

# Electromigration in systems with additives in background electrolytes

## II. Ionic admixture

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### ABSTRACT

A theoretical model is presented for isotachophoretic migration in systems where both the leading and terminating electrolytes contain another common component (admixture) (*e.g.*, a difficult to remove impurity). The effective mobility of the admixture is lower than that of the leading ion and higher than that of the terminating ion. The model system includes a further two samples. The mathematical simulation of the separation dynamics of this system was obtained by solution of basic physico-chemical laws. This facilitated a comprehensive formulation of the principles of the steady state and calculations of the parameters of all zones. Based on the theoretical model, a method for the determination of admixture concentrations in the leading and terminating electrolytes from isotachophoretic measurements and accurate determination of the limiting mobilities of the separated compounds from experimental data affected by the presence of the admixture are described.

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### INTRODUCTION

Various electrophoretic methods are based on the same separation principle and differ in the initial arrangement or the composition of the electrophoretic system. Real electrophoretic systems frequently cannot be distinguished unambiguously according to the conventional classification of electrophoretic methods. The systems, although designated as isotachophoretic but fulfilling only some demands of classical isotachopheresis (ITP), can be mentioned as examples.

Systems including a mixture of several components instead of one counter ionic species constitute one group [1,2]. The second large group is formed by systems in which the leading

electrolyte (LE) or the terminating electrolyte (TE) contains more components migrating in the same direction as the separated substances. So-called combined systems [3,4], where the LE contains some amount of terminating ions or the TE contains some amount of leading ions, are typical examples of this group. A mathematical model was formulated for anionic systems where terminating ions are present in the LE [5] and were denoted as systems with two leading ions. Based on this model, steady-state parameters of separated and terminating zones were calculated. Further, it was shown that at a certain concentration ratio of the two leading ions, also dependent on the effective mobilities of the ions, ITP changes into zone electrophoresis. In contrast, in zone electrophoresis some ions can migrate under certain conditions in the ITP mode [6]. The cationic system, in which a weak or strong acid is used as the LE and the mixture of this

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acid and its salt with a weak or strong base serves as the TE [7], also belongs to the group with mixed background electrolytes. It was shown that the parameters of all zones are affected not only by the composition of the LE but also by that of the TE.

The main effects, e.g., a decrease in the specific resistance and an increase in the zone length of separated components due to the presence of an admixture in their zones originating from the leading or the terminating electrolyte, are well known [5,7–11].

The purpose of this paper is to contribute to the theory of ITP with admixtures in background electrolytes and to the evaluation of the experimental data affected by the admixture.

## THEORY

### Fundamental characterization of the model system

Let us consider a model system in which both the LE and the TE contain another common ionic component, an admixture. The admixture migrates in the same direction as separated ionic species and its effective mobility is lower than that of the leading ion and higher than that of the terminating ion. Two ionic components, A and B, fulfilling the following relationship of the effective mobilities,  $m^{ef}$ , were chosen as samples:

$$m_{T,T}^{ef} < m_{B,B}^{ef} < m_{X,X}^{ef} < m_{A,A}^{ef} < m_{L,L}^{ef}$$

The first subscript indicates the substances: A and B are the samples, L is the leading ion, T is the terminating ion and X is the admixture. The second subscript specifies the zone (see Fig. 1). The arrangement of the model system at the initial state (at time  $t=0$ ) and at the steady state (at time  $t$ ) is illustrated schematically in Fig. 1. Not only the classical zones in the separation column (zones L, A, X, B and T) but also the electrolyte in the sampling compartment (zone TS) and the original terminating electrolyte in the electrode compartment (zone TE) are denoted as zones. Zones T and TS differ from each other in the concentration which is determined by the initial state. Zone T is created in the

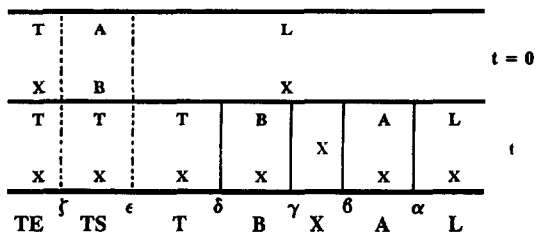


Fig. 1. Schematic illustration of the model system at the initial state (at time  $t=0$ ) and at the steady state (at time  $t$ ). The Greek letters denote boundaries and the dashed lines distinguish the stationary boundaries ( $\epsilon, \zeta$ ) from the migrating boundaries ( $\alpha, \beta, \gamma, \delta$ ). The counter ion R, which is considered identical in all zones, is not marked.

capillary where the leading electrolyte was initially and zone TS is created in the sampling compartment.

This model system enables one to study the instances where the admixture is separated from the leading and/or terminating electrolyte and creates not only the pure zone itself (zone X) but also the mixed zones with the samples (zones A and B).

In the theoretical model no complex equilibria are taken into account. The temperature changes resulting from the Joule heat and osmotic and convection flows are also omitted. The composition of the TE is maintained constant.

### Separation dynamics of the model system

The separation dynamics of the model system were solved by means of the simulation program of electromigration [12]. The graphical depiction of the concentration distribution and of the specific resistance along the capillary are obtained as the result. The algorithm presents the solution in a so-called "observational window", that shifts owing to the migration velocity of the leading ion and shows only the interesting part of the capillary.

The demonstration of the separation dynamics of the anionic model system consisting of uni-univalent strong electrolytes is shown in Fig. 2. It can be clearly seen from Fig. 2b that during the separation the admixture from the LE remains behind the leading ion and the admixture in the TE outruns the terminating ion. Simultaneously, samples A and B separate from each other and their concentrations are changed when they

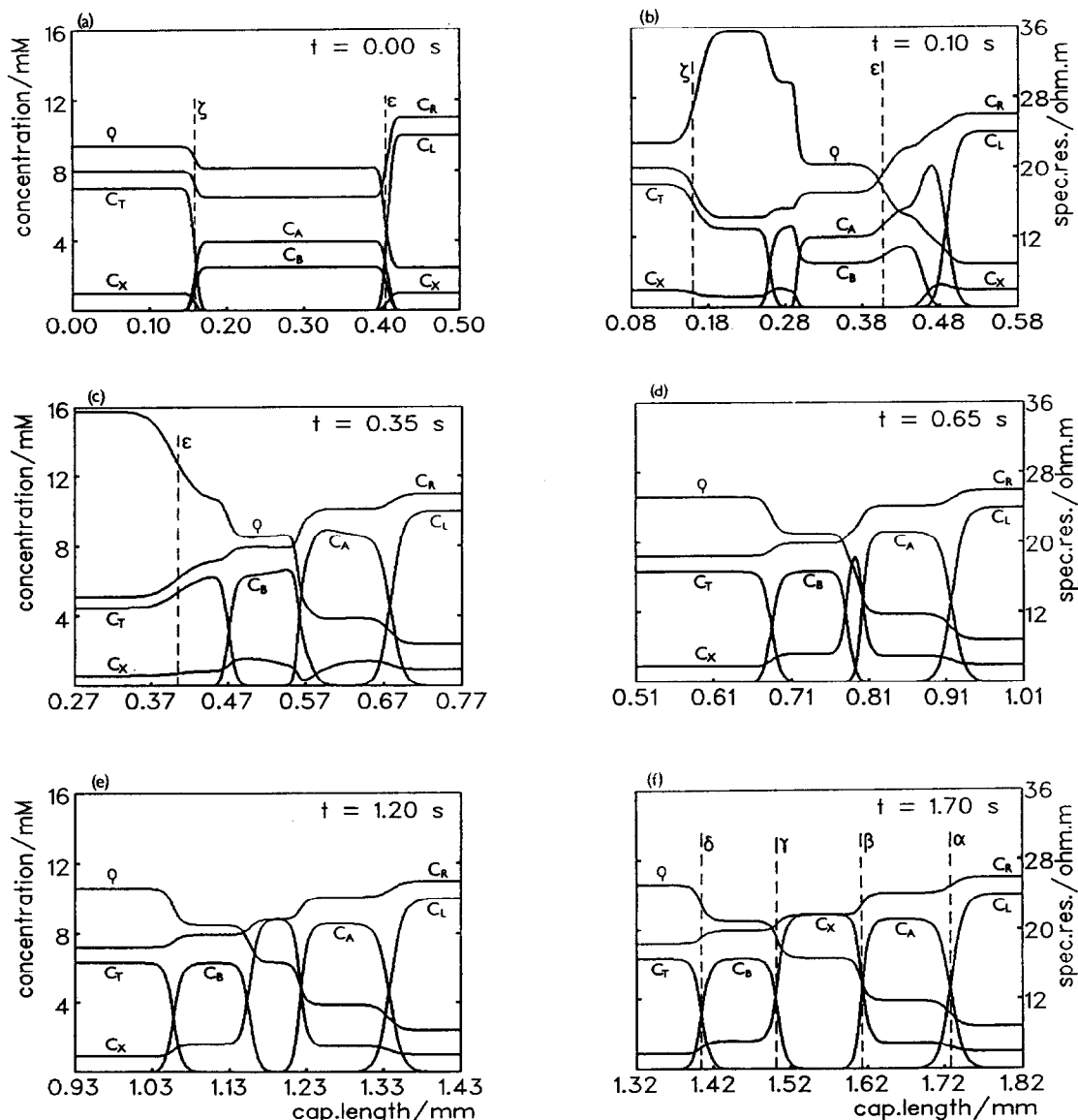


Fig. 2. Separation dynamics of the anionic model system. The mobility of the ions ( $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) are  $m_L = 80$ ,  $m_A = 60$ ,  $m_X = 40$ ,  $m_B = 30$ ,  $m_T = 25$  and  $m_R = 30$ . The initial concentrations (mM) [see also (a)] are  $c_L = 10$  and  $c_X = 1$  in both the LE and the TE,  $c_A = 4$ ,  $c_B = 2.5$  and  $c_T = 7$ . The current density is  $1100 \text{ A m}^{-2}$ .  $\rho$  = Specific resistance;  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  = migrating boundaries;  $\epsilon$ ,  $\zeta$  = stationary boundaries, which demarcate the sampling compartment (see also Fig. 1).

leave the sampling compartment. Similarly, the concentrations of the terminating ion and of the admixture are changed when they migrate into the sampling compartment or when they leave it (see Fig. 2b and c). The admixture further outruns the sample B and remains behind the sample A and so forms its pure zone (see Fig. 2d). After the steady state has been reached

(Fig. 2e and 2f), the concentrations of all components in all zones are constant both with time and along the zones. The lengths of zones A and B also do not change with time. On the other hand, the length of zone X increases with time.

Further, it can be concluded that the boundaries  $\alpha$  and  $\beta$  migrate at the same velocity equal to the velocity of the leading ion. The

boundaries  $\gamma$  and  $\delta$  also have the same velocity but different from that of  $\alpha$  and  $\beta$ . With the chosen values of the initial concentrations and mobilities, this separation represents a combination of ITP with the method of moving boundaries. The concept of a "steady state", taken over the theory of classical ITP, is understood as the state where the concentrations of all components in all zones are constant.

#### Steady state of the model system

The mathematical models are formulated for both strong and weak uni-univalent electrolytes. The concentrations of all components in all zones and the zone lengths are obtained as solution of these models.

In the theory of classical ITP, a uniform mathematical description can be used for all separated zones. In the given model system, the zones have different characteristics and, therefore, the mathematical description of every zone must be done separately.

#### Model system formed by strong uni-univalent electrolytes

In this mathematical model, mobility is considered as a quantity independent on ionic strength. This assumption enables one to acquire the solution in the analytical form.

Zone A includes the ionic species of sample A, the ionic species of admixture X and the counter ionic species R. The concentration of the admixture in the zone A,  $c_{X,A}$ , can be calculated from the equation describing the mass balance of the admixture for the boundary  $\alpha$ :

$$c_{X,L}(m_X E_L - m_L E_L) = c_{X,A}(m_X E_A - m_L E_L) \quad (1)$$

where  $m$  is the ionic mobility and  $E$  is the potential gradient. The subscripts on  $m$  and  $E$  denote the ionic species and the zone, respectively. Because the ITP condition

$$m_L E_L = m_A E_A \quad (2)$$

is fulfilled for zone A, eqn. 1 can be expressed in the form

$$c_{X,A} = c_{X,L} \cdot \frac{m_X - m_L}{m_L} \cdot \frac{m_A}{m_X - m_A} \quad (3)$$

The concentration  $c_{A,A}$  can be obtained from the

Kohlrausch regulating function (KRF) including the electroneutrality condition (EN):

$$c_{A,A} = c_{L,L} \cdot \frac{m_L + m_R}{m_L} \cdot \frac{m_A}{m_A + m_R} + c_{X,L} \cdot \frac{m_X + m_R}{m_X} \cdot \frac{m_A}{m_A + m_R} - c_{X,A} \cdot \frac{m_X + m_R}{m_X} \cdot \frac{m_A}{m_A + m_R} \quad (4)$$

The second term on the right-hand side expresses the increase in the concentration  $c_{A,A}$  due to the presence of the admixture in the LE (it is analogous to the first term) and the third term represents the decrease in the concentration  $c_{A,A}$  due to the presence of the admixture in zone A.

The simplest is zone X, containing only the ionic species of the admixture and counter ionic species. Therefore, we use the KRF and the EN for expressing  $c_{X,X}$ :

$$c_{X,X} = c_{L,L} \cdot \frac{m_L + m_R}{m_L} \cdot \frac{m_X}{m_X + m_R} + c_{X,L} \quad (5)$$

If  $c_{X,L} = 0$  (so the admixture will be only in the TE), eqn. 5 will be reduced to the form known from classical ITP for a sample.

In zone T, as in the previous instances, the KRF including the EN serves as the first equation for the unknown concentrations  $c_{T,T}$  and  $c_{X,T}$ . The mass balance for the immobile concentration boundaries  $\epsilon$  and  $\zeta$ :

$$\frac{c_{T,T}}{c_{X,T}} = \frac{c_{T,TS}}{c_{X,TS}} = \frac{c_{T,TE}}{c_{X,TE}} \quad (6)$$

can be used as the second equation. This mass balance expresses, that all ionic species are diluted or concentrated over the immobile boundary to the same extent. The solution of these two equations leads to

$$c_{T,T} = \left( c_{L,L} \cdot \frac{m_L + m_R}{m_L} + c_{X,L} \cdot \frac{m_X + m_R}{m_X} \right) \times \frac{c_{T,TE}}{c_{T,TE} \cdot \frac{m_T + m_R}{m_T} + c_{X,TE} \cdot \frac{m_X + m_R}{m_X}} \quad (7)$$

A similar relationship can be obtained for the

concentration of the admixture in zone T by combining eqns. 6 and 7. It is clear that the composition of zone T depends not only on the parameters of the LE but also on those of the TE.

An analogous approach to that for zone A can be taken for the expression of parameters of zone B. The concentration  $c_{B,B}$  is given by

$$c_{B,B} = c_{L,L} \cdot \frac{m_L + m_R}{m_L} \cdot \frac{m_B}{m_B + m_R} + c_{X,L} \cdot \frac{m_X + m_R}{m_X} \cdot \frac{m_B}{m_B + m_R} - c_{X,B} \cdot \frac{m_X + m_R}{m_X} \cdot \frac{m_B}{m_B + m_R} \quad (8)$$

For the boundary  $\delta$  the mass balance of admixture can be written as

$$c_{X,B}(m_X E_B - m_T E_T) = c_{X,T}(m_X E_T - m_T E_T) \quad (9)$$

and after rearrangement by means of

$$m_B E_B = m_T E_T \quad (10)$$

we obtain the relationship for the concentration of the admixture in zone B:

$$c_{X,B} = c_{X,T} \cdot \frac{m_X - m_T}{m_T} \cdot \frac{m_B}{m_X - m_B} \quad (11)$$

The parameters of zone B are also dependent on the composition of the TE owing to the concentration  $c_{T,T}$  in eqn. 11.

If steady-state concentrations are known, the relationships for the zone length can be derived. Generally, the length of the separated zone  $k$ ,  $l_k$ , is given by

$$l_k = \frac{n_{k,k}}{c_{k,k} S} \quad (12)$$

where  $S$  is the inner cross-section of the capillary and  $n_{k,k}$  is the amount of component  $k$  in the  $k$ th zone. The amounts  $n_{A,A}$  and  $n_{B,B}$  correspond to the sampling amounts  $n_{A,0}$  and  $n_{B,0}$ , respectively. For the admixture the amount  $n_{X,X}$  is dependent on time. The following consideration can be applied for its derivation. The admixture goes to zone X from both the leading and the terminating zones. The contribution from the LE (the

TE) is given by the amount  $n_{X,\alpha}$  ( $n_{X,\zeta}$ ) that passes the boundary  $\alpha$  ( $\zeta$ ) during the time  $t$ :

$$n_{X,\alpha} = c_{X,L}(m_L - m_X)E_L t S \quad (13)$$

$$n_{X,\zeta} = c_{X,TE} m_X E_{TE} t S \quad (14)$$

These contributions must be deducted by the amount of the admixture contained in the zones A, B, T and TS. These amounts can be generally described as

$$n_{X,k} = c_{X,k} l_k S \quad (15)$$

The following relationship can be derived for the length of the zone T, which is dependent on time:

$$l_T = m_T E_T t - l_{TS} \cdot \frac{E_T}{E_{TS}} \quad (16)$$

Combining the set of eqns. 12–16, we obtain

$$l_X = \frac{c_{X,L}}{c_{X,X}} (m_L - m_X) E_L t + \frac{c_{X,TE}}{c_{X,X}} (m_X - m_T) E_{TE} t - \frac{c_{X,A} n_{A,0}}{c_{X,X} c_{A,A} S} - \frac{c_{X,B} n_{B,0}}{c_{X,X} c_{B,B} S} \quad (17)$$

One important conclusion can be drawn from eqn. 17: the calculation of the zone length of the admixture can be done without a knowledge of the concentrations of the constituents in the zone TS.

#### Model system formed by weak uni-univalent electrolytes

In this theoretical model, the dependence of ionic mobilities on ionic strength is taken into account. As most of the zones in our model system are mixed zones, the Onsager–Fuoss theory [13], which incorporates also the so-called mixing effect, was used. The McInnes approximation of the activity coefficient was chosen for recalculation of the thermodynamic dissociation constants for a given ionic strength.

The pH and concentrations of ionic forms of given constituents must be calculated in all zones (even in the LE and TE). For the calculation of these unknown parameters, we have the electroneutrality condition and the set of equations describing dissociation equilibria. In the separated zones (zones A, X, B and T) the analytical

concentrations,  $c^{\text{tot}}$ , are also unknown, so that the equations of the mass balance must be added. The parameters of the LE determine the parameters of the subsequent zones. This is expressed in all zones by the mass balance of the buffer:

$$c_{R,k}^{\text{tot}}(m_{R,k}^{\text{ef}}E_k + v_{k/k-1}) = c_{R,k-1}^{\text{tot}}(m_{R,k-1}^{\text{ef}}E_{k-1} + v_{k/k-1}) \quad (18)$$

where  $v_{k/k-1}$  represents the velocity of the boundary between the  $k$ th and  $(k-1)$ th zone. For the boundary velocities we can write

$$v_\gamma = v_\delta \quad \text{so} \quad m_{B,B}^{\text{ef}}E_B = m_{T,T}^{\text{ef}}E_T \quad (19)$$

$$v_\alpha = v_\beta \quad \text{so} \quad m_{L,L}^{\text{ef}}E_L = m_{A,A}^{\text{ef}}E_A \quad (20)$$

Another equation must give the information about the migration flow of the admixture:

$$c_{X,k}^{\text{tot}}(m_{X,k}^{\text{ef}}E_k - v_{k/k-1}) = c_{X,k-1}^{\text{tot}}(m_{X,k-1}^{\text{ef}}E_{k-1} - v_{k/k-1}) \quad (21)$$

Eqns. 18 and 21 together with the EN and the dissociation equilibria are sufficient to describe the five parameters in zone X. Eqns. 19 and 20 must be added to this set for the description of the seven unknown parameters in zones A and B, respectively. In zone T there are also seven unknown parameters. Eqns. 18 and 21 cannot be used for zone T and the closely preceding zone B, because the parameters of zone B depend on zone T and therefore they must be solved subsequently. Considering that the steady state is being described and eqn. 20 is valid, the mass balance of the buffer and the admixture for zones T and X can be used. The dependence of the parameters of zone T on the parameters of the TE is expressed by means of the mass balance of the immobile boundaries  $\epsilon$  and  $\zeta$ :

$$c_{X,TE}^{\text{tot}}m_{X,TE}^{\text{ef}}E_{TE} = c_{X,T}^{\text{tot}}m_{X,T}^{\text{ef}}E_T \quad (22)$$

The relationships for the zone length are analogous to those for the strong electrolytes:

$$l_k = \frac{n_{k,k}}{c_{k,k}^{\text{tot}}S} \quad (23)$$

The considerations leading to the derivation of  $n_{X,X}$  are also the same. Nevertheless, for strong

electrolytes the mobility of an ionic species is considered to be constant in all zones. Consequently, the zone length  $l_X$  is not influenced by the parameters of zone TS. This simplifying assumption is not used for weak electrolytes. Because the calculation of the steady-state parameters of zone TS is feasible only by considering similar assumptions, for the zone length  $l_X$  the following approximate relationship is used:

$$l_X = \frac{c_{X,L}^{\text{tot}}}{c_{X,X}^{\text{tot}}} (m_{L,L}^{\text{ef}} - m_{X,L}^{\text{ef}})E_L t + \frac{c_{X,TE}^{\text{tot}}}{c_{X,X}^{\text{tot}}} \left( m_{X,TE}^{\text{ef}} - m_{T,T}^{\text{ef}} \frac{m_{X,TE}^{\text{ef}}}{m_{X,T}^{\text{ef}}} \right) E_{TE} t - \frac{c_{X,A}^{\text{tot}}n_{A,0}}{c_{X,X}^{\text{tot}}c_{A,A}^{\text{tot}}S} - \frac{c_{X,B}^{\text{tot}}n_{B,0}}{c_{X,X}^{\text{tot}}c_{B,B}^{\text{tot}}S} \quad (24)$$

The zone length  $l_k$  is not experimentally available. The detector records the time during which the zone is passing through. This value, proportional to the zone length, will be called the time zone length,  $\Delta t_k$ . For the time length of the sample zone the following simple equation is valid:

$$\Delta t_k = \frac{l_k}{m_{k,k}^{\text{ef}}E_k} \quad (25)$$

For the time length of the admixture zone the calculation is more complicated because the admixture does not migrate at the same velocity as the boundaries that demarcate this zone. As was mentioned above, the length  $l_X$  is elongated during passage through the detector. The time length  $\Delta t_X$  can be expressed as

$$\Delta t_X = \frac{l_X}{m_{B,B}^{\text{ef}}E_B} \quad (26)$$

where  $l_X$  is the zone length of the admixture in time  $t_\beta$ , when the boundary  $\beta$  is missing the detector. For  $t_\beta$  we can write

$$t_\beta = \frac{l_c}{m_{L,L}^{\text{ef}}E_L} + \Delta t_A \quad (27)$$

where  $l_c$  is the length of the capillary from the sampling compartment to the detector.

An interesting difference exists in the characteristics of the zone of samples and of admix-

tures: the time zone lengths of samples are independent of the capillary inner cross-section, but the time zone length of the admixture is influenced.

#### Calculation procedure

Various properties of the zone are reflected in the mathematical description, but the procedure of numerical evaluation is the same for all zones. The set of non-linear algebraic equations are solved by the Newton–Raphson iteration method [14]. Another iterative cycle consists of the recalculation of the limiting mobilities and the dissociation constants for the ionic strength in the respective zone. The sequence in which the zones are solved corresponds to their sequence in the capillary only for the first three, *i.e.*, for zones L, A and X. Then zone TE must be computed because the subsequently solved zones T and B are dependent on its parameters.

#### EXPERIMENTAL

The computational algorithm was programmed in Pascal and runs on PC computers.

A laboratory-made ITP apparatus equipped with a high-frequency contactless conductivity detector [15] was used in all experiments. The electrode compartments and sampling valve were from the commercial apparatus AGROFOR

(JZD, Odra Krmelín, Czech Republic). The measuring cell was thermostated at 25°C by means of a water thermostat. Non-linear calibration of the detector was performed without an electric current. A constant current of 30  $\mu\text{A}$  was applied in all experiments. The maximum change of the specific resistance caused by passing the electric current was less than 0.3%.

Chemicals used for the preparation of the leading and terminating electrolytes were of analytical-reagent grade.

#### RESULTS AND DISCUSSION

##### Verification of the theoretical model for strong electrolytes

The theoretical model of a steady state for strong electrolytes was verified by means of the simulation program of electromigration that gives information not only about the concentrations of all constituents but also about zone lengths in a given time. The system on which the simulation was demonstrated (input data are given in Fig. 2) was explicitly used for the comparison. The results of the steady-state model together with those obtained by the simulation are presented in Table I.

It can be concluded that both methods of solution afford the same results.

TABLE I

VALUES OF THE STEADY-STATE CONCENTRATION AND THE ZONE LENGTH CALCULATED ON THE BASIS OF THE MATHEMATICAL MODEL OF THE STEADY STATE (1) AND THOSE OBTAINED USING THE SIMULATION PROGRAM OF ELECTROMIGRATION (2)

The input data of the model system are given in Fig. 2. The time  $t = 1.7$  s.

Calculation method	Steady-state concentration (mM)						
	$c_{A,A}$	$c_{X,A}$	$c_{X,X}$	$c_{B,B}$	$c_{X,B}$	$c_{T,T}$	$c_{X,T}$
1	8.583	1.500	8.857	6.327	1.627	6.327	0.904
2	8.579	1.500	8.856	6.326	1.627	6.327	0.903
	Zone length (mm)						
	$l_A$		$l_X$		$l_B$		
1	0.0117		0.0109		0.0099		
2	0.0118		0.0110		0.0099		

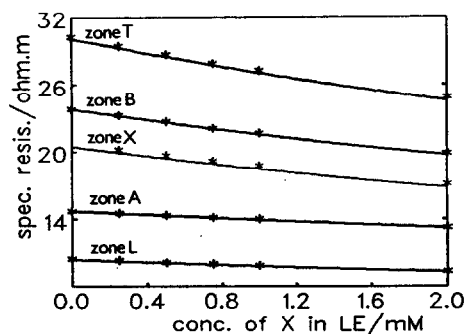


Fig. 3. Calculated (lines) and experimental (asterisks) relationships between the specific zone resistance and the concentration of the admixture in the LE. The concentration of the admixture in the TE is zero.

#### Verification of the theoretical model for weak electrolytes

The theoretical model for weak electrolytes was verified experimentally. The dependences of the specific resistance and the time length of the zones on the concentration of the admixture in the LE and the TE were both computed theoretically and measured experimentally. The results were processed graphically and are demonstrated in the Figs. 3–6.

The leading electrolyte consists of 0.01 M  $\text{Cl}^-$  as the leading ion, histidine as the buffer counter ion with a total concentration of 0.02 M and various concentrations of acetic acid as the admixture X. The terminating electrolyte contains 0.005 M glutamic acid, 0.01 M histidine and various concentrations of acetic acid. A mixture of formic and propionic acid is used as the

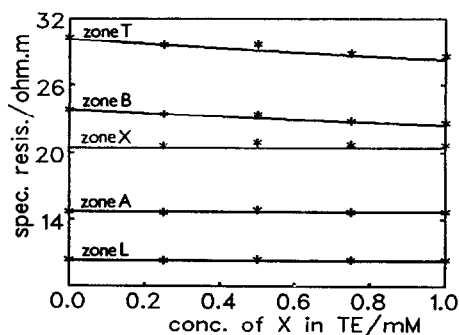


Fig. 4. Calculated (lines) and experimental (asterisks) relationships between the specific zone resistance and the concentration of the admixture in the TE. The concentration of the admixture in the LE is zero.

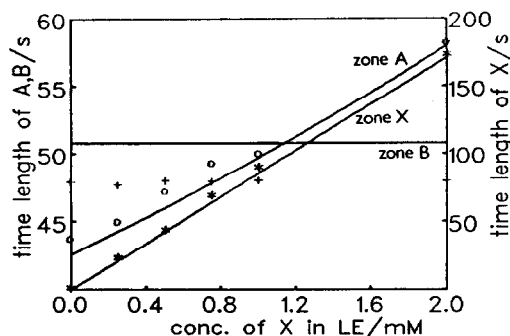


Fig. 5. Calculated (lines) and experimental (points: O = zone A, \* = zone X; + = zone B) relationships between the time zone length and the concentration of the admixture in the LE. The concentration of the admixture in the TE is zero.

sample, both at a concentration of 0.0004 M. Formic acid represents sample A in the model system and propionic acid corresponds to the sample B. All ionic limiting mobilities and pK values used in the calculation are given in Table II.

As it is clearly seen from Figs. 3 and 4, the agreement between the calculated and measured values of the specific zone resistance is very good. Poorer agreement was achieved between the measured and calculated time zone lengths (see Figs. 5 and 6), but the character of the experimental relationships corresponds to that of the theoretical relationships. For example, in both instances, the time zone length of propionic acid is independent of  $c_{X,LE}$  but the measured  $\Delta t_B$  value is ca. 5% lower than theoretical value even for zero concentration of the admixture

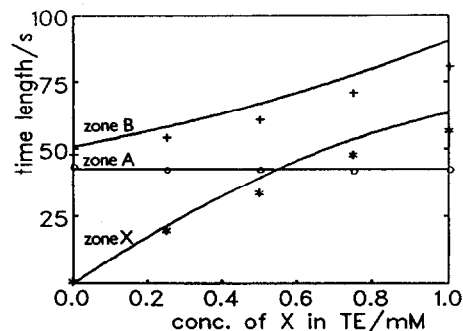


Fig. 6. Calculated (lines) and experimental (points: O = zone A, \* = zone X, + = zone B) relationships between the time zone length and the concentration of the admixture in the TE. The concentration of the admixture in the LE is zero.



TABLE II  
pK VALUES AND LIMITING MOBILITIES FOR THE IONIC SPECIES USED IN THE CALCULATION

Component	pK	$m^0$ ( $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ )
Acetic acid (-1)	4.76	42.4
Formic acid (-1)	3.75	56.6
Glutamic acid (-1)	4.32	28.9
Histidine (+1)	6.03	29.7
Hydrochloric acid (-1)	-2.00	79.1
Propionic acid (-1)	4.87	37.1

$c_{X,LE}$ . The explanation may lie in the inconvenient construction of the sampling valve. The broken shape of the valve gives rise to a “dead space” (the space without a potential gradient) and consequently detains some amount of the sample. This amount can depend on the sample mobility and therefore the differences between the measured and the calculated values can have different magnitudes for different samples.

#### Determination of admixture concentration in background electrolytes

If the admixture is contained only in the LE or in the TE, the determination is easy on the basis of the known relationships for  $\Delta t_X$  versus  $c_{X,LE}$  and  $\Delta t_X$  versus  $c_{X,TE}$ , respectively, if no sample is applied.

If the admixture is present in both the LE and the TE simultaneously, then the time length of

the admixture zone is given by the sum of the contributions from the LE and the TE. In this more complicated case we can utilize the fact that the parameters of zone A (the zone of the sample with a higher effective mobility than that of the admixture) are influenced only by parameters of the LE. The admixture concentration in the LE,  $c_{X,LE}$  is determined from the difference between the time length of zone A with zero concentration of the admixture (computed theoretically) and that with the investigated concentration of the admixture (measured experimentally). From the known concentration  $c_{X,LE}$  the contribution of the LE to the time length of the admixture zone is computed and the remainder of this time zone length corresponds to the admixture concentration in the TE.

A computer program for the calculation of the concentrations of the admixture existing simultaneously in the LE and the TE from data from one experiment (the time lengths of zones A and X) was set up. The results of this program together with input experimental data for the three known values of  $c_{X,LE}$  and  $c_{X,TE}$  are given in Table III.

The principle of the determination utilizes the difference between two values, so the accuracy is lower than that of quantitative ITP analyses. This method can be used (especially if  $c_{X,LE}$  is less than 10% of  $c_{L,LE}$ ) only for rough estimations. However, on the other hand it must be realized that if, e.g.,  $c_{X,LE}$  equals 10% of  $c_{L,LE}$

TABLE III  
DETERMINED VALUES OF THE CONCENTRATIONS OF THE ADMIXTURE FROM THE EXPERIMENTALLY OBTAINED TIME ZONE LENGTHS FOR THREE KNOWN CONCENTRATIONS  $c_{X,L}^{tot}$  AND  $c_{X,TE}^{tot}$

The theoretically computed time zone length of sample A (formic acid) for zero concentration of the admixture in the LE is  $\Delta t_A = 42.60$  s.

No.	$\Delta t$ (s)		$c$ (mM)			
	$\Delta t_A$	$\Delta t_X$	$c_{X,L}^{tot}$		$c_{X,TE}^{tot}$	
			Determined	Real	Determined	Real
1	50.15	142.83	1.06	1.00	0.44	0.50
2	49.61	146.49	0.99	0.75	0.53	0.75
3	58.06	199.81	2.01	2.00	0.21	0.25

and is determined within a 20% deviation, we are able to correct the parameters of the LE within an accuracy of ca. 2%.

#### Determination of limiting mobilities

In classical ITP a relative qualitative characteristic  $R_E$  (ratio of potential gradients of sample and leading zones) [16] is used for the determination of the limiting mobilities of a sample from experimental ITP data. The advantage of  $R_E$  over other relative characteristics used in ITP is given by the ITP condition stating that the  $R_E$  is directly equal to the ratio of the effective mobilities of the leading and sample constituents. In our model system, the ITP condition is not generally fulfilled. Nevertheless, it holds that sample A migrates at the same velocity as the leading ion and the migrating velocity of the sample B is equal to that of the terminator in zone T. This means that for the determination of the limiting mobility of sample A (the sample with a higher effective mobility than that of the admixture), the  $R_E$  value can be used:

$$R_{E,A} = \frac{E_A}{E_L} = \frac{m_{L,L}^{ef}}{m_{A,A}^{ef}} \quad (28)$$

For expression of the effective mobility of sample B (the sample with a lower effective mobility than that of the admixture) we can analogously introduce a relative qualitative characteristic

referred to the adjusted terminating zone T which we shall denote by the symbol  $R_{ET}$ :

$$R_{ET,B} = \frac{E_B}{E_T} = \frac{m_{T,T}^{ef}}{m_{B,B}^{ef}} \quad (29)$$

The computing algorithm results from the mathematical description of the model system mentioned above. First the value of  $m_{L,L}^{ef}$  is found by the solution of zone L. From the experimentally obtained value of  $R_{E,A}$ , the effective mobility of substance A is expressed by using eqn. 28. In a similar way, the effective mobility of substance B is determined from  $R_{ET,B}$  and  $m_{T,T}^{ef}$ . For calculation of the  $m_{T,T}^{ef}$ , the parameters of zones X, TE and T must be known. Then the limiting mobilities of components A and B (approximate values must be given as an initial estimate) are computed from the effective values by iterative calculation of the parameters of the respective zones. It must be emphasized that for the determination of the limiting mobility of sample A, the input data consist only of the parameters of the LE (the limiting mobilities, pK and the total concentrations of the leading ionic species, of the buffer and of the admixture), but for sample B the parameters of the TE must also be known.

The determined values of the limiting mobilities of formic and propionic acid from the experimentally obtained values of  $R_{E,A}$  and  $R_{ET,B}$ , respectively, are given in Table IV for the

TABLE IV

DETERMINED VALUES OF THE LIMITING MOBILITIES ( $m^0$ ) OF FORMIC AND PROPIONIC ACID FROM THE EXPERIMENTALLY OBTAINED VALUES OF  $R_{E,A}$  AND  $R_{ET,B}$ , RESPECTIVELY, (1) USING THE EXPERIMENTALLY ACQUIRED CONCENTRATIONS  $c_{X,L}^{tot}$  AND  $c_{X,TE}^{tot}$  (SEE TABLE III) AND (2) SUPPOSING THE CONCENTRATIONS OF THE ADMIXTURE ARE UNKNOWN AND THEREFORE ZERO VALUES WERE USED FOR THEM

The tabulated limiting mobilities are for formic acid  $m_A^0 = 56.6 \cdot 10^{-9}$  and for propionic acid  $m_B^0 = 37.1 \cdot 10^{-9} \text{ V}^{-1} \text{ m}^2 \text{ s}^{-1}$ .

Real concentration (mM)		$R_{E,A}$	$m_A^0$ ( $10^{-9} \text{ V}^{-1} \text{ m}^2 \text{ s}^{-1}$ )		$R_{ET,B}$	$m_B^0$ ( $10^{-9} \text{ V}^{-1} \text{ m}^2 \text{ s}^{-1}$ )	
$c_{X,L}^{tot}$	$c_{X,TE}^{tot}$		(1)	(2)		(1)	(2)
1.00	0.50	1.421	56.65	56.58	0.794	37.24	37.02
0.75	0.75	1.419	56.72	56.66	0.794	37.23	37.02
2.00	0.25	1.422	56.67	56.54	0.798	37.26	36.85

three different concentrations of acetic acid in the LE and the TE. As can be seen, the differences between the determined and the tabulated values are less than 0.5%. On the other hand, using the  $R_{E,B}$  value for calculation of the limiting mobility for propionic acid would give, e.g., for 0.75 mM acetic acid in both the LE and TE the value  $m_B^0 = 40.22 \cdot 10^9 \text{ V}^{-1} \text{ m}^2 \text{ s}^{-1}$ , which means an 8% deviation from the tabulated value.

For the concretely used substances of our model system, the quantities  $R_{E,A}$  and  $R_{ET,B}$  show an interesting feature. As Figs. 7 and 8 illustrate, their values are hardly influenced by the presence of the admixture in the LE and in the TE. Beckers and Everaerts [5] considered this feature of  $R_E$  to be as a consequence of the validity of the ITP condition. However, from the ITP condition it only follows that the quantities  $R_E$  and  $R_{ET}$  are equal to the ratio of the effective mobilities of the respective pair (see eqns. 28 and 29). Therefore, for uni-univalent electrolytes it can be expected that the quantities  $R_E$  and  $R_{ET}$  will not be dependent on the presence of the admixture in the LE and the TE if any of the following conditions is fulfilled: (a) the pH of the LE is maintained at a constant value; (b) the effective mobilities of both members of the respective pair do not depend on pH (e.g., it holds for the chloride-formate pair, where HCl is a strong acid and HCOOH is fully dissociated at the pH of the LE); (c) the dependence of the effective mobilities on pH is roughly the same

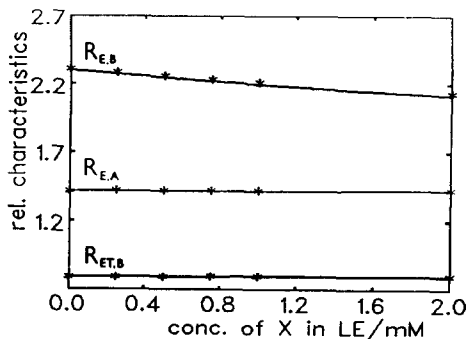


Fig. 7. Calculated (lines) and experimental (asterisks) relationships between the relative characteristics  $R_{E,A}$ ,  $R_{E,B}$  and  $R_{ET,B}$  and the concentration of the admixture in the LE. The concentration of the admixture in the TE is zero.

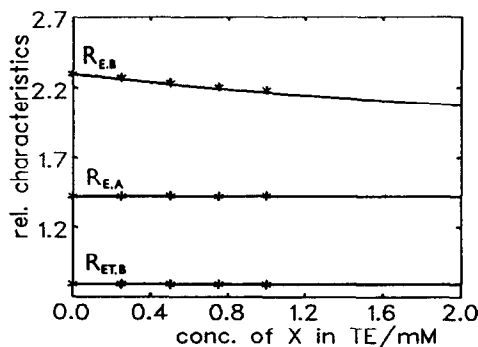


Fig. 8. Calculated (lines) and experimental (asterisks) relationships between the relative characteristics  $R_{E,A}$ ,  $R_{E,B}$  and  $R_{ET,B}$  and the concentration of the admixture in the TE. The concentration of the admixture in the LE is zero.

for both components (e.g., glutamic and propionic acid, which have similar pK values).

The above-mentioned consideration results in an important conclusion. If the LE and the TE can be chosen or prepared in such a way that some of the given demands are fulfilled, the limiting mobilities of samples can be determined without a knowledge of the concentrations of the admixture in the LE and the TE. This is also demonstrated by the results given in Table IV.

## CONCLUSIONS

Isotachophoretic migration in systems with an admixture in the background electrolytes has been described. The approach for the determination of the limiting mobilities of separated compounds from experimental data affected by presence of the admixture was introduced. It was shown the attention must be paid to such separated compounds which have effective mobilities lower than that of the admixture. For evaluation of these compounds it is necessary to use the newly established experimental characteristic  $R_{ET}$ .

ITP anionic separation at  $\text{pH} > 7$ , when carbonate anions are an unremovable admixture, is a typical situation when this effect must be considered. The presented mathematical model for uni-univalent electrolytes can be applied to this problem only up to  $\text{pH} 8.5$ . In forthcoming work this model will be extended to polyvalent electrolytes and used at higher pH for the

accurate determination of anionic limiting mobilities of amino acids and peptides.

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