Ideal model of chromatography applied to charged solutes in reversed-phase liquid chromatography

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Abstract

The ideal model of chromatography assumes that the equilibration of a solute between the mobile and stationary phase is an infinitely rapid process. Under these assumptions, and for a given adsorption isotherm the model can be used to theoretically describe the evolution of a solute zone (peak), as it migrates along the chromatographic column. In this paper hydrophobic charged solutes are injected on a RP-18 column and the ideal model of chromatography is used to calculate the eluting peak shape. The advantage of using charged hydrophobic solutes is that the no linearity of the adsorption isotherm occurs at very low mobile phase concentrations of the solute. Another advantage is that from a theoretical point of view the adsorption isotherm is very well described by the electrostatic surface potential modified linear adsorption isotherm and the physical origin of the non-linearity is well understood. The application of this adsorption isotherm to the ideal model of chromatography is, however, mathematically more complex than for the Langmuir or Freundlich adsorption isotherms which previously have been combined with the ideal model of chromatography. It is shown that for negatively charged solutes the ideal model of chromatography combined with the electrostatically modified linear adsorption isotherm describes well the peak shape of the eluting peak. Since the non-linearity of the used adsorption isotherm is physically well defined, the experimental and theoretical study of eluting peak shapes of charged analytes may be a tool for the characterisation of the chromatographic column and also for the study of the interaction between charged solutes and the stationary phase surface.

Keywords: Adsorption isotherms; Band profiles; Charged solutes; Toluenesulfonate

1. Introduction

The ideal model of chromatography assumes that the column efficiency is infinite implying that the mass transfer for a solute between the mobile and stationary phase is infinitely fast. For a solute with a linear adsorption isotherm the ideal model states that the concentration profile of a solute travels unchanged through the column, this case is therefore usually not of theoretical interest. For high solute

concentration the adsorption isotherm often becomes non-linear and the ideal model is the simplest possible model which can calculate the change in zone profile, caused by the non-linearity, as the solute travels through the column. Furthermore, the efficiency of modern chromatographic columns is such that for concentration pulses that are strongly non-linear during the migration through the column, the change in concentration profile calculated from the ideal model strongly dominates other effects which cause dispersion of a migrating peak. The expected difference between concentration profiles

calculated from the ideal model and those experimentally measured is that the former predicts profiles with sharp angles while in practice they are smooth.

The mass balance equation forms the mathematical basis of the ideal model of chromatography and was originally developed by Wilson [1]. A more rigorous mathematical analysis of the mass balance equation was later made by DeVault [2] who showed that when a pulse of a solute with a non-linear adsorption is injected a concentration shock is formed. Further theoretical and experimental contributions to this field was later made by Glueckauf [3,4] who discussed several types of adsorption isotherms and also by Weiss [5] who treated the change in band profile for a Langmuir and a Freundlich isotherm. More recently Golshan-Shirazi and Guiochon have in a series of papers theoretically and experimentally investigated the properties of the ideal model of chromatography applied to the Langmuir isotherm and the bi-Langmuir isotherm, e.g., references [6–8]. A very good summary of their work as well as of others in the field of theoretical models for the prediction of band profiles is found in ref. [9].

The adsorption isotherm of charged amphiphilic molecules to an oil–water interface is known to follow the electrostatically modified Langmuir isotherm [10] and has also been shown to apply to reversed-phase surfaces [11–15]. In the domain of low surface concentration of the amphiphile it is simplified to the electrostatically modified linear adsorption isotherm. The electrostatic repulsion created by the adsorbed amphiphile itself causes a physically well defined non-linear relation between the mobile phase concentration and surface concentration of the amphiphile. In the electrostatically modified linear adsorption isotherm this repulsion is quantified; in the absence of electrostatic repulsion the adsorption isotherm becomes linear. In this paper the theoretical and experimental band profile for a negatively charged amphiphile is studied in the domain of the electrostatically modified linear adsorption isotherm. The advantage of using a negatively charged analyte is that no specific interactions with underivatised silica groups on the reversed-phase surface are expected and that the adsorption isotherm is non-linear at very low concentrations of

amphiphile in the mobile phase. Another advantage is that the non-linearity is physically well defined since it is caused by the electrostatic repulsion created by the adsorbed amphiphile itself. This means that the non-linearity as well as the band profile is described by a number of physical constants and in principle it does not involve any unknown fitting parameters. Because of these advantages charged amphiphilic molecules are very well suited for a theoretical study of non-linear effects in the ideal model of chromatography.

Apart from the already discussed theoretical aspects, this study is also of practical chromatographic significance. As will be discussed below, the peak profile for a charged solute is dependent on the surface area of the column while its capacity factor at infinite dilution is related to both the hydrophobicity and the surface area of the stationary phase surface. It is therefore possible to characterise important column parameters by comparing experimentally obtained with theoretically calculated eluting peak profiles. Another practical aspect of this work is the calculation of eluting peak profiles for charged solutes in reversed-phase chromatography. It is shown that for sufficiently high solute concentrations, a very good agreement between the experimental and calculated band broadening is obtained for charged solutes which do not have specific interactions with silanol groups or any other type of active surface sites. This also implies that when these types of interactions are present, they can be detected and characterised by using the presented approach.

2. Theory

2.1. General theory of ideal chromatography

An infinite column efficiency is assumed in the ideal model of chromatography, i.e., the solute is in instantaneous equilibrium between the mobile and stationary phase and there is therefore no axial dispersion due to mass transfer. The starting point for the ideal model is the mass balance equation for a solute:

$$\frac{\partial c}{\partial t} + \Phi \frac{\partial n}{\partial t} + u_0 \frac{\partial c}{\partial z} = 0 \tag{1}$$

where u_0 is the velocity of the mobile phase (m/s) given by $u_0 = L/t_0$, where L is the column length (m) and t_0 the column dead time (s); n is the concentration of the solute on the stationary phase surface (mol/m^2) and c its concentration in the mobile phase (mol/m^3). When the mass transfer of the solute is infinitely fast between the two phases, n and c are at each point in the column related through the adsorption isotherm to the stationary phase surface, $n = f(c)$. Φ is the column phase ratio which in this case is defined as A/V_0 where A is the area of the stationary phase surface in the column (m^2) and V_0 is the column dead volume (m^3); z is the column length coordinate (m) and t is the time (s). Under the conditions of ideal chromatography Eq. (1) can be rewritten:

$$\frac{\partial c}{\partial t} + \frac{u_0}{\left(1 + \Phi\dfrac{dn}{dc}\right)} \frac{\partial c}{\partial z} = 0 \tag{2}$$

The solution to this equation describes the concentration velocity [16], u, of the solute in the z-direction on the diffuse boundary of a chromatographic band profile:

$$u = \frac{u_0}{1 + \Phi\dfrac{dn}{dc}} \tag{3}$$

Under normal chromatographic conditions the width of the injected peak is negligible compared to the eluting peak, under these conditions the elution time for concentration c on the eluting peak is obtained from Eq. (3):

$$t_R(c) = \frac{L}{u_0} \cdot \left(1 + \Phi\frac{dn}{dc}\right) \tag{4}$$

From this equation follows that the elution time of the peak for an analyte with a non-linear adsorption isotherm will vary with the amount injected on the column. It was shown by de Vault [2] that when the analyte concentration is in the non-linear regime of the adsorption isotherm its peak profile develops to a concentration discontinuity during the migration through the column. It is important to remember that Eq. (4) can only be used for the continuous part of the peak profile and can not be used to calculate the elution time of the discontinuity. The elution time of the end of a diffuse peak profile of an analyte with a convex isotherm, i.e., in the limit $c \to 0$, is obtained from Eq. (4) and becomes:

$$t_{R,0} = t_0 \cdot \left(1 + \Phi\left(\frac{dn}{dc}\right)_{c \to 0}\right) \tag{5}$$

where $t_{R,0}$ is the retention time of an infinitely small pulse of the analyte.

For the calculation of the retention time of the concentration shock Golshan-Shirazi and Guiochon [7] used the fact that the sample mass of the analyte is constant during the migration along the column. They calculated the retention time of the discontinuity from the mass conservation integral:

$$U \int_{t_R}^{t_{R,0}} c(t)dt = M \tag{6}$$

where U is the volumetric flow-rate of the mobile phase (m^3/s); t_R is the retention time of the discontinuity for an analyte with a convex adsorption isotherm and M is the injected mass of the analyte (mol). By solving the integral in Eq. (6) the maximum concentration of the eluting band is obtained, the retention time for the maximum concentration is obtained from Eq. (4).

2.2. Ideal model of chromatography for amphiphilic ions

An adsorption isotherm for charged amphiphilic molecules which considers both the electrostatic surface potential created by the amphiphile, its hydrophobic interaction as well as its maximum possible surface concentration has previously been used in the electrostatic theory for ion-pair chromatography [11,12,14]. The adsorption isotherm is based on a Langmuir isotherm for describing the competition for available surface area in combination with a simple expression for the electrostatic interaction between the charged surface, which is generated by the amphiphile itself, and the charged amphiphile. The equation is generally called the electrostatically modified Langmuir isotherm:

$$n = \frac{n_0 K_{AS} \cdot c \cdot e^{-\frac{z_A F \Psi_0}{RT}}}{1 + K_{AS} \cdot c \cdot e^{-\frac{z_A F \Psi_0}{RT}}} \tag{7}$$

where n is the surface concentration of amphiphile (mol/m^2) and n_0 is the monolayer capacity of the surface (mol/m^2), c is the amphiphile concentration in the liquid phase (mol/m^3), F is the Faraday constant (C/mol), z_A is the charge of the amphiphile and Ψ_0 is the electrostatic potential (V) of the stationary phase surface. K_{AS} is the "chemical" binding constant of the amphiphile to the surface, i.e.,:

$$K_{AS} = \exp - \left(\frac{\mu_{AS}^0 - \mu_A^0 - \mu_S^0}{RT} \right) \qquad (8)$$

μ_i^0 is the electrochemical potential of the standard state for the surface S, the amphiphile in the mobile phase A and the amphiphile–surface complex, AS. When $n/n_0 < 0.2$ the denominator in Eq. (7) is close to 1 and to a first approximation the linear form of the adsorption isotherm is valid:

$$n = n_0 K_{AS} \cdot c \cdot e^{-\frac{z_A F \Psi_0}{RT}} \qquad (9)$$

This is the electrostatically modified linear isotherm and it is used in the ensuing calculations for the peak profile and the retention time.

When the charged amphiphile adsorbs on the stationary phase surface a change in the electrostatic surface potential is created. By solving the linearised Poisson–Boltzmann equation in cylindrical coordinates the relation between the surface concentration of amphiphile in the stationary phase pores and the change of the electrostatic surface potential is obtained [10]:

$$\Delta \Psi_0 = \frac{z_A \cdot F \cdot n}{\kappa \varepsilon_0 \varepsilon_r} \cdot \frac{I_0(\kappa r)}{I_1(\kappa r)} \qquad (10)$$

where ε_0 is the permittivity of vacuum, ε_r the dielectric constant of the fluid medium, $I_0(\kappa r)$ and $I_1(\kappa r)$ is the modified Bessel function of the first kind of order zero and one, respectively, and r is the pore radius of the stationary phase. $1/\kappa$ is the Debye length which is a measure of the thickness of the electrical double layer and is defined as

$$\kappa = F \cdot \left(\frac{2I}{\varepsilon_0 \varepsilon_r RT} \right)^{1/2} \qquad (11)$$

where I is the ionic strength of the mobile phase. If the stationary phase surface has no chemically bound charged groups, the change in surface potential

calculated from Eq. (10) is, for this particular case, equal to the net potential, Ψ_0 in Eq. (9). Under neutral or basic conditions the silanol groups are partly dissociated creating a net charged surface with a non-zero electrostatic potential, $\Psi_{0,s}$, so that the net surface potential, $\Psi_{0,t}$, becomes

$$\Psi_{0,t} = \Psi_{0,s} + \Delta \Psi_0 \qquad (12)$$

Under these conditions the net electrostatic potential given by Eq. (12) is used in the adsorption isotherm Eq. (9).

When applying the theory for non-linear ideal chromatography to a given adsorption isotherm the usual procedure is to start with the derivative dn/dc for the known adsorption isotherm, $n = f(c)$. For the adsorption of charged amphiphilic molecules there is a coupling between the electrostatic potential created by the adsorbed amphiphile, Eq. (10), and the adsorption isotherm, Eq. (9). It is therefore mathematically more convenient to express c as a function of n. Rewriting Eq. (9) and combining it with Eq. (10) gives the mobile phase concentration of the amphiphile, c, as a function of its surface concentration, n.

$$c = \frac{n}{n_0 K_{AS}} \cdot e^{B \cdot n} \qquad (13)$$

where, for simplicity, the physical constants in the exponential term has been abbreviated into B;

$$B = \frac{z_A^2 F^2}{\kappa \varepsilon_0 \varepsilon_r RT} \cdot \frac{I_0(\kappa r)}{I_1(\kappa r)} \qquad (14)$$

Because of the coupling between the surface concentration of amphiphile and the generated electrostatic surface potential, the theoretically calculated shape of the continuous part of the peak profile, $c = f(t_R)$, the procedure previously used for a Langmuir isotherm can not be applied [6–9]. Since it is easy to obtain a closed equation for $c = f(n)$ we have chosen to make the derivation in terms of the surface concentration of the amphiphile instead of in terms of its mobile phase concentration. By using Eq. (4) the result is then converted into the measurable mobile phase concentration at the column outlet. Consequently, we express the retention time for a given concentration in terms of n by taking the

derivative (dc/dn) of Eq. (13), which is inserted into Eq. (4).

$$t_R = \frac{L}{u_0} \cdot \left(1 + \frac{\Phi}{\frac{dc}{dn}}\right)$$

$$= t_0 \cdot \left(1 + \frac{\Phi \cdot n_0 K_{AS}}{e^{Bn(t)}(1 + Bn(t))}\right) \qquad (15)$$

This equation gives the retention time for the diffuse part of the peak profile in terms of the surface concentration of the solute. The experimentally measured peak profile is equal to the concentration of the solute in the mobile phase leaving the column and this concentration is calculated by combining Eq. (15) with Eq. (13). The maximum concentration of the eluted peak is obtained through the mass conservation integral, Eq. (6), which is combined with Eq. (13) to

$$M = U \int_{t_R}^{t_{R,0}} c(t)dt = U \int_{t_R}^{t_{R,0}} \frac{n(t)}{n_0 K_{AS}} \cdot e^{B \cdot n(t)} dt \qquad (16)$$

where M is the number of moles of solute injected on the column. From Eq. (15) we obtain dt as a function of n:

$$dt = -t_0 \Phi \frac{n_0 K_{AS} \cdot Be^{-Bn(t)} \cdot (2 + Bn(t))}{(1 + Bn(t))^2} dn \qquad (17)$$

Since $U \cdot t_0 = V_0$, the integral in Eq. (16) becomes

$$M = -V_0 \Phi \cdot B \cdot \int_{n_m}^{0} \frac{n \cdot (2 + B \cdot n)}{(1 + B \cdot n)^2} dn$$

$$= V_0 \Phi \cdot B \cdot \frac{n_m^2}{(1 + B \cdot n_m)} \qquad (18)$$

where n_m is the surface concentration of solute in equilibrium with the mobile phase at the peak maximum. By solving the second order equation we obtain n_m as a function of M:

$$n_m = \frac{1}{V_0 \Phi} \left(\frac{M}{2} + \sqrt{\frac{M^2}{4} + \frac{V_0 \Phi \cdot M}{B}}\right) \qquad (19)$$

The experimentally measured parameter is the corresponding solute concentration in the mobile phase,

c_m, which is obtained by inserting Eq. (19) into the expression for the adsorption isotherm, Eq. (13):

$$c_m = \frac{\left(\frac{M}{2} + \sqrt{\frac{M^2}{4} + \frac{V_0 \Phi \cdot M}{B}}\right)}{V_0 k_0}$$

$$\cdot \exp\left(\frac{B}{V_0 \Phi}\left(\frac{M}{2} + \sqrt{\frac{M^2}{4} + \frac{V_0 \Phi \cdot M}{B}}\right)\right) \qquad (20)$$

where we have introduced the capacity factor of the solute at infinitely low concentration, k_0, given by

$$k_0 = \Phi \cdot n_0 K_{AS} \qquad (21)$$

Eq. (20) can be used only to calculate the concentration at the peak maximum but can not be used to obtain its retention time, or in other words, it can not be used for calculating the peak profile $c = f(t_R)$.

The peak profile can be obtained from several equivalent procedures, for the calculations presented in Section 4 the following procedure is used: In the first step the value for M is varied from zero to its actual value. Then, for each of the selected M values the corresponding n_m value is calculated from Eq. (19). By inserting the obtained n_m values into Eq. (13) the function $c = c(n_m)$ is found. By inserting the same n_m values into Eq. (15) the function $t_r = t_r(n_m)$ is found. Finally, the peak profile, i.e., $c = f(t_r)$ is obtained by plotting $c(n_m)$ as a function of $t_r(n_m)$. An alternative procedure is to determine n_m from the experimentally known M, then calculate t_r for n values varying from zero to n_m and finally plot $c = f(n_m)$ as a function of $t_r(n_m)$. Both procedures are easily performed by using a computer.

3. Experimental

A LC system from Perkin-Elmer was used for the chromatography and it was composed of a LC-gradient pump series 200, a LC sample processor ISS 200, a LC oven 101 and a diode array detector 235 C. The system was controlled by a data system, Turbochrom 4, also from Perkin-Elmer. The column was a LiChrospher 100 from Merck, Darmstadt, Germany (RP-1, particle diameter = 5 μm) with the dimensions 12 cm × 4 mm I.D., the same column was used in all experiments. The pH measurements were

performed using a Radiometer PHM 64 Research pH meter.

As solute toluene-4-sulfonic acid (Merck) was used. For the buffer solutions sodium dihydrogen-phosphate monohydrate GR (Merck) and o-phosphoric acid 85% GR (Merck) were used. The water used was from a Milli-Q and with a resistivity of 18.2 MΩ cm. All the buffer solutions were filtered through a 0.45 μm filter, from Millipore, before use.

The analyte ions were injected after dissolution in the mobile phase and the injection volume was 20 μl. The flow-rate was 1.0 ml/min throughout all of the studies and the column temperature was 298 K. The dead time, t_0, was determined by injection of nitrate ions and subsequent measurement of the retention time, and it was found to be between 0.88–0.93 min for the different experimental series.

4. Results and discussion

When the theoretical equations are applied to experimental data there are two unknown parameters: the surface area of the stationary phase in the column and the capacity factor, k_0, of the solute at infinitely low concentration. By measuring the adsorption isotherm for paratoluenesulfonate as well as other negatively charged amphiphiles under various conditions on this particular column, the measured surface area of the column is 135 m^2 [13]. This value is therefore used in the ensuing calculations. From these measurements it is also shown that in the range of injected concentrations of p-toluenesulfonate, the adsorption isotherm equation, Eq. (13) as well as the validity of the linearised Poisson–Boltzmann equation (i.e., $\Delta\psi_0 < 30$ mV), are fulfilled during the whole migration process of the solute through the column. That the depletion of p-toluenesulfonate, due to electrostatic repulsion, in the region of the mobile phase close to the charged stationary phase is negligible is briefly discussed in Ref. [13].

An accurate independent value for k_0 is difficult to obtain because even at the lowest concentrations of p-toluenesulfonate used in this study the peak profiles are skewed because of the non-linear isotherm. From measurements of the adsorption isotherm for p-toluenesulfonate under the same experimental conditions as the zone elution experiments, an indepen-

dent value for k_0 is obtained [13] and this value is used in the following figures.

In Fig. 1 is shown the experimentally obtained and theoretically calculated elution peaks for different concentrations of injected p-toluenesulfonate using a mobile phase with ionic strength 50 mol/m^3 and pH 3. In the experiments 20 μl of the solute solution is injected and the concentration of p-toluenesulfonate in the solution varies by a factor of ten, from 0.53 mol/m^3 to 5.3 mol/m^3. At high solute concentrations the non-linearity in the adsorption isotherm is more pronounced during the whole migration process through the column and therefore the agreement between the experimental and theoretical profile is generally best for the highest injected solute concentration. It is also seen that, due to dispersion effects, the experimentally measured profile shows a stronger tailing than the theoretically calculated profile. As the concentration of the injected solute decreases the non-linearity of the adsorption isotherm decreases and the relative importance of the band broadening due to other dispersion effects increases. This leads to relatively broader and more symmetrical profiles and is very clearly seen in Fig. 1.

From Eq. (13) follows that the non-linearity of the adsorption isotherm of the charged amphiphile becomes more pronounced when the ionic strength of the mobile phase decreases. A critical test of the proposed theory for the adsorption isotherm and its effect on the peak profile is therefore to vary the ionic strength of the mobile phase. Fig. 2 shows the experimentally measured and the theoretically calculated concentration profiles for a mobile phase with ionic strength 25 mol/m^3 and pH 3. The volume injected and the solute concentrations are the same as in Fig. 1 so that a direct comparison between the figures can be made. It is seen that also in this case there is a good agreement between the theoretical and experimental profiles at the highest injected solute concentration and that the deviation between the two becomes larger as the solute concentration decreases.

Comparing the retention time of the solute at infinitely low concentration between the two experiments it is seen that the retention time increases with about 100 s when going from 25 to 50 mol/m^3 ionic strength. In an experiment (not shown) using a
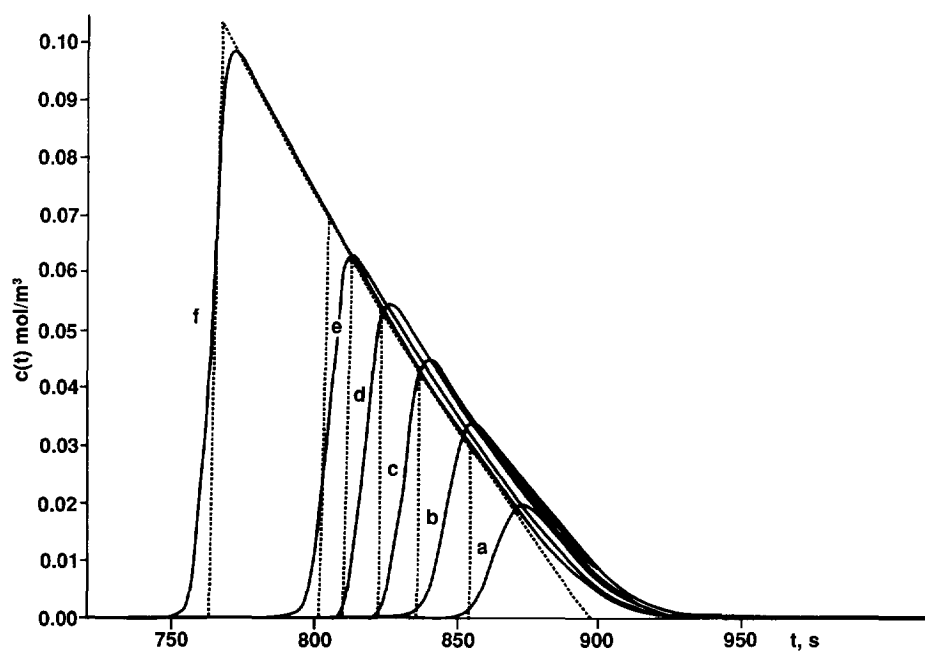
Fig. 1. Experimentally measured (——) and theoretically calculated (- - -) eluting peak profiles for paratoluenesulfonate of different amounts injected on a reversed-phase column. The amount injected is (a) $1.06 \cdot 10^{-8}$ mol; (b) $2.12 \cdot 10^{-8}$ mol; (c) $3.18 \cdot 10^{-8}$ mol; (d) $4.24 \cdot 10^{-8}$ mol; (e) $5.30 \cdot 10^{-8}$ mol and (f) $10.6 \cdot 10^{-8}$ mol. Mobile phase composition: sodium phosphate buffer at pH 3 with ionic strength 50 mol/m^3.
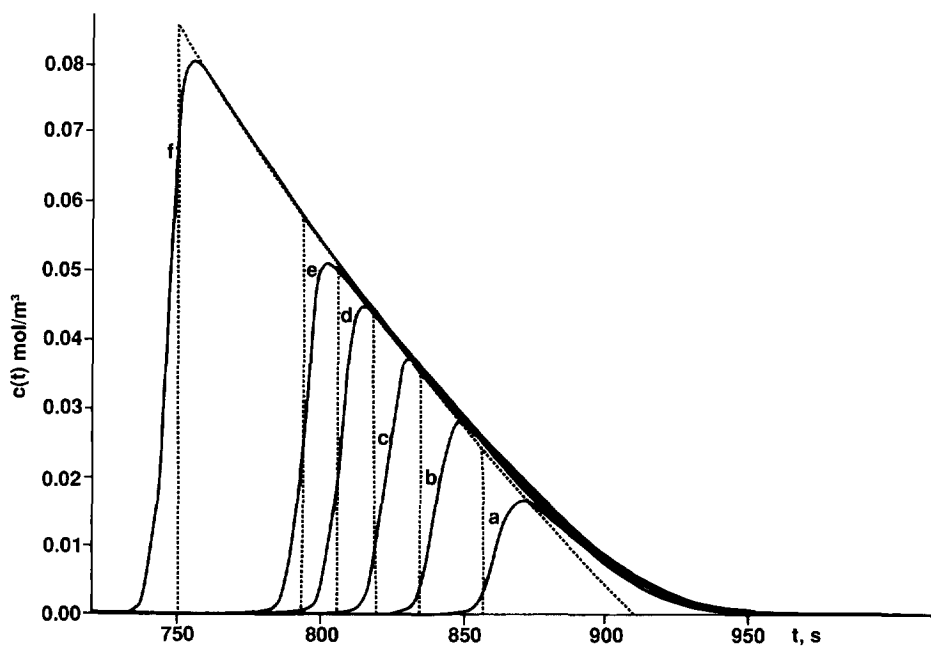

Fig. 2. Experimentally measured (——) and theoretically calculated (- - -) eluting peak profiles for p-toluenesulfonate of different amounts injected on a reversed-phase column. The amount injected is (a) $1.06 \cdot 10^{-8}$ mol; (b) $2.12 \cdot 10^{-8}$ mol; (c) $3.18 \cdot 10^{-8}$ mol; (d) $4.24 \cdot 10^{-8}$ mol; (e) $5.30 \cdot 10^{-8}$ mol and (f) $10.6 \cdot 10^{-8}$ mol. Mobile phase composition: sodium phosphate buffer at pH 3 with ionic strength 25 mol/m^3.

mobile phase with ionic strength 100 mol/m^3 the retention time increased further by about 90 s in comparison with the retention time using ionic strength 50 mol/m^3. An explanation may be that the activity of the solute in the mobile phase increases as the ionic strength increases, a "salting out" effect. Another possibility is that the surface properties of the stationary phase changes causing an increase in the interfacial tension between the stationary and mobile phase so that, according to Gibbs adsorption isotherm [10], adsorption of the analyte becomes more energetically favorable.

To more clearly demonstrate the effect of ionic strength on the concentration profile for *p*-toluenesulfonate, the eluting profiles and the corresponding theoretical curves for the highest solute concentration are assembled in Fig. 3. It can be seen that the retention time at infinitely low solute concentration is slightly longer at ionic strength 25 mol/m^3 compared to 50 mol/m^3 and that the concentration profile is more asymmetric in the former case, illustrating the effect of the stronger non-linearity of its adsorption isotherm.

5. Conclusions

In the electrostatic theory for ion-pair chromatography the electrostatically modified Langmuir adsorption isotherm is used to describe the adsorption properties of the ion pair reagent or amphiphile. At low surface concentrations of the amphiphile this adsorption isotherm can be simplified to its linearised form, the electrostatically modified linear adsorption isotherm. This adsorption isotherm consists of two parts; a binding constant which is related to the "chemical" part of the interaction between the solute and the stationary phase surface and a term which takes into account the electrostatic interaction which varies with the surface concentration of the solute. The latter term gives rise to a physically well defined non-linearity of the adsorption isotherm at very low surface concentrations. This means that charged amphiphilic molecules are ideal for studying non-linear effects in reversed-phase chromatographic systems.

By combining the electrostatically modified linear adsorption isotherm with the theory for ideal chro-
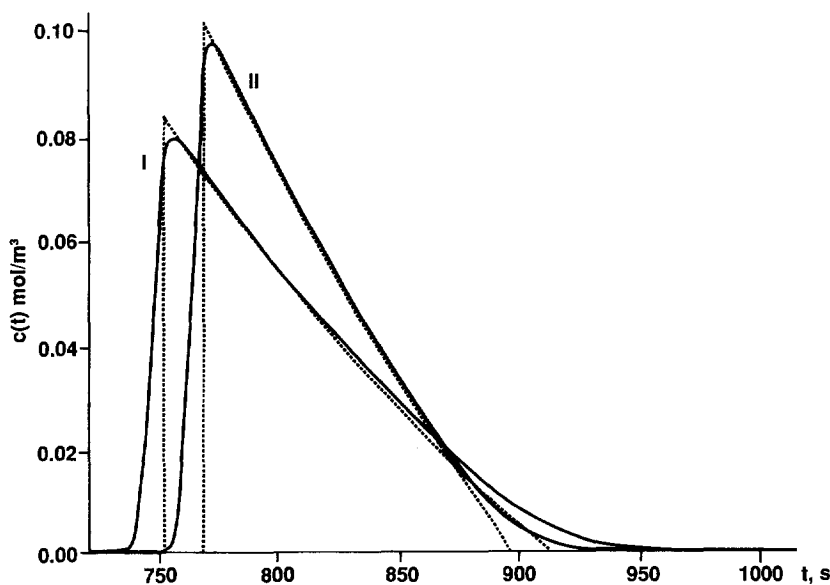

Fig. 3. Experimentally measured (———) and theoretically calculated (- - -) eluting peak profiles for $10.6 \cdot 10^{-8}$ mol injected *p*-toluenesulfonate on a reversed-phase column. Mobile phase composition: (I) sodium phosphate buffer at pH 3 with ionic strength 25 mol/m^3; (II) sodium phosphate buffer at pH 3 with ionic strength 50 mol/m^3.

matography, the concentration profile of an eluting peak from a reversed-phase column can be described for an amphiphilic ion. From independent measurements of the adsorption isotherm of p-toluenesulfonate using the same column it is known that the electrostatically modified linear adsorption isotherm is followed. The eluting peak shape of an injected concentration zone is analysed by applying this adsorption isotherm to the ideal model of chromatography. Comparison between the theoretically calculated and the experimentally obtained concentration profiles shows a very good agreement at the highest paratoluenesulfonate concentrations used in this study. Due to increasing importance of normal peak dispersion effects, the agreement between the two sets of profiles gradually decreases as the injected solute concentration decreases.

The experiments show that the proposed theory can be used to describe changes in the eluting peak profile caused by changing the ionic strength of the mobile phase. Because the peak profile depends only on two unknown and physically well defined parameters, k_0 and column surface area, the study of peak profiles can be used to characterise both the column and the interactions between the charged solute and the stationary phase surface. Furthermore, by using the proposed theory it is possible to predict the peak shape and retention time under overloading conditions for charged solutes.

In reversed-phase chromatography the adsorbent surface consists of chemically bound alkyl groups with a high saturation capacity and unreacted silanol groups with low capacity. These two different sites may interact with a solute with very different strength, e.g., it is often assumed that the strong peak tailing of amines is due to a strong interaction with

the unreacted silanol groups. By comparing the peak shape of a solute with that of a solute which behaves ideally, e.g., p-toluenesulfonate, the peak tailing due to few active sites on the surface can be quantified. It can therefore be concluded that the study of eluting peak profiles of charged solutes in combination with the proposed theory is a tool for both the understanding of the chromatographic process and the characterisation of the chromatographic column.

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