Theory for migration of ions in capillary electrochromatography

Jan Ståhlberg[1]

AstraZeneca, Pharmaceutical Production Gärtuna Södertälje, S-151 85 Södertälje, Sweden

Abstract

The fundamental migration theories for chromatography and electrophoresis are both based on a solution of the mass balance equation. The corresponding analysis for an electrochromatographic system has previously been published and is analysed in more detail in this paper. It is shown that the resulting equation, Eq. (8) in this paper, is in agreement with both electrophoretic and chromatographic theories and that when these migration modes are mixed a complicated migration behaviour emerge. These complications arise, if the comparison is done with electrophoretic theory, because the presence of the stationary phase creates a number of new restrictions on the system (electroneutrality on the stationary phase and simultaneous equilibrium for all components between the eluent and stationary phase). From a mathematical point of view, these restrictions make it difficult for the system to satisfy the coherence condition and this in turn may lead to an anomalous behaviour. To minimise the possibility for a complicated behaviour it is advisable to avoid too much mixing of the two migration mechanisms and/or to match the mobilities of the ionic components in the eluent phase with the mobility of the analyte ion.   © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Electrochromatography

1. Introduction

The first electrochromatographic experiments were made by Pretorius et al. in 1974 [1] and some years later Jorgenson and Lukacs [2,3] described the separation of neutral compounds in packed capillaries to which an electric potential difference across the length of the column was applied. In a series of papers Knox and Grant [4–6] analysed and discussed the advantages of the electrochromatographic technique compared to chromatography and electrophoresis. Since then, and particularly during the last 5 years, the research in electrochromatography has increased dramatically and it has been demonstrated to be a very powerful separation technique. In contrast to liquid chromatography and electrophoresis, the physicochemical properties of an electrochromatographic system are not well known. Of particular importance is the generation and control of electroosmotic flow (EOF) in packed columns and this is therefore an area which recently have been more closely investigated [7–12]. In capillary electrochromatography (CEC) the EOF is caused by a combination of electrical and viscous forces in a packed bed. The physics of this process is very complex and the existing theoretical models for these systems were recently reviewed by Rathore and Horváth [13].

With some exceptions, most of the literature on CEC treats the separation and migration of uncharged eluites. Such eluites migrate through a CEC column due to the flow of the eluent phase only and generally have the same velocity and retention factor as in a corresponding chromatographic system. For uncharged eluites there is therefore no difference in

[1]Affiliated to the Department of Analytical Chemistry, Uppsala University, Uppsala, Sweden.

the basic retention principles between these two techniques. The only difference is that the flow profile in the column can be different so that the band spreading mechanism is different. The migration of an ionic eluite, on the other hand, is influenced by both the electrical field in the column and the adsorption to the stationary phase. This leads to the complex non-linear coupling between the electrophoretic and the chromatographic migration which it is the purpose of this paper to investigate. The effect resembles the effect of the Kohlrausch regulating function in electrophoresis, i.e. the fact that the electrolyte composition in the zone containing the eluite is different from the composition in the background electrolyte. However, in CEC of ionic eluites this effect becomes much more complicated than in electrophoresis. The reason is that the condition of electroneutrality in both the stationary phase and the eluent, in combination with the equilibrium condition for the species between the two phases, must also be fulfilled.

The migration rate of an eluite through a chromatographic column as a function of its adsorption isotherm to the stationary phase and the velocity of the eluent phase is obtained from a mass balance analysis. The analysis forms the basis of the ideal model for chromatography and was first performed by Wilson [14] and later in a more rigorous form by DeVault [15]. From the analysis the migration rate of the eluite as a function of its adsorption isotherm is obtained and it is shown that when the eluite has a non-linear adsorption isotherm the eluting peak will be asymmetric. The ideal model for chromatography has more recently been theoretically and experimentally studied in a series of papers by Golshan–Shirazi and Guiochon for the case of a Langmuir isotherm [16–19]. A detailed discussion of the mass balance analysis in chromatography is found in the classical book by Helfferich and Klein [20].

The migration rate of an eluite zone in electrophoresis is also obtained from a mass balance analysis. From the analysis Svensson [21] formulated a method to calculate the number of boundaries and the ionic composition in the zones which are created in moving boundary electrophoresis. The Kohlrausch regulating function [22] is another fundamental result in electrophoretic theory which is obtained from a mass balance analysis. From this equation the ionic composition of the solution containing the eluite ion can be calculated in terms of the ionic composition of the background electrolyte. It is found that the conductivity is a function of the eluite concentration in the column and that this leads to inhomogeneous field strength within an eluting zone. At high eluite concentrations these inhomogeneities in field strength leads to sharpening and broadening effects at the boundaries between the eluite containing zone and the background electrolyte. This effect is the main cause for the non-Gaussian peak shape encountered in electrophoresis and has been discussed in detail by Poppe [23] and Mikkers et al. [24,25].

In analogy with chromatography and electrophoresis, the migration theory for a ionic eluite in CEC is based on a mass balance analysis [26]. Since electrochromatography is a hybrid between chromatography and electrophoresis the resulting theory for CEC is more general and contains chromatographic and electrophoretic migration theory as limiting cases. The combination of chromatographic and electrophoretic migration theories means that the assumptions made in chromatographic theory (e.g. equilibrium distribution of the eluite between the stationary and eluent phase, electroneutrality in both these phases etc.), is combined with the assumptions in electrophoretic theory (constant current through the column cross-section etc.). The combination of these assumptions and restrictions implies that it is more difficult for the CEC system to fulfil the mass balance equation than it is for the 'simple' systems. Therefore the result of a mass balance analysis in CEC becomes much more complicated and shows many more features than the corresponding 'simple' theories. It has, for example, previously been shown [26] that for a non-linear adsorption isotherm of the analyte a focusing effect may occur which offers an explanation for the extremely sharp peaks that has been reported for ionic analytes in CEC [27,28]. In its general form, the migration theory for an eluite in CEC contains an arbitrary number of eluent phase components, where each of the components may adsorb to the stationary phase. To simplify the analysis, a model system consisting of just three ionic components is discussed here. It is reasonable to assume that the theoretical results for this model system also reflect the behavior of a more complex system.

A mass balance analysis is performed in either differential or finite form. The former form is

required to calculate the peak shape at different locations in the column. For CEC this mathematical approach results in a set of coupled differential equations which are probably not possible to solve analytically. The finite form, on the other hand, can be solved analytically for a three-component system and gives qualitative information about both the migration rate and the peak shape. In this paper the previously [26] derived algebraic equation for a three-component system with finite concentration differences is treated. The purpose is to further investigate and analyse the properties and implications of this equation.

2. Theory

The three-component model system discussed in this paper consists of a sharp boundary located between two different eluent phase compositions and is schematically shown in Fig. 1. In a general three-component system all three ionic species may occur on both sides of the boundary. In this theoretical analysis only two components (denoted 1 and 2) are present on both sides and the third component is present on the left hand side of the boundary only. In analytical applications of CEC this system corresponds to the case where components 1 and 2 are eluent ions and component 3 is the analyte ion. A mass balance analysis for this system has previously

been performed and the resulting equation has been solved [26]. The solution gives the velocity of the moving boundary as a function of the concentration of component 3 on the S side of the boundary. From the velocity the composition of the solution on the S side of the boundary as a function of the concentration of component 3 is calculated.

In the mass balance analysis the system must be carefully specified and some assumptions introduced. In the ensuing discussion the stationary phase is assumed to be a cation exchanger, components 1 and 3 are positively charged and component 2 is negatively charged, all ionic species are assumed to be monovalent. The boundary moves along the column due to a flow of eluent phase [with the velocity v_0 (m/s)] and current [current density I (A/m^2)] through the column. In the system the direction of the electrophoretic movement of component 3 and the direction of the eluent phase flow are therefore the same. The current density and the volumetric flow of the eluent phase are both assumed to be constant and the same through all column cross-sections.

We also assume that components 1 and 2 do not adsorb specifically to the stationary phase. However, since component 1 is counterion to the fixed charges on the stationary phase, electroneutrality in this phase requires that component 1 is nonspecifically adsorbed to this surface. Component 3, on the other hand, may adsorb specifically to the stationary phase and in order to maintain electroneutrality, desorption of component 1 occurs to the same extent as component 3 is adsorbed. The assumptions used in the ideal model of chromatography is also used, i.e. there is an instantaneous equilibrium between the composition in the eluent phase and the stationary phase, respectively. It is also assumed that the current is transported only by the ions in the eluent phase, i.e. there is no surface conduction. Another assumption is that the elecrophoretic mobility of the ionic components is constant and independent of the ionic strength in the eluent phase.

Under these assumptions the solution of the mass balance equation results in the following equation for the velocity v (in m/s) of the boundary (see Appendix A for a brief summary of the derivation of Eq. (1) and Ref. [26] for the derivation of a more general equation):
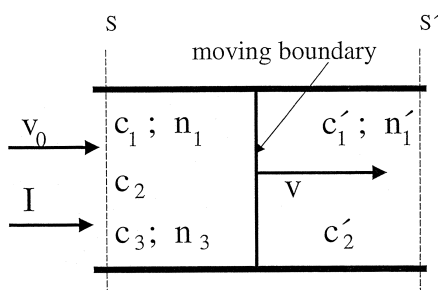

Fig. 1. Schematic description of the three-component electrochromatographic system which is discussed in this paper. The system consists of a moving boundary that separates two different compositions of the eluent phase. Ions 1 and 2 are present on both sides of the boundary but component 3 is present on one side only, c_i and n_i represents the eluent and stationary phase concentration of component i, respectively.

$$v = \frac{\kappa_K v_0 (2c_3 + \phi n_3) + v_0 \beta + Ic_3 u_3 \pm \sqrt{(v_0 \beta - \kappa_K v_0 \phi n_3 + Ic_3 u_3)^2 - 4v_0 \beta Ic_3 u_3 (c_3 + \phi n_3)}}{2(c_3 + \phi n_3)(\kappa_K + \beta)} \tag{1}$$

In Eq. (1) c_3 (mol/m^3) is the concentration of component 3 in the eluent phase and n_3 (mol/m^2) is the corresponding equilibrium concentration on the stationary phase. In the equation F is the Faraday constant (C/mol), ϕ the column phase ratio (1/m) and u_i (m^2/V s) the electrophoretic mobility of component i. κ_K is the conductivity of the eluent phase on the S side of the boundary when there is no adsorption of component 3 to the stationary phase, i.e. in a corresponding pure electrophoretic system:

$$\kappa_K = F \left[c_3 \frac{(u_3 - u_1)(u_3 - u_2)}{u_3} - K_u u_1 u_2 \right] \tag{2}$$

where K_u is the Kohlrausch regulating function:

$$K_u = \frac{c_1'}{u_1} - \frac{c_2'}{u_2} \tag{3}$$

Here c_1' and c_2' (mol/m^3) are the concentrations of components 1 and 2 on the S′ side of the boundary. At this side of the boundary these are the only ionic components and electroneutrality in the eluent phase requires that $c_1' = c_2'$. In analytical applications c_1' is experimentally known since it is the ionic composition of the eluent phase flowing through the column.

The term β in Eq. (1) has its origin in the exchange between component 3 and 1 on the stationary phase when the boundary moves along the column. Its numerical value is equal to the contribution to the eluent phase conductivity from this exchange:

$$\beta = u_2 u_1 F \phi n_3 \cdot \left(\frac{1}{u_3} - \frac{1}{u_1} \right) \tag{4}$$

When the adsorption isotherm of component 3 to the stationary phase surface is known, all the parameters appearing on the right hand side of Eq. (1) are known from the experimental conditions of the system.

In analogy with both electrophoresis and chromatography, the composition on the S side of the boundary is not the same as on the S′ side and a mass balance analysis gives that:

$$c_1 = \frac{c_3 \cdot \dfrac{u_1}{u_3} \cdot (u_3 - u_2) + K_u u_1 u_2 - \dfrac{v}{v - v_0} \cdot \dfrac{\beta}{F}}{u_2 - u_1} \tag{5}$$

and that:

$$c_2 = \frac{u_1 u_2 \cdot \left[K_u - c_3 \left(\dfrac{1}{u_3} - \dfrac{1}{u_1} \right) \right] - \dfrac{v}{v - v_0} \cdot \dfrac{\beta}{F}}{u_2 - u_1} \tag{6}$$

By inserting the velocity calculated from Eq. (1) into these two equations, the ionic composition on the S side of the boundary can be calculated as a function of c_3.

In this paper we will assume that the adsorption isotherm of component 3 is linear. For this case we can set:

$$\phi n_3 = k c_3 \tag{7}$$

where k is the chromatographic retention factor of component 3. The assumption of linearity is probably the most serious assumption made in the ensuing examples. The reason is that in an ion-exchange system the adsorption isotherm of the analyte ion depends on the ionic strength of the eluent phase. This simplification is in principle not needed but is necessary to introduce in order to obtain a closed form algebraic solution to the mass balance equation. The behaviour of a real system will be more complex than the simplified systems which are discussed here. The presented examples can be considered as obtained from an idealised system that has a similar behaviour as a real system.

Eq. (1) in combination with Eq. (7) can after some rearrangement be written so that the terms representing chromatography, electrophoresis and the mixing between these two are separated:

$$v = \frac{v_0(2 + k) + v_0 \cdot \dfrac{\beta}{\kappa_K} + \dfrac{Iu_3}{\kappa_K} \pm \sqrt{\left(v_0 \dfrac{\beta}{\kappa_K} - v_0 k + \dfrac{Iu_3}{\kappa_K} \right)^2 - 4v_0 \dfrac{\beta}{\kappa_K} \cdot \dfrac{Iu_3}{\kappa_K} \cdot (1 + k)}}{2(1 + k) \cdot \left(1 + \dfrac{\beta}{\kappa_K} \right)} \tag{8}$$

Here the term Iu_3/κ_K represents the velocity of component 3 in a pure electrophoretic system and the term β/κ_K represents the change in conductivity, relative to a pure electrophoretic system, caused by the adsorption of component 3.

Eq. (8) is of fundamental importance and it is necessary to investigate its properties more closely. First we note that Eq. (8) has two roots which both satisfy the mass balance equation. It is important to investigate if both roots are physically realistic because this would imply that two different velocities for the boundary are allowed. Secondly, the term under the root sign may become negative so that an imaginary root is obtained. This is not physically realistic and implies that the mass balance equation can not be satisfied by the system, i.e. a constant composition on the S side of the boundary does not exist. Thirdly, the denominator may approach zero implying that the velocity may approach infinity. To rationalise the multitude of possibilities, the ensuing discussion is divided into some limiting cases.

(A) No current through the column, i.e. $I = 0$: this corresponds to a pure chromatographic system and inserting $I = 0$ into Eq. (8) the following two roots are obtained:

$$\text{root 1: } v = \frac{v_0}{1 + k} \tag{9a}$$

$$\text{root 2: } v = \frac{v_0}{1 + \dfrac{\beta}{\kappa_K}} \tag{9b}$$

Root 1 corresponds to the velocity of a chromatographic zone but root 2 has no counterpart in chromatographic theory. From these two equations alone it is, in principle, not possible to judge if both roots are physically meaningful. A necessary physical condition is that the concentration of component 1 in the zone is higher than zero, i.e. $c_1 > 0$. By inserting the two different roots into Eq. (5) we obtain that:

$$\text{root 1: } c_1 = c_1' - c_3 \tag{10a}$$

$$\text{root 2: } c_1 = c_3 \cdot \frac{(u_3 - u_2)}{(u_2 - u_1)} \tag{10b}$$

As expected, root 1 is the same as that obtained according to the chromatographic theory for this particular system. This contrasts to root 2 which is

physically unattainable, the reason being that the numerator is always positive and the denominator always negative resulting in a negative value for c_1.

(B) No adsorption of the analyte ion to the stationary phase, i.e. $k = 0$: when the analyte ion does not adsorb to the stationary phase we have a pure electrophoretic system. When $k = 0$ is inserted into Eq. (8), the following two roots are obtained:

$$\text{root 1: } v = v_0 + \frac{Iu_3}{\kappa_K} \tag{11a}$$

$$\text{root 2: } v = v_0 \tag{11b}$$

The velocity according to root 1 is the same as that obtained from electrophoretic theory and the velocity according to root 2 is equal to the eluent phase velocity through the column. Inserting these two roots into Eq. (5) gives:

$$\text{root 1: } c_1 = c_3 \cdot \frac{u_1(u_3 - u_2)}{u_3(u_2 - u_1)} + c_1' \tag{12}$$

As expected root 1 gives the same c_1 value as that obtained from the Kohlrausch regulating function in electrophoretic theory.

The value for c_1 from root 2 is more complicated because when $v = v_0$ and $k = 0$ (i.e. $\beta = 0$) the third term in the numerator in Eq. (5) is undetermined. The limiting behaviour for $v - v_0$ when $k \to 0$ can be investigated by performing a series expansion of the square root term ($\sqrt{(1 + x)} \approx 1 + 1/2x$) as well as of the terms in the denominator $[1/(1 + x) \approx (1 - x)]$ in Eq. (8). It is easily shown that for small k, the $v - v_0$ term contains only terms which are quadratic and of higher order in k. When this result is inserted into Eq. (5), the concentration of component 1 approaches infinity when $k \to 0$. The physical interpretation of this result is that the conductivity in the zone that contains component 3 approaches infinity, so that the electric field strength in the zone approaches zero. There is therefore no electrophoretic migration of component 3 and it moves under the influence of the eluent phase only.

(C) No flow of eluent phase, i.e. $v_0 = 0$: inserting $v_0 = 0$ into Eq. (8) gives the following two roots:

$$\text{root 1: } v = \frac{Iu_3}{(1 + k)(\kappa_K + \beta)} \tag{13a}$$

root 2: $v = 0$ (13b)

The velocity obtained from root 1 is the velocity of the analyte due to electrophoretic migration retarded by adsorption. The first term in the denominator is the adsorptive retardation and the second term represents the conductivity of the eluent phase.

The corresponding concentrations for component 1 becomes:

$$\text{root 1: } c_1 = \frac{c_3 \cdot \dfrac{u_1}{u_3}(u_3 - u_2) + K_u u_1 u_2 - \dfrac{\beta}{F}}{u_2 - u_1} \quad (14)$$

The corresponding concentration for root 2 is more complicated since 'zero is divided by zero' when both v and v_0 are equal to zero. It can be shown by series expansion of the square root term (see case C) for small v_0 that $v - v_0$ contains only quadratic v_0 terms. This implies that for this root the concentration of component 1 approaches infinity so that the electric field strength in the zone approaches zero, i.e. in analogy with case C, there is no electrophoretic migration of component 3.

(D) The mobility of the analyte and its co-ion are equal, i.e. $u_1 = u_3$: inserting $u_1 = u_3$ into Eq. (8) gives:

$$\text{root 1: } v = \frac{v_0 + \dfrac{Iu_3}{\kappa_K}}{1 + k} \quad (15a)$$

and

root 2: $v = v_0$ (15b)

The velocity given by root 1 is the linear addition of the chromatographic and electrophoretic velocity retarded by adsorption. The reason why the simple adding of velocities applies here is that the conductivity in the eluent phase is the same as in a pure electrophoretic system. The physical explanation for this result is that, since the electrophoretic mobility of component 1 and 3 are equal, the conductivity of the eluent phase is independent of which of the two components is present in the eluent phase.

The concentration of component 1 according to root 1 is:

$$\text{root 1: } c_1 = c_3 \cdot \frac{u_1(u_3 - u_2)}{u_3(u_2 - u_1)} + c_1' \quad (16)$$

Again, the second root is more complicated and is in this case investigated by letting $(1/u_3 - 1/u_1)$, i.e. β, approach zero. By series expansion it can be shown that:

$$\left(\frac{v}{v - v_0} \cdot \frac{\beta}{F} \right)_{\beta \to 0} \to \frac{Iu_3 - v_0 k \kappa_K}{F v_0 k} \quad (17)$$

which, when inserted into Eq. (5), gives that:

$$\text{root 2: } c_1 = \frac{\dfrac{Iu_3}{F v_0 k} - c_3(u_3 - u_2)}{u_1 - u_2} \quad (18)$$

(E) Small mixing of chromatographic and electrophoretic propagation mechanisms: inspection of Eq. (8) shows that the last term under the root sign contains the mixing of chromatographic and electrophoretic parameters. When either the current, I, or the retention factor, k, is small, either a chromatographic or a electrophoretic transport mechanism will dominate. Under these conditions the last term under the root sign is small compared to the squared term. Series expansion of the square root term gives that Eq. (8) can be approximated as:

$$\text{root 1: } v \approx \frac{v_0 + \dfrac{Iu_3}{\kappa_K + \beta}}{1 + k}$$
$$- \frac{v_0 \beta Iu_3}{(\kappa_K + \beta)(v_0 \beta - \kappa_K v_0 k + Iu_3)} \quad (19a)$$

$$\text{root 2: } v \approx \frac{v_0 \cdot \left[1 + \dfrac{\beta Iu_3}{\kappa_K(v_0 \beta - \kappa_K v_0 k + Iu_3)} \right]}{1 + \dfrac{\beta}{\kappa_K}}$$
$$(19b)$$

The first term in Eq. (19a) is the sum of retarded chromatographic and electrophoretic velocities and the second term is the first order mixing term. Eq. (19b) shows that the velocity corresponding to the second root will be close to v_0 at low concentrations of component 3.

3. Results and discussion

The theory section concentrated on a discussion of some special cases where Eq. (8) can be simplified. Most of these are treated because their properties are known from the theories of chromatography and electrophoresis and it is important that Eq. (8) is consistent with these. In the analysis of the general properties of Eq. (8) it is convenient to distinguish between the cases where $u_3 < u_1$ or $u_3 > u_1$. In the first case β is always negative which implies that the term under the root sign in Eq. (8) is always positive. However, a negative β value also implies that β/κ_K has a negative numerical value and may become equal to -1, the denominator in Eq. (8) becomes zero. This would lead to an infinite zone velocity, which of course is physically unrealistic, and the mathematical properties of this particular situation needs to be further investigated. When $u_3 > u_1$ the numerical value of β becomes positive which implies that the term under the root sign in Eq. (8) may become negative and this gives two imaginary roots for the zone velocity. The physical meaning of an imaginary root is that no solution to the mass balance equation exists so that the coherence condition for a migrating zone can not be fulfilled. These unusual properties of Eq. (8) are illustrated and further discussed in the ensuing three examples.

In the first example $u_3 < u_1$ and the numerical value for the rest of the parameters in Eq. (8) are chosen so that they are of the same order of magnitude as in experimental practice. The velocity of the boundary as a function of the concentration of component 3 according to the two existing roots is shown in Fig. 2a. The figure shows that at low c_3 values one of the roots (root 1) gives the velocity $2.67 \cdot 10^{-3}$ (m/s) and that the other root (root 2) approaches the velocity v_0 ($=5 \cdot 10^{-3}$ m/s) The value for root 1 is close to the velocity in corresponding pure chromatographic system [$2.5 \cdot 10^{-3}$ (m/s)] and shows that, with this particular set of values for the different parameters, the contribution from the electric field to the migration is very small. It is also interesting to note that the velocity is more or less independent of the concentration of component 3 in the zone. The reason for this independence can be understood by examining the concentration of component 1 in the zone as a function
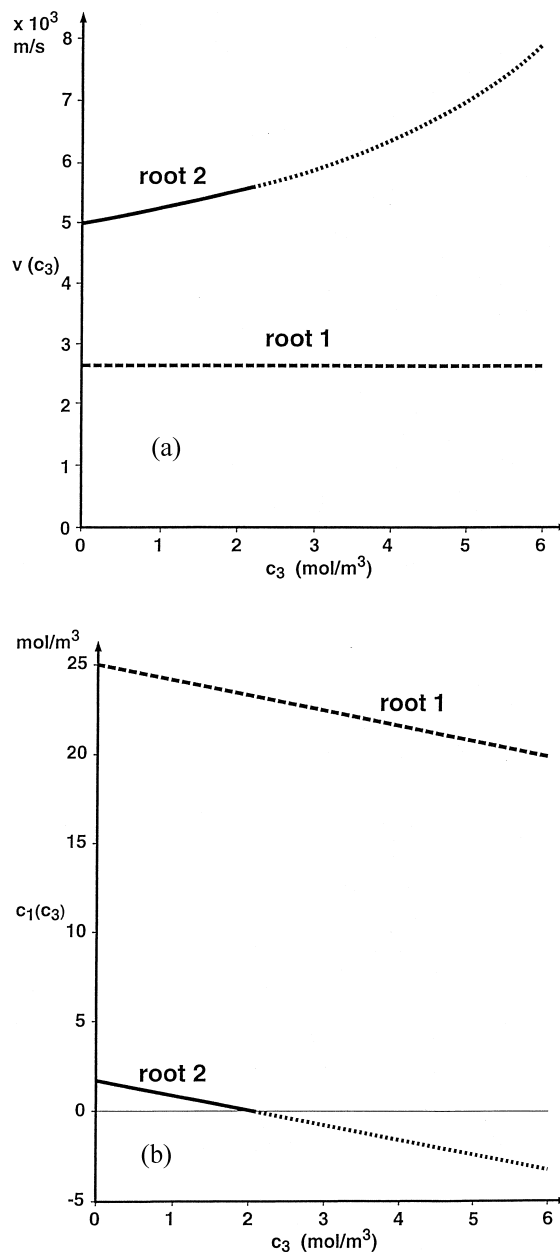

Fig. 2. (a) Velocity of the moving boundary as a function of the concentration of component 3 according to the two roots of Eq. (8). (b) Concentration of component 1 in the zone which satisfy the coherence condition, as a function of the concentration of component 3. In both figures all other parameters in Eq. (8) are held constant with the following values: $k = 1$, $v_0 = 5 \cdot 10^{-3}$ (m/s), $u_1 = 6 \cdot 10^{-8}$ (m^2/V s), $u_2 = -6 \cdot 10^{-8}$ (m^2/V s), $u_3 = 2 \cdot 10^{-8}$ (m^2/V s), $c_1' = c_2' = 25$ (mol/m^3) and $I = 5000$ (C/m^2 s).

of c_3, Fig. 2b. The figure shows that when the concentration of component 3 increases, the concentration of component 1 in the zone changes in such a way that $(c_1 + c_3)$ is approximately constant. The net result is that, in order to fulfil the coherence condition, the conductivity and the created electric field strength in the eluent phase are almost independent of c_3.

The properties of root 2 are more complicated. Despite the relatively high chromatographic retardation the zone velocity is higher than the eluent phase velocity when $c_3 > 0$. The reason is that the coherence condition gives very low concentrations of components 1 and 2 in the zone. This results in a very low conductivity and a corresponding high field strength in the zone. The relatively high zone velocity is therefore caused by a high electrophoretic velocity of component 3. The figure also shows that when the concentration of component 3 increases above 2 mol/m^3, the velocity according to root 2 becomes physically unrealistic because the concentration of component 1 is negative. Thus, when the concentration of component 3 is < 2 mol/m^3, the mass balance analysis alone cannot discriminate between these two roots. New criteria need to be found to determine which of the two roots a particular system obeys. Such criteria have no counterpart in either chromatographic or electrophoretic theories and therefore remains to be formulated. It seems reasonable to believe that the starting conditions determine which of the two roots a particular system follows. Root 1 is followed when the concentration of component 1 in the starting solution is close to its value in the eluent phase, i.e. in this case 25 mol/m^3. When the concentration of component 1 is very low in the starting solution it is reasonable to assume that it follows root 2. However, more experimental and theoretical work are needed to solve this interesting problem.

In the example shown in Fig. 3 the numerical value of the parameters are the same as in the previous example, except that the chromatographic retention factor of the analyte increases from unity to ten. The value for β therefore increases by a factor of ten and when $c_3 \approx 2.2$ mol/m^3 its value is equal to $-\kappa_K$. At this point the denominator in Eq. (8) becomes zero. The zone velocity for both roots as a function of c_3 is shown in Fig. 3a where it is seen
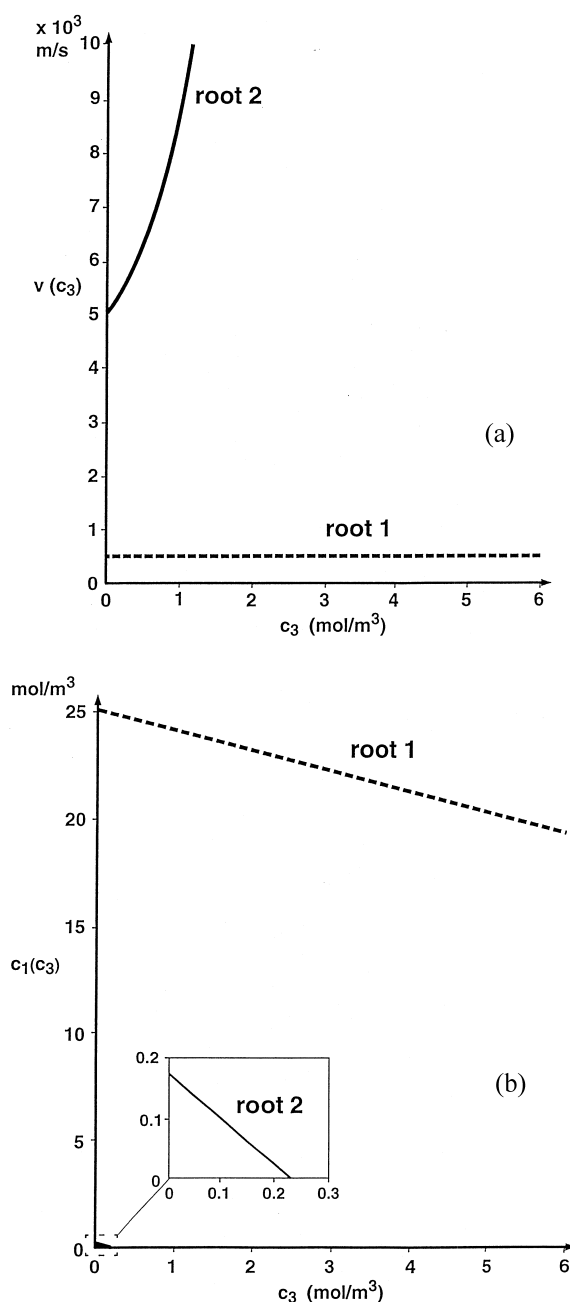

Fig. 3. (a) Velocity of the moving boundary as a function of the concentration of component 3 according to the two roots of Eq. (8). (b) Concentration of component 1 in the zone which satisfy the coherence condition, as a function of the concentration of component 3. In both figures all other parameters in Eq. (8) are held constant with the following values: $k = 10$, $v_0 = 5 \cdot 10^{-3}$ (m/s), $u_1 = 6 \cdot 10^{-8}$ (m^2/V s), $u_2 = -6 \cdot 10^{-8}$ (m^2/V s), $u_3 = 2 \cdot 10^{-8}$ (m^2/V s), $c_1' = c_2' = 25$ (mol/m^3) and $I = 5000$ (C/m^2 s).

that the velocity according to root 2 approaches infinity at the point where $\beta = -\kappa_K$. The corresponding concentration of component 1 as a function of c_3 is shown in Fig. 3b and shows that for this root the value for c_1 is negative at very low concentrations of component 3. When $\beta = -\kappa_K$ the conductivity in the zone is zero, which corresponds to the hypothetical case of negative concentrations of component 1 and 2 which, of course, is a physically unattainable state.

Fig. 3a also shows that even when the denominator in Eq. (8) passes through zero the velocity for root 1 is continuous in the studied concentration interval for component 3. The reason is that for this root the numerator also becomes zero when $\kappa_K = -\beta$ so that the ratio between the two has a finite value at this point. As seen in Fig. 3b the concentration of component 1 is positive in the whole c_3 interval which means that this root is physically realistic. This system therefore has root 1 as the only physically attainable root, except possibly for very low c_3 values, and will therefore migrate with the velocity given by this root.

When $u_3 > u_1$ the denominator in Eq. (8) has positive values for all concentrations of component 3 and its numerical value will increase with increasing c_3. However, the last term under the root sign in the numerator, representing a mixing between electrophoretic and chromatographic migration, is negative for all c_3 values and the whole term under the root sign may become negative. This case is illustrated in Fig. 4a. Compared to the example shown in Fig. 2, the numerical value of the mobility for component 3 have been interchanged with the mobility of the ions in the eluent phase. The value of the other parameters are the same in the two examples. A comparison of the velocity given by root 1 with that in Fig. 2a shows that its value has increased. There are two reasons for that: firstly, the mobility of components 1 and 2 has decreased and, since the current is the same in the two examples, there is a higher field strength in the present case. Secondly, the electrophoretic mobility of component 3 has increased which results in a higher electrophoretic migration velocity.

The concentration of component 1 in the zone as a function of c_3, which corresponds to the velocity according to root 1 Fig. 4a, is shown in Fig. 4b. The
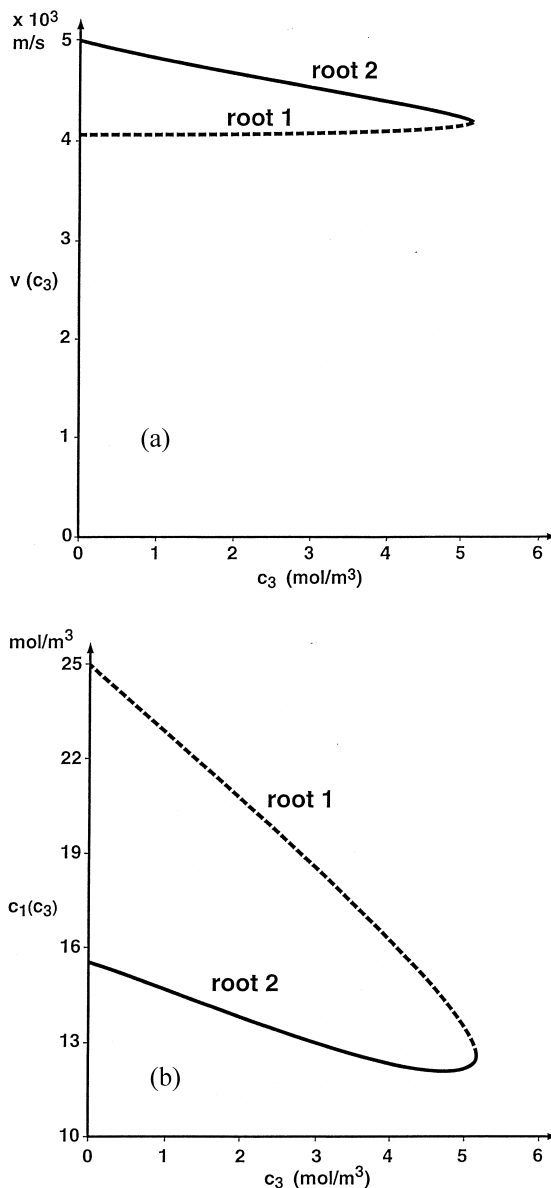

Fig. 4. (a) Velocity of the moving boundary as a function of the concentration of component 3 according to the two roots of Eq. (8). (b) Concentration of component 1 in the zone which satisfy the coherence condition, as a function of the concentration of component 3. In both figures all other parameters in Eq. (8) are held constant with the following values: $k = 1$, $v_0 = 5 \cdot 10^{-3}$ (m/s), $u_1 = 2 \cdot 10^{-8}$ (m^2/V s), $u_2 = -2 \cdot 10^{-8}$ (m^2/V s), $u_3 = 6 \cdot 10^{-8}$ (m^2/V s), $c_1' = c_2' = 25$ (mol/m^3) and $I = 5000$ (C/m^2 s).

figure shows that its value approaches the ionic composition of the eluent phase at low concentrations of component 3. As the c_3 value increases the concentration of both component 1 and 2 decrease and is lower than in the eluent phase. The conductivity in the zone therefore decreases slightly when the concentration of component 3 increases and this explains the slight increase in zone velocity with increasing c_3.

The second root in Fig. 4a approaches the value v_0 at low c_3 values and, in contrast to the behaviour of root 1, its value decreases with increasing c_3. The higher velocity for root 2 is achieved by a lower concentration of both component 1 and 2 for low c_3 values. This gives a lower conductivity in the zone and a corresponding higher field strength. As the concentration of component 3 increases the concentration of both component 1 and 2 increases. This results in an increase in the zone conductivity and decreased field strength. This explains the decrease in velocity of the zone with increasing concentration of component 3.

Fig. 4a also illustrates that at a certain c_3 value the sum of terms under the root sign becomes zero and at this point the numerical value of root 1 and 2 are the same. Fig. 4b shows, as expected, that at this point both roots gives the same ionic composition in the zone. For higher c_3 values the term under the root sign in Eq. (8) is negative and the solution is imaginary. The existence of an imaginary root is interesting because it implies that there exists no solution to the mass balance equation for certain c_3 values. The physical interpretation of this state is that the coherence condition cannot be satisfied so that no stable composition in the zone can develop. If this state arises in an electrochromatographic system the peak broadens strongly and the peak shape becomes very unsymmetrical. For high enough c_3 values the term under the root sign becomes positive again. There is therefore an interval of c_3 values for which the root is imaginary (not shown in the figure) and the width of the interval varies from case to case.

For c_3 values lower than 5 mol/m^3, Fig. 4b shows that both existing roots to Eq. (8) give a positive value for the concentration of component 1 in the zone. This implies that both roots satisfy the coherence condition in the zone and it is therefore not possible to reject any of the roots from the mass balance analysis alone.

4. Conclusions

The velocity of a zone through a chromatographic or an electrophoretic column is obtained by solving the mass balance equation for the system. For an electrochromatographic system containing three ionic components, a mass balance analysis gives that the velocity of a composition boundary is expressed by Eq. (8). This equation is analysed in this paper with respect to its general properties and its properties at certain limits. The conclusion from the performed analysis is that in the limit of no current or no adsorption, Eq. (8) is consistent with the existing theories for chromatography and electrophoresis, respectively. However, when chromatographic and electrophoretic migrations are mixed the equation has complex properties. The reasons for the complexity are

1. The solution has two roots, and under some conditions both of the roots are physically attainable. This implies that a zone (or a peak) consisting of one single analyte ion may migrate through the column with two different velocities which both satisfy the coherence condition.
2. When the mobility of the analyte ion (component 3 in this paper) is smaller than the mobility of its co-ion (component 1) the denominator of Eq. (8) may approach zero. At the zero point only one of the solutions of Eq. (8) is physically attainable
3. When the mobility of the analyte ion is higher than the mobility of its co-ion the numerical value of the term under the root sign in Eq. (8) may be negative. Under this circumstance the coherence condition can not be satisfied by the system and no stable zone or peak exists. This leads to an extraordinary peak broadening and asymmetry.

From the analysis of the mass balance equation, two general rules emerge in order to avoid the complex conditions that usually are detrimental in an analytical application:

1. The value for β in Eq. (8) shall be chosen so that it takes a small value. In an ion-exchange system this is achieved by choosing the mobility of the co-ion (component 1 in this paper) to be as close as possible to the mobility of the analyte ion.
2. The last term under the root sign in Eq. (8) shall have a relatively small value. This means that too much mixing of chromatographic and electrophoretic migration shall be avoided. However,

this does not mean that it is impossible to successfully run a CEC system with mixed migration, only that it is rather difficult to find efficient and stable experimental conditions.

5. Nomenclature

A_s	specific surface area of the stationary phase (m^2/kg)
A_c	column cross section area (m^2)
c_i	mobile phase concentration of component i on the S side of the boundary (mol/m^3)
c_i'	mobile phase concentration of component i on the S′ side of the boundary (mol/m^3)
F	Faraday constant (C/mol)
i	(in the presented examples) $1 =$ positively moncharged ion; $2 =$ negatively moncharged ion; $3 =$ positively moncharged ion, the eluite ion
I	current density through the column (C/m^2 s).
I_0	current through the column (A)
k	chromatographic retention factor of component 3
K_u	analogue of the Kohlrausch regulating function, defined in Eq. (12), (mol s V/m^5)
n_i	surface concentration of component i on the stationary phase surface (mol/m^2)
T_i	transference number of component i (dimensionless)
t	time (s)
u_i	electrophoretic mobility of component i in the mobile phase $(m^2/V\ s)$
x	length coordinate along the column axis (m)
z_i	charge number of component i (dimensionless)
b	defined by Eq. (4)
ϵ	total porosity of the packed column (dimensionless)
ϕ	column phase ratio (1/m)
κ	conductivity of the mobile phase on the S side of the moving boundary $(S\ m^{-1})$
κ'	conductivity of the mobile phase on the S′ side of the moving boundary $(S\ m^{-1})$
κ_K	conductivity of the mobile phase in a corresponding electrophoretic system $(S\ m^{-1})$
ρ	density of the stationary phase material (kg/m^3)

Appendix A

Consider two fixed cross-sections of the column, S and S′ in Fig. 1, between these there is a distinct boundary dividing two different compositions of the eluent phase. The boundary moves along the column with the linear velocity v (m/s) due to a flow, v_v (m^3/s) of the eluent phase and to a current I_0 (A) passing through the column. The eluent phase consists of three different components; each of them may adsorb to the stationary phase. The amount of component i that is accumulated between the two cross-sections during the time interval δt and due to the propagation of the eluent phase and the transport of current is:

$$\text{Amount accumulated} = c_i v_v \delta t + \frac{T_i I_0}{z_i F} \delta t - c_i' v_v \delta t$$
$$- \frac{T_i' I_0}{z_i F} \delta t \quad (A1)$$

where c_i and c_i' are the concentrations (mol/m^3) of component i in the eluent phase and T_i and T_i' are the transference numbers in the two different eluent phase compositions, respectively. F is the Faraday constant (C/mol) and z_i the charge of the component. Since the composition on each side of the boundary is constant, the amount accumulated equals the change in amount caused by the change δx in position of the boundary:

$$\text{Amount accumulated} = [(n_i - n_i')A_s$$
$$+ (c_i - c_i)V_0]A_c(1 - \epsilon)\rho\delta x \quad (A2)$$

where n_i and n_i' are the surface concentrations (mol/m^2) of component i on each side of the moving boundary, V_0 is the volume of the mobile phase per weight unit of the stationary phase (kg/m^3) and A_s is the specific surface area of the stationary phase

(m²/kg). A_c is the area of the cross-section of the column, ϵ is the porosity of the packed column and ρ is the density (kg/m³) of the stationary phase material. Since Eqs. (A1) and (A2) express the same accumulation they are identical and recognising that $\delta x / \delta t$ is equal to linear the velocity, v (m/s) of the boundary we obtain that:

$$\frac{\delta x}{\delta t} = v$$

$$= \frac{\left[(c_i - c_i')v_v + \frac{(T_i - T_i')I_0}{z_i F} \right]}{[(n_i - n_i')A_s + (c_i - c_i')V_0]A_c(1 - \epsilon)\rho} \quad (A3)$$

We now introduce the following definitions and identities:

$$\phi = \frac{A_s}{V_0} \qquad \phi \text{ is the column phase ratio (1/m)}$$

$$\rho V_0 = \frac{\epsilon}{1 - \epsilon} \quad \text{(dimensionless)}$$

$$v_0 = \frac{v_v}{A_c \epsilon} \qquad \begin{array}{l} v_0 \text{ is the linear velocity of an uncharged} \\ \text{non-retained marker molecule (m/s)} \end{array}$$

$$I = \frac{I_0}{A_c \epsilon} \qquad \text{(C/m²s)}$$

Eq. (A3) can now be written as:

$$v = \frac{v_0(c_i - c_i') + \frac{I}{Fz_i}(T_i - T_i')}{\phi(n_i - n_i') + (c_i - c_i')} \quad (A4)$$

The transference number of a component is equal to:

$$T_i = \frac{Fz_i c_i u_i}{\kappa} \quad (A5)$$

where κ is the conductivity of the mobile phase, defined as:

$$\kappa = F \sum_{i=1}^{n} c_i z_i u_i \quad (A6)$$

The definition of the transference number is introduced into Eq. (A4) to give:

$$v = \frac{v_0(c_i - c_i') + \frac{I}{Fz_i}\left(\frac{Fz_i c_i u_i}{\kappa} - \frac{Fz_i c_i' u_i'}{\kappa'} \right)}{(c_i - c_i') + \phi(n_i - n_i')} \quad (A7)$$

where v is the velocity (m/s) of the boundary

through the column. When the boundary is stable, i.e. the coherence condition is fulfilled, Eq. (A7) applies simultaneously for all the components present in the system. For the three component system discussed in this paper there are therefore three equations of the form A7 and, in principle, this set of equations can be used to solve for v as a function of c_3. However, it is possible to simplify the algebra by using the electroneutrality condition in the eluent phase and to rewrite Eq. (A7), see Ref. [26] for details.

References

[1] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[2] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 23.
[3] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[4] J.H. Knox, I. Grant, Chromatographia 24 (1987) 135.
[5] J.H. Knox, I. Grant, Chromatographia 32 (1991) 317.
[6] J.H. Knox, Chromatographia 26 (1988) 329.
[7] Q. Wan, J. Phys. Chem. 101 (1997) 4860.
[8] Q. Wan, J. Phys. Chem. 101 (1997) 8449.
[9] Q. Wan, J. Chromatogr. A 782 (1997) 181.
[10] A.S. Rathore, Cs. Horváth, Anal. Chem. 70 (1998) 3069.
[11] A.S. Rathore, Cs. Hovárth, Anal. Chem. 70 (1998) 3271.
[12] Th. Adam, S. Lüdtke, K.K. Unger, Chromatographia 49 (1999) S49.
[13] A.S. Rathore, Cs. Horváth, J. Chromatogr. A 781 (1997) 185.
[14] N.J. Wilson, J. Am. Chem. Soc. 62 (1940) 1583.
[15] D.J. deVault, J. Am. Chem. Soc. 65 (1943) 532.
[16] S. Golshan-Shirazi, G. Guiochon, Anal. Chem. 60 (1988) 2364.
[17] S. Golshan-Shirazi, G. Guiochon, J. Phys. Chem. 94 (1990) 495.
[18] S. Golshan-Shirazi, G. Guiochon, Anal. Chem. 61 (1989) 462.
[19] S. Golshan-Shirazi, G. Guiochon, J. Phys. Chem. 93 (1989) 4143.
[20] F. Helfferich, G. Klein, Multicomponent Chromatography — A Theory of Interference, Marcel Dekker, New York, 1970.
[21] H. Svensson, Ark. Kemi Mineral. Geol. 17A (1943) 1.
[22] F. Kohlrausch, Ann. Phys. 62 (1897) 209.
[23] H. Poppe, Anal. Chem. 64 (1992) 1908.
[24] F.E.P. Mikkers, F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 1.
[25] F.E.P. Mikkers, F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[26] J. Ståhlberg, Anal. Chem. 69 (1997) 3812.
[27] N.W. Smith, M.B. Evans, Chromatographia 38 (1994) 649.
[28] N.W. Smith, M.B. Evans, Chromatographia 41 (1995) 197.