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Calculation of the composition of sample zones in capillary zone electrophoresis
IV. Weak acids and system characteristics

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Abstract

In this final part, Part IV, of a series of papers about the calculation of parameters in sample zones in capillary zone electrophoresis by a repeated application of a steady-state model, the mathematical model is extended for the calculation of parameters in sample zones of weak anionic analytes, applying background electrolytes (BGEs) with more than one co- and/or counter-ionic species. From the calculations, it appeared to be practically impossible to formulate general rules for the description of the characteristics of anionic and cationic sample peaks in a particular BGE, especially applying more co-ionic species. However, it is possible to describe the most important system characteristics by means of a set of eight figures, which will be called a SystChart. Four figures of such a SystChart are used for the visualization of the relationships between the mobilities at infinite dilution of cationic analytes and the pH, the ratio $E_1 m_1 / E_2 m_2$, the total concentrations of the cations and of the anions of the BGE in a sample zone with a sample concentration of $5 \cdot 10^{-4}$ M, respectively, and four figures for the description of the same relationships for anionic analytes. In this way, the most important parameters, from which several peak characteristics can be deduced, such as the fronting/tailing character and peaks/dips for both cationic and anionic sample ionic species, are visualised for a particular BGE. Descriptions are given of SystCharts for BGEs consisting of (1) 0.01 M imidazole and 0.02 M acetic acid at pH 4.7, (2) 0.02 M imidazole and 0.01 M acetic acid at pH 7.0 and (3) 0.02 M histidine, 0.005 M butyric acid and 0.005 M hydrochloric acid at pH 6.1 (two anionic co-ions).

Keywords: Sample zone composition; Background electrolytes; Mathematical models; Fronting; Tailing; Weak acids

1. Introduction

Under the assumption that in a solution of an electrolyte, if an electric current is passed, at each point of time and space, the laws of electroneutrality and Ohm hold and the mass balances for all ionic species must be satisfied, all electrophoretic processes can be described by some simple mathematical equations. For steady-state processes, such as isotachophoresis (ITP), these models are generally known [1–4], but they also can be applied for non-

steady-state processes. In Part I [5] of this series of papers, it has been described already that non-steady-state electrophoretic processes can be treated by a repeated application of a steady-state model and a mathematical model is given for the calculation of all parameters in sample zones in capillary zone electrophoresis (CZE). In this model, sample zones in zone electrophoresis are divided into segments with varying sample concentrations and by solving the set of equations describing the relationships between all parameters of two adjacent peak segments, all pa-

rameters of a specific segment can be calculated from those of the preceding segment (with lower analyte concentration), starting from the composition of the background electrolyte (BGE). The model is valid if electrodispersive effects predominate because other peak-broadening effects, such as diffusion and the influence of temperature, are neglected. In Part II [6], it is shown how temporal electropherograms can be simulated with this mathematical model and simulated and measured electropherograms are strikingly similar concerning the fronting/tailing character of sample peaks, the question of peaks and dips and migration times. In Part III [7], rules of thumb are derived from calculations with this model for peak characteristics and for the change of the pH and electric field strengths in sample zones for weak bases and, furthermore, the model is extended for calculations in sample zones of weak bases by applying BGEs with two co-ionic species. For BGEs with two co-ions, all concepts concerning peaks/dips, fronting/tailing character and transfer ratio lose their meaning, whereas the existence of system peaks appeared from the calculations. With this part, Part IV, the tetralogy will be completed, by describing the mathematical model for the calculation of parameters in sample zones of weak anionic analytes. By this, characteristics for sample peaks of both weak cationic and anionic analytes in any BGE can be calculated. It appears from the calculations that it is practically impossible to derive general rules of thumb for sample peak characteristics of anionic and cationic sample peaks. Therefore, we will describe all "system characteristics" in a set of eight figures (called a SystChart), visualizing all sample peak parameters for a particular BGE. Three SystCharts will be described for BGEs consisting of (1) 0.01 M imidazole and 0.02 M acetic acid at pH 4.7, (2) 0.02 M imidazole and 0.01 M acetic acid at pH 7.0 and (3) 0.02 M histidine, 0.005 M butyric acid and 0.005 M hydrochloric acid at pH 6.1 (two anionic co-ions). Furthermore, I will describe how SystCharts can be used.

2. Theory

When working with BGEs consisting of a single co- and counter-ionic species, the number of parame-

ters describing the BGE in CZE experiments is four, viz. the total concentrations of the co-ion, c_A, and of the counter-ion, c_C, the pH and the electric field strength, E. Because the composition of the BGE is known, all parameters in the BGE can be calculated. In a sample zone, the number of parameters is five, viz. the total concentrations of the sample component c_S, of the co-ions c_A and of the counter-ions c_C and the pH and E. If sample zones are divided into peak segments with varying sample component concentrations, the parameters of these segments can be calculated from the parameters of the BGE by applying the electroneutrality equation, the modified Ohm's law and the mass balances of the co- and counter-ions, if the total concentration of the sample component c_S is assumed. For negatively charged sample components, these equations are as follows (see Refs. [5–7] for a complete description of the equations).

2.1. The 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.

2.2. Modified 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, respectively.

2.3. Mass balance of the co-ions A

Under the assumption that no electroosmotic flow (EOF) is present and that the mobility of the sample ions, S, m_S, is higher than that of the co-ions, m_A, the mass balance can be derived for negatively charged sample ionic species (see Fig. 1A). The notation m refers to the absolute values of the effective mobilities of the ionic components! The velocity of the zone boundary between two segments 1 and 2, whereby 1 and 2 can refer to two adjacent peak
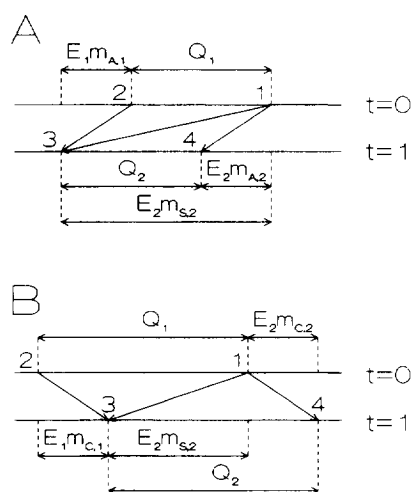
Fig. 1. Migration paths over a zone boundary between segments 1 and 2 for (A) the co-ions, A, and (B) the counter-ions, C. For a further explanation, see text.

segments or to the BGE and the first segment of a sample zone, 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 $t=0$ to point 3 at time $t=1$, over a distance $E_2 m_{S,2}$. The co-ions A present at point 2 at time $t=0$ will just reach the zone boundary at point 3 at time $t=1$. The distance between points 2 and 3 is $E_1 m_{A,1}$. Co-ions in zone 2 present at point 1 at time $t=0$ move over a distance $E_2 m_{A,2}$ to point 4 at time $t=1$. This means that the co-ions, present in zone 1 at a total concentration of $c_{A,1}$ at time $t=0$ between the points 1 and 2 will be present between the points 4 and 3 at a total concentration of $c_{A,2}$ at time $t=1$, i.e. 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:

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

or

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

Identical formulae are obtained if the co-ions have a mobility higher than that of the sample ions and they are even valid in the presence of an EOF.

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. 1B) to point 3 at time $t=1$ over a distance $E_2 m_{S,2}$. 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 points 2 and 3 is $E_1 m_{C,1}$. The counter-ions present at point 1 at time $t=0$ will move over a distance $E_2 m_{C,2}$ and will reach point 4 at time $t=1$. All counter-ions C present between points 1 and 2 in zone 1 at a total concentration of $c_{C,1}$ at time $t=0$, will be present in zone 2 between points 3 and 4 at a total concentration of $c_{C,2}$ 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, therefore, be:

$$c_{C,1}(E_2 m_{S,2} + E_1 m_{C,1}) = c_{C,2}(E_2 m_{S,2} + E_2 m_{C,2}) \quad (3)$$

or

$$c_{C,2} = c_{C,1} \frac{m_{S,2} + \dfrac{E_1}{E_2} m_{C,1}}{m_{S,2} + m_{C,2}} \quad (4)$$

Identical formulae are obtained in the presence of an EOF.

2.5. Procedure for the calculation

All parameters of the BGE can be calculated because the composition of the BGE is known.

Solving the set of equations, describing the relationships between the parameters of segments 1 and 2, respectively, whereby segment 1 refers to the BGE and segment 2 to the first peak segment, all parameters of the first sample peak segment can be calculated from the parameters of the BGE. In a similar manner, all parameters of a peak segment can be calculated from those of the preceding peak segment. In this way, the parameters of all segments can be calculated from these of the BGE. In the first instance (Part I, Ref. [5]), the calculation procedure, A, of Fig. 2 was chosen. The calculation of the parameters of segment 2, starting from those of the BGE is as follows. The concentration $c_{S,2}$ of the sample component is assumed. Then a pH_2 is assumed whereby all pH-dependent parameters, such
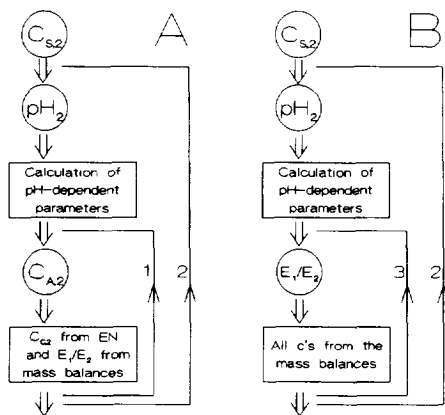
Fig. 2. Two possible iteration procedures for the calculation of parameters in sample zones in capillary electrophoresis. The mass balances of the co- and counter-ions, the electroneutrality equation and the modified Ohm's law are always used in the calculations. In the iteration, step 1 is iterated between a low and a high $c_{A,2}$ value, step 2 is iterated between a low and a high pH value and step 3 is iterated between a low and a high value of the ratio E_1/E_2. For further information, see text.

as the effective mobilities, can be calculated. If a concentration of the co-ions of $c_{A,2}$ is assumed, the concentration of the counter-ions, $c_{C,2}$, can be calculated from the EN equation. Iterating between a high and low value of the $c_{A,2}$, the correct value of $c_{A,2}$ can be obtained whereby the two values of the ratios E_1/E_2, obtained from the mass balances of the co-ions and of the counter-ions, are equal (iteration procedure 1). The correct value for pH_2 can be found by iterating between a high and low value of pH_2 until the modified Ohm's law is obeyed (iteration procedure 2). Afterwards, in solving the set of equations for two co-ionic species (Part III, Ref. [7]), the procedure for calculating B in Fig. 2 seems to be more suitable. In this procedure, the composition of the BGE is calculated first. A concentration, $c_{S,2}$, of the sample component in a particular sample segment 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 all ionic species from mass balances can be calculated using Eqs. (2,4). 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 (iteration procedure 3). The correct value of pH_2 can be found

by iterating over a low and high pH value until Ohm's law is obeyed (iteration procedure 2). The latter procedure has the big advantage that the same procedure can always be followed, independently of the number of co-ionic and counter-ionic species. This procedure can be applied for all calculations of anionic sample ions. Comparison with the calculation procedures in Part III shows that the calculations are identical for anionic and cationic sample species, on the understanding that in the case of cationic sample species, the cations of the BGE are the co-ions and the anions of the BGE are the counter-ions in the calculations and vice versa for anionic sample species.

3. Experimental

For all CZE experiments a P/ACE System 2000 HPCE (Beckman, Fullerton, CA, USA) was used. All experiments were carried out with 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, with a constant voltage of 10 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.

4. Results and discussion

In Part III, the calculation of parameters in cationic sample zones is discussed and rules of thumb are formulated for the change in pH and in the concentrations of co- and counter-ions in the sample zones, for the prediction of the fronting and tailing character of the sample peaks and for the question of peaks and dips for UV-transparent analytes. This is possible for BGEs with a single co- and counter-ionic species, even for weak bases. However, when using BGEs with more co- and counter-ions, all of these rules lose their meaning. In theory, all calcula-

tions should be carried out separately for each sample component in a particular BGE. Calculations for anionic analytes show the same results and the most important question is how to present calculated parameters in such a way that they can be used in a suitable way by non-specialist readers.

The parameters that are of interest for the description of sample peak characteristics in CZE are, besides all the parameters of the BGE, the mobility and pK values of the sample component and the pH, the electric field strength, E, and the total concentrations of the sample component, of the co-ions and of the counter-ions in the considered sample peak segment. Fortunately, all of these parameters often change proportionally to the concentration of the sample component. This is illustrated in Fig. 3

for the cations potassium ($\triangle$), sodium ($\bigcirc$) and lithium ($\triangledown$) (A and C) and for the anions chloride ($\blacktriangle$), formate ($\bullet$) and benzoate ($\blacktriangledown$) (B and D) for a BGE consisting of a mixture of 0.01 M imidazole and 0.02 M acetic acid. In Fig. 3A–B, the calculated relationships are given between the pH (straight lines, left-hand scale) and the ratio $E_1 m_1 / E_2 m_2$ (dashed lines, right-hand scale) of the sample zones, respectively, and the concentrations (see Table 1 for mobilities and pK values) of the sample ions, c_S, in the sample peak segments. In Fig. 3C–D, the relationships between the total concentration of imidazole, c^+ (straight lines, left-hand scale), that of acetate c^- (dashed lines, right-hand scale) and c_S are given. Nearly all peak characteristics can be deduced from these relationships. The pH increases in a
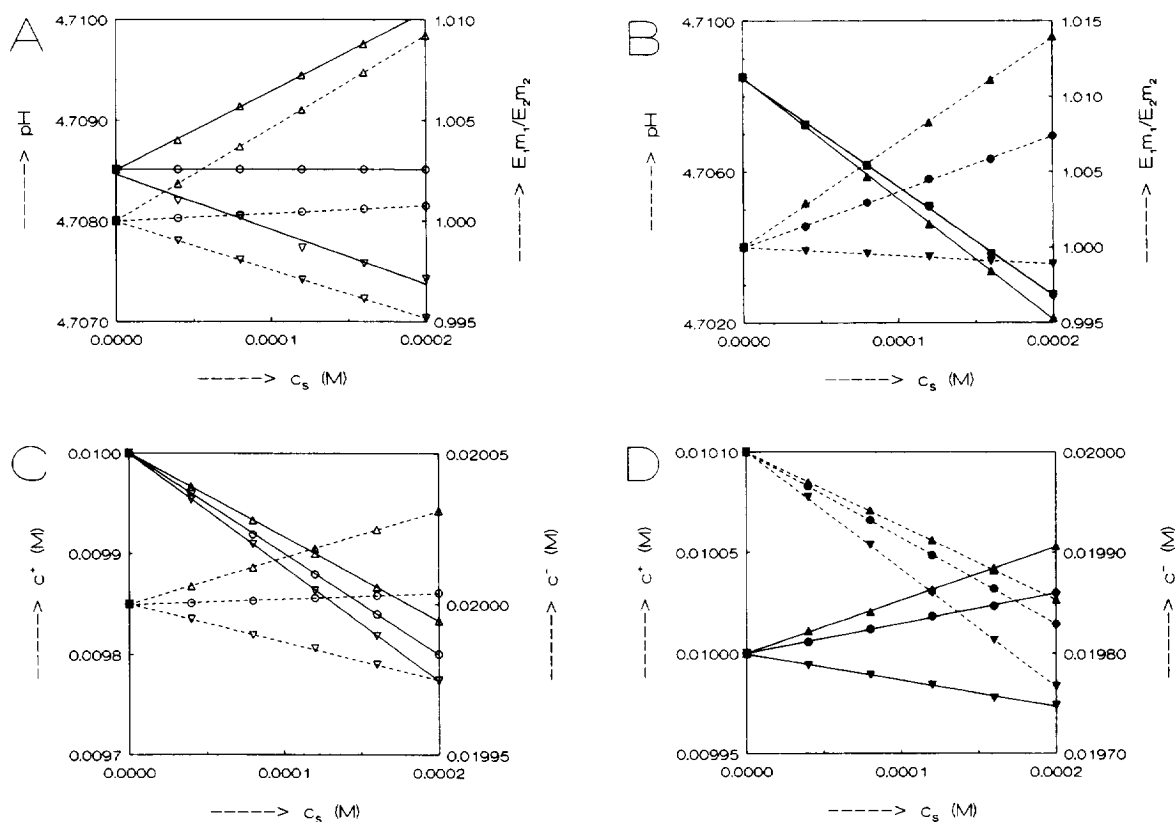

Fig. 3. Calculated relationships between several parameters of the sample zones and the concentration of the sample ions, c_S, in that sample zone, using a BGE consisting of 0.01 M imidazole and 0.02 M acetic acid. In A and B the pH (straight lines, left-hand scale) and the ratios $E_1 m_1 / E_2 m_2$ (dashed lines, right-hand scale) are given and in C and D, the total concentrations of imidazole, c^+ (straight lines, left-hand scale) and of acetate, c^- (dashed lines, right-hand scale) are given for the sample cations ($\triangle$) K$^+$, ($\bigcirc$) Na$^+$ and ($\triangledown$) Li$^+$ (A,C) and for the anions ($\blacktriangle$) Cl$^-$, ($\bullet$) formate and ($\blacktriangledown$) benzoate (B,D). For further information, see text.

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

Ionic species	$m \cdot 10^9$	pK
Acetic acid	−42.4	4.76
Benzoic acid	−33.6	4.203
Butyric acid	−33.8	4.82
Formic acid	−56.6	3.7
Histidine	29.7	6.03
Hydrochloric acid	−79.1	−2.0
Imidazole	50.4	6.953
Lithium	40.1	14.0
Nitric acid	−74.3	−2
Sodium	51.9	14.0
Potassium	76.2	14.0

sample peak of a fully ionized cation with a mobility higher (potassium) than that of the co-ion (imidazole) of the system and decreases for a sample ion with a lower mobility (lithium). This is because the counter-ions of the BGE are the buffering ionic species (see Part III, Ref. [7]). For the anionic analytes, the pH always decreases because the co-ions (acetate) are the buffering ionic species. Fronting peaks can be expected if the peak segments with a higher sample concentration move more slowly than those with a lower concentration. Because the migration speed of a specific segment is determined by the product Em, we use the ratio $E_1 m_1 / E_2 m_2$ for predicting the fronting/tailing character of sample peaks. Here, E_1 refers to the electric field strength in the BGE and the m_1 is the calculated effective mobility that the sample ionic species would have in the BGE. The term $E_1 m_1$ indicates the velocity of the diffuse edge of a sample peak. The subscript 2 refers to the composition of the peak segment considered. For fully ionized ionic species, the mobility is generally fairly constant, i.e., the ratio E_1 / E_2 can also be used, however, sometimes the change in mobility of the component overrules the effect of the change in E, and it is better to use the ratio $E_1 m_1 / E_2 m_2$. Fronting peaks are obtained if the ratio $E_1 m_1 / E_2 m_2$ is larger than unity and from Fig. 3A–B, it can be concluded that this is true if the mobilities of the sample ions are higher than those of the co-ions by applying BGEs with a single co-ionic species. From Fig. 3C–D, it can be concluded that the concentrations of the co-ions always decrease for increasing

concentrations of sample ions. The concentrations of the counter-ions can both increase and decrease, depending on the mobilities of the sample ions and the transfer ratio [8]. Applying UV-absorbing counter-ions can lead to peaks for UV-transparent sample ions [9]. Although all of the presented sample analytes are nearly fully ionized, the linear relationships also hold for partially ionized cationic and anionic species, as shown in Fig. 4. In Fig. 4, the same parameters as described in Fig. 3 for fully ionized cations and anions (and using the same BGE; 0.01 M imidazole acetate at a pH of 4.7) are given for cationic and anionic analytes with mobilities of $60 \cdot 10^{-9}$ m^2/V s and different pK values. To study the effect of pK values of the sample ions for the cationic analyte, pK values of 14 (△), 5.2 (○) and 4.2 (▽) (A and C) and for the anions, pK values of −2 (▲), 4.2 (●) and 5.2 (▼) (B and D) are assumed. In a system with a pH of approximately 4.7, the cations with a pK value of 14 can be considered as completely ionized, those with a pK value of 5.2 as ionized for the larger part and those with a pK value of 4.2 as ionized for a minor part. For anions, pK values are taken of −2 (fully ionized), 4.2 (ionized for the larger part) and 5.2 (ionized for the minor part). Because the calculated relationships between peak parameters and the total concentration of the sample ions in the sample peak are linear, over a large concentration range, it is sufficient to present the calculated values for a single sample concentration. They must, however, be given for different mobilities and pK values of the sample components. By this method, it is possible to present the most important parameters, describing all sample peak characteristics in a specific background electrolyte with a set of eight figures, that we will denote as a SystChart.

4.1. SystCharts in electrophoresis

With the mathematical model described, all parameters in sample zones can be calculated. To visualize the calculated parameters, a so-called SystChart will be used. A SystChart is a set of eight figures in which all important peak characteristics for anionic and cationic analytes are presented for a particular BGE. Calculations for SystCharts are carried out at a constant ionic strength, viz., that of
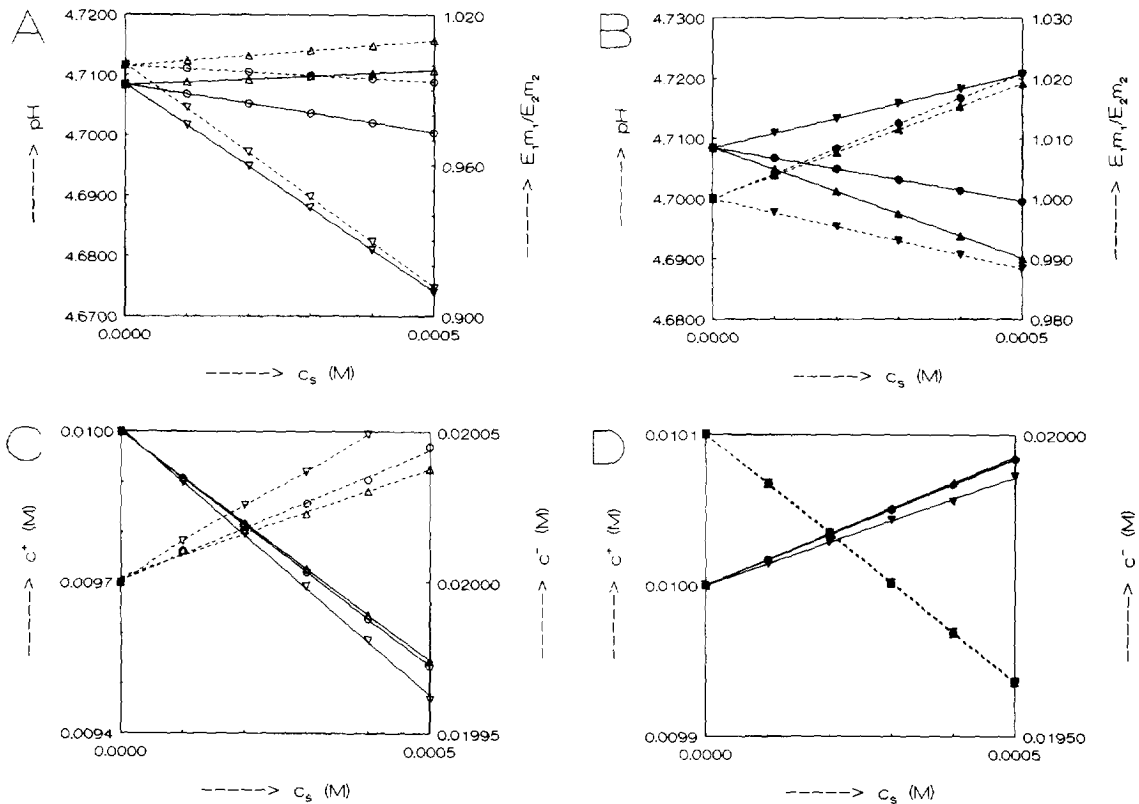
Fig. 4. The same parameters in the same BGE as shown in Fig. 3 for (A,C) sample cations with an ionic mobility at infinite dilution of $60 \cdot 10^{-9}$ m^2/V s and pK values of ($\triangle$) 14 (almost fully ionized, ($\bigcirc$) 5.2 (ionized for a major part) and ($\triangledown$) 4.2 (ionized for a minor part) and (B,D) for anions with an ionic mobility of $60 \cdot 10^{-9}$ m^2/V s and pK values of ($\blacktriangle$) -2 (almost fully ionized), ($\bullet$) 4.2 (ionized for a major part) and ($\blacktriangledown$) 5.2 (ionized for a minor part). For further information, see text.

the BGE, and all parameters are calculated for a peak segment with a total concentration of the sample component of $5 \cdot 10^{-4}$ M. In the calculations, corrections are made for activity coefficients and concentration effects on mobilities. The relationships between a specific parameter and the mobility, at infinite dilution of the sample ions, are always given. If the calculated parameter strongly depends on the pK value of the sample ions, the relationships are given for pK_S values of 14 (fully ionized cation, $\triangle$), pH + 0.5 (ionized for the larger part, $\bigcirc$) and pH − 0.5 (ionized for a minor part, $\triangledown$) for cationic analytes and pK_S values of −2 (fully ionized anions, $\blacktriangle$), pH − 0.5 (ionized for a larger part, $\bullet$) and pH + 0.5 (ionized for a minor part, $\blacktriangledown$) for anionic analytes. From these relationships, the effect of the pK value of the sample ions can be estimated. The design of a

SystChart is always as follows (see Fig. 5 Fig. 6 Fig. 7). In the figures, panels A–D describe the electrophoretic behaviour of cationic analytes and panels E–H those of the anionic analytes for the specified BGE. In panels A and E, the pH values in the sample zones are given. In panels C and G, the ratios $E_1 m_1 / E_2 m_2$ are given, where the subscripts 1 and 2 refer to the BGE and sample zone with a c_S of $5 \cdot 10^{-4}$ M, respectively, and in this formula, the m refers to the values of the effective mobilities of the ionic species. If the ratio is larger than unity, the sample component in the BGE moves faster than that in the sample zone at a concentration of $5 \cdot 10^{-4}$ M, through which a fronting peak will be obtained and if the ratio is smaller than unity, tailing peaks are the result. In panels B and F, the total concentrations are given of all cationic species of the BGE present in
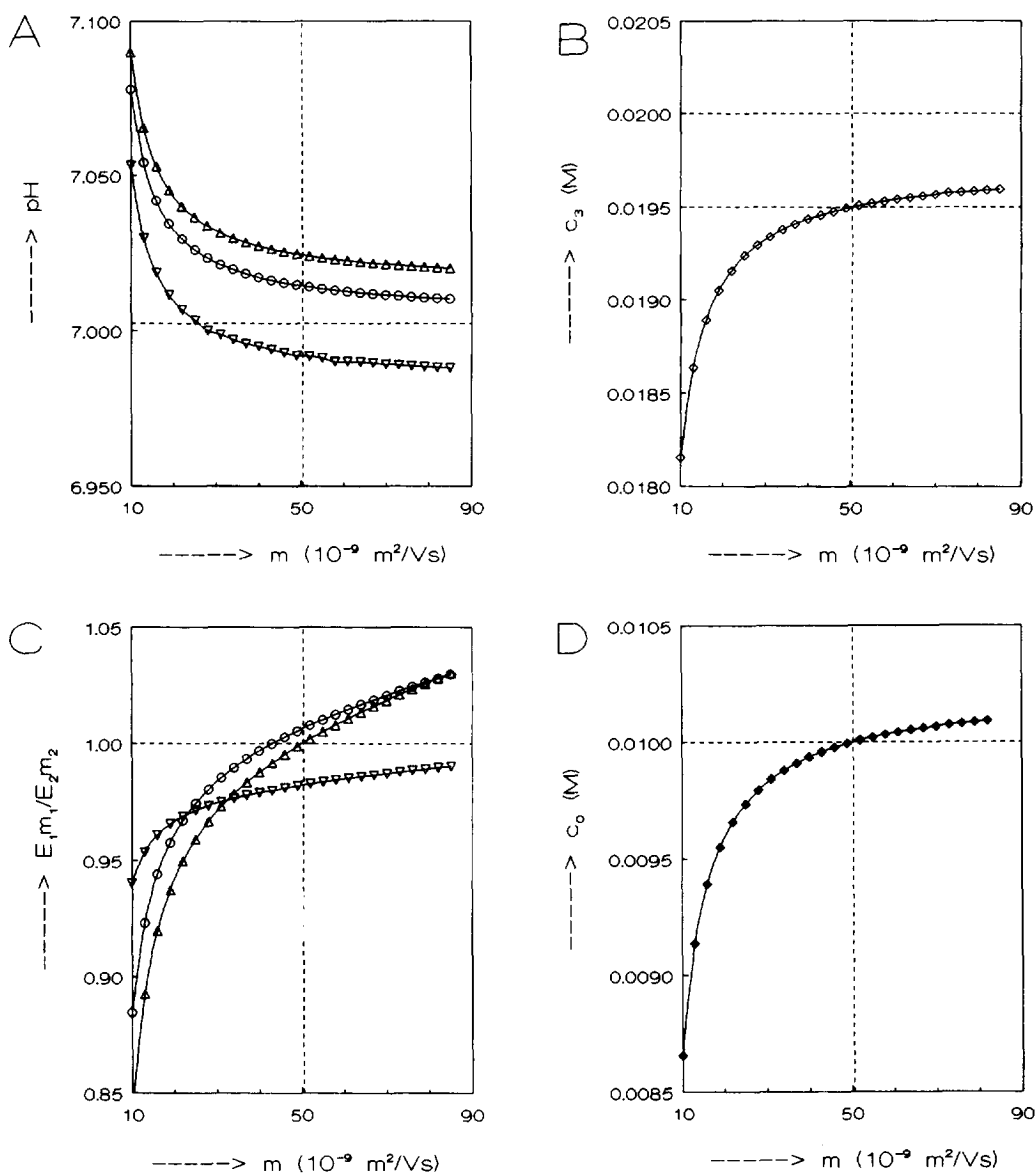
Fig. 5. SystChart for a BGE consisting of 0.02 M imidazole and 0.01 M acetic acid at a pH of 7.0. A–D refer to the parameters of sample zones of cationic analytes with pK values of ($\triangle$) 14, ($\bigcirc$) 7.5 and ($\triangledown$) 6.5 and E–H refer to those of anionic analytes with pK values of ($\blacktriangle$) -2, ($\bullet$) 6.5 and ($\blacktriangledown$) 7.5 in sample peaks with a sample concentration of $5 \cdot 10^{-4}$ M. The terms c_3 and c_0 refer to the cations of imidazole, and the anions acetate (of the BGE), respectively. For further information, see text.

the sample peaks, and in panels D and H, the total concentrations of all anionic species of the BGE present in the sample peaks are given. Panels B and H describe the concentrations of the co-ions, and panels D and F describe those of the counter-ionic species. Because the relationships between the calcu-

lated total concentrations of the co- and counter-ions versus the concentration of the sample ions often do not differ much for cationic and anionic analytes with equal mobilities at infinite dilution and different pK values, a single relationship generally will be given, viz., the relationships for the fully ionized
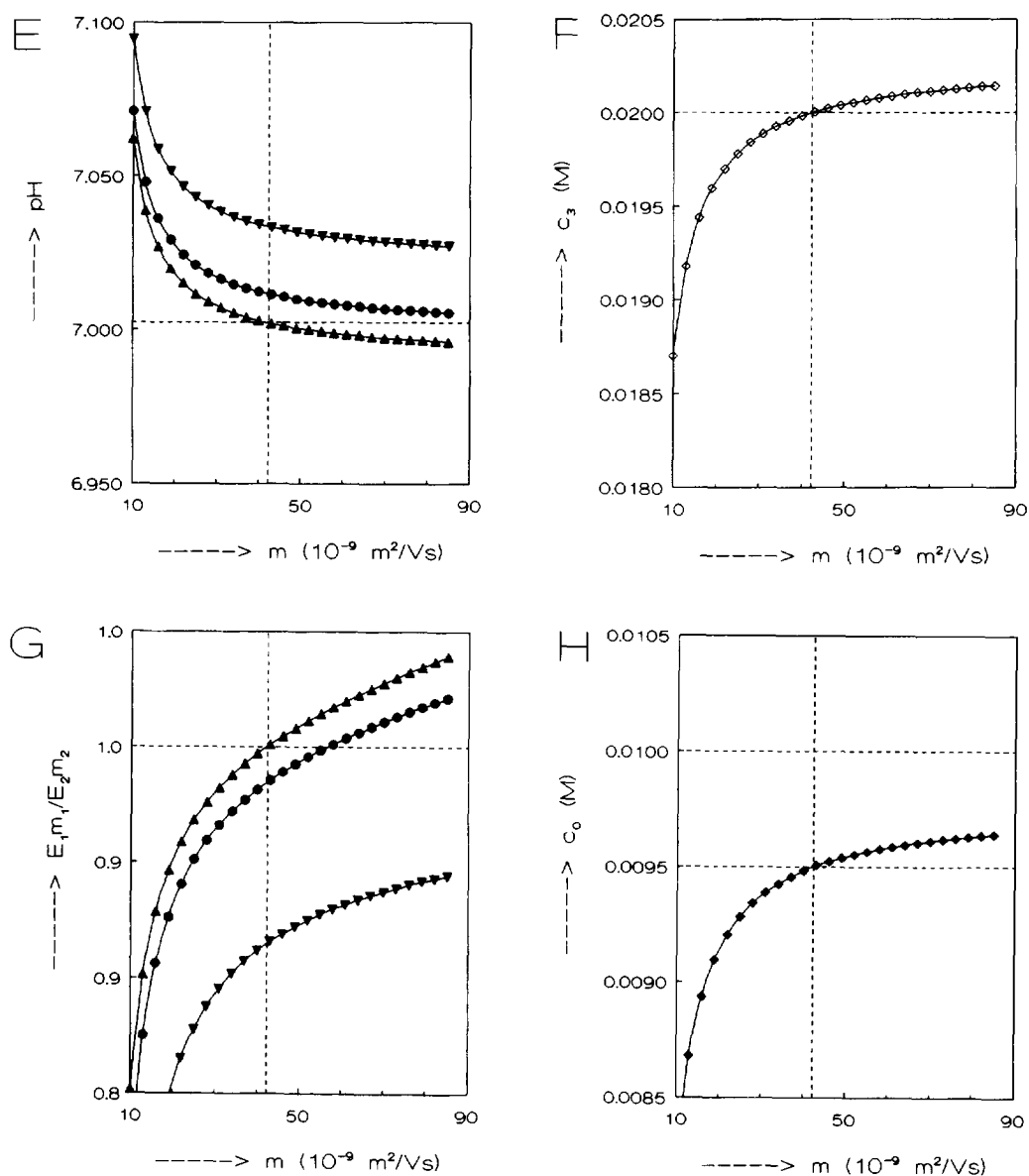
Fig. 5. (continued)

sample ionic species. For a good understanding, details of some SystCharts will be discussed further.

4.1.1. The SystCharts ImAc4.7 and ImAc7

SystCharts for BGEs containing a single co- and counter-ion, will be described first, namely those for BGEs consisting of 0.02 M imidazole and 0.01 M acetic acid at a pH of 7.0 (called ImAc7) and consisting of 0.01 M imidazole and 0.02 M acetic acid at a pH of 4.7 (ImAc4.7). In ImAc7, the buffering component is imidazole, i.e. buffering co-ions for cations and buffering counter-ions for anions. For ImAc4.7, the buffering component is acetic acid. The set of figures for ImAc7 are given in Fig. 5 and those for ImAc4.7 in Fig. 6. Considering
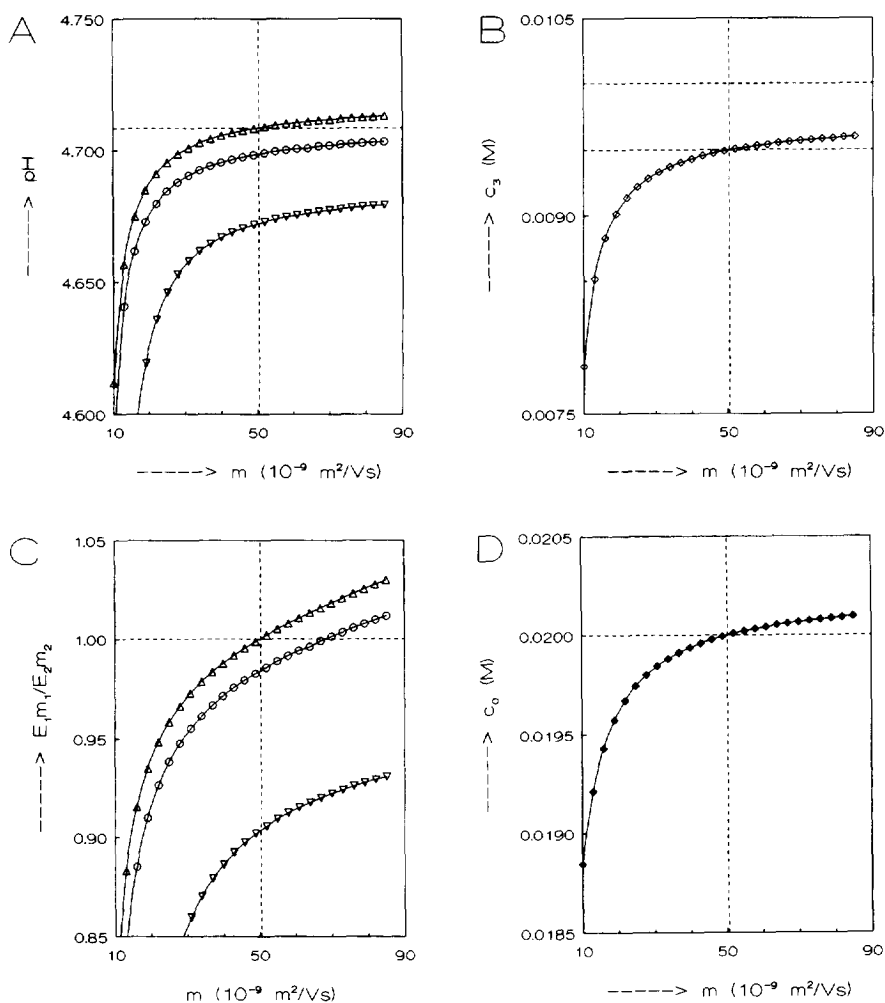
Fig. 6. SystChart for a BGE consisting of 0.01 M imidazole and 0.02 M acetic acid, at a pH of 4.7, for cationic analytes with pK values of ($\triangle$) 14, ($\bigcirc$) 5.25 and ($\triangledown$) 4.25 and for anionic analytes with pK values of ($\blacktriangle$) -2, ($\bullet$) 4.25 and ($\blacktriangledown$) 5.25. For further information, see text.

the SystCharts ImAc7 and ImAc4.7, several similarities are obvious and these will be discussed.

In all figures, the dashed vertical lines indicate the mobilities of the co-ionic species at infinite dilution and the dashed horizontal lines indicate the values of the given parameter in the BGE. In B and H, a second dashed line sometimes indicates the total concentration of the co-ions for a transfer ratio of unity, i.e., with a concentration value that is $5 \cdot 10^{-4}$ M lower than that of the concentration in the BGE. The transfer ratio is defined as the number of molecules of the BGE displaced by each analyte

molecule. If the BGE contains more co- and counter-ionic species, I will use the symbols c_0 ($\blacklozenge$), c_1 ($\blacksquare$) and c_2 ($+$) in B, D, F and H to indicate the concentrations of the anionic species of the BGE and c_3 ($\lozenge$), c_4 ($\square$) and c_5 ($+$) for the cationic species. In SystCharts ImAc7 and ImAc4.7, c_0 and c_3 refer to acetic acid and imidazole, respectively.

Panels B, D, F and H

In all of these figures, only a single relationship is given for the fully ionized cationic or anionic
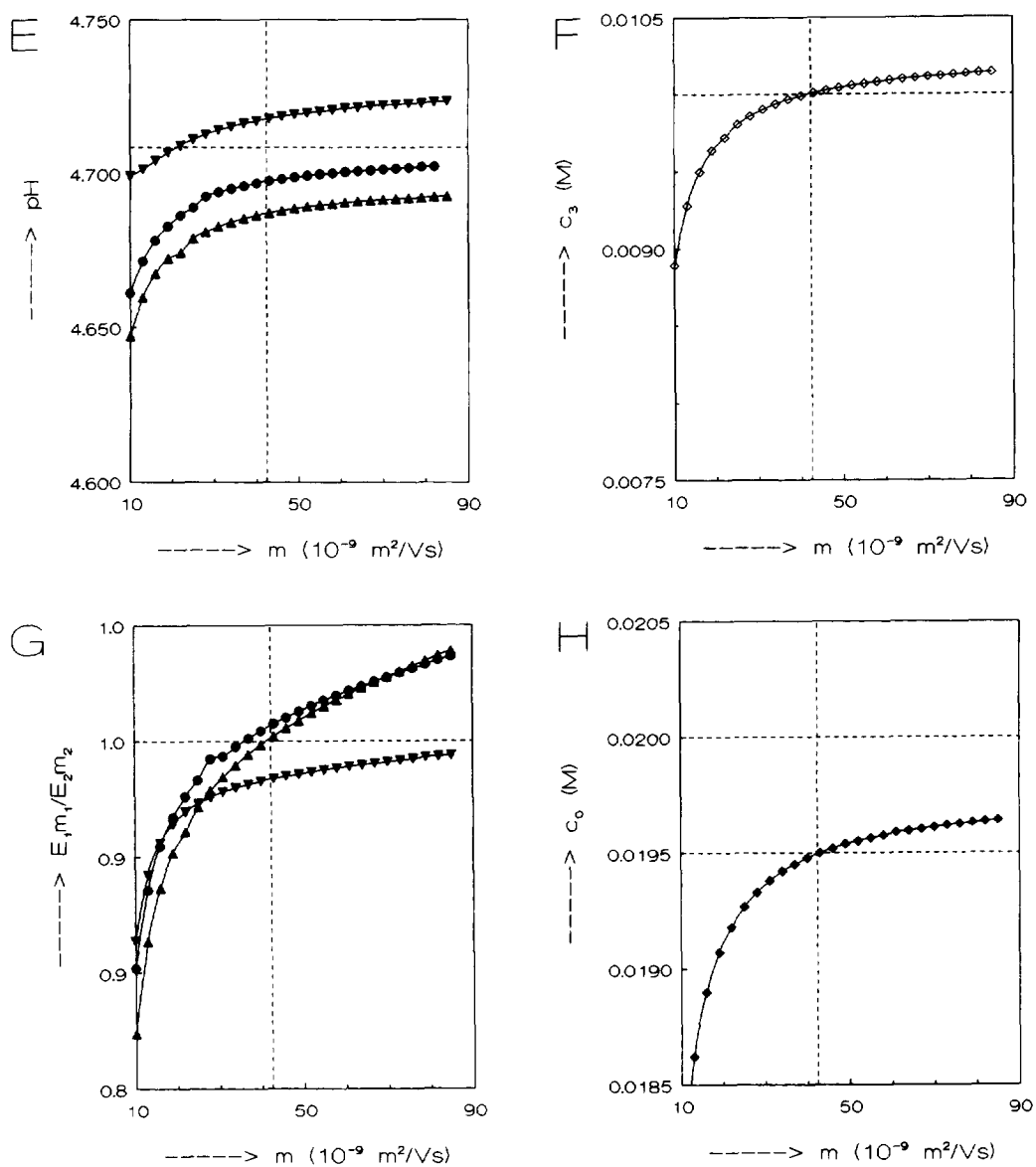
Fig. 6. (continued)

analytes because different pK values of the analytes do not strongly affect these relationships. Thus, the turn-over point for the transfer ratio T_R, i.e., the mobility of the sample ions when the value of T_R is unity, is generally not affected by the pK values of the sample ions. In all cases, the total concentrations of the co-ions (see panels B and H) are always

smaller than those of the co-ions in the BGE. For sample components with an ionic mobility lower than that of the co-ions, the transfer ratio is larger than unity, which means that the decrease in the concentration of the co-ions is larger than $5 \cdot 10^{-4}$ M. If ionic mobilities of sample components and co-ions are equal, there is a one-to-one displacement of
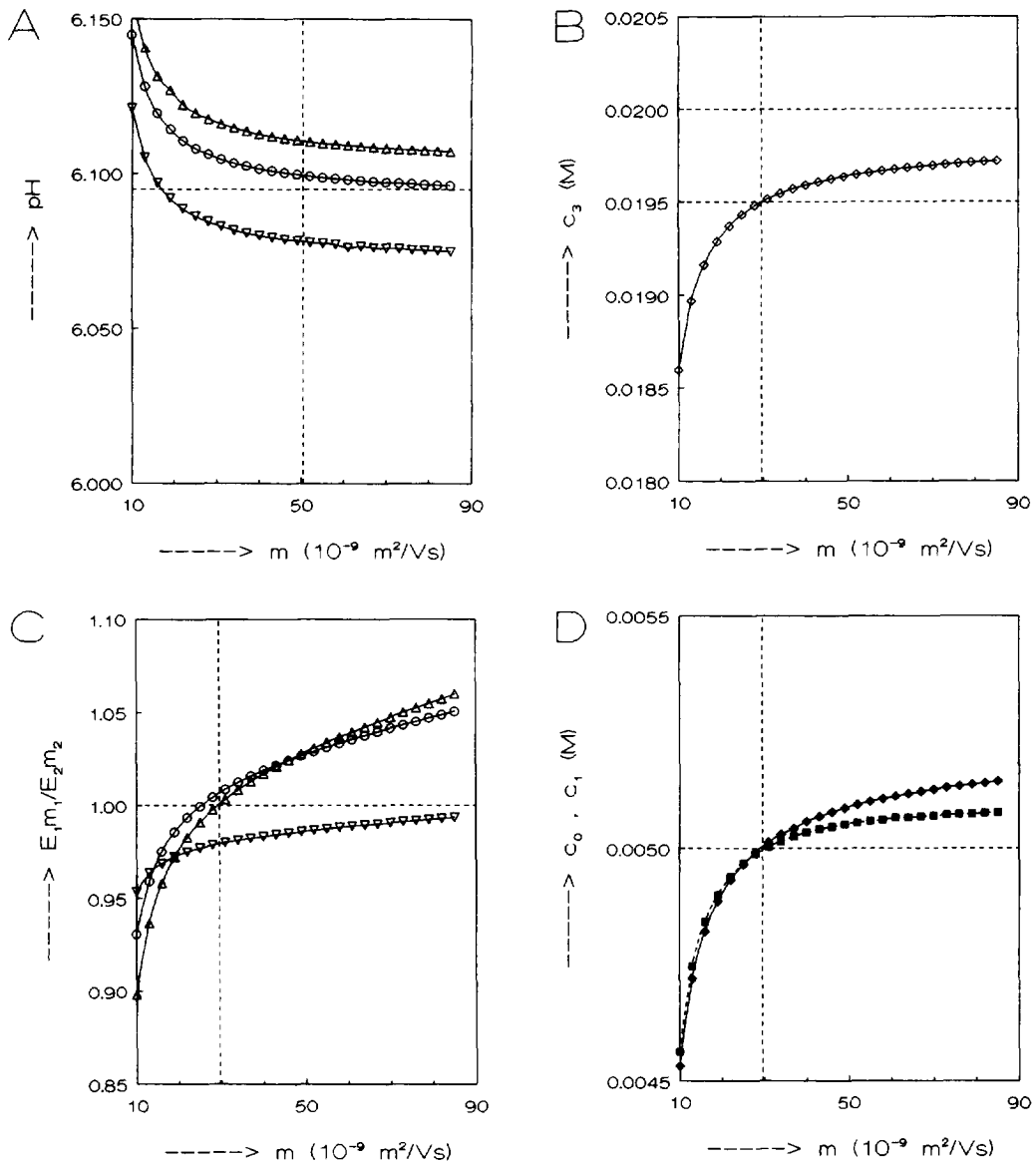
Fig. 7. SystChart for a BGE consisting of a mixture of 0.02 M histidine, 0.005 M hydrochloric acid and 0.005 M butyric acid, at a pH of 6.1, for cationic analytes with pK values of ($\triangle$) 14, ($\bigcirc$) 6.5 and ($\bigtriangledown$) 5.5 and for anionic analytes with pK values of ($\blacktriangle$) -2, ($\bullet$) 5.5 and ($\blacktriangledown$) 6.5. The terms c_0, c_1 and c_3 refer to the total concentrations of ($\blacklozenge$) hydrochloric acid, ($\blacksquare$) butyric acid and ($\lozenge$) histidine, respectively. For further information, see text.

sample and co-ions and, in this case, the concentration of the counter-ions equals that of the BGE. If the ionic mobility of the sample ions is higher than that of the co-ions, the transfer ratio is smaller than unity, implying that the decrease in concentration of

the co-ions is smaller than $5 \cdot 10^{-4}$ M, and this results in an increase in the ionic strength and the concentration of the counter-ions. The use of UV-absorbing counter-ions leads to peaks for UV-transparent sample components in the latter case [9].
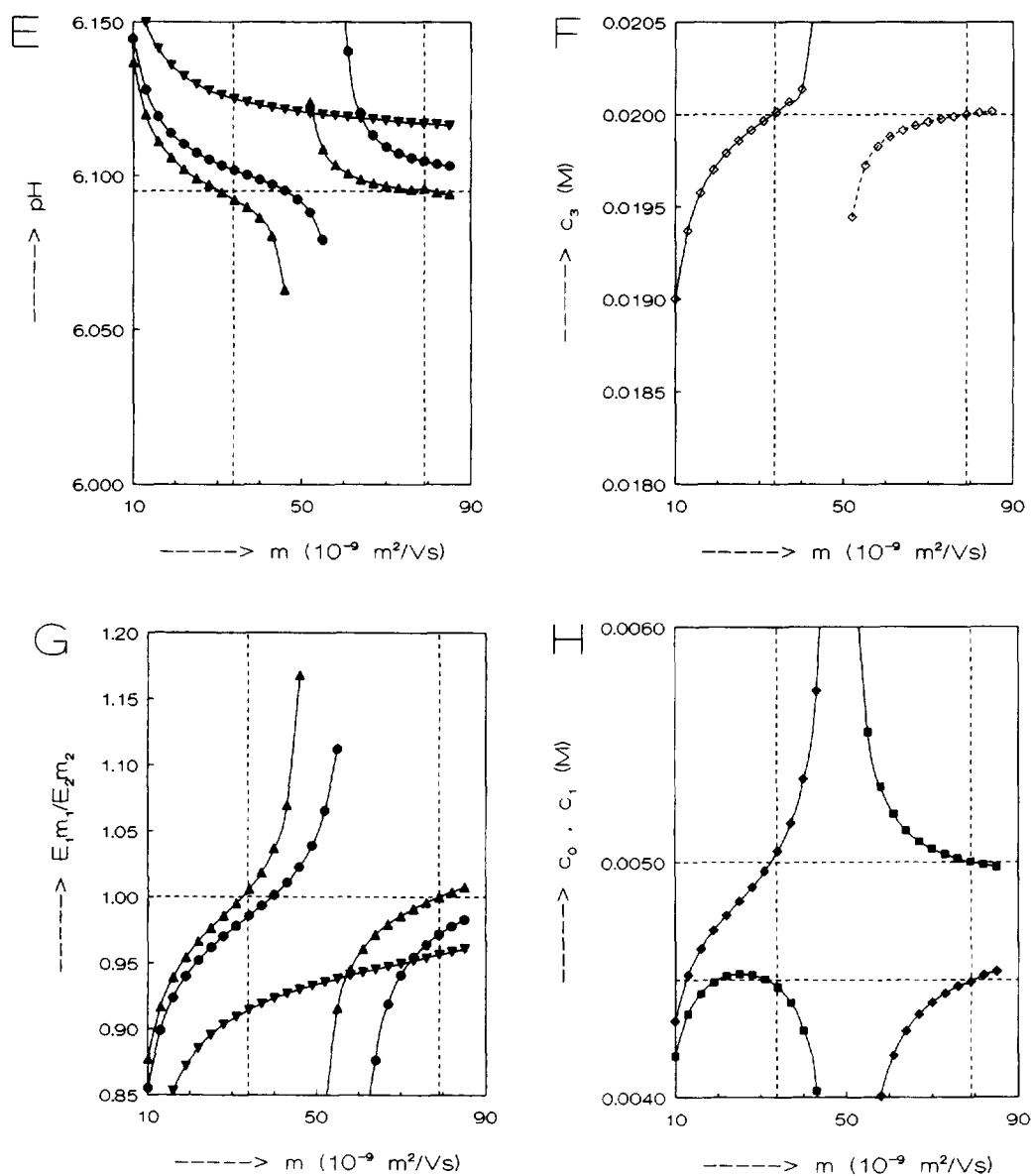
Fig. 7. (continued)

Panels A and E

The pH values are always highest for the fully ionized cations and lowest for the fully ionized anions. For buffering co-ions, the pH is always higher than that of the BGE for cations (see Fig. 5A) and lower for anions (see Fig. 6E) if the sample ions are properly ionized. Otherwise ($pK_s < pH$ for cat-

ions and $pK_s > pH$ for anions) the pH is nearly always lower and higher than the pH of the BGE, for cations and anions, respectively. In the case of a buffering counter-ion (see Fig. 5E and Fig. 6A), the pH in the sample zone equals the pH in the BGE if $m_S = m_{co-ion}$ for fully ionized sample ions. For partially ionized cations and anions, the pH values are

always lower and higher than for fully ionized
analytes.

C and G

In the ratio $E_1 m_1 / E_2 m_2$, the numbers 1 and 2 refer
to the composition of the BGE and the sample zone
of $5 \cdot 10^{-4}$ M, i.e., the ratio represents the quotient of
the velocities of the sample ions in the BGE and the
sample zone, respectively. In this formula, m refers
to effective mobilities. If the ratio >1, peaks will be
fronting because the lowest concentration segment
will move fastest, whereas tailing peaks will be
obtained if the ratio <1. This ratio is better than the
ratio E_1 / E_2, used earlier, because for "weak" acids
and bases, small shifts in pH can result in a large
change in effective mobility and this effect can often
overrule changes in the E ratio. There is remarkable
similarity between Fig. 5C and Fig. 6G, and between
Fig. 5G and Fig. 6C. In all cases, the ratio is unity
for fully ionized cations and anions if $m_S = m_{co-ion}$.
Partially ionized cationic and anionic are mostly
tailing.

4.1.2. The SystChart HiClBu6.1

The BGE for the SystChart HiClBu6.1 consists of
a mixture of 0.02 M histidine, 0.005 M hydrochloric
acid and 0.005 M butyric acid at a pH of approxi-
mately 6.1 and for cationic analytes a single co-ion is
present and for anionic analytes two co-ions are
present. The buffering ionic species is histidine. All
figures of the SystChart HiClBu6.1 are given in Fig.
7. A–D of SystChart HiClBu6.1 strongly resemble
the A–D of SystChart ImAc7, with the understand-
ing that in D of HiClBu6.1, two relationships are
given for the total concentration of the counter-ions
($\blacklozenge$) chloride and ($\blacksquare$) butyrate. That is, the presence
of two anionic counter-ions does not affect the
behaviour of the cationic analytes in the system and
does not lead to system peaks. For anionic analytes,
the situation is totally different (see E–H) because
two co-ions are present. In all figures, the relation-
ships are discontinuous. From Fig. 7H, it can be
concluded that selective displacement will occur for
sample anions with mobilities that are equal to one
of the mobilities of the two co-anionic species. If a
component has a mobility lower than that of the
co-ion with the highest mobility, the concentration of
that co-ion in the sample zone decreases considera-

bly and if the component has a mobility higher than
that of the co-ion with the lowest mobility, the
concentration of that co-ion decreases (selective
displacement). If the concentration of a co-ion
decreases, the concentration of the other one in-
creases considerably. There is discontinuity between
the mobilities of the two co-ions and this means that
the set of equations describing the electrophoretic
process can not be solved for a particular mobility
domain of the anionic analytes and a system peak
will be present in the electropherogram. This mobili-
ty domain is in between sample mobilities of approx-
imately 43 to $57 \cdot 10^{-9}$ m^2/V s in Fig. 7H and the
mobility of the system peak will be approximately
$50 \cdot 10^{-9}$ m^2/V s. As it is dependent on the UV-
absorbing properties of the co-ions, a sample zone of
a UV-transparent ion can be present as a dip or as a
peak in the UV signal, as already observed [7,9]. A
decrease in the concentration of one of the co-ions
means that a system peak will be present with an
increase in that co-ion and vice versa. From E and G
it can be concluded that for analytes that are barely
ionized, the relationships are continuous, indicating
that no system peak would be present. Sample anions
with mobilities in between the mobilities of the
system peak and of the co-ion with the highest
mobility are tailing (see G) and those with mobilities
in between the mobilities of the system peak and the
mobility of the co-ion with the lowest mobility are
fronting again! We have shown only the concen-
trations of fully ionized analytes in F and H, for the
sake of simplicity, although different relationships
are sometimes obtained for weak anions. Selective
displacement can be observed for analytes with
mobilities in the vicinity of those of the co-ions,
although both co-ions are transferred to a certain
degree and often an increase in concentration occurs.

4.2. How to use SystCharts

In SystCharts, the most important parameters that
play a part in electrophoretic processes are visual-
ised, for both fully and partially ionized cationic and
anionic analytes for a particular BGE. To decide
which BGE would be preferable for a particular
separation, several things must be considered. In
order to minimize the electrodispersive effect and to
minimize peak-broadening, the ratio $E_1 m_1 / E_2 m_2$

must be as close to unity as possible. If the value of the ratio is larger or smaller than unity, fronting and tailing peaks are obtained. An optimal UV signal is obtained if the change in concentration is maximal for the UV-absorbing component present in the sample peak. Regarding SystCharts ImAc7 and ImAc4.7, applying BGEs with a single anion and cation, the narrowest peaks are obtained for fully ionized analytes with a mobility that is close to that of the co-ions present [10]. When analysing two components with large differences in mobility, one of the sample ions will always show a broad and diffuse sample peak. When using a BGE containing two co-ions (see SystChart HiClBu6.1), the ratio $E_1 m_1 / E_2 m_2$ will be unity if the anionic mobility equals the mobility of one of the co-ions, i.e., for anionic analytes with different mobilities, sharp peaks can be obtained by choosing two appropriate co-ions. Furthermore, it can be concluded from the SystChart that system peaks are present in cases where there are two co-ions, but the presence of two counter-ions does not seem to affect the separations. The application of two co-ions (see SystChart HiClBu6.1 for anions and Part III [7] for cations) results in a reversal of the fronting/tailing character for sample peaks with mobilities in the vicinity of that of the system peak and, in this case, the concentrations of the co-ions decrease considerably for co-ions with the highest mobilities and increase for those with the lowest mobilities.

To demonstrate some of these effects, we have studied the separation of a sample containing a 0.0005 M concentration of the anionic analytes nitrate, formate, acetate and benzoate. The measured electropherograms for 10 s pressure injections of the sample are shown in Fig. 8 where the BGEs consisted of a mixture of 0.02 M histidine and (a) 0.01 M hydrochloric acid, (b) 0.01 M butyric acid and (c) 0.005 M hydrochloric acid and 0.005 M butyric acid. The electropherograms were measured in the anionic mode (anode at the outlet, applied voltage 10 kV) and, in order to suppress the EOF, 0.025% mowiol poly(vinyl alcohol) (PVA) and $5 \cdot 10^{-4}$ M cetyltri-methylammoniumhydroxide (CTA) were added to all BGEs [11]. Using the single co-ion, chloride (electropherogram a), with high mobility, the first sample peak nitrate is sharp, whereas sample peaks of components with lower mobilities show peak-
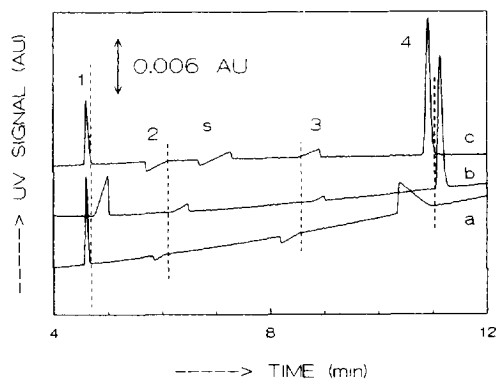

Fig. 8. Measured electropherograms for the separation of a mixture of 0.0005 M (1) nitrate, (2) formate, (3) acetate and (4) benzoate (10 s pressure injection) using BGEs consisting of a mixture of 0.02 M histidine and (a) 0.01 M HCl, (b) 0.01 M butyric acid and (c) 0.005 M HCl and 0.005 M butyric acid. For further information, see text.

broadening due to the increasingly electrodispersive effect. Because a UV-absorbing counter-ionic species is applied (histidine), the UV-transparent components, formate and acetate, are dips because their mobilities are lower than that of the co-ion, chloride [9]. All sample peaks are tailing because the mobilities of the samples are lower than that of chloride. Using the co-ion butyrate (see electropherogram b), with low mobility, all sample peaks are fronting and the UV-transparent sample ions, formate and acetate, are peaks when using a UV-absorbing counter-ion, because their mobilities are higher than that of the co-ion, butyrate. The sample peak of benzoate is sharp because its mobility is close to that of butyrate. When two co-ions are used, the situation is totally different. According to Fig. 7G, both the nitrate and the benzoate peaks are sharp. There is a system peak, S, with a mobility that is in between the mobilities of chloride and butyrate. Again, formate is tailing and acetate is fronting, according to Fig. 7G (all sample ionic species can be considered to be fully ionized). The UV-transparent component, formate, is a dip and the UV-transparent component, acetate, is a peak conform Fig. 7F. All of the sample's peak characteristics that were measured and are shown in Fig. 8 are in accordance with and can be deduced from the SystChart HiClBu6.1. From Fig. 8, it can be concluded that it is advisable to use the migration of the

diffuse edge of a sample peak for determination of the mobilities.

5. Conclusion

In a series of four papers, a mathematical model is discussed for the calculation of all parameters in sample zones in capillary zone electrophoresis for strong and weak anionic and cationic analytes. The model is based on a repeated steady-state model, using all mass balances of the ionic species, the modified Ohms' Law and the electroneutrality equation. Results of calculations confirm all sorts of rules of thumb concerning peaks/dips and the fronting/tailing character of sample peaks for BGEs with a single co-ion and provides further information about changes in the pH and in the concentration of all ionic species present in a sample peak. For BGEs with more co-ionic species, the set of equations describing the electrophoretic process could not be solved for a mobility-domain of the sample components and the centre of that mobility-domain corresponds to the mobility of system peaks lying in-between the mobilities of the co-ions. At that mobility, there was often discontinuity in the relationships between the parameters in the sample zones and the mobility of the sample component. Although all parameters in the sample zone for all concentrations of the sample component can be calculated, for a particular sample ionic species in a particular BGE, it is not possible to formulate general rules of thumb. In order to visualize information about anionic and cationic analytes in CZE in an appropriate way, SystCharts are presented so that in a set of eight figures, the most important parameters of sample peaks are visualized for a particular BGE. To date, all experimental data have been in accordance with calculated values and a comparison with other simulation programs is important to determine and compare their possibilities [12–20].

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