Computer-aided simulation of electromigration

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ABSTRACT

Equations that describe the electrophoretic migration of monovalent ionic substances in solution with a significant presence of H^+ or OH^- ions are formulated. A derivation of the Kohlrausch regulating function for these conditions is presented. The model of electromigration consists of a set of continuity equations, together with a set of algebraic equations describing the chemical equilibria involved, and is implemented on a personal computer. Simulation of some experimental phenomena in electrophoretic methods, *e.g.*, the sharpening effect in capillary zone electrophoresis or anomalous spikes in isotachophoretic systems, is presented.

INTRODUCTION

Considerable attention has been paid to the description of the migration of ions in an electric field. Efforts of theoreticians are directed towards two aspects: first, the use of fundamental laws describing mass transport, chemical equilibria or the electroneutrality principle and the derivation of explicit conclusions useful in a particular application, and second, attempts to solve the basic laws "from the beginning", which leads in practice to the necessity for a solution of partial differential and non-linear algebraic equations. In most instances, these equations are not analytically soluble and therefore are solved numerically using computers. The results are obtained in a numerical or graphical form.

It is not the aim of this paper to attempt a comprehensive review of previous theoretical work, so only a selection will be mentioned. Kohlrausch's study [1] belongs to the first group and established a basis for many subsequent studies. It is possible to refer to, *e.g.*, the *RFQ* method of evaluation of the steady state in isotachophoresis introduced by Beckers and co-workers [2,3] and the work of Mikkers and co-workers dealing with transient states in isotachophoresis [4–6] and some aspects of electrophoresis [7]. A series of papers by Boček and co-workers [8–12] have shown the usefulness of zone existence diagrams in isotachophoresis and introduced the concept

of the effective mobility of the hydrogen ion. Hirokawa and co-workers described the dynamics of separations in isotachophoresis using a transient state model [13,14] and applied the steady-state theory in isotachophoresis [15,16] to the determination of basic physico-chemical constants.

Many papers by Mosher, Palusinski and co-workers [17–29] belong to the second group. They have developed a general model of electrophoresis of ampholytes in solution and have been able to implement the model on a computer, solve it numerically and simulate many electrophoretic techniques.

Our laboratory has also been engaged in an effort to understand electromigration separation processes. The dynamics of the motion of the boundary between two strong electrolytes was solved on a computer using the net method [30]. The isotachophoretic separation of four strong electrolytes was conveniently simulated on a hybrid computer [31] and later published [32,33].

The purpose of this paper is to present a relatively simple model of migration of weak uni–univalent electrolytes in an electric field. In spite of its simplicity, the model is able to describe or explain many phenomena appearing in electromigration methods. The model is easily implementable on personal computers and is also instructive from a pedagogical point of view.

MATHEMATICAL MODEL

Let us consider a solution of weak uni–univalent electrolytes which are able to migrate in an electric field in one direction, mostly in a capillary tube. The limitation of these considerations to uni–univalent electrolytes is not a serious drawback because most features of electromigration methods could be described in spite of this restriction. It is also known that many important relationships holding in the theory of electromigration can then be easily formulated [20,34]. Let us also neglect the diffusional dispersion. Even this restriction [7,20] will not cause a serious limitation of the model, especially in describing isotachophoresis. Nevertheless, when the presence of H^+ or OH^- ions originating from a solvent and weak electrolytes is significant we shall take these ions into consideration, therefore, we shall use a new term in this context, viz., substance.

By the term substance we shall understand an ionic species together with an eventually related undissociated compound. Three examples of such substances are acetate plus acetic acid, H^+ ions plus propionic acid or OH^- ions plus NH_3.

Concentrations and electrophoretic mobilities are assumed to be positive numbers. The total concentration of substance j is denoted c_j, the relative charge of ionic species j is denoted z_j and can be $+1$ for a cation and -1 for an anion, the concentration of the ionic part of substance j is denoted c_j^z and that of the undissociated compound j is denoted c_j^0. It is then possible to write $c_j = c_j^z + c_j^0$. Further, for simplicity, we shall use the term "weak ion" instead of "ion of a weak electrolyte".

Our aim is to find the time course of the distributions in the capillary tube of all the substances in either the dissociated or the undissociated form. The derivation of the basic equations can start from the mass conservation law in an infinitesimal volume or continuity equation.

First, for simplicity, let us assume a negligible concentration of the H^+ or OH^- ions produced by ionization compared with other substances. These requirements can

be met by performing the separation in the so-called "safe region" [9] at a pH within the range *ca.* 5–10. Fig. 1 shows a system of two weak anions and one weak counter cation. The electric field orientation and consequently the direction of the electric current are assumed to follow the direction of increasing values on abscissa. S_j^z represents the mass flow of j ions. The horizontal arrows denote the mass flow through the depicted volume and the bent arrows symbolize the time changes of the concentration of the substances. The conservation equations can be written as (*cf.*, ref. 20)

$$\frac{\delta c_j}{\delta t} = - z_j \, i \, U_j \frac{\delta}{\delta x} \left(\frac{c_j^z}{\varkappa} \right) \tag{1}$$

and

$$\varkappa = F \sum_{j=1}^{n} c_j^z \, U_j \tag{2}$$

where $j = 1, 2, \ldots, n$, t is time, i is the current density in the capillary tube, $\varkappa$ is the specific conductivity, U_j is the ionic mobility of ion j, x is the length coordinate along the capillary tube, n is the number of substances and F is the Faraday constant.

We assume that ionic mobility U_j (which generally depends on the ionic strength) can be approximated by the limiting ionic mobility in our model. From the set of eqn. 1 we can obtain the regulating function connected with the mass conservation law:

$$\sum_{j=1}^{n} z_j \, c_j = \text{constant} \, (x) \tag{3}$$

where constant (x) is a function of the length coordinate x, independent of time. If the eletroneutrality condition

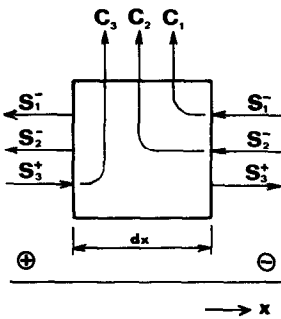$$\sum_{j=1}^{n} z_j \, c_j^z = 0 \tag{4}$$

Fig. 1. Graphical illustration of the transport equations for the system of two weak anions and one weak counter cation.

is fulfilled at any length point (it is a good approximation [27]), then the Kohlrausch regulating function holds:

$$\sum_{j=1}^{n} \frac{c_j}{U_j} = \text{constant}\ (x) \tag{5}$$

Eqns. 3, 4 and 5 are the bonding conditions for the solution of the initial and boundary problem of the set of eqn. 1. Because migration of weak electrolytes is generally considered, we must take into account additional bonding conditions, which are equations describing chemical dissociation equilibria:

$$f(c_1, c_2, \ldots, c_n) = K \tag{6}$$

where f is a non-linear function (the Guldberg–Waage law) and K is the dissociation constant.

For illustration, let us consider a system consisting of five substances: two weak and two strong anions and one common weak counter cation. There are eight unknown variables in this system: two concentrations of the charged and uncharged part of the counter substance, four concentrations of the charged and uncharged part of the weak anionic substances and two concentrations of the strong anionic substances. It follows that we need eight equations to solve this system: three are eqns. 3–5 and two are equations describing dissociation equilibria [of the three original relationships for chemical equilibria (eqn. 6) of the three weak substances, one is cancelled after elimination of the hydrogen ion concentration]. Consequently, three differential equations of the set of eqn. 1 remain.

The situation is more complicated if H^+ or OH^- ions participate in the migration. First, let us assume a significant role of the H^+ ions. For illustration, Fig. 2 shows a system consisting of three substances: acetate (which is composed of acetate anion, acet$^-$, and acetic acid, Hacet), H^+ (which consists of H^+ cation and acetic acid, Hacet) and K^+. The notation of the symbols and arrows is the same as in Fig. 2. An equation describing the transport of hydrogen (either as H^+ ion and hydrogen bonded in acetic acid) can be written in the form

$$\frac{\delta(c_H^+ + c_{Hacet}^0)}{\delta t} = -i\ U_H \frac{\delta}{\delta x}\left(\frac{c_H^+}{\varkappa}\right) \tag{7}$$

The Kohlrausch regulating function is then

$$\frac{c_{Hacet}^0}{U_{acet}} + \frac{c_{acet}^-}{U_{acet}} + \frac{c_K^+}{U_K} + \frac{c_H^+}{U_H} + \frac{c_{Hacet}^0}{U_H} = \text{constant}\ (x) \tag{8}$$

It can be shown that all the relationships describing the migrating reaction boundary of the zone of H^+ ions in isotachophoresis [8,9,35] can be readily derived from the regulating function in eqn. 8.

The following relationship holds for the general system with significant contribution of H^+ ions:

$$\frac{\delta\left(c_H^+ - \sum_{j=1}^{n} z_j\ c_j^0\right)}{\delta t} = -i\ U_H \frac{\delta}{\delta x}\left(\frac{c_H^+}{\varkappa}\right) \tag{9}$$
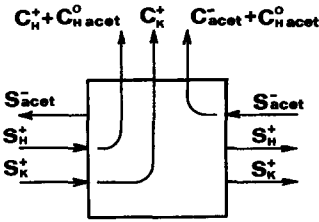
Fig. 2. Graphical illustration of the transport equations for the system potassium acetate–acetic acid.

where n is the number of substances except hydrogen. The Kohlrausch regulating function takes the form

$$\sum_{j=1}^{n} \frac{c_j}{U_j} + \frac{c_H^+}{U_H} - \sum_{j=1}^{n} z_j\, c_j^0/U_H = \text{constant } (x) \tag{10}$$

and the condition of electroneutrality is

$$\sum_{j=1}^{n} c_j^z z_j + c_H^+ = 0 \tag{11}$$

There is no simple relationship analogous to eqn. 3.

For the solution of, *e.g.*, a four-substance system containing two weak cations, H^+ cation and weak counter anion, there are seven unknown variables. Three eqn. 6 describe the dissociation equilibria of the weak ions, two equations follow from eqns. 10 and 11 and, consequently, two partial differential equations remain. It is useful to realize that the number of remaining partial differential equations equals the number of boundaries realizable in an isotachophoretic system or the number of peaks in a system modelling zone electrophoresis.

Considering the contribution of the OH⁻ ions to migration, the following equations can be written:

$$\frac{\delta\left(c_{OH}^- + \sum_{j=1}^{n} z_j\, c_j^0 \right)}{\delta t} = i\, U_{OH} \frac{\delta}{\delta x}\left(\frac{c_{OH}^-}{\varkappa} \right) \tag{12}$$

where n is the number of substances except OH⁻ substance, and the Kohlrausch regulating function,

$$\sum_{j=1}^{n} \frac{c_j}{U_j} + \frac{c_{OH}^-}{U_{OH}} + \sum_{j=1}^{n} z_j\, c_j^0/U_{OH} = \text{constant } (x) \tag{13}$$

is obtained.

It is apparent from the above that a simulation of migration in an electric field (in the framework of the described model) requires the solution of the set of partial differential and linear plus non-linear algebraic equations for various initial and

boundary conditions. These conditions can circumscribe several current electro-migration methods, *e.g.*, isotachophoresis or capillary zone electrophoresis.

NUMERICAL METHOD

The same method as in previous work [31] was chosen for the solution of the partial differential equations, namely the CSDT (continuous space, discrete time) method of lines. However, computation was carried out on a personal instead of a hybrid computer.

The CSDT method consists in discretizing the time derivatives at a set of grid points to generate a set of ordinary differential equations instead of partial differential equations with length as the independent variable. The finite-difference approximation is based on the first-order backward difference approximation:

$$\frac{\delta c}{\delta t} = \frac{c_i - c_{i-1}}{\Delta t} \tag{14}$$

where $t = i\Delta t, i = 1, 2, \ldots$ and c_0 is the initial concentration distribution in a capillary tube at time $t = 0$.

The Hamming modification of the fourth-order predictor–corrector method [36] was chosen for integration of the set of ordinary differential equations. The method exhibits the same accuracy as the standard fourth-order Runge–Kutta method, but it is almost twice as fast. In addition, the method permits an easy estimation of its error, which is convenient from the numerical point of view.

The numerical method of solution of the differential equations also necessitates discretization of the x-coordinate. The left-hand side of eqn. 1 must be calculated at each point of the x-coordinate; in fact it requires a set of non-linear algebraic equations to be solved at each point for systems including weak electrolytes, because the bonding conditions resulting from the laws of chemical equilibria include products and quotients of the concentrations. The set of non-linear algebraic equations was solved by the Newton–Raphson iteration method [36] and the concentrations computed in the preceding time step are taken as the initial estimates.

All the bonding conditions are linear if only strong electrolytes are considered, and therefore the Gauss elimination method can be used to solve the left-hand side of eqn. 1 in this instance.

EXPERIMENTAL

The computational algorithm for the solution of the whole model was programmed in Pascal language and runs on IBM PC AT computers or other compatible types. The result of computation is a graphical presentation of the time-dependent evolution of the distribution of all the substances and their charged and uncharged parts along the x-coordinate in a capillary tube.

The algorithm allows an "observational window" to be advanced along the capillary tube at the migration rate of a substance of interest and thus enables excessive computer time and memory requirements to be avoided. It was used in simulations of all isotachopherograms, where the window shifts at the migration velocity of the leading ion.

TABLE I

INPUT DATA FOR COMPUTER SIMULATIONS

Substance	pK_a	Ionic mobility $(10^{-9}$ m^2 V^{-1} s$^{-1})$
Acetate	4.76	42.4
Benzoate	4.20	36.3
Cl$^-$	—	79.1
Bis–Tris^a	6.4	26
Aniline	4.8	32.5
Pyridine	5.18	30
TBA	14	18
K$^+$	—	76.2
H$^+$	—	363

^{a} Bis[tris(hydroxyethylaminomethane)].

Ionic mobilities and pK values of substances used as input data for simulations are listed in Table I.

A CS ZKI 01 isotachophoretic analyser assembled in the column coupling configuration of the separation unit was used for experiments. Chemicals used for the preparation of the leading and terminating electrolytes were of analytical-reagent grade and some were purified by conventional methods.

RESULTS AND DISCUSSION

As pointed out above, the model of electrophoretic migration neglects diffusion of substances. However, during numerical calculation, the first-order approximation eqn. 14 causes an error that imitates the diffusional term in the continuity equations. This error can in principle be decreased by diminishing of Δt in eqn. 14 but it involves more computer time and memory requirements. A reasonable medium value of Δt should always be chosen.

Fig. 3 shows the solution of the isotachophoretic separation of two weak anionic substances in a system of strong leading and terminating anion and a weak buffering counter cation, where the contribution of H$^+$ or OH$^-$ ions to migration is neglected. A pH inversion occurs between the second weak substance and the terminating anion.

Isotachophoretic systems with a mobility inversion [11] are more interesting. Inversion of mobility can appear in routine practice more often than one might expect, especially in analyses of real samples where related samples could often be present. Anomalies in the conductivity recording of the separation are often considered as artifacts of the contact conductivity detector. The mobility inversion mostly occurs in practice in systems with a low buffering capacity of the counter substance (as, e.g., in the 0.01 M potassium acetate system for cationic separation [11]) or in buffer-free systems [12]. Such systems allow a wide pH swing between subsequent zones. In systems with a maximum buffering capacity of the counter substance, the inversion of the mobilities is a subtle phenomenon observable only within a narrow range of the pH of the leading electrolyte.

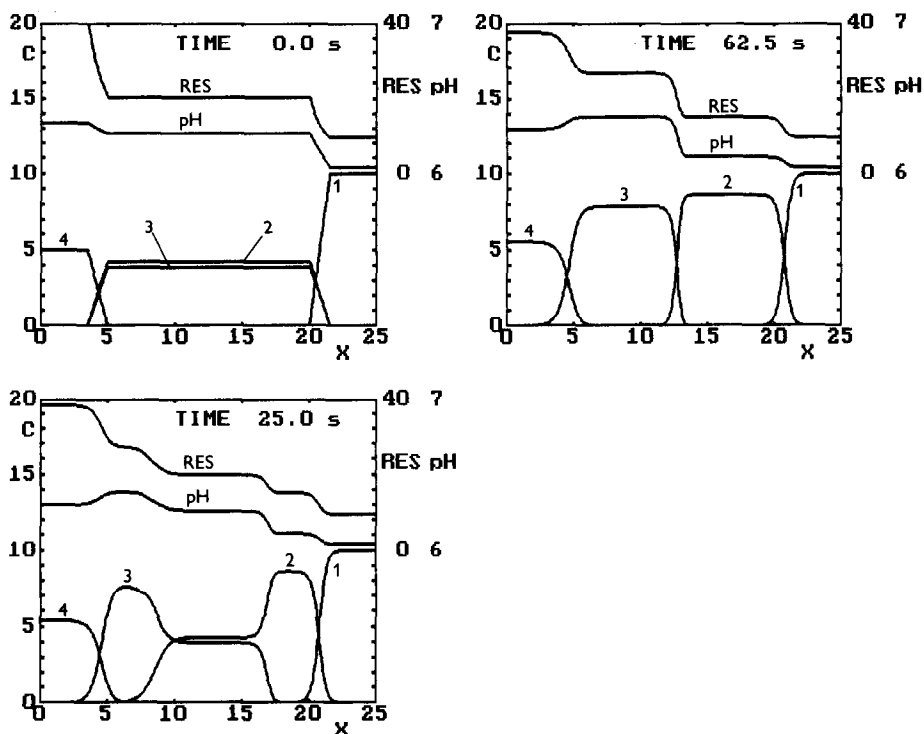A condition for isotachophoretic migration of two weak substances with

Fig. 3. Simulation of anionic isotachophoresis. 1 = Strong leading anion chloride, $u_1^- = 79.1 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; 2 = weak anion, $u_2^- = 50 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, pK = 4; 3 = weak anion, $u_3^- = 40 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, pK = 6; 4 = strong terminating anion, $u_4^- = 20 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. Weak counter cation, histidine, $u_5^+ = 29.6 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, pK = 6.04. Leading electrolyte, 10 mM chloride–20 mM histidine. Current density, 709 A m^{-2}. C = concentration (mM), RES = specific resistance (Ω m), x = coordinate (mm).

mobility inversion is the intersection of their mobility curves. Fig. 4 displays the mobility curves of acetic and benzoic acid, which intersect at pH 5.15, and Fig. 5 shows some parameters of this pair migrating in the system 15 mM chloride as leading anion
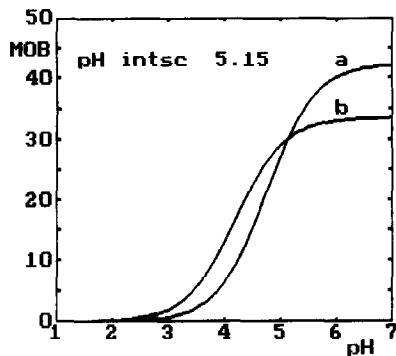

Fig. 4. Mobility curves of (a) acetic acid and (b) benzoic acid. MOB = mobility (10^{-9} m^2 V^{-1} s^{-1}).
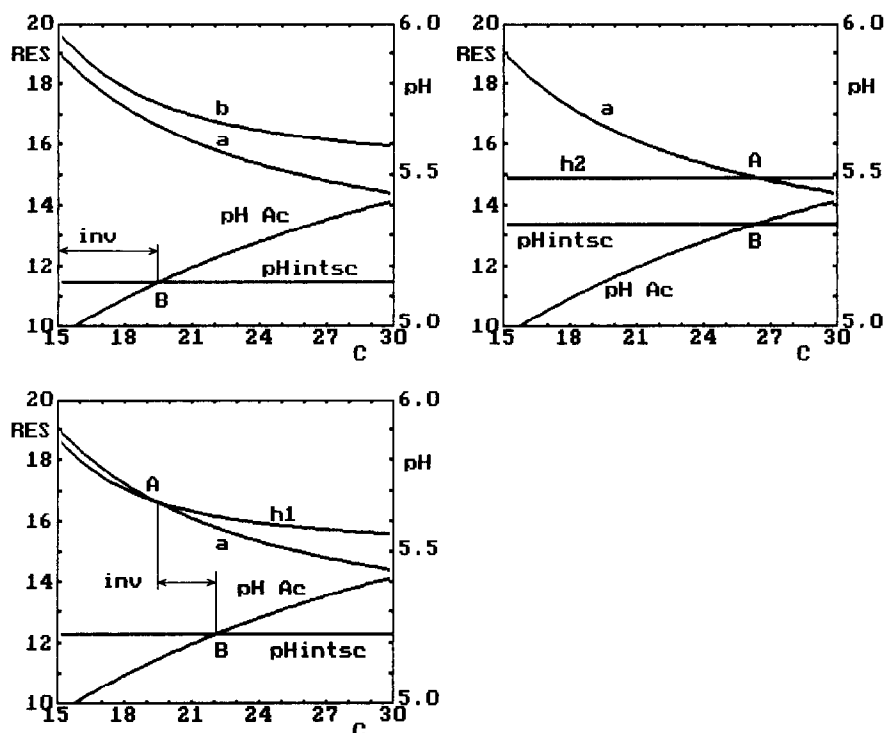
Fig. 5. Influence of pyridine concentration on the specific resistance and pH of zones in the system 15 mM Cl$^-$ plus pyridine. a = Specific resistance of acetate zone; b = specific resistance of benzoate zone; h1 = specific resistance of a hypothetical substance with pK = 4.0; h2 = specific resistance of a hypothetical substance with pK = −1. pH Ac = pH of acetate zone; pHintsc = pH of intersection of mobility curves; inv = interval of mobility inversion; C = concentration of pyridine (mM); RES = specific resistance (Ω m).

and pyridine as buffering counter ion. If instead of benzoic acid with pK = 4.2 another hypothetical acid with pK = 4 is used, migration with mobility inversion can occur in a narrower interval, between the intersection A on the conductivity curves and the intersection B on the pH curves. When a strong acid with pK of, $e.g.$, −1 is used, then, in spite of intersection of the mobility curves, mobility inversion is impossible because there is no space between points A and B. Hence, migration with mobility inversion can occur if the mobility curves intersect but the pK values are not very different. For buffered systems exhibiting a maximum buffering capacity (which are usually employed in practice) this means that the pK difference lies in the range $ca.$ 0.3–1 if mobility inversion occurs.

These conclusions are illustrated in Figs. 6 and 7, depicting a computer simulation (see also ref. 11) and an isotachopherogram of the separation of acetic and benzoic acid with a mobility inversion in 15 mM hydrochloric acid + 16 mM pyridine as the leading electrolyte system. Both the computer simulation and the experimental verification on a double-column isotachophoretic analyser have shown that the boundary between benzoate and acetate zones is stable and both are zones of pure substances.
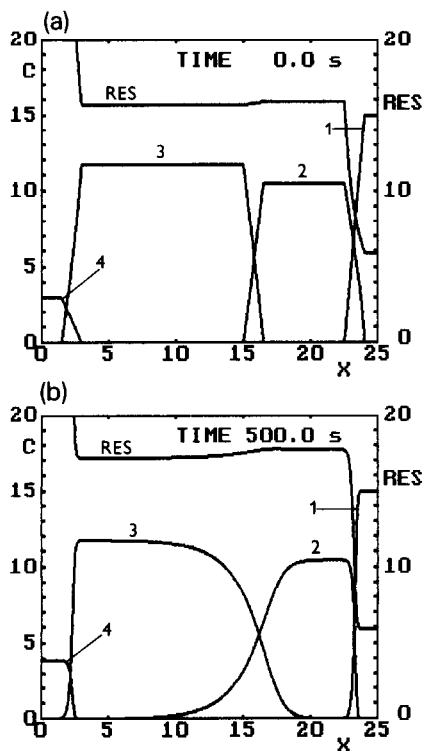
Fig. 6. Simulation of the isotachophoretic system with mobility inversion. $1 = Cl^-$; $2 = $ benzoate; $3 = $ acetate; $4 = $ strong terminating anion with $u_4^- = 8 \cdot 10^{-9}$ m^2 V^{-1} S^{-1}. Leading electrolyte, 15 mM Cl$^-$–16 mM pyridine. Current density, 1064 A m^{-2}. $C = $ concentration (mM), RES = specific resistance (Ω m); $x = $ coordinate (mm).
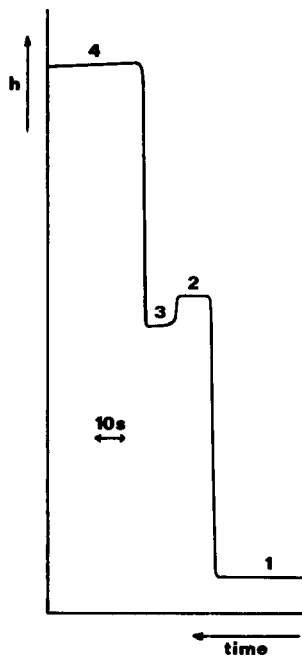
Fig. 7. Isotachopherogram of the separation of acetic and benzoic acids with mobility inversion. $1 = Cl^-$; $2 = $ benzoate; $3 = $ acetate; $4 = $ caproic acid. Leading electrolyte, 15 mM Cl$^-$–16 mM pyridine.

The stable mixed zones can also originate in isotachophoretic systems with intersection of the mobility curves, as already described by Gebauer and Boček [11]. One experiment from this work is simulated in Fig. 8 for a system consisting of potassium acetate + acetic acid with a mixed zone created between aniline and tetrabutylammonium (TBA). It can be seen that the model also depicts a conductivity bump on the boundary between the pure zone of tetrabutylammonium and the mixed zone of tetrabutylammonium + aniline.

Various "irregularities" and "bumps" are often present in conductivity records of isotachopherograms. Mosher et al. [22] showed that even the conductivity profile between two pure zones need not always be monotonous but could also exhibit such a bump. This bump can be especially distinct when one of the zones is the zone of hydrogen ions. Fig. 9 shows a simulated conductivity profile of the boundary between zones of aniline and hydrogen ions in the system consisting of 2 mM Bis–Tris as the leading cation and different concentrations of acetic acid as the counter anion. Aniline and hydrogen ions migrate in this system with inverted mobilities. Computed
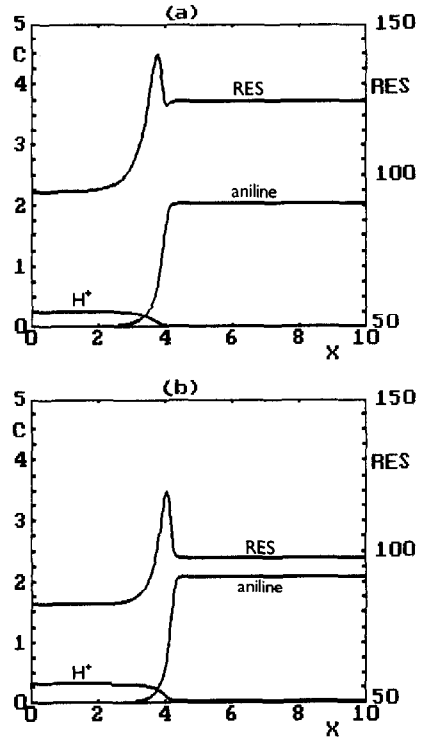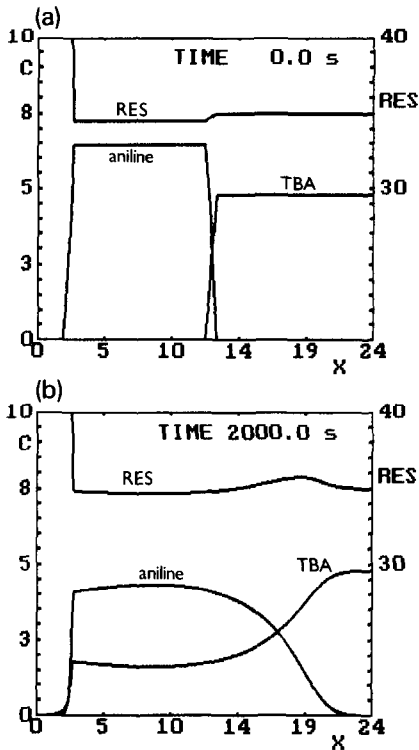
Fig. 8. Simulation of the isotachophoretic system with the stable mixed zone. TBA = tetrabutylammonium. Leading electrolyte, 10 mM potassium acetate–4 mM acetic acid. Current density, 238 A m^{-2}. C = concentration (mM); RES = specific resistance (Ω m); x = coordinate (mm).

Fig. 9. Simulation of the isotachophoretic boundary between aniline and H$^+$ zone. Leading electrolyte, 2 mM Bis–Tris plus (a) 1.74 mM acetic acid, pH 5.82 or (b) 3 mM acetic acid, pH 5.06. C = concentration (mM); RES = specific resistance (Ω m); x = coordinate (mm).

conductivity profiles for two different pH values of the leading electrolyte are shown. Fig. 10 depicts the results of experimental verification. The strange shape of the conductivity profile at pH 5.8 could cause an incorrect impression that a trace impurity is present in the boundary. Nevertheless, this is the natural shape of the boundary. Similar conductivity profiles of the boundaries between zones have already been published [11].

Models of electromigration that originate from basic physical and chemical laws provide a unifying basis for the description of all electrophoretic processes used in practice [17]. The described model can be employed to explain some phenomena in capillary zone electrophoresis described by Verheggen et al. [37]. It has been reported [38] that the method of introducing the sample into the capillary tube is important because only diffusional spreading of the peaks can develop during migration in a homogeneous electric field. It is advantageous if the conductivity of the initial sample area is less than that of the carrier electrolyte because the sample sharpens on the leading edge of the conductivity profile between the sample area and the remainder of
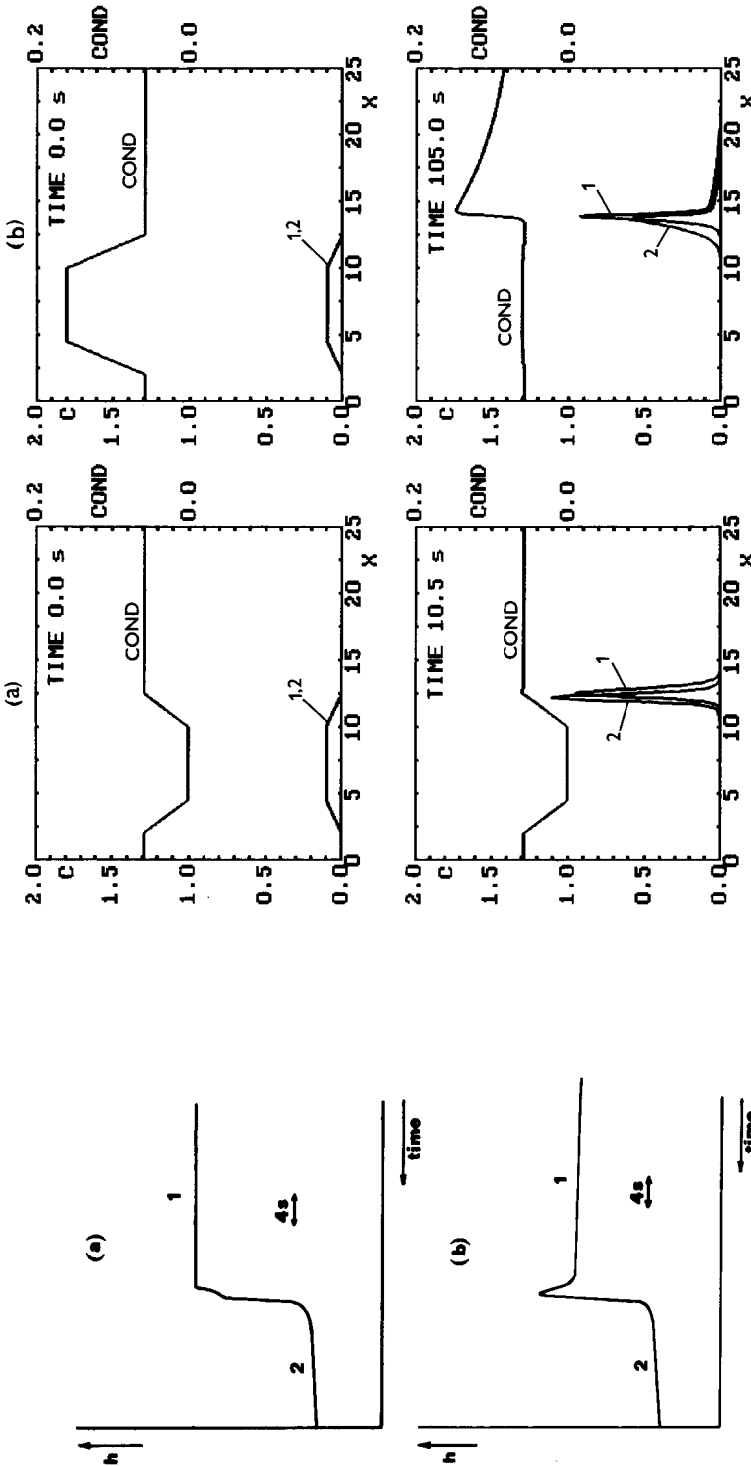
Fig. 10. Registration of the conductivity profile between aniline and H^+ zone. $1 = $ Aniline; $2 = H^+$. Leading electrolyte, 2 mM Bis–Tris plus acetic acid: (a) pH 5.8; (b) pH 5.0. Diameter of capillary tube, 0.3 mm; current, 10 μA.

Fig. 11. Simulation of the sharpening and separation of the sample in CZE, with 10 mM carrier strong electrolyte, anionic mobility, $u = 30 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; cationic mobility, $u = 29.6 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. $1 = $ First sample, $u = 40 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. $2 = $ second sample, $u = 30 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. COND $= $ specific conductivity (Ω^{-1} m^{-1}). Current density, 200 A m^{-2}. Concentration of Cl$^-$ in sample area: (a) $c_{Cl}^- = 0$; (b) $c_{Cl}^- = 15$ mM.

the carrier electrolyte. This is shown in Fig. 11, where the sharpening and separation of two hypothetical anionic substances in a strong electrolyte system is simulated.

Nevertheless, it is possible to use another means of sharpening the sample, namely by addition of an excess of some inactive, fastest or slowest substance. By an inactive substance is meant a substance that does not give a detector response. Fig. 11 shows the separation of the two substances if 15 mM chloride is added to the sample. It can be seen that sharpening could also be efficient in this instance. This sharpening effect may explain the very high plate numbers found for some early eluting peaks in Fig. 9 in ref. 37.

REFERENCES

1　F. Kohlrausch, *Ann. Phys. Chem.*, 62 (1897) 209.
2　J. L. Beckers, *Thesis*, Technical University of Eindhoven, Eindhoven, 1973.
3　F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis —Theory, Instrumentation and Application*, Elsevier, Amsterdam, Oxford, New York, 1976.
4　F. E. P. Mikkers, *Thesis*, Technical University of Eindhoven, Eindhoven, 1980.
5　F. E. P. Mikkers, F. M. Everaerts and J. A. F. Peek, *J. Chromatogr.*, 168 (1979) 293.
6　F. E. P. Mikkers, F. M. Everaerts and J. A. F. Peek, *J. Chromatogr.*, 168 (1979) 317.
7　F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 1.
8　P. Boček, P. Gebauer and M. Deml, *J. Chromatogr.*, 217 (1981) 209.
9　P. Boček, P. Gebauer and M. Deml, *J. Chromatogr.*, 219 (1981) 21.
10　P. Gebauer and P. Boček, *J. Chromatogr.*, 242 (1982) 245.
11　P. Gebauer and P. Boček, *J. Chromatogr.*, 267 (1983) 49.
12　L. Křivánková, F. Foret, P. Gebauer and P. Boček, *J. Chromatogr.*, 390 (1987) 3.
13　T. Hirokawa, K. Nakahara and Y. Kiso, *J. Chromatogr.*, 463 (1989) 51.
14　T. Hirokawa, K. Nakahara and Y. Kiso, *J. Chromatogr.*, 470 (1989) 21.
15　T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi and J. Akiyama, *J. Chromatogr.*, 271 (1983) D1.
16　T. Hirokawa, M. Nishino and Y. Kiso, *J. Chromatogr.*, 252 (1982) 49.
17　M. Bier, O. A. Palusinski, R. A. Mosher and D. A. Saville, *Science*, 219 (1983) 1281.
18　W. Thormann and R. A. Mosher, *Trans. Soc. Computer Simulation*, 1 (1984) 83.
19　W. Thormann, R. A. Mosher and M. Bier, *Electrophoresis*, 6 (1985) 78.
20　W. Thormann and R. A. Mosher, *Electrophoresis*, 6 (1985) 413.
21　R. A. Mosher and W. Thormann, *Electrophoresis*, 6 (1985) 477.
22　R. A. Mosher, W. Thormann and M. Bier, *J. Chromatogr.*, 320 (1985) 23.
23　D. A. Saville and O. A. Palusinski, *AIChE J.*, 32 (1986) 207.
24　O. A. Palusinski, A. Graham, R. A. Mosher, M. Bier and D. A. Saville, *AIChE J.*, 32 (1986) 215.
25　W. Thormann, R. A. Mosher and M. Bier, *J. Chromatogr.*, 351 (1986) 17.
26　R. A. Mosher and W. Thormann, *Electrophoresis*, 7 (1986) 395.
27　Y. Su, O. A. Palusinski and P. C. Fife, *J. Chromatogr.*, 405 (1987) 77.
28　R. Kuhn, H. Wagner, R. A. Mosher and W. Thormann, *Electrophoresis*, 8 (1987) 503.
29　R. A. Mosher, D. Dewey, W. Thormann, D. A. Saville and M. Bier, *Anal. Chem.*, 61 (1989) 362.
30　L. Juška, *Thesis*, Charles University, Prague, 1973.
31　B. Gaš, *Diploma Thesis*, Charles University, Prague, 1975.
32　V. Fidler, J. Vacík and Z. Fidler, *J. Chromatogr.*, 320 (1985) 167.
33　Z. Fidler, V. Fidler and J. Vacík, *J. Chromatogr.*, 320 (1985) 175.
34　F. B. Dismukes and R. A. Alberty, *J. Am. Chem. Soc.*, 76 (1954) 191.
35　R. A. Alberty and J. C. Nichol, *J. Am. Chem. Soc.*, 70 (1948) 2297.
36　A. Ralston, *A First Course in Numerical Methods*, McGraw-Hill, New York, 1965.
37　Th. P. E. M. Verheggen, A. C. Schoots and F. M. Everaerts, *J. Chromatogr.*, 503 (1990) 245.
38　F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.