Review

Retention models for ions in chromatography[1]

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Abstract

Since chromatography of ions is a widely used technique in analytical chemistry a basic understanding of the retention mechanism is important. The principles of the different retention models that have been proposed are examined in this paper. The focus is on those models that are derived from the physical chemistry of charged surfaces immersed in an electrolyte solution. In the first two sections the theory for the electrical double layer and the Donnan potential are presented together with experimental results from surface and colloid chemistry. In Section 3 a comparison between stoichiometric and non-stoichiometric models is made. In this section the physical meaning of the retention factor is also examined. The Donnan model and the different double layer models developed for ion exchange chromatography of small ions are discussed in Section 4. The next section presents the corresponding models that have been developed for ion pair chromatography and compares them with the experimental findings. The theoretical modifications needed when going from small ions to ionic macromolecules are discussed in the last section and the developed models are compared with the experimental results. © 1999 Elsevier Science B.V. All rights reserved.

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Contents

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1. Introduction

Many different types of chromatographic techniques are used for the analytical separation of ions and in each of them a number of different stationary phases, eluents, type of eluite ions, etc., are used. In this review retention models for three main chromatographic techniques is treated; ion-exchange chromatography of small ions, ion pair chromatography using reversed-phase stationary phases and ion-exchange chromatography of ionic macromolecules with emphasis on proteins. A common feature of these three techniques is the dominant role played by the electrostatic interaction. It is the purpose of this review to discuss, from a physical point of view, the background, advantages and limitations of the different theoretical models that are used to describe the retention of ions in analytical applications of chromatography.

The equilibrium distribution of ions between an ion exchanger and the external solvent has been extensively studied for many years. The experimental data have been interpreted according to equations derived from a theoretical model or, alternatively, fitted to an empirical relation. The drawback of an empirical model, which has been found to suit a particular system, is that it is likely that the found empirical coeffients, etc., vary with the experimental conditions. A fundamental approach, on the other hand, has value in that the results demonstrate the validity of a particular mechanism and enables the underlying processes of ion-exchange to be better understood and interpreted. However, it is important to recognise that an agreement between experiments and theory does not imply conclusively that the theory is correct. In order for the theory to be acceptable, it must also be consistent with our general physical knowledge. Since electrostatic interactions governs the ion-exchange process, the fundamental approach implies the study of the physical chemistry of charged surfaces and polyelectrolytes. Such an approach has been taken in surface and colloid chemistry where the interaction between ions and surfaces is an important field.

Few retention models are based on a strictly physical approach, instead the conceptually simpler, and in some cases more practical, stoichiometric models are widely used. A short discussion regarding

why the stoichiometric models not are appropriate for describing the electrostatic interaction is presented in Section 3.2. It is the purpose of this review to summarise the retention models that have been proposed for ionic eluites for the three mentioned chromatographic techniques, focussing on models that have their origin in surface and colloid chemistry. Recommended texts in colloid and surface chemistry are the books by Israelachvili [1], Hiemenz [2], Hunter [3] and Evans and Wennerström [4]. Hopefully this review will make a contribution to narrow the gap between chromatography and surface and colloid chemistry and to facilitate the incorporation of the knowledge accumulated in the latter field into chromatography.

This paper is organized as follows: First some basic knowledge about electrostatic interactions and charged surfaces in contact with an electrolyte solution is briefly presented, e.g. the Gouy–Chapman (G–C) theory for the electrical double layer. This section includes a discussion of the limitations of the G–C theory and also how it can be extended to include the adsorption of ions to the surface. Other double layer models and the basic concept of the Donnan potential are also presented. A short summary of results from the experimental testing of the different models that have been performed in surface and colloid chemistry is also included. The intention of the section is to provide a theoretical background to the models used in ion-exchange chromatography of small ions and in ion pair chromatography.

The remaining sections deal with the application of the various types of retention theories for ions in chromatography. The Donnan potential and double layer models, respectively, developed for ion-exchange chromatography of small ions and ion pair chromatography are examined. For ion-exchange chromatography of macromolecules these models needs to be extended. In the last section the theoretical background of the non-stoichiometric models that have been applied to ion-exchange chromatography of proteins is discussed.

Within each of the discussed techniques a great variety in the experimental conditions exists. When developing a retention model for a particular system it is important to identify the physical properties which are characteristic for that particular system and include these in the model, e.g. it can not a priori

be assumed that the same physical description applies to the retention of Na^+ in ion-exchange chromatography when different types of columns or different eluent compositions are used. Each chromatographic system is in this sense unique. Since the experimental conditions within each of these techniques may differ widely it is not possible to include all theoretical aspects in this review. Only the simplest and most typical retention models and cases are discussed. Furthermore, only the most basic results that has been found in the physical chemistry of charged surfaces can be included.

2. Charged surfaces in electrolyte solutions

2.1. General introduction to electrostatic interactions

In chromatography the two most important types of interactions are the electrostatic and van der Waals interaction, the latter is the sum of the Keesom, the Debye and the London dispersion energy. They are usually referred to as physical interactions and it is important to recognise that they lack the specificity, directionality and stoichiometry of the covalent bond. There are two properties of the electrostatic interaction which makes it important, one is that the interaction energy is long ranged and the second is its strength. For example, Coulombs law gives that the binding energy for Na^+ and Cl^- at contact ($r=2.76$ Å) in vacuum is approximately 500 kJ/mol [1], i.e. similar to the energies for covalent bonds. When the two ions are 5000 Å apart the interaction energy is approximately the same as between two Ne atoms at contact. When the electrostatic interaction takes place in a medium the interaction energy is reduced by a factor of ε, where ε is the dielectric constant of the medium. The dielectric constant for water is unusually high, its value is 80, which explains its high solubilizing power for ions. Yet, the electrostatic interaction is still strong in water and dominates the behavior of charged bodies also in this medium.

Although no exact physical law exists for the electrostatic interaction in a medium the electrostatic interaction between charged species can be more

rigorously treated than other intermolecular interactions. The combination of strength, long ranged nature and a relatively rigor physical law for the electrostatic interaction gives a unique possibility to understand the behavior of charged species in chemical systems. In classical physics the electrostatic interaction is usually calculated between charged bodies with a fixed position in space. In chemistry, on the other hand, we are often faced with the problem of calculating the electrostatic interaction in a salt solution. In such solution the ions are constantly in thermal motion and, due to the strength and long ranged nature of the electrostatic interaction, the motion of a particular ion is affected by the presence and movements of the other ions or other charged bodies in the system. In most chemical systems, the electrostatic interaction is therefore a many body interaction and its description requires a combination of theories from classical electrostatics and statistical mechanics. The simplest and most frequently used combination is the Poisson–Boltzmann (P–B) equation which is a second order differential equation [5]. It has been possible to obtain a closed form analytical solution to the P–B equation for one geometry only, the interaction between a charged planar surface in contact with an electrolyte solution, the Gouy–Chapman (G–C) theory.

In the P–B equation three important assumptions are made; first, the ions are considered to be point charges which means that the size of the ions is set to zero; second, the solvent is considered to be a continuum with a given dielectric constant and third, it is a mean field theory which means that the interaction between an ion in a given point and the surrounding ions is calculated as its mean value. These assumptions impose some restrictions on the validity of the P–B equation, the restrictions are dependent of the geometry of the system but generally speaking the deviation from the theory increases with increasing salt concentration in the solution and when the charge density of the bodies increases.

When the strength of the electrostatic interaction between charged bodies and ions in the system is small compared to the thermal energy of the ions, it is possible to simplify the P–B equation into the linearised P–B equation. This is a second order differential equation which is easier to solve than the original equation and has consequently been solved

for several geometries. A well known example is the Debye–Hückel theory for calculating activity coefficients of ions in an electrolyte solution, this theory is based on a solution of the linearised P–B equation for a sphere in contact with surrounding point charges.

In chromatography the retention of an ion may only partly be due to the electrostatic interaction, other types of intermolecular interactions may also be of importance. In the process of developing and investigating retention models for ionic species it is important to distinguish the electrostatic interaction from other interactions which may contribute to the retention. A proper model of the electrostatic interaction must be accomplished first and after that the role of the other types of interactions can be investigated. In many chromatographic systems the role of the electrostatic interaction can be studied through the ionic strength dependence of eluite retention. If the retention varies significantly with the ionic strength of the mobile phase the electrostatic interaction must be included in the retention model.

2.2. Introduction to charged surfaces in contact with an electrolyte solution

When a surface with ionisable groups, e.g. $-SO_3H$, is immersed in an electrolyte solution, e.g. Na^+ and $H_2PO_4^-$, a charged surface is created. Due to the thermal motion (i.e. the entropy) the counterions corresponding to the oppositely charged surface group are not bound as stoichiometric 1:1 complexes to the surface. Instead, they are distributed in a diffuse layer close to the surface, the so-called diffuse double layer. The final distribution of the counterions in the double layer is a result of the balance between the electrostatic attraction to the charged surface, the way the counterions shield each other and the (smearing out) effect of the thermal motion. In order to keep electroneutrality, the number of charges on the surface is balanced by an equivalent number of counterions located in the layer close to the surface plane. The excess of negatively charged SO_3^- groups on the surface results in both a positive excess of counterions in the electrical double layer as well as a negative excess, or depletion, of ions with the same charge as the surface charge. Since the ions in the double layer are not situated in

the same plane as the charges on the surface, a difference in electrostatic potential between the surface and a point in the solution is created.

The main problem in describing the electrostatic interaction in charged systems is to find an exact description of the magnitude of the electrostatic potential at different points in the system. In systems of chromatographic interest the simplest possible approach is to consider the charges constituting the stationary phase as a separate phase and assuming that the electrostatic potential has a constant value in this phase, the Donnan potential. In this model the concentration change of counter and co-ions between the two phases is considered to take place at a sharp boundary, and it does not give rise to the above described diffuse double layer. The difference between the two models is schematically illustrated in Fig. 1. Retention models based on the Donnan
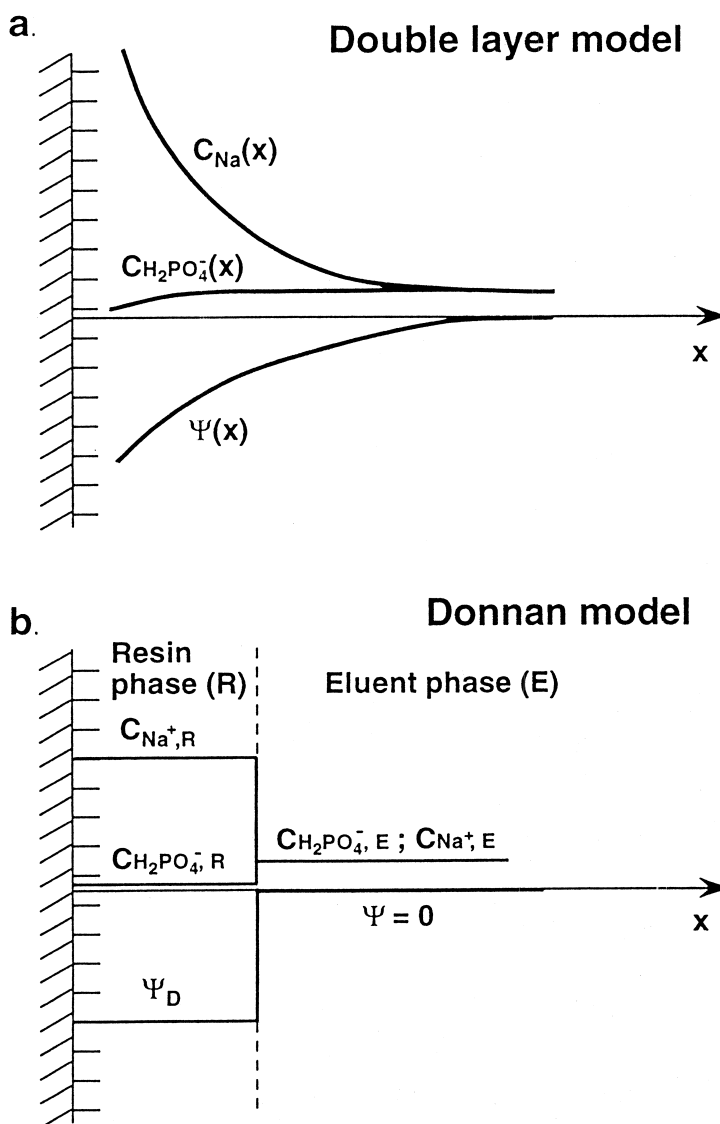

Fig. 1. Schematic description of the concentration profiles for a counterion, c_{Na}, a co-ion, $c_{H_2PO_4}$, and the electrostatic potential profile in the vicinity of a negatively charged surface according to (a) the double layer model and (b) the Donnan model.

potential concept are often used in ion (exchange) chromatography and its physical background is outlined in Section 2.3.

2.3. The Donnan potential

Consider a cation exchanger, with SO_3^- groups chemically bound to its surface and Na^+ as counter-ions placed in a dilute water solution of NaH_2PO_4. Assume that a distinct boundary exists between the electrolyte solution and the resin phase and that the total ionic concentration in the resin phase (including the charged surface groups) is higher than in the electrolyte solution. This is depicted in Fig. 1b where the electrolyte solution is denoted E and the resin phase R. Due to the thermal motion of the Na^+ and $H_2PO_4^-$ ions, a redistribution of these ions will occur between the two phases. Before the resin phase comes in contact with the electrolyte solution the number of Na^+ ions in the resin phase is equal to the number of SO_3^- groups. When the two phases are in contact, a net migration of Na^+ ions into the solution and $H_2PO_4^-$ ions into the resin phase, respectively, will occur. The result is an accumulation of positive charge in the solution and negative charge in the resin phase which creates a difference in electrostatic potential between the two phases, the Donnan potential, Ψ_D. In the final ionic distribution, an equilibrium is established in which the tendency of the ions to level out the existing concentration differences between the two phases is balanced by the difference in Donnan potential [6]. Electrostatic considerations show that only an extremely small imbalance in the charge distribution is needed to create the Donnan potential, in fact, too small to be measurable. Since there is a (very small) excess of negative charges in the resin phase it has a negative potential relative to the electrolyte phase.

A quantitative treatment of the distribution of the ions between the two phases is obtained from thermodynamics which states that at equilibrium the electrochemical potential, μ, of an ionic species is the same in the two phases. The electrochemical potential differs from the chemical potential by a term which depends on the work needed to reversibly transfer a charged species between the two phases, and which is due to the difference in electrostatic potential between the phases, i.e. in this case the Donnan potential.

For the Na^+ ions the thermodynamic condition for equilibrium between the resin and elelctrolyte phase, respectively, is written as

$$\mu_{Na}^0 + RT \ln \gamma_{Na,R} \cdot c_{Na,R} + z_{Na} F \Psi_D = \mu_{Na}^0$$
$$+ RT \ln \gamma_{Na,E} \cdot c_{Na,E} \qquad (1)$$

where μ_{Na}^0 is the chemical potential of the Na^+ ion in its standard state, its value is assumed to be equal in the two phases; $c_{Na,R}$ and $c_{Na,E}$ are the sodium concentration in the resin and eluent phase, respectively; $\gamma_{Na,R}$ and $\gamma_{Na,E}$ are the corresponding activity coefficient; z_{Na} is the charge of the species, i.e. $+1$ for Na^+; and F is the Faraday constant which is a conversion factor from electrical to molar units. A more detailed thermodynamic analysis shows that in this equation a term for the difference in osmotic pressure between the two phases shall be included, see e.g. Ref. [7]. It can be shown, however, that in most ion-exchange chromatographic systems such a term is usually relatively small, therefore it is ignored in this simplified presentation. Rearrangament of Eq. 1 gives that

$$c_{Na,R} \gamma_{Na,R} = c_{Na,E} \gamma_{Na,E} \cdot e^{-(z_{Na} F \Psi_D / RT)}$$
$$\text{where } z_{Na} = +1 \qquad (2)$$

The analogous equilibrium condition for H_2PO_4 ions gives that their distribution between the two phases is described by Eq. 3

$$c_{H_2PO_4,R} \gamma_{H_2PO_4,R} = c_{H_2PO_4,E} \cdot e^{-(z_{H_2PO_4} F \Psi_D / RT)}$$
$$\text{where } z_{H_2PO_4} = -1 \qquad (3)$$

Eq. 2 can be rearranged to

$$\Psi_D = \frac{RT}{z_{Na} F} \cdot \ln \frac{\gamma_{Na,E} c_{Na,E}}{\gamma_{Na,R} c_{Na,R}} \qquad (4)$$

If there are several types of cations in the system the above thermodynamic analysis, i.e. Eqs. 1, 2 and 4, is applicable to each of them. Consider e.g. the above discussed cation exchanger system to which a very small amount of KH_2PO_4 is added. In analytical chromatography this corresponds to a NaH_2PO_4 electrolyte as eluent phase and K^+ as an eluite ion.

Applying the same thermodynamic analysis to the K^+ ion gives that

$$\Psi_D = \frac{RT}{z_K F} \cdot \ln \frac{\gamma_{K,E} c_{K,E}}{\gamma_{K,R} c_{K,R}} \quad (5)$$

Since the Donnan potential experienced by the two species is the same, Eqs. 5 and 4 are equal, i.e.

$$\frac{\gamma_{K,R} c_{K,R}}{\gamma_{Na,R} c_{Na,R}} = \left(\frac{\gamma_{Na,E} c_{Na,R}}{\gamma_{Na,E} c_{Na,E}} \right)^{(z_K/z_{Na})} \quad (6)$$

In an analogous manner a general expression can be obtained for a system consisting of salt ion, $C^z c$, which constitutes the electrolyte as well as being the counterion to the surface charge groups, and an eluite ion B. The final result is

$$\frac{c_{B,R}}{c_{B,E}} = \frac{\gamma_{B,E}}{\gamma_{B,R}} \left(\frac{\gamma_{C,R} c_{C,R}}{\gamma_{C,E} c_{C,E}} \right)^{(z_B/z_C)}$$

$$= K_\gamma \left(\frac{c_{C,R}}{c_{C,E}} \right)^{(z_B/z_C)} \quad (7)$$

where K_γ includes all the activity coefficients.

Since the quotient $c_{C,R}/c_{C,E}$ usually is higher than unity, an important consequence of Eq. 7 is that a multiple charged eluite will be more strongly attracted to the resin phase than a single charged. The physical explanation is that the ions experience an energy difference between the resin and eluent phase, respectively, which is proportional to both the charge of the ion and the Donnan potential. In chromatography this implies that a divalent ion is more retained than a monovalent, this is usually referred to as electroselectivity. Another consequence of the equation is that when the counterion concentration is increased in the solution phase, the quotient $c_{C,R}/c_{C,E}$ decreases and the distribution of the eluite to the resin phase decreases. Physically this is caused by a decrease in Donnan potential as the counterion concentration increases. From the equation also follows that when the eluite and counterions have charge of opposite sign, the eluite will be excluded from the resin phase, i.e. its concentration is lower in the resin phase than in the solution. This follows from the negative sign in the exponent in Eq. 7 and the physical explanation for this is that the eluite ion and the Donnan potential are both of the same sign, i.e. the eluite ion is repelled from the resin phase.

When all eluite ions of the same sign have the same activity coefficients in the solution and in the resin phase there is no chromatographic selectivity between the ions. A chromatographic thermodynamic model such as the Donnan model therefore can not explain the physical cause for the selectivity of equally charged ions. Formally, chromatographic selectivity is represented in the Donnan model as differences in activity coefficients and to explain the physical cause of these differences additional assumptions are needed. Factors that usually are proposed are the size and hydration number of the ion, ion pair formation and specific electrostatic interactions [7]. A presentation of different models that have been proposed to explain selectivity is discussed in the section treating the models used in ion-exchange chromatography of small ions. Since the Donnan model forms the basis of many important theories for ion-exchange chromatography it is important to more closely investigate its properties and limitations.

For a symmetrical electrolyte solution, i.e. 1:1, 2:2, etc. electrolytes, a relation between the counterion concentration in the resin phase, the salt concentration in the solution and the volume concentration of surface groups in the resin phase, C_R, can be obtained from the Donnan model. Eq. 8 is obtained by combining the condition of electroneutrality in both the solution as well as in the resin phase with the expression for the concentration of both the counter and co-ion in the two phases, i.e. Eqs. 2 and 3.

$$c_{C,R} = \frac{|z_R|}{|z_C|} \cdot \frac{C_R}{2} + \sqrt{\frac{z_R^2}{4 z_C^2} \cdot C_R^2 + \frac{\gamma_{\pm C,E}^2}{\gamma_{\pm C,R}^2} c_{C,E}^2} \quad (8)$$

Where z_R is the charge of the surface groups bound to the surface and z_C the charge of the counterion. $(\gamma_{\pm C,E}^2/\gamma_{\pm C,R}^2)$ is the quotient between the square of the mean molar activity coefficient of the counterion in the electrolyte solution and the resin phase, respectively. The corresponding equation for the co-ion concentration in the resin as a function of the salt concentration in the solution is obtained in an analogous manner [7]:

$$c_{D,R} = -\frac{|z_R|}{|z_D|} \cdot \frac{C_R}{2} + \sqrt{\frac{z_R^2}{4 z_D^2} \cdot C_R^2 + \frac{\gamma_{\pm E}^2}{\gamma_{\pm R}^2} c_{D,E}^2} \quad (9)$$

Where $c_{D,R}$ and $c_{D,E}$ are the co-ion concentration in the resin and eluent phase, respectively. A general problem with the Donnan model is that it assumes that the electrostatic potential is constant throughout the resin phase, i.e. it neglects the presence of any electrostatic potential gradient in this phase. This is a rather serious assumption which leads to a discrepancy between the model and experimental results (Ref. [7] and references therein, Ref. [8]). Since the reason for this discrepancy is of great importance regarding the applicability of the approach taken in the Donnan model it is of interest to analyse it more closely.

At low eluent salt concentrations it is easily shown that Eq. 9 can be written (using the series expansion $(1+x)^{1/2} \approx 1 + \frac{1}{2} x$ for x values $\ll 1$).

$$c_{D,R} = c_{D,E}^2 \, \frac{|z_R|}{|z_D|} \cdot \frac{\gamma_{\pm E}^2}{\gamma_{\pm R}^2} \frac{1}{C_R} \tag{10}$$

According to Eq. 10 the Donnan model predicts that the co-ion concentration in the resin phase will drop with the square of its concentration in the eluent. However, experimental data for the co-ion concentration in the resin phase is found to be much higher than what is predicted from Eq. 10 (c.f. Ref. [7] and references therein). The reason for this discrepancy, which is contrary to the assumptions made in the model, is that the mean molar activity coefficient in the resin phase drops to values lower than unity at great dilution. The physical reason for this drop in activity coefficient is still a subject of debate and of great interest in ion-exchange chromatography [8]. However, already in 1956 it was pointed out by Overbeek that this effect is expected when the charge and the potential are not evenly distributed within a phase and that the effect can be predicted from the Poisson–Boltzmann equation [9]. In an important, but often neglected paper, Marcus [10] derived in an elegant way general expressions for the thermodynamic properties of polyelectrolyte solutions. Since his expressions also apply to the present problem and are instructive to the understanding of the limitations of the Donnan model, a short, but less rigorous, derivation is presented here. To this authors knowledge the analysis presented below has not been previously applied to ion-exchange chromatographic theory.

Consider a resin phase containing fixed charged groups in equilibrium with an inert salt solution. The difference in electrostatic potential between a given point r in the resin phase and the salt solution is $\Psi(r)$. When the concentration of the counterion in the salt solution (where $\Psi = 0$) is $c_{C,E}$, its concentration at point r, $c_{C,R}(r)$, is given by

$$c_{C,R}(r) = c_{C,E} \cdot e^{-(z_C F \Psi(r)/RT)} \tag{11}$$

The number of counterions in the resin phase, i.e. the mean concentration in the resin phase, $c_{C,R}$, multiplied by the volume of this phase, is obtained by integrating Eq. 11 over the volume of the resin phase, V_R:

$$c_{C,R} \cdot V_R = c_{C,E} \cdot \int_{V_R} e^{-(z_C F \Psi(r)/RT)} \, dV \tag{12}$$

Analogous arguments give that the mean concentration of co-ions in the resin phase, $c_{D,R}$ is

$$c_{D,R} \cdot V_R = c_{D,E} \cdot \int_{V_R} e^{-(z_D F \Psi(r)/RT)} \, dV \tag{13}$$

The mean molar activity coefficient, $\gamma_{\pm,R}$, in the resin phase for a monovalent 1:1 salt (for the general case the reader is referred to the original paper by Marcus) can be defined as:

$$c_{C,R} \cdot c_{D,R} \cdot \gamma_{\pm,R}^2 = c_{C,E} \cdot c_{D,E} \tag{14}$$

Inserting Eqs. 12 and 13 into Eq. 14 gives that

$$\gamma_{\pm,R}^2 = \frac{V_r^2}{\displaystyle\int_{V_R} e^{-(z_C F \Psi(r)/RT)} \, dV \cdot \int_{V_R} e^{-(z_D F \Psi(r)/RT)} \, dV} \tag{15}$$

Eq. 15 is an expression for the mean activity coefficient of the electrolyte ions in the resin phase and it shows that its numerical value depends on the value of the two integrals. If it is assumed, as is done in the Donnan model, that the electrostatic potential has a constant value, ψ_D at all points in the resin phase Eq.15 can be evaluated according to:

$$\gamma_{\pm,R}^2 = \frac{V_r^2}{\displaystyle\int_{V_R} e^{-(z_C F \Psi_D / RT)} \, dV \cdot \int_{V_R} e^{-(z_D F \Psi_D / RT)} \, dV}$$

$$= \frac{V_r^2}{e^{(z_D F \Psi_D / RT)} \cdot e^{-(z_D F \Psi_D / RT)} \displaystyle\int_{V_R} dV \cdot \int_{V_R} dV}$$

$$= 1 \tag{16}$$

where in the second equality we have used the fact that $z_C = -z_D$. Thus, applying the assumption made in the Donnan model to Eq. 15 correctly gives that the mean activity coefficient is unity. In the general case, however, where the electrostatic potential vary from point to point in the resin phase this simple result is not correct.

For the general case a numerical value for $\gamma_{\pm R}$ cannot be found because it will depend on the resin properties. However, a general consequence of the theory can be obtained by using Schwarz's inequality which states that:

$$\int f^2 \, dV \cdot \int g^2 \, dV \geq \left(\int fg \, dV \right)^2 \tag{17}$$

It can be shown that the equality sign in Eq. 17 holds only when $f = \text{constant} \cdot g$ [11]. Setting $f = e^{-(z_D F \Psi(r)/2RT)}$ and $g = e^{-(z_C F \Psi(r)/2RT)}$ in Eq. 17, inequality means that the product of the integrals in the denominator is greater than V_R^2 So, if we let the electrostatic potential vary within the resin phase the activity coefficient will be less than unity, in accordance with the experimental observation.

The above analysis clarifies that the Donnan concept fails because it does not correctly calculate the mean concentration of both the counter and co-ion in the resin phase. One can argue that, in principle, it would be possible to find a mean potential in the resin phase, Ψ_D, which, for a certain salt concentration of salt in the eluent phase, correctly calculates the mean concentration of, e.g. the counterion. However, using the same numerical value for Ψ_D would incorrectly calculate the corresponding coion concentration. It is therefore not possible to use the same value for Ψ_D to simultaneously calculate both the counterion and co-ion concentration, respectively, in the resin phase.

The strength of the analysis by Marcus is that it is based on a minimum of assumptions and that it applies to any geometry of the resin phase. It is this authors opinion that these results provide further insights into the complex problem of ion-exchange chromatography. An example based on this analysis is outlined in Appendix A which dicusses the retention of an eluite ion as a function of electrolyte concentration in the eluent. From this example follows that the experimentally found retention of a monovalent eluite ion as a function of the eluent concentration of monovalent electrolyte can be explained for almost any geometry of the resin phase.

2.4. The diffuse double layer

The classical description of the diffuse double layer was first made by Gouy [12] and, independently, by Chapman [13] and the result is referred to as the Gouy–Chapman (G–C) model. The model is based on the solution of the P–B equation for a planar charged surface in contact with an electrolyte solution and forms the starting point for more elaborate descriptions of the double layer. Therefore, it is appropriate to discuss its results briefly, more detailed presentations of the properties of the electrical double layer can be found in, e.g. Refs. [1–4,14]. Retention models based on double layer theories proposed for ion-exchange and ion pair chromatography are discussed in Sections 4 and 5.

The basic assumptions of the G–C theory are:
  the charged surface is impenetrable for the electrolyte ions.
  the surface charge is uniformly spread over the surface.
  the electrolyte ions behave as point charges and are able to approach right up to the plane of the surface.
  the solvent is considered to be a continuum of constant dielectric constant up to the surface.

Using these assumptions the P–B equation can be solved giving mathematical expressions for (i) the electrostatic potential at the planar surface as a function of both the surface charge density and the concentration of salt in the electrolyte solution and (ii) the electrostatic potential at any distance x from the surface as a function of the electrostatic potential at the planar surface ($x = 0$) and the salt concen-

tration. The exact equations can be found in the textbooks [1–4,14], only some qualitative aspects of the theory will be discussed here.

In Fig. 2a the calculated electrostatic potential is shown as a function of the distance from the planar surface for three different concentrations of a monovalent salt in the solution (=water); 1, 100 and 500 mole/m^3 and a surface charge density of -0.1 C/m^2 ($=1.04 \cdot 10^{-6}$ mol/m^2). The theoretical curves obtained from the G–C theory show that the electrostatic potential at the surface, $\Psi(x=0) = \Psi_0$, decreases from -200 to -50 mV as the salt concentration increases from 1 to 500 mole/m^3. The curves also show that the electrostatic potential drops more rapidly with the distance from the surface for a more concentrated salt solution. The physical explananation for this is that the surface charges become more shielded by the counterions in the solution when their concentration is higher. It is also seen that the distance from the surface at which the potential approaches zero may become large, e.g. for the 1 mol/m^3 solution it is around 30 nm.

When the electrostatic surface potential, Ψ_0, is below 25 mV it can be approximated by solutions of the linearised form of the P–B equation. In this limit, known as the Debye–Hückel approximation, simpler mathematical expressions describing the potential in a point situated the distance x from the surface are obtained:

$$\Psi(x) = \Psi_0 \cdot e^{-\kappa x} \qquad (18)$$

where

$$\kappa = F \left(\frac{1000 \cdot \sum_i z_{i,E}^2 c_{i,E}}{\varepsilon_0 \varepsilon_r RT} \right)^{1/2} \qquad (19)$$

where summation over i means that the concentration of all ionic species is added, $1/\kappa$ is the Debye length and is a measure of the double layer thickness. From its definition follows that its thickness increases with the reciprocal square root of the ionic strength of the electrolyte solution. In the exact G–C theory the algebraic relation between the electrostatic surface potential and the salt concentration is complex but in the Debye–Hückel approximation it is simplified to

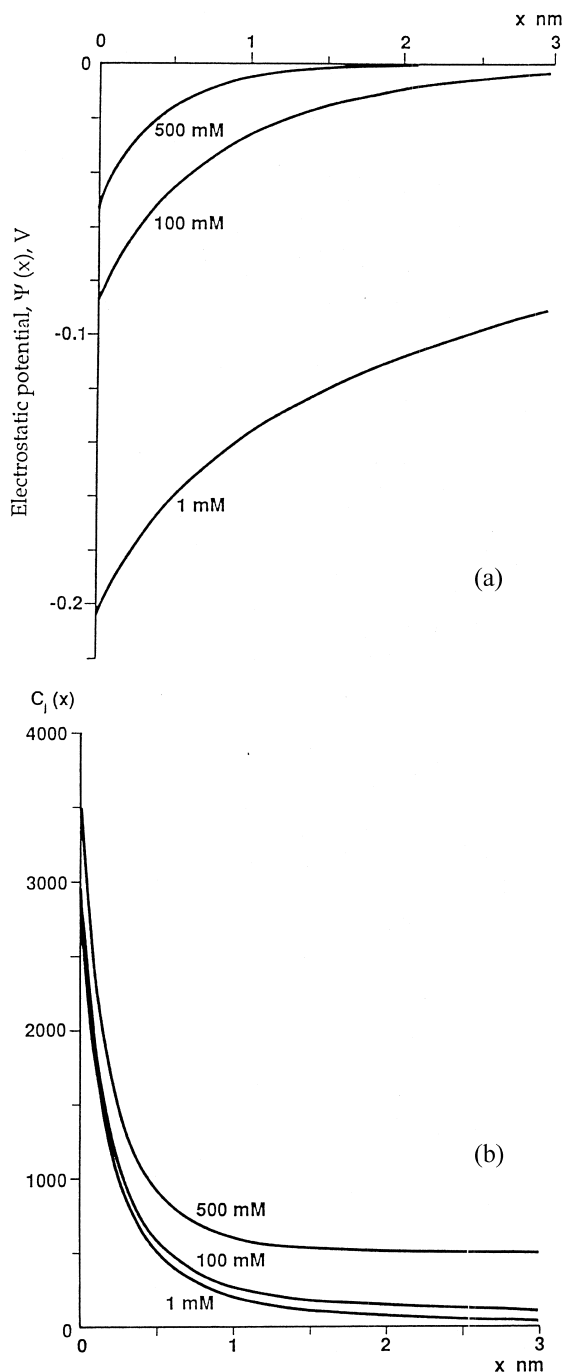$$\Psi_0 = \frac{\sigma}{\kappa \varepsilon_0 \varepsilon_r} \qquad (20)$$

Fig. 2. (a) The electrostatic potential and (b) the counterion concentration as a function of the distance from a negatively charged surface (charge density -0.1 C/m^2) according to the Gouy–Chapman theory, for three different concentrations of a 1:1 electrolyte (1, 100 and 500 mol/m^3). (The figures are reprinted from Ref. [58] with permission).

where σ is the surface charge density (C/m^2), ε_0 is the permittivity of vacuum and ε_r is the dielectric constant of the eluent.

In the previous presentation of the Donnan model it was shown that the distribution of ions due to the difference in electrostatic potential between the two phases follows Eqs. 2 and 3. In contrast to the Donnan model, where the ion distribution within each phase is homogenous, the G–C theory gives a continous *electrostatic potential* profile from which the resulting *concentration* profile is calculated, i.e. for each and every distance there is a potential to which Eqs. 2 or 3 applies. In the G–C theory it is assumed that the 'chemical' properties in the bulk solution and the diffuse layer are identical and consequently the quotient of activity coefficients in these equations is set to unity. For example, in the Debye–Hückel approximation the concentration profile for an ion outside the charged surface is obtained by combining Eqs. 2 or 3 and 18:

$$c_i(x) = c_i \cdot e^{-(z_i F \Psi_0 e^{-\kappa x}/RT)} \qquad (21)$$

here c_i is the concentration of ion i in the bulk phase, the ion may be a counterion or a coion. Since the sign of the surface potential, Ψ_0, is of opposite (same) sign as the counterion (co-ion), z_i, the equation describes the accumulation (depletion) of counterions (co-ions) to the surface.

Fig. 2b shows the exact concentration profile calculated from the G–C theory for monovalent counterions calculated from the potential profile in Fig. 2a. An interesting consequence of the G–C theory, is also illustrated in this figure; the counterion concentration at the surface is only about 20% higher when going from 1 mol/m^3 to 500 mol/m^3 concentration in the bulk solution. The counterion concentration at the surface is therefore rather insensitive to changes in the salt concentration of the bulk electrolyte.

2.5. Ion binding to charged surfaces

The G–C theory describes the accumulation of point charges into a diffuse double layer close to a charged surface. According to the theory these point charges interact with the surface only through 'pure' electrostatic or Coulomb interactions. However,

when real ions interact with a surface the possibility of other types of interactions between the surface and the ions must be considered. These additional interactions are usually referred to as the chemical part of the total interaction. The chemical interaction is short ranged implying that only ions very close to the surface may bind 'chemically' to the surface while ions situated in the double layer further away from the surface are too far away to interact. Consequently, it is the local concentration of a given ion close to or at the surface that determines the concentration of specifically or 'chemically' bound ions to the surface. In this case a mass action law for association to the surface can be used [1]:

$$K_{ch} = \frac{[is]}{c_i(0) \cdot [s]} \qquad (22)$$

where $c_i(0)$ is the concentration of the ion i at the surface (i.e. $x \approx 0$), [s] is a measure of the concentration of free surface groups to which the ion can bind, [is] is a measure of the concentration of surface groups to which i is bound and K_{ch} is the association constant for the i–s complex. The concentration of ion i at the surface is, as before, calculated by the equation:

$$c_i(0) = c_i \cdot e^{-(z_i F \Psi_0/RT)} \qquad (23)$$

where Ψ_0 is the electrostatic potential (relative to the bulk solution) at the surface.

A lot of theoretical work has been done in order to find appropriate models to describe the adsorption of different ionic species to different kind of surfaces. To obtain a quantitative description of the adsorption of ions to the surface the main problem is to locate the position of the counterions in relation to the charged surface. In the above model the adsorbed counterions are assumed to be located in the plane of the surface charges. The exact position of the adsorbed ions is modelled differently in different models and consequently the plane at which the electrostatic surface potential is calculated also differ. It is beyond the scope of this review to treat all models that have been proposed, the physical background to those that are related to chromatographic retention models will primarily be discussed. It is important to recognise that the basis for each model depends on the purpose for which it is used. Some

models are used with the prospect of gaining insight into the physical nature of the electrical double layer while others are used to obtain numerical description of specific experimental data.

A large part of the knowledge regarding charged surfaces in electrolyte solutions comes from investigations of the interface between mercury and a water solution. This surface is considered to be molecularly smooth and impenetrable for the counterions, therefore very detailed and physically realistic assumptions concerning the location of the ions at the surface can be made. For this particular interface it is often assumed that there is a charge free region near the surface confined between the surface and a plane close to the surface, the Inner Helmholtz Plane (IHP), see Fig. 3. A physical reason for the existence of this charge free region is that due to their own size, the counterions are not able to get closer to the metal surface than a certain distance. The thickness of this region varies from about 0.1 nm for a bare ion to 0.5 nm for a hydrated ion. There is a drop in the electrostatic potential potential in this region from Ψ_0 at the surface to Ψ_{IHP} at the IHP. It was proposed by Stern [15] that apart from the electrostatic attraction (repulsion) the ions situated at the IHP may be specifically (or chemically) adsorbed with a free energy of adsorption, ΔG_{ch}^0. There are a limited

number of sites available for ions in the IHP, and the saturation of the IHP is described with a Langmuir type of adsorption isotherm. Beyond the IHP there is another plane which usually is called the Outer Helmholtz Plane (OHP) which is the innermost plane of the diffuse part of the double layer. In the original Stern treatment no specific chemical interactions occur between ions situated at the OHP and the surface. Consequently, the region between IHP and OHP is free of charges and there may or may not (dependent of the version of the model) be a drop in electrostatic potential between the IHP and OHP. In this theory, the Stern–Gouy–Chapman (SGC) theory, the diffuse part of the double layer extends outward from the OHP and its properties are given by the Gouy–Chapman theory where Ψ_{OHP} replaces Ψ_0 in all equations.

The free energy of interaction between the surface and the specifically adsorbed ions, which are situated at the IHP and interacts 'chemically' with the surface, is expressed as

$$\Delta G_{i,ads}^0 = z_i F \Psi_{IHP} + \Delta G_{i,ch}^0 \tag{24}$$

where $\Delta G_{i,ads}^0$ is the total free energy of adsorption and is divided into an electrostatic part, $z_i F \Psi_{IHP}$, and a chemical part, $\Delta G_{i,ch}$. The electrostatic part corre-
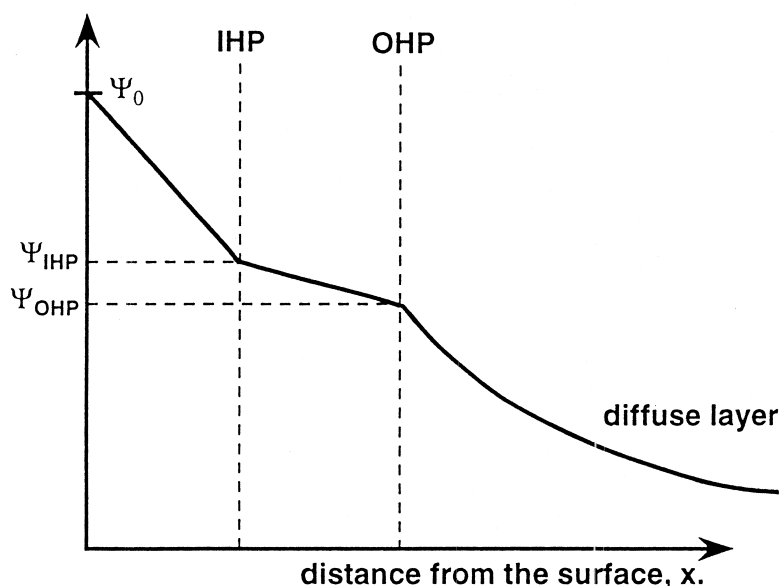

Fig. 3. A schematic representation of the electrostatic potential as function of the distance from the surface according to the model by Stern.

sponds to the elecrostatic energy needed to move the charged ion from the bulk to the IHP. In this model the difference in adsorption properties between different ions of the same charge, z_i, is due to a difference in the chemical part of the adsorption energy. The competition for the available space in the IHP is accounted for by using a Langmuir type of isotherm which is combined with Eq. 24 for describing the total free energy of adsorption. Assuming that only counterions are adsorbed at the IHP, the equation is:

$$n_{C,IHP} = \frac{n_{0IHP} \cdot K_{C,ch} \cdot c_{C,E} \cdot e^{-(z_C F \Psi_{IHP}/RT)}}{1 + K_{C,ch} c_{C,E} \cdot e^{-(z_C F \Psi_{IHP}/RT)}} \qquad (25)$$

where $n_{C,HP}$ is the surface concentration of counterions (in mol/m^2) at IHP, n_{0IHP} is the monolayer capacity in the IHP, $c_{C,E}$ is the bulk concentration of counterions and where

$$K_{C,ch} = e^{-(\Delta G^0_{C,ch}/RT)} \qquad (26)$$

Since the term $c_{C,E} \cdot e^{-(z_C F \Psi_{IHP}/RT)}$ is equal to the volume concentration of counterions at IHP, Eq. 25 is closely related to Eq. 22.

A large amount of theoretical and experimental work has been done to evaluate and refine the SGC theory for the mercury–solution system. The resulting theories become complex with a number of detailed assumptions about the properties of the ions and the solvent in the compact part of the double layer, i.e. the region between the surface and the OHP. For example, some theories account for a change in dielectric constant of the solvent (water) due to high electrical fields at the surface. Experimental results show that simple cations, Li^+, Na^+, K^+, etc. follow the simple G–C theory whereas the less hydrated anions, Cl^-, I^-, etc. often are specifically adsorbed in the compact part of the double layer. In contrast to the mercury–solution interface, the surfaces in neither chromatography nor in colloid chemistry are atomically smooth and there is therefore no point in trying to give a detailed description of the physical properties of the solvent or ions situated in the innermost part of the double layer in these systems.

Several models have been proposed to describe the adsorption of ions to charged surfaces of inorganic oxides such as SiO_2, TiO_2, Fe_2O_3, different clay minerals, etc. On these materials the surface charge density varies when changing the pH of the solution, the H^+ ion therefore plays a very important role in these systems. When counterions other than H^+ are present in the bulk solution there is a mixture of counterions and H^+ ions both in the double layer and in the inner layers. We shall here only describe the basis for the simplest version of a set of models which usually are called the surface complexation models. The basis of this model is again an equation of the form of Eq. 22 for both the H^+ and counterion.

The dissociation of an acidic surface group may be represented as:

$$AH \rightleftharpoons A^- + H^+ \qquad (27)$$

with a dissociation constant given by

$$K_a = \frac{[A^-] \cdot [H^+]_{sH}}{[AH]} \qquad (28)$$

where $[H^+]_{sH}$ is the hydrogen concentration at the plane sH which is the adsorption plane of this ion. Using the Stern model the constant K_a is related to the chemical part of the free energy of adsorption. According to the Boltzmann distribution in Eq. 3 $[H^+]_{sH}$ is given by:

$$[H^+]_{sH} = [H^+]_E \cdot e^{-(F \Psi_{sH}/RT)} \qquad (29)$$

$[H^+]_E$ is the hydrogen ion concentration in the bulk of the eluent and Ψ_{sH} is the electrostatic potential at the adsorption plane for the hydrogen ion. A corresponding equilibrium constant for, e.g. the Na^+ ion is

$$K_{Na} = \frac{[A^-] \cdot [Na^+]_{sNa}}{[ANa]} \qquad (30)$$

where $[Na^+]_{sNa}$ is the concentration of Na^+ ions at its adsorption plane sNa which again is given by the Boltzmann distribution

$$[Na^+]_{sNa} = [Na^+]_E \cdot e^{-(F \Psi_{sNa}/RT)} \qquad (31)$$

In this model the adsorption of the two ions to take place at planes which are at different distances from the surface. The electrostatic potential at this plane, Ψ_{sNa}, is consequently different from the potential at the sH plane. However, since the surface usually not

is atomically smooth, the location of the planes is not geometrically well defined.

By combining the set of Eqs. 28–31 the following equation can be obtained:

$$K_e = \frac{K_H}{K_{Na}} = \frac{[ANa][H^+]_E}{[HA][Na^+]_E} \cdot e^{-(F(\Psi_{sH} - \Psi_{sNa})/RT)} \quad (32)$$

An additional assumption usually made in the different surface complexation models is that the total number of sites per unit area is limited and equal to $[AH]+[A^-]+[ANa]$.

These mass law and material balance equations represents the simplest case of a number of different surface complexation models (see Ref. [16] and references therein)). The difference between the models lies in the description of the location of the various planes of adsorption and the electrostatic equations used to describe the relation between surface charge and surface potential. In some of the models the plane of adsorption of the hydrogen ion is the surface and for the counterions it is the IHP. The different models that have been proposed contain a number of parameters which in principle can be adjusted to fit experimental data. In the study by Westall and Hohl [16] it was concluded that many combinations of the adjustable parameters could be used to represent the experimental data. It was also concluded that no unique description exists for chemical and electrostatic energies at the oxide surface. Recently, Horst and coworkers adapted the surface complexation theory to ion-exchange equilibria [17,18]. They applied the model to describe the complete adsorption isotherm of metal ions in a multicomponent system on a weak cation exchanger. The purpose of their work is not to describe chromatographic retention and the model has therefore not been compared with such data.

Another system which has been thoroughly investigated in colloid chemistry is the AgI(s)–solution interface. When AgI crystals are placed in water a very small concentration of Ag^+ and I^- exist in the solution at equilibrium. A small shift in the Ag^+ or I^- concentration in the solution leads to small changes in the surface concentration of Ag^+ or I^-, respectively. The charge density on the crystal surface can thus be varied by varying the concentration of these two ions. When the thermody-

namic expression for equilibrium is applied to Ag^+-ions the following equation is obtained;

$$\mu_{Ag}^{0}(w) + RT \ln c_{Ag}(w) \cdot \gamma_{Ag}(w) = \mu_{Ag}^0(s)$$
$$+ RT \ln a_{Ag}(s) + F\Delta\Psi_0 \quad (33)$$

where $\mu_{Ag}^0(w)$ and $\mu_{Ag}^0(s)$ are the chemical potential in the standard state in water solution and in the solid state, respectively; a_{Ag} is the activity of the adsorbed silver ion at the surface and $\Delta\Psi_0$ is the difference in electrostatic potential between the solution and the surface of the AgI crystal. For a certain concentration of Ag^+ in the solution $[Ag^+]_{pzc}$ the net charge of the AgI crystals is zero, the point of zero charge (pzc). Thermodynamic equilibrium at this point gives that

$$\mu_{Ag}^0(w) + RT \ln c_{Agpzc}(w) \cdot \gamma_{Agpzc}(w) = \mu_{Ag}^0(s)$$
$$+ RT \ln a_{Ag,pzc}(s) \quad (34)$$

Since an extremely small relative change in the surface concentration of the silver ion is required to establish an electrostatic potential, the activity of the silver ions at the surface is the same on the charged and uncharged surface, i.e. $a_{Ag}(s) = a_{Ag,pzc}(s)$ for all concentrations. This equality in combination with Eqs. 33 and 34 gives Nernst equation, Eq. 35:

$$\Delta\Psi_0 = \Psi_0 = \frac{RT}{F} \cdot \ln \frac{c_{Ag}(w)\gamma_{Ag}(w)}{c_{Agpzc}(w)\gamma_{Agpzc}(w)} \quad (35)$$

The electrostatic surface potential as a function of $c_{Ag}(w)$ is determined from this equation by measuring the silver activity in the solution with a Ag/AgCl-electrode and relate it to the activity at the pzc and a given silver concentration. It is important to be aware of that Nernst equation can only be used when the relative change in surface concentration of the ion which creates the surface charge is vanishingly small. The use of Nernst equation is therefore limited to surfaces where the ion which creates the surface charge is a component of the surface lattice. Surfaces which meet this criterion are called Nernstian surfaces.

There are several other areas in surface and colloid chemistry where a lot of knowledge has been accumulated in the field of electrostatic interactions in ionic systems. Some examples where electrostatic interaction are of prime importance are: the prop-

erties of ionic micelles; liquid crystals and polyelec-trolytes; the adsorption of ions to the water–air (or oil) interface; stability of colloids etc. Micelles, proteins, adsorbed layers at the air–water interface etc., belong to the category of 'soft' surfaces, i.e. where the charge is confined to a chemical group and is part of a molecule which is localised on the surface. Experimental results show that solutions of the P–B equation describes the properties of these systems too. Specific interactions between the ions and the surface can easily be accounted for by introducing an equilibrium constant according to Eq. 22. The charge on these surfaces may protrude somewhat from the surface and the counterion may therefore penetrate somewhat in between the charged groups so that the plane of adsorption becomes closer than one atomic radius (Ref. [3], p. 737). It is not possible to cover any of these areas in this review paper and the interested reader is encouraged to look into the literature for further details.

2.6. Concluding remarks concerning double layer models

Double layer models have been applied to various kinds of charged surfaces; 'soft' surfaces, smooth hard surfaces and irregular hard surfaces, respective-ly. From the presentation it is clear that many different models have been developed and that the details of each model reflect the physical properties of the system under investigation. The common basis for all the discussed models is solutions of the P–B equation, which for a planar surface is the G–C model, which assumes that the ions in the solution are point charges and that they can reach up to the surface plane.

Experimental studies of the mercury–water inter-face were some of the earlier and the most important application of the G–C theory. The interpretation of the experimental results led to the development of different kinds of layers where the ions are situated. Various double layer models have been applied to describe the properties of charged irregular surfaces such as inorganic oxides. These models can to a large extent be considered as modifications of the models developed for the mercury–solution inter-face. The complete models may contain many adjust-able parameters that are difficult to unambiguously

determine from experimental data. However, Sposito [19] has presented a thermodynamic analysis which describes how the thermodynamically correct value for equilibrium constant of binding to the surface can be obtained from experimental data.The physical meaning of various kinds of counterion-layers which have been used for hard surfaces becomes unclear when used for 'soft' surfaces. In fact only a set of unknown constants are introduced which allows for adjustment to the experimental data. For soft sur-faces there is a tendency in colloid and surface science to use a suitable solution of the P–B equation (e.g. the G–C theory), complemented with a single binding constant to the surface according to Eq. 22.

There are limitations regarding the applicability of the G–C theory and other solutions of the P–B equation. For example its use for divalent ions is more restricted than for monovalent ions and for higher charged small ions the model shall not be used. It is also restricted to low and moderate salt concentrations and electrostatic potentials. When these criteria are not fulfilled a detailed description of the inner part of the double layer must be introduced which is a difficult task from a physical point of view. Despite all of the work done in this area much remains until a consistent picture of the physical properties of the inner part of the double layer can be given. Despite the above limitations, solutions of the P–B equation have greatly con-tributed to our understanding and quantitative de-scription of the physical properties of charged bodies in contact with an electrolyte solution.

3. Retention theory for ions: general considerations

3.1. A brief introduction to the physical meaning of the retention factor

In column chromatography the retention of an eluite is characterized by its retention or capacity factor, k. In textbooks the relation between the retention factor and the distribution coefficient, K, for the eluite to the stationary phase is usually derived as:

$$k = \frac{V_R}{V_0} \cdot K = \frac{V_R}{V_0} \cdot \frac{c_{B,R}}{c_{B,E}} \qquad (36)$$

Where V_R and V_0 are the volume of the stationary and mobile phase in the column, respectively. This equation assumes that the eluite is distributed between two distinct phases and that each phase has a certain volume, V_R and V_0, respectively, and that the eluite is distributed homogenously within the two phases. Furthermore, the experimental determination of k requires that the marker molecule, which is used to measure the column dead volume, does not interact with the stationary phase or penetrate into it, i.e. the marker molecule does not enter into the volume V_R during its migration through the column. From the discussion in previous sections it is clear that when an eluite ion interacts with a charged surface its concentration varies within the volume designated as the resin or stationary phase. Therefore, the prerequisite for using Eq. 36 are not fulfilled and in order to describe eluite retention due to electrostatic interactions a more general interpretation of the retention factor is needed.

In the literature of chromatographic theory several formulations of the retention factor can be found [20–24]. In this review a formulation adapted to the case when retention occurs because the eluite is held in a diffuse layer close to a planar stationary phase surface is presented. A complete derivation of the more general equation can be found in Refs. [23,24]. In analytical applications of chromatography, the eluite concentration is low and it can be assumed that the adsorption isotherm of the eluite to the stationary phase is linear.

Within the moving eluite zone the concentration of the eluite ion, B, varies as a result of the interaction with the stationary phase surface. If it is assumed that the distribution is in equilibrium, i.e. the eluite concentration is Boltzmann distributed, the eluite concentration at a distance x from the planar surface, $c_B(x)$, is related to its concentration in the bulk phase, $c_{B,E}$, by the following expression:

$$c_B(x) = c_{B,E} \cdot e^{-(\Delta G^0(x)/RT)} \qquad (37)$$

Where $\Delta G^0(x)$ is the standard free energy of transfer of the eluite from the bulk solution to a point at the distance x from the stationary phase surface. From Eq. 37 and the operational definition of the capacity factor, $(t_r - t_0)/t_0$, (t_r is the elution time for the eluite and t_0 for the marker molecule) it can be shown that the expression for the capacity factor becomes, see e.g. Ref. [23]:

$$k = \frac{A_s}{V_0} \int_0^l (\exp(-\Delta G^0(x)/RT) - 1)\, dx \qquad (38)$$

where A_s is the surface area of the stationary phase. The value for l is such that it is situated in the bulk of the mobile phase, i.e. $\Delta G(l) = 0$. The integral in Eq. 38 represents a sum of relative surface excesses of the eluite over the distance from the planar surface. This is illustrated for a hypothetical case in Fig. 4 where the curve represents the concentration of an eluite ion relative to its concentration in the bulk solution as a function of the distance from the surface. The shaded area corresponds to the integral in Eq. 38. In the derivation of this equation it is assumed that the mobile phase is stagnant in the region where interaction between the eluite and the stationary phase surface occurs.

It is important to note that the experimental determination of the retention factor depends on the properties of the marker molecule used to measure the column dead volume. In Eq. 38 it is assumed that the marker molecule has access to the same volume as the eluite ions. If, on the other hand, it is assumed that the marker molecule does not penetrate at all into the volume designated as the resin or the stationary phase, the retention factor is represented by Eq. 39:

$$k = \frac{A_s}{V_0} \int_0^l \exp(-\Delta G^0(x)/RT)\, dx \qquad (39)$$

It can easily be shown that if the eluite is homogeneously distributed between two distinct phases Eq. 39 is reduced to Eq. 36, Eq. 39 is therefore a generalisation of the commonly used interpretation of the retention factor. For the evaluation of the integral in Eqs. 38 or 39 the mathematical form of the interaction energy vs. distance, $\Delta G^0(x)$ must be known or assumed.
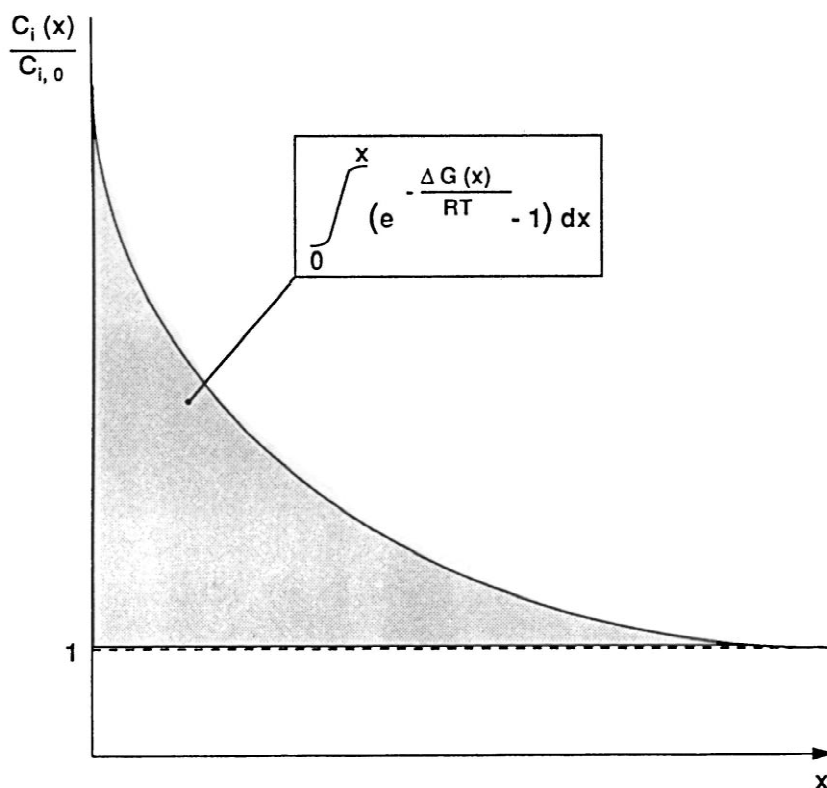
Fig. 4. Concentration of a solute ion, $c_i(x)$ relative to its bulk concentration, c_{i0}, as a function of the distance from the surface. The shaded area corresponds to the integral in Eq. 38. (The figure is reprinted from Ref. [58] with permission).

## 3.2. Stoichiometric vs. non-stoichiometric models

In the previous parts of this review paper the physical approach often used in surface and colloid chemistry to describe the properties of charged surfaces in contact with an electrolyte solution has been presented. It has been shown that solutions of the P–B equation, with the GC model as an important example, has greatly contributed to a quantitative understanding of the behavior of many different types of charged systems. Retention of ions in various forms of chromatography is governed by the same types of principles and consequently solutions of the P–B equation is a good theoretical starting point. However, retention models based on stoichiometric arguments are still dominating in the field of analytical chemistry. Since misconceptions regarding the theoretical treatment of electrostatic interactions appear regularly, it is appropriate to compare the theoretical background of the stoichio-

metric and electrostatic models in more detail. In the discussion we will use ion-exchange chromatography of small ions as an example, the reason is that the system is relatively easy to discuss (i.e. a stationary phase with a constant charge density, an electrolyte with common inorganic salt and small eluite ions which can be considered as point charges). Many other techniques are more complex and show even more 'non-stoichoinetric' properties, but the same type of arguments can be applied to all techniques involving electrostatic interactions.

In the modern literature of analytical applications of ion (exchange) chromatography several formulations of the ion-exchange equilibrium exists, however, the exact meaning of the used formulation is usually not discussed. This is in contrast to the early days of the development of this technique when there was more concern about the physical interpretation of the ion-exchange process. In this section some possible interpretations of the stoichiometric ap-

proach are discussed and compared with the general knowledge obtained in colloid and surface chemistry for electrostatic interactions. As will be illuminated in the ensuing paragraphs, an examination of the meaning of the stoichiometric approach leads to two fundamental problems; first, the validity of its assumptions for describing electrostatic interactions and, second, the problems connected with the interpretation of chromatographic retention using the stoichiometric approach.

The ion-exchange equilibrium between two cations on a cation exchanger is sometimes written as:

$$z_C B^{z_B} + z_B C^{z_C} - S(z_C) \rightleftharpoons z_C B^{z_B} - S(z_B) + z_B C^{z_C} \quad (40)$$
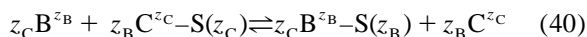
where S is the charged surface group (monovalent) and B and C the eluite and the counterion, respectively, carrying charge z_B and z_C, respectively. The literal interpretation of this equation would be that one C^{z_C} ion is bound to z_C number of charged surface groups as a complex and that a stoichiometric substitution takes place at the surface when ion B^{z_B} is added to the mobile phase. When we are considering electrostatic forces in a medium of high dielectric constant (e.g. water), such an interpretation is physically incorrect. The reason is that the formation of complexes at the surface decrease the entropy of the system too much, i.e. the system has a too high degree of order. Another way to express this is to say that the thermal energy of the ions is too high for them to be bound as complexes by electrostatic forces alone. Instead, as has been extensively discussed in the previous sections, an electrical double layer exists close to the surface.

The equilibrium constant for the exchange expressed by Eq. 40 is usually written as:

$$K_{exc} = \frac{\{S-B^{z_B}\}^{z_C}\{C^{z_C}\}^{z_B}}{\{S-C^{z_C}\}^{z_B}\{B^{z_B}\}^{z_C}}$$
$$= \frac{[S-B^{z_B}]^{z_C}[C^{z_C}]^{z_B}}{[S-C^{z_C}]^{z_B}[B^{z_B}]^{z_C}} \cdot \frac{\gamma_{S-B}^{z_C}\gamma_C^{z_B}}{\gamma_{S-C}^{z_B}\gamma_B^{z_C}} \quad (41)$$

where the {} brackets signify the activity, the squared brackets signify the concentration and γ_i is the activity coefficient of respective species. Instead of making the literal interpretation of species bound as complexes to the stationary phase, Eqs. 40 and 41 are discussed in terms of the activity of the species in a resin phase. Except for the fact that the activity

coefficient for individual ionic species is not measurable, equation of the form of Eq. 41 is physically correct. However, the equation is of no interest as long as the variation of the activity coefficients with the experimental conditions is unknown or can be theoretically described.

In most treatments no attempt is made to estimate the activity coefficients in Eq. 41, instead it is usually assumed that the ratio of activity coefficients in Eq. 41 is constant. However, it has been known for a long time that this is not generally true as the electrolyte concentration in the eluent phase changes [7]. The reason for this can be understood from the thermodynamic theory for polyelectrolytes of Marcus [10]. According to this theory the contribution from electrostatics to the activity coefficients in the resin relative to that in the eluent is expressed as:

$$\left(\frac{\gamma_{S-B}}{\gamma_B}\right)^{z_C} = \left(\frac{V_R}{\int\limits_{V_R} e^{-(z_B F \Psi(r)/RT)} dV_R}\right)^{z_C} \quad (42)$$

$$\left(\frac{\gamma_{S-C}}{\gamma_C}\right)^{z_B} = \left(\frac{V_R}{\int\limits_{V_R} e^{-(z_C F \Psi(r)/RT)} dV_R}\right)^{z_B} \quad (43)$$

where $\Psi(r)$ is the electrostatic potential at a point r in the resin phase. Combining these two equations it is readily found that the ratio of activity coefficients in Eq. 41 is:

$$\frac{\gamma_{S-B}^{z_C}\gamma_C^{z_B}}{\gamma_B^{z_C}\gamma_{S-C}^{z_B}} = \frac{V_R^{z_C-z_B}\left(\int\limits_{V_R} e^{-(z_C F \Psi(r)/RT)} dV_R\right)^{z_B}}{\left(\int\limits_{V_R} e^{-(z_B F \Psi(r)/RT)} dV_R\right)^{z_C}} \quad (44)$$

For the case when $z_B = z_C$ the ratio of activity coefficients is constant and equal to unity. However, this is not so when z_B and z_C have different values, to illustrate this point we consider the case where $z_B = 2$ and $z_C = 1$. Under this condition Eq. 44 becomes:

$$\frac{\gamma_{S-B}\gamma_C^2}{\gamma_B\gamma_{S-C}^2} = \frac{\left(\int\limits_{V_R} e^{-(F\Psi(r)/RT)} dV_R\right)^2}{V_R \cdot \int\limits_{V_R} e^{-(2F\Psi(r)/RT)} dV_R} \quad (45)$$

a ratio which can not a priori be considered to be constant. Since $V_R = \int 1 \cdot dV_R$, Schwarz's inequality (see Section 2.2, Eq. 17) can be applied for this particular case and we obtain that

$$\frac{\gamma_{S-B}\gamma_C^2}{\gamma_B\gamma_{S-C}^2} = \frac{\left(\int\limits_{V_R} e^{-(F\Psi(r)/RT)} \, dV_R\right)^2}{V_R \cdot \int\limits_{V_R} e^{-(2F\Psi(r)/RT)} \, dV_R} \leq 1 \qquad (46)$$

The physical interpretation of this inequality is that the mean concentration of the divalent ion in the resin phase, relative to that of the monovalent ion, generally will be higher than what is predicted from pure stoichiometry. This example illustrates that the polyelectrolyte theory of Marcus is a good starting point for investigating under which conditions the assumption of constant activity coefficient ratio is reasonable. More theoretical as well as experimental work are needed to investigate the properties of Eq. 44 and its applicability to ion-exchange systems.

A numerical example illuminate some of the aspects discussed above. Consider a ion-exchange system consisting of a planar negatively charged surface (charge density -0.02 C/m^2) in contact with a solution consisting of a mixture of mono and divalent cations and monovalent anions. By using numerical methods the Poisson–Boltzmann equation can be solved for this system [25]. The result for a constant concentration of monovalent ions (10 mol/

m^3) and varying concentrations of divalent ions in the eluent phase (0.1, 1 and 10 mol/m^3) is presented in Table 1. In order to make a comparison with a stoichiometric model, we are forced to assign a certain volume for the resin phase. In one of these calculations this is assumed to be $2 \cdot 10^{-8}$ m^3/m^2 stationary phase surface, i.e. equivalent to a 200 Å thick resin phase, and in the other it is 50 Å thick, respectively. Table 1 shows the mean concentration of the different ions in the assigned resin phase and also the value of an apparent stoichiometric constant for distribution of the divalent ion between the phases. It is clearly seen that the predictions from a stoichiometric approach fails, e.g. when the concentration of divalent ions increases the value of the stoichiometric constant decreases reflecting the changes in the ratio of activity coefficients. Table 1 also shows that the values strongly depend on the assumed volume of the resin phase which illustrates that an unambiguous designation of a resin phase volume can not be done in this case.

In the stoichiometric approach it is sometimes stated that the reaction scheme in Eq. 40 implies that the exchange of ions takes place with mathematical exactness, i.e. when one divalent ion is entering into the 'resin' phase, electroneutrality in this phase requires that exactly two monovalent ions leave the 'resin' phase. This argument is generally not true; the reason is that in the resin phase the concentration of co-ions to the surface charges increases when the ionic strength in the eluent phase increases. The data

Table 1
The mean concentration of C$^+$, B^{2+} and D$^-$, respectively, in the 'resin phase' ([S–C$^+$], [S–B^{2+}] and [D]$_R$, respectively), calculated by a numerical solution of the Poisson–Boltzmann equation[a,b]

[C$^+$] (mol/m^3)	[B^{2+}] (mol/m^3)	[D$^-$] (mol/m^3)	[S–C$^+$] (mol/m^3)	[S–B^{2+}] (mol/m^3)	[D$^-$]$_R$ (mol/m^3)	K_{exc}
200 Å thick resin phase						
10	0.1	10.2	46.6	32.3	7.5	14.9
10	1	12	24.5	44.4	9.6	7.4
10	10	30	14.2	58.3	27.1	2.9
50 Å thick resin phase						
10	0.1	10.2	157.5	129.8	2.0	5.2
10	1	12	68.1	175.4	3.8	3.8
10	10	30	26.7	203.5	18.5	2.9

[a] The calculations are made for different bulk concentrations of B^{2+} (0.1, 1 and 10 mol/m^3, respectively) keeping both the bulk concentration of C$^+$ (10 mol/m^3) and the surface charge density (-0.02 C/m^2) constant.

[b] K_{exc} is the apparent stoichiometric exchange constant calculated from Eq. 41.

in Table 1 also illustrates this fact since the mean concentration of the anion in the resin phase increases when the concentration of divalent ion increases.

From the preceding discussions regarding the physical background of the electrostatic interaction it is clear that the arguments leading to the mass action law do not apply to ion-exchange chromatography. Although this has been recognised for many years it was only recently demonstrated that this model gives inconsistent experimental results [26]. Stoichiometric models based on the mass action law shall therefore be considered as empirical models and will therefore not be further discussed in this review paper. This does not in any way mean that empirical approaches to ion-exchange equilibria are not valuable. On the contrary, they have contributed greatly to our understanding of the subject. Furthermore, many systems are so complex that data fitting based on multiparametric retention equations from stoichiometric models is sometimes necessary for practical description of measured retention data.

4. Ion (exchange) chromatography of small ions

4.1. Introduction

When applying the previously treated theories to analytical ion-exchange chromatography it is appropriate to discuss the chromatography of small and large ions separately. The reasons for this is that small eluite ions can be considered as point charges and that in analytical applications their concentration can be assumed to be small enough to not disturb the electrostatic potential gradient created within the column packing by the fixed charges and the ions constituting the eluent. However, this idealisation can not be made for large ions, e.g. proteins, because the size of the eluite becomes important and the interaction involves the overlap of the double layers associated with both the protein and the stationary phase. Clearly, there is a diffuse border between cases of small and large ions and theoretical models for these intermediate cases are still scarce.

Previously we discussed the physical properties of charged surfaces in contact with an electrolyte solution. This discussion shows that the geometrical distribution of the fixed charges in space is of importance for the type of model that can be used to describe the ionic distribution in the stationary phase. Therefore, when discussing retention theories for small ions, a distinction between two types of stationary phases must be made; (i) phases where the fixed charges can be considered to be situated at a surface and (ii) phases where the fixed charges form a random three-dimensional network, i.e. a gel. For the former case, physical models for the description of the ionic distribution based on the Poisson–Boltzmann equation are well developed and fairly well understood. The geometrical distribution of charges is less defined in the gel type of phases, therefore the corresponding retention theories are less exact. Still, as shown in Appendix A, it is possible to formulate a retention theory which is more or less independent of the geometry and therefore holds for both types of phases. The gel type phases dominated during the early development of ion-exchange chromatography. The corresponding theories for ion-exchange chromatography treated the electrostatic component in terms of Donnan potential to which the effect of swelling was added. Although the types of stationary phases used in modern analytical applications of ion-exchange chromatography have changed, the Donnan potential approach or the stoichiometric ion-exchange theory are still frequently used.

In analytical applications of ion-exchange chromatography two aspects of retention are of interest: (i) How is the capacity factor of an eluite ion affected by the concentration of the eluent salt? and (ii) How is the selectivity between two eluite ions affected by the type and concentration of the eluent salt? In the following sections, the theories that have been proposed to answer these two questions are discussed. Several models exist which in a more or less acceptable way answer the first question. The second question is much more difficult and a brief summary of the different attempts to explain the selectivity differences are collected in a separate section.

In analytical applications of ion-exchange chromatography the concentration of the eluite is usually very small compared to the other ion(s). In the ensuing sections we will therefore restrict the discussion to the linear part of the adsorption isotherm for the eluite ion. In many other areas where ion-ex-

change chromatography is used, e.g. in chemical engineering, this is not the case. In this area the goal of most of the theories has been to describe the complete exchange adsorption isotherm. This is a much more complicated issue which will not be treated here.

4.2. Experimental results for ion-exchange chromatography of small ions

The main purpose of a theory is to, in a consistent manner, explain experimental data obtained under well-defined and controlled conditions. It is therefore very important to compare the theoretical predictions with experimental results. When discussing theoretical models, a natural starting point is therefore to show the general behavior of experimental data.

Many investigations have been performed in order to study how retention of an eluite ion varies as a function of eluent salt concentration and how the selectivity is influenced by various parameters [26–37]. From a physical point of view, there is an important difference between these two types of investigations. When studying variation of the capacity factor with the concentration of salt in the eluent, the physical parameter that is measured is the change in total standard free energy of transfer of the eluite ion between the eluent and the resin phases, with changing eluent salt concentration. On the other hand, selectivity studies measure the difference in this total standard free energy of transfer, between two different eluites.

Experimental data for the capacity factor of an eluite as a function of salt concentration in the eluent is usually plotted in the form:
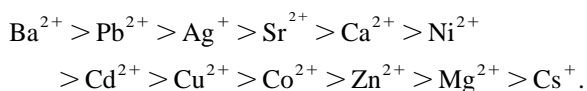
$$\log k = -s \log[c_{C,E}] + \text{constant} \qquad (47)$$

where s is a constant and $c_{C,E}$ is the mobile phase concentration of the counterion to the charged groups on the stationary phase. An example of experimental data plotted according to Eq. 47 is shown in Fig. 5, the plots usually show good linearity. The slope, s, is usually found to be close to the ratio z_B/z_C, where z_B is the charge of the eluite and z_C the charge of the counterion. When the net charge of any of the ions involved in the 'exchange' process is higher than three, the value of s may depart significantly from the z_B/z_C ratio, the ratio will in these cases depend

on the properties of both the eluent ions and the stationary phase [38], respectively. It was recognised early that there is an order of preferred affinity for both cations as well as anions to conventional ion-exchange stationary phases. For a strong acid cation exchanger the following affinity sequences are often found [8]:

$$Ag^+ > Cs^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$$

and

$$Ba^{2+} > Pb^{2+} > Ag^+ > Sr^{2+} > Ca^{2+} > Ni^{2+}$$
$$> Cd^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+} > Cs^+.$$

For a strong anion exchanger the experimentally found affinity sequence is usually:

$$SO_4^{2-} > HSO_4^- > I^- > NO_3^- > Br^- > Cl^-$$
$$> HCO_3^- > HSiO_3^- > F^- > OH^-.$$
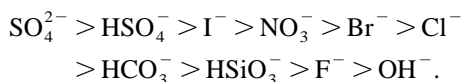
These sequences are not absolute and especially the sequence for anion exchange vary widely [39]. The affinity order on weak cation or anion exchangers is similar to those for strong exchangers, the exceptions are the H^+ and OH^- ion, respectively, which have distinctly higher affinity to weak exchangers. A general trend in both cation and anion exchange chromatography is that divalent ions have higher affinity to the stationary phase than monovalent ions. This effect is usually called electroselectivity [39] and is easily explained from both the Donnan and the double layer models, vide infra.

A classical subject in colloid chemistry is the study of the influence of type and concentration of salt on the critical coagulation concentration of a colloidal sol. It is worth noting that there are strong similarities between the physical effects of different salts on coagulation and the affinity sequence in ion-exchange chromatography. The effect of valence on the critical coagulation concentration is in colloid chemistry known as the Schulze Hardy rule [3] and corresponds to the electroselectivity concept. In colloid chemistry there are many processes where the properties of different ions of the same valency are important. A series resembling to the affinity sequence ion-exchange chromatography is the lyotropic or Hofmeister series for coagulation. For anions this series is [3]
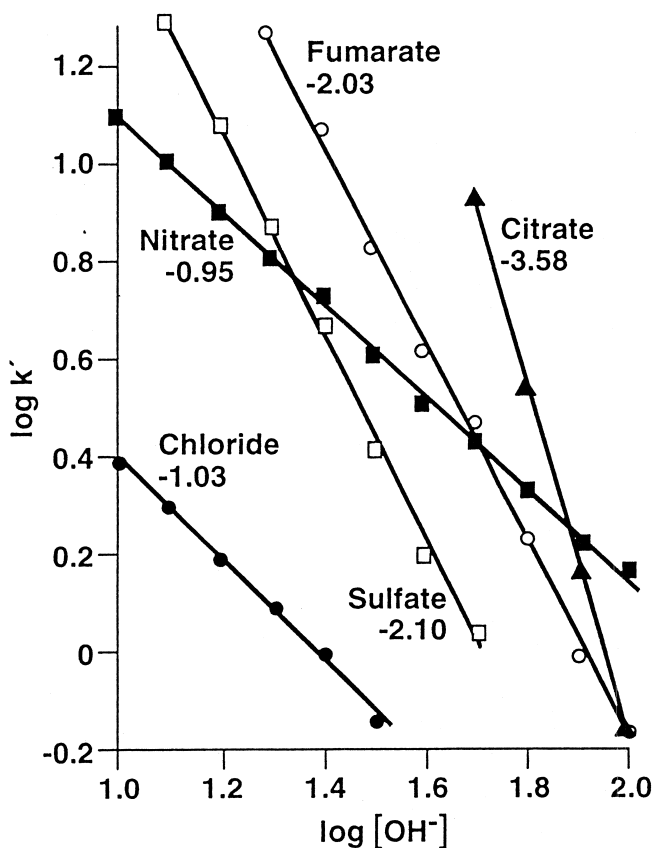
Fig. 5. Plots of log k_B vs. log [C$^-$] for NaOH eluents of different concentration. The column used was Dionex HPICAS5A. (The figure is reprinted from Ref. [37] with permission).

$$CNS^- > I^- > Br^- > Cl^- > F^- > NO_3^- > ClO_4^-$$
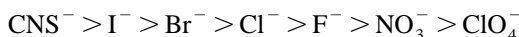
whilst for cations it is

$$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$$

and

$$Ca^{2+} > Mg^{2+}$$
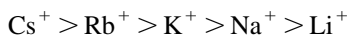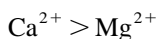
In colloid and surface chemistry these series are sometimes ascribed to the influence of the ions on the water structure. A more appropriate explanation seems to be their relative affinity to interfaces [4].

4.3. Application of the Donnan potential model to ion-exchange chromatography

As a starting point for the discussion of the Donnan potential approach we use the previously derived Eq. 6. This equation describes the distribution of Na$^+$ and K$^+$ between the eluent and the resin phase. An assumption often made is that the concentration of co-ions to the charged surface groups is negligible in the resin phase [39]. For dilute solutions and when the eluite ion, in our case the K$^+$ ions, is in trace amounts electroneutrality in the resin phase requires that the concentration of Na$^+$ in the resin phase, $c_{Na,R}$, is equal to the concentration of sulfonate groups and has therefore a constant value, C_R. Using this assumption Eq. 6 becomes:

$$\frac{c_{K,R}}{c_{K,E}} = \frac{\gamma_{K,E}}{\gamma_{K,R}} \left(\frac{\gamma_{Na,R} c_{Na,R}}{\gamma_{Na,E} c_{Na,E}} \right)^{z_K/z_{Na}}$$

$$= K_\gamma \left(\frac{C_R}{c_{Na,E}} \right)^{z_K/z_{Na}} \tag{48}$$

where K_γ represents the quotient of the different

activity coefficients. For an arbitrary eluite ion, B, and counterion, C, this assumption gives Eq. 49 as a generalised form of Eq. 48:

$$\frac{c_{B,R}}{c_{B,E}} = \frac{\gamma_{B,E}}{\gamma_{B,R}} \left(\frac{\gamma_{C,R} c_{C,R}}{\gamma_{C,E} c_{C,E}} \right)^{z_B/z_C}$$

$$= K_\gamma \left(\frac{C_R}{c_{C,E}} \right)^{z_B/z_C} \tag{49}$$

Introducing the traditional definition for the capacity factor, Eq. 36 into Eq. 49 the following expression for log k is obtained

$$\log k = \log\left(\frac{V_R \cdot C_R^{z_B/z_s}}{V_0} \right) + \log K_\gamma - \frac{z_B}{z_C} \cdot \log c_{C,E} \tag{50}$$

Comparison with the experimentally obtained relation, Eq. 47, shows that Eq. 50 correctly represents experimentally obtained log k vs. log $c_{C,E}$ plots.

There are two problems associated with this theoretical approach. First, the assumption of zero concentration of co-ions in the resin phase, i.e. Eq. 9 is zero, is in principle never met in practice. Second, the Donnan model is valid only when the electrostatic potential is equal at each and every point in the resin phase. These assumptions are analysed in detail in Section 2.2 where it is shown to lead to incorrect results in the calculation of the mean concentration of both the counter and co-ion in the resin phase.

The strength of the previously presented analysis by Marcus is that it is based on a minimum of assumptions and that it applies to any geometry of the resin phase. It is this authors opinion that further insights into the complex problem of ion-exchange chromatography can be gained from this approach. An example is outlined in Appendix A which discusses the retention of an eluite ion as a function of salt concentration in the eluent. From the treatment follows that the experimentally found retention dependence of salt concentration for a monovalent eluite ion can be explained for almost any geometry of the resin phase. To the authors knowledge the theory of Marcus has not previously been used in the field of ion-exchange chromatography.

4.4. Selectivity: a brief discussion

The chromatographic selectivity caused by differences in eluite charge, i.e. electroselectivity, is readily explained by electrostatic arguments in, e.g. the Donnan model. For eluite ions of equal charge the selectivity between different eluite ions is attributed in the Donnan model to differences in the activity quotient coefficient K_γ, Eq. 49. In the earlier development of theories for selectivity the desire for thermodynamic rigor was great. Experiments were carried out to correlate the activity coefficients with other quantities which are accessible by independent measurements. Classical work in the field of thermodynamics of ion-exchange was made by Glueckauf [40], Myers and Boyd [41,42] and others [43–48]. It is in this context important to remember that activity coefficients are strictly empirical quantities and as soon as theoretical explanations are put forward the introduction of activity coefficients does not make any contribution to the solution of the problem. Thermodynamics alone can therefore not be used to gain information concerning the actual value of K_γ, and to explain selectivity the Donnan based models must be complemented with a molecular model.

On a molecular scale there are probably several physical mechanisms which causes selectivity;

- the size of the eluite ion; the size is of importance because it determines the distance of closest approach for the pure Coulombic interaction between the fixed resin ions and the eluite ions. Since cations usually are stronger hydrated than anions, the size of the hydrated ion in these cases is the principal parameter. In the model by Pauley [49] selectivity is explained solely by differences in size. Reichenberg [50] considered both the size and the free energy of solvation as factors causing selectivity. Another aspect of the influence of the size of the ion on selectivity is proposed in the model by Gregor [51,52] where counterions with the larger molar volumes are preferentially squeezed out from the resin phase by the osmotic pressure in the resin.
- specific (or chemical) interaction between the eluite ion and the fixed ionic groups. This is one of the corner stones in the rather elaborate model by Harris and Rice [53,54].
- the polarisability of the ion, a higher polarisability gives a stronger attraction to the fixed ions by charge-induced dipole interaction.
- the van der Waals interaction between the resin phase and the eluite ion. This interaction is

probably of great importance for selectivity between different organic ions.

- the interaction between the eluite ion and eluent ions adsorbed in the resin phase. This effect has been proposed by Bokx and Boots [55,56] as the principal factor causing selectivity.

For the selectivity between organic ions two additional factors are important; (i) the hydrophobic effect (i.e. the structuring of water molecules around a hydrophobic molecule) and (ii) the hydrophobic interaction (i.e. the unusual strong interaction between hydrophobic molecules in water).

This list indicates that many physical effects give rise to chromatographic selectivity between ions. It is therefore a very difficult task to quantitatively include all of them (and possibly other effects as well) in one single model. A tremendous amount of work has been performed also in surface and colloid chemistry to investigate the origin of the lyotropic or Hofmeister series, a series which has the same origin as selectivity. For example, a review paper from 1985 by Collins and Washabaugh [57] on this subject lists over 900 references. Also in this field the origin of the affinity sequence is under debate and it is generally recognised that there is insufficient knowledge of the physical chemistry of concentrated electrolyte solutions to explain its cause.

4.5. Application of double layer models to ion-exchange chromatography

When the chemically bound charges, which comprise the stationary phase, are ordered so that they on a molecular scale can be considered as situated on a two-dimensional surface, the previously discussed double layer models become applicable to describe the retention of ions in ion-exchange chromatography. For chemical engineering purposes the previously mentioned model by Horst and coworkers [17,18] is one electrostatic model. We will here focus on analytical applications and the two double layer models that have been proposed in this area: the model developed by Cantwell and coworkers and the model by Ståhlberg. The model by Ståhlberg is conceptually easier and follows from a straightforward application of the previously discussed principles. We therefore begin with a presentation of this

model followed by a discussion of the model by Cantwell.

4.5.1. The double layer model by Ståhlberg

In the model by Ståhlberg [58] the effect of electrolyte concentration in the eluent on the capacity factor is described. The model is based on the Gouy–Chapman (G–C) theory complemented with the possibility for specific adsorption of both the eluite and the eluent counterions to the stationary phase surface. The specifically adsorbed counterions are located in the same plane as the fixed charges where the electrostatic potential is Ψ_0, see Fig. 6. The specific binding of both types of ions to the stationary phase is described by an association constant according to Eq. 22. Beyond this plane, the ion concentration of *non-specifically* adsorbed counterions is assumed to follow G–C theory. It is assumed that the concentration of eluite ions is negligible compared to the concentration of eluent ions, i.e. the ion-exchange process takes place under trace conditions. This implies that the eluite ion distribution is determined by the potential profile created by the eluent ions and the charged stationary phase. For a particular combination of stationary phase surface and type of eluent salt, changes in retention with the electrolyte concentration in the eluent is consequently due to an altered electrostatic potential profile in the double layer.

According to this model the retention of an eluite ion is due to accumulation of the ion in the double layer, k_{DL}, and possibly also due to specific adsorption to the surface, k_{ADS}, i.e. the capacity factor can be divided into two parts

$$k = k_{DL} + k_{ADS} \tag{51}$$

Where k_{DL} is the contribution from accumulation and k_{ADS} due to specific adsorption. Let us first consider the contribution from accumulation. In Section 3.1 the mathematical treatment of the capacity factor for distance dependent interactions is discussed and it is shown there that Eq. 38 describes k_{DL}. In this model it is assumed that:

$$\Delta G^0(x) = z_B F \Psi(x) \tag{52}$$

where $\Psi(x)$ is the difference in electrostatic potential between a point at distance x from the surface plane

Fig. 6. A schematic illustration of the double layer model for ion-exchange chromatography of small ions according to Såhlberg. A negatively charged surface is in contact with a solution containing eluent sal C^+D^- and an eluite ion B^+. The model uses the electrostatic potential profile $\Psi(x)$ which is calculated from the G–C theory. Ions situated in the plane of the surface charges may be specifically adsorbed to the surface.

and the bulk solution (where the potential is set to zero). We therefore obtain that

$$k_{DL} = \frac{A_s}{V_0} \int_0^l \left(e^{-(z_B F \Psi(x)/RT)} - 1\right) dx \qquad (53)$$

In this model $\Psi(x)$ is calculated from the Gouy–

Chapman theory implying that the 'chemical' environment for the eluite ion, e.g. water structure, hydration etc., in the double layer is the same as in the bulk solution.

The capacity factor due to specific adsorption, k_{ADS}, is accounted for by introducing an association constant, $K_{ch,B}$, according to Eq. 22. It is important

to keep in mind that in this equation $c_i(0)$ is the 'local' concentration of the eluite ion at the surface plane, $c_B(0)$. At this plane the concentration depends on the electrostatic potential at the surface plane, Ψ_0, and its bulk concentration, $c_{B,E}$, according to:

$$c_B(0) = c_{B,E} \cdot e^{-(z_B F \Psi_0 / RT)} \qquad (54)$$

In Ref. [58] it is shown that the final results for k_{ADS} becomes

$$k_{ADS} = \frac{A_s}{V_0} \cdot \frac{\sigma \cdot K_{ch,B} e^{-(z_B F \Psi_0 / RT)}}{-z_B F} \qquad (55)$$

where σ is the density of free charges (in C/m^2) on the stationary phase surface, i.e. it corresponds to the term [s] in Eq. 22. This charge density is not necessarily equal to the total number of chemically bounded charges per unit area on the stationary phase surface. The reason is that the counterions to these charges, constituting the eluent, may also bind specifically to the surface, reducing the effective charge density. In the model this effect is corrected for by again using Eq. 22, this time $c_j(0)$ means the counterion concentration at the surface plane, $c_c(0)$, which is calculated analogous to Eq. 54. Assuming that the sum of [s] and [is] corresponds to the maximum surface concentration of specifically bound counterions on the surface, a Langmuir type adsorption isotherm is obtained for the counterions. It is shown in Ref. [58] that under these assumptions the charge density of free charges is

$$\sigma = \frac{\sigma_0}{\left(1 + K_{ch,C} c_{C,E} e^{-(z_C F \Psi_0 / RT)}\right)} \qquad (56)$$

where σ is the total surface concentration of bound charged groups on the stationary phase surface (in C/m^2) and $K_{ch,C}$ is the association constant for binding of the eluent counterion to the surface. In all these equations the electrostatic potential at the surface plane, Ψ_0, is a parameter for which the numerical value depends on the parameter σ. Eq. 56 is therefore not a closed form equation but can be solved numerically by using the relation between Ψ_0 and σ from the Gouy–Chapman theory [58].

The final equation for the capacity factor is the sum of Eqs. 53 and 55

$$k = \frac{A_s}{V_0} \cdot \left(\frac{\sigma \cdot K_{ch,B} \, e^{-(z_B F \Psi_0 / RT)}}{-z_B F} + \int_0^l \left(e^{-(z_B F \Psi(x)/RT)} - 1 \right) dx \right) \qquad (57)$$

In this model the selectivity between eluite ions is due to different values of the association constant, $K_{ch,B}$. Also the difference in properties between different eluent salts are due to different association constant, $K_{ch,C}$. According to Eq. 57 the slope of a log k vs. log $c_{C,E}$ plot is influenced by the surface concentration of fixed charges, the association constant and the charge of both the analyte and the eluent counterion, respectively. The validity of the model was tested by numerical evaluation of Eq. 57 for different values of these parameters and plotting the result in a log k vs. log $c_{C,E}$ plot. The theoretically calculated curves are in good agreement with the general experimental observation, i.e. the plots are linear with a slope close to the quotient z_B/z_C. It was furthermore demonstrated that the slope only slightly depends on the properties of the eluite ion, eluent counterion and the properties of the stationary phase.

4.5.2. The double layer model by Cantwell et al.

In the retention model by Cantwell and coworkers [59] the following description for the charged surface–electrolyte system is used. The plane of closest approach for non-adsorbed counterions is the OHP (see Fig. 7) where the electrostatic potential is Ψ_{OHP}. Between the charged surface groups and the OHP there is a compact part of the double layer and beyond OHP there is a diffuse double layer. The electrostatic potential at the surface, Ψ_0, is related to Ψ_{OHP} via the capacitance of the compact part of the double layer, C_1. No electrolyte counterions, C, penetrate into the compact part and the capacitance of this layer is therefore constant and independent of the electrolyte concentration. In the model, the electrostatic potential at OHP, Ψ_{OHP}, as a function of the electrolyte concentration in the eluent is calculated according to the Gouy–Chapman theory.

Retention of eluite ions is, as in the model by Ståhlberg, considered to take place under trace conditions. In contrast to the eluent counterions, the
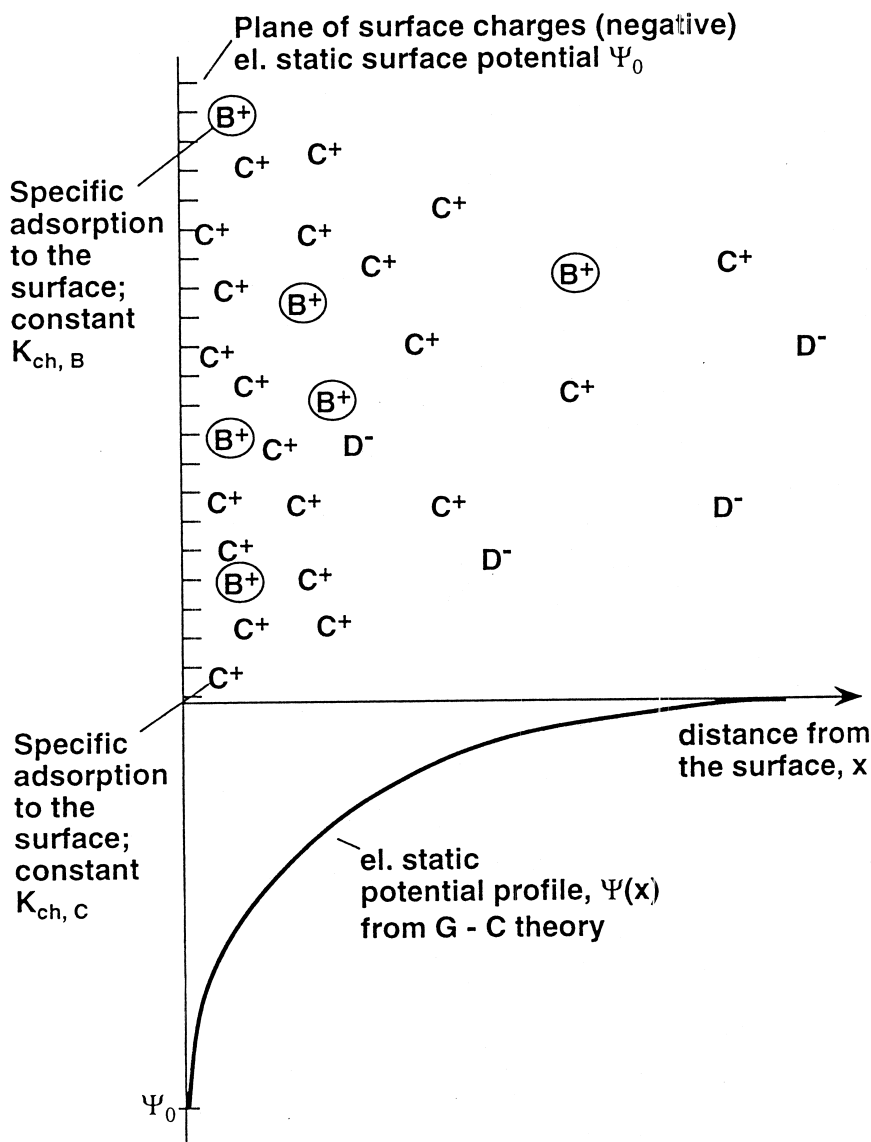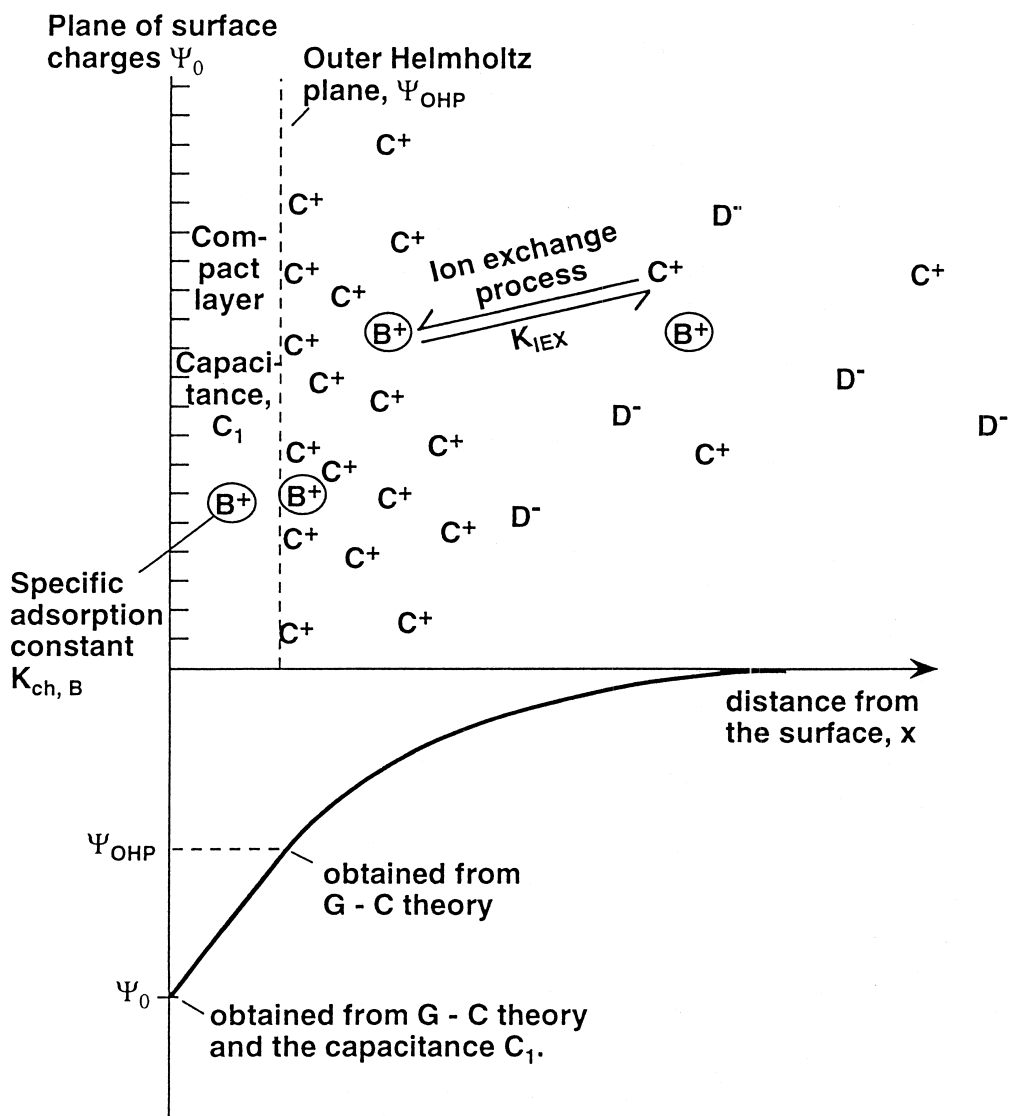
Fig. 7. A schematic illustration of the double layer model for ion-exchange chromatography of small ions according to the model by Cantwell and coworkers. The value for Ψ_0 is calculated from the properties of the double layer, which reaches up to OHP, and the capacitance of the compact layer, C_1. Retention of an eluite ion is governed by adsorption in the compact layer or by an ion-exchange process taking place between the diffuse double layer and the bulk of the eluent.

eluite ions may penetrate into the compact layer. In this layer they are considered to be specifically adsorbed and experiencing the electrostatic potential Ψ_0, i.e. the potential at the plane of the surface charges. Retention is due to both adsorption of the eluite ion in the compact layer and to ion-exchange in the diffuse layer. Cantwell writes for the retention due to accumulation in the compact layer:

$$k_{ADS} = \frac{V_R}{V_0} \cdot K_{ch,B} \cdot e^{-(z_B F \Psi_0 / RT)} \qquad (58)$$

In order to use Eq. 58 it is necessary to calculate the

value for Ψ_0 as a function of the surface charge density, σ_0, and the electrolyte concentration in the eluent. For low surface potentials the following relation is used (for higher Ψ_{OHP} values the exact equation from the Gouy–Chapman theory is used):

$$\Psi_0 = \frac{\sigma_0}{C_1} + \frac{\text{constant} \cdot \sigma_0}{\sqrt{c_{C,E}}} \qquad (59)$$

The 'constant' includes only physical constants and its value depends on the units which are used. The capacitance of the compact layer is estimated from the dielectric constant of the eluent (water) and its thickness is set to be equal to the radius of the hydrated eluit ion. σ_0 is determined from the ion-exchange capacity of the column stationary phase and its area.

In the model by Cantwell the retention of the eluite ion due to accumulation in the diffuse part of the double layer is described as an ion-exchange process between $B^{\pm}$ and $C^{\pm}$ taking place between the diffuse layer and the eluent. The resulting expression for k_{DL} for univalent electrolyte ions as well as eluite ions is

$$k_{DL} = -\frac{A_s}{V_0 F z_B} K_{IEX} \cdot \frac{\sigma_0}{c_{C,E}} \qquad (60)$$

where K_{IEX} is a stoichiometric exchange constant and σ_0 is the surface charge density of the stationary phase. As previously, the resulting capacity factor is obtained by adding Eqs. 58 and 60.

Cantwell and coworkers performed experiments with a styrene–divinylbenzene copolymer adsorbent which was chemically modified on the surface with either sulfonate or quarternary ammonium groups, in both cases the surface charge density was low (<0.01 C/m^2). By studying the capacity factor of various organic univalent ions as a function of eluent salt concentration, the authors were able to obtain the individual contributions of each of the processes described by Eqs. 58 and 60, respectively. The reason is that the ion-exchange process (Eq. 60) is inversely proportional to $c_{C,E}$ while the adsorption process has a more complex ionic strength dependence, i.e. given by a combination of Eqs. 58 and 59. By performing a numerical fitting of the experimental data to the theoretical equations, it was found that

the ion-exchange in the diffuse double layer is the principal mechanism responsible for the retention.

4.6. Summary and concluding remarks concerning models for retention of small ions in ion-exchange chromatography

Three models for the dependence of retention on eluent salt concentration have been discussed; the Donnan model and two models based on the diffuse electrical double layer. The strength of the Donnan approach is that it does not specify a certain geometry for the stationary or resin phase but only requires that retention is due to accumulation in the stationary phase. The weakness, as is demonstrated through the use of the Marcus theory for polyelectrolytes, is that it requires the electrostatic potential to be constant throughout the volume designated as the stationary phase. Furthermore, the experimentally observed retention equation, Eq. 47, can only be derived from the Donnan model if it is assumed that the co-ion concentration in the resin phase is zero.

The two diffuse double layer models discussed, on the other hand, require that the fixed charged groups bound to the stationary phase can be considered to be smeared out on a two-dimensional plane. This assumption is acceptable when the charged groups are bound to a surface and when the radius of the stationary phase pores are greater than 4–6 Debye lengths. The advantage of these models is that when this assumption is fulfilled, the models contain more information than the Donnan model regarding the influence of various parameters on retention. There are important differences between the two double layer models. In the model by Ståhlberg the selectivity is due to different adsorption properties of the eluite ions to the stationary phase surface. In the model by Cantwell and coworkers selectivity is due to both an ion-exchange process taking place in the double layer as well as different adsorption properties. Another difference is that in the model by Ståhlberg the diffuse double layer reaches up to the plane of the fixed charges while in the model by Cantwell there is a few Angstrom thick compact layer separating the plane and the diffuse layer. This leads to different expressions for the dependence of retention on eluent electrolyte concentration.

In Appendix A a fourth model is presented. This

model is based on Marcus analysis for polyelectrolytes and can to some extent be considered to be a generalisation of the other three models. This model has the same advantages as the Donnan model but it does not suffer from its weaknesses. The only additional assumption used is the additivity law for polyelectrolytes with added salt to the solution, which holds very well for a 1:1 salt.

The models for explaining selectivity differences are briefly discussed. The physical mechanism behind selectivity is very complicated, there is therefore no single model which can comprise all the parameters that are necessary to describe this complex phenomenon. More knowledge about the properties of concentrated polyelectrolyte solutions and the properties of the innermost part of the double layer is still needed to understand the physical cause of selectivity.

5. Ion pair chromatography

5.1. Introduction to ion pair chromatography

The most frequently used chromatographic technique today is reversed-phase liquid chromatography (RPLC) in which a polar mobile phase in combination with a hydrophobic stationary phase is used. The stationary phase usually consists of porous silica particles which have been chemically modified by attaching an alkyl group to the surface silanol groups. In RPLC the retention of an eluite is regulated by varying the relative amount of water and a water miscible organic solvent, e.g. methanol or acetonitrile. For ionic compounds an additional mode for retention regulation has been developed, the technique is based on the addition of amphiphilic (surface active) ions to the mobile phase. A number of different names have been proposed for this technique, e.g. ion interaction chromatography [63], soap chromatography [64], dynamic solvent generated ion-exchange chromatography [65], ion pair chromatography [66], ion-modified RPLC [67].

Ion pair chromatography was early applied to LLC (Liquid Liquid Chromatography), in which a hydrophobic liquid layer coated the inert silica support [68]. In this application it can be assumed that the change in retention of the eluite ion, when the concentration of the amphiphilic ion varies, is due to a distribution of an ion pair between the two liquid phases. The name ion pair chromatography derives from this early application. When the liquid layer was substituted by covalently bound alkyl groups, the ion pair model still influenced the formulation of the retention mechanism. Without implications for the actual mechanism of this chromatographic mode, the term ion pair chromatography (IPC) for the technique and pairing ion (IP-reagent) for the amphiphilic ion will be used in this paper.

The theory for IPC became a controversial subject and many theories have been proposed. Following Melander and Horváth [69] the theories can be divided into two categories; stoichiometric theories (e.g. [64,65,69–71]) and non-stoichiometric theories (e.g. [59–63,71–82]). The stoichiometric models are based on modifications of the ion pair extraction model and they have provided an easy-to-understand picture of solute retention for many analysts and have promoted the practical use of IPC. Several review papers treating stoichiometric models have been published [83–86]. Three of the non-stoichiometric models use solutions of the P–B equation to describe the role of elecrostatics on retention in IPC. The name given for each of these models is [67]: Liquid partition double layer model [76,77], Surface adsorption, diffuse layer ion-exchange double layer model [72–75] and Surface adsorption double layer model [78–82].

This chapter is intended to be an introduction to these three models and as complementary reading Refs. [67,92] are recommended. In Ref. [92] a more detailed presentation of the theory of the surface adsorption double layer model and comparison with experimental data is found. This reference also provides further details on how this theory can be formulated to be used in practical work. Ref. [67] is a readable review paper by Weber, Cantwell and coworkers discussing non-stoichiometric theoretical models for IPC. To get an overview of practical applications in IPC the reader is referred to a recent review paper by Gennaro and references therein [87]. Before going into the details of each model some basic concepts which are more or less common to the three models will be treated.

Since the surface layer of the bonded phases is very thin (e.g. <20 Å for a C_{18} phase [88]) it is the

surface properties of the layer, and not its bulk properties, which is of importance for the distribution of ionic species between the polar mobile and the nonpolar stationary phase. The physical reason is that the energy required to transfer a charged species from a medium of high to a medium of low dielectric constant is high. Therefore, charged hydrophobic species, e.g. alkylsulfonates or alkylsulfates which usually are used as IP-reagents, are oriented at the interface so that the charged part of the molecule remains in the polar phase and the hydrophobic part try to penetrate into the hydrophobic medium, see Fig. 8. The IP-reagent is therefore adsorbed at the interface between the stationary and mobile phase creating a charged surface, while the inorganic counterions form a corresponding diffuse layer. This implies that the IP-reagent creates an electrostatic surface potential, Ψ_0, and that the magnitude of this potential is primarily determined by the surface concentration of the IP-reagent. As a first approxi-

mation it can be assumed that the adsorption of the IP-reagent does not change any other property of the stationary phase than its electrostatic potential.

The capacity factor of an eluite ion in absence of an IP-reagent can be related to its standard free energy of adsorption, ΔG_B^0, according to:

$$k_{0B} = \phi \cdot e^{-(\Delta G_B^0/RT)} \tag{61}$$

where the subscript zero denotes that the concentration of IP-reagent is zero. When the IP-reagent is adsorbed an electrostatic surface potential is created. Assuming that the plane of adsorption of the eluite ions is the same as for the adsorbed IP-reagent, the free energy of adsorption, ΔG_B^0, of the eluite ions is changed to

$$\Delta G_{t,B}^0 = \Delta G_B^0 + z_B F \Psi_0 \tag{62}$$

where z_B is the charge of the eluite ion and Ψ_0 is the electrostatic surface potential created by the adsorbed
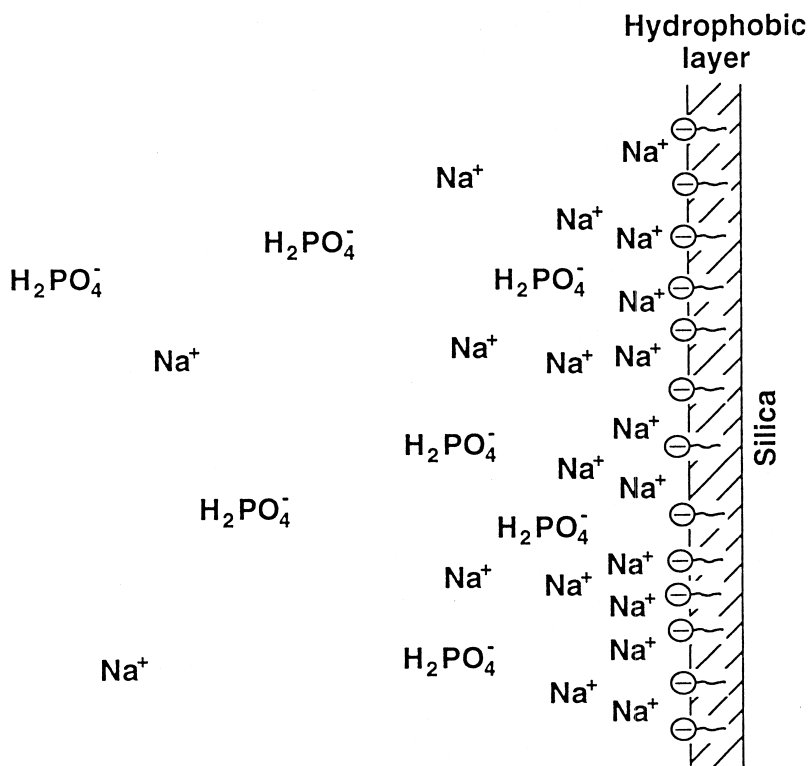

Fig. 8. An idealised picture of the electrical double layer in reversed-phase ion pair chromatography. (The figure is reprinted from Ref. [92] with permission).

Table 2
The capacity factor for different analytes with tetrabutylammonium as IP-reagent[a]

A^+ (mM)	Phenol ▲	Benzene-sulfonate ■	Naphtalene-2-sulfonate ●	Bupivacaine △	Etidocaine ⊗	Bensyltriethyl ammonium □	1,5-Naphtalene disulfonate ◐	1-Naphtol-3,6-disulfonate ◨
0	5.94	0.82	10.0	42.7	28.8	5.88	–	–
0.10	5.45	1.58	19.8	20.1	13.9	3.03	0.79	1.13
0.25	5.55	2.08	27.2	15.4	10.7	2.32	1.45	2.05
0.50	5.41	2.70	36.5	11.2	7.8	1.66	2.75	3.86
1.00	6.28	4.39	57.2	–	–	1.43	6.37	8.79
2.00	6.05	5.75	78.7	6.7	4.4	0.96	13.1	17.5
4.00	5.76	7.16	99.5	4.3	3.1	0.53	24.2	31.7

[a] Experimental conditions: mobile phase, acetonitrile–water (10:90); phosphate buffer pH=2.1.

IP-reagent. The parameter which determines Ψ_0 at constant eluent ionic strength is the surface charge density, i.e. the concentration of charges on the surface.

5.2. Experimental behaviour

It is appropriate to start the discussion with some examples showing the typical experimental behaviour found in IP-chromatography. Many parameters influence the retention of an eluite ion, the ensuing treatment is limited to three of them; (i) the concentration of the IP-reagent in the eluent, (ii) the type of IP-reagent and (iii) the mobile phase ionic strength.

In Table 2 a typical example is shown for the influence of the concentration of the IP-reagent (tetrabutylammonium ion, TBA) on the capacity factor for an uncharged eluite (Phenol), two negative moncharged ions (Benzenesulfonate and Naphtalenesulfonate), two negative divalent ions (1,5-Naphtalenedisulfonate and 1-Naphtol-3,6-disulfonate) and three positive moncharged ions (Bupivacaine, Etidocaine and Bensyltriethylammonium ion) [79]. The experimental system consists of an RP-18 column and acetonitrile–phosphate buffer (pH=2.1) 10:90 as eluent phase. The data in Table 2 show that when the concentration of tetrabutylaminonium ion increases in the eluent, the retention of an uncharged eluite is unchanged, the retention of negatively charged ions increases and decreases for positively charged ions. Thus, the addition of an ion pair reagent to the mobile phase has a characteristic influence on the retention of eluites; it has generally a negligible effect on the retention of uncharged eluites, increases the retention of oppositely charged eluites and decreases the retention of eluites with the same sign of charge. To illustrate the effect of the type of ion pair reagent, experimental results for another IP-reagent, tetraethylammonium ion [79] are shown in Table 3 using

Table 3
The capacity factor for different analytes with tetraethylammonium as IP-reagent[a]

A^+	Phenol ▲	Benzene-sulfonate ■	Naphtalene-2-sulfonate ●	Bupivacaine △	Etidocaine ⊗	Bensyltriethyl ammonium □	1,5-Naphtalene disulfonate ◐	1-Naphtol-3,6-disulfonate ◨
0	5.94	0.82	10.0	42.7	28.8	5.88	–	–
10	5.56	1.05	12.7	30.9	20.8	3.72	–	–
20	5.50	1.24	14.4	27.3	18.6	3.21	0.42	0.62
40	5.30	1.34	15.8	24.2	16.6	2.68	0.62	0.82
80	5.29	1.52	17.7	22.1	15.2	2.30	0.81	1.03
120	5.00	1.60	17.2	20.5	14.1	2.06	0.86	1.05

[a] Experimental conditions: mobile phase, acetonitrile–water (10:90); phosphate buffer pH=2.1.

the same eluites and experimental conditions as in Table 2. Comparison between the two tables shows that, to obtain the same retention shifts, a much higher eluent concentration of tetraethylammonium ion than TBA is required.

Many examples can be found in the literature regarding the effect of eluent ionic strength on the retention of charged eluites at a constant ion pair reagent concentration (see e.g. Refs. [80,81,90,91]). Some typical examples are shown in Fig. 9 where retention data for negatively charged hydroxybenzoic acids [91] are plotted as a function of ionic strength at constant eluent concentration of a positively charged IP-reagent. The displayed data are from van de Venne et al. and the experiments were performed on a RP-18 column with a phosphate buffer (pH=7) and a constant concentration of hexylamine (9.2 mM). It can be seen in the figure that, at a given

IP-reagent concentration, the retention of oppositely charged eluites decreases with increasing eluent ionic strength. For similarly charged eluites it is found that the retention increases with increasing ionic strength.

The parameters discussed above are just a few examples of parameters that can be varied in IP-chromatography. Other variables are pH, type of RP-column, type and concentration of organic modifier, type of salt in the eluent, respectively. A more detailed and complete presentation and analysis of the influence of these parameters on retention in IP chromatography is found in Ref. [92]. The experimental results presented here shall be considered as representative for the change in retention with the chosen parameters. Under extreme conditions, e.g. very high surface concentration of IP-reagent, high organic modifier content of the mobile phase etc., deviations from the presented behavior may occur.
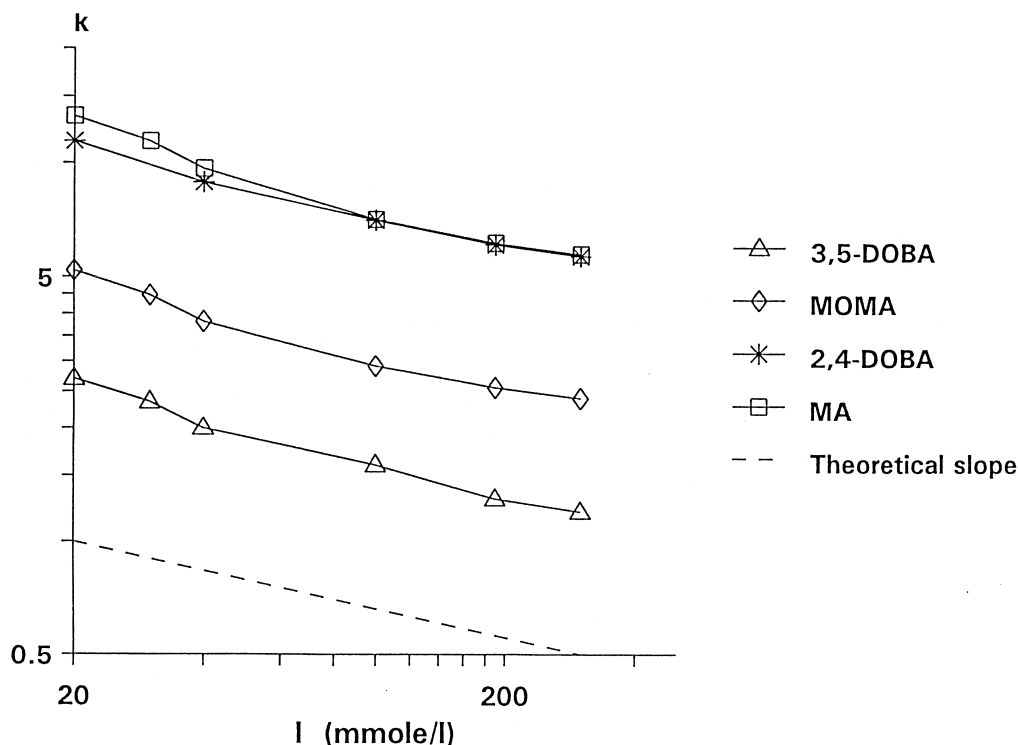
Fig. 9. Capacity factor for dissociated carboxylic acids as a function of ionic strength (I) of the phosphate buffer mobile phase (pH=7) at a constant concentration of hexylamine (9.2 mM). Data were measured by van de Venne et al. [91] using a LiChrosorb RP-18 column. Solutes: △=3,5-dihydroxybenzoic acid; ◇=4-hydroxymandelic acid; *=2,4-dihydroxybezoic acid; □=mandelic acid. The dashed line is the theoretical slope. For other experimental conditions, see Ref. [91]. (The figure is reprinted from Ref. [91] with permission).

5.3. Surface adsorption double layer model for reversed-phase ion pair chromatography

Of the three models using solutions of the P–B equation for describing electrostatic effects, the surface adsorption double layer model has been most extensively studied and experimentally tested [78]. This model has the same theoretical foundation as the previously treated double layer model for ion-exchange chromatography developed by Ståhlberg. In this section the basic theory of this model is briefly discussed and compared with the experimentally found behavior presented above.

The capacity factor in the presence of the concentration c of IP-reagent in the eluent, $k_{c,B}$, is obtained by combining Eqs. 61 and 62:

$$k_{c,B} = k_{0,B}\, e^{-(z_B F \Psi_0 / RT)} \tag{63}$$

In the derivation of this equation it is assumed that the chemical part of the free energy of adsorption is unchanged by the presence of adsorbed IP-reagent. Furthermore, the usually small contribution to the capacity factor from accumulation or depletion of the eluite ions in the double layer region is neglected. This equation implies that the retention increases for eluite ions with opposite charge as the IP-reagent, and that it decreases for similarly charged ions. From the equation also follows that the relative increase (decrease) in capacity factor for homovalent oppositely (similarly) charged eluites is the same for all eluites upon adding the IP-reagent. The equation also states that for divalent eluites the relative increase (decrease) is equal to the square of this factor. For example, if the capacity factor doubles for a mono-charged eluite upon adding the IP-reagent, all other eluites with the same charge also double their capacity factor. Under these conditions the capacity factor for divalent ions increases with a factor of four and all monovalent eluites with the same sign of charge as the IP-reagent will halve their capacity factor. Furthermore, the retention of an uncharged eluite, $z_B = 0$, should be unaffected by the presence of the IP-reagent in the eluent.

Comparison of these theoretical predictions with experimental data (c.f. Table 2) shows that there is in general a very good agreement. For example, when eluent concentration of TBA is increased from zero to 4 mM TBA in the mobile phase, the capacity factor for naphtalene-2-sulfonate increases by a factor of 10 ($k = 10.0 \rightarrow 99.5$) and for bupivacaine it decreases by the same factor ($k = 42.7 \rightarrow 4.3$). These are just a few examples demonstrating the validity of Eq. 63, many other examples can be found in the literature. Deviations from these simple rules may occur at high surface concentration of the IP-reagent. A possible explanation is that there is competition for the limited surface area between the IP-reagent and the eluite ion, an effect that can easily be included in the model [81]. The agreement between this theory and experimental results implies that under the conditions which IP-chromatography is used, changes in the surface potential modulate the retention of ions. Strong deviations from these rules indicates that other retention mechanisms also are operating.

Another consequence of Eq. 63 is that it is the surface concentration of IP-reagent, and not the type of IP-reagent, that determines the retention changes, when other eluent parameters are kept constant. This was also found experimentally for a series of alkylsulfates [71] and alkylsulfonates [93] used as IP-reagents at constant ionic strength in the eluent phase. An example is shown in Fig. 10 where 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, respectively. It is seen that although the pairing ions have different chain length, the same surface concentration results in identical eluite retention, i.e. creates the same electrostatic surface potential. In practice, it is the eluent concentration of the IP-reagent that is controlled by the chromatographer and not its surface concentration. These two are related through the adsorption isotherm of the IP-reagent to the stationary phase surface. Of the three discussed models, the surface adsorption double layer model is the only model that incorporates an adsorption isotherm into the retention theory.

The free energy of adsorption of the IP-reagent is, in analogy with the discussion above, divided into a chemical part and an electrostatic part. 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 electro-
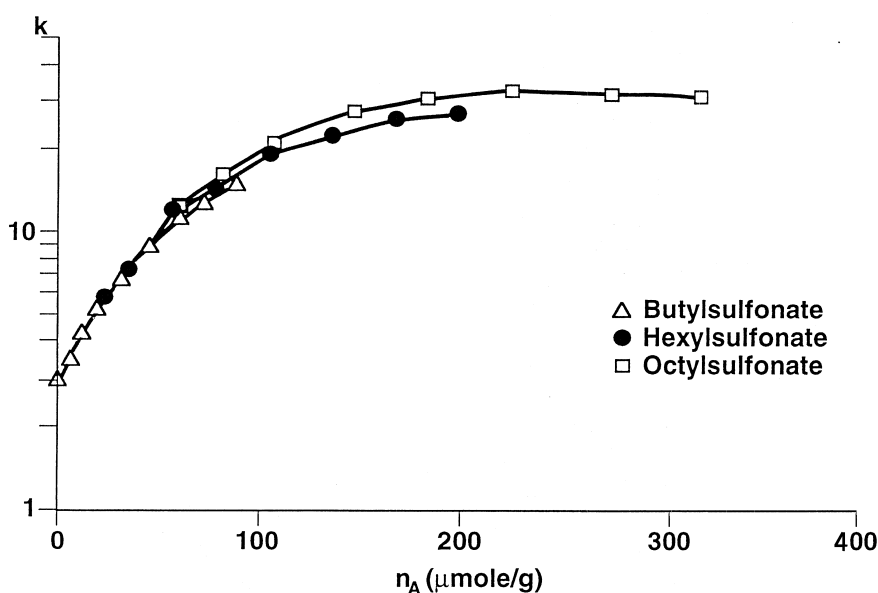
Fig. 10. Capacity factor (k) data for adrenaline as a function of stationary phase concentration (n_A) sodium ($\triangle$) butyl-, ($\bullet$) hexyl-, and ($\square$) octylsulphonate pairing ion. The data are obtained at constant ionic strength (175 mM) of the phosphate buffer (pH=2.1) mobile phase on an ODS-Hypersil column. See Ref. [89] for experimental details. (The figure is reprinted from Ref. [91] with permission).

statically repel itself from the surface so that a non-linear relation between eluent and stationary phase concentrations are obtained. The simplest adsorption isotherm is the surface potential modified linear isotherm:

$$n_A = n_0 K_A c_A \cdot e^{-(z_A F \Psi_0 / RT)} \tag{64}$$

where n_A is the surface concentration of the IP-reagent, c_A its eluent concentration, n_0 the monolayer capacity of the stationary phase and K_A the association constant to the stationary phase. In Eq. 64 the product $n_0 K_A c_A$ is a linear adsorption isotherm and the exponential term accounts for the electrostatic repulsion which gives rise to the non-linearity.

As the surface concentration increases, the area accessible for additional molecules decreases and the molecule finds it more and more difficult to find an adsorption site. This effect is taken into account in the Langmuir adsorption isotherm which in combination with the effect of the electrostatic surface potential forms the surface potential modified Langmuir adsorption isotherm:

$$n_A = \frac{n_0 K_A c_A \cdot e^{-(z_A F \Psi_0 / RT)}}{1 + K_A c_A \cdot e^{-(z_A F \Psi_0 / RT)}} \tag{65}$$

In practice, the non-linearity of the adsorption isotherm, due to the limited monolayer capacity, is not detected for surface concentrations below $0.3 n_0$ while the non-linearity due to the electrostatic repulsion is significant at much lower surface concentrations.

By making several well defined mathematical approximations in the equations used in the surface adsorption double layer model it is possible to understand and describe the experimentally found behavior presented in the previous section [92]. It can be shown that the retention of an eluite ion can be approximated by the following equation:

$$\ln k_{c,B} = \ln k_{0,B} + \frac{-z_A z_B}{(z_B^2 + 1)} \left[\ln \left(\frac{n_0 K_A c_A}{\kappa} \right) \right.$$
$$\left. + \ln \left(\frac{F^2}{RT \varepsilon_0 \varepsilon_r} \right) + 1 \right] \tag{66}$$

This equation is obtained from the electrostatically modified linear adsorption, isotherm, Eq. 64, the electrostatic surface potential as a function of surface charge density, Eq. 20, combined with the capacity factor as a function of electrostatic surface potential, Eq. 63 [92]. A closer inspection of the equation

shows that it semiquantitavively can describe the experimental behavior discussed in the previous section. E.g.;

The eluent concentration and type of IP-reagent mainly influence the eluite retention through its surface concentration which in turn is governed by the association constant, K_A, in Eqs. 64 and 66. This implies that molecules with a lower association constant to the stationary phase require higher eluent concentrations to induce a certain retention change.

An increasing eluent salt concentration increases the shielding of the surface charges and therefore lowers the electrostatic surface potential. The lowering of the surface potential means that the electrostatic repulsion of the IP-reagent from the surface decreases, this allows for a further increase of the surface concentration of IP-reagent. The increase of IP-reagent surface concentration only partly compensates the decrease in Ψ_0 caused by the salt. This is quantitatively described for low surface potentials by Eq. 20, while for higher potentials it can be obtained from, e.g. the Gouy–Chapman theory. In Eq. 66 the influence of eluent salt is included in the κ-term, defined by Eq. 19. For oppositely monocharged IP-reagent and eluite ion, respectively, this leads to a slope $-1/4$ in a $\ln k$ vs. $\ln I$ plot. The experimentally found behavior closely follows this theoretical prediction as shown in Fig. 9 where the dotted line represents the theoretical slope.

In the surface adsorption model it is assumed that retention of an eluite ion is due to adsorption to the stationary phase surface only, the effect of accumulation in the double layer is therefore neglected. Numerical calculations show that the accumulation usually can be neglected for $k_{0,B}$ values higher than 0.5 [92]. The effect of accumulation of eluite ions in the double layer can be taken into account for by adding the integral in Eq. 53, or by using a simplified algebraic solution [92], to the original version of the model.

The adsorption isotherm in Eq. 63 was recently applied to the determination of the chromatographically accessible surface area of RP surfaces [94] and also for the description of peak shapes of charged eluites under overloading conditions [95]. The surface adsorption double layer model has later been complemented with a model in which the heterogenity of the stationary phase surface is considered [96]. This implies that the chemical part of the free energy of adsorption is not assumed to take one single value, instead a range of values is assumed.

5.4. Surface adsorption, diffuse layer ion-exchange double layer model

From both a conceptual and practical point of view this model is more complex than the previous. The basic assumptions are the same as in the already presented ion-exchange model of Cantwell and co-workers. Its application to ion pair chromatography is, however, not straightforward and a rather complicated and elaborated procedure has been developed in order to determine all the unknown constants. It is not possible to give a detailed presentation of all theoretical and practical aspects of the theory in this paper, the purpose is to present the general picture only and to point out some difficulties. A short description of the evaluation procedure can be found in Ref. [75] which should be complemented with the information given in the Appendix in Ref. [72]. The evaluation procedure can be divided into two parts; (i) the calculation of the surface potential, Ψ_0 and (ii) the calculation of the capacity factor of an eluite ion as a function of mobile phase ionic strength. In order to use the model the following data are necessary: (a) a family of adsorption isotherms of the IP-reagent, each isotherm is measured at constant mobile phase concentrations of electrolyte and (b) retention data for the eluite ion for the same sets of IP-reagent and electrolyte concentrations, respectively.

For the calculation of Ψ_0 the main difference between Cantwells ion-exchange model and the corresponding IP-model is that the fixed charges, c.f. Fig. 7, are absent and substituted by the IP-reagent ion. The adsorbed IP-reagent is therefore located at the interface between the stationary and eluent phase, respectively, creating a charged surface and an electrostatic surface potential. For a given mobile phase ionic strength, the surface potential is related, through e.g. Eq. 59 to the surface concentration of charges and the capacitance of the empty compact part of the double layer. However, the experimentally obtained isotherm is not exactly equal to the surface concentration of ions, the reason is a deple-

tion of IP-reagent ions close to the surface. This depletion is primarily of importance when the column phase ratio is high in combination with a low affinity of the IP-reagent to the stationary phase surface. The depletion is related to the potential at OHP and therefore an iterative procedure is needed to simultaneously solve for the surface charge density and Ψ_{OHP}. An important requirement for the evaluation of experimental data is the knowledge of the activity of the IP-reagent in the mobile phase. This is obtained from the Debye–Hückel theory or from literature data. As a result different mobile phase compositions of IP-reagent and electrolyte, respectively, may give the same activity of the IP-reagent. In the next step in the evaluation procedure it is assumed that compositions of equal IP-reagent activity create the same surface potential. The argument for this assumption derives from the previously discussed Nernst equation, Section 2.6. Cantwell and coworkers plot the surface concentration of the IP-reagent, n_A, as a function of its activity in the mobile phase. From this family of plots a certain activity is chosen. For each chosen activity, the set of equations are formulated so that linear plots are expected when $1/n_A$ is chosen as the ordinata and a function containing Ψ_{OHP} and Ψ_0 as abscissa. From the slope and intercept of this plot the value for the capacitance, C_1, and Ψ_0 may be obtained. After this procedure the surface potential as a function of the activity of the IP-reagent in the mobile phase has been obtained.

Retention of the eluite ion is assumed to be the sum of two processes; (i) adsorption to the same plane as the adsorbed IP-reagent ion described by Eq. 58; (ii) an ion-exchange process in the diffuse double layer, characterised by a stoichiometric exchange constant according to Eq. 60. Since Ψ_0 is known (from the previous analysis) as well as the properties of the stationary phase, each of these equations contains only one unknown parameter, $K_{ch,B}$, and K_{IEX}, respectively. Furthermore, the ionic strength dependence of the two equations are different from one another. The final step in the analysis is to plot the retention of the eluite as a function of the electrolyte concentration in the mobile phase. The value for each of the unknown parameters is obtained by numerical fitting of this curve to the sum of Eqs. 58 and 60.

This model is complex and requires an extensive data set for its evaluation and comparison with experimental retention data. It is therefore very difficult to compare the previously presented experimental data with what is predicted from this theory. The theory has been experimentally tested [74] with TBA$^+$ as IP-reagent and p-nitrobenzenesulfonate (NBS$^-$) as eluite ion using a C$_{18}$-RP column. For mobile phase ionic strengths less than 0.1 M the activity of the TBA$^+$ ion was calculated from the Debye–Hückel theory and for higher ionic strengths it was obtained from the literature. From numerical fitting of the experimental data to the model, the authors concluded that in this particular system the retention was mainly due to ion-exchange in the double layer between the TBA$^+$ counterion (Cl$^-$) and NBS$^-$, the numerical value for the stoichiometric ion-exchange constant was evaluated to 221 ± 10.

5.5. The liquid partition double layer model

In the liquid partition double layer model by Weber the RP surface is assumed to be a bulk liquid in which the eluite and IP-reagent ions are dissolved. The model therefore considers the partitioning of the ions as taking place between two bulk liquid systems. As an example we consider the case where sodiumoctylsulfonate (OctSO$_3^-$Na$^+$) is added to a system consisting of a polar mobile phase and a non-polar liquid phase. The OctSO$_3^-$ ions tend to dissolve preferentially in the non-polar phase while the sodium ions tend to remain in the polar mobile phase. This must cause the non-polar phase to be negatively charged with respect to the bulk of the mobile phase. Therefore, in general, there is an excess electrical charge on one side of the interface, which in view of the electroneutrality condition has to be compensated by an excess of opposite charge on the other side, see Fig. 11. The excess of electrical charge creates an electrostatic potential difference between the bulk of the two phases and an accompanying potential profile in the region close to the interface, see Fig. 11. For further reading concerning some aspects of electrochemical phenomena at liquid–liquid interfaces the reader is referred to a review paper by Samec [97].

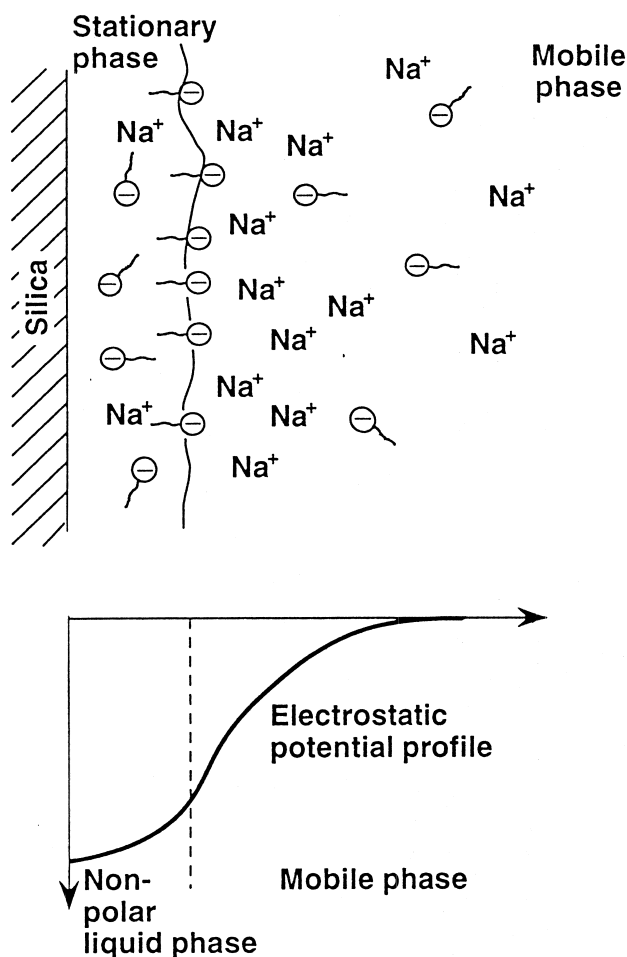Weber studied two different geometries; in one

Fig. 11. A schematic presentation of the liquid partition double layer model for ion pair chromatography and the electrostatic potential profile generated by negatively charged ion pair reagent ions. In this model both the ion pair reagent and the eluite ion are considered as being distributed between two liquid phases.

case the pore of the stationary phase is treated as an infinitely long cylinder of radius R where the silica surface constitutes the wall. Inside this cylinder another central cylinder cavity of radius a is situated representing the stagnant mobile phase. The RP-layer is represented by the difference between these two giving it the thickness $R-a$. In the second case the pore is considered to have a 'sandwich' geometry with two planar parallel silica walls separated by a distance $2R$. The thickness of the RP-layer is again denoted $R-a$ so that a represents one half of the thickness of the mobile phase layer. The distribution of the IP-reagent between the mobile phase and the

RP-layer is obtained by solving the P–B equation or its linearised form in each layer. The solution for each layer is connected by using a set of boundary conditions which must be satisfied at the interface. The tendency for each ion in the system to be solvated in respective phase is considered in the P–B equation by introducing a standard free energy of transfer between the two phases. The limited volume in the RP-phase for sorption of the IP-reagent is taken into account by using a volume based analog of the electrostatic potential modified Langmuir adsorption isotherm, Eq. 65. From these calculations Weber obtains the electrostatic potential profile from

the middle of the pore up to the wall created by the IP-reagent, the calculations allows the pore wall, at *R*, to have a net charge even when no IP-reagent is added.

The capacity factor for an eluite ion is in principle obtained by taking an integral of the form of Eq. 38 over the mobile phase and the stationary phase volumes, respectively. From the theoretical electrostatic profile in the pore Weber calculated a volume weighted average potential difference between the two phases, $\Delta\Psi$. The model was tested by comparing the model predicted value of $\Delta\Psi$ with an experimentally measured value. The latter value was obtained by making the tetraphenylarsonium–tetraphenylborate reference electrolyte assumption (see e.g. Refs. [98–100]) which relies on the assumption that in the absence of electrostatic potential differences the solubility of these two ions is the same in two phases. As a consequence of this assumption the capacity factor for these two eluites is expected to be the same in the absence of elecrostatic effects. When electrostatic effects are present Weber made the assumption that the potential profile integral in Eq. 38 can be substituted by $\Delta\Psi$ so that

$$\Delta\Psi = \frac{RT}{2F} \ln \frac{k_{\phi_4 B}}{k_{\phi_4 As}} \qquad (67)$$

Comparison of the theoretically predicted $\Delta\Psi$ values with the experimentally measured values showed similar trends and the observed deviations were attributed to a significant contribution from fixed charge sites on the silica surface.

There is a similarity in basic properties between this model and the surface adsorption double layer model. Even though the physical meaning of the electrostatic potential difference between the stationary phase and the mobile phase is distinctly different in the two models, both use Eq. 63 for the capacity factor. Therefore, there is a good agreement between predictions from the liquid partition double layer model and the experimental data shown in Tables 2 and 3. Furthermore, in agreement with the data shown in Fig. 10, the model will predict that the same stationary phase concentration of different IP-reagents will give the same retention. However, the change in retention with changing salt concentration in the mobile phase is very complex in this model. It

is therefore not possible to make a comparison between the model and the data shown in Fig. 9.

5.6. Concluding remarks concerning ion pair chromatography

The models for IP-chromatography which in the most consequent way have adopted the surface science view to describe the retention process are the three models treated above. The surface adsorption double layer model is the model which has been most extensively tested against experimental data and its formulation has also been adapted to practical use. Since the accumulation of the eluite ion in the double layer region is neglected, the model can not be used for accurate description of the retention for eluites with low capacity factors, $k_{0,B} < 0.3$, in the absence of the IP-reagent [92]. In a previous review [67] it was pointed out that the surface adsorption double layer model depends on the validity of the electrostatic modified adsorption isotherms. Experimental data show that for RP-stationary phases this isotherm will be obeyed for the most commonly used IP-reagents. However, for stationary phases other than RP-silica this may not be true and need to be separately investigated.

The experimental testing of the surface adsorption, diffuse layer ion-exchange double layer model has been focussed on the determination of the composite adsorption isotherm of the eluite ion and the IP-reagent using SDVB-based [59–61] or a silica based C_{18}-phase [73–75]. The main difference between this model and the surface adsorption double layer model can be summarised as follows:

- The model requires a large experimental data set and the evaluation procedure is complex, involving, e.g the knowledge of the activity coefficient of the IP-reagent in the mobile phase.
- The model does not include any theoretical adsorption isotherm for the IP-reagent to the stationary phase surface. Instead the adsorption isotherm is experimentally measured.
- The model assumes the existence of an empty compact part in the double layer with a capacitance determined from experimental data. The electrostatic potential at the Helmoltz outer plane, Ψ_{OHP}, is related to the experimentally obtained capacitance and to Ψ_0.

- It is assumed that the IP-reagent is a potential determining ion, i.e. that Ψ_0 is constant provided that the ionic activity of the IP-reagent in the solution is constant, regardless of the ionic strength of the eluent. As is discussed in the last parts of Section 2.5, this equation assumes a vanishingly small *relative* change in the surface concentration of the IP-reagent with varying mobile phase salt concentration.
- Retention of the eluite ion is assumed to be due to both adsorbtion in the same plane as the adsorbed IP-reagent ions as well as to accumulation in the diffuse double layer. Retention caused by the former process is described in the same way as in the surface adsorption double layer model and the accumulation is described by a stoichiometric ion-exchange constant between the eluent salt and the eluite ion in the double layer.

The liquid partition double layer model has not been applied to describe the retention behavior of eluites in IP-chromatography. It has been directed towards the understanding and measuring interfacial electrostatic potential differences. In principle, the advantage of this model over the other two is the inclusion of an electrostatic potential profile in the stationary phase layer. At the same time, however, this is also the main problem in this model. The reason is that in a medium of low dielectric constant the P–B equation does not hold because of the high correlation existing between the ions, i.e. what is usually called ion pair formation. Another problem is that Weber in the evaluation of the data calculated a volume averaged potential difference. As has been discussed in connection with the Donnan model, this averaging procedure is not applicable for potentials for which the Debye–Hückel approximation is not valid.

6. Retention models for ion-exchange chromatography of proteins

6.1. Introduction

Ion exchange chromatography of proteins for preparative and analytical separation has been a standard technique for many years. The popularity of this technique is due to the easy methodology and to the preservation of biological activity of the protein during the ion-exchange process. Its importance has further increased during the last decade with the rapid developments in biotechnology. In this separation technique the stationary phase consists of silica or a polymer to which charged functional groups are grafted. To minimise silanophilic or hydrophobic interactions between the protein and the support matrix, the stationary surface is covered with a hydrophilic polymer layer. The mobile phase consists of a buffer solution and the retention of the protein is modulated by adding an eluting salt. In most cases the separation is performed by gradient elution with increasing salt concentration.

The adsorption of proteins to solid surfaces is an important topic in many scientific areas; in biology the interaction between the protein and the cell membrane is of fundamental interest; in medicine the adsorption of proteins is of importance, e.g. for the biocompatibility of synthetic materials; in technical applications proteins are often used as emulsifiers and/or stabilizers and protein adsorption causes the undesirable biofouling in, e.g food process equipment. Despite the large amount of work performed to investigate protein adsorption, a quantitative description of the processes governing the adsorption has not been achieved. This is therefore an area of high scientific activity in several branches and has been summarised in a number of review papers [101].

In Section 2 the interaction between a charged surface and the ions constituting the electrolyte is discussed. The theoretical models for this system consider the electrolyte ions as well as the eluite ions as point charges interacting with a charged surface. When discussing the interaction between charged macromolecules and a charged stationary phase in an electrolyte solution, both the size, geometric form and the charge of the macromolecule must be considered. Since globular proteins are the most important and most extensively studied eluites, the ensuing discussion is focussed on retention models for these. Retaining the assumption that the electrolyte ions in the eluent are point charges, interactions in the protein–electrolyte-stationary phase system can be considered as the interaction between two oppositely charged bodies, which both are surrounded by an electrical double layer, taking place in an electrolyte solution.

The successful application of a solution of the P–B equation to many areas in physical chemistry makes it natural to use this approach also in the field of chromatography of proteins. In colloid and surface chemistry such theories have successfully been applied to describe the interaction between charged bodies in a variety of systems. In most cases, e.g. the DLVO theory [105,106], the sign of the charge of the two bodies is the same giving an elecrostatic repulsion between them. Theories for describing the situation in which the two bodies bear opposite sign of charge, as in ion-exchange of proteins, have mainly been neglected in surface and colloid science. Retention theories of charged macromolecules therefore need new theoretical developments also from a physico-chemical point of view. This means that retention theories for charged macromolecules are continuously developing and tested against experimental data and that little knowledge can be gained from physical chemistry regarding the physical behavior of these systems.

The solution of the P–B equation depends on the geometry of the chosen system. For the interaction between two charged bodies in an electrolyte solution the simplest possible geometry is the interaction between two charged parallel planar surfaces or slabs. Even for this simple case no closed form solution of the P–B equation has been accomplished, only the solution of its linearised form is available [107]. Although the geometry is too idealised to be applied to many real situations, this solution contains basic physical information about the principles of electrostatic interaction between oppositely charged bodies in an electrolyte solution. A short presentation of the physical properties of the slab model and its application to chromatography are presented in Section 6.3 and 6.5, respectively.

A retention model based on the slab model has been shown to describe the change in retention of proteins in ion-exchange chromatography as a function of salt concentration in the mobile phase [108]. The 'slab' model has later been complemented with other physical properties, i.e. van der Waals interactions [109] and the effect of charge regulation [110]. There are still uncertainties regarding which geometry is best suited to describe ion-exchange chromatography of proteins. One possibility is to consider the protein as a sphere which interacts with an oppositely charged planar surface. For this geometry the linearised Poisson–Boltzmann equation can be solved albeit the calculations can only be done using a computer program [111,112]. In a series of papers Lenhoff and coworkers used numerical solutions of this geometry to describe the protein–surface interaction [113,114]. They found that the ionic strength dependence of adsorption of lysozyme and chymotrypsinogen A to a charged quartz surface, was equally well described by a charged sphere as a model using the complete protein structure and charge distribution. They have also found a good correlation between the mean surface potential of a protein and its retention time in ion-exchange chromatography [115].

More recently Roth et al. [116] suggested that the linear superposition approximation for a charged sphere and an oppositely charged surface can be used to describe the electrostatic interaction between a protein molecule and the stationary phase. In this approximation the basic assumption is that there is a weak overlap between the double layers connected to the two bodies and it is therefore possible to calculate the potential at a given point in space by adding the potentials created by each body separately. This approximation is mainly valid at long separation distances between the protein and the surface. Another approach has been taken by Noinville et al. who used the AMBER force field to find the elution order of α-lactalbumin and lysozyme in ion-exchange chromatography [117]. Before discussing ion-exchange chromatography of proteins some general conclusions regarding protein adsorption to solid surfaces will be summarised.

6.2. Brief discussion of protein adsorption to solid surfaces

As has been pointed out by W. Norde [118] protein adsorption can be viewed upon as the transfer of the protein molecule from the solution phase, usually water–salt solution, to a phase where parts of the environment of the molecule is close to or in contact with a solid-phase. In order to get some basic understanding of the physical processes which causes protein adsorption the state of the protein molecule in these two environments must be considered. A recommended review paper which treats the present

physico-chemical view of protein adsorption is found in Ref. [101].

The structure of proteins is usually discussed at various levels where the amino acid sequence constituting the backbone is referred to the primary structure. This backbone adopts different conformations, e.g. α-helix, β-sheet or random coil, the so-called secondary structure. The tertiary structure is the way the folded segments of the polypeptide chain are arranged in space. The globular proteins contain helix, pleated sheet and randomly structured parts ordered into a compact structure in which 70–80% of the volume is occupied by atoms, a value which is comparable to what is found in glasses and polymer crystals [119]. The major driving force for formation of the compact protein structure is the entropy increase of water upon dehydrating the hydrophobic side groups bound to some of the amino acids, i.e. the hydrophobic effect. Both the α-helix and the β-sheet structures are characterised by hydrogen bond formation between the peptide units reducing the rotational freedom and hence the conformational entropy of the protein by several hundreds of kJ/mol [120].

Around the isoelectric pH of the protein, the electrostatic interaction between the positively and negatively charged groups bound to the protein may contribute to a stabilisation of the compact structure. Away from this pH the excess of charge leads to a net repulsion between the charges promoting an expanded structure. Other interactions which favor a compact structure are hydrogen bonds between groups in the protein molecule and the van der Waals interaction, however, these interactions are believed to have only minor effects on the structure of a protein molecule in water [118]. From the discussion above it can be concluded that the two major effects which determine the stability of a protein in water solution are the hydrophobic effect, which promotes a compact structure, and the decrease in rotational mobility, which oppose this structure. These two contributions tend to compensate each other and as a result the tertiary structure of proteins is only marginally stable in aqueous solutions.

The hydrophobic side chains strives to minimise their contact with the surrounding water and these groups therefore prefer to be situated in the interior of the protein. Not all hydrophobic chains can be hidden in the interior and, because the surface to volume ratio increases when the protein size decreases, the smaller molecules have a relatively larger portion of hydrophobic parts on the surface than larger molecules. For example, ribonuclease, lysozyme and myoglobin, with molecular masses between 13 700 and 17 800, have ca. 40–50% of the area covered with non-polar atoms [121]. The charges of the protein molecule are mainly situated in the perifery and are surrounded by a diffuse layer of counterions on the aqueous side. These two properties of protein molecules imply that when it interacts with a solid surface the most important factors are the hydrophobic effect and the electrostatic interaction. Upon transferring the protein from the aqueous solution to the surface, the physical properties of its environment are changed, which may lead to structural rearrangements of the marginally stable protein molecule [122–124]. This may result in a decrease in α-helix or β-sheet content and a more random structure giving an increase of the entropy in the adsorbed state. This entropy gain may give a significant contribution to a spontaneous protein adsorption.

6.3. The interaction between two oppositely charged bodies

The electrostatic interaction between two oppositely charged bodies in contact with an electrolyte solution is of fundamental interest in many areas. Important insights into the phenomena which occurs in such systems can be obtained from solutions of the P–B equation. In this section results from such calculations for the interaction between two oppositely charged planar surfaces or slabs and for the interaction between a charged sphere and a charged planar surface are briefly discussed. As will be shown, the solution of the linearised P–B equation for these systems leads to results that are difficult to obtain from an intuitive reasoning.

6.3.1. The electrostatic interaction between two oppositely charged slabs

Consider a system consisting of two oppositely charged planar surfaces in contact with an electrolyte solution, see Fig. 12. When the surfaces are at an infinite distance from each other there is an undis-
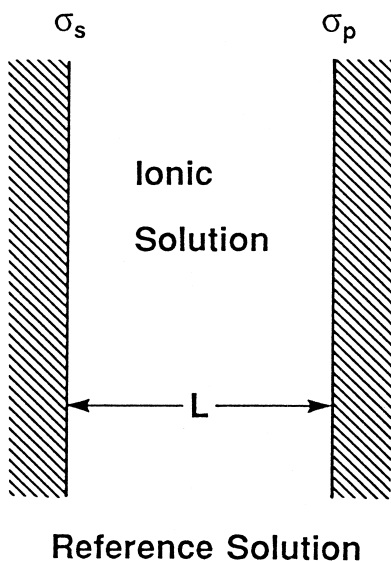
Fig. 12. A schematic view of the geometry of the slab model for the electrostatic interaction between the charged protein and stationary phase surface. (The figure is reprinted from Ref. [108] with permission).

turbed electrical double layer connected to each surface. As the two surfaces move towards each other the two double layers start to overlap and there is a concomitant change in the free energy of the system. Solving the linearised Poisson–Boltzmann equation for the system, the specific work, i.e. the change in free energy per surface area, $\Delta G_{es}/A_p$, needed to move the surfaces from infinity to a distance L from each other is found to be:

$$\frac{\Delta G_{es}(L)}{A_p} = \frac{1}{\kappa \varepsilon_0 \varepsilon_r} \left(\frac{(\sigma_p^2 + \sigma_s^2) \cdot e^{-\kappa L} + 2\sigma_s \sigma_p}{e^{\kappa L} - e^{-\kappa L}} \right)$$

(68)

Here σ_s and σ_p, are the respective charge densities of the two surfaces, these values are assumed to be constant and independent of the distance, L, between the surfaces.

In order to illustrate Eq. 68, the dependence of the specific work on the distance between the surfaces is calculated at various ionic strengths and for a given set of conditions ($\sigma_p = 0.03$ C/m^2, $\sigma_s = -0.16$ C/m^2, and $\varepsilon_r = 80$ (water)) and shown in Fig. 13. As expected, there is a range of separating distances, L,

where the work required is negative so that there is an attraction between the slabs. Most of the attraction does not have pure electrostatic origin but is due to the increase in entropy when ions from the two double layers are released to the bulk electrolyte solution. At a certain separation distance, L_m, the free energy goes through a minimum and when the two surfaces approach each other further, the free energy increases strongly so that repulsion occurs as the surfaces move closer to each other. This interesting result shows that even for slabs carrying opposite charges, there is an equilibrium distance between the surfaces at which the free energy is minimum. The explanation for this behavior comes from the difference in charge density between the two surfaces, which require, in order to maintain electroneutrality, the presence of electrolyte ions between the surfaces. As the surfaces come closer than the equilibrium distance, the volume available for these ions becomes so small that the entropy decrease of these ions offset the gain in energy.

By setting the derivative of the right hand side of Eq. 68 with respect to L to zero, we can express the separation distance, L_m, for the free energy minimum by

$$L_m = \frac{1}{\kappa} \ln \left(\frac{-\sigma_p}{\sigma_s} \right) \qquad \text{when} \quad -\sigma_p > \sigma_s$$

$$L_m = -\frac{1}{\kappa} \ln \left(\frac{-\sigma_p}{\sigma_s} \right) \qquad \text{when} \quad -\sigma_p < \sigma_s$$

(69)

From these equations it follows that the position of the minimum in the $\Delta G_{es}(L)/A_p$ curve is proportional to the Debye length and to the logarithm of the relative surface charge density on the two surfaces. When the surface charge densities are the same on the two surfaces, the equations gives that $L = 0$ and no minimum in the curve occurs. Furthermore, inserting these expressions into Eq. 68, we can express the minimum value of the free energy as

$$\left(\frac{\Delta G_m}{A_p} = \frac{\sigma_s^2}{\kappa \varepsilon_0 \varepsilon_r} \right) \qquad \text{when} \quad -\sigma_p > \sigma_s$$

$$\left(\frac{\Delta G_m}{A_p} = \frac{\sigma_p^2}{\kappa \varepsilon_0 \varepsilon_r} \right) \qquad \text{when} \quad -\sigma_p < \sigma_s$$

(70)

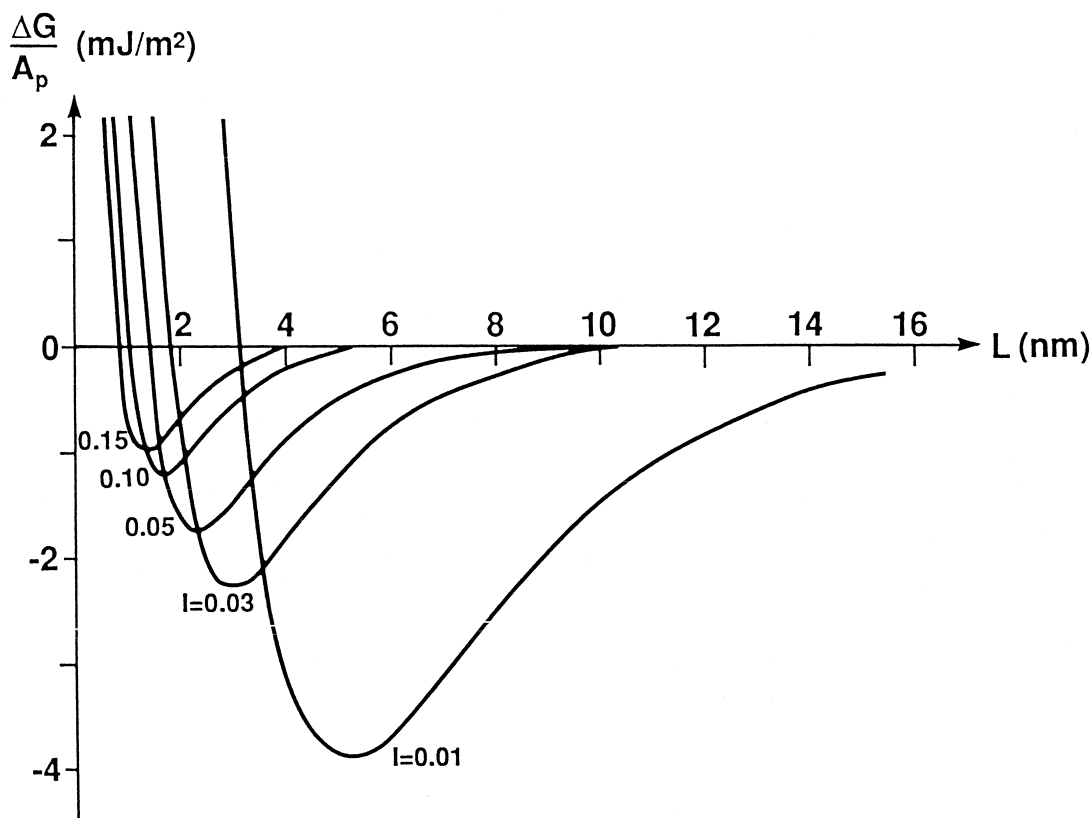These equations show that at L_m, the strength of the

Fig. 13. Plots according to the slab model for the Gibbs free energy per unit area, $\Delta G/A_p$, as a function of the distance between the two oppositely charged surfaces, L, with the ionic strength as the parameter. The plots are obtained from Eq. 69 with $\varepsilon_r = 80$ and by setting -0.16 and 0.03 C/m^2 for the charge densities σ_s and σ_p, respectively. (The figure is reprinted from Ref. [108] with permission).

interaction is entirely determined by the surface carrying the lowest charge density and is independent of the charge density of the other surface.

6.3.2. The electrostatic interaction between a charged sphere and a charged surface

The solution of the linearised P–B equation for a system consisting of a charged sphere and a charged planar surface is complex. We will show here that the exact solution can partly be rationalised and physically understood by using an approximative approach. For this purpose it is useful to divide the interaction process into three different components [111].

- The interaction between the charged planar surface and a point charge of the same net charge as

the sphere situated in the center of the sphere. The interaction energy for this process can easily be obtained from the electrostatic potential profile calculated from the Gouy–Chapman theory.

- The interaction between the charged planar surface and a hypothetical *uncharged* sphere of the same size and dielectric constant as the sphere in question. This interaction energy is positive, i.e. repulsive, because the sphere penetrates into the double layer of the charged planar surface and compresses it. The uncharged sphere also displaces the counterions and creates a charge free region. The repulsive energy increases when the size of the sphere increases and when its dielectric constant decreases.

- The interaction between the charged sphere and an *uncharged* planar surface. The effect is similar

to that described above where in this case the role of the surface and the sphere is interchanged.

By summing the interaction energy for these three separate interactions an estimate of the total interaction energy is obtained. This is illustrated in Fig. 14 where the interaction energy between a positively charged planar surface and a positively and negatively monocharged 25 Å sphere, respectively, vs. the separation distance between the bodies, is depicted. The full curves are the interaction energy calculated from the exact solution of the linearised P–B equation and the dotted lines are the result from adding the three components. An important observation is that, at a given distance, the repulsive energy for the similarly charged case is much stronger than the corresponding attractive energy for the oppositely charged case. This contrasts to the situation for

corresponding point charges interacting with the surface, in such cases the magnitude of the interaction is the same irrespective of the sign of the charge. Since the full and dotted curves are similar, the asymmetry between the curves in Fig. 14 reflects the influence of the two latter parameters on the total interaction. It is interesting to note that also for a sphere interacting with an oppositely charged planar surface a minimum in the interaction energy may occur.

6.3.3. van der Waals interactions

Besides the electrostatic interaction, the interaction between a charged macromolecule and a charged surface is likely to entail van der Waals interactions. In the well known DLVO theory the distance depen-
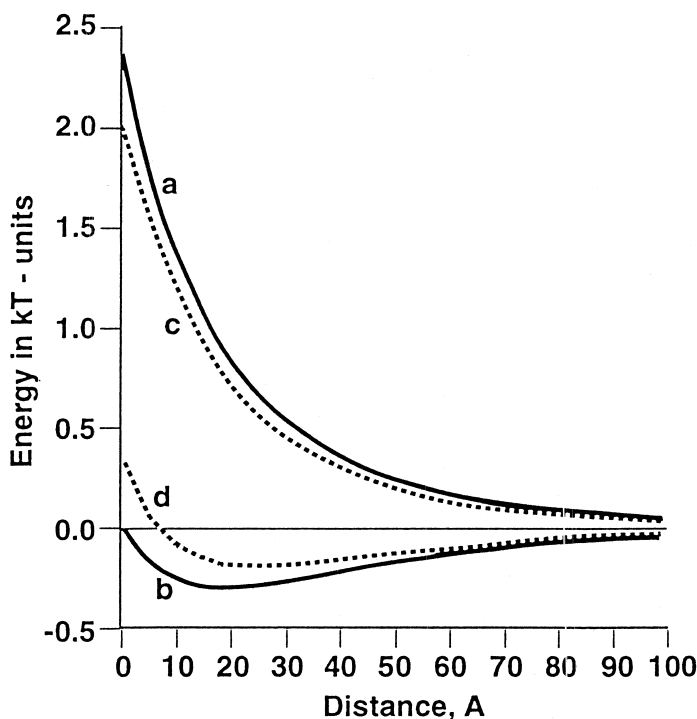
Fig. 14. Gibbs free energy as a function of separation distance between a charged planar surface ($\sigma_p = 0.0118$ C/m^2) and a monocharged 25 Å sphere ($\sigma_s = \pm 0.00204$ C/m^2). The full lines are calculated from the numerical solution of the linearized P–B equation and the dotted lines are obtained from the summation procedure discussed in the text. (a) $\sigma_s = 0.00204$ C/m^2, (b) $\sigma_s = -0.00204$ C/m^2, (c) parameters as for line (a); (d) parameters as for line (b). Other calculation parameters are $1/\kappa = 30 \cdot 10^{-10}$ m; $T = 298$ K and dielectric constant of the solvent phase $= 80$ and for the sphere $= 2$. (The figure is reprinted from Ref. [111] with permission).

dence of the interaction between colloidal particles is described as the sum of these two interactions. It can be shown that for the van der Waals interaction the free energy change per unit surface area, $\Delta G_{\mathrm{vdW}}/A_{\mathrm{p}}$, as a function of the distance between two slabs can be approximated as:

$$\frac{\Delta G_{\mathrm{vdW}}}{A_{\mathrm{p}}} = -\frac{H}{12\pi L^2} \qquad (71)$$

Where H is the Hamaker constant, its value depends on the dielectric properties of both the intervening medium as well as on the two slabs, its numerical value is of the order $1 \cdot 10^{-20}$ J. Unlike the double layer interaction, the van der Waals interaction is insensitive to variations in electrolyte concentration and may be considered as constant. Since it is a power law interaction, the van der Waals interaction always exceed any double layer repulsion at small enough separation distances between the slabs.

The combined electrostatic and van der Waals interaction is known to describe the long range interaction (i.e. separation distances >1 nm) in systems studied in colloid and surface chemistry. At shorter separation distances between the two bodies these two interactions do not entirely explain the experimentally measured values, the reason is the structural behavior of the solvent remaining between the two approaching surfaces [1]. A detailed treatment of such a complex binding process has not yet been accomplished and as a simplifying approach it is often assumed that all major features involved are implicit in Eqs. 68 and 71. This means that the numerical value for the Hamaker constant is regarded as an effective value and is therefore not solely due to pure van der Waals interactions. The overall free energy change for two interacting slabs, ΔG_{t}, is expressed as the sum of the free energy change associated with electrostatic and van der Waals interactions:

$$\frac{\Delta G_{\mathrm{t}}}{A_{\mathrm{p}}} = \frac{\Delta G_{\mathrm{es}}}{A_{\mathrm{p}}} + \frac{\Delta G_{\mathrm{vdW}}}{A_{\mathrm{p}}}$$

$$= \frac{1}{\kappa \varepsilon_0 \varepsilon_r} \left(\frac{(\sigma_{\mathrm{p}}^2 + \sigma_{\mathrm{s}}^2) \cdot \mathrm{e}^{-\kappa L} + 2\sigma_{\mathrm{s}}\sigma_{\mathrm{p}}}{\mathrm{e}^{\kappa L} - \mathrm{e}^{-\kappa L}} \right) - \frac{H}{12\pi L^2} \qquad (72)$$

6.4. Introduction to retention models for ion-exchange chromatography of proteins

As is discussed above, the adsorption of proteins to solid surfaces is mainly determined by the following factors: (i) the electrostatic interaction, (ii) the hydrophobic effect, (iii) van der Waals interactions and possibly also (iv) structural rearrangement of the protein structure. Since the surfaces used in ion-exchange chromatography of proteins are covered with a hydrophilic layer the three latter effects are minimised so that the electrostatic interaction dominates.

Also in ion-exchange chromatography of proteins the traditional retention theory has been based on a stoichiometric exchange of ions between the protein and the counterions to the fixed charges on the stationary phase. This theory was first formulated by Boardman and Partridge [125] and has since then been used in several alternative formulations, all based on stoichiometric concepts [126,127]. The result of this model is a linear dependence of the logarithm of the capacity factor on the logarithm of the concentration of counterions to the fixed stationary phase charges. In such plots the experimental data indeed give linear relations with negative slopes, which in the model can be interpreted as the number of binding sites between the protein and stationary phase surface, A further modification of the stoichiometric approach was taken by Cramer in which the possible effect of sterically hindered exchange sites is included [128].

The retention model which use a solution of the P–B equation (linearised) for describing chromatographic behavior of macromolecules is the slab model for ion-exchange chromatography of proteins [108–110]. A summary of the results from the utilisation of the solutions of the Poisson–Boltzmann equation for describing the retention properties of proteins in ion-exchange chromatography is presented in the next section. Two other nonstoichiometric retention models for proteins in ion-exchange chromatography have been developed by using the Manning ion condensation theory. In the last section a summary of these theories is found as well as a presentation of the experimental data which have been evaluated according to these models.

6.5. A retention model based on the solution of the linearised Poisson–Boltzmann equation

A critical test for models of ion-exchange chromatography of proteins is to compare the theoretically predicted and the experimentally obtained salt dependence for retention under various conditions, e.g. protein size and net charge, surface charge density of the stationary phase etc. The simplest possible geometry for describing the electrostatic interaction between two charged macroscopic bodies is two interacting slabs. It was previously shown that solution of the linearised Poisson–Boltzmann equation for this geometry results in simple algebraic equations for the interaction energy. A basic assumption in Eq. 68 is that the charge density on the two surfaces is constant and independent of the distance between the surfaces, i.e. the charge density of the two slabs is independent of whether the two slabs are far away or close to each other. However, as two oppositely charged surfaces approach each other, the electrical field from one surface penetrates the intervening salt solution and reaches the opposite surface causing a change in the electrostatic potential on the respective surface.

When ions in the intervening electrolyte bind specifically to the surface, the change in surface potential may lead to a change in the surface charge density of that surface, e.g. hydrogen ions bind specifically to the acidic and basic groups on a protein giving a pH dependent protein net charge. This effect is usually called charge regulation. In ion-exchange chromatography of proteins, the protein net charge will therefore vary with the distance between the protein and the stationary phase surface. It can be shown that charge regulation causes an increase in the adsorption energy and that it is possible to quantitatively correct for this effect by introducing a constant in the set of Eqs. 68–70.

Eq. 68 describes the free energy of interaction as a function of the separation distance, L, between the surfaces. As is discussed in Section 3.1, the thermodynamic definition of the capacity factor involves the integral of the exponential interaction energy over the separation distance, L, Eq. 38 (x in Eq. 38 is the distance from the surface to a point, in Eq. 68 this is equivalent to the distance between the surfaces). When there is a minimum in the free energy vs.

separation distance curve, ΔG_{m}, it can be shown that the integral in Eq. 38 can be approximated as:

$$
\begin{aligned}
k &= \frac{A_{\mathrm{s}}}{V_0} \cdot \int_0^\infty \left(e^{-(\Delta G(L)/RT)} - 1 \right) \mathrm{d}L \\
&\approx \frac{A_{\mathrm{s}}}{V_0} \cdot d \cdot e^{-(\Delta G_{\mathrm{m}}/RT)}
\end{aligned}
\tag{73}
$$

where A_{S} is the surface area of the stationary phase and V_0 the dead volume of the column, d is the characteristic width of the adsorption layer and its numerical value can be estimated from the model. When the charge density of the stationary phase surface is higher than on the protein surface, the charge regulated slab model gives that ΔG_{m} is

$$
\frac{\Delta G_{\mathrm{m}}}{A_{\mathrm{p}}} = -\frac{\sigma_{\mathrm{p}}^2}{\kappa \varepsilon_0 \varepsilon_r (1 - K_{\mathrm{p}})}
\tag{74}
$$

where σ_{p} is the protein surface charge density at infinite distance between the protein and the stationary phase and K_{p} is a constant with a characteristic value for a certain protein at a given pH value and eluent ionic strength. A thermodynamic analysis shows that its value is proportional to the slope of a pH titration curve of the protein so that its value increases when the change in protein charge per pH unit increases.

By combining Eqs. 73 and 74 an expression for the logarithmic capacity factor is obtained:

$$
\ln k = \frac{\sigma_{\mathrm{p}}^2 \cdot A_{\mathrm{p}}}{F(2RT\varepsilon_0 \varepsilon_r)^{1/2}(1 - K_{\mathrm{p}})\sqrt{I}} + \ln\left(\frac{A_{\mathrm{s}}d}{V_0}\right)
\tag{75}
$$

For moderate changes in ionic strength, e.g. from 0.05 to 0.5 M, the variation in $\ln(A_{\mathrm{s}}d/V_0)$ with ionic strength is small compared to the variation in ΔG_{m} given in Eq. 74. Eq. 75 therefore predicts a linear relation between the logarithmic retention factor and the reciprocal square root of the ionic strength of the eluent. In Table 4 a large body of retention data obtained in ion-exchange chromatography of proteins is summarised. The data cover a wide range of experimental conditions and was plotted as $\ln k$ vs. $1/\sqrt{I}$, from the slope of the plots the net charge of the different proteins, q_{chr}, were estimated using the titrimetrically determined K_{p} values and fundamental physico-chemical constants. The ratio between q_{chr}

Table 4
Chromatographic conditions and calculated net charge of proteins, q_{chr}, from chromatographic experiments and a comparison between q_{chr} and q_{titr}

Protein	Eluent pH	Stationary phase	Eluting salt	K_p	q_{chr}	q_{chr}/q_{titr}
Ovalbumin	5.5	Mono Q (SAX)	NaCl	0.77	−5.6	0.58
	6.0	SynChropak Q 300	NaCl	0.42	−11.5	0.88
	6.5	Mono Q (SAX)	NaCl	0.28	−14.8	0.97
	7.0	SynChropak Q 300	NaCl	0.31	−13.8	0.81
	7.5	Mono Q (SAX)	NaCl	0.15	−16.0	0.89
	7.8	Zorbax Bio Series WAX 300	$(NH_4)_2SO_4$	0.08	−14.7	0.78
	7.8	Zorbax Bio Series SAX 300	$(NH_4)_2SO_4$	0.12	−12.5	0.66
	8.0	SynChropak 0300	NaCl	0.09	−18.5	0.96
	9.6	Mono Q (SAX)	NaCl	0.57	−13.0	0.59
Lysozyme	4.9	in-housemade WCX	$Ca(OAc)_2$	0.33	+7.5	0.71
	6.0	Zorbax Bio Series WCX 300	$(NH_4)_2SO_4$	0.12	+9.9	1.10
	6.0	Zorbax Bio Series SCX 300	$(NH_4)_2SO_4$	0.13	+8.4	0.93
	6.4	Zorbax Bio Series WCX 300	NaOAc	0.13	+10.2	1.18
	6.4	Zorbax Bio Series SCX 300	NaOAc	0.12	+9.7	1.12
Cytochrome *c*	4.9	in-housemade WCX	$Ca(OAc)_2$	0.38	+6.8	0.76
	6.0	Zorbax Bio Series WCX 300	$(NH_4)_2SO_4$	0.13	+10.0	1.39
	6.0	Zorbas Bio Series SCX 300	$(NH_4)_2SO_4$	0.15	+7.9	1.10
Ribonuclease	6.0	Zorbax Bio Series WCX 300	$(NH_4)_2SO_4$	0.63	+5.3	0.88
	6.4	Zorbax Bio Series WCX 300	$(NH_4)_2SO_4$	0.71	+5.1	0.95
	6.4	Zorbax Bio Series SCX 300	$(NH_4)_2SO_4$	0.79	+3.8	0.70
Myoglobin	6.0	Zorbax Bio Series WCX 300	$(NH_4)_2SO_4$	0.50	+4.9	1.02
β-Lactoglobulin	7.0	Synchropak SAX 300	NaCl	0.44	−15.9	1.18
	8.0	Synchropax SAX 300	NaCl	0.37	−16.8	0.91
Bovine serum albumin	7.8	Zorbax Bio Series WAX 300	$(NH_4)_2SO_4$	0.24	−16.1	1.08
	7.8	Zorbax Bio Series SAX 300	$(NH_4)_2SO_4$	0.23	−13.0	0.87
Human serum albumin	6.5	Mono Q (SAX)	NaCl	0.34	−15.3	1.56
	7.5	Mono Q (SAXI)	NaCl	0.31	−16.7	1.03
	9.6	Mono Q (SAX)	NaCl	0.8S	−9.10	0.26

and the titrimetrically determined protein net charge shown in the Table 4 indicates that the chromatographically measured protein charges compare well to those obtained from titrimetric experiments [110]. In conclusion, the slab model or its more developed version, the charge regulated slab model, both very well describe the salt dependence of protein retention and they also give protein net charges in reasonable agreement with the titrimetrically determined net charge.

In an interesting work by Cai et al. [129] protein retention in mixed mode size exclusion and ion-exchange chromatography was studied over a wide range of ionic strength. The effect of ionic strength on retention of four different proteins was studied and the experimental results were compared with predictions from the stoichiometric model by Kopaciewicz et al. [126] and the slab model [108]. It was found that the ionic strength dependence of

retention more closely fitted the slab model and, since the charge density of the stationary phase is lower than on the proteins used, that the stationary phase charge density calculated from the slab model agrees well with the experimentally measured value.

6.6. Retention models based on Manning condensation theory

Two models have used Manning condensation theory for describing the retention of proteins in ion-exchange chromatography [130,131]. Before going into the details of these theories a short introduction to Manning condensation theory is presented.

The purpose of the Manning condensation or the counterion condensation theory (CC theory) [132,133] is to describe the physical properties of locally cylindrical chain polyelectrolytes such as

DNA. The theory is a simple analytical alternative to the P–B equation for a charged cylinder immersed in an electrolyte solution, analytical solution of the P–B equation for this geometry has been achieved for the linearised form only. In the CC theory, as well as in the corresponding P–B theory, the chain polion is modelled as an infinitely long cylinder with the charge density expressed by the non-dimensional parameter:

$$\xi = \frac{e^2}{\varepsilon_0 \varepsilon_r kTb} \tag{76}$$

where b is the average axial distance between polion structural charges, e is the charge of the electron and k is the Boltzmann constant. The central tenet of the CC theory is that, when a cylindrical polion with a ξ value greater than unity is immersed in an electrolyte consisting of monovalent ions, the effective ξ value, ξ_{eff}, is reduced to unity. The physical interpretation of this is that a certain fraction, Θ, of the polion charges are shielded by counterions, the counterions are 'condensed' or associated with the polion. Since $\xi_{eff} = 1$ this fraction becomes

$$\Theta = 1 - \frac{\xi_{eff}}{\xi} = 1 - \xi^{-1} \tag{77}$$

When $\xi > 1$ the fraction of 'condensed' counterions is insensitive to the electrolyte concentration in the bulk solution and remains constant also at infinite dilution of electrolyte. On the other hand when $\xi < 1$ the theory assumes that the concentration of 'condensed' counterions approaches zero at infinite dilution of the bulk electrolyte.

In the CC theory a distinction is made between the physical counterion binding parameter, Θ, and the thermodynamic counterion binding parameter, ψ. The difference between the two is that the latter includes the additional accumulation or 'binding' of counterions caused by the remaining unshielded polion charges. The value of ψ can be obtained from the P–B theory under limiting law conditions to Ref. [134]:

$$\psi = \Theta + \frac{1}{2\xi} = 1 - (2\xi)^{-1} \tag{78}$$

Since Mannings formulation of the CC theory pre-

dicts that Θ remains fixed, this equation is used over the range of salt concentrations where the CC model is applicable (up to at least 0.1 M) [134].

Record et al. used the results from the CC model in the formulation of a theory for the binding of oligopeptides and proteins to a nucleic acid [134,135]. The theory is based on Wymans linkage theory [136] into which the condensation and screening caused by electrolyte ions are incorporated. The complete theory involves a set of parameters which determines the effect of an electrolyte on the binding of, e.g. a protein to a nucleic acid. After making some approximations, Record et al. [134] conclude that the binding constant changes with the concentration of monovalent ions in the electrolyte solution according to Eq. 79:

$$\frac{d \ln K}{d \ln c_{C,E}} \approx -Z_p \psi \tag{79}$$

where ψ is the thermodynamic ion binding parameter defined in Eq. 78, Z_p is the number of protein charges interacting with the nucleic acid and K the association constant between the protein and the nucleic acid.

In the model proposed by Mazsaroff et al. the theory of Record is used to describe the influence of mobile phase electrolyte concentration on protein retention [130]. Instead of considering the interaction between the nucleic acid and the protein, Mazsaroff et al. used the same theory to describe the interaction between the charged stationary phase and the protein.

The role of the nucleic acid in the Record theory is therefore substituted by the stationary phase, and consequently Mazsaroff et al. used Eq. 79 to describe the change in capacity factor with varying eluent salt concentration. Since the numerical format of this equation is the same as that obtained from a stoichiometric model, i.e. a linear $\ln k$ vs. $\ln c_{C,E}$ relation, the model is in numerical agreement with experimental data. Isocratically determined data for ovalbumin, using a quaternized polyethyleneimine column, were evaluated according to the model. Some non-linearities were observed at pH values close to the pI of the protein and these deviations were interpreted according to a more elaborated version of the model by Record.

In a model proposed by Melander et al. [131] the Manning condensation theory is used in combination with the solvophobic theory by Sinanoglu [137,138]. The Manning theory is adapted to evaluate the layer thickness of the condensed ions on both the protein and the stationary phase. The obtained layer thickness, in combination with the surface charge density of the stationary phase, is used to define the volume of the 'bonding' region between the protein and the stationary phase. They find that the effect of salt on retention is described by a three parameter equation

$$\log k = A - B \log m_s + C m_s \qquad (80)$$

where m_S is molal salt concentration in the eluent. The electrostatic part of the interaction is characterised by the constant $B(=Z_p/Z_0)$ and the hydrophobic interaction by the constant C. In agreement with the model experimentally measured k values for different eluent salt concentrations did yield a U-shaped $\log k$ vs. $\log m_s$ plot to which Eq. 80 could be numerically fitted.

6.7. Concluding remarks regarding retention models of charged macromolecules

The complexity of the adsorption process for proteins to solid surfaces makes it very difficult to construct physically models describing the interaction. The main features of the electrostatic part of the interaction, which dominates the salt dependence of retention in ion-exchange chromatography, should be possible to achieve from solutions of the P–B equation for a suitable geometry. In this paper one model which uses the solution of the linearised P–B equation for two interacting slabs and two models which uses adapted versions of the Manning condensation theory are briefly presented.

The slab model correctly describes the functional form of the dependence of retention on eluent ionic strength, the model often also gives reasonable values for the net charge of the protein. The model may therefore be of practical value, as demonstrated in the paper by Cai et al. [129]. From a theoretical point of view the model suffers mainly from three weaknesses: (i) the geometry of the model is too simplistic, (ii) the assumptions made in the linearised P–B equation are usually not fulfilled and (iii) the

model assumes an evenly smeared out charge distribution on the protein surface. The surprisingly good agreement between the theory and experiments is probably due to the fact that the errors caused by the geometry and linearisation (points (i) and (ii) above) tend to compensate each other. Despite the limitations of the linearised P–B equation, its solutions are of interest for discovering and understanding basic phenomena occuring in these systems. Note added in proof. Recently, a model which to a large extent does not have weaknesses (i) and (ii) was presented by Jönsson and Ståhlberg (B. Jönsson, J., Ståhlberg, Colloids and Surfaces B, in press).

Both models based on the Manning condenstation theory predict a linear $\log k$ vs. $\log c_{C,E}$ relation, a relation often observed in practice. Major problems common in these models are (i) the Manning condensation theory is developed for a linear array of charges and not for a two-dimensional charge distribution; and (ii) the physical background for the Manning condensation theory is much less rigorous than the P–B equation.

In conclusion, the models proposed so far for describing the electrostatic interaction between a protein and a charged surface are not as well developed as for the other chromatographic techniques treated in this review paper. Further development in this area is therefore needed and would be of interest not only in analytical chromatography but also in other areas where protein adsorption is of fundamental interest.

7. List of symbols

A	IP reagent, an amphiphilic ion.
A_p	Interacting surface area, (m^2).
A_s	The area of the stationary phase in a column, (m^2).
a_i	Activity of species i.
B	Eluite ion (analyte ion).
b	Average axial distance between cylindrically polyion structural charges (m).
C	An ion which constitutes the electrolyte and is counterion to the charges bound to the stationary phase.
C_1	Capacitance of the compact part of the double layer (C/Vm^2)

C_R	Volume concentration of charged groups bound to the stationary phase (mol/m^3).
$c_{i,j}$	Concentration of species i in phase j (mol/m^3).
D	An ion which constitute the electrolyte and is co-ion to the charges bound to the stationary phase, i.e. counterion to C in the electrolyte phase.
E	Eluent phase (mobile phase).
F	Faraday constant (96 485 C/mol).
G	Gibbs free energy (J/mol.)
H	Hamaker constant (J).
I	Ionic strength (mol/m^3).
K	Stoichiometric equilibrium constant.
K_γ	Quotient of activity coefficients.
k	Retention factor (capacity factor).
k	Boltzmanns constant (J/molecule*K).
L	Distance between two interacting bodies (m).
n_i	Surface concentration of species i, (mol/m^2).
n_0	Monolayer capacity (mol/m^2)
R	Resin phase.
R	Universal gas constant (J/mol/K).
r	Distance, (m).
r	A point.
S	Charged surface groups bound to the stationary phase surface.
T	Temperature, (K).
V	Volume (m^3).
V_0	Column dead volume (m^3).
V_R	Volume of the resin phase available to the eluent phase (m^3).
x	Distance (m).
z_i	Charge of species i.
$\gamma_{\pm,j}$	Mean molar activity coefficient of species in phase j.
$\gamma_{i,j}$	Activity coefficient of species i in phase j.
ε_0	Permittivity of vacuum (F/m).
ε_r	Dielectric constant.
Θ	Fraction of shielded polyion charges.
κ	Inverse Debye length, defined in Eq. 19 (1/m).
$\mu_i^{\,0}$	Chemical potential of species i in the standard state (J/mol).
ξ	Non-dimensional charge density, defined in Eq. 77.

Ψ	Electrical potential (V).
Ψ_D	Donnan potential (V).
ψ	Thermodynamic ion binding parameter, used in Manning condensation theory.

Appendix

By using the previously discussed theory by Marcus, see Section 2.2, a relation for the capacity factor of the eluite ion as a function of eluent salt concentration is derived in this Appendix.

Consider a positive monovalent eluite ion which is distributed between a resin phase, containing negatively charged chemically bound groups, and an eluent phase containing a monovalent eluent salt. According to the discussion in Section 3.1 the general expression for the capacity factor is:

$$k = \frac{\int\limits_{V_R} \left(e^{-(\Delta G^0(r)/RT)} - 1 \right) dV}{V_0} \tag{A1}$$

where $\Delta G^0(r)$ is the standard free energy of transfer of the eluite ion from the bulk of the mobile phase to a point r in the resin phase. V_0 and V_R is the volume of the mobile and resin phase, respectively. The elecrostatic work required to transfer one mole of eluite ions to a point r in the resin phase, i.e. the standard free energy of transfer, is equal to $z_B F \Psi(r)$, where $\Psi(r)$ is the difference in electrostatic potential between the bulk of the eluent and point r and z_B is the charge of the eluite ion ($+1$ in our case). Note that the electrostatic potential in the resin phase is negative because the fixed. groups are negatively charged. Eq. A1 is therefore equal to:

$$k = \frac{\int\limits_{V_R} \left(e^{-(\Delta G^0(r)/RT)} - 1 \right) dV}{V_0}$$

$$= \frac{\int\limits_{V_R} e^{-(z_C F \Psi(r)/RT)} dV - V_R}{V_0} \tag{A2}$$

where in the last equality the fact that $z_B = z_C$ has been used. We want to find how k, i.e. the integral in Eq. A2, depends on the salt concentration in the eluent phase. A first step is to recognise that Eq. 12 can be written:

$$\int\limits_{V_R} e^{-(z_C F \Psi(r)/RT)} dV = \frac{c_{C,R}}{c_{C,E}} \cdot V_R \tag{A3}$$

which gives a relation between the integral and the salt concentration in both the resin and eluent phase, respectively. To solve the problem, the mean concentration of counterion in the resin phase as a function of its concentration in the eluent is needed. In polyelectrolyte theory there is a relation called the additivity law for polyelectrolyte solutions with added salt [139], this law states that

$$c_{C,R} \cong c_{C,R}^0 + c_{C,E} \tag{A4}$$

where $c_{C,R}^0$ is the mean concentration of the monovalent counterion in the resin phase when there is no added salt to the eluent phase, i.e. only counterions to the charges bound in the resin phase are present. Inserting Eq. A4 into A3 we obtain that the capacity factor in Eq. A2 becomes:

$$k = \frac{(c_{C,R}^0 + c_{C,E})}{c_{C,E} \cdot V_0} \cdot V_R - \frac{V_R}{V_0} = \frac{V_R}{V_0} \cdot \frac{C_R}{c_{C,E}} \tag{A5}$$

in the last equality we have used the fact that the mean concentration of counterions in the resin phase with no added salt, is equal to the mean concentration of fixed charged groups, C_R. For monovalent ions Eq. A5 is the same as Eq. 49 obtained from the Donnan model when activity coefficients are neglected (these can be included in this treatment as well). The strength of this derivation, compared to the derivation of Eq. 49, is that it does not rely on the assumption that the co-ion concentration is zero in the resin phase and independent of its concen-

tration in the eluent phase. Instead it is based on the additivity law, Eq. A4, which is less drastic and holds well in many cases.

References

[1] J. Israelachvili, Intermolecular & Surface Forces, Academic Press, London, 1991.
[2] P.G. Hiemenz, Principles of Colloid and Surface Chemistry, Dekker, New York, 1986.
[3] R.J. Hunter, Foundations of Colloid Science, Vol. I and II, Oxford University Press, 1986.
[4] D.F. Evans, H. Wennerström, The Colloidal Domain, VCH Publishers, 1994.
[5] D.A. McQuarrie, Statistical Mechanics, Harper & Row, New York, 1976.
[6] F.G. Donnan, Z. Electrochem. 17 (1911) 572.
[7] F. Helfferich, Ion Exchange, McGraw-Hill, New York, 1962.
[8] C.E. Harland, Ion Exchange: Theory and Practice, The Royal Society of Chemistry, Cambridge, England, 1994.
[9] J.Th.G. Overbeek, Progr. Biophys. 6 (1956) 57.
[10] R.A. Marcus, J. Chem. Phys. 23 (1955) 1057.
[11] G. Arfken, Mathematical Methods for Physicists, 2nd ed, Academic Press, New York, 1970.
[12] G. Gouy, J. Phys. 9 (1910) 457.
[13] D.L. Chapman, Philos. Mag. 25 (1913) 475.
[14] R.J. Hunter, Foundations of Colloid Science, Vol. II, Oxford University Press, 1986.
[15] O. Stern, Z. Electrochem. 30 (1924) 508.
[16] J. Westall, H. Hohl, Adv. Colloid Interface Sci. 12 (1980) 265.
[17] J. Horst, W.H. Holl, S.H. Eberle, Reactive Polymers 13 (1990) 209.
[18] W.H. Holl, M. Franzreb, J. Horst, S.H. Eberle, in: J. Marinsky, Y. Marcus (Eds.), Ion Exchange and Solvent Extraction. A Series of Advances, Marcel Dekker, New York, 1993.
[19] G. Sposito, J. Colloid Interface Sci. 91 (1983) 329.
[20] D. deVault, J. Am. Chem. Soc. 65 (1943) 532.
[21] F. Riedo, E.sz. Kováts, J. Chromatogr 239 (1982) 1.
[22] G.H. Findenegg, F. Köster, J. Chem. Soc., Faraday Trans. I 82 (1986) 2691.
[23] J. Ståhlberg, B. Jönsson, C. Horváth, Anal. Chem. 64 (1992) 3118.
[24] F. Riedo, E. sz. Kováts, in: F. Dondi, G. Guiochon (Eds.), Theoretical Advancement in Chromatography and Related Separation Techniques, NATO ASI Series, Kluwer Academic, Dordrecht, 1992.
[25] The author is indebted to Bengt Jönsson for performing the calculations.
[26] G. Fóti, G. Révész, P. Hajós, G. Pellaton, E. Kováts, Anal. Chem. 68 (1996) 2580.
[27] D.T. Gjerde, J.S. Fritz, J. Chromatogr. 176 (1979) 199.
[28] G.J. Sevenich, J.S. Fritz, J. Chromatogr. 371 (1986) 361.
[29] D.T. Gjerde, J. Chromatogr. 439 (1988) 49.

[30] D.T. Gjerde, G. Schinuckler, J.S. Fritz, J. Chromatogr. 187 (1980) 35.
[31] P.R. Haddad, C.E. Cowie, J. Chromatogr. 303 (1984) 321.
[32] A. Rahman, N.E. Hoffman, J. Chromatogr. Sci. 28 (1990) 157.
[33] L.G. Daignault, D.P. Rillema, D.C. Jackinan, J. High Resolut. Chromatogr. 13 (1990) 293.
[34] M. Caude, R. Rosset, J. Chromatogr. Sci. 15 (1977) 405.
[35] R.C.L. Foley, P.R. Haddad, J. Chromatogr. 366 (1986) 13.
[36] R.W. Slingsby, C.A. Pohl, J. Chromatogr. 458 (1988) 241.
[37] R.D. Rocklin, C.A. Pohl, J.A. Schibler, J. Chromatogr. 411 (1987) 107.
[38] M. Lederer, J. Chromatogr. 452 (1988) 265.
[39] H. Small, Ion Chromatography, Plenum Press, New York, 1989.
[40] E. Glueckauf, Proc. Roy. Soc. A214 (1952) 207.
[41] G.E. Myers, G.E. Boyd, J. Phys. Chem. 60 (1956) 521.
[42] G.E. Boyd, S. Lindenbaum, G.E. Myers, J. Phys. Chem. 65 (1961) 577.
[43] W.J. Argersinger, A.W. Davidson, J. Phys. Chem. 56 (1952) 92.
[44] O.D. Bonner, W.J. Argersinger, A.W. Davidson, J. Am. Chem. Soc. 74 (1952) 1044.
[45] O.D. Bonner, J. Phys. Chem. 59 (1955) 719.
[46] E. Ekedahl, E. Högfeldt, L.G. Sillén, Acta Chem. Scand. 4 (1950) 556.
[47] E. Högfeldt, E. Ekedahl, L.G. Sillén, Acta Chem. Scand. 4 (1950) 828.
[48] G.L. Gaines, H.C. Thomas, J. Chem. Phys. 21 (1953) 714.
[49] J.L. Pauley, J. Am. Chem. Soc. 76 (1954) 1422.
[50] D. Reichenberg, in: J. Marinsky (Ed.), Ion Exchange and Solvent Extraction. A Series of Advances, Marcel Dekker, New York, 1966.
[51] H.P. Gregor, J. Am. Chem. Soc. 70 (1948) 1293.
[52] H.P. Gregor, J. Am. Chem. Soc. 73 (1951) 642.
[53] F.E. Harris, S.A. Rice, J. Chem. Phys. 24 (1956) 1258.
[54] S.A. Rice, F.E. Harris, Z. Phys. Chem. 8 (1956) 207.
[55] H.M.J. Boots, P.K. Bokx, J. Phys. Chem. 93 (1989) 8240.
[56] P.K. Bokx, H.M.J. Boots, J. Phys. Chem. 93 (1989) 8243.
[57] K.D. Collins, M.W. Washabaugh, Q. Rev. Biophys. 18 (1985) 323.
[58] J. Ståhlberg, Anal. Chem. 66 (1994) 440.
[59] F.F. Cantwell, S. Puon, Anal. Chem. 51 (1979) 623.
[60] S. Afrashtehfar, F.F. Cantwell, Anal. Chem. 54 (1982) 2422.
[61] R.A. Hux, F.F. Cantwell, Anal. Chem. 56 (1984) 1258.
[62] F.F. Cantwell, in: J.A. Marinsky, Y. Marcus (Eds.), Ion-Exchange and Solvent Extraction, Vol. 9, Marcel Dekker, New York, 1985.
[63] B.A. Bidlingmeyer, S.N. Deming, W.P. Price Jr., B. Sachok, M.J. Petrusek, J. Chromatogr. 186 (1979) 419.
[64] J.H. Knox, G.R. Laird, J. Chromatogr. 122 (1976) 17.
[65] J.C. Kraak, K.M. Jonker, J.F.K. Huber, J. Chromatogr. 142 (1977) 671.
[66] B. Fransson, K.-G. Wahlund, I.M. Johansson, G. Schill, J. Chromatogr. 125 (1976) 327.
[67] J.G. Chen, S.G. Weber, L.L. Glavina, F.F. Cantwell, J. Chromatogr. A 656 (1993) 549.
[68] I.M. Johansson, K.-G. Wahlund, G. Schill, J. Chromatogr. 149 (1978) 281.
[69] W.R. Melander, Cs. Horváth, in: M.T.W. Hearn (Ed.), Ion Pair Chromatography: Theory and Biological and Pharmaceutical Applications, Vol. 31, Marcel Dekker, New York, pp. 27–275.
[70] A. Tilly Melin, Y. Askemark, K.-G. Wahlund, G. Schill, Anal. Chem. 51 (1979) 976.
[71] J.H. Knox, R.A. Hartwick, J. Chromatogr. 204 (1981) 3.
[72] F.F. Cantwell, J. Pharm. Biomed. Anal. 2 (1985) 153.
[73] H.-J. Liu, F.F. Cantwell, Anal. Chem. 63 (1991) 993.
[74] H.-J. Liu, F.F. Cantwell, Anal. Chem. 63 (1991) 2032.
[75] L.L.M. Glavina, F.F. Cantwell, Anal. Chem. 65 (1993) 268.
[76] S.G. Weber, J.D. Orr, J. Chromatogr. 322 (1985) 433.
[77] S.G. Weber, Talanta 36 (1989) 99.
[78] J. Ståhlberg, J. Chromatogr. 356 (1986) 231.
[79] J. Ståhlberg, A. Furängen, Chromatographia 24 (1987) 783.
[80] J. Ståhlberg, I. Hägglund, Anal. Chem. 60 (1988) 1958.
[81] J. Ståhlberg, Á. Bartha, J. Chromatogr. 456 (1988) 253.
[82] Á. Bartha, J. Ståhlberg, F. Szokoli, J. Chromatogr. 552 (1991) 13.
[83] G. Schill, in: J.A. Marinsky, Y. Marcus (Eds.), Ion-Exchange and Solvent Extraction, Vol. 6, Marcel Dekker, New York, 1974.
[84] M.T.W. Hearn (Ed.), Ion-Pair Chromatography – Theory and Biological and Pharmaceutical Applications, Marcel Dekker, New York, 1985.
[85] Cs. Horváth (Ed.), High Performance Liquid Chromatography – Advances and Perspectives, Vol. 1 and 2, Academic Press, New York, 1980.
[86] R.H.A. Sorel, A. Hulshoff, in: E. Grushka, P.R. Brown (Eds.), Advances in Chromatography, Vol. 21, Marcel Dekker, New York, 1983.
[87] M.C. Gennaro, in: E. Grushka, P.R. Brown (Eds.), Advances in Chromatography, Vol. 35, Marcel Dekker, New York, 1995.
[88] L.C. Sander, C.J. Glinka, S.A. Wise, Anal. Chem. 62 (1990) 1099.
[89] Á. Bartha, Gy. Vigh, H.A.H. Billiet, L. de Galan, J. Chromatogr. 303 (1984) 29.
[90] Á. Bartha, Gy. Vigh, H.A.H. Billiet, L. de Galan, J. Chromatogr. 291 (1984) 91.
[91] J.L.M. Van de Venne, J.L.H.M. Hendrikx, R.S. Deelder, J. Chromatogr. 167 (1978) 1.
[92] Á. Bartha, J. Ståhlberg, J. Chromatogr. A 668 (1994) 255.
[93] Á. Bartha, Gy. Vigh, H.A.H. Billiet, L. de Galan, J. Chromatogr. 303 (1984) 29.
[94] I. Hägglund, J. Ståhlberg, J. Chromatogr. A 761 (1996) 3.
[95] I. Hägglund, J. Ståhlberg, J. Chromatogr. A 761 (1996) 13.
[96] J. Narkiewicz-Michalek, Chromatographia 35 (1993) 527.
[97] Z. Samec, Chem. Rev. 88 (1988) 617.
[98] R. Alexander, A.J. Parker, J. Am. Chem. Soc. 89 (1967) 5549.
[99] I.M. Kolthoff, M.K. Chantooni Jr., J. Phys. Chem. 76 (1972) 2024.
[100] J.F. Coetzee, W.R. Sharpe, J. Phys. Chem. 75 (1971) 3141.
[101] W. Norde, in: M. Malmsten (Ed.), Biopolymers at interfaces, Marcel Dekker, Inc, New York, 1998.

[102] C.M. Roth, A.M. Lenhoff, in: M. Malmsten (Ed.), Biopolymers at Interfaces, Marcel Dekker, Inc, New York, 1998.

[103] J.D. Andrade, V. Hlady, Adv. Polym. Sci. 79 (1986) 1.

[104] A. Sadana, Chem. Rev. 92 (1992) 1799.

[105] B. Deryaguin, Trans. Faraday Soc. 36 (1940) 203.

[106] E.J.W. Verwey, J.Th.G. Overbeek, Theory of the Stability of Lyophobic Colloids, Elsevier, Amsterdam, 1948.

[107] V.A. Parsegian, D. Gingell, Biophys. J. 12 (1972) 1192.

[108] J. Ståhlberg, B. Jönsson, Cs. Horváth, Anal. Chem. 63 (1991) 1867.

[109] J. Ståhlberg, B. Jönsson, Cs. Horváth, Anal. Chem. 64 (1992) 3118.

[110] J. Ståhlberg, B. Jönsson, Anal. Chem. 68 (1996) 1536.

[111] J. Ståhlberg, U. Appelgren, B. Jönsson, J. Colloid Interface Sci. 176 (1995) 397.

[112] S.L. Carnie, D.Y.C. Chan, J.S. Gunning, Langmuir 10 (1994) 2993.

[113] C.M. Roth, A.M. Lenhoff, Langmuir 9 (1995) 962.

[114] C.M. Roth, A.M. Lenhoff, Langmuir 11 (1995) 3500.

[115] L. Haggerty, A.M. Lenhoff, J. Phys. Chem. 95 (1991) 1472.

[116] C.M. Roth, K.K. Unger, A.M. Lenhoff, J. Chromatogr. 726 (1995) 45.

[117] V. Noinville, C. Vidal-Madjar, B. Sebille, J. Phys. Chem. 99 (1995) 1516.

[118] W. Norde, J. Lyklema, J. Biomater. Sci. Polymer Edn. 2 (1991) 183.

[119] K. Dill, Biochemistry 29 (1990) 7133.

[120] C.A. Haynes, W. Norde, J. Colloid Interface Sci. 169 (1995) 313.

[121] B. Lee, F.M. Richards, J. Mol. Biol. 55 (1971) 379.

[122] W. Norde, J.P. Favier, Colloids Surf. 64 (1992) 87.

[123] C.R. McMillan, A.G. Walton, J. Colloid Interface Sci. 48 (1974) 345.

[124] A. Kondo, S. Oku, K. Higashitani, J. Colloid Interface Sci. 143 (1991) 214.

[125] N.K. Boardman, S.M. Partridge, Biochem. J. 59 (1955) 543.

[126] W. Kopaciewicz, M.A. Rounds, J. Fausnaugh, F.E. Regnier, J. Chromatogr. 283 (1984) 37.

[127] A. Velayudhan, Cs. Horváth, J. Chromatogr. 367 (1986) 160.

[128] C.A. Brooks, S.M. Cramer, AIChE J. 38 (12) (1992) 1969.

[129] C. Cai, V.A. Romano, P.L. Dubin, J. Chromatogr. A 693 (1995) 251.

[130] I. Mazsaroff, L. Vàrady, G.A. Mouchawar, F.E. Regnier, J. Chromatogr. 499 (1990) 63.

[131] W.R. Melander, Z. ElRassi, Cs. Horváth, J. Chromatogr. 469 (1989) 3.

[132] G.S. Manning, J. Chem. Phys. 51 (1969) 924.

[133] G.S. Manning, J. Chem. Phys. 51 (1969) 3249.

[134] M.T. Record Jr., C.F. Anderson, T.M. Lohinan, Q. Rev. Biophys. 11 (1978) 103.

[135] M.T. Record Jr., Biopolymers 14 (1975) 2137.

[136] J. Wyman, Adv. Protein Chem. 19 (1964) 223.

[137] O. Sinanoglu, in: H. Ratajczak, W.J. Thomas-Orville (Eds.), Molecular Interactions, Vol. 3, Wiley, New York, 1982.

[138] Cs. Horváth, W. Melander, I. Molnár, J. Chromatogr. 125 (1976) 129.

[139] F. Oosawa, Polyelectrolytes, Marcel Dekker, New York, 1971.

[140] A. Katchalsky, Z. Alexandrowics, O. Kedem, in: B.E. Conway, R.G. Barradas (Eds.), Chemical Physics of Ionic Solutions, John Wiley, New York, 1966.

[141] B. Jönsson, H. Wennerström, Chem. Scr. 15 (1980) 40.

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