988) 253.
20 Á. Bartha, Gy. Vigh and Z. Varga-Puchony, *J. Chromatogr.*, 499 (1990) 423.
21 D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solutions*, Butterworth, London, 1965.
22 G. Kortum, W. Vogel and K. Andrussow, *Dissociation Constants of Organic Acids in Aqueous Solution*, Butterworth, London, 1961.
23 B. Sachok, R. C. Kong and S. N. Deming, *J. Chromatogr.*, 199 (1980) 317.
24 P. M. J. Coenegracht, N. V. Tuyen, H. J. Metting and P. M. J. Coenegracht-Lamers, *J. Chromatogr.*, 389 (1987) 351.
25 P. Wester, J. Gottfries, K. Johansson, F. Klinteback and B. Winblad, *J. Chromatogr.*, 415 (1987) 261.
26 F. Szokoli, Zs. Nemeth and J. Inczedy, *Chromatographia*, 29 (1990) 265.
27 P. J. Schoenmakers and T. Blaffert, *J. Chromatogr.*, 384 (1987) 117.