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Electrostatic retention model of reversed-phase ion-pair chromatography

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Abstract

The theoretical foundation of the electrostatic theory of ion-pair chromatography derives from colloid and surface chemistry. In the first part of this paper, the basic concepts of the theory are discussed with emphasis on the physical principles. The theory can predict retention changes of a charged solute when varying experimental parameters in ion-pair chromatographic systems. However, because of the interplay between the different parameters, such a prediction is only feasible when using iterative numerical procedures. Therefore, a simplified theory is developed in the second part where a relationship is derived which separates the contributions of various parameters, such as type and concentration of ion-pairing reagent, ionic strength, concentration of organic modifier and eluent pH. At high surface concentrations of the ion-pairing reagent, competition between the solute and ion-pairing reagent for the limited area of the stationary phase available may occur. It is shown in the third part of the paper that this results in a maximum in the relationship between capacity factor and concentration of ion-pairing reagent in the eluent. In the final section, an extended version of the electrostatic theory is developed. It accounts for the effect of accumulation of solute ions in the electrical double layer on the capacity factor. The extended form of the electrostatic theory provides the most complete treatment of the retention of charged solutes. However, this is achieved at the cost of developing a complex mathematical formulation.

1. Introduction

Reversed-phase ion-pair chromatography (RP-IPC) is a popular separation mode of HPLC [1]. It is primarily used for the separation of mixtures of ionic and/or ionizable compounds, often in the presence of neutral solutes. The technique is based on the addition of amphiphilic (surface-active) ions to the mobile phase in order to enhance the retention of ionic sample components. Other important application areas of IPC include the separation of inorganic ions, detection enhancement with UV-active ionic additives and the separation of enantiomers.

Although a number of alternative names exist (*e.g.*, ion-interaction chromatography, dynamic ion-exchange chromatography), ion-pair chromatography is the most commonly used name for the technique, and it derives from the historical application of ion-pair extraction principles to liquid chromatography by Schill [2]. Without implications for the actual mechanism of this chromatographic mode, we shall use the term IPC for the technique and pairing ion (IP reagent) for the amphiphilic ion throughout this paper.

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In IPC systems, numerous mobile phase variables (pairing ion type and concentration, ionic strength, eluent pH, organic solvents) can be used to control solute retention and separation selectivity. The broad choice and combination of these variables allow for the separation of complex sample mixtures containing ionic/ionizable and neutral solutes.

However, method development in IPC is often difficult owing both to the uncertainties concerning the interpretation of the underlying retention processes and to the large number of variables. In spite of many mechanistic studies, the theory of ion-pair chromatography has remained a subject of debate over many years. In order to develop a better understanding of ion-pair chromatographic systems, the physical background of the different theories must be examined.

Historically, two groups of retention theories can be distinguished, stoichiometric and non-stoichiometric [3]. At the introduction of the technique, stoichiometric theories were developed. These suggested that solute ions and pairing ions form stoichiometric complexes either in the mobile phase (ion-pair model) or at the stationary phase (dynamic ion-exchange model). The ion-pairing adsorption model assumes the formation of an ion pair in the polar mobile phase followed by the adsorption of this uncharged complex on the hydrophobic stationary phase. The dynamic ion-exchange model presumes that the amphiphilic IP reagent molecules adsorb together with their inorganic counter ions on the stationary phase and cause the column to behave as a dynamically generated ion exchanger. The retention of solute ions is then assumed to be due to ion exchange with the inorganic counter ions. In a fundamental study on stoichiometric models, Knox and Hartwick [4] pointed out that formally both models lead to identical retention equations. Many variants and combinations of such stoichiometric models have been published. They have provided an easy-to-understand qualitative picture of solute retention for many analysts and promoted the practical use of IPC. The reader is referred to a number of review papers for a more extensive discussion of stoichiometric models [3–7].

The common feature of stoichiometric models is to derive retention relationships from a number of equilibria between the mobile phase constituents. In chemical thermodynamics, stoichiometric relationships describe the behaviour of a system fairly well when the concentrations are low and the interactions are short range, *e.g.*, Van der Waals forces and London forces. In this case, the standard free energy of adsorption (ΔG_t^0) for the retention process is independent of the concentrations of the reactants and products in the system. However, this does not apply to electrostatic interactions resulting from long-range forces implying multi-body interactions. In Fig. 1 we illustrate the long-range nature of forces operating in typical ion-pair chromatographic systems. The electrostatic attraction and the electrostatic repulsion forces between molecules of pairing ions, solute ions and inorganic counter ions are represented by simple arrows, although more rigorously they result from the potential fields of all these ions. These forces are effective over a long range, which implies multi-body interactions, and they cannot be described by stoichiometric relationships. Therefore, the long-range nature of the electrostatic interactions

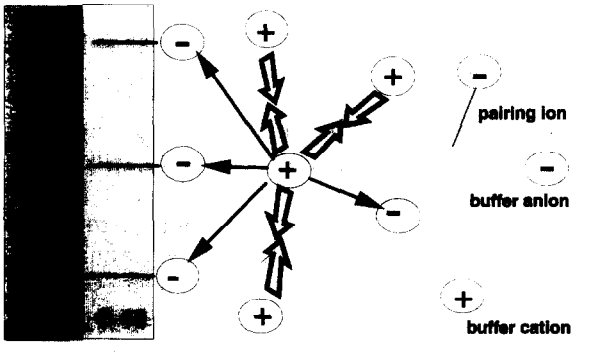

Fig. 1. Schematic illustration of the long-range nature of electrostatic forces between ions in typical ion-pair chromatographic systems. Open and full arrows represent electrostatic repulsion and attraction forces, respectively. See text for details.

makes the stoichiometric models fundamentally limited in interpreting electrostatic interactions involved in IPC systems. The remedy to describe the interaction between charged species is to turn to electrostatics and statistical thermodynamics which form the basis of the Poisson–Boltzmann equation.

Non-stoichiometric models describe the retention of ionic analytes without the formation of chemical complexes. These models assume that the retention of solute ions is partly determined by their interaction with the electric field created by the adsorbed pairing ion. Therefore, the effect of the pairing ion is assumed to be indirect and acts through establishing a certain electrostatic surface potential. The development of non-stoichiometric theories was largely stimulated by experimental evidence of the adsorption of the IP reagent on the hydrophobic stationary phase.

A qualitative description of electrostatic interactions in IPC systems was given by Bidlingmeyer [8] in the ion-interaction theory. According to this hypothesis, the pairing ion is adsorbed on the stationary phase surface forming a primary ion layer. The electrolytic (inorganic) counter ions form a secondary ion layer between the charged surface and the bulk eluent. The analyte ions are attracted or repelled by the primary ion layer depending on the sign of their charge and that of the layer. This simple qualitative picture, which did not assume the formation of complexes (ion pairs), has become popular and the term "ion-interaction" chromatography is still used at the time of writing [9]. A number of attempts have been made to formulate the model quantitatively [10,11]. However, none of them provided a rigorous description of the system, and some [9,12] even reduced to a stoichiometric model.

A theory based on two retention processes, *i.e.*, ion exchange and interaction with the electrical double layer, has been proposed by Cantwell and co-workers [13–15]. Based on their experimental results they suggested that the main process that determines retention is ion exchange between solute ions and inorganic counter ions in the diffuse part of the electrical double layer. Testing and application of the model are not straightforward and require the simultaneous measurement of the adsorption of the solute ion and the pairing ion [15] at different eluent concentrations. Further discussion of this model is given at the end of this paper.

Qualitatively, the electrostatic retention model as suggested by Ståhlberg [16] is similar to all these non-stoichiometric models. It assumes the formation of a surface potential between the bulk mobile phase and the hydrophobic stationary phase, resulting from the selective adsorption of amphiphilic pairing ions. The retention of ionic solutes depends on both their hydrophobicity and the electrical surface potential. The surface potential is obtained from solving the Poisson–Boltzmann equation. A theory based on similar concepts and on the solution of the Poisson–Boltzmann equation has been developed for the electrostatic interaction chromatography of proteins [17] and most recently for the ion-exchange chromatography of inorganic ions [18].

The major advantage of the electrostatic retention model for ion-pair chromatography is that it is well founded in physical chemistry and also that it provides equations for practical tests and retention prediction. It assumes that the primary contribution to the retention of ionic analytes is their increased (attraction) or decreased (repulsion) adsorption at the electrically charged surface. Therefore, it accounts for the retention behaviour of both oppositely and similarly charged solute ion–pairing ion combinations. Since it was introduced, the electrostatic model has been thoroughly tested [19,20] to describe the adsorption of the pairing ion and its effects on solute retention [21,22]. Based on this model, Ståhlberg and Hägglund [23] were able to explain the effects of different mobile phase electrolytes; Bartha and co-workers have extended the model to include the effect of organic solvent [24] and eluent pH [25]. However, the model has been considered to be too complex for practical work [1].

In this paper, we discuss and illustrate the basic concepts of the electrostatic retention

theory, especially that involving the electrostatic double layer and surface potential, and their effects on the adsorption isotherm of the pairing ion and on the capacity factor of charged solute ions.

We present a full framework to predict retention in ion-pair chromatographic systems based on a simplified form of the electrostatic model. A retention equation is developed to include the charges of solute and pairing ions and to provide an explicit expression for the effect of the ionic strength. This form of the model separates and delineates the contributions of solute charges, pairing ion concentration and hydrophobicity, organic solvent concentration, pH and ionic strength of the mobile phase, and also that of the reversed-phase packing material. In order to reach a practically useful equation, a number of simplifications have necessarily been made. These include the use of a solution of a linearized form of the Poisson–Boltzmann equation and a linearized form of the potential modified adsorption isotherm of the pairing ion. The simplified theoretical equations will therefore be applicable when the electrical surface potential (and the surface concentration of the adsorbed pairing ion) is relatively low (*i.e.*, less than ±50 mV). In practice, this corresponds to an approximately tenfold change in the capacity factor of analyte ions. The theoretical equations are accompanied by illustrations and comparison with experimental data both from the literature and our own work. The discussion of the simplified retention model aims to provide the information required to understand the basic features of the electrostatic theory and to utilize or test its predictions in practical work.

A more rigorous version of the retention model is also discussed when the surface concentration of the adsorbed pairing ion is high and solute retention is affected by competition for the adsorption sites. This extension of the model includes a full form of the potential-modified adsorption isotherm of the pairing ion. It considers the possible effects of competition for the adsorption sites of the stationary phase and uses the non-linear form of the Poisson–Boltzmann equation. Finally, we discuss the possible contributions of the diffuse part of the electrical double layer to the retention of analyte ions. This extended version of the electrostatic model, however, results in mathematically complex expressions and requires numerical evaluation.

2. Theory

2.1. General introduction

The addition of an ion-pairing (IP) reagent to the mobile phase has a characteristic influence on the retention of ionic analytes; it increases the retention of analytes with the opposite charge to that of the IP reagent, decreases the retention when the charges are of the same sign and has a negligible effect when the analyte is uncharged. The electrostatic theory for ion-pair chromatography (IPC) gives a quantitative and consistent physico-chemical explanation for this general behaviour by applying well known principles from colloid and surface chemistry. Its quantitative formulation is useful for prediction and optimization purposes. In this section, a brief introduction of some key concepts of the theory is outlined, while a more detailed discussion of the interplay between various parameters is presented in the following sections.

Consider a reversed-phase chromatographic system with an aqueous mobile phase in which a sodium phosphate buffer and octylsulphonate are dissolved and equilibrated with the stationary phase surface. As the octylsulphonate ions are more hydrophobic than their corresponding counter ions, *i.e.*, the sodium ions, they have a higher affinity for the hydrophobic surface and will therefore be bound to the surface in higher concentrations than the counter ions. It is a well established fact that in such a case the sodium ions are not bound to the IP reagent as stoichiometric 1:1 complexes, but are distributed in a layer close to the surface, the so-called diffuse double layer. The distribution of the sodium ions in the double layer is a result of the balance between the electrostatic attraction to the charged surface, the way the sodium ions shield

each other and the (smearing out) effect of thermal motion (entropy).

A well known consequence of the double layer is the electroosmotic flow in CZE, which of course would not exist if there were a complete formation of "ion pairs" between the surface charges and the counter ions. The important point is that a higher concentration of negatively charged octylsulphonate ions than of sodium ions at the hydrophobic surface implies that the surface carries a negative net charge. The argument may be put in a generalized form: when there is a difference in adsorption tendency between the IP reagent and its counter ions, a net surface charge is created. It is important to realise that this does not violate the principle of electroneutrality because the number of charges on the surface is balanced by an equivalent number of sodium ions located in a layer close to the surface (see Fig. 2).

Qualitatively, the effect of the negative net surface charge created by octylsulphonate ions on the retention of charged analytes is very simple: positively charged analytes are attracted to the negatively charged surface, while negatively charged analytes are repelled, resulting in an increase or decrease, respectively, in retention. The principle will, of course, hold irrespective of the charge of the IP reagent; a positively charged IP reagent will repel positively charged analytes from the surface and attract negatively charged analytes. This intuitive view of the effect of the IP reagent on retention is a useful starting point for a preliminary understanding of ion-pair chromatography. However, in order to formulate a theory for ion-pair chromatography it must be complemented with a quantitative formulation of the influence of the physical parameters which are of importance. Answers are sought to questions such as the following. How is the strength of attraction and repulsion affected by the nature and surface concentration of ion-pairing reagent on the surface? What is the relationship between the surface concentration of IP reagent and its concentration in the mobile phase? What is the effect of the concentration of organic modifier in the mobile phase? What is the effect of ionic strength and pH in the mobile phase? These questions must be answered quantitatively in physically consistent and realistic terms. In the rest of this paper they will be answered within the framework of the electrostatic theory of ion-pair chromatography and illustrated with experimental data. Although the mathematical treatment of the theory is awkward, it is possible to make some well defined, reasonable approximations and to obtain simple relationships which are useful in practical work. The complete theory, however, is readily evaluated by a computer.

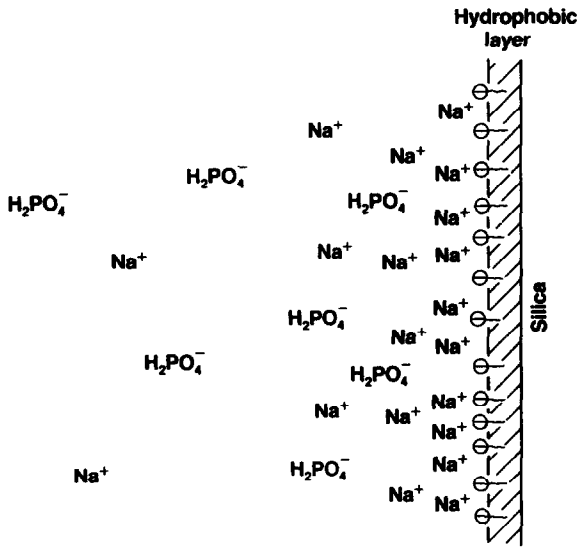

Fig. 2. An idealised picture of the electrical double layer in reversed-phase ion-pair chromatography.

2.2. Concept of electrostatic surface potential

As a first step to formulate a quantitative theory of ion-pair chromatography, the concept of electrostatic surface potential and its role in the determination of the magnitude of retention must be understood. From physics we know that a charged body creates an electrical field which causes similarly charged bodies to repel and oppositely charged bodies to attract each other. The electrical field around a charged body is a vector but it can also be described by a scalar quantity, the electrostatic potential. The electro-

static potential difference between two points is equal to the amount of work needed to move a positive unit test charge between these points.

In ion-pair chromatography, a difference in electrostatic potential is created between the bulk of the mobile phase, which is electroneutral, and the net charged surface. The difference is a result of the separation of charges in space originating from the difference in adsorption characteristics of the IP reagent and its counter ions. The electrostatic repulsion and attraction of analytes to the charged stationary phase surface are quantified as the amount of work (*i.e.*, free energy) needed to transport the charge of the analyte ion from the bulk of the mobile phase to the surface. If this work is positive the surface repels the analyte, whereas if it is negative it attracts it. There are several factors that determine the work of transporting a charge between two phases; we shall discuss only the surface potential changes caused by the adsorption of IP reagent, because the other factors are, to a first approximation, constant. The electrostatic work is, however, only one part of the total work involved in the process of transporting the analyte from the bulk of the mobile phase to the stationary phase surface. As will be discussed in the next section, it is the total work that ultimately determines the magnitude of the retention.

2.3. Preliminary discussion of the capacity factor in ion-pair chromatography

The thermodynamic interpretation of the capacity factor in ion-pair chromatography can be discussed on several theoretical levels. The first level discussed in this section is valid under most practical conditions. The complexity of the arguments will then gradually increase in subsequent sections.

The assumption at this level is that the capacity factor is a measure of the distribution of the analyte between the mobile phase and the surface of the stationary phase. Basic chromatographic theory relates the capacity factor to the equilibrium constant for adsorption according to

$$k_B = \phi K_{t,B} = \phi e^{-\frac{\Delta G^0_{t,B}}{RT}} \quad (1)$$

where ϕ is the column phase ratio and $K_{t,B}$ is the equilibrium constant for adsorption. As is known from thermodynamics, this equilibrium constant depends on the change in free energy of adsorption of the solute, $\Delta G^0_{t,B}$, which is equal to the work needed to transfer the molecule from the bulk of the mobile phase to the stationary phase surface. As discussed for the electrostatic surface potential, the electrostatic work involved in the transfer of a charged analyte to the charged surface constitutes one part of the total work, $\Delta G^0_{t,B}$, which is quantitatively expressed as a difference in electrostatic potential between the surface and the bulk of the mobile phase. We can therefore define the electrostatic contribution in $\Delta G^0_{t,B}$,

$$\Delta G^0_{t,B} = \Delta G^0_B + \Delta G^0_{el,B} = \Delta G^0_B + z_B F \Delta \Psi_0 \quad (2)$$

where z_B is the charge of the analyte ion, F the Faraday constant and $\Delta\Psi_0$ is the difference in electrostatic potential between the bulk of the mobile phase and the stationary phase surface, induced by adsorption of the IP reagent. The sign of $\Delta\Psi_0$ is the same as the sign of the IP reagent, so that the product $z_B\Delta\Psi_0$ is positive when the analyte and the IP reagent are of the same sign and negative when they are of opposite sign. Physically this means that the adsorption of the analyte is enhanced when the IP reagent is of opposite sign and decreases when they are of the same sign.

As indicated in Eq. 2, the electrostatic part of the free energy change constitutes only one part of the total free energy change of adsorption, $\Delta G^0_{t,B}$, of the charged analyte. The other part is usually called the "chemical" part, ΔG^0_B, of the total free energy change and in RP chromatography it is a measure of the hydrophobicity of the analyte. The physical meaning of this latter term corresponds to the free energy of adsorption of the analyte in the absence of IP reagent in the chromatographic system, and is related to the capacity factor of the analyte (Eq. 1) in an IP reagent-free mobile phase. A chromatographic

system without IP reagent is therefore a suitable reference point from which IP reagent-induced changes in the capacity factor of the analyte can be studied. Bearing in mind that all changes in capacity factor are related to a reference point, we can set the electrostatic potential in the bulk of the mobile phase to zero so that the difference in electrostatic potential between the bulk and the surface, $\Delta\Psi_0$, becomes numerically identical with Ψ_0, the electrostatic surface potential.

Fig. 3 (left-hand side) illustrates the thermodynamic relationships for one positively and one negatively charged analyte in the absence and presence of ion-pairing reagent. The capacity factor measured in the absence of IP reagent represents a reference point to which changes induced by the addition of IP reagent are related. When the IP reagent is added to the mobile phase, the stationary phase surface becomes electrically charged, as is illustrated on the right-hand side of Fig. 3 for a positively charged IP reagent. The capacity factor is related to the total free energy of adsorption, which also includes the free energy (=work) required to transfer the charge of the charged analyte to the charged surface. For positively and singly-charged analytes the capacity factor in the presence of a positively charged IP reagent is

$$k_{cB^+} = k_{0B^+} e^{-\frac{F\Psi_0}{RT}} \quad (3a)$$

and for negatively and singly-charged analytes the corresponding equation is

$$k_{cB^-} = k_{0B^-} e^{\frac{F\Psi_0}{RT}} \quad (3b)$$

where k_{0B} is the capacity factor of the respective analyte for the reference composition of mobile phase, *i.e.*, in the absence of IP reagent.

A generalisation of these equations can be obtained by combining Eqs. 1 and 2:

$$k_{cB} = k_{0B} e^{-\frac{z_B F\Psi_0}{RT}} \quad (4a)$$

bearing in mind that the electrostatic surface potential is positive when a positively charged IP reagent is used and negative when the IP reagent is negatively charged. The equation is therefore consistent with the fact that when the IP reagent and analyte ions are of opposite (or the same) charge, the retention increases (decreases). A

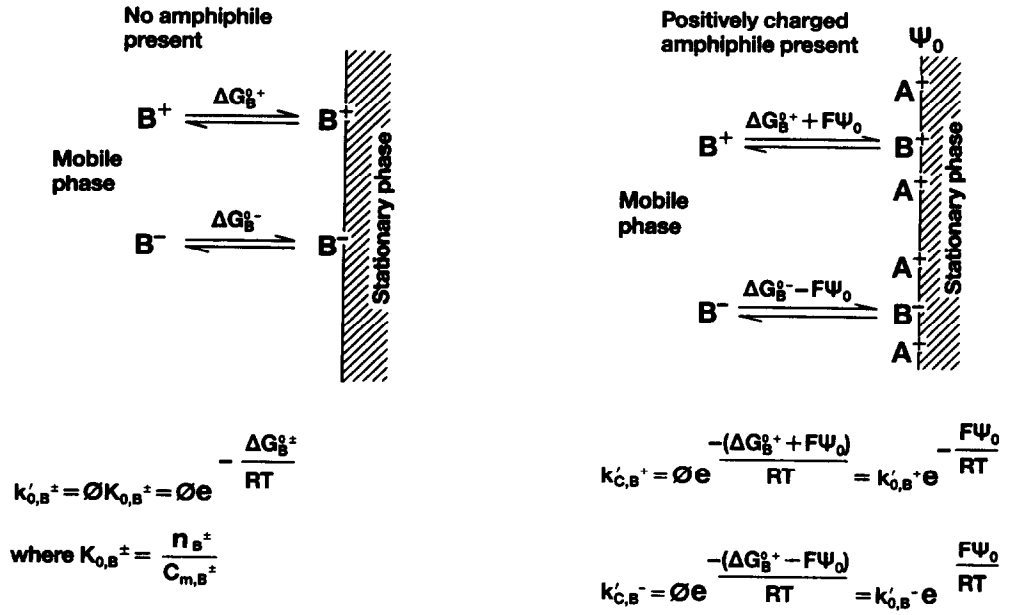

Fig. 3. Schematic illustration of the thermodynamics of the electrostatic theory applied to the distribution of analytes between the mobile phase and the stationary phase.

practical consequence of this equation is that the factor by which oppositely (or similarly) charged solutes increase (decrease) its capacity factor is the same for all oppositely (similarly) charged analytes. For example, if the capacity factor doubles for a solute on adding IP reagent, all other solutes with opposite charge to the IP reagent will also double their capacity factor. Further, all solutes with the same charge as that of the IP reagent will halve their capacity factor. Some of the experimental evidence of this symmetrical behaviour is given in ref. 19, where the theoretical implications of the equation are discussed. As discussed later, Eq. 4a is often limited to low surface concentrations of IP reagent.

By using Eq. 4a, it is possible to estimate the magnitude of the surface potential created by the IP reagent solely from the capacity factor data for a fully ionized solute, a feature that is used in the discussion of the adsorption isotherm of the IP reagent. By rearranging Eq. 4a we obtain

$$\Psi_0 = -\frac{z_B F}{RT} \cdot \ln \frac{k_{cB}}{k_{0B}} \tag{4b}$$

2.4. Gouy–Chapman theory of the electrical double layer

We have seen that it is the electrostatic surface potential created by the IP reagent that causes the changes in retention of charged analytes. The logical question is therefore: What factors influence the magnitude of the electrostatic surface potential? The answer is found in the theory for the electrical double layer, the Gouy–Chapman theory, and its coupling to the adsorption isotherm for the IP reagent. In this section a brief discussion of relevant parts of the Gouy–Chapman theory is presented.

From the discussion of the physical background of the electrostatic surface potential, it is intuitively clear that its magnitude *inter alia* is dependent on the concentration of charges at the surface. A rigorous theoretical treatment for a planar surface gives the following relationship:

$$\Psi_0 = \frac{2RT}{F} \ln \left\{ \frac{n_A z_A F}{\left(8\varepsilon_0 \varepsilon_r RT \sum_i c_{0i}\right)^{\frac{1}{2}}} + \left[\frac{(n_A z_A F)^2}{8\varepsilon_0 \varepsilon_r RT \sum_i c_{0i}} + 1 \right]^{\frac{1}{2}} \right\} \tag{5}$$

where n_A is the surface concentration of the charged species in mol/m^2, ε_0 is the electrical permittivity of vacuum, ε_r is the dielectricity constant of the mobile phase and Σc_{0i} is the mobile phase concentration of electrolyte ions, which are assumed to be singly charged. This equation shows that the electrostatic surface potential primarly depends on two parameters: the surface concentration of the IP reagent and the electrolyte concentration in the mobile phase. For low surface potentials its value is linearly dependent on the surface concentration and in this region Eq. 5 can be approximated by

$$\Psi_0 = \frac{z_A n_A F}{\kappa \varepsilon_0 \varepsilon_r} \tag{6}$$

where κ is called the inverse Debye length and is given by

$$\kappa = F \left[\frac{\sum_i (z_i^2 c_{0i})}{\varepsilon_0 \varepsilon_r RT} \right]^{1/2} \tag{7}$$

An interesting consequence of this theory is that it is the surface concentration and not the type of IP reagent that is of importance for retention. This was also found experimentally for alkylsulphate [4] and alkylsulphonate [26] pairing ions at a constant ionic strength of the mobile phase. In Fig. 4 the capacity factor of positively charged adrenaline is shown as a function of the experimentally measured surface concentration of butyl-, hexyl- and octylsulphonate pairing ions at a constant ionic strength (0.175 *M*) of the mobile phase [26]. It can be seen that pairing ions having different chain lengths result in identical

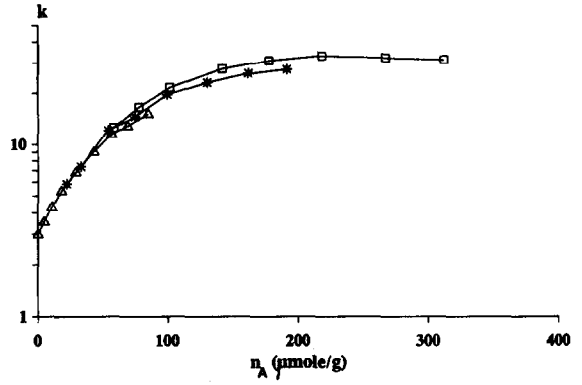

Fig. 4. Capacity factor (k) data for adrenaline *vs.* stationary phase concentration (n_A) of sodium (△) butyl-, (*) hexyl- and (□) octylsulphonate pairing ions measured at constant ionic strength (175 m*M* Na^+) of the phosphate buffer (pH 2.1) mobile phase on an ODS-Hypersil column. See ref. 26 for experimental details.

solute retention at the same surface concentration (electrostatic surface potential).

2.5. Adsorption isotherm of the IP reagent

In practical chromatographic work, the experimenter can choose the mobile phase concentration of the IP reagent and not its surface concentration. These two parameters are related through the adsorption isotherm of the IP reagent.

Thermodynamically, the adsorption is determined by the change in free energy of adsorption, which at low surface concentrations is divided into an electrostatic part and a chemical part, analogously to the treatment described earlier for the adsorption of an analyte. Physically this means that the electrostatic potential created by the IP reagent must be included in its own adsorption isotherm. The adsorbed IP reagent will electrostatically "repel itself" from the surface so that a non-linear relationship between mobile phase concentration and surface concentration is obtained. Another factor that influence the adsorption isotherm is the limited surface area or the monolayer capacity of the stationary phase surface. As the surface concentration increases, the area accessible for additional molecules on the surface decreases and the molecule will find it more and more difficult to find an adsorption site. This effect forms the background to the Langmuir adsorption isotherm, which, combined with the effect of the electrostatic surface potential, forms the basis of the surface potential modified Langmuir isotherm:

$$n_A = \frac{n_0 K_A C_A e^{-\frac{z_A F \Psi_0}{RT}}}{1 + K_A c_A e^{-\frac{z_A F \Psi_0}{RT}}} \quad (8)$$

where n_A is the surface concentration of IP reagent, n_0 the monolayer capacity, c_A its concentration in the mobile phase and K_A the adsorption constant, given by

$$K_A = e^{-\frac{\Delta G_A^0}{RT}} \quad (9)$$

where ΔG_A^0 is the "chemical" part of the free energy of adsorption, in an analogous fashion to the chemical free energy of adsorption for the analyte in Eq. 2. The non-linearity of the adsorption isotherm due to limited monolayer capacity is in practice not detected for surface concentrations lower than $0.3n_0$. On the other hand, the non-linearity caused by the electrostatic repulsion is usually noticeable for much lower surface concentrations, a point addressed later in this paper.

Substituting the expression for Ψ_0 as a function of n_A (Eq. 5) into Eq. 8 and solving for n_A should in principle give the desired adsorption isotherm for the IP reagent. However, because of the complex algebraic form of the resulting equation, this has not been accomplished without introducing the approximations discussed in the next section.

On the other hand, Ψ_0 data determined from the capacity factor of fully ionized solutes (*cf.*, eqn 4b) can be used to interpret the adsorption data of pairing ions. In Fig. 5a two adsorption isotherms are shown for butylsulphonate on a

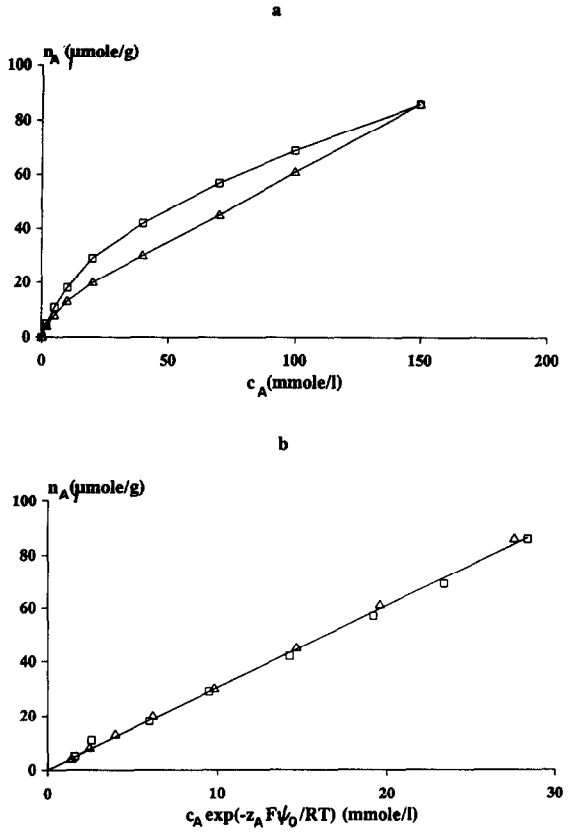

Fig. 5. Adsorption isotherms of sodium butylsulphonate pairing ion on an ODS-Hypersil (5 μm) reversed-phase column from a phosphate buffer at (△) varying (25–175 m*M* Na^+) and (□) constant ionic strength (175 m*M* Na^+): (a) without correction and (b) with correction for the surface potential. See ref. 21 for experimental details.

reversed-phase packing. The lower isotherm was obtained at a varying ionic strength (0.025–0.175 *M*), whereas higher adsorption is obtained for a constant ionic strength (0.175 *M*). As the surface concentration is relatively low, the linearized form of Eq. 8 can be used (the denominator is taken as 1). After correcting the mobile phase concentration with the electrical surface potential term (see Fig. 5b), calculated from simultaneously measured capacity factor data for adrenaline, the two isotherms coincide. This means that the effect of ionic strength on the adsorption data is fully accounted for by the induced variations in the surface potential (see refs. 21 and 23 for more details).

3. Simplified electrostatic model

3.1. Simplified treatment of the capacity factor as a function of mobile phase variables in ion-pair chromatography

The interplay between the surface concentration of IP reagent and the created electrostatic surface potential makes it difficult to obtain a rigorous yet simple relationship between the capacity factor and mobile phase concentration of IP reagent. However, by making a series of well defined approximations it is possible to obtain an equation (see Appendix for details of the derivation) which is of interest in practical work. For $c_A > 0$:

$$\ln k_{cB} = \ln k_{0B} + \left(\frac{-z_A z_B}{z_A^2 + 1}\right)\left[\ln\left(\frac{n_0 K_A c_A}{\kappa}\right) + \ln\left(\frac{F^2}{RT\varepsilon_0\varepsilon_r}\right) + 1\right] \quad (10)$$

The most important feature of this equation is that the different contributions to the capacity factor of a fully ionized analyte are separately identified:

(i) the capacity factor of the analyte in absence of IP reagent, k_{0B}, which is determined mainly by the hydrophobicity of the solute and the concentration of the organic modifier;

(ii) the effect of the charges of the solute ion and the pairing ion (z_B and z_A);

(iii) the influence of the mobile phase concentration of the IP reagent, c_A;

(iv) the influence of the electrolyte concentration in the mobile phase, included in the inverse Debye length, κ (see Eq. 7);

(v) the monolayer capacity of the stationary phase, n_0, and the free energy of adsorption of the IP reagent, $K_A = \exp(-\Delta G_A^0/RT)$, which depend on the type of IP reagent and the organic modifier concentration of the mobile phase.

The explicit expression makes it possible to

discuss and understand the contributions of different chromatographic variables in physicochemically meaningful terms, bearing in mind the limitations of this simplified treatment.

Eq. 10 can be used at relatively low electrostatic surface potentials (from 5 to about 50 mV) where the approximations of using linearized solutions for the Poisson–Boltzmann Eq. (6) and for the surface potential modified adsorption isotherm of the pairing ion, as well as the series expansion discussed in the Appendix, are applicable. This corresponds to *ca.* 0.08–0.8 μmol/m^2 surface concentration of adsorbed pairing ion on regular reversed-phase packings with surface areas of 150–200 m^2/g and ionic strengths of 0.05–0.1 *M*. Further, in order to use Eq. 6 for the approximation of the surface potential, the pore diameter of the stationary phase should be at least 100 Å and the electrolyte concentration of the mobile phase should exceed *ca.* 50 m*M*. As a first approximation, it is also assumed that the reversed-phase retention of solute *B* (k_{0B}), *i.e.*, the chemical part of the free energy adsorption, is not influenced by the presence of the pairing ion. The retention equations for the organic modifier were derived assuming that both ΔG^0_A and ΔG^0_B are linear functions of the concentration of the organic modifier, and that variations in the dielectric constant due to changing mobile phase conditions can be neglected.

Within the framework of these assumptions, the addition of the ion-pairing reagent usually results in a tenfold change in retention. Owing to the extensive linearization of the relationships, retention predicted from Eq. 10 may have a relative error up to 15–25%. In terms of the surface concentration of the pairing ion, Eq. 10 corresponds to the earliest and steepest part of solute retention curve (see Fig. 4). At high surface concentrations of the pairing ion (comparable to the monolayer capacity of the stationary phase), the retention curve levels off, which can be (at least partly) accounted for the competition of solute and pairing ion molecules for the hydrophobic surface, as discussed later. In the following subsections we discuss the retention-modifying effect of the individual chromatographic variables. Several examples of the utility of Eq. 10 in practical chromatographic work are presented.

3.2. Effect of electrical charge of the solute ion and the pairing ion

The sign of the ionic charge of the adsorbing pairing ion determines the sign of the electrostatic surface potential. Analyte ions with opposite charge are attracted whereas ions with identical charge are repelled by the charged surface. When the eluent (and surface) concentration of the pairing ion increases, the retention of analyte ions decreases for identical charges and increases for opposite charges. The steepness of this change is, however, determined by the actual number of charges on the solute and the pairing ion. Qualitatively, multiply charged ions would be expected to show larger changes than singly charged ions. In accordance with this qualitative picture, Eq. 10 predicts steeper changes for multiply charged ions if any of the mobile phase parameters (pairing ion concentration, ionic strength or organic modifier concentration) is varied.

At a given constant ionic strength and organic modifier concentration, Eq. 10 can be simplified as

$$\ln k_{cB} = K_2 - \left(\frac{z_A z_B}{z_A^2 + 1}\right) \cdot \ln c_A \qquad (11)$$

where K_2 is a constant depending on the hydrophobicity and the charge of solute and pairing ion, organic modifier and ionic strength. According to this equation, $\ln k_{cB}$ is a linear function of $\ln c_A$ with a slope and sign determined by the charge of solute and pairing ions. A simplified version of this equation for singly charged pairing ions has been published earlier [22]. The theoretical slope values for a few singly and doubly charged solute ion–pairing ion combinations are given in Table 1.

Predictions for singly charged pairing ions and singly and doubly charged solute ions based on this equation agree well with experimental data. Over a moderate concentration range of common octylsulphonate and tetrabutylammonium

Table 1
Theoretical slope values for the ln k_{cB} *versus* ln c_A relationship at different solute ion (z_B)–pairing ion (z_A) charges

z_A	z_B	$-z_A z_B/(z_A^2+1)$
+1	−1	+1/2
+1	+1	−1/2
+1	−2	+1
+1	+2	−1
+2	−1	+2/5
+2	+1	−2/5
+2	−2	+4/5
+2	+2	−4/5

pairing ions, experimental data for singly and doubly charged solute ions are characterized by the theoretical slopes of ±1/2 and ±1, respectively [22].

An interesting prediction of Eq. 11 is that the combination of a singly charged solute with a doubly charged pairing ion only gives a slope of 0.4, compared with a slope value of 1 for a doubly charged solute with a singly charged pairing ion. Experimental data for multiply charged solutes and pairing ions at constant ionic strength are scarce in the literature. In Fig. 6, we replotted retention data from Pettersson and Schill [27] for some naphthalene sulphonates and disulphonates against the eluent concentration of doubly charged hexamethonium ion as a pairing ion. The theoretical slope is shown by the solid lines (+0.4 for singly and +0.8 for doubly charged solutes) for the experimental data. No attempt was made to fit the data to the theoretical behaviour, rather the agreement between the predicted and experimental retention change for the differently charged solutes is interesting. The retention at low pairing ion concentrations was higher than expected (not shown on the plot). In fact, non-linear behaviour is expected at both low and high pairing ion concentrations where Ψ_0 is outside the range 5–50 mV (*cf.*, discussion for Eq. 10). However, the qualitative agreement between the predicted and experimental behaviour supports the applicability of Eq. 11 not only in interpreting but also in approximating the retention for multiply charged

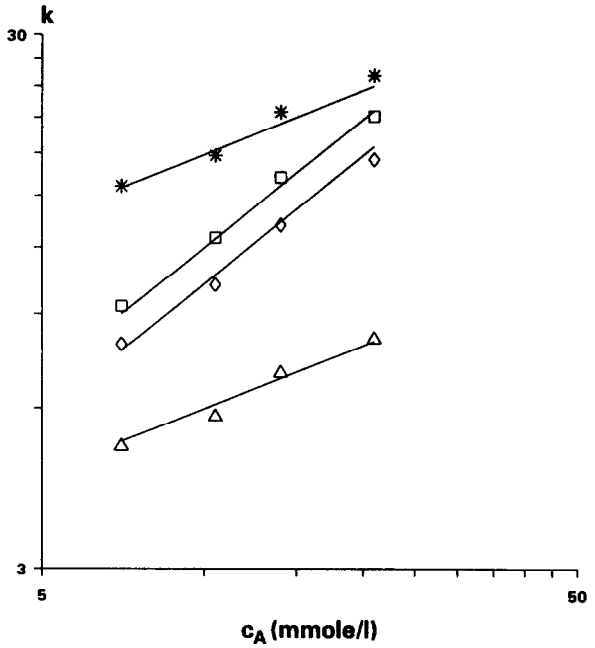

Fig. 6. Capacity factor (k) data for singly (△ = 1-naphthylamine-4-sulphonic acid; * = 6-naphthol-2-sulphonic acid) and doubly charged sulphonic acids (□ = 2-naphthol-6,8-disulphonic acid; ◇ = naphthalene-2,7-disulphonic acid) as a function of eluent concentration (c_A) of a doubly charged pairing ion, hexamethonium bromide ($z_A = +2$). Data were measured by Petterson and Schill [27] using a phosphate buffer (pH 5.5) at constant ionic strength (0.1 *M*) on a LiChrosorb RP-18 column.

solute–pairing ion combinations. It must be pointed out that owing to the theoretical limitations of using the Poisson–Boltzmann equation for multiply charged ions, retention estimates may result in larger errors than for singly charged solutes.

Recently, Zhang *et al.* [28] have reported on the correlation between retention data measured in a reversed-phase mode (ln k_{0B}) and in the ion-pairing mode (ln k_{cB}). They found a linear correlation between retention data for sulphonic acids in the two modes of chromatography. The intercept parameters of the correlation were strongly dependent on the number of negative charges (from 1 to 3) of the solute ions, which is theoretically to be expected according to Eqs. 10 and 11.

3.3. Effect of pairing ion hydrophobicity

One of the important parameters to control retention in ion-pair chromatography is the hydrophobicity of the pairing ion. Generally, the retention of oppositely charged solutes increases with increasing hydrophobicity of the pairing ions when they are used at identical mobile phase concentrations. The retention change can be attributed to the higher adsorption of more hydrophobic pairing ions and a corresponding higher electrostatic surface potential, at a constant value of the ionic strength and other chromatographic variables. In terms of the electrostatic model, the hydrophobicity of the pairing ion influences the free energy of adsorption (ΔG_A^0), and the size of the paring ion affects the monolayer capacity (n_0), *i.e.*, it changes the factor $n_0 K_A(\Delta G_A^0)$ (which will be referred to as the "adsorption term" for convenience) in the adsorption isotherm of the pairing ion. At constant ionic strength and organic modifier concentration, Eq. 10 can be rewritten as

$$\ln k_{cB} = K_3 - \left(\frac{z_A z_B}{z_A^2 + 1}\right) \cdot \ln (n_0 K_A) - \left(\frac{z_A z_B}{z_A^2 + 1}\right) \cdot \ln c_A \quad (12)$$

where K_3 is a constant.

Eq. 12 predicts similar retention behaviour for ionic analytes as a function of the eluent concentration of the pairing ion (c_A), as long as the number and sign of the charges of the pairing ions are identical. Any change in pairing ion hydrophobicity results in an incremental change of its adsorption term ($\ln n_0 K_A$), *i.e.*, it causes a parallel shift of $\ln k_{cB}$ *vs.* $\ln c_A$ curves of the analyte ions. The more hydrophobic the pairing ion, the larger is the retention increase (oppositely charged solutes) or decrease (similarly charged solutes). Examples are shown in Fig. 7a and b for both positively (dopamine) and negatively (naphthalene-2-sulphonic acid) charged solute ions, respectively, with different alkylsulphonate pairing ions [26]. Note that the sign of the retention shift (due to increasing hydrophobicity of the pairing ion) depends on the combination of charges are predicted by Eq. 12.

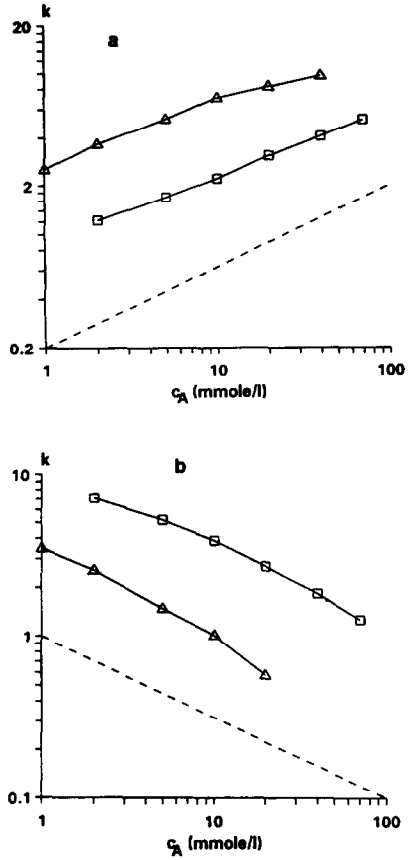

Fig. 7. Capacity factor (k) data for (a) dopamine and (b) naphthalene-2-sulphonic acid as a function of the eluent concentration of sodium (□) hexyl- and (△) octylsulphonate pairing ions. Measurements were made in methanol–aqueous phosphate buffer (pH 2.1) (10:90, v/v) eluents of constant ionic strength (175 m*M* Na^+, adjusted with sodium bromide) on an ODS-Hypersil column. The dashed line is the theoretical slope. See ref. 26 for experimental details.

The adsorption term $\ln n_0 K_A$ depends both on the hydrophobicity of the pairing ion and the concentration of the organic modifier. In practice, when using higher organic modifier concentrations one needs to use more hydrophobic pairing ions to reach a high enough retention. In other words, one has to select a pairing ion which can establish a sufficiently high electrostatic surface potential. Recommendations based

on measured adsorption data have been published by Bartha *et al.* [29] for the practical selection of the most common pairing ions (alkylsulphonates and tetraalkylammonium ions) and their eluent concentration in combination with increasing concentration of the organic modifier.

3.4. Effect of type and concentration of organic modifier in the mobile phase

In reversed-phase chromatography, the logarithm of the capacity factor for an uncharged analyte is often described as a linear function of the concentration of organic modifier in the mobile phase (φ):

$$\ln k_B(\varphi) = \ln k_{wB} - S_B\varphi \quad (13)$$

where k_{wB} is the capacity factor for analyte B in water and S_B is a constant for a given analyte–solvent combination. The physical interpretation of this relationship is that the free energy of adsorption is a linear function of the organic modifier concentration. For an uncharged analyte ($z_B = 0$) there is no electrostatic term in the total free energy of adsorption (Eq. 2), and by applying Eq. 1 we can rewrite Eq. 13 as

$$-\frac{\Delta G_B^0(\varphi)}{RT} = -\frac{\Delta G_B^0(\varphi = 0)}{RT} - S_B\varphi \quad (14)$$

For IP chromatography this relationship therefore means that the "chemical" part of the free energy of adsorption for the analyte (Eq. 2) decreases linearly with increasing concentration of organic modifier in the mobile phase. An analogous linear relationship for the "chemical" component of the free energy of adsorption for the pairing ion as a function of organic modifier concentration in the mobile phase is therefore to be expected.

$$\ln K_A(\varphi) = -\frac{\Delta G_A^0(\varphi)}{RT} = -\frac{\Delta G_A^0(\varphi = 0)}{RT} - S_A\varphi \quad (15)$$

The electrostatic component of the free energy of adsorption is, as before, governed by the electrostatic surface potential. We have shown earlier [24] from the analysis of adsorption isotherm data for alkylsulphonate pairing ions that this assumption is reasonable. A major advantage of the simplified equation (Eq. 10) for the capacity factor is that it separates the contributions from electrostatics, type of analyte and IP reagent from each other. After applying Eqs. 14 and 15 to Eq. 10, the following equation is obtained when $c_A > 0$:

$$\ln k_{cB}(\varphi) = \ln k_{0B}(\varphi = 0) - S_B\varphi + \left(\frac{-z_A z_B}{z_A^2 + 1}\right) \times \left[\ln K_A(\varphi = 0) - S_A\varphi + \ln\left(\frac{n_0 c_A}{\kappa}\right) + \ln\left(\frac{F^2}{RT\varepsilon_0\varepsilon_r}\right) + 1\right] \quad (16)$$

Although this equation relates the capacity factor for the analyte at a given concentration of organic modifier, φ, to its capacity factor in a mobile phase in which $\varphi = 0$, the relationship can equally well be applied to estimate the changes in k_{cB} fro any starting concentrations of organic modifier.

Assuming constant ionic strength and neglecting the effect of variations in the dielectric constant on the surface potential (note that the inverse Debye length, κ, also contains the dielectric constant), Eq. 16 can be rearranged for φ:

$$\ln k_{cB}(\varphi) = K_4 + \ln k_{0B}(\varphi = 0) - \left(S_B - \frac{z_A z_B}{z_A^2 + 1}\cdot S_A\right)\varphi + \left(\frac{-z_A z_B}{z_A^2 + 1}\right) \times \{\ln [n_0 K_A(\varphi = 0)] + \ln c_A\} \quad (17)$$

where K_4 is a constant depending on the charges, ionic strength and the dielectric constant. According to this equation, the reversed-phase retention dependence for ionized solutes on the organic modifier concentration (*i.e.*, slope of the $\ln k_{0B}$ *vs.* φ relationship, S_B) is modified in ion-pair chromatography by the dependence of the adsorption of the pairing ion (S_A) on φ and by the actual number of ionic charges. When the sample ion is oppositely charged to the pairing ion, the slope of the $\ln k_{cB}$ *vs.* φ relationship becomes steeper ($S_B + 0.5\ S_A$, for singly charged ions). If they have identical charges, the slope

becomes less steep ($S_B - 0.5\ S_A$, for singly charged ions) compared with the original reversed-phase slope (S_B).

Fig. 8a and b show the ln k_{cB} *vs.* φ plots for a positively charged (phenylalanine) and a negatively charged (naphthalene-2-sulphonic acid) solute ion in the absence (dashed lines) and presence (solid lines) of a negatively charged octylsulphonate (5 mmol/l) pairing ion [24]. It can be seen that the shifts of the slopes are in agreement with predictions from Eq. 17.

The polarity of the organic modifier influences the slope values for the dependence of solute retention (S_B) and pairing ion adsorption (S_A) as a function of φ. Generally, the less polar is the organic modifier, the steeper are the slopes

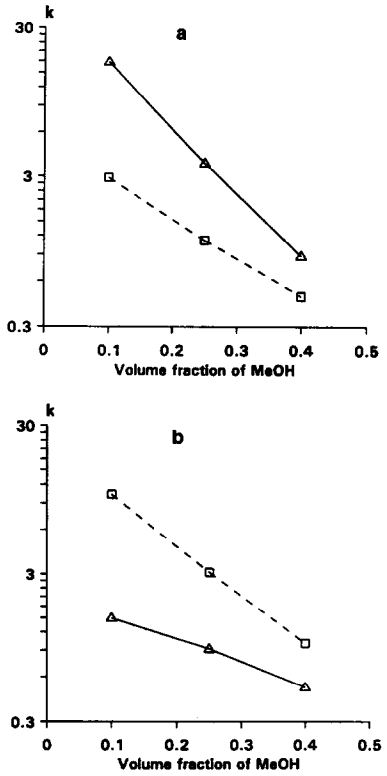

Fig. 8. Capacity factor (k) data for (a) phenylalanine and (b) naphthalene-2-sulphonic acid as a function of the methanol concentration (φ) in the phosphate buffer (pH 2.1, constant ionic strength, 175 mM Na^+) mobile phase in (□) the absence and (△) the presence (5 mM) of sodium octylsulphonate ion-pairing reagent. See ref. 24 for experimental details.

(larger absolute value). Further discussion of the organic modifier effects in IPC can be found in refs. 24 and 32.

A practical consequence of the above observation is that under IPC conditions the slope of the ln k_{cB} *vs.* φ relationship becomes steeper for oppositely charged solute ion–pairing ion combinations and the separation is less robust (while the opposite is expected for similarly charged solutes and pairing ion combinations). Further, the size of this effect is amplified by higher number of charges of the solute ions.

3.5. Effect of mobile phase electrolyte

Changing the mobile phase ionic strength induces effects that partly cancel each other out with respect to the capacity factor. The influence of ionic strength can be understood on the basis of the thermodynamic principles involving the adsorption isotherm of the IP reagent and the capacity factor of the analyte.

The free energy of adsorption of the analyte and of the IP reagent can be partitioned into a "chemical" free energy and an electrostatic free energy component. The influence of moderate variation in the ionic strength on the "chemical" component is usually very small and can be neglected in relation to the changes in the electrostatic part of the free energy of adsorption, *i.e.*, changes in the electrostatic surface potential. Assuming that there are no specific interactions, *i.e.*, no ion pairing or adsorption of the counter ions, Eq. 5 can be used to determine the influence of ionic strength on the surface potential at fixed concentration of charges on the surface. As the mobile phase salt concentration term appears in the denominator of the equation, an increase in salt concentration for this case results in a decrease in the magnitude of the electrostatic surface potential. On the other hand, when the magnitude of the surface potential decreases the adsorption of the IP reagent increases owing to there being less electrostatic "self"-repulsion, *i.e.*, the surface concentration of charges increases. The net effect is to lower the magnitude of the electrostatic surface potential, but because of the increased surface

concentration of the IP reagent the decrease is less than that simply predicted from Eq. 5. As discussed for the adsorption isotherm of the pairing ion, the effect of ionic strength on the adsorption data can be fully accounted for by the variations induced in the surface potential [21,23].

Another important question is the effect of the nature of the electrolyte ions. In the ideal case only the amphiphilic pairing ion (*e.g.*, tetrabutylammonium) adsorbs to the surface, thereby creating the surface potential. However, electrolytic counter ions with slight hydrophobic properties (*e.g.*, bromide, acetate) can also adsorb on the surface layer, thus reducing the effective surface charge concentration. Ståhlberg and Hägglund [21] studied the adsorption of tetrabutylammonium ion in the presence of different electrolytic counter ions. They concluded that the effect can be described by a term in the "chemical" component of the free energy of adsorption, which is added to the non-specific electrostatic energy (see ref. 21 for details), *i.e.*, by applying the same principles as for the adsorption of analyte and IP reagent, respectively.

In conclusion, the concentration of the electrolyte influences the retention of ionic analytes through modifying the surface potential established by the adsorbing pairing ion. The higher the ionic strength, the lower is the surface potential. With increasing ionic strength both the attraction of oppositely charged analytes and the repulsion of similarly charged analytes decreases.

When the surface concentrations are relatively low and no specific adsorption of the electrolytic counter ions occurs, Eq. 10 can be used to predict the effect of the ionic strength on the capacity factor. The ionic strength effect on the surface potential and the capacity factor is embedded in the inverse Debye length (κ). Therefore, by substituting Eq. 7 into Eq. 10 for κ, one can obtain an expression for the capacity factor which is explicit for the ionic strength (I):

$$\ln k_{cB} = K_5 + \frac{1}{2} \cdot \left(\frac{z_A z_B}{z_A^2 + 1} \right) \cdot \ln I - \left(\frac{z_A z_B}{z_A^2 + 1} \right) \cdot \ln c_A \quad (18)$$

where K_5 is a constant depending on the respective charges and hydrophobicity of the solute and the pairing ion and on the organic modifier concentration. According to Eq. 18, with increasing ionic strength the retention of oppositely (similarly) charged solute ions decreases (or increases) at a given pairing ion concentration. The slope of the $\ln k_{cB}$ *vs.* $\ln I$ relationship depends on the number of charges of the solute and pairing ions. The retention of singly charged analyte ions that are opposite in charge to the pairing ion is expected to *decrease* with a slope of $-1/4$. The retention of similarly charged ions is expected to *increase* with a slope of $+1/4$.

A typical experimental example is shown in Fig. 9, where retention data from Van de Venne *et al.* [31] are plotted for negatively charged hydroxybenzoic acids as a function of ionic strength (varying concentration of phosphate buffer in the eluent) as a constant mobile phase concentration of the positively charged ion-pairing reagent (5 m*M* hexylamine). There is generally good agreement between the theoretical slope (shown by the dashed line) and the experimental behaviour. Many other examples can

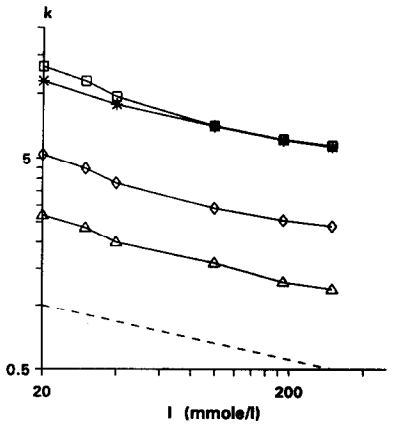

Fig. 9. Capacity factor (k) data for dissociated carboxylic acids as a function of ionic strength (I) of the phosphate buffer (pH 7) mobile phase at a constant concentration (9.2 m*M*) of hexylamine as ion-pairing reagent. Data were measured by Van de Venne *et al.* [31] using a LiChrosorb RP-18 column. Solutes: △ = 3,5-dihydroxybenzoic acid; ◇ = 4-hydroxymandelic acid; * = 2,4-dihydroxybenzoic acid; □ = mandelic acid. The dashed line is the theoretical slope. For other conditions, see ref. 31.

be found in the literature for decrease (and increase) in retention of oppositely (and similarly) charged solutes and pairing ions with the addition of inorganic salts (*e.g.*, [26,32]).

Another interesting feature of Eq. 18 is its prediction of the simultaneous effect of the pairing ion concentration and the ionic strength. When the eluent concentration of the pairing ion is varied, the ionic strength can either be kept constant by the addition of an inorganic salt or can be left to vary. In the first case, the slope of the $\ln k_{cB}$ *vs.* $\ln c_A$ relationship is constant and proportional to the charges as shown above. In the second case the ionic strength becomes a function of the concentrations of the initial buffer and the pairing ion and the slope of the $\ln k_{cB}$ *vs.* $\ln c_A$ will vary between 1/2 and 1/4 for singly charged ions. A special case when there is no buffer, or where its concentration is negligible compared with the concentration of the pairing ion, and the ionic strength will be practically equal to c_A, so that Eq. 18 simplifies to

$$\ln k_{cB} = K_5 - \frac{1}{2} \cdot \left(\frac{z_A z_B}{z_A^2 + 1} \right) \cdot \ln c_A \qquad (19)$$

According to Eq. 19, the slope of the $\ln k_{cB}$ *vs.* $\ln c_A$ relationship will be only half of the values given in Table 1. Some experimental data taken from Jandera *et al.* [33] and theoretical slope values predicted from Eq. 16 for multiply charged solutes are given in Table 2. Only strong acids (naphthalenesulphonic acids) were selected as examples as these are fully ionized and have a well defined number of charges.

Although the concentration range of the pairing ion (and the ionic strength) is fairly low, the experimental slopes show relatively good agreement with those predicted from theory. Again one must remember that quantitative predictions from the model are expected to agree within 10–25% and are mainly applicable in the concentration ranges discussed in connection with Eq. 10.

An important consequence of the above analysis is that (as for all other parameters discussed) the effect of the ionic strength is amplified by the number of charges on the solute and the pairing ions. Zhang *et al.* [28,34] recently published capacity factor *vs.* salt concentration data for multiply charged solutes. Their data show good qualitative agreement with predictions from Eq. 18, *i.e.*, the retention of oppositely and multiply charged analyte ions decreases more steeply than that for singly charged ions.

3.6. Effects of the stationary phase

Pore size of the reversed-phase packing

The stationary phase in RP-IPC typically consists of porous particles. The adsorbed pairing ion establishes an electrostatic potential both in the pores and on the outside of the particles. The simplified treatment of the capacity factor (*cf.*,

Table 2
Experimental (fitted by linear regression, $r > 0.98$) and theoretical (calculated from Eq. 19) slope values of the $\ln k_{cB}$ *versus* $\ln c_A$ relationship for some differently charged naphthalenesulphonic acids ($z_B = -1$ to -3) in the presence of 1.5–4.0 m*M* tetrabutylammonium bromide IP reagent ($z_A = +1$) in methanol–water (35:65, v/v) as mobile phase on an octadecylsilica column

Compound	z_B	Experimental slope	Theoretical slope
2-Naphthalenesulphonic acid	−1	0.298	0.25
1,5-Naphthalenedisulphonic acid	−2	0.497	0.50
1,6-Naphthalenedisulphonic acid	−2	0.582	0.50
2,6-Naphthalenedisulphonic acid	−2	0.454	0.50
2,7-Naphthalenedisulphonic acid	−2	0.532	0.50
1,3,5-Naphthalenetrisulphonic acid	−3	0.800	0.75
1,3,6-Naphthalenetrisulphonic acid	−3	0.687	0.75
1,3,7-Naphthalenetrisulphonic acid	−3	0.721	0.75

See ref. 33 for more details.

Eq. 10) is based on the solution of the linearized Poisson–Boltzmann equation for planar surfaces (see Eq. 6) rather than in the pores. In order to examine the possible effect of pores, they may be considered as a cylindrical surface. For such surfaces the relationship between the surface potential, Ψ_0, and the concentration of surface charge, n_A, can be obtained [20] as

$$\Psi_0 = \frac{z_A n_A F I_0(\kappa r)}{\kappa \varepsilon_0 \varepsilon_r I_1(\kappa r)} \quad (20)$$

where $I_0(\kappa r)$ and $I_1(\kappa r)$ are modified Bessel functions of the first kind (of order zero and one), r is the pore radius of the stationary phase and κ is the inverse Debye length. The solution of the Poisson–Boltzmann equation for planar and cylindrical surfaces differs in the factor of $I_0(\kappa r)/I_1(\kappa r)$. When the Debye length ($1/\kappa$) is relatively small compared with the pore radius, this factor becomes a constant close to unity and Eq. 20 reduces to that for planar surfaces. Table 3 gives some typical data for ionic strength and the corresponding Debye length, in addition to nominal pore sizes for reversed-phase packings.

It can be seen that if the ionic strength is at least 10 m*M* (which is a minimum requirement for buffer concentration in IPC [4]) and the pore diameter of the reversed-phase packing material is at least 100 Å, the ratio $I_0(\kappa r)/I_1(\kappa r)$ changes less steeply with ionic strength and converges to unity. This conclusion is supported by calculations by Weber [35], who determined the theoretical potential profile from an analytical solution of the Poisson–Boltzmann equation for idealized (200 Å diameter) pores of the stationary phase. The results showed that the pore geometry plays a significant role below 10 m*M* electrolyte concentration.

Table 3
Typical values of $I_0(\kappa r)/I_1(\kappa r)$ as a function of ionic strength and pore radii

Ionic strength (m*M*)	$1/\kappa$ (Å)	r (Å)	κr	$I_0(\kappa r)/I_1(\kappa r)$
1	97	50	0.52	4.01
5	43	50	1.15	2.01
10	31	50	1.63	1.63
25	19	50	2.58	1.29
50	14	50	3.60	1.18
100	9.7	50	5.15	1.11

Calculations were made assuming a pore diameter of 100 Å and a dielectric constant of $\varepsilon_r = 80$ for the aqueous mobile phase and room temperature (25°C).

In conclusion, under realistic experimental conditions when the ionic strength and pore size of the stationary phase are reasonably large, the ratio $I_0(\kappa r)/I_1(\kappa r)$ can be considered as constant, and therefore the simplified retention model (Eq. 10) can be used for the discussion of stationary phase effects.

Adsorption capacity of the reversed-phase packing

The use of stationary phases with higher hydrophobicity (*e.g.*, longer alkyl chains or higher bonded-phase ligand density) influences the retention of ionic analytes by providing higher $-\Delta G_A^0$ and $-\Delta G_B^0$ values. Increased adsorption of the pairing ion results in higher electrostatic surface potentials and consequently larger changes in solute retention.

At constant ionic strength and organic modifier concentration, a simplified equation can be derived from Eq. 10:

$$\ln k_{cB} = K_6 + \ln k_{0B} - \left(\frac{z_A z_B}{z_A^2 + 1}\right) \cdot \ln (n_0 K_A) - \left(\frac{z_A z_B}{z_A^2 + 1}\right) \cdot \ln c_A \quad (21)$$

where K_6 is a constant depending on the charge of the solute and the pairing ions, ionic strength and organic modifier of the eluent.

Stationary phases with different hydrophobicities and monolayer capacities will influence both the second and third terms of Eq. 21, *i.e.*, the adsorption of the solute without pairing ion ($\ln k_{0B}$) and the adsorption term (monolayer capacity, n_0, and adsorption constant, K_A) of the pairing ion. Therefore, the retention of ionic solutes, $\ln k_{cB}$, should be a linear function of the mobile phase concentration of the pairing ion, $\ln c_A$, on any of the reversed-phase columns at constant ionic strength and organic modifier

concentration (provided that the pairing ion surface concentration is well below the monolayer capacity of the respective stationary phase). In similar fashion to the effect of the pairing ion hydrophobicity, changing the reversed-phase packing material will influence only the position of the ln k_{cB} *vs.* ln c_A plot but not its slope.

In Fig. 10a and b, the retention dependence of dopamine ($z_B = +1$) and 2-naphthalenesulphonic acid ($z_B = -1$) is shown as a function of the eluent concentration of sodium octylsulphonate ($z_A = -1$) as pairing ion on seven different reversed-phase columns at constant ionic strength (0.175 *M*) [30]. The theoretical slopes (+1/2 and −1/2) are indicated by dashed lines. Generally, good agreement is found between the theoretically expected and the experimental behaviour irrespective of the silica base or ligand chain length of the packings.

A practical consequence of this behaviour is that solute retention changes with pairing ion concentration can be easily predicted on any of the stationary phases (without a knowledge of the actual adsorption isotherm data) after measuring the capacity factor at only one pairing ion concentration. Differences in the adsorption of the pairing ion do not influence the slope (robustness) of the logarithmic retention plot. This interesting observation can be expected to have profound practical implications for methodological design in RP-IPC.

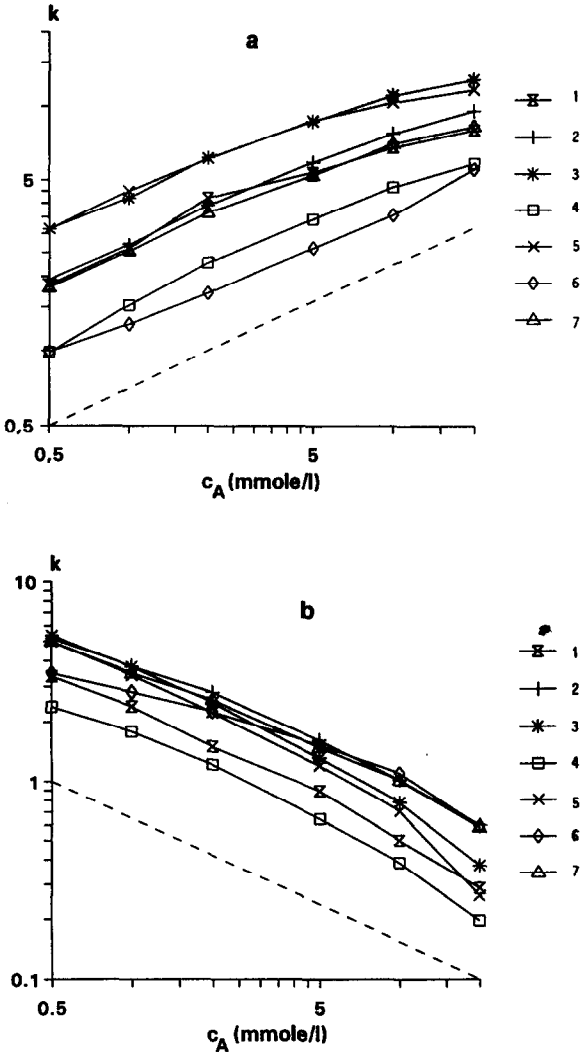

Fig. 10. Capacity factor (k) data for (a) dopamine and (b) naphthalene-2-sulphonic acid as a function of the mobile phase concentration (c_A) of sodium octylsulphonate on seven reversed-phase columns: 1 = LiChrosorb RP-18; 2 = Nucleosil C_{18}; 3 = Dimethyl-ODS; 4 = LiChrosorb RP-8; 5 = Supelco S C_{18}; 6 = BST-C_{18}; 7 = ODS-Hypersil. All measurements were made in methanol–aqueous phosphate buffer (pH 2.1) (10:90, v/v) eluents of constant ionic strength (175 m*M*, adjusted with sodium bromide). The theoretical slope predicted from the electrostatic model is shown by the dashed line.

Dissociation of silanol groups

The retention data of acids and especially bases measured on different silica-based reversed-phase columns often show large variations. Deviations are usually accounted for by the effect of silanol groups remaining on the silica surface after the chemical bonding process. The largest effect on the retention of basic (positively charged) substances usually occurs in buffers at pH values higher than the pK_a value of the silica gel, where the residual silanol groups become partly ionized, so that the negatively charged silanol groups establish a certain (negative) surface potential even in the absence of amphiphilic ions. This means that the reference point for the capacity factor (k_{0B}) in the electrostatic model corresponds to non-zero surface potential and in the ion-pair chromatographic

mode the adsorbing amphiphilic pairing ion meets a negatively charged surface.

When the pairing ion is negatively charged, its adsorption will add to the negative potential. When positively charged pairing ions adsorb on the negatively charged surface, the surface potential will be determined by the net surface concentration of charges ($[n_{A^+}] - [n_{SiO^-}]$). Therefore, the adsorption of the positively charged pairing ion has first to counterbalance this negative potential, before the surface can become positively charged.

As Eq. 21 is based on relative surface potential, it will probably be applicable as a first approximation for low and moderate concentrations of dissociated silanols. However, further experimental data are needed to clarify the effect of dissociated silanol groups on the adsorption isotherm of the ion-pairing reagents.

3.7. Effect of eluent pH

In the discussion so far, we have assumed a constant eluent pH and fully ionized solute (and pairing) ions. However, variation of the eluent pH can introduce large changes in the degree of ionization and consequently in the retention of weak acids and bases in both the regular reversed-phase and the ion-pair chromatographic modes. In reversed-phase chromatography, the capacity factor of partly ionized analytes, k_B, is the weighted sum of the capacity factor for the charged, k_{iB}, and the uncharged, k_{uB}, species, respectively

$$k_B = (1-f)k_{uB} + fk_{iB} \quad (22)$$

where f is the fraction of charged analyte at the given pH value. At a constant pH the addition of IP reagent influences only the retention of the ionized fraction of the analyte. Retention in the ion-pair chromatographic mode can be expressed by substituting Eq. 4a for the capacity factor in the presence of the IP reagent into Eq. 22:

$$k_B = (1-f)k_{uB} + fk_{0iB}e^{-\frac{z_i F\Psi_0}{RT}} \quad (23)$$

where k_{0iB} is the capacity factor of the fully ionized form of the analyte in the absence of IP reagent. Expressing the fraction of the charged analyte as a function of the hydrogen ion concentration ($[H^+]$) and the dissociation constant(s) (K_a), one can derive retention equations for weak acids and bases [25].

It has been demonstrated that expressions analogous to those used in RP chromatography can be obtained, and the experimental retention data agreed reasonably well with model predictions both for weak and strong acids and bases [25]. In order to use the model for predictive purposes, one needs the pK value, the k values of the charged and uncharged forms of the solute ion, and the retention or the value of the surface potential at a given pairing ion concentration (the latter can be obtained from Eq. 4b by measuring the retention of one fully ionized solute in the absence and in the presence of the pairing ion). As a result, measurements in three eluents provide a starting point to estimate the magnitude of retention shifts at other eluent compositions. As the retention estimates have an error margin of 10–20%, the above equations can be utilized in estimating the initial mobile phase conditions which provide reasonable retention for certain solute ion–pairing ion combinations, rather than for describing collected retention data.

3.8. Summary of the simplified electrostatic model

The major advantage of the simplified electrostatic model is that it allows us to discuss and understand the contributions of different chromatographic variables in physico-chemically meaningful terms. While the limitations of this simplified treatment must be kept in mind (*cf.*, discussion of Eq. 10), the explicit expression for the capacity factor can be utilized to predict the magnitude and direction of retention changes brought about by the major variables in ion-pair chromatographic systems. To conclude the discussion of individual parameters, Eq. 10 can be rewritten in the following form, at constant pH and organic modifier concentration:

$$\ln k_{cB} = \ln k_{0B} + \left(\frac{-z_A z_B}{z_A^2 + 1}\right) \cdot \left[\ln (n_0 K_A) + \ln c_A - \frac{1}{2} \cdot \ln I + \ln \left(\frac{e^2 F^2}{RT\varepsilon_0 \varepsilon_r}\right)^{1/2}\right] \quad (24)$$

or by including the effect of the organic modifier concentration:

$$\ln k_{cB}(\varphi) = \ln k_{0B}(\varphi = 0) - \left(S_B - \frac{z_A z_B}{z_A^2 + 1} \cdot S_A\right)\varphi + \left(\frac{-z_A z_B}{z_A^2 + 1}\right) \cdot \left[\ln (n_0 K_A(\varphi = 0) + \ln c_A - \frac{1}{2} \cdot \ln I + \ln \left(\frac{e^2 F^2}{RT\varepsilon_0 \varepsilon_r}\right)^{1/2}\right] \quad (25)$$

where the temperature (T) and the dielectric constant of the mobile phase (ε_r) are collected in the last logarithmic term together with other constants. The effect of the chromatographic variables on the capacity factor of ionized solutes is shown in Table 4.

It must be pointed out that throughout this discussion we have concentrated on understanding and predicting retention and not separation selectivity in ion-pair chromatographic systems. One must realize that often very small differences in the chemical structure of the solutes can result in specific interactions with the different components of the chromatographic system. Prediction of small changes in retention (and selectivity) would require more sophisticated retention models, both for the underlying reversed-phase system and for the IPC system.

4. Capacity factor when competition with IP reagent for the limited surface area occurs

It was stated earlier that there are several theoretical levels at which the capacity factor of the analyte can be described. In the first discussion, the effect of the chemical and electrostatic free energies of adsorption of the analyte are considered. After discussion of the adsorption isotherm of the IP reagent, the effect of the limited monolayer capacity of the stationary phase can be included.

A theoretical analysis shows [21] that when there is a one-to-one competition between the analyte and the IP reagent for the limited area accessible, the following expression is obtained for the capacity factor:

$$k_{cB} = \frac{k_{0B} e^{-\frac{z_B F \Psi_0}{RT}}}{1 + K_A c_A e^{-\frac{z_A F \Psi_0}{RT}}} \quad (26)$$

where the definitions of the symbols are as before. The degree of competition between the analyte and IP reagent for the surface area may, however, depend on the type of IP reagent and analyte used. The capacity factors of *p*-toluenesulphonate and adrenaline were found to follow this equation with a series of alkylsulphonates as IP reagents [21]. On the other hand, the simpler Eq. 4 could be used over the entire surface concentration range when tetrabutylammonium ion was used [23]. This difference in behaviour can be explained by the difference in the adsorbed layer: the alkylsulphonate ions adsorb so that the charged polar sulphonate group is oriented towards the polar mobile phase and thereby the hydrophobic contact area between these two phases is reduced, whereas the symmetrically placed alkyl chains of tetrabutylammonium ion intermingle with the alkyl chains of the stationary phase.

An often debated phenomenon in the literature of ion-pair chromatography is the parabolic concentration dependence of retention, *i.e.*, with increasing concentration of IP reagent the capacity factor of an oppositely charged analyte increases to a maximum followed by a decrease at higher concentrations of IP reagent [3]. The understanding of such behaviour is straightforward if micelle formation has occurred in the mobile phase. If the ionic strength is not kept constant when increasing the IP reagent concentration, it can also counteract the retention increase (see earlier discussion). However, a maximum in k'_{cB} may still occur for concentrations of IP reagent below the critical micelle formation concentration (CMC) even when the ionic strength is kept constant, although it is not as pronounced as under non-constant conditions.

Table 4
Effect of increasing the value of different chromatographic variables in reversed-phase ion-pair chromatographic systems according to the simplified electrostatic model

Variable	Effect	k (oppositely charged solute)	k (similarly charged solute)
Charge of analyte ion ($z_B = \pm 1, 2, \ldots$)	Amplifies the effect of all parameters below	Increasing number of charges of the solute ion increases the absolute slope of the ln k_{cB} *vs.* ln c_A relationship	
Charge of pairing ion ($z_A = \pm 1, 2, \ldots$)	Determines the sign of the electrostatic surface potential	Increasing number of charges of the pairing ion slightly decreases the absolute slope of the ln k_{cB} *vs.* ln c_A relationship	
Concentration of pairing ion (c_A)	Increases the absolute value of the electrostatic surface potential through the adsorption of pairing ion molecules on the hydrophobic stationary phase	Increases owing to attraction to the charged surface	Decreases owing to repulsion from the charged surface
Hydrophobicity of pairing ion (K_A)	The adsorption constant of the pairing ion increases with increasing hydrophobicity. The more hydrophobic the IP reagent, the higher is its adsorption under identical eluent conditions	Increases owing to higher electrostatic surface potential	Decreases owing to higher electrostatic surface potential
Concentration of organic modifier (φ)	Decreases both the adsorption of the pairing ion (lower surface potential) and the reversed-phase retention of the analyte (lower polarity of the mobile phase)	Decreases. The slope of the ln k_{cB} *vs.* φ relationship becomes steeper compared with the regular RP slope	Decreases. The slope of the ln k_{cB} *vs.* φ relationship becomes less steep compared with the regular RP slope
Type of organic modifier (S_A, S_B)	Less polar organic modifiers lead to larger decreases in both pairing ion adsorption and solute retention	The slope of the ln k_{cB} *vs.* φ relationship becomes even steeper	The slope of the ln k_{cB} *vs.* φ relationship becomes even less steep
Ionic strength (I)	The adsorption of the pairing ion slightly increases while the electrostatic surface potential decreases	Decreases owing to lower electrostatic surface potential	Increases owing to lower electrostatic surface potential
Type of stationary phase	The higher the adsorption capacity of the stationary phase, the higher are the surface concentration of the pairing ion and the electrostatic surface potential. It affects the RP retention of the solute (k_{0B}) and the adsorption term ($n_0 K_A$) of the pairing ion	A parallel shift of the ln k_{cB} *vs.* c_A relationship occurs for both positively and negatively charged analytes when using different columns	
Eluent pH	Influences the ionization of solute (and pairing) ions. If the solute becomes more ionized, the retention contribution of the electrostatic interactions increases	Retention of the ionized form increases; it can become even larger than the regular RP retention of the non-ionized form	Retention of the ionized form decreases

One major reason for this retention behaviour is the competition for available surface area as described by Eq. 26.

In Fig. 11, the experimentally found capacity factor of adrenaline is shown as a function of the eluent concentration of octylsulphonate [26] at two different (constant) ionic strengths, 0.095 and 0.175 *M*. Model calculations were performed by a numerical computation solving simultaneously the Gouy–Chapman equation (Eq. 5) for the electrostatic surface potential and Eq. 26 for the capacity factor using Mathcad ver. 3.0. In order to obtain a reasonable fit to the experimental values, the previously reported value for n_0 ($1.81 \cdot 10^{-6}$ mol/m^2) [21] and a slightly adjusted value of K_A (2.5 m^3/mol) were used. Note that the same set of values is used for both data series and that the value of K_A agrees well with that obtained for the adsorption isotherm of octylsulphonate, as reported in ref. 21. It can be seen that the extended model (indicated by the lines) predicts the experimentally observed fold

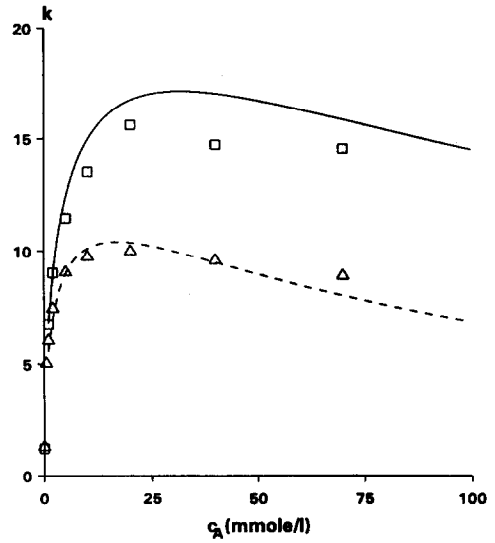

Fig. 11. Capacity factor (k) data for adrenaline as a function of the mobile phase concentration (c_A) of sodium octylsulphonate at two different (constant) ionic strengths, (□) 95 m*M* and (△) 175 m*M*. Other conditions as in ref. 26. The lines were calculated from the electrostatic model for high pairing ion surface concentrations (see text) using as parameter values $k_{0B} = 1.2$, $n_0 = 1.81 \cdot 10^{-6}$ mol/m^2 and $K_A = 2.5$ m^3/mol. See text for details.

over of the capacity factor at high pairing ion concentrations, while using realistic (experimental) values for the monolayer capacity and the adsorption constant.

One of the simplifications made in the derivation of this model is the assumption that the analyte ion and the pairing ion require approximately the same surface area for their adsorption. According to Eq. 26, the ratio k_{cB}/k_{0B} for two different solutes having the same charge should be constant (*i.e.*, the ln k_{cB} *vs.* ln c_A plots should run parallel). Whereas at low surface concentrations of the pairing ion this assumption proved to be reasonable [21,22], at high concentrations solutes (even with similar charge and chemical structure [36]) can show different retention behaviour [37]. Recently, Narkiewicz-Michalek [37] suggested that such behaviour can be accounted for by differences in the required surface area for adsorption of the analyte ion and the pairing ion, and extended the electrostatic model by including a multi-site occupancy model for adsorption.

In conclusion, according to the extended electrostatic model, competition for the available surface area on the stationary phase between the analyte ions and pairing ions will decrease the overall retention when the surface concentration of the pairing ion is high (*ca.* $n_A > 0.3n_0$). For oppositely charged solute ion–pairing ion combinations, this will result in a fold over of the ln k_{cB} *vs.* ln c_A plots. For similarly charged solute ion–pairing ion combinations it will decrease retention even further. A better fit to retention data can be obtained by refining the model with the different surface area requirements of solute ion and pairing ion adsorption.

5. Extended theory of ion-pair chromatography

5.1. Discussion of the distance-dependent capacity factor

The theory presented so far has treated the capacity factor as a result of the distribution between the mobile phase and the stationary phase surface. Close to the charged stationary

phase there is the double layer in which oppositely charged ions (both analytes and bulk electrolyte ions) are accumulated and similarly charged ions are depleted. The effect of accumulation (or depletion) on the capacity factor of analyte ions is not considered in the original version of the theory and is usually of minor importance for organic analyte ions. There are, however, conditions under which this effect cannot be neglected and it is therefore pertinent to include it to complete the treatment.

For porous packing materials the contribution to the capacity factor from the small fraction of the surface that lies outside the particle can be neglected. The retention of analyte ions is mainly due to the adsorption of ions at the pore surface and, to a certain extent, to accumulation in the double layer extending from the pore surface into the pore volume, where the mobile phase is stagnant. The mathematical relationship between the excess (or deficiency) of analyte in the stagnant mobile phase in the pores and its capacity factor has recently been formulated and applied to the ion-exchange chromatography of proteins [17] and small ions [18]. The detailed derivation of the final equation is complex and can be found in ref. 17; the final result is

$$k_{DL} = \frac{A_s}{V_0} \cdot \int_0^{x'} \{\exp[-\Delta G_t(x)/RT] - 1\}\, dx \quad (27)$$

where A_s is the area of the stationary phase and V_0 the column dead volume. The integral represents the sum of surface excesses of the analyte over the distance, x, from the surface. $\Delta G_t(x)$ represents the total free energy change in moving the analyte from the bulk of the mobile phase to a point at a distance x from the stationary phase surface. In ion-pair chromatography, $\Delta G_t(x)$ is entirely due to the change in electrostatic potential between the bulk of the mobile phase and the point x, $\Delta\Psi(x)$. Since the convention is that the electrostatic potential is zero in the bulk of the mobile phase, we can substitute $\Delta\Psi(x)$ by $\Psi(x)$ so that $\Delta G_t(x) = z_B F\Psi(x)$ in Eq. 27. The measured capacity factor, k_{cBt}, is the sum of contributions from surface adsorption, k_{cB} (Eq. 26), and accumulation in the double layer, k_{DL}:

$$k_{cBt} = k_{cB} + k_{DL} = \frac{k_{0B} e^{-\frac{z_B F\Psi_0}{RT}}}{1 + K_A c_A e^{-\frac{z_A F\Psi_0}{RT}}} + \frac{A_s}{V_0} \cdot \int_0^{x'} \{\exp[z_B F\Psi(x)/RT] - 1\}\, dx \quad (28)$$

For a planar geometry the potential at a point situated a distance x from the surface is approximately $\Psi(x) = \Psi_0 \exp(-\kappa x)$, which can be used in Eq. 27. The resulting integral can only be solved by numerical methods but, as discussed in the Appendix, after making the series expansion $\exp(-\kappa x) \approx (1 - \kappa x)$, the integral can be solved approximately:

$$k_{DL} = \frac{A_s}{V_0} \cdot \int_0^{x'} (e^{-\frac{z_B F\Psi_0 e^{-\kappa x}}{RT}} - 1)\, dx \approx \frac{A_s}{V_0} \cdot \frac{1}{\kappa} \left[\frac{RT(1 - e^{-\frac{z_B F\Psi_0}{RT}})}{z_B F\Psi_0} - 1 \right] \quad (29)$$

The final approximate equation for the capacity factor is obtained by substituting Eq. 29 into Eq. 28:

$$k_{cBt} = k_{cB} + k_{DL} = \frac{k_{0B} e^{-\frac{z_B F\Psi_0}{RT}}}{1 + K_A c_A e^{-\frac{z_A F\Psi_0}{RT}}} + \frac{A_s}{V_0} \cdot \frac{1}{\kappa} \left[\frac{RT(1 - e^{-\frac{z_B F\Psi_0}{RT}})}{z_B F\Psi_0} - 1 \right] \quad (30)$$

From this equation it is inferred that the contribution from accumulation in the double layer becomes of primary importance when k_{0B} is very small, *e.g.*, for inorganic ions, or for low ionic strengths, *i.e.*, when $1/\kappa$ is large.

The contribution of the accumulations of ions in the double layer to the final capacity factor as a function of mobile phase ionic strength or its κ value is shown in Fig. 12a and b for analyte ions with two different k_{0B} values (0.1 and 0.3) (for a constant surface potential of 50 mV, neglecting any competition for available surface area), *i.e.*, the denominator in the first term is equal to unity. For each k_{0B} value (using a constant

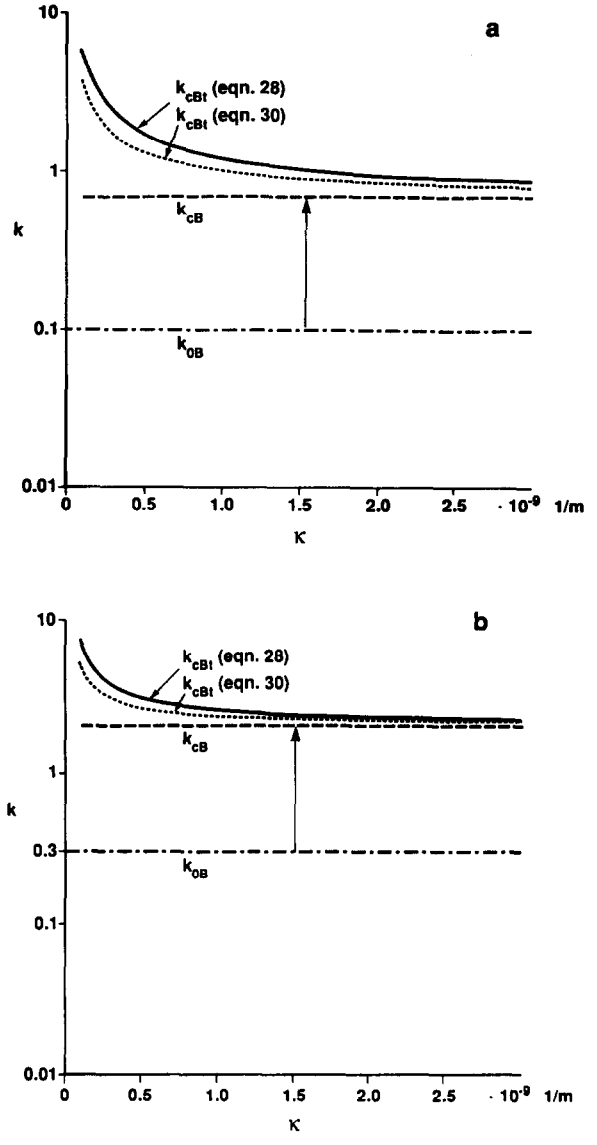

Fig. 12. Contribution of the accumulation of ions in the double layer to the capacity factor (k) at a constant surface potential (50 mV) as a function of the inverse Debye length (κ) (or ionic strength). Retention of hypothetical positively charged analyte ions with a reversed-phase retention (k_{0B}) of (a) 0.1 and (b) 0.3. Other parameters: phase ratio (A_s/V_0), $\phi = 1.5 \cdot 10^8$ m^2/m^3; $z_A = -1$; $z_B = +1$; temperature, $T = 300$ K. See text for discussion.

surface potential of 50 mV), the resulting k_{cB} value was calculated and connected to the respective k_{0B} value with an arrow as shown. The term k_{cB} corresponds to the capacity factor calculated from the simple version of the theory, calculated from Eq. 4a. The resulting k_{cBt} value as a function of the inverse Debye length (κ) was calculated by numerical integration of Eq. 28. The difference between the lines of k_{cBt} and k_{cB} in Fig. 12 is equal to k_{DL} as a function of κ. To compare the values of k_{cBt} obtained by the numerical evaluation of the integral in Eq. 28 with those obtained from the approximate analytical solution in Eq. 30, both sets of data are plotted in Fig. 12 using the same set of calculation parameters.

Fig. 12 shows that, under these conditions, the influence of accumulation in the double layer can be neglected when the k_{0B} value is larger than 0.3 (Fig. 12b). It can be seen that for lower k_{0B} values the effect of accumulation becomes increasingly important and dominates the retention for k_{0B} values lower than 0.1 (Fig. 12a). It is found that the approximate Eq. 30 underestimates the contribution of double-layer accumulation, but that it can be used to make a first estimate of its importance relative to the surface adsorption term, k_{cB}. For practical applications of Eq. 30 a value for the column phase ratio is needed and, unless it is well known, any calculation of k_{DL} becomes approximate. When using Eq. 30 it should also be borne in mind that it is based on an assumption of planar geometry. If the pore radius is smaller than about three times the Debye length, the effect of overlapping double layers and surface curvature must be considered. Inclusion of such effects follows the same principles as used above, but becomes mathematically more complex and is therefore omitted in this presentation. An illuminating study of the combined effect of pore radius and salt concentrations has been made by Weber [35].

5.2. Brief comparison with the model for RP-IPC developed by Cantwell

A retention theory based on an ion-exchange process in the diffuse double layer combined with surface adsorption, which includes electrostatic interactions, has been developed by Cantwell and co-workers [13–15]. Without going into

detail, we shall briefly discuss two crucial points where the Cantwell model differs from that discussed in this paper.

(i) The basic assumption made by Cantwell in both the theoretical and experimental analysis is that a constant activity of the IP reagent in the mobile phase results in a constant electrostatic potential at the stationary phase surface. The argument for this assumption derives from the Nernst equation for an ion that determines the potential and Cantwell consequently uses the term potential-determining ion for the IP reagent. In this context it is appropriate to cite the discussion of potential determining ions in the AgI–water system in the classical book by Hunter ([38], p. 19):

"The important assumption in deriving Eq. 4" (*i.e.* the Nernst equation) "is that ... when the bulk activity of Ag^+ is altered, the surface activity remains constant. The justification for this assumption is that the surface of the AgI crystals contains a large number of Ag^+ and I^- ions and the few extra ions which are adsorbed in order to establish the potential Ψ_0 are not likely to affect the activity of those surface ions. The special role of the crystal lattice ions is recognized by referring to them as the *potential-determining ions* for the system, to distinguish them from ions like K^+ and NO_3^- which are not expected to enjoy a special interaction with the surface. These latter are called *indifferent ions*. Intermediate between these extremes are ions which appear to interact in some special (*e.g.*, chemical) way with the surface and these are referred to as *specifically adsorbed ions*".

When discussing the theoretical implications of the Nernst equation, Hunter [38] writes (p. 238): "For the silver halide–solution interface we shall assume the validity of the Nernst equation: ... and a similar equation should hold for any system in which the potential-determining ions are themselves constituents of the crystal lattice (*e.g.*, $BaSO_4$) so that the assumption in deriving ..." (*i.e.*, the Nernst equation) "can be assumed to hold ... *In all other cases it will be necessary to set up a more elaborate expression for* Ψ_0, *and indeed this will prove to be one of the more difficult aspects of the problem*" (present authors' italics).

In the electrostatic theory as presented in this paper, the IP reagent is treated as specifically adsorbed ions (in contrast to Cantwell and co-workers, who used the potential-determining ion concept) and Ψ_0 is calculated from the Gouy–Chapman theory or related theories describing the relationship between surface concentration of the IP reagent and Ψ_0.

(ii) Cantwell and co-workers assign a stoichiometric constant for the exchange of ions between the bulk of the mobile phase and the diffuse part of the double layer and obtained numerical values for this constant as high as 900 for the exchange between *p*-nitrobenzenesulphonate and chloride ions [39]. From their investigations, they conclude that this ion-exchange process is the dominant contribution to retention at low ionic strengths or high surface potentials. As the exchange constant is a measure of differences in solvent–analyte interactions (*i.e.*, no electrostatic interactions are included) between the bulk phase and the "double-layer phase", Cantwell and co-workers' description is tantamount to a transfer of ions between two different phases.

In the theory described in this paper, the role of the double layer in the retention process is only accounted for in the extended version, where it is considered as an accumulation of ions in the diffuse layer. It is also possible, however, to introduce an exchange constant in this treatment, which then takes the value of unity. For the moment there are no physical reasons to believe that the value of this constant should depart significantly from unity.

6. Conclusions

The retention of organic ions in RP-IPC is influenced by the choice of mobile phase parameters such as concentration of organic modifier, ionic strength, pH and type and concentration of ion-pairing reagent. The electrostatic theory of IPC offers a physically consistent and quantitative description of retention when varying these

parameters. The theoretical foundation of the electrostatic theory has its origin in surface and colloid chemistry and the basic principles may therefore fall outside the customary expertise of chromatographers. In the first section the elementary concepts of the theory are presented, focusing on a qualitative understanding of the physical principles.

The complete theory, as discussed in the theoretical sections, is mathematically complex and can only be solved using numerical methods; consequently, it is less useful for practical work. To provide a relationship that is easy to use in practice, an equation has been developed that separates the originally complicated interdependence between the chromatographic parameters into its constituent components. The limitations of the simplified theory and its usefulness have been thoroughly discussed and illustrated by using many practical examples. From these examples it can be concluded that the simplified theory is an effective tool for the understanding and prediction of the retention of solute ions of differing type and charge when the mobile phase composition is varied. The generality of the simplified theory with respect to different stationary phases has also been illustrated.

At high surface concentrations of the ion-pair reagent, competition between the solute ion and the ion-pair reagent for the limited surface area of the stationary phase occurs. As a result of this competition there is a maximum in the plot of capacity factors *versus* ion-pair reagent concentration in the mobile phase. This was illustrated for octylsulphonate as IP reagent and keeping the ionic strength constant at 0.095 and 0.175 *M*.

When the solute ion has a small adsorption constant to the stationary phase, *i.e.*, $k_{0B} < 0.3$, the contribution of accumulation in the electrical double layer to its capacity factor in the presence of the IP reagent cannot be neglected. The ion-pair chromatography of inorganic ions is a typical example for such systems. An extended version of the electrostatic theory has been presented where this contribution to the capacity factor is included. Although the extension is based on the Gouy–Chapman theory for a planar surface, the principles presented can in fact be used for any suitable geometry, *e.g.*, if the stationary phase pores are approximated by cylinders.

7. Symbols

A	ion pair reagent
(A^-)	concentration of weak acid
A_s	surface area of the stationary phase, m^2/g
B	analyte ion
c_A	concentration of ion pair reagent in the mobile phase, mol/m^3 (=m*M*)
$c_{i,0}$	bulk concentration of electrolyte ion *i*, mol/m^3 (=m*M*)
f	fraction of ionized weak acid or base analyte
F	Faraday constant, C/mol
G	Gibbs free energy, J/mol
$G(x)$	Gibbs free energy at a point located a distance x from the stationary phase surface
I	ionic strength of the mobile phase
I_0	modified Bessel functions of the first kind of zero order
I_1	modified Bessel functions of the first kind of first order
k_{0B}	capacity factor for analyte B at zero concentration of ion pair reagent
k_{cB}	capacity factor for analyte B at a non-zero concentration of ion pair reagent in the mobile phase due to surface adsorption of the analyte
k_{cBt}	capacity factor for an analyte due to both surface adsorption and accumulation in the diffuse double layer, *i.e.*, $k_{cBt} = k_{cB} + k_{DL}$
k_{DL}	capacity factor for an analyte due to accumulation in the diffuse double layer
K_A	binding constant for the binding of the ion pair reagent to the surface, m^3/mol
K_B	binding constant for the binding of the analyte ion *i* to the surface, m^3/mol
K_n	numerical constant, $n = 1–6$
n_A	surface concentration of ion pair reagent, mol/m^2

n_0 monolayer capacity of the stationary phase surface for the ion-pair reagent, mol/m^2
R gas constant, $J/mol \cdot K$
S_A slope of a log $(n_0 K_A)$ *vs.* percentage of organic modifier (in the mobile phase) plot
S_B slope of a log k' *vs.* percentage of organic modifier (in the mobile phase) plot
T temperature, K
V_0 column dead volume $(=V_s + V_m)$, m^3
x distance from the stationary phase surface, m
z_A charge of ion pair reagent ion
z_B charge of analyte ion B
z_i charge of the mobile phase electrolyte ions

Greek letters

ε_0 permittivity of vacuum, F/m
ε_r dielectricity constant of the mobile phase
κ inverse Debye length, m^{-1}
σ surface charge density on the stationary phase, C/m^2
φ percentage of organic modifier in the mobile phase
ϕ column phase ratio, m^2/m^3
Ψ_0 electrostatic potential of the surface relative to the bulk of the electrolyte, V
$\Psi(x)$ electrostatic potential at distance x from the stationary phase surface, V

8. Appendix

8.1. Derivation of Eq. 10

The capacity factor of the analyte B, k_{cB}, when the IP reagent concentration in the mobile phase is c, is related to its capacity factor at zero concentration of IP reagent, k_{0B}, through the relationship

$$k_{cB} = k_{0B} e^{-\frac{z_B F \Psi_0}{RT}} \quad \text{(A1)}$$

where z_B is the charge of the analyte and Ψ_0 is the electrostatic surface potential. According to the Debye–Hückel approximation, Ψ_0 is a linear function of the surface concentration of IP reagent, n_A (mol/m^2);

$$\Psi_0 = \frac{z_A n_A F}{\kappa \varepsilon_0 \varepsilon_r} \quad \text{(A2)}$$

where κ is the inverse Debye length defined in Eq. 7 and z_A is the charge of the IP reagent. For moderate surface concentrations of the IP reagent, n_A is related to the mobile phase concentration, c_A, through the equation

$$n_A = n_0 K_A c_A e^{-\frac{z_A F \Psi_0}{RT}} \quad \text{(A3)}$$

Substituting Eq. A1 into the right-hand side of Eq. A3 and Eq. A2 into the left-hand side and rearranging gives

$$\Psi_0 = \frac{z_A F}{\kappa \varepsilon_0 \varepsilon_r} \cdot n_0 K_A c_A \cdot \left(\frac{k_{cB}}{k_{0B}}\right)^{\frac{z_A}{z_B}} \quad \text{(A4)}$$

The value for Ψ_0 is also obtained by rewriting Eq. A1:

$$\Psi_0 = -\frac{RT}{z_B F} \cdot \ln\left(\frac{k_{cB}}{k_{0B}}\right) \quad \text{(A5)}$$

which is substituted into Eq. A4, giving

$$\left(\frac{k_{cB}}{k_{0B}}\right)^{\frac{-z_A}{z_B}} \cdot \frac{-1}{z_A z_B} \cdot \ln\left(\frac{k_{cB}}{k_{0B}}\right) = \frac{F^2}{RT\varepsilon_0 \varepsilon_r} \cdot \frac{n_0 K_A c_A}{\kappa} \quad \text{(A6)}$$

After taking the natural logarithm of Eq. A6, we obtain

$$\ln\left(\frac{k_{cB}}{k_{0B}}\right)^{\frac{-z_A}{z_B}} + \ln\left[\ln\left(\frac{k_{cB}}{k_{0B}}\right)^{\frac{-1}{z_A z_B}}\right] = \ln\left(\frac{n_0 K_A c_A}{\kappa}\right) + \ln\left(\frac{F^2}{RT\varepsilon_0 \varepsilon_r}\right) \quad \text{(A7)}$$

The double logarithmic term on the left-hand side is series expanded using the expression $\ln(x+1) = x$ for small x values, where

$$x = \ln\left(\frac{k_{cB}}{k_{0B}}\right)^{\frac{-1}{z_A z_B}} - 1 \quad \text{(A8)}$$

or

$$\ln\left\{\left[\ln\left(\frac{k_{cB}}{k_{0B}}\right)^{\frac{-1}{z_A z_B}}-1\right]+1\right\}\approx\ln\left(\frac{k_{cB}}{k_{0B}}\right)^{\frac{-1}{z_A z_B}}-1 \quad (A9)$$

which is substituted into Eq. A7 for the double logarithmic term. The final equation is obtained after rearrangement of the exponents containing z_A and z_B terms:

$$\ln k_{cB}=\ln k_{0B}+\left(\frac{-z_A z_B}{z_A^2+1}\right)\cdot\left[\ln\left(\frac{n_0 K_A c_A}{\kappa}\right)+\ln\left(\frac{F^2}{RT\varepsilon_0\varepsilon_r}\right)+1\right] \quad (A10)$$

When the capacity factor ratio k_{cB}/k_{0B} varies between 2 and 10, approximating the double logarithmic term results in a relative error below 15% when the whole Eq. A10 is compared with Eq. A7. Keeping this in mind, Eq. A10 can be used to estimate the influence of different parameters on the capacity factor of a completely ionized analyte.

8.2. Derivation of Eq. 27

The electrostatic surface potential as a function of the distance from a planar surface in contact with an electrolyte solution is approximately

$$\Psi(x)=\Psi_0 e^{-\kappa x} \quad (A11)$$

The change in free energy when transporting an ion from the bulk of the mobile phase, where $\Psi=0$, to a point located at a distance x from the surface is

$$\Delta G^0_{t,B}(x)=z_B F\Psi(x) \quad (A12)$$

The capacity factor for a distance-dependent interaction is described by the following equation, into which Eqs. A11 and A12 are substituted:

$$k_{DL}=\frac{A_s}{V_0}\cdot\int_0^{x'}\left[\exp(-z_B F\Psi_0 e^{-\kappa x}/RT)-1\right]dx \quad (A13)$$

where x' is formally chosen so that

$$A_s\cdot\int_0^{x'}dx=V_p \quad (A14)$$

where V_p is the pore volume. In the ensuing numerical evaluation of the integral in Eq. A13 it is assumed that the Debye length is much smaller than the pore radius, so that the actual value of x' becomes unimportant as long as it is larger than about three Debye lengths. The integral A13 has no closed-form solution and is therefore evaluated numerically in Fig. 12a and b. The approximate closed-form solution shown in Eq. 30 is obtained by straightforward integration after using the series expansion $\exp(-kx)\approx 1-\kappa x$ in Eq. A13 and consequently changing the upper integral limit to $1/\kappa$:

$$k_{DL}=\frac{A_s}{V_0}\cdot\int_0^{\frac{1}{\kappa}}\{\exp[-z_B F\Psi_0(1-\kappa x)/RT]-1\}dx=\frac{A_s}{V_0}\cdot\frac{1}{\kappa}\left[\frac{RT(1-e^{-\frac{z_B F\Psi_0}{RT}})}{z_B F\Psi_0}-1\right] \quad (A15)$$

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