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Steady-state models in electrophoresis: From isotachopheresis to capillary zone electrophoresis

Although all electrophoretic techniques are closely allied and controlled by the same rules, we often distinguish between steady-state and dynamic models in the modeling of electrophoretic processes, whereby steady-state models are applied for isotachopheresis (ITP) and dynamic models are applied for other electrophoretic processes, wherein a steady-state is not reached. This paper shows how, starting from a mathematical model for the steady-state in ITP, mathematical models can be derived for several modifications of ITP and that even nonsteady-state processes can be estimated by a repeated application of a steady-state model. In this way all parameters in sample zones in capillary zone electrophoresis (CZE), through which temporal electropherograms can be simulated, can be calculated. Realistic simulations can be obtained for zone electrophoretic processes, for which the electrodispersive character is the dominating peak broadening mechanism, and simulated electropherograms resemble measured electropherograms concerning migration times, peak shapes, fronting or tailing character and the question of peaks and dips.

1 Introduction

In electrophoresis often four main techniques [1–5] are distinguished, viz., (i) moving boundary electrophoresis (MBE), (ii) isotachopheresis (ITP), (iii) zone electrophoresis (ZE) and (iv) isoelectric focusing (IEF), besides several hybrid techniques such as micellar electrokinetic capillary chromatography (MECC), where the electrophoretic and chromatographic character are combined. In a single electrophoretic run more than one electrophoretic mode can occur and generally the first step in an electrophoretic separation process is a moving boundary procedure followed by an ITP or a ZE process. The affinity between all main techniques in electrophoresis is apparent from the fact that all electrophoretic equipment can be applied for all electrophoretic techniques, whereby only the choice of the electrolytes in the separation and electrode compartments determines which electrophoretic mode is actually applied. Nevertheless, in modeling, we often distinguish between steady-state and dynamic models. For the calculation of parameters in ITP usually a steady-state mathematical model is applied, based on modified Ohm's law, the electroneutrality equation, the ITP condition and the mass balance of the counter ions. For simulation of the CZE process the basic transport equations are generally solved. Yet, non-steady-state CZE processes can also be estimated by a repeated application of a steady-state model. In that case, sample peaks are divided into small segments with varying sample component concentrations and all parameters of a segment can be calculated from the parameters of the preceding segment with the steady-state model.

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Nonstandard abbreviations: BGE, background electrolyte; CZE, capillary zone electrophoresis; EN, electroneutrality; IC, isotachopheretic condition; MBE, moving boundary electrophoresis; ZE, zone electrophoresis

Keywords: Steady-state models / Isotachopheresis / Capillary zone electrophoresis

In this paper we discuss a mathematical model for the steady state in ITP and we will show how the model can be modified for the application of hydrogen ions as terminator, and for the use of two leading and two counter ions in ITP. Further, this model will be applied for the estimation of sample peaks in CZE. With this model, electropherograms can be simulated and the simulated electropherograms are compared with measured electropherograms.

For all experiments the P/ACE System 5000 HPCE instrument (Beckman, Fullerton, CA, USA) was used. All experiments were carried out applying Beckman eCap capillary tubing (75 μm ID) with a total length of 46.7 cm and a distance between injection and detection of 40.0 cm. The wavelength of the UV detector was set at 214 nm and the operating temperature was 25°C. All CZE experiments were carried out applying a constant voltage of 10 kV, whereas all ITP experiments were carried out applying a constant current of 5 μA , unless otherwise given. Sample injection was performed by applying pressure injection where a 1 s pressure injection equals an injected amount of ca. 6 nL and an injected length of 0.136 cm. Data analysis was performed using the laboratory-written data analysis program CAESAR.

2 Notations, symbols and units

Symbols and units

A	coionic species	
C	counterionic species	
c	total concentration of a substance	(mol/m ³)
E	electric field strength	(V/m)
F	Faraday constant	(C/equiv)
K	equilibrium constant	
L	leading ionic species	
m	effective mobility	(m ² /Vs)
m ^c	ionic mobility at finite dilution	(m ² /Vs)
m ^o	ionic mobility at infinite dilution	(m ² /Vs)
n	number of pK values	

SZR_{25}	specific zone resistance at 25°C	(Ωm)
T	terminating ionic species	
v	velocity of a zone boundary	(m/s)
z	charge of an ionic species	(equiv/mol)
α	degree of dissociation	
σ	zone conductivity	($\Omega^{-1} m^{-1}$)

First subscripts

A, C, T, and L	according to substance A, C, T and L
t	total
z-i	refers to the charge of ionic species

Second subscripts

A, C, T and L	in the zone of substance A, C, T and L
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Superscripts

z	maximum charge of an ionic species
$(..)^i$	to the i^{th} power

Example

$m_{C,A}$	effective mobility of ionic species C in zone A
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3 Basic concepts in electrophoresis

In all mathematical models for electrophoretic processes, the same basic concepts are applied, viz. the effective mobility, the principle of electroneutrality, the modified Ohm's law and mass balances. In this paragraph these concepts are considered for polyvalent ampholytes.

3.1 The effective mobility of a substance

In electrophoresis, ionic constituents can be separated under the influence of an applied electric field according to differences in the effective mobilities. Hereby the mobility is defined as the velocity per unit electric field strength. Mobilities are signed values, positive for cationic and negative for anionic species. The ionic mobility at finite dilution, m^c , can be calculated from the ionic mobility at infinite dilution, m^o , by correcting for the relaxation effect and the electrophoretic retardation [6, 7]. Tiselius [8] pointed out that a substance that consists of several forms with different mobilities in equilibrium with each other will generally migrate as a uniform substance with an effective mobility given by

$$m = \sum_i \alpha_i m_i^c = \sum_{i=0}^n \frac{[A^{z-i}]}{[A]_t} m_{z-i}^c \quad (1)$$

For an amphiprotic polyvalent molecule with the highest charge of z , A^z , with n pK values (only proton interactions are taken into account), the following equilibria can be set up:

The general expression for the i^{th} concentration equilibrium equation will be

$$K_i = \frac{[A^{z-i}][H_3O^+]}{[A^{z-i+1}]} \text{ or } [A^{z-i}] = [A^{z-i+1}] \frac{K_i}{[H_3O^+]} \quad (6)$$

The concentration equilibrium constants can be calculated from the thermodynamic constants correcting for the activities. Applying the expressions for equilibria, all ionic concentrations can be expressed as the concentration of A^z :

$$[A^{z-i}] = [A^z] \frac{\prod_{j=1}^i K_j}{[H_3O^+]^i} \quad (7)$$

The total concentration of a substance A with n pK values is:

$$[A]_t = \sum_{i=0}^n [A^{z-i}] = [A^z] + [A^z] \frac{K_1}{[H_3O^+]} + [A^z] \frac{K_1 K_2}{[H_3O^+]^2} + \dots$$

$$= [A^z] \left(1 + \sum_{i=1}^n \frac{\prod_{j=1}^i K_j}{[H_3O^+]^i} \right) \quad (8)$$

For a substance with n pK values there exist $n+1$ ionic forms and their concentrations can be calculated from the total concentration of that substance by applying

$$[A^{z-i}] = [A]_t \frac{\frac{\prod_{j=1}^i K_j}{[H_3O^+]^i}}{1 + \sum_{i=1}^n \frac{\prod_{j=1}^i K_j}{[H_3O^+]^i}} \quad (9)$$

For the effective mobility of substance A, Eq. (1) can be transformed to

$$m_A = \frac{m_z^c + \sum_{i=1}^n m_{z-i}^c \frac{\prod_{j=1}^i K_j}{[H_3O^+]^i}}{1 + \sum_{i=1}^n \frac{\prod_{j=1}^i K_j}{[H_3O^+]^i}} \quad (10)$$

If for a substance all pK values and ionic mobilities are known, the total composition and effective mobility of that substance can be calculated for a given pH if the total concentration of that substance is known. The reduced number of parameters [1] for that substance is one. All formula are identical for both cationic and anionic species.

3.2 The principle of electroneutrality

According to the principle of electroneutrality (EN), the arithmetic sum of all products of the concentrations of all

forms for all ionic species and the corresponding valences must be zero in all zones and can be written as:

$$[H_3O^+] - [OH^-] + \sum_{i=0}^{n_A} (z-i) [A^{z-i}] + \sum_{i=0}^{n_C} (z-i) [C^{z-i}] = 0 \quad (11)$$

For convenience, only one coionic species A and counter-ionic species C are assumed. If more ionic species are present they must be taken into account in the EN and in Ohm's law.

3.3 The modified Ohm's law

Working at a constant current density the product of $E\sigma$ is constant in all zones or the function:

$$RFQ = \frac{E_1\sigma_1}{E_2\sigma_2} - 1 \quad (12)$$

must be zero. The overall electrical conductivity, σ , of a zone is the sum of the values $c|z|F$ for all ionic species and consequently:

$$E \left\{ |m_{H^+}| [H_3O^+] + |m_{OH^-}| [OH^-] + \sum_{i=0}^{n_A} |m_{z-i}| (z-i) [A^{z-i}] + \sum_{i=0}^{n_C} |m_{z-i}| (z-i) [C^{z-i}] \right\} \quad (13)$$

must be constant in all zones.

3.4 Mass balances in electrophoresis

For ionic species passing a zone boundary, two cases can be distinguished, viz., the ionic species migrates against the direction of the zone boundary (see Fig. 1A) or it migrates in the same direction as the zone boundary (see Fig. 1B). In Fig. 1A the cationic zone boundary between electrolyte 1 and 2 moves in a unit of time from point C to B, over a distance v . The counterions C at time $t = 0$, present at point A in electrolyte 1, will just reach the zone boundary at $t = 1$. The distance between point A and B is $-E_1m_{C,1}$. Because the mobility of the counterions C is negatively signed, a positive value for the displacement is obtained by the negative sign in this expression. The counterions C present at point C at $t = 0$ will reach point D at $t = 1$ and will move over a distance $-E_2m_{C,2}$. All counterions C present between point A and C (amount Q_1) in electrolyte 1 with a total concentration of $c_{C,1}$ will be present between point B and D (amount Q_2) in electrolyte 2 at $t = 1$ with a concentration of $c_{C,2}$. The mass balance will therefore be:

$$c_{C,1} (v - E_1m_{C,1}) = c_{C,2} (v - E_2m_{C,2}) \quad (14)$$

In Fig. 1B the situation is given for coions A migrating in the same direction as the zone boundary between electrolyte 1 and 2, moving from point C to B over a distance v in unit time. Ions A present at point D at time $t = 0$ just reach the zone boundary at point B at time $t = 1$. The distance between point D and B is $E_2m_{A,2}$. The ions A present at point C at time $t = 0$ will reach point A at time $t = 1$ and will migrate over a distance $E_1m_{A,1}$. The ions A present between point C and D (Q_2) in electrolyte 2 with a concentration of $c_{A,2}$ will be present between

Figure 1. Migration paths of (A) the counterionic species C and (B) coionic species A over a moving zone boundary between electrolyte 1 and 2. For further explanation see Section 3.4.

point A and B (Q_1) in electrolyte 1 with a concentration $c_{A,1}$. The mass balance will be:

$$c_{A,1} (E_1m_{A,1} - v) = c_{A,2} (E_2m_{A,2} - v) \quad (15)$$

The mathematical expressions for anionic (Eq. 14) and cationic species (Eq. 15) are identical and the same equations are obtained if the mobilities of the coions and counterions are lower or higher than that of the moving boundary. In the derivation the mobility of the EOF is neglected; identical expressions are obtained, however, in the presence of an EOF because the effect of the EOF on both sides in the equations is canceled.

4 Mathematical models for steady-state processes

With the basic concepts already described, all mathematical models for steady states in electrophoresis can be described. The first step in all models is to define the first electrolyte and to calculate all parameters in this electrolyte. For example, in ITP this will be the leading electrolyte. For all other zones, the reduced number of parameters can be counted. In this reduced number, those parameters are not taken into account that can be calculated from another parameter by a specific relationship, such as ionic concentrations, which can be calculated from the total concentration and pK values of a substance, and the pOH, which can be calculated from the pH value. The reduced number of parameters determines how many known parameters and/or equations

are necessary in order to calculate all unknown parameters. Generally the solution can be found in an iterative way. For the ITP model and modifications of the ITP model we only describe the reduced number of parameters and the necessary parameters and/or equations in the calculations. For the calculation procedure the reader is referred to the literature.

4.1 ITP

In a single run in ITP, only cations or anions can be separated. In the classical ITP mode, sample ionic species are introduced between leading and terminating ions, with mobilities higher and lower, respectively, than those of any of the sample ionic species. If the steady state is reached and the ionic species of the sample are separated, all sample zones migrate with equal speed in the order of decreasing effective mobilities. The reduced number of parameters is four for all ITP zones [1], viz., the electric field strength E , the pH and the total concentrations of the coions and counterions in the zone. For all zones, four known parameters and/or equations are necessary in order to calculate all parameters. For the leading zone the known parameters are, e.g., the total concentrations of the leading ions L and the counterions C and the equations are Ohm's law and the EN. For all other zones, the four available equations are the EN (Eq. 11), Ohm's law (Eq. 12), the mass balance of the counterions C and the isotachophoretic condition (IC). The mass balance of the counterions C can be deduced from Eq. (14) by substituting for the velocity of the zone boundary, $E_L m_{L,L}$:

$$c_{C,L} (E_L m_{L,L} - E_L m_{C,L}) = c_{C,A} (E_L m_{L,L} - E_A m_{C,A}) \quad (16)$$

and after applying the IC (see Eq. 18):

$$c_{C,L} \left(\frac{-m_{C,L}}{m_{L,L}} + 1 \right) = c_{C,A} \left(\frac{-m_{C,A}}{m_{A,A}} + 1 \right) \quad (17)$$

The IC can be derived from the condition that all zones move with the speed of the leading zone L and therefore

$$E_L m_{L,L} = E_A m_{A,A} \quad (18)$$

for the leading zone and a zone with sample ions A , respectively. The equations can be solved in an iterative way [9]. The features of ITP separations are the self-correcting property of zone boundaries and the adaptation of the concentration of the sample components to that of the leading ions, through which diluted sample ions are concentrated in very narrow bands. For that reason ITP is often applied as concentrating pretreatment in CZE analysis. For cationic separations the pH generally decreases for lower mobile cations, whereas the pH increases in anionic separations. As an example, in Fig. 2A the relationships between the calculated concentrations (dashed lines, right-hand scale) and specific zone resistance at 25°C (solid lines, left-hand scale), respectively, and the absolute value of the mobility of fully ionized anions (solid symbols) and cations (open symbols) are given for an anionic leading electrolyte consisting of 0.01 M HCl adjusted to pH 6 by adding histidine, and a cationic leading electrolyte consisting of 0.01 M HCl adjusted to pH 6 by adding histidine.

Table 1. Ionic mobilities at infinite dilution, m (m^2/Vs), and pK values for ionic species used in the calculations and experiments

Ionic species	$m \times 10^9$	pK
Acetic acid	-42.4	4.76
Benzoic acid	-33.6	4.203
Butyric acid	-33.8	4.82
Caproic acid	-30.2	4.857
Creatinine	37.2	4.828
Formic acid	-56.6	3.75
Histidine	29.7	6.03
Hydrochloric acid	-79.1	-2.0
Imidazole	50.4	6.953
Lithium	40.1	-
MES	-28.0	6.095
Nitric acid	-74.10	-1.0
Potassium	76.2	-
Sodium	51.9	-
Propionic acid	-37.1	4.874
Tris	29.5	8.1

Figure 2. Relationships between the calculated (A) concentrations (dashed lines, right-hand scale) and specific zone resistance at 25°C (solid lines, left-hand scale) and (B) zone pH, respectively, and the absolute values of the mobility of fully ionized sample anions (solid symbols) and sample cations (open symbols) for an anionic leading electrolyte consisting of 0.01 M HCl, adjusted to pH 6 by adding histidine, and a cationic leading electrolyte consisting of 0.01 M KOH, adjusted to pH 5 by adding acetic acid.

Figure 3. ITP separations of 25 s pressure injections of 0.002 M equimolar mixtures of anions applying a leading electrolyte, consisting of 0.01 M HCl, adjusted to pH 6 by adding histidine, with addition of 0.001 M CTAB and 0.04% Mowiol for suppression of the EOF and a terminating solution of 0.01 M MES/histidine. For UV transparent anions, the decreasing UV signal corresponds to the decreasing concentration of the counterionic species histidine in the zones. (L) chloride, (1) nitrate, (2) formate, (3) acetate, (4) propionate, (5) butyrate, (6) hexanoate and (T) MES. Applied constant current 3 μ A. For further information see Section 4.1.

dine and a cationic leading electrolyte consisting of 0.01 M KOH adjusted to pH 5 by adding acetic acid. In Table 1 all ionic mobilities at infinite dilution and the pK values for the ionic species used in the calculations and experiments are given. For sample ions with lower mobilities the zone concentrations decrease whereas the specific zone resistances increase [10]. In Fig. 2B the zone pH is given for the same electrolyte systems. Several investigators in the field of electrophoresis have worked with steady-state models and much of data concerning isotachophoretically obtained pK and mobility data have been published, both in aqueous and non-aqueous systems [11–15]. Generally ITP is applied in closed systems, but it can also be applied in open capillaries, although some precautions have to be taken to avoid a strong shift in electroosmotic flow during the analysis, owing to the fact that at first the EOF is determined by the leading electrolyte, whereas later on the capillary will be filled more and more with terminating solution. The best way is to suppress the EOF by adding additives to the solutions. As an example, isotachopherograms for the separations of some anions are given (see Fig. 3), applying a leading electrolyte consisting of 0.01 M HCl adjusted to pH 6 by adding histidine and a terminating solution of 0.01 M MES/histidine (constant current 3 μ A). Because all anions are UV transparent (except nitrate), the UV signal corresponds to the concentration of the counter ionic species histidine in the zones. The decreasing steps confirm decreasing concentrations of histidine in zones for sample ions with lower mobilities. To suppress the EOF, 0.001 M CTAB and 0.04% Mowiol (Hoechst, Frankfurt, Germany) were added to the background electrolyte (BGE) [16]. Besides the classical ITP model, several modifications of the ITP mode can be carried out. For all modifications, a mathematical model can be set up by adapting the set of parameters/equations needed for the calculations. Some examples are given, such as the models for hydrogen

Figure 4. Calculated relationships between pH (left-hand scale, solid line) and mobility (right-hand scale, dashed line) of the terminating hydrogen zone and the pH of leading electrolytes consisting of 0.01 M KOH, adjusted to different pH by adding benzoic acid.

ions as terminator, the use of more than one counterion and the application of two leading ions.

4.2 Isotachophoresis with terminating hydrogen ions

In cationic ITP at low pH, hydrogen ions are often used as terminator; Boček *et al.* [17] defined theoretical conditions for the ITP migration of cations with a controlled migration behavior of the hydrogen ions and formulated the concept of the effective mobility of the terminating hydrogen ions in cationic ITP [18] for monovalent counterionic species. Starting from the steady-state ITP model, a general model can be formulated for polyvalent counterionic species. The calculation of the leading zone is identical with the calculation in the classical ITP mode and for the terminating hydrogen zone, the question is whether a zone can be established whereby hydrogen ions migrate as the only positive ions in the terminating zone. If such a steady state is possible, the reduced number of parameters for the terminating hydrogen zone is only three, viz., the E , pH and total concentration of the counter ions C in the terminating hydrogen zone. Hence the EN , Ohm's law and the mass balance of the counterions C are sufficient to calculate all parameters [9]. The ITP condition gives no further information, but can be used for the calculation of the effective mobility of the hydrogen ions, in conformity with the concept of Boček *et al.* [18]. Calculations with the model indicate that the pHs in the terminating hydrogen zones are very low, which can result in enforced ITP. In enforced ITP sample ionic species migrate to a zone with a low effective mobility and high pH, in front of a zone with higher mobility at low pH. If the sample ionic species in the enforced mode stays behind in the terminating zone at low pH, their effective mobilities increase, through which they return to their own zone. Gebauer *et al.* [19] calculated existence diagrams indicating which sample ionic species can migrate in the enforced ITP mode. The mobilities of the terminating hydrogen zones rapidly increase for leading electrolytes at low pH. As an example the calculated relationships between mobility (right-hand scale, dashed line) and pH

Figure 5. Measured isotachopherograms for the separation of (A) 1 s and (B) 5 s pressure injections of an equimolar mixture of 0.01 M (1) sodium, (2) lithium and (3) Tris ions, applying leading electrolytes of 0.01 M KOH, adjusted to pH 3.9 by adding benzoic acid. It can be seen clearly that in (A) the Tris ions migrate in the (T) terminating H^+ zone in a zone electrophoretic way whereas sodium and lithium migrate in the ITP mode behind the (L) leading ions. If a large sample amount is introduced it takes a long time before the H^+ ions have overtaken the sample ions. Applied direct constant current, 5 μA .

Figure 6. Migration paths of L_2 ions over a zone boundary between the leading electrolyte consisting of L_1 and L_2 ions and a sample zone consisting of sample ions A and L_2 ions. For further information see Section 4.4.

(left-hand scale, solid line) of the terminating hydrogen zone and pH of the leading electrolytes consisting of 0.01 M KOH and benzoic acid adjusted at different pHs are given in Fig. 4. In Fig. 5 the isotachopherograms are given for the separations of (A) 1 s and (B) 5 s pressure injections of a mixture of 0.01 M of (1) sodium, (2) lithium and (3) Tris ions, applying leading electrolytes of 0.01 M KOH, adjusted to pH 3.9 by adding benzoic acid. It can be seen clearly that, in (A), sodium and lithium migrate in the ITP mode behind the (L) leading ions whereas Tris migrates zone electrophoretically in the (T) terminating hydrogen zone. For larger sample amounts the terminating hydrogen zone has not overtaken the Tris zone although a decrease in the UV signal of the terminating zone indicates that Tris ions are staying behind.

4.3 Isotachophoresis with two counterions

Sometimes two counterionic species are applied, e.g. one ionic species with buffering capacity and the other as complexing agent. For an electrolyte system with two

counterions the reduced number of parameters will be five in all ITP zones, viz., the E , pH and total concentrations of coions and two counterions. Therefore five known parameters and/or equations are necessary for the calculation of all parameters. If the composition of the leading zone is known, all parameters can be calculated with Ohm's law and the EN. For all other zones the five available equations are the EN, Ohm's law, the IC and the mass balances for the two counterions. Calculations carried out for the determination of Al^{3+} in enforced systems with α -hydroxyisobutyric acid (HIBA) as complexing agent can be found in [9].

4.4 Isotachophoresis with two leading ions

An interesting modification of ITP is ITP with two leading ions (2L-ITP) [20, 21], because ITP changes in CZE for particular ionic species and because it forms the basis for ITP superimposed on CZE (ITP/CZE) [22]. In 2L-ITP, the leading ion with the lowest effective mobility, L_2 , remains partially behind the leading ion with the highest mobility, L_1 , and creates a terminating L_2 zone with a fixed E gradient and specific zone resistance (SZR_{25}) and R_E value [11]. Sample ions migrate in the ITP mode if the values of SZR_{25} or R_E are smaller than those of the terminating L_2 zone. In fact, an ITP system is created with the leading ions L_1 , superimposed on a zone electrophoretic system consisting of the L_2 ions and the counterions. Applying a leading electrolyte with two leading ions, the reduced number of parameters is five in all zones. For the leading zone the parameters are the E , pH and the total concentrations of L_1 , L_2 and C, where L_1 is the leading ion with the highest mobility, L_2 the leading ion with the lowest mobility and C is the counterion. If the composition of the leading electrolyte is known, all parameters can be calculated with the EN and Ohm's law. For the sample zones the unknown parameters are the E , pH and the total concentrations of the counterions C, the leading ions L_2 and sample ions A. Consequently, five known parameters and/or equations are needed for the calculations, viz., the IC, Ohm's law and EN and the mass balances for L_2 and the counterions C. For the IC the principal question is whether the sample component will migrate in an ITP fashion behind the leading zone L_1 , in spite of the presence of the L_2 ions. If the amount of L_2 remaining behind the L_1 zone is sufficient to form a terminating L_2 zone without any sample ionic species and if this terminating L_2 zone has an E gradient smaller than that of the sample zone, the IC is no longer valid. In that case the ITP mode changes into a ZE mode and the sample ionic species migrates in a ZE manner to the terminating L_2 zone. If the E of a sample zone is smaller than that of a terminating L_2 zone, the sample zone migrates in the ITP mode. The mass balance for L_2 ions in a sample zone of ionic species A can be derived from Fig. 6. The velocity of the zone boundary between the leading zone, consisting of the ions L_1 and L_2 , and the sample zone, consisting of the ions A and L_2 , is determined by the L_1 ions and moves in a unit of time from point E at $t=0$ to point B at $t=1$ over a distance EB of $E_L m_{L1,L}$. The leading ionic species L_2 (and also terminating ion at the same time) at time $t=0$ present at point F will reach point C at $t=1$ and migrates over a distance $E_A m_{L2,A}$.

Figure 7. Mobility window (hatched area), indicating which positive components migrate in the ITP mode in 2L-ITP systems for varying concentrations of the leading ions potassium and Tris. The left part of the diagram concerns electrolytes consisting of 0.01 M KOH (L_1) and varying concentrations of Tris (L_2) whereas the right part concerns electrolytes consisting of 0.01 M Tris and varying concentrations of KOH, adjusted to a pH of 5.5 by adding acetic acid. For further information see Section 4.4.

The leading ions L_2 present at point D at $t = 0$ will reach point A at $t = 1$ and migrate over a distance $E_L m_{L2,L}$. The amount Q_1 of ionic species L_2 (partially present in the leading and partially in the sample zone) must be equal to the amount Q_2 , and the mass balance will therefore be:

$$c_{L2,L} E_L m_{L1,L} + c_{L2,A} E_A m_{L2,A} = c_{L2,L} E_L m_{L2,L} + c_{L2,A} E_L m_{L1,L} \quad (19)$$

The other equations equal Eqs. (18), (12), (11) and (14), respectively. For a terminating L_2 zone, the reduced number of parameters is only four, viz., the E , pH and the total concentrations of L_2 and the counterions, and for the calculation of all parameters the needed equations are the mass balances of the counterions and the L_2 ions and Ohm's law and the EN. The mass balance of the L_2 ions for the terminating L_2 zone can be derived from Eq. (19) by exchanging the A indices with T indices. In the mathematical model two calculations always have to be made, viz. the calculation of the parameters of a terminating L_2 zone and those of the parameters in the sample zone. With the mathematical models we can calculate which ionic components with a specific mobility can migrate in the ITP mode between the leading electrolyte $L_1 + L_2$ and the terminating zone L_2 . As an example, the mobility window (hatched area), indicating which components migrate in the ITP mode when applying leading electrolytes consisting of potassium and Tris, is given in Fig. 7. The left part of the mobility window concerns leading electrolytes of 0.01 M KOH (L_1) and varying concentrations of Tris (L_2) and the right part of the diagram represents electrolytes consisting of 0.01 M Tris (L_2) and varying concentrations of KOH (L_1). The upper limit of the mobility window is always the mobility of the leading ion L_1 with the highest mobility (here potassium ions) whereas the lower limit is determined by both the mobilities and concentrations of the leading ions L_1 and L_2 . This contrasts with the classical ITP where only the mobility of the terminating ions are important. Beyond the lower and upper limit of the mobility window, ITP changes in CZE.

Figure 8. Electropherograms of 5 s pressure injections of 0.01 M imidazole, applying leading electrolytes of (A) 0.01 M potassium (L_1) and 0.002 M Tris (L_2) and (B) 0.002 M potassium (L_1) and 0.01 M Tris (L_2) with the terminating electrolyte 0.01 M Tris. All electrolytes were adjusted to pH 5.5 by adding acetic acid. Applied constant current, 5 μ A. For further information see Section 4.4.

The mobility window is reduced with decreasing values of the concentration of the L_1 ions and increasing values of the concentration of L_2 and has a maximum width in the pure ITP mode (left-hand side of Fig. 7) and a minimum width in the pure CZE mode (right-hand side of Fig. 7). As an example, the electropherograms for 5 s pressure injections of 0.01 M imidazole are given in Fig. 8, applying leading electrolytes of (A) 0.01 M potassium (L_1) and 0.002 M Tris (L_2) and (B) 0.002 M potassium (L_1) and 0.01 M Tris (L_2) with the terminating electrolyte 0.01 M Tris. All electrolytes were adjusted to pH 5.5 by adding acetic acid. The Applied current was 5 μ A. According to the mobility window of Fig. 7, imidazole migrates in the ITP mode between the leading and terminating zones in electropherogram A with a concentration adapted to that of the leading ions L_1 and self-correcting zone boundaries, whereas it migrates in a CZE way in the terminating electrolyte behind the L/T boundary in diagram B. Besides supplying models for several modifications of ITP, a great deal of work has been done for all kinds of subjects such as, e.g., mixed zones in ITP [23–25].

5 Mathematical models for nonsteady-state processes

The previous section showed how mathematical models can be set up for the description of steady-state electrophoretic processes. In all models, Ohm's law, the EN and mass balances are applied, besides the IC, in ITP. In many electrophoretic processes no steady state will be reached, however; in CZE, for example, sample peaks are continuously becoming broader during the process by diffusion and electrodispersive effects. Further, mass transport could take place through the system if mass balances do not fit. Nevertheless, such nonsteady-state processes can be estimated by a repeated application of a steady-state model, if diffusion is neglected. The shift in the base line, caused by e.g. the presence of a sample

Figure 9. For calculations of sample peaks in CZE, the peaks are thought to be built up of small steps of increasing concentrations of the sample component. The calculations are started from the leading zone with parameters E_1 , pH_1 , $c_{C,1}$ and $c_{A,1}$. For each step the concentration of the sample ions $c_{S,2}$ is assumed and the other parameters can be calculated with the given model.

ionic species in the sample peak whereby the sample peak may be of an irregular shape, is divided up into small steps of varying concentrations of one of the ionic species present and all parameters of a segment can be calculated from the parameters of the preceding segment with a steady-state model. Such treatment produces good results in the calculation of all parameters in sample peaks in CZE and this treatment can be used for the simulation of electropherograms in CZE.

5.1 CZE

If in CZE peak broadening is mainly caused by the electrodispersive character, triangle-shaped sample peaks are obtained; this means that the different segments of the sample peak with different sample ion concentrations do not migrate with equal speed, owing to differences in mobility and local electric field strength. During the separation process, the sample peaks will broaden and the separation will not lead to a steady state. Yet, such a non-steady-state process can be estimated by a repeated application of a steady-state model in the following way. Sample peaks (see Fig. 9) are divided into segments with different concentrations of the sample component. The calculations start from the BGE where no sample ions are present. Therefore the reduced number of parameters in the BGE is generally four, viz., the E_1 , pH_1 and the total concentrations of the coions $c_{A,1}$ and counterions $c_{C,1}$, and if the composition of the BGE is known, all parameters can be calculated with the EN and modified Ohm's law. For each following segment in the sample peak the reduced number of parameters is five, viz., the E_2 , pH_2 and the total concentrations of the sample ions $c_{S,2}$, coions $c_{A,2}$ and counterions $c_{C,2}$. If the concentration of the sample ions $c_{S,2}$ in a segment is assumed, the reduced number of parameters is four and all parameters can be calculated with the EN, Ohm's law and the mass balances for the coions and counterions.

The EN and modified Ohm's law equal Eqs. (11) and (12) and the mass balances for the coions and counterions over segments 1 and 2 are, respectively:

$$c_{A,1} (E_1 m_{A,1} - E_2 m_{S,2}) = c_{A,2} (E_2 m_{A,2} - E_2 m_{S,2}) \quad (20)$$

$$c_{C,1} (E_2 m_{S,2} - E_1 m_{C,1}) = c_{C,2} (E_2 m_{S,2} - E_2 m_{C,2}) \quad (21)$$

according to the Eqs. (15) and (14), because the velocity of the zone boundary between segments 1 and 2 is $E_2 m_{S,2}$. The procedure of calculation is relatively easy [26, 27]. If the composition of the BGE is known, all parameters of the BGE zone can be calculated. All parameters of the proceeding sample peak segments can be calculated from those of the preceding segment in the following way. The concentration in segment $c_{S,2}$ is assumed. Then pH_2 is assumed, through which all pH-dependent parameters can be calculated. Assuming a $c_{A,2}$, the $c_{C,2}$ can be calculated from the EN equation. From Ohm's law, E_2 can be obtained and with an iteration procedure the correct values of pH_2 and $c_{A,2}$ can be found, whereby Eqs. (20) and (21) are satisfied.

5.2 Significance of the model

With a computer program based on the foregoing mathematical model, all sample zone parameters are calculated for BGEs consisting of coions, with a pK value of 7 and ionic mobilities at infinite dilution of (1) 70, (2) 50, (3) 30 and (4) $10 \times 10^{-9} \text{ m}^2/\text{Vs}$, adjusted to pH 5.5 by adding acetic acid for the sample component potassium. In Fig. 10A the calculated relationship between pH and concentrations c_S in the sample zone of the sample ion potassium is given (solid line, left-hand scale). In all cases, the pH increases for increasing concentrations of the sample component, because the ionic mobility at infinite dilution of the sample component potassium is higher than that of the coions of the BGE, especially for a large difference between the mobilities of potassium and coion, just as in ITP. For sample components with a mobility lower than that of the coions the pH decreases. The electrophoretic mobility for the sample potassium is nearly constant in the zones. The local electric field strength E_i differs considerably for varying sample concentrations and is lower for increasing concentrations of the sample if the sample ionic mobility is higher than that of the coions. The apparent mobility of a specific segment i in the sample zone can be defined as:

$$m_{\text{app},i} = m_i \frac{E_i}{E_{\text{BGE}}} + m_{\text{EOF}} \quad (22)$$

In Fig. 10A the relationships between apparent mobilities (dashed lines, right-hand scale) and sample concentrations are given. It can be seen clearly that the apparent mobility of a sample peak segment decreases for increasing concentrations of the sample component if the mobility of the sample component is higher than that of the coions of the BGE, resulting in fronting peaks. If the mobility of the sample component is lower than that of the coions, the apparent mobility increases for increasing concentrations of the sample component, causing tailing peaks. In Fig. 10B the relationships between the total concentrations of the coions (solid

Figure 10. Calculated relationships between the concentrations in sample zones for the component potassium (c_s) and (A) the pH (solid line, left-hand scale) and apparent mobility (dashed line, right-hand scale) and (B) total concentrations of the coions (solid lines, left-hand scale) and total concentrations of the counterions (dashed lines, right-hand scale) for BGEs consisting of coions with ionic mobilities of (1) 70, (2) 50, (3) 30 and (4) $10 \times 10^{-9} \text{ m}^2/\text{Vs}$ ($m_{\text{EOF}} = 0$). For further information see Section 5.2.

lines, left-hand scale) and of the counterions (dashed lines, right-hand scale) and the concentration of the sample component are given. As can be expected, the concentration of the coions decreases for increasing concentrations of the sample component. For BGE (1) where the mobility of the coions does not differ much from that of potassium, the transfer ratio [28] is nearly unity and the ionic strength and concentration of the counterions are nearly constant. If the difference between the mobilities of sample and coions differs much ($m_s > m_i$), the transfer ratio is smaller than unity, through which the ionic strength and concentration of the counterions increase. If the mobility of the sample ions is smaller than that of the coions of the BGE, the transfer ratio is larger than unity, resulting in a larger decrease of the concentration of the coions, a smaller ionic strength and a decrease of the concentration of the counterions. Applying this model, electropherograms can be simulated.

5.3 Simulation of electropherograms

Electropherograms are generally measured on a temporal basis, which means that for the simulation of electropherograms we must calculate how a specific parameter changes in time at the position of the detector. Calculations with the given mathematical model show that different segments of the sample peak migrate with a different mobility by the combined effect of differences in effective mobility of the component and differences in the local electric field strength. In this case different segments of the sample peak migrate with different velocities, causing the typical triangle-shaped peaks in CZE and, in the course of time, the sample peak broadens, whereby the peak heights decrease owing to the electrodispersive character. The sample peak is in dynamic equilibrium, the peak shape changes continuously and a mass transport takes place over the peak segments. The calculation of the simulated electropherograms on a temporal basis is therefore as follows. With the mathematical model the relationships between the concentration of the sample component in a sample peak and all other parameters, such as the apparent mobility of the segment, all concentrations and pH and electric field strength, are calculated. Starting from the diffuse side of the sample peak, we can calculate which sample peak segment passes the detector position; this calculation is repeated until the amount of sample ions that have passed the detector position is equal to the injected amount. In this treatment the front or backside of the sample peak is diffuse whereas the other side is assumed to be upright. For the simulated electropherogram, that parameter is given in time, as registered by the detector chosen. As an example, the (A) measured and (B) simulated electropherograms are given in Fig. 11, for the separation of 10 s pressure injections of a mixture of $5 \times 10^{-4} \text{ M}$ imidazole and histidine, applying a BGE of 0.01 M Tris, adjusted to pH 7 by adding acetic acid, a mixture of $5 \times 10^{-4} \text{ M}$ imidazole, histidine and creatinine, applying a BGE of 0.01 M Tris, adjusted to pH 5 by adding acetic acid, and a mixture of $5 \times 10^{-4} \text{ M}$ imidazole and creatinine, applying a BGE of 0.01 M Tris, adjusted to pH 3.8 by adding formic acid. In the simulated electropherograms the total concentrations of the sample components are given because all components are measured in the classical direct UV mode and the mobility of the EOF is calculated from the measured electropherograms.

Simulated electropherograms bear a striking resemblance to the measured electropherograms and predict a reversal from fronting to tailing character for imidazole between pH 5 and 7, and for creatinine between pH 3.8 and 5, confirming that peaks will front if the effective mobility of the sample component is higher than that of the coions of the BGE. Note the curving peak shape for histidine at pH 7 in measured and calculated electropherograms. All calculated migration times are smaller than those measured. The first reason is that the chosen voltage across the capillary is not immediately reached; a second reason is that the resistance of the sampling zone (low concentration) is higher than that of the BGE in the separation capillary, through which the effective electric field strength over the separation capillary is

Figure 11. (A) Measured and (B) simulated electropherograms for the separation of 10 s pressure injections of a mixture of 5×10^{-4} M imidazole and histidine, applying a BGE of 0.01 M Tris acetate, pH 7, of a mixture of 5×10^{-4} M imidazole, histidine and creatinine, applying a BGE of 0.01 M Tris acetate, pH 5, and of a mixture of 5×10^{-4} M imidazole and creatinine applying a BGE of 0.01 M Tris formate, pH 3.8. Peak heights of histidine are larger than those for imidazole because of its higher molar absorptivity. For further information see Section 5.3. (1) Imidazole, (2) histidine and (3) creatinine.

lower than calculated. The mobility of the creatinine, used in the calculations, seems to be too high. Further, all calculated peaks are narrower than the measured ones because other peak broadening mechanisms are neglected. In Fig. 12, the (A) measured and (B) simulated electropherograms are given for the separation of 5 s pressure injections of an 0.001 M equimolar mixture of the cations potassium, sodium and lithium, applying a BGE consisting of 0.01 M imidazole, adjusted to pH 5 and 7 by adding acetic acid. In the simulated electropherogram the concentration of the total concentration of imidazole is presented because all ions are measured in the classical indirect UV mode. At a pH of 5, imidazole will be fully protonated, and the potassium and sodium peaks have a fronting character because their effective mobilities are higher than that of the coion imidazole, whereas the lithium peak is tailing. At pH 7 the effective mobility of imidazole is lower than those of all sample ions. Nevertheless, the lithium zone is still tailing, indicating that the effective mobility of the sample ions must be compared with the ionic mobility of weak ionic species of the BGE to answer the question whether fronting or tailing sample peaks can be expected. The simulated electropherograms resemble the measured electropherograms although in the simulations, peaks are a little bit narrower and the peak height of sodium is overestimated because diffusion is neglected in the calculations.

If BGEs are applied with UV absorbing counterions, UV transparent ionic species can be visible in electropherograms both as peaks or as dips. If the mobilities of the sample ions are higher than those of the coions, they are peaks, whereas sample dips are obtained if their mobilities are lower than those of the coions. To demonstrate

this effect, the electropherograms of 5 s pressure injections of an 0.001 M equimolar mixture of the anions formate, acetate, propionate, butyrate and caproate are given in Fig. 13, applying BGEs of (A) 0.01 M HCl and 0.02 M histidine, (B) 0.01 M butyric acid and 0.02 M histidine, and (C) 0.01 M MES and 0.02 M histidine. The experiments are carried out in the anionic mode and for a reversal of the EOF 0.001 M CTAB and 0.04% Mowiol are added to the BGEs. Applying the (A) coions chloride, all sample anions have lower mobilities than those of the coions chloride and all sample zones are visible as tailing dips in the electropherogram. Applying the (B) coions butyrate, the sample caproate is present as a tailing dip, whereas formate, acetate and propionate are present as fronting peaks because their mobilities are higher than those of the coions butyrate. The sample ions butyrate are not visible in the electropherogram because the BGE contains the coions butyrate. Note the presence of a system peak, S. By the addition of CTAB in the BGE, a BGE is created with the two coions butyrate and bromide. In such a case, system peaks can be expected [29] with a mobility between that of the two coions. Because the concentration of the bromide ions is the lowest, the mobility of the system peak is near that of bromide. In the case of the two coions chloride and bromide (electropherogram A) with nearly equal mobilities, no system peak can be expected. In case (C), the coions MES and bromide are present, again resulting in a system peak S. The mobilities of formate, acetate, propionate and butyrate are higher than that of the coions MES and therefore they are present in the electropherogram as fronting peaks. Caproate with a mobility nearly equal to that of MES is not visible. As a demonstration that the mathematical model is able to predict peaks and dips we give in Fig. 14 the calculated (a) concentration

Figure 12. (A) Measured and (B) simulated electropherograms for the CZE separation of 5 s pressure injections of a mixture of an equimolar solution of 0.001 M potassium, sodium and lithium ions, applying a BGE consisting of 0.01 M imidazole, adjusted to pH 5 and 7 by adding acetic acid. Applied voltage, 15 kV. For further information see Section 5.3.

of the counterions histidine (left-hand scale) and (b) concentration of the sample components for the separation of the anionic mixture of Fig. 13 applying the BGE of histidinebutyrate, indicating that ionic species with high mobilities indeed give peaks whereas ionic species with low mobilities are dips. The concentration of histidine corresponds to the detector signal of the electropherogram of Fig. 13.

6 Discussion

Steady-state mathematical models, based on the modified Ohm's law, the electroneutrality equation, mass balances and the isotachophoretic condition, can be applied for the description of isotachophoretic processes and it has been shown that this model can be modified for several different modes of ITP. The usefulness of these models has been proven, witness the fact that many parameters published in the literature were calcu-

Figure 13. Electropherograms for the separations of 5 s pressure injections of 0.001 M equimolar mixtures of the anionic species (1) formate, (2) acetate, (3) propionate, (4) butyrate and (5) caproate applying BGEs of (A) 0.01 M HCl and 0.02 M histidine, (B) 0.01 M butyric acid and 0.02 M histidine and (C) 0.01 M MES and 0.02 M histidine. Experiments are carried out in the anionic mode with reversed EOF (voltage 5 kV) and adding 0.001 M CTAB and 0.04% Mowiol to the BGE. For further information see Section 5.3.

Figure 14. Simulated electropherograms for the separation of the anionic mixture and BGE of histidine butyrate from Fig. 13. For further information see Section 5.3.

lated from experimental values with these models. Non-steady-state processes in electrophoresis are generally treated by means of a numerical solution of the basic transport equations [30–32] or the application of the concept of the “eigenvector” [33] but nonsteady-state processes can also be estimated by steady-state models. For example, sample peaks in CZE can be divided into small segments whereby all parameters from a segment can be calculated from the preceding segment with a steady-state model, starting from the BGE composition of the diffuse side of a sample peak. Calculations with this model show that the combined effect of a change in pH, resulting in changing effective mobilities for weak acids and bases, and the change in the local electric field strength E leads to a change in apparent mobilities of the different segments of the sample peaks, resulting in the triangle-shaped peaks in CZE. With this model, electropherograms based on a temporal basis can be simu-

Figure 15. Peak shapes for 20 s pressure injections of 5×10^{-4} M imidazole solutions, mixed with 0.001 M Tris and adjusted to different pH (pH_s) by adding acetic acid. BGEs were 0.01 M Tris, adjusted to different pH (pH_b) by adding acetic acid. Applied voltage 7 kV. For further information see Section 6.

lated; also it can be calculated whether sample zones are fronting or tailing and whether sample ionic species are present in electropherograms as peaks or dips. For the fronting or tailing character, the effective mobilities of the sample ionic species must be compared with the ionic mobilities of the coions of the BGE. Realistic simulations can be obtained, for zone electrophoretic processes whereby the electrodispersive character is the dominating peak broadening mechanism.

The model can be handled for weak ionic species whereby pH effects are included; even system peaks and moving boundaries, originating from discontinuities in the concentration and/or the pH of the BGE, can be treated [34]. In this model peak broadening effects due to diffusion and differences in temperature are neglected and all peaks are considered apart, *i.e.* initial effects connected with comigration of the different sample components and sample stacking cannot be treated. That other parameters can also strongly affect peak shape in CZE is demonstrated in Fig. 15; the measured peak shapes are given for 20 s pressure injections of 5×10^{-4} M imidazole solutions, mixed with 0.001 M Tris and adjusted to different pH (pH_s) by adding acetic acid. BGEs were 0.01 M Tris, adjusted to different pH (pH_b) by adding acetic acid (applied voltage 7 kV). For increasing pH of the BGE the imidazole peak changes from a fronting to a tailing character at a pH_b of 6.5, where the effective mobility of imidazole equals the ionic mobility of Tris. If

the pH in the sample solution is higher than the pK value of the sample component, strongly tailing peaks are obtained owing to the fact that the buffering capacity of the BGE is not able to maintain its pH in the sample zone, through which the effective mobility of the backside of the sample peak is very low.

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