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Calculation of the composition of sample zones in capillary zone electrophoresis

III. Weak bases and the application of background electrolytes with two co-ions

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Abstract

All parameters of the sample zones in electrophoretic processes can be calculated by using a mathematical model based on the repeated application of steady-state equations, viz., the modified version of Ohm's law, electroneutrality and all mass balances. From calculations with this model, rules of thumb have been deduced to predict the fronting/tailing character of sample peaks, the question of peaks and dips, the pH and the transfer ratio for weak bases in capillary zone electrophoresis. Furthermore, the mathematical model is extended in order to calculate the parameters of sample zones in capillary zone electrophoresis by applying background electrolytes with two co-ions, through which insight can be obtained concerning mobility-selective displacement and system peaks. In applying background electrolytes with two co-ions, all concepts concerning peak/dips, transfer ratio and fronting/tailing character lose their meaning.

Keywords: Sample zone composition; Background electrophoresis; Mathematical model; Fronting; Tailing; Weak bases

1. Introduction

In capillary zone electrophoresis (CZE), the mobilities and pK values of the sample, co- and counterions often play a prominent part in the phenomena encountered. The fronting/tailing character of sample peaks, for example, is determined by the mobilities of the components. It may be generally stated that if the mobility of the sample component is higher than that of the co-ions of the background electrolyte (BGE), fronting sample peaks may be expected, in which case the pH in the sample zone increases for bases. In order to enhance the sensitivity in UV detection, the electrodispersive effect has to be minimized by choosing a BGE, with a co-ion

whose mobility is as close as possible to that of the sample component [1]. Applying indirect UV detection, UV-transparent components may be present in the electropherograms, both as peaks or dips [2], depending on the transfer ratio T_R [3], which can be calculated from the mobilities of the components. If the T_R is smaller than one, the ionic strength in the sample peak increases, causing an increase in the concentration of counter ions, through which a sample peak is obtained for UV-transparent components, applying UV-absorbing counter ions [2]. For BGEs with two co-ions, even a mobility-selective displacement was observed [4] and a model was set up to explain this phenomenon. If fully-ionized ionic species are considered in theoretical and experimen-

tal studies, the simplified version of Kohlrausch's law can be applied for calculations and to explain observed phenomena, in which case ionic mobilities have to be used. For weak acids and bases, however, the situation is complicated and very different. To answer the question of whether ionic or effective mobilities should be used, non-steady-state electrophoretic processes have to be studied. Generally, these processes are described by differential equations [5–7], or by the application of "eigenvectors" [8], but solutions are often difficult to obtain. If an electric current is passed in an electrolyte solution, at each point of time and space the law of electro-neutrality and the modified version of Ohm's law ($E\sigma = \text{constant}$, see Section 3.2) must be satisfied and applying the mass balances for all ionic species, all electrophoretic processes can be described by some simple mathematical equations. For steady-state processes, such as ITP [9], these models are generally known but they also can be applied for non-steady-state processes. For capillary zone electrophoresis, sample peaks can be divided into small segments with different sample concentrations and starting from the composition of the BGE, the composition and all parameters of proceeding segments can be calculated with the above-mentioned equations. With such a mathematical model [10], realistic simulated electropherograms can be obtained [11]. In this paper we will apply this model in order to discuss the change of the pH in sample zones, the fronting/tailing character of sample peaks and to answer the question of "peaks and dips" in indirect UV detection. Furthermore, rules of thumb will be derived for the change of these parameters in sample zones of weak bases for several cases. The mathematical model for the calculation of parameters in sample zones will further be extended to explain some interesting phenomena in the application of two co-ions.

2. Experimental

For all CZE experiments a P/ACE System 2000 HPCE (Beckman, Fullerton, CA, USA) was used. All experiments were carried out with a Beckman eCAP capillary tubing (75 μm I.D.) 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. All experiments were carried out in the cationic mode applying a constant voltage of 5 kV, unless stated otherwise, and the operating temperature was 25°C. Sample introduction was performed by applying pressure injection where a 1-s pressure injection represents an injected volume of ca. 6 nl and an injected length of 0.136 cm. Data analysis was performed using the laboratory-written data analysis program CAESAR.

3. Theory

All parameters in sample zones are determined by Kohlrausch's regulation function [12], often applied in the form

$$\sum_i \frac{c_i}{m_i} = \omega \quad (1)$$

for fully-ionized monovalent ionic species, where c_i and m_i represent the ionic concentrations and absolute values of the ionic mobilities at finite dilution of all ionic constituents. The numerical value of ω is locally invariant with time [13], i.e., the initial conditions determine all parameters in passing sample zones. With this formula, it can be calculated that in sample zones with mobilities higher than that of the co-ions of the BGE, the local electric field (E) gradient is lower than that in the BGE, causing fronting peaks. No definite answer can be obtained concerning the pH in sample zones. The behaviour of sample peaks in UV detection can be described with the transfer ratio T_R [3] or response factor, defined as the number of molecules of the co-ions A of the BGE displaced by each analyte molecule i

$$T_R = \frac{c_A^{\text{BGE}} - c_A^{\text{S}}}{c_i^{\text{S}}} = \frac{\Delta c_A}{c_i^{\text{S}}} = k_i \quad (2)$$

with k_i [5]

$$k_i = \frac{m_i + m_C}{m_A + m_C} \cdot \frac{m_A}{m_i} \quad (3)$$

where the subscripts A, C and i refer to the co-ions A, counter ions C and sample ions i , and the superscripts S and BGE refer to the composition of the sample peak and BGE, respectively. In accord-

ance with this definition, T_R values are always positive and the concentrations of the co-ions in sample zones always decrease. If the sample ions i , co-ions A and counter ions C are considered to be UV-transparent or UV-absorbing with equal molar absorptivities ϵ , then [2] eight different cases can be distinguished, applying for the UV signal the expression

$$\Delta A = A^S - A^{BGE} = c_i^S l [\epsilon_i - k_i \epsilon_A - (k_i - 1) \epsilon_C] \quad (4)$$

where ϵ is the molar absorptivity ($1/\text{mol} \cdot \text{cm}$) and from Eq. (4) it will be clear that the transfer ratio ($T_R = k_i$) plays an important role in the signal of UV detection. In Fig. 1 the relationship between the k_i values [calculated using Eq. (3)] and the ionic mobilities at finite dilution of sample ions is given,

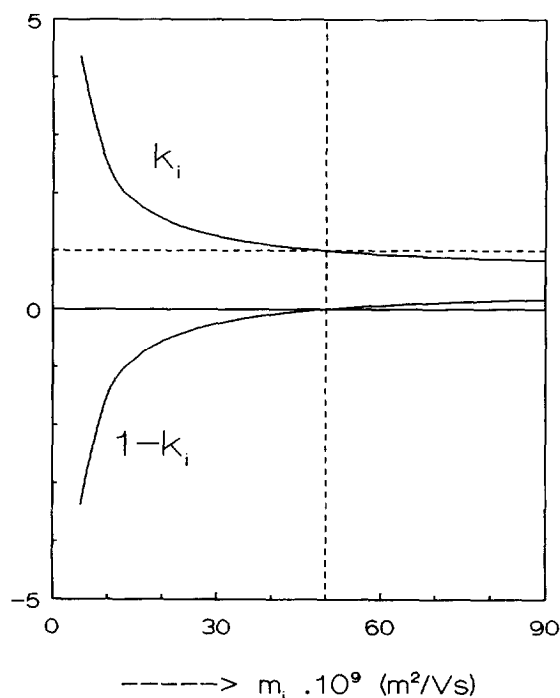


Fig. 1. Calculated relationships between k_i and $1 - k_i$, respectively, and the mobility of sample ions assuming mobilities for the co- and counter ions of 50 and $-30 \cdot 10^{-9} \text{ m}^2/\text{Vs}$. The k_i equals the transfer ratio T_R for BGEs with a single co-ion and indicates that the ionic strength increases if the mobility of the sample ions is higher than that of the co-ions. In that case positive peaks are obtained in the electropherograms for UV-transparent sample ions, applying BGEs with UV-absorbing counter ions, because the UV signal is proportional to the factor $1 - k_i$.

assuming for the mobilities of co- and counter-ion values of 50 and $-30 \cdot 10^{-9} \text{ m}^2/\text{Vs}$ respectively. If the mobility of the sample ion is higher than that of the co-ion (vertical dashed line), the transfer ratio is smaller than unity (horizontal dashed line), i.e., the total concentration of co-ions and sample ions is larger than that of the co-ions in the BGE. The ionic strength in the sample peak increases, as does the concentration of the counter ions. If indirect UV detection is applied with UV-absorbing counter ions [$\Delta A = -c_i^S l (k_i - 1) \epsilon_C$, see Eq. (4)], the UV signal will be proportional to the factor $1 - k_i$ (see Fig. 1). This means that UV-transparent components with mobilities higher than that of the co-ions of the BGE are present as peaks in the electropherograms. For fully-ionized monovalent ionic constituents, all parameters, such as the T_R , can easily be calculated.

3.1. Weak bases

For weak acids and bases, the situation can be totally different and we will apply a mathematical model [10,11] through which all parameters in the sample zones can be calculated and information regarding the character of sample peaks of weak bases obtained. The principle of the mathematical model is that all sample peaks are divided into small segments with varying sample component concentrations and that all basic equations between two adjacent segments must be fulfilled. If for a specific sample peak segment, the concentration of the sample component is assumed, the number of unknown parameters is four, viz., the concentrations of the co- and counter ions and the pH and E . All parameters can be calculated from the conditions of the preceding segment, starting from the composition of the BGE and solving the set of four equations, viz. Ohm's law, the principle of electroneutrality and the mass balances for the co- and counter ions. Calculations with this model indicate that the $\text{p}K_a$ values of the sample, co- and counter ions are very important in determining the parameters of the sample zones, particularly the question as to which component is the buffering component. If sample, co- and counter ions are considered to be strong or weak acids and bases, whereby a base and an acid can be treated as strong if their $\text{p}K_a$ values are much higher and lower, respectively, than the pH in the system, eight differ-

Table 1

If acids and bases are considered as strong (S) or weak (W), eight cases can be distinguished for cationic separations

	Sample ions	Co-ion	Counter ion	
1	S	S	S	Non-buffering system
2	S	S	W	Buffering counter ions
3	S	W	S	Buffering co-ions
4	S	W	W	Superbuffer
5	W	S	S	Non-buffering system
6	W	S	W	Buffering counter ions
7	W	W	S	Buffering co-ions
8	W	W	W	Superbuffer

ent cases can be distinguished as summarised in Table 1 for cationic separations. By the term “strong” and “weak” what is actually meant is that the component is ionized for the larger part or is only partially ionized. Cases 1 and 5 are non-buffering systems and will not be considered in the discussions. For case 8, all components affect all parameters and general rules of thumb can not be formulated. The remaining cases are nearly fully-ionized cationic species (cases 2–4) and partially ionized cationic species (cases 6 and 7) in BGEs with buffering co- or counter ions. From many calculations for a variety of sample, co- and counter ions, some general rules of thumb are deduced for the electrophoretic behaviour of weak bases which will be discussed successively. The ionic mobilities and pK_a values for all ionic species used in calculations and experiments are given in Table 2.

Table 2

Ionic mobilities at infinite dilution, m (m^2/Vs) and pK_a values for ionic species used in the calculations and experiments (25°C)

Ionic species ^a	$m \cdot 10^9$	pK_a
Acetic acid	-42.4	4.762
Creatinine	+37.2	4.83
Formic acid	-56.6	3.75
Histidine	+29.7	6.03
Imidazole	+50.4	6.953
Lithium	+40.1	14.0
Potassium	+76.2	14.0
Sodium	+51.9	14.0
TEA	+32.5	>9.0
TMA	+43.4	>9.0
Tris	+29.5	8.10

^a TEA = tetraethylammonium; TMA = tetramethylammonium; Tris = Tris(hydroxymethyl)aminomethane.

3.1.1. Change in pH in sample zones

For BGEs with buffering co-ions (cases 3 and 7), the value of the pH of the BGE will be close to that of the pK value of the co-ions A, pK_A , whereas the counter ions C can be considered as being fully-ionized anionic species. If the pK value of the sample ions S, pK_S , is larger than pK_A , the pH in the sample zone always increases and the pH generally decreases if $pK_S < pK_A$, independent of the values of effective or ionic mobilities of sample ions and co-ions. If buffering counter ions are applied for fully-ionized cationic sample species (case 2), the pH increases if the ionic mobility of the sample ions is higher than that of the co-ions and decreases for lower ionic mobilities. If the pK_S approaches the pH or is even lower than the pH (case 6), the pH in the sample zone decreases independently of the values of effective or ionic mobilities of sample ions and co-ions. If all pK values are close to the pH (case 8), the case is very complex, has to be calculated with the model and no general rule of thumb can be given.

3.1.2. Fronting or tailing character of sample peaks

Fronting sample peaks are obtained if the mobility of the sample ions is higher than that of the co-ions in the BGE. Sometimes effective mobilities and sometimes ionic mobilities have to be compared. For BGEs with buffering co-ions (cases 3 and 7), the value of the pH of the BGE is close to that of the pK value of the co-ions A and if $pK_S > pK_A$, ionic mobilities of sample ions and co-ions have to be compared. If $pK_S < pK_A$, effective mobilities have to be compared and fronting peaks are obtained if the concerned mobilities of the sample ions are higher than that of the co-ions and vice versa. If the pK_S and pK_A are higher than the pH for BGEs with buffering counter ions (case 2), ionic mobilities have to be compared. If the pK_S approaches the pH, and is smaller than the pK_A (case 6), effective mobilities have to be compared for the fronting character where fronting peaks are obtained if the concerned mobilities of the sample ions are higher than that of the co-ions and vice versa, and in this case it is very important which component determines the pH in the sample zone. To demonstrate the fronting/tailing character of peaks, some typical measured elec-

tropherograms are given in Fig. 2. In Fig. 2a, the electropherograms for the separations of 10-s pressure injections of $5 \cdot 10^{-4}$ M lithium ions are given, applying BGEs of 0.01 M imidazole acetate adjusted to a pH of 5 and 7, respectively. At pH 5 (case 2), ionic mobilities have to be compared and because the mobility of imidazole is higher than that of lithium, tailing peaks are obtained. At a pH of 7 (case 3), once again ionic mobilities have to be compared, and although the effective mobility of the co-ion imidazole is lower than that of lithium, tailing peaks are obtained. In Fig. 2b the electropherograms of 10-s pressure injections of $5 \cdot 10^{-4}$ M imidazole with BGEs of 0.01 M Tris acetate at a pH of 5 and 7, respectively, are given. At pH 5 (case 2), ionic mobilities have to be compared and imidazole is fronting. At a pH of 7 (case 7), effective mobilities have to be compared because $pK_S < pK_A$ and the imidazole peak is tailing. It is difficult to decide whether this example is case 5 or 7, because Tris has a higher pK value than imidazole. Assuming, how-

ever, that the pH is determined largely by the co-ion Tris, because of its higher concentration, case 7 is arbitrarily assumed. In Fig. 2c electropherograms for the 5-s pressure injections of $5 \cdot 10^{-4}$ M creatinine are given, applying BGEs of 0.01 M Tris adjusted to a pH of 3.5 and 4 (with formic acid) and to pH 4.5 and 5 (with acetic acid). Applying BGEs with buffering counter ions (case 6), effective mobilities have to be compared and because the effective mobility of creatinine decreases at higher pH values ($pK_S = 4.83$), the fronting peaks of creatinine become tailing at a higher pH. Because for strong bases effective mobilities equal the ionic mobilities, it seems that for all described systems, effective mobilities of the sample ions have to be compared with ionic mobilities of the co-ions.

3.1.3. Transfer ratio

The question of peaks and dips in UV detection can be answered by the calculation of the T_R value, indicating how many co-ions are transferred by a single sample molecule. Because in UV detection often both charged and neutral forms of components are UV-absorbing, we use the expression from Eq. (2) for the calculation of the T_R value on the understanding that by concentrations, we mean the total concentrations of the components. Sometimes we will refer to the T_R value according to Eq. (2) calculated with ionic concentrations, and in that case we will talk about the ionic T_R or $T_{R,i}$ value. For all calculations carried out, the turnover point, i.e., the mobility of the sample ions whereby the value of the T_R value is unity, was generally equal for both values of T_R and $T_{R,i}$ and corresponds to the point where the concentration of the counter ions of the sample peak equals that of the BGE. However, if $pK_S < pH$, extremely large and/or negative values for the $T_{R,i}$ were obtained. For the transfer ratio, generally ionic mobilities of sample and co-ions have to be compared. As an example, the calculated relationships between the pH in the sample zone (straight line, left-hand scale) and the ratio E_1/E_2 (dashed line, right-hand scale) and the ionic mobility at infinite dilution (assumed $c_S = 5 \cdot 10^{-4}$ M and $pK_S = 8$) are given in Fig. 3 for a BGE of 0.01 M imidazole adjusted to a pH of (A) 5 and (B) 7 by adding acetic acid. The ratio E_1/E_2 refers to the ratio of the

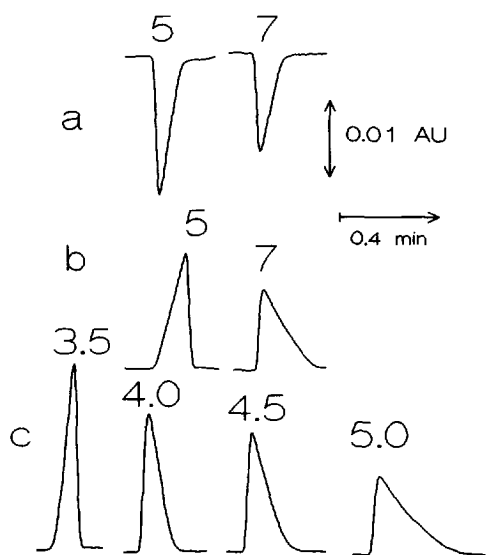


Fig. 2. Measured electropherograms for 10-s pressure injections of $5 \cdot 10^{-4}$ M of the sample ions; (a) lithium in BGEs of 0.01 M imidazole acetate at a pH of 5 and 7, (b) imidazole with BGEs of 0.01 M Tris acetate at a pH of 5 and 7 and for 5-s pressure injections of (c) $5 \cdot 10^{-4}$ M creatinine with BGEs of 0.01 M Tris formate at pH 3.5 and 4 and Tris acetate at pH 4.5 and 5. For the fronting/tailing character, effective sample mobilities have to be compared with ionic mobilities of the co-ions. The numbers refer to the pH of the BGEs. Applied voltage 10 kV.

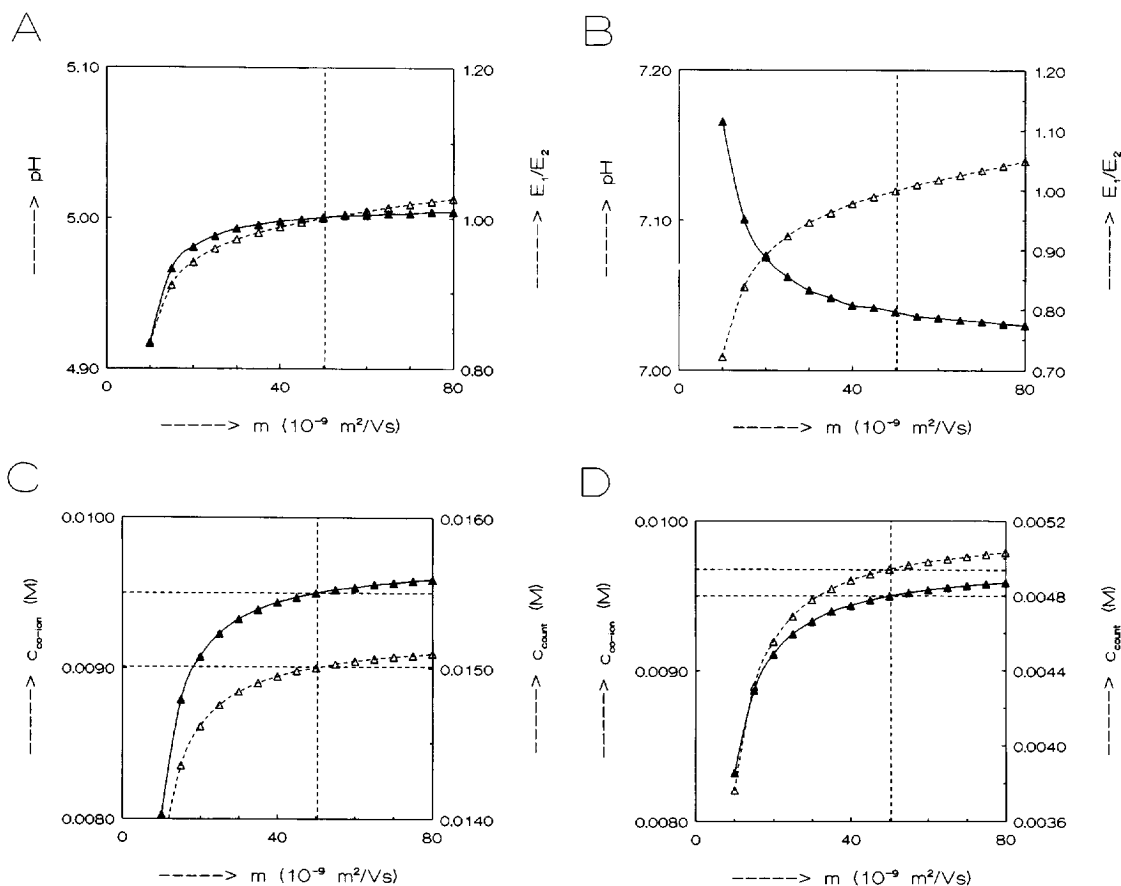


Fig. 3. Calculated relationships between the pH in the sample zone (straight line, left-hand scale) and the ratio E_1/E_2 (dashed line, right-hand scale) and the ionic mobility of sample ions at infinite dilution (assumed $c_s = 5 \cdot 10^{-4} \text{ M}$ and $\text{p}K_s = 8$) for a BGE of 0.01 M imidazole adjusted to a pH of (A) 5 and (B) 7 by adding acetic acid and the relationships between the total concentration of the co-ions (straight line, left-hand scale) and concentration of the counter ions (dashed line, right-hand scale) and the ionic mobility of the sample ions for the BGEs 0.01 M imidazole acetate at pH (C) 5 and (D) 7. The vertical dashed lines indicate the ionic mobility of imidazole and the horizontal dashed lines the concentrations of the co-ions for a transfer ratio of one and for the concentrations of the counter ions in the BGE. For further information see text.

electric-field strengths in the BGE (zero concentration of the sample ions) and in the sample zone (c_s is $5 \cdot 10^{-4} \text{ M}$), respectively. In conformity with the rules of thumb, $\text{pH} > 5$ for sample mobilities higher than the ionic mobility of imidazole (vertical dashed line at a mobility of 50.4) and < 5 for sample ions with a lower ionic mobility in Fig. 3A. In Fig. 3B the pH is always > 7 . In both cases the peaks are fronting ($E_1/E_2 > 1$) if the mobilities of the sample ions are higher than that of the ionic imidazole mobility. In Fig. 3C and Fig. 3D the relationships

between the total concentration of the co-ions (straight line, left-hand scale), the concentration of the counter ions (dashed line, right-hand scale) and the sample mobilities are given for 0.01 M imidazole acetate as BGE at pH 5 and 7, respectively. In both cases the transfer ratio is unity if the sample mobility equals the ionic mobility of imidazole. In that case the $c_{\text{co-ion}}$ is 0.0095 M and, simultaneously, the total concentration of the counter ions is equal to that in the BGE, as indicated by horizontal dashed lines in the figures.

3.2. Background electrolytes with two co-ions in cationic separations

For the calculation of all parameters in sample zones, as already indicated in Section 3.1, sample peaks are divided into small segments and, starting from the BGE, parameters of a segment are calculated from those of the preceding segment by applying the electrophoretic basic equations. If BGEs are applied with two co-ions, the number of parameters in the BGE is five (see Fig. 4), viz. the concentrations of the two co-ions A and B and of the counter ions C, and the pH and *E* gradient. Because the composition of the BGE is known, all parameters of the BGE can be calculated. In all peak segments,

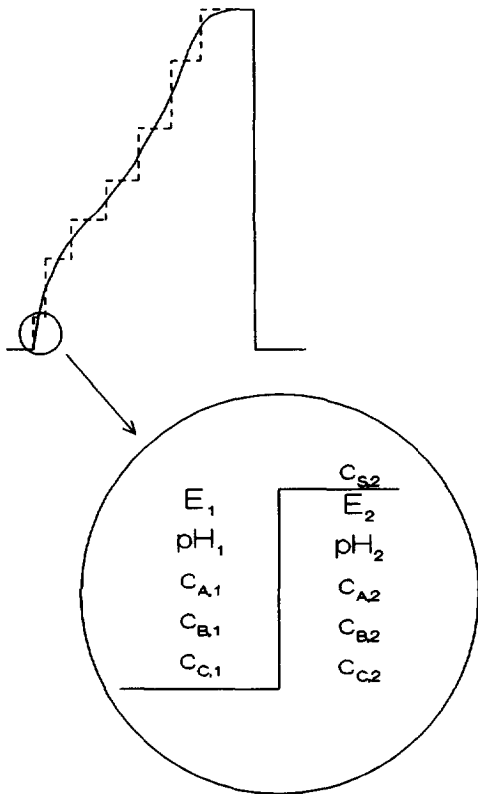


Fig. 4. For the calculation of all parameters in sample zones of weak bases in a BGE with two co-ions, the sample peak is divided in small segments with varying sample component concentrations. Calculations are started from the BGE with the known parameters E_1 , pH_1 , $c_{A,1}$, $c_{B,1}$ and $c_{C,1}$. For each following segment the $c_{S,2}$ is assumed and the parameters E_2 , pH_2 , $c_{A,2}$, $c_{B,2}$ and $c_{C,2}$ can be calculated with the given mathematical model.

there are 6 unknown parameters, viz. the concentrations of the ionic species A, B and C and that of the sample component S, and the pH and *E*. If for a specific peak segment, the concentration of the sample component S is assumed, five parameters of the sample segment have to be calculated by applying the electroneutrality equation, Ohm's law and the mass balances of the ionic species A, B and C. The calculation starts from the diffuse edge of the peak with the BGE parameters and the parameters of a specific segment are calculated from those of the preceding segments. This calculation is valid for all peak shapes. The set of equations to be solved (see Refs. [10,14] for a complete description of all equations) is:

3.2.1. Principle of electroneutrality

In accordance with the principle of electroneutrality (EN), the arithmetic sum of all products of the concentration of all forms for all ionic species and the corresponding valences, present in each zone, must be zero [14].

3.2.2. Modified version of Ohm's law

According to Ohm's law, the product of the electric-field strength *E* and electrical conductivity σ must be constant for all zones. The electrical conductivity, σ , of a zone is the sum of the values $c|mz|F$, where *z* and *F* represent the valency of the ionic species and the Faraday constant.

3.2.3. Mass balances of the co-ions A and B

The following derivation is given under the assumption that no electroosmotic flow (EOF) is present and that the mobility of the sample ions m_S , is higher than that of the co-ions m_A and m_B , respectively. Identical formulae are obtained, however, if the co-ions have a mobility higher than that of the sample ions and are even valid in the presence of an EOF. The notation *m* refers to the absolute values of the effective mobilities of the ionic components. The velocity of the zone boundary between the two segments 1 and 2 is determined by the velocity of the sample component S in zone 2, and this zone boundary moves in a unit of time from point 1 at time $t=0$ (see Fig. 5A) to point 3 at time $t=1$, over a distance $E_2 m_{S,2}$. The co-ions A present

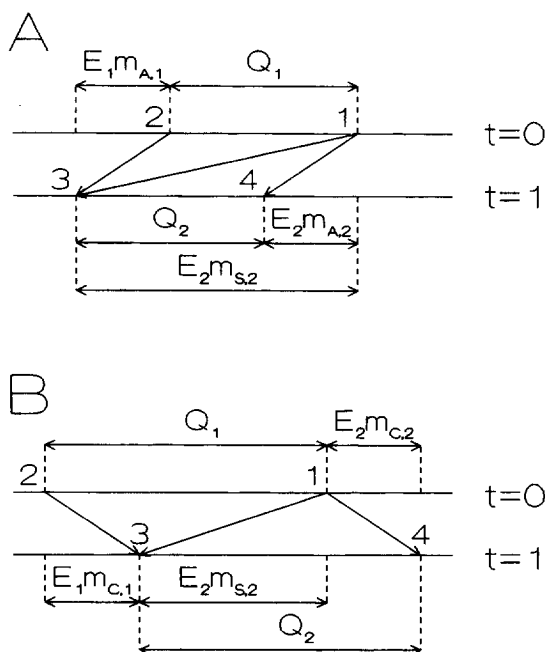


Fig. 5. Migration paths over a zone boundary between the segments 1 and 2 for (A) the co-ions A and (B) the counter ions C. For further explanation, see text.

at point 2 at time $t=0$ will just reach the zone boundary at point 3 at time $t=1$. The distance between point 2 and 3 is $E_1m_{A,1}$. Co-ions in zone 2 present at point 1 at time $t=0$ move over a distance $E_2m_{A,2}$ to point 4 at time $t=1$. This means that the co-ions, present in zone 1 at a total concentration $c_{A,1}$ at time $t=0$ between the points 1 and 2 will be present between the points 4 and 3 at a total concentration $c_{A,2}$ at time $t=1$, i.e., that the amounts of the co-ions Q_1 and Q_2 are equal. Therefore the mass balance of the co-ions over the zone boundary will be:

$$c_{A,1}(E_2m_{S,2} - E_1m_{A,1}) = c_{A,2}(E_2m_{S,2} - E_2m_{A,2}) \quad (5)$$

or

$$c_{A,2} = c_{A,1} \frac{m_{S,2} - \frac{E_1}{E_2}m_{A,1}}{m_{S,2} - m_{A,2}} \quad (6)$$

For the second co-ion B can be derived the mass balance

$$c_{B,2} = c_{B,1} \frac{m_{S,2} - \frac{E_1}{E_2}m_{B,1}}{m_{S,2} - m_{B,2}} \quad (7)$$

3.2.4. Mass balance of the counter ions C

The zone boundary moves in a unit of time from point 1 at time $t=0$ (see Fig. 5B) to point 3 at time $t=1$ over a distance $E_2m_{S,2}$. The counter ions C present at point 2 at time $t=0$, will just reach the zone boundary at point 3 at time $t=1$. The distance between point 2 and 3 is $E_1m_{C,1}$. The counter ions present at point 1 at time $t=0$ will move over a distance $E_2m_{C,2}$ and will reach point 4 at time $t=1$. All counter ions C present between point 1 and 2 in zone 1 at a total concentration $c_{C,1}$ at time $t=0$, will be present in zone 2 between 3 and 4 at a total concentration $c_{C,2}$ at time $t=1$. The amount of counter ions Q_1 and Q_2 will be equal and the mass balance of the counter ions C will be therefore:

$$c_{C,1}(E_2m_{S,2} + E_1m_{C,1}) = c_{C,2}(E_2m_{S,2} + E_2m_{C,2}) \quad (8)$$

or

$$c_{C,2} = c_{C,1} \frac{E_1m_{C,1} + m_{S,2}}{m_{C,2} + m_{S,2}} \quad (9)$$

Identical formulae are obtained in the presence of an EOF.

3.2.5. Procedure of calculation

All parameters of the BGE can be calculated because the composition of the BGE is known. The calculation of the parameters of segment 2, starting from those of the BGE, is as follows (see Fig. 6). The concentration $c_{S,2}$ of the sample component is assumed. Then a pH_2 is assumed whereby all pH-dependent parameters, such as the effective mobilities, can be calculated. Furthermore, the ratio E_1/E_2 is assumed, through which the concentration of A, B and C can be calculated using Eqs. (6,7,9). Iterating between a high and low value of E_1/E_2 , the correct value of E_1/E_2 can be found whereby the EN equation is valid. The correct value of pH_2 can be found by iterating over a low and high pH value until Ohm's law is obeyed. Parameters for each following segment can be calculated in the same way from the parameters of the preceding segment.

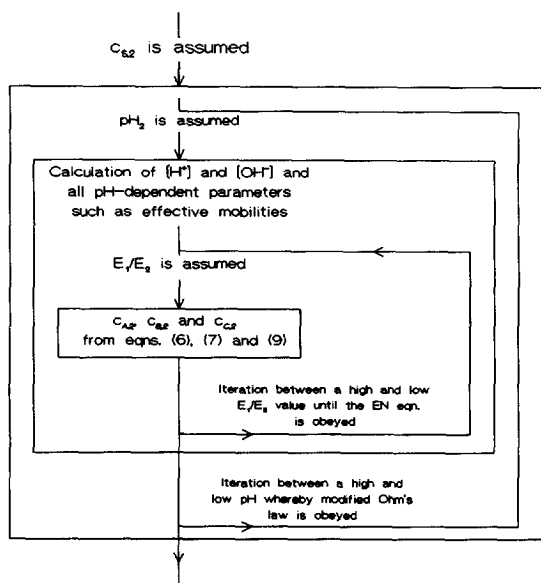


Fig. 6. Calculation procedure for the parameters in sample zones of weak bases in capillary zone electrophoresis applying a BGE with two co-ions.

4. Results and discussion

Calculations obtained from the mathematical model lead to interesting and remarkable results for weak bases applying BGEs with two co-ions. To demonstrate this, in Fig. 7 the calculated relationships between the change in concentration of the co-ions Δc (left-hand scale), calculated as $c^{BGE} - c^S$, and the transfer ratio T_R (right-hand scale), respectively, and the ionic mobility at infinite dilution of sample ions (assumed pK_s is 8) are given for sample peak segments at a concentration of $5 \cdot 10^{-4} M$ for BGEs with a single co-ion consisting of (K) 0.01 M potassium acetate and (Hist) 0.01 M histidine acetate respectively, adjusted to a pH of 5 (see Fig. 7A) and 6 (see Fig. 7B) by adding acetic acid. Remember that positive values for Δc implicate smaller concentrations of the co-ions in the sample zones compared with that in the BGE. It can clearly be seen that T_R values of unity are obtained if the ionic mobilities of the sample ions equal those of the co-ions of the BGEs (line I for histidine and line II for potassium), both applying buffering counter ions (Fig. 7A) and buffering co-ions (histidine in Fig. 7B), indicating that ionic mobilities have to be compared in these

cases. If the mobility of the sample ions is higher than that of the co-ions, beyond lines I and II, the T_R is smaller than unity, implicating an increase in the ionic strength and in concentration of the counter ions. The same parameters from Fig. 7A and Fig. 7B are given in Fig. 7C and Fig. 7D for BGEs with two co-ions consisting of a mixture of 0.005 M potassium acetate and 0.005 M histidine acetate adjusted to a pH of 5 and 6 respectively by adding acetic acid. The changes in Δc and T_R for the co-ion histidine are given as dashed lines and as straight lines for the co-ion potassium. From Fig. 7C and Fig. 7D, it can be seen that the concentrations of both co-ions appear to change for all mobilities of the sample ions, i.e., that the concept of "selective displacement" [4] is attractive but seems to be incorrect. At point I (mobility of histidine) histidine is predominantly transferred ($T_R \approx 1$) and at point II (mobility of potassium) the T_R is exactly unity, but for very low and high sample mobilities, both co-ions are transferred. Between the mobilities of the two co-ions, there is a mobility domain where no solution for the set of equations can be obtained. The width of this mobility domain depends on several parameters, such as the pK values of all ionic species and pH of the system, and is in Fig. 7C situated between mobilities of about 43 and $47 \cdot 10^{-9} m^2/Vs$ and in Fig. 7D between mobilities of about 30 and $41 \cdot 10^{-9} m^2/Vs$. By applying sample ionic species with mobilities lying in that domain, the mass balances are not satisfied, which indicates that a disturbance in the composition of the BGE is present. This domain is also found for calculations with very low concentrations of the sample ions. In fact this is in accordance with earlier experiments [2], where it was observed that a system peak (the disturbance in the composition of the BGE) migrates through the separation capillary, applying BGEs with two co-ions, with a mobility lying between the mobilities of the co-ions. The mobility of the system peak corresponding to the centre of the mobility domain where the set of equations cannot be solved, is dependent on the concentrations of the co-ions and, as can be concluded from Fig. 7C and Fig. 7D, also on the pH of the system. In the vicinity of the mobility of the system peak, the "selectivity" of displacement increases greatly. The concentration of one of the two co-ions decreases to such a strong degree that the

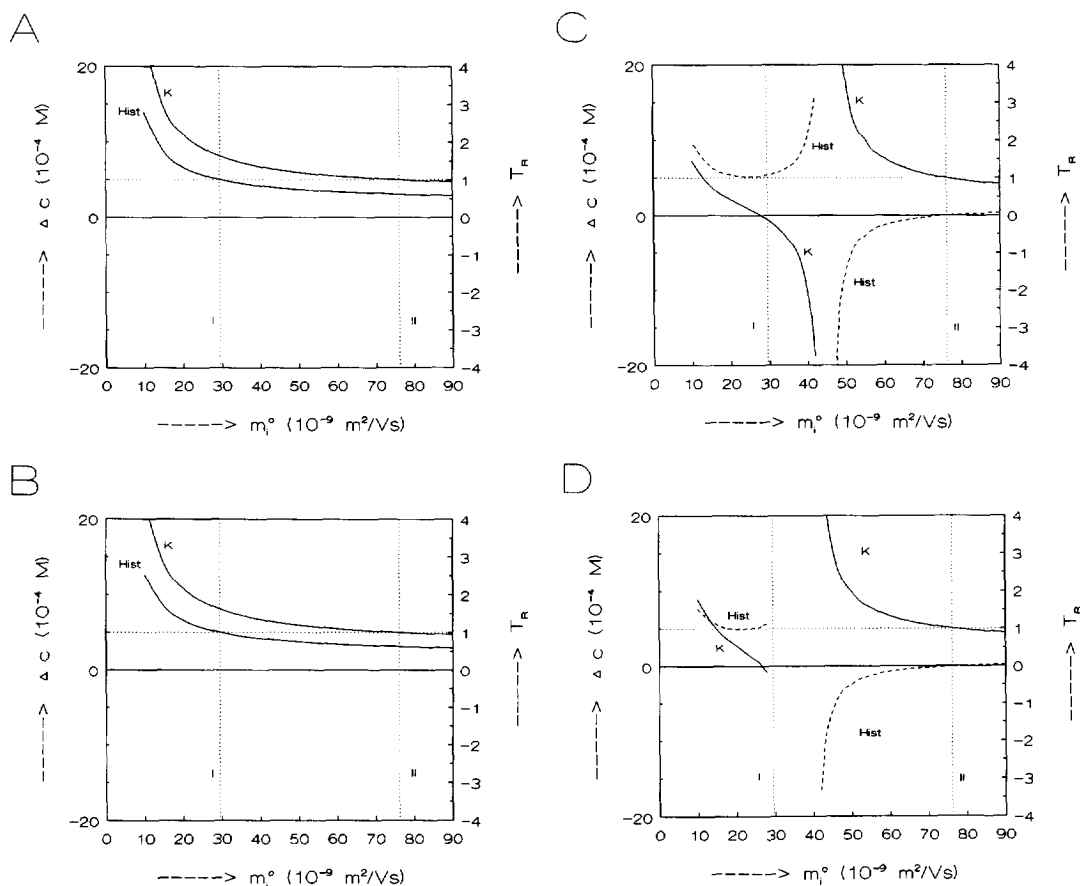


Fig. 7. Calculated relationships between the change in concentrations of the co-ions Δc (left-hand scale) and the transfer ratio T_R (right-hand scale) and the ionic mobility of sample ions at infinite dilution (assumed pK_s is 8), for sample peak segments at a concentration of $5 \cdot 10^{-4}$ M for BGEs consisting of 0.01 M potassium acetate and 0.01 M histidine acetate, adjusted to a pH of (A) 5 and (B) 6 by adding acetic acid and for BGEs with two co-ions consisting of a mixture of 0.005 M potassium acetate and 0.005 M histidine acetate, adjusted to a pH of (C) 5 and (D) 6 by adding acetic acid. For BGEs with two co-ions the change in concentration of the co-ion histidine is a dashed line and for potassium a straight line.

concentration of the other co-ionic species strongly increases, showing a negative T_R value. In Fig. 7C, for example, the histidine concentration decreases greatly for sample mobilities up to about $40 \cdot 10^{-9}$ m²/Vs corresponding with an increase in the concentration of potassium. These sample peaks will be present as large dips in the electropherograms, applying UV detection. Beyond a system-peak mobility of about $45 \cdot 10^{-9}$ m²/Vs, the sample peaks will be present as large peaks because the concentration of histidine strongly increases, whereas the concentration of potassium decreases. These phenomena have already been observed [2]. For a

pH of 6, the mobility domain where a solution of the set of equations cannot be found is larger than at a pH of 5 and the mobility of the system peak is lower.

For a complete check of the model given, for BGEs with two co-ions, calculations and measurements are carried out for BGEs consisting of 0.005 M of the co-ions imidazole or sodium and Tris or histidine adjusted to pH 4.75 by adding acetic acid. The mobilities of imidazole and sodium (about $50 \cdot 10^{-9}$ m²/Vs), and Tris and histidine (about $30 \cdot 10^{-9}$ m²/Vs), respectively, are nearly equal. Because imidazole and histidine are UV-absorbing and sodium and Tris are UV-transparent, we can make a

BGE at will with a UV-absorbing co-ion with a high or low mobility. For all combinations of two co-ions, viz., the BGEs imidazole/Tris acetate, sodium/Tris acetate, imidazole/histidine acetate and sodium/histidine acetate at pH 4.75, all calculated parameters are nearly equal, with small differences owing to the differences in the mobilities of the co-ions. As an example, in Fig. 8A the calculated relationships between the change in concentration of the co-ions Δc (left-hand scale) and the transfer ratio T_R (right-hand scale) respectively and the ionic mobility at

infinite dilution of sample ions (assuming pK_s is 8) are given for sample peak segments at a concentration of $5 \cdot 10^{-4} M$ for a BGE with two co-ions, consisting of a mixture of (Im) 0.005 M imidazole acetate (straight lines) and (Tris) 0.005 M Tris acetate, respectively (dashed lines), adjusted at a pH of 4.75 by adding acetic acid. The vertical lines I and II indicate the mobilities of the co-ions Tris and imidazole. Just as in Fig. 7, there is a mobility domain where no solution can be found for the set of equations of the mathematical model corresponding

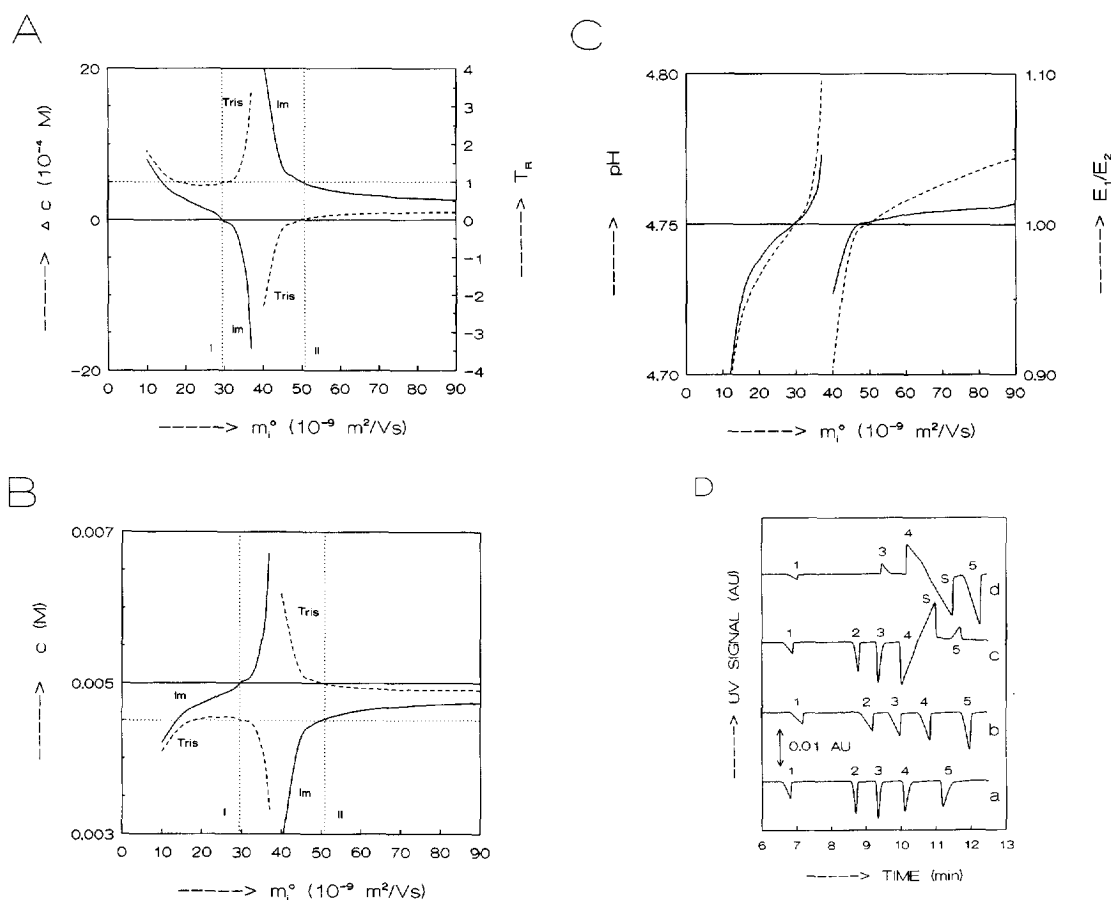


Fig. 8. Calculated relationships between (A) the change in concentrations Δc (left-hand scale) of the co-ions imidazole (straight lines) and Tris (dashed lines) and the transfer ratio T_R (right-hand scale), (B) concentration of the two co-ions and (C) pH (straight line, left-hand scale) and ratio E_1/E_2 (dashed line, right-hand scale) versus the ionic mobility at infinite dilution of sample ions (assumed pK_s is 8), for sample peak segments at a concentration of $5.10^{-4} M$ for a BGE consisting of a mixture of 0.005 M imidazole and 0.005 M Tris adjusted to pH 4.75 by adding acetic acid. (D) Measured electropherograms for the separation of 5-s pressure injections of a mixture of $5.10^{-4} M$ (1) potassium, (2) sodium, (3) TMA, (4) lithium and (5) TEA applying BGEs of (a) 0.01 M imidazole, (b) 0.01 M histidine, (c) 0.005 M imidazole and 0.005 M Tris and (d) 0.005 M sodium and 0.005 M histidine, all BGEs adjusted to pH 4.75 by adding acetic acid. For further information see text. Applied voltage 5 kV.

with a system peak. The mobility of the system peak must be $38 \cdot 10^{-9} \text{ m}^2/\text{Vs}$. In Fig. 8B, the concentrations of the co-ions imidazole (straight line) and Tris (dashed line) are given, and in Fig. 8C the pH (straight line, left-hand scale) and the ratio E_1/E_2 (dashed line, right-hand scale) are given as function of the ionic mobility at infinite dilution of sample ions. The meaning of Fig. 8A and Fig. 8B is clear. Applying the BGE imidazole/Tris acetate, both the concentrations of Tris and imidazole are lower in the sample zone than in the BGE, for sample ions with a mobility higher than that of imidazole. UV-transparent sample ions are present as dips in the electropherogram, because imidazole is UV-absorbing. If the mobility is between those of the system peak and imidazole, the UV-transparent sample ions are also dips. If the mobility of sample ions approaches that of the system peak, the concentration of imidazole in the sample peak strongly decreases, implying an extremely large dip. UV-transparent sample ions with mobilities between the mobility of Tris and the system peak are peaks, and if the mobility is lower than that of Tris they are dips again. If a BGE of sodium-histidine acetate is used, all values in Fig. 8A–C are nearly identical, bearing in mind that the dashed lines correspond with the values for histidine and the straight lines those for sodium. In that case, UV-transparent sample ions with mobilities higher than sodium are dips, sample ions with mobilities between sodium and the system peak are peaks and sample ions with mobilities lower than the system peak are dips. From Fig. 8C, the fronting and tailing character of sample peaks can be deduced. If $E_1 m_1 > E_2 m_2$, sample segments with low sample concentrations migrate faster than with higher concentrations and these sample peaks are fronting. For sample components with assumed $\text{p}K_s$ values of 8, the mobilities are nearly constant, i.e. (see Fig. 8C), sample components with a mobility higher than that of imidazole are fronting, with a mobility between imidazole and the system peak are tailing, with a mobility between those of Tris and the system peak are fronting, and with a mobility lower than that of Tris are tailing again. This character is similar for all BGEs with two co-ions mentioned before. To check all these points separations are carried out, of a sample mixture consisting of $5 \cdot 10^{-4} \text{ M}$ (1) potassium, (2) sodium, (3) TMA, (4) Li and (5) TEA ions in water and the electropherograms are given in Fig.

8D for the BGEs (a) 0.01 *M* imidazole acetate, (b) 0.01 *M* histidine acetate, (c) a mixture of 0.005 *M* imidazole and 0.005 *M* Tris acetate and (d) a mixture of 0.005 *M* sodium and 0.005 *M* histidine acetate. All BGEs had pH 4.75. In BGEs with a single co-ion all sample peaks are present as dips, in conformity with the theory. In (a) imidazole acetate, potassium and sodium are fronting because their mobilities are higher than that of the co-ion imidazole, whereas the dips of TMA, Lithium and TEA are tailing. In (b) histidine acetate, all dips are fronting. In electropherograms (c) and (d), the huge peaks and dips of the sample component lithium and the system peak are striking, but are in complete agreement with Fig. 8A and Fig. 8B. Because the mobility of lithium is only a little bit higher than that of the system peak the concentration of the co-ion with the highest mobility is greatly decreased and the concentration of the co-ion with the lowest mobility greatly increased. This means a huge dip in the system imidazole/Tris acetate and a huge peak in the system sodium/histidine acetate. If the sample component is a peak, the system peak is a dip and if the sample peak is tailing, the system peak is fronting and vice versa [2]. By the way, if the difference between the mobilities of the system peak and sample peak is small, it is difficult to find out experimentally, which peak is the sample peak and which is the system peak. In sodium/histidine acetate the sample component sodium is invisible. According to Fig. 8C the potassium and sodium peaks are fronting, TMA and lithium peaks are tailing and the peak of TEA is fronting again. According to Fig. 8A and Fig. 8B potassium, sodium, TMA and lithium components are dips and TEA is a peak in the system imidazole/Tris acetate. In sodium/histidine acetate, potassium is a dip, TMA and lithium are peaks and TEA is a dip again. In both systems we can see first a fronting potassium component, tailing TMA and lithium components and again a fronting TEA component. In conformity with Fig. 8B, peak 3 in electropherogram (d) is smaller than the dip in electropherogram (c).

5. Conclusion

In Refs. [10,11], a mathematical model is described through which all parameters in the sample

zones in capillary zone electrophoresis can actually be calculated. From calculations with this model, rules of thumb are deduced for the fronting/tailing character of peaks, the question of peaks and dips, the pH and the transfer ratio for sample zones of weak bases in CZE. The model is extended for BGEs consisting of two co-ions showing remarkable results. For a specific mobility domain of the sample ions, the set of equations of the mathematical model cannot be solved and this mobility domain corresponds to the mobility of system peaks present in electropherograms applying BGEs with two co-ions. Furthermore, the concept of transfer ratio is not useful anymore, because the concentration of one of the co-ions decreases whereas the other one increases. Depending on the UV-absorbing properties of the co-ions, UV-transparent sample ionic species can be present as dips or peaks in the electropherograms. The concept of selective displacement is of comparative value. In a single run in CZE, peaks can

alternately be fronting, tailing and fronting again. Moreover, the first peak in an electropherogram can be tailing, whereas a second peak can be fronting. In Fig. 9, the electropherograms for the separation of 10-s pressure injections of a mixture of 0.005 M imidazole and creatinine are given, applying as BGE (a) 0.01 M sodium formate, (b) 0.01 M Tris formate and (c) a mixture of 0.005 M sodium and 0.005 M Tris formate. All BGEs were adjusted to pH 3.5 by adding formic acid. In electropherogram (a), the mobilities of both sample ions are lower than that of sodium ions and the peaks are tailing. In electropherogram (b), the sample mobilities are higher than that of Tris and the peaks are fronting. In electropherogram (c), the imidazole peak (before the invisible system peak) is tailing and creatinine, with a mobility lower than that of the system peak and higher than that of Tris, it is fronting, showing that in BGEs with two co-ions all concepts concerning peaks/dips, T_R values and fronting/tailing character lose their meaning. The examples given in this paper often cannot be classed in Table 1, because in fact the eight limiting cases are described. Some other examples for case 7 have recently been published by J. Collet and P. Gareil [15].

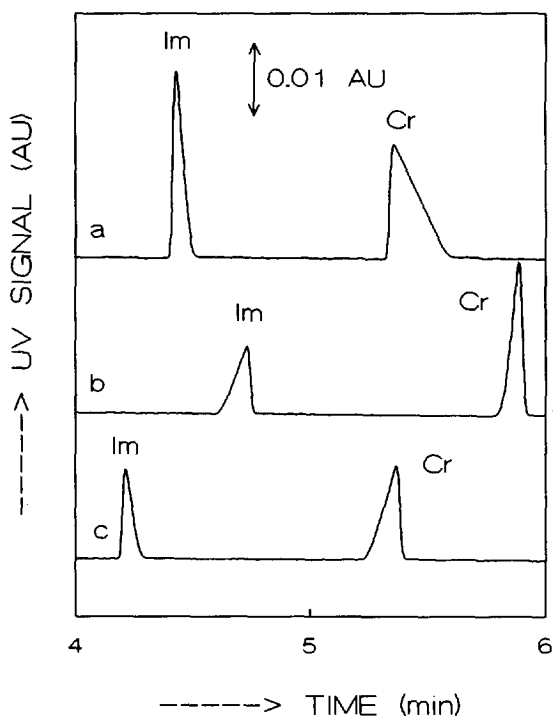


Fig. 9. Measured electropherograms for the separations of 10-s pressure injections of a mixture of 0.0005 M imidazole (Im) and creatinine (Cr) applying BGEs of (a) 0.01 M sodium formate, (b) 0.01 M Tris formate and (c) a mixture of 0.005 M sodium and 0.005 M Tris formate. All BGEs were adjusted to pH 3.5 by adding formic acid.

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