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Retention prediction based on the electrostatic model of reversed-phase ion-pair high-performance liquid chromatography: effect of pairing ion concentration

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ABSTRACT

Based on the electrostatic model of reversed-phase ion-pair chromatography, a practical equation was developed to describe the relationship between the capacity factor of ionic solutes and the eluent concentration of the amphiphilic pairing ion. At constant ionic strength and organic modifier concentration, the logarithm of solute capacity factor can be approximated as a linear function of the logarithm of the pairing ion concentration. The slope of this relationship is determined by the sign and number of charges of the solute ion and the pairing ion. The limitations and the practical use of the proposed model are discussed in detail.

INTRODUCTION

Reversed-phase ion-pair liquid chromatography (RP-IPC) is a well established method for the separation of ionic organic compounds. One of the main chromatographic variables for controlling the retention of ionic solutes is the mobile phase concentration of the amphiphilic ion-pairing reagent. When the pairing ion concentration increases, the retention of the ionic solutes decreases for charges of identical sign and increases for charges of opposite sign.

In a number of studies [1–7], when the capacity factor of the solute ion (k'_{cB}) was plotted against the mobile phase concentration of the pairing ion (c_A) on a logarithmic scale, an approximately linear relationship was obtained. The linearity of this relationship allowed Billiet and co-workers [5,6] to apply an efficient iterative optimization strategy to the selectivity optimization of ion-pair liquid chromatographic separations.

In fact, a linear log k'_{cB} *vs.* log c_A relationship can be derived from some of the retention models suggested for ion-pair chromatography. However, these models disagree on the slope of this log–log relationship.

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Schoenmakers [8] has shown that in an ideal case the ion-pair partition model predicts a slope of unity. Van de Venne *et al.* [3] assumed a Freundlich-type adsorption isotherm for the pairing ion together with a dynamic ion-exchange mechanism. According to their retention equation [3], the slope of the log k'_{cB} *vs.* log c_A relationship is identical with the slope of the Freundlich isotherm of the pairing ion at constant ionic strength.

The analysis of experimental IPC data from different studies indicated that the slope of the log k'_{cB} *vs.* log c_A relationship is usually less than unity [3,5,7], is less sensitive to variations in the adsorption properties of the pairing ions [9,10] and is more sensitive to the sign and number of charges carried by the respective solute ions [4]. Ståhlberg [11] developed an electrostatic retention model for RP-IPC. Since its introduction, the electrostatic theory has been thoroughly tested [12,13] and extended to account for the eluent concentration of the electrolyte [14,15] and the organic modifiers [15,16].

In this paper, we discuss the relationship between ionic solute retention and pairing ion concentration on the basis of the electrostatic retention model. A general retention equation is derived to approximate the retention of ionic solutes when they have either single or multiple charges with similar or opposite sign as compared with that of a monocharged pairing ion. Theoretical predictions are compared with experimental retention data for differently charged analytes measured in the presence of either positively (tetrabutylammonium) or negatively charged (octylsulphate) pairing ions.

THEORY

The basic assumption of the electrostatic theory [11–13] is that the adsorbed amphiphilic pairing ions and the counter ions of the buffer form an electrical double layer at the interface between the stationary and mobile phase, creating an electrostatic surface potential. This surface potential will influence both the adsorption isotherm of the pairing ion and the retention of the ionic solutes. Owing to this complicated interrelationship [13] (adsorption–surface potential–retention), it is not possible to develop a closed-form expression describing the solute capacity factor (k'_{cB}) as a function of the eluent concentration of the pairing ion (c_A).

However, an approximate expression can be obtained by combining the surface potential-modified Langmuir adsorption isotherm of the pairing ion [13–15] and the linear form of the Gouy–Chapman equation [13]. Assuming non-zero pairing ion concentrations ($c_A > 0$) and using a series expansion, separate expressions can be obtained for the oppositely and for the similarly charged ionic solutes [16]. When there is a unit charge of opposite sign on both the solute ion and the pairing ion,

$$\log k'_{cB} = \log k'_{oB} + 1/2 \log c_A + K_1 - K_0 \tag{1}$$

and when there is a unit charge of identical sign on both the solute ion and the pairing ion,

$$\log k'_{cB} = \log k'_{oB} - 1/2 \log c_A - K_1 - K_0 \tag{2}$$

where k'_{cB} is the capacity factor of solute ion B in the presence of the pairing ion, k'_{oB} is

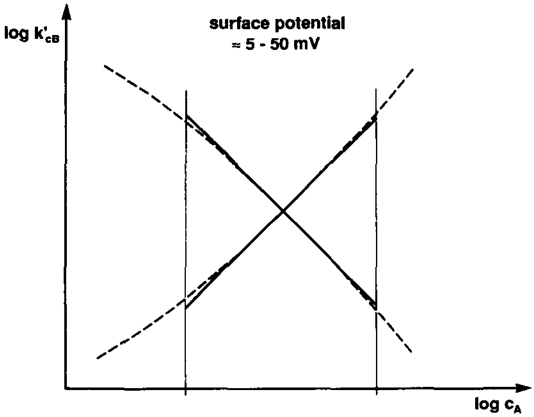


Fig. 1. Schematic illustration of log k'_{cB} as a function of log c_A obtained from the complete electrostatic theory (dashed lines) and from the approximate theory, eqn. 3 (solid lines).

the capacity factor of solute ion B in the absence of the pairing ion and c_A is the mobile phase concentration of pairing ion A. According to the electrostatic retention model, K_0 and K_1 are independent of the solute. The value of K_1 decreases when the ionic strength increases [13], and the value of K_0 decreases when less hydrophobic pairing ions and higher organic modifier concentrations [16] are used.

Eqns. 1 and 2 can be combined into a more general form which holds for any value and sign of the charge of the solute ions and for monocharged pairing ions:

$$\log k'_{cB} = K_2 - \tfrac{1}{2} z_B/z_A \log c_A \qquad (3)$$

where $z_B = \pm 1,2,\ldots$ is the charge of solute ion B, $z_A = \pm 1$ is the charge of pairing ion A and K_2 is a constant, depending on the solute, pairing ion, organic modifier concentration and ionic strength. According to this simplified equation, log k'_{cB} is a linear function of log c_A with a slope of $\tfrac{1}{2}z_B$ for opposite-signed charges and $-\tfrac{1}{2}z_B$ for similar-signed charges of the solute ion and the pairing ion.

Eqn. 3 is approximately valid when the surface potential-modified Langmuir adsorption isotherm is linear and when the surface potential lies between 5 and 50 mV [15]. Practical chromatographic work is often performed under such conditions. Therefore, reasonably wide applicability can be foreseen for eqn. 3. Above and below the imposed limits, *i.e.*, at high and low eluent concentrations of the pairing ion, non-linear retention behaviour is theoretically expected. This is illustrated schematically in Fig. 1, where log k'_{cB} is plotted as a function of log c_A for the complete electrostatic theory and compared with the approximate theory, *i.e.*, eqn. 3.

EXPERIMENTAL

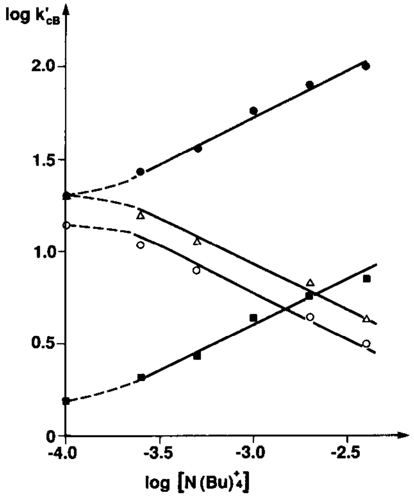
Analytical-reagent grade benzenesulphonic acid, naphthalene-2-sulphonic acid, anthraquinone-2-sulphonate, 1,5-naphthalenedisulphonate, tetrabutylammonium bromide and Orange G were purchased from Fluka (Buchs, Switzerland) and benzyltrimethylammonium bromide, benzyltriethylammonium bromide, sodium octylsulphate and sodium octylsulphonate from Merck (Darmstadt, F.R.G.). Pharmacopeial-grade bupivacaine and etidocaine were supplied by Astra Pharmaceutical Production AB (Södertälje, Sweden).

A Model LC 5000 liquid chromatograph (Varian Aerograph, Walnut Creek, CA, U.S.A.), equipped with a UV detector (254 nm) and with Model 7010 and 7126 injection valves (Rheodyne, Cotati, CA, U.S.A.), was used. The analytical columns were packed with μBondapak C_{18} (150 mm × 3.9 mm I.D.) (Waters Assoc., Milford, MA, U.S.A.) and ODS-Hypersil (200 mm × 4.6 mm I.D.) (Shandon, Runcom, U.K.). The eluents were prepared with 25 mM phosphoric acid and 25 mM sodium dihydrogenphosphate (pH 2.1), and various concentrations of acetonitrile and ion-pairing reagents. Eluents for the experiments with octylsulphonate also contained sodium bromide (I = 0.15 M).

RESULTS AND DISCUSSION

Retention behaviour of monocharged solute ions

In Figs. 2 and 3, the capacity factors (log k'_{cB}) of positively and negatively charged analytes are plotted against the mobile phase concentration (log c_A) of positively charged (tetrabutylammonium) and negatively charged (octylsulphonate and octylsulphate) pairing ions, respectively. Full lines show the theoretical retention behaviour with a slope of $\pm 1/2$ as predicted by eqn. 3. The theoretical lines were placed



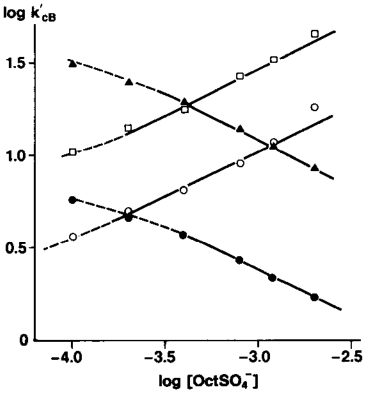


Fig. 2. Log k'_{cB} *vs.* log c_A relationships for positively and negatively charged analytes and positively charged tetrabutylammonium bromide $[N(Bu)_4^+]$ as pairing ion. Solutes: (■) benzenesulphonate, (●) naphthalene-2-sulphonate, (△) bupivacaine and (○) etidocaine. Solid lines indicate the theoretically predicted behaviour. Column, μBondapak C_{18}; eluent, 10% acetonitrile in 0.10 M phosphate buffer (pH 2.1); temperature, 27°C.

Fig. 3. Log k'_{cB} *vs.* log c_A relationships for positively and negatively charged analytes and negatively charged octylsulphate ($OctSO_4^-$) as pairing ion. Solutes: (▲) anthraquinone-2-sulphonate, (●) naphthalene-2-sulphonate, (○) benzyltrimethylammonium and (□) benzyltriethylammonium. Solid lines indicate the theoretically predicted behaviour. Other conditions as in Fig. 2.

as close to the experimental points as possible (*i.e.*, with arbitrary intercept values along the ordinate), using the theoretical slope values.

In accordance with the electrostatic model of ion-pair chromatography [11], solute ions with opposite and similar charges show symmetrical behaviour. The experimental log k'_{cB} values agree well with the predictions made from eqn. 13 for all solute ion–pairing ion combinations. At low c_A values some deviation can be observed from the linear model. Obviously, these deviations between the complete and approximate equation do not allow the accurate description of ionic solute retention over a broad range of pairing ion concentrations. However, the simplified model can be still used in practice to estimate the magnitude of retention changes for ionic solutes in RP-IPC.

An important area of such applications is the determination of initial mobile phase compositions, which lead to chromatograms with reasonable analysis times, prior to systematic selectivity optimization [17,18]. In this early stage of optimization parameter selection, predictions must be made from limited number of chromatographic experiments while larger errors in the predicted retention times are still acceptable.

When the charge type (z_B) and the retention (log k'_{cB}) of the solute are known at a single eluent concentration (log c_A) of the pairing ion (z_A), eqn. 3 can be used to calculate K_2 and estimate the solute capacity factors at other pairing ion concentrations.

Retention behaviour of doubly-charged solute ions

Another important test of eqn. 3 is the comparison of the retentions of singly- and multiply-charged solute ions under typical ion-pair chromatographic conditions. In Figs. 4 and 5, the retention (log k'_{cB}) of singly-charged (benzenesulphonate, naphthalene-2-sulphonate) and doubly-charged (1,5-naphthalenedisulphonate, Orange G) negative solute ions is plotted against the eluent concentration (log c_A) of a positively (tetrabutylammonium) and a negatively charged (octylsulphonate) pairing ion, respectively.

The experimental data closely follow the theoretically predicted behaviour (solid lines), for both increasing (Fig. 4) and decreasing (Fig. 5) solute retention. The slopes doubled (from $\pm 1/2$ to ± 1) with doubling the charge of the solute ions. Again, slight deviations from linearity indicate some of the limitations of this simplified model, as discussed above.

However, the physically consistent and unambiguous difference in the values for the two slopes for the singly- and doubly-charged solutes allows other practical applications of eqn. 3. For example, by simply measuring the retention of a (unknown) solute at two different pairing ion concentrations and calculating the slope, information can be obtained not only about the charge type (positive, negative, zero) of the analyte but also about the number of charges carried.

It should be noted that the data in Figs. 4 and 5 were measured on different columns, which gives further support to the generality of the model described.

It must be emphasized that the application of eqn. 3 requires a constant ionic strength and organic modifier concentration in the mobile phase, otherwise K_2 (and K_1) will not be constant. When the pairing ion and the organic modifier concentration are varied simultaneously, the extended form of eqns. 1 and 2 must be used [16].

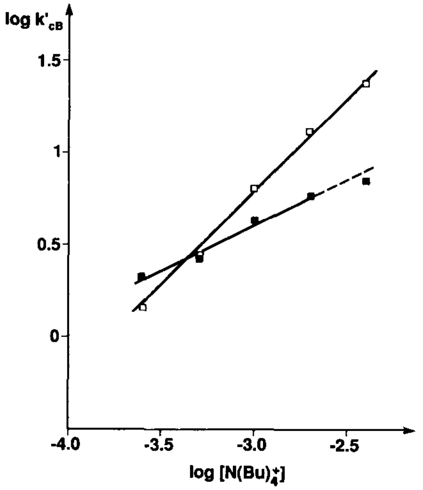


Fig. 4. Log k'_{cB} *vs.* log c_A relationships for singly- and doubly-charged negative solute ions and positively charged tetrabutylammonium bromide $[N(Bu)_4^+]$ as pairing ion. Solutes: (■) benzenesulphonate and (□) 1,5-napthalenedisulphonate. Solid lines indicate the theoretically predicted behaviour. Other conditions as in Fig. 2.

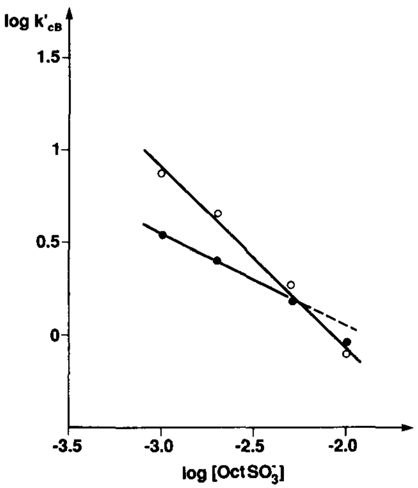


Fig. 5. Log k'_{cB} *vs.* log c_A relationships for singly- and doubly-charged negative solute ions and negatively charged octylsulphonate ($OctSO_3^-$) as pairing ion. Solutes: (●) naphthalene-2-sulphonate and (○) Orange G. Solid lines indicate the theoretically predicted behaviour. Column, ODS-Hypersil; eluent, 6% acetonitrile in 50 m*M* phosphate buffer (pH 2.1); temperature, 25°C.

CONCLUSIONS

The electrostatic theory of ion-pair chromatography has been used to derive simple equations to approximate the retention of ionic solutes as a function of the eluent concentration of the pairing ion. At constant ionic strength and organic modifier concentration, a linear relationship is predicted between the logarithm of the capacity factor of the ionic solute and the logarithm of the eluent concentration of the amphiphilic pairing ion.

According to the electrostatic theory, the slope of this relationship depends only on the sign and number of charges of the solute ion and the pairing ion. For singly-charged pairing ions, the slope is $+ \frac{1}{2}z_B$ for oppositly and $- \frac{1}{2}z_B$ for similarly (singly- and multiply) charged solute ions, where z_B is the charge of the analyte.

Predictions of the retention model agreed well with experimental data for all solute ion–pairing ion combinations. When the eluent concentration of the pairing ions varied over a broad range, the log(retention) *vs.* log(pairing ion concentration) relationship may become non-linear. Such deviations from the theoretical behaviour limit the practical use of the simplified retention model to the approximation rather than the precise description of the retention of ionic solutes.

In conclusion, the electrostatic retention model gives a physically consistent description of the retention effect of the sign and number of charges of the ionic solutes (in ion-pair chromatography) and allows the rationalization of experimental data.

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