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Transfer ratio in indirect UV detection in capillary zone electrophoresis
A mathematical approach

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Abstract

Generally, the transfer ratio (TR) is defined as the number of molecules of the background electrolyte (BGE) with UV absorbing properties, displaced by each analyte molecule, and this concept plays an important part in the choice of the BGE in capillary zone electrophoresis (CZE) in the indirect UV mode. Less transparent is this concept and its importance, applying BGEs with more than one co-ions, in the application of UV absorbing counterions and for the use of BGEs at low or high pH values. In this paper, the concept of the TR is discussed for these cases and applying a mathematical model, with which all parameters in the sample zones can be calculated, the TR values are calculated for several BGEs. Hereby, different values are obtained for anionic and cationic TR values for all ionic species of the BGE. Discontinuities in the relationships of the calculated TR values versus the mobilities of the sample components indicate that system peaks are present in the electropherograms applying BGEs with two co-ions and also applying BGEs with a single co-ion at both high and low pH values. This means that in the latter cases OH^- and H^+ ions act as a second co-ion, whereby the mobilities of the system peaks generally increase for higher and lower pH values, respectively. If the mobilities of the analytes approach the mobility of a system peak, the TR values increase considerably. These phenomena are confirmed experimentally.

Keywords: Transfer ratio; Background electrolyte composition; Mathematical modelling; Inorganic cations; Organic acids; Imidazole; Histidine; Ethanolamine

1. Introduction

In order to detect UV transparent analytes in capillary zone electrophoresis (CZE), the indirect UV mode is often applied. In the indirect UV mode, generally a background electrolyte (BGE) is chosen, whereby the co-ion – having a similar charge as the analytes – has UV absorbing properties and an optimal tuning of the indirect UV signal can be obtained if the mobilities of the analytes match that of the co-ion [1]. A concept playing an important part in the indirect UV mode is the so-called transfer ratio (TR) sometimes called the displacement ratio (D_R) or response factor (RF).

Originally, the indirect UV mode has been applied in ion-chromatography where negative peaks are created in the UV detector signal, by the ion-exchange mechanism, resulting in a one-to-one displacement of the eluent UV absorbing ions of the

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same charge [2]. Afterwards the indirect UV mode was applied in electrophoresis [3,4], where generally not a one-to-one displacement will occur. From Kohlrausch regulation function (KRF) [5] an expression for the TR, with relation to the displacement of the co-ions, can be derived for strong ionic species and neglecting the presence of H^+ and OH^- ions [6–8]:

$$TR = \frac{|\Delta c_A|}{c_S^S} = \frac{|c_A^{BGE} - c_A^S|}{c_S^S} = \frac{m_A}{m_S} \times \frac{m_S + m_C}{m_A + m_C} \quad (1)$$

In this formula *m* refers to the absolute values of the mobilities, the superscripts S and BGE refer to the sample zones and BGE respectively, and the subscripts S, A and C refer to the sample ionic species S, the co-ions A and the counterions C. The value of TR depends on the mobilities of all ions and clearly can be seen that only a one-to-one displacement occurs if the mobilities of the analytes are equal to that of the co-ions (TR=1). In that case the electrodispersion is minimal resulting in a minimal peak broadening and large plate numbers. If $m_S > m_A$ the TR values are smaller than unity and for $m_S < m_A$ this value is larger than unity. For large differences between the mobilities of analytes and co-ions the electrodispersion strongly increases. Therefore better separations can often be obtained applying two co-ions whereby a selective displacement occurs [9].

The concept of the TR is more complicated applying BGEs with more co-ions or applying BGEs containing UV absorbing counterions. In these cases UV transparent analytes can be present in the electropherograms both as dips and peaks, depending on the TRs of all ionic species of the BGE [10,11]. A lot of experimental work has been done studying the influence of the use of two co-ions, the use of counterions and for the verification for expressions for TR, derived from the KRF [12–14]. In all these treatments the effects of extreme pHs are not considered, however. With a model based on a repeated application of a steady-state model, all parameters in a sample zone can be calculated and they can be visualised in a so-called SystChart [15]. This model is not based on the KRF, the effects of H^+ and OH^- are taken into account and corrections are made according to Debye–Huckel–Onsager. If all parameters in the sample zones can be obtained applying such a mathematical model, all TR values can be calculated. In this paper the concept of the TR will be discussed and TR values will be calculated for several cases, such as BGEs with more co-ions, BGEs at low and high pH values and the application of UV absorbing counterions. Experiments are carried out to verify the calculated values.

2. Experimental

2.1. Chemicals

Chemicals were all of analytical-reagent grade and purchased from Merck (Darmstadt, Germany). For the preparation of all solutions, ultra-pure water was used.

2.2. Instrumentation

For all CZE experiments a P/ACE system 2200 HPCE system (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 47.2 cm and a distance between injection and detection of 40.6 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, and the operating temperature was 25°C. Sample introduction was performed by applying pressure injection. Data analysis was performed using the laboratory-written data analysis program CAESAR.

3. Results and discussion

3.1. The concept of the transfer ratio

If in capillary zone electrophoresis a BGE is applied containing just one co-ion, always co-ions are displaced by sample ions, i.e. that the concentration of the co-ions in the sample zone is always lower than that in the BGE, although the so-called transfer ratio, TR, varies and is only unity if the mobilities of sample ions and co-ions are equal. The situation is more complicated if BGEs are applied containing more co-ions. Sometimes both the

co-ions are displaced in a degree, but often one of the co-ions is displaced, i.e. that its concentration in the sample zone is lower than that in the BGE, whereas the concentration of the second co-ion increases (see Section 3.3). So the second co-ion is not displaced, although the change in its concentration is connected to the presence of the sample ions. Even the concentration of the counterions in the sample zone differs from that in the BGE. All the changes in concentrations of all ionic species of the BGE, in combination with their molar absorption coefficients, determines the UV signal. For a discussion about the UV signal applying the indirect UV mode, the transfer ratio is important and in the following we will use for the transfer ratio, TR, the expression:

$$TR_i = \frac{c_i^S - c_i^{BGE}}{c_S^S} \quad (2)$$

With the symbol *c*, the total concentration of an ionic species is meant, the subscript i refers to a specific ionic species of the BGE (this can be both a co-ionic species and a counterionic species) and the subscript S refers to the sample ionic species. The superscripts S and BGE refer to the sample zone and pure BGE. This means that if the concentration of an ionic species i of the BGE in a sample zone is lower than that in the BGE, a negative TR is obtained which corresponds with a dip in the electropherogram if that ionic species is UV absorbing. An increased concentration of ionic species i in a sample zone results in a positive TR and a peak will be present in the electropherogram for a UV absorbing ionic species i. This is true for both co-ions and counterions. Applying a specific BGE, there exists two TR_i values for each ionic constituents i, because the TR_i values are different for anionic or cationic analytes in that BGE. We will distinguish between these TR values by using the terms anionic TR_i and cationic TR_i, respectively. These TR_i values will be considered for several BGE-compositions. The TR_i values are calculated applying a mathematical model as mentioned before [15,16]. In Table 1 the ionic mobilities and p*K* values for all ionic species, used in the calculations are given. For the calculation of the TR values a sample concentration of 0.0005 *M* is assumed in the sample peak. In fact this parameter is not important as generally the TR values are independent to the sample concentrations in the sample zone.

Table 1
Ionic mobilities at infinite dilution and p*K* values for all ionic constituents used in the calculations

Ionic species[a]	$m \times 10^9$ (m^2/V s)	p*K*
Acetic acid	−42.4	4.756
Benzoic acid	−33.6	4.203
Ethanolamine	44.3	9.458
Formic acid	−56.6	3.752
Histidine	29.7	6.03
Imidazole	50.4	6.953
Lithium	40.1	>14
Monochloroacetic acid	−43.7	2.865
Potassium	76.2	>14
Sodium	51.9	>14
TBA	25.0	>9
TEA	32.5	>9
TMA	43.4	>9

[a] TBA = tetrabutylammonium; TEA = tetraethylammonium; TMA = tetramethylammonium.

3.2. A BGE consisting of a single cationic and anionic species

A BGE, consisting of a single cationic and anionic species at a pH where the effects of H^+ and OH^- ions can be neglected, is a mixture of 0.01 *M* imidazole and 0.02 *M* acetic acid (pH = 4.7). For this BGE, four TR values can be calculated, viz. the cationic and anionic TR of both imidazole and acetic acid. In Fig. 1A the relationships between the anionic TR values of imidazole (Im) and acetic acid (Ac) are given versus the mobilities, m_S, at infinite dilution of the sample anions (absolute values). From Fig. 1A it can be seen that a one-to-one displacement of acetic acid takes place if the m_S equals the mobility of acetate (vertical dashed line), i.e. the TR_{Ac} is −1 and at that point the TR_{Im} is zero. For higher values of the m_S, the absolute values of TR_{Ac} are smaller than 1, i.e. the ionic strength increases and also the concentration of imidazole increases (positive value of the TR_{Im}). These sample ions can be detected in the indirect mode with UV absorbing counterions imidazole and are then present in the electropherograms as peaks. For lower values of the m_S, the ionic strength decreases and by this also the imidazole

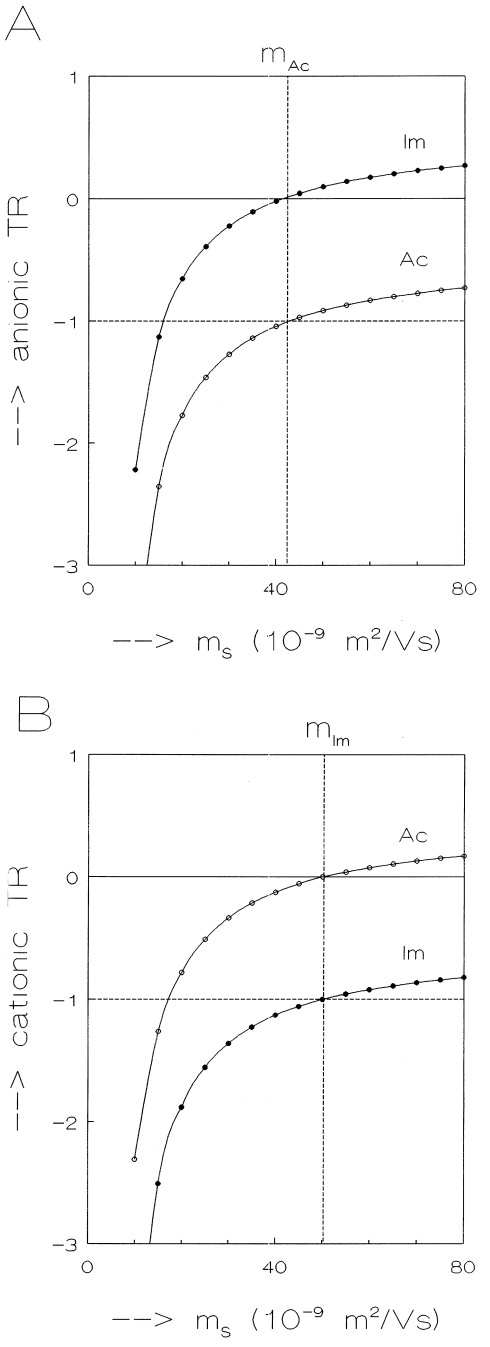

Fig. 1. Calculated relationships between (A) anionic and (B) cationic TR values for imidazole (Im) and acetic acid (Ac) versus the absolute values of the mobilities m_S at infinite dilution of respectively the anionic and cationic analytes. A one-to-one displacement occurs if m_S equals the mobility of the co-ion. For further information see text.

concentration in the sample zone resulting in negative values for TR_{Im}. These sample ions are always dips in the electropherograms for UV absorbing counterions. An optimal UV signal would be obtained for sample anions with $m_S < m_{Ac}$ and applying both UV absorbing counterions and UV absorbing co-ions. In Fig. 1B the relationships between the cationic TR values of imidazole (Im) and acetic acid (Ac) are given versus the mobilities m_S at infinite dilution of the sample cations. All phenomena observed in Fig. 1A are also valid for Fig. 1B. The one-to-one displacement takes place if m_S equals m_{Im} (vertical dashed line). All these conclusions agree with the results of the calculations applying Eq. (1).

3.3. A BGE with two cationic and a single anionic species

A mixture of 0.005 *M* histidine, 0.005 *M* potassium and 0.02 *M* acetic acid was chosen as a BGE with two cationic and a single anionic species (pH 4.7). For this BGE, six TR values can be calculated, viz. the cationic and anionic TR values of histidine, potassium and acetic acid. In Fig. 2A the relationships between the anionic TR values of histidine (Hist), potassium (K) and acetic acid (Ac) are given against the mobilities m_S at infinite dilution of the sample anions (absolute values). This BGE contains only a single co-ion for anionic analytes and because there are no discontinuities visible in the calculated relationships, no system peaks will be present in the electropherograms [15]. The anionic TR_{Ac} values are practically identical with these in Fig. 1A. The anionic TR values for histidine and potassium are nearly equal and therefore about half the values of imidazole in Fig. 1A, although the TR values of potassium are a little bit larger than those of histidine (absolute values). A totally different pattern is obtained for the cationic TR values in Fig. 2B. For cationic analytes this BGE contains two co-ions. In all cationic TR relationships there exists a discontinuity, indicating that a system peak (SP) will be present in the electropherograms. With a mathematical model described earlier [16] the mobility at infinite dilution of this SP is calculated as to be ca. 46.3×10^{-9} m^2/Vs and this value is indicated by a dashed vertical line in Fig. 2B. This value fits the place of the discontinuities. If the mobility of the

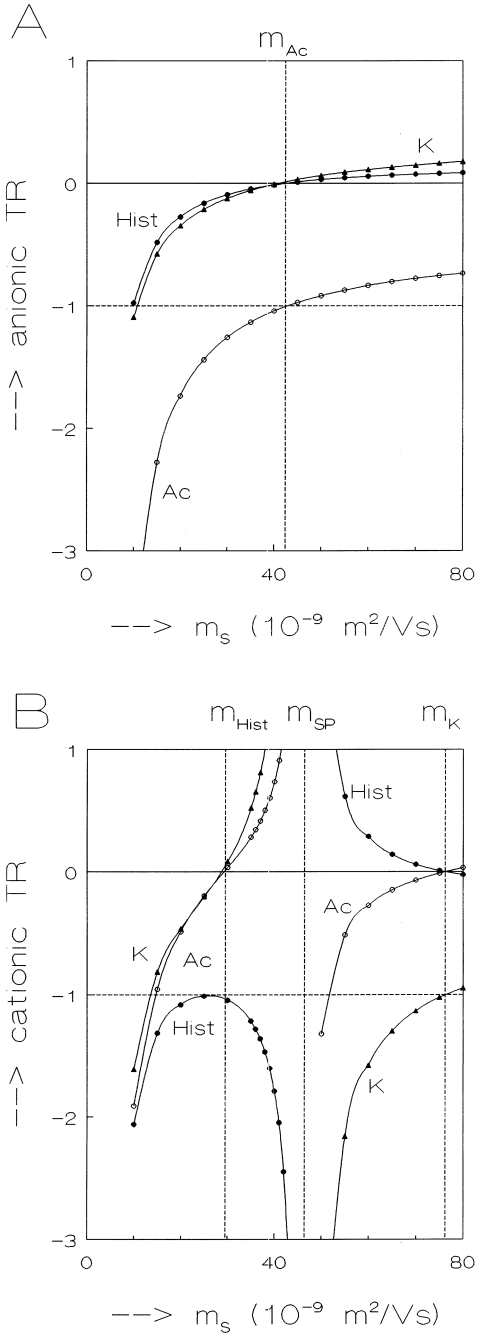

Fig. 2. Calculated relationships between (A) anionic and (B) cationic TR values for a BGE consisting of two cationic species potassium (K) and histidine (Hist) and a single anion acetic acid (Ac) versus the absolute values of the mobilities m_S at infinite dilution of, respectively, the anionic and cationic analytes. In the relationships of the cationic TR values, discontinuities are present indicating the presence of system peaks in the electropherograms. For further information see text.

analyte equals one of the mobilities of the co-ions, a selective displacement occurs, i.e. that the cationic TR_{Hist} is -1 and the TR_K value is zero if the sample mobility equals m_{Hist} and v.v for m_K. As already mentioned in Section 3.1, the TR_{Hist} strongly decreases just below the m_{SP}, i.e. that a UV transparent cationic species will appear in the electropherogram as a huge dip and at the same time the TR_K strongly increases. Just above the m_{SP} a reversed behaviour can be seen. For a cationic separation, applying this BGE (histidine is the chromophore), sample analytes are always dips if their mobilities are smaller than the m_{SP}, peaks if $m_{SP}<m_S<m_K$ and dips again for $m_S>m_K$.

3.4. Concentration dependence of TR values

For a BGE with a single co-ion and counterion, at intermediate pH values, the TR_i values are practically constant and do not vary for different concentrations of the components of the BGE. For BGEs with a more complex composition it is more complicated. The application of more co-ions and/or counterions leads to selective displacement and often SPs are present, which can influence migration times and peak areas and it is of interest to know whether and how TR_i values depend on the concentrations of the ionic species of the BGE. Although several authors already presented both experimentally obtained results and simulated electropherograms, no TR values are given. To study the concentration dependence of TR values, the TR values are calculated for BGEs consisting of mixtures of histidine, potassium and acetic acid, whereby the sum of the concentrations of histidine and potassium was always 0.01 *M* and the concentration of acetic acid was always 0.02 *M*. The pH of all BGEs was ca. 4.7. The anionic TR values show a behaviour similar to that of Figs. 1A and 2B, viz. the TR_{Ac} values are practically identical and the anionic TR_{Hist} and TR_K values follow a curve as Fig. 2A, with the understanding that the 'displacement' is larger for a higher concentration of that cationic species in the BGE. The cationic TR values show a complex behaviour. In Fig. 3A and B relationships between the cationic TR_{Hist} and TR_K values versus the mobilities m_S of the cationic analytes are given for different compositions of the BGEs. In Fig. 3A the numbers refer to the concentration of histidine in

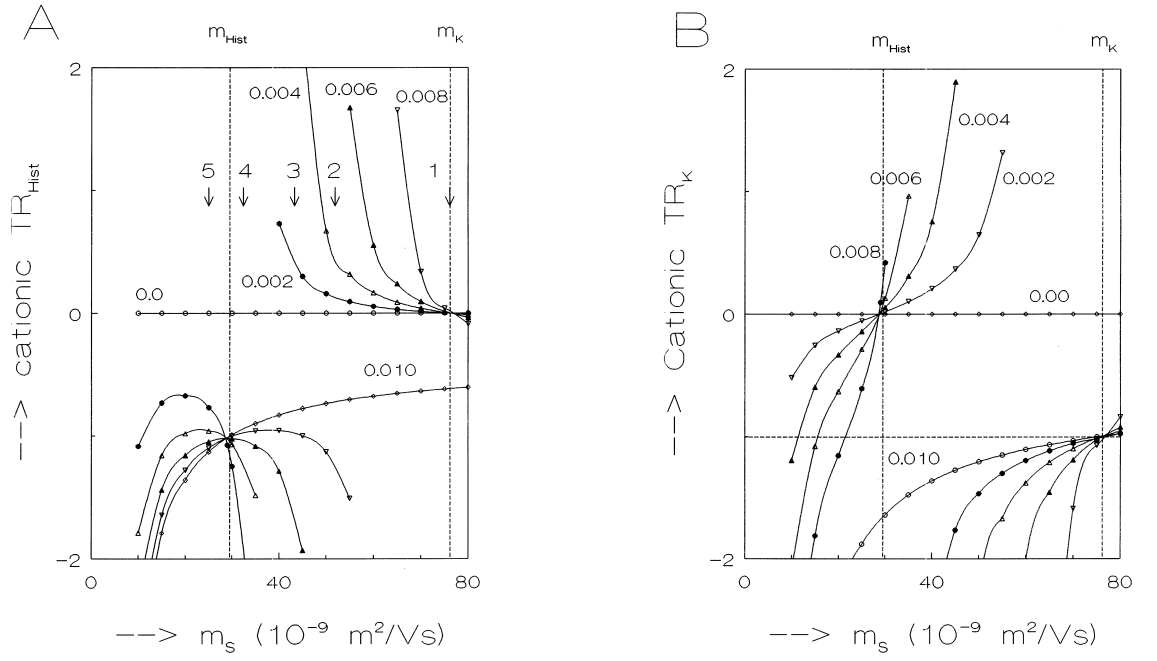

Fig. 3. Calculated relationships between cationic TR values of (A) histidine and (B) potassium versus the mobilities m_S at infinite dilution of cationic analytes applying BGEs consisting of varying concentrations of potassium, histidine and acetic acid at pH values of 4.7. The sum of the concentrations of potassium and histidine is always 0.01 *M* and the concentration of acetic acid is always 0.02 *M*. The numbers in (A) refer to the concentration of histidine in the BGEs and the numbers in (B) to the concentration of potassium. The numbered arrows in (A) refer to mobilities of the analytes separated in the electropherogram of Fig. 4. For further information see text.

the BGEs and the numbered arrows refer to the mobilities of the analytes applied in the electropherogram of Fig. 4. If the concentration of histidine in the BGE is 0.01 *M* (then the concentration of potassium in the BGE is zero, thus a BGE with a single co-ion) all TR values are negative, i.e. all cations are dips. If the concentration of histidine is zero, all TR_{Hist} values are zero. For all other cases there is a discontinuity in the relationship indicating that applying these BGEs, system peaks are present in the electropherogram. For higher concentrations of histidine, the mobility of the SP approaches that of potassium (vertical dashed line), whereas for decreasing concentrations of histidine the m_{SP} approaches the value of m_{Hist}. In Table 2 calculated and measured values are given for several applied electrolyte systems. If the mobility of the sample cations equals that of histidine there is a selective displacement of histidine and this means that all relationships intersect each other near the mobility of histidine at a TR value of -1. If the m_S equals the mobility of potassium, there is a selective displacement of potassium and all relationships of TR_{Hist} intersect each other at the m_K with a TR value of zero. At the mobility of the system peak the relationships show a discontinuity and for cationic mobilities near the m_{SP}, extremely large TR values are obtained. For a $m_S < m_{SP}$ the TR values are very negative (huge dips) and for $m_S > m_{SP}$ the TR values are very positive. In Fig. 3B the cationic TR_K values are shown and in this figure the numbers refer to the concentrations of the potassium ion in the BGEs. Similar phenomena as visible in Fig. 3A can be observed. For the relationship with 0.01 *M* K^+ (the concentration of histidine in the BGE is then zero, i.e. a single co-ion K^+) all TR values are negative and for a concentration of K^+ of zero this TR value is zero, as a matter of course. If m_S is just smaller than m_{SP} the TR values are very positive and if they are just larger than m_{SP} they are very negative. All the relationships intersect each other at the m_{Hist} ($TR = 0$) and at m_K ($TR = -1$) due to a selective

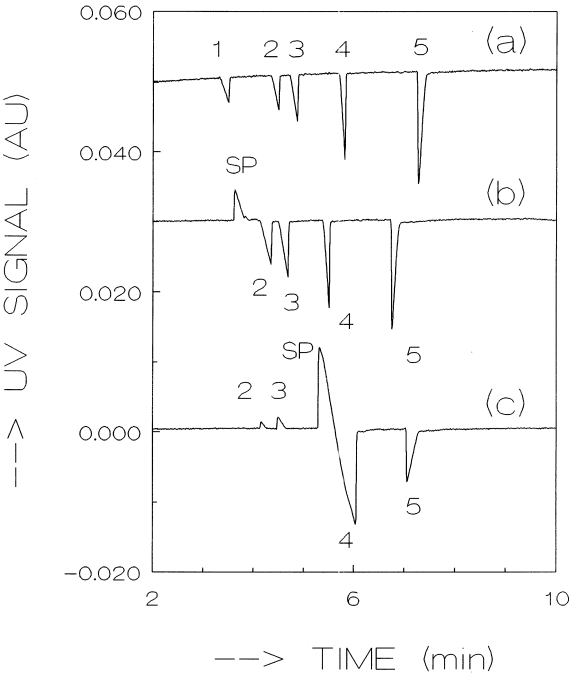

Fig. 4. Measured electropherograms for 5-s pressure injections of equimolar sample solutions of 5×10^{-4} M of (1) potassium, (2) sodium, (3) TMA, (4) TEA and (5) TBA ions applying BGEs at pH 4.7 consisting of potassium, histidine and acetic acid at concentrations of (a) 0.0, 0.01 and 0.02 *M*, (b) 0.002, 0.008 and 0.02 *M* and (c) 0.008, 0.002 and 0.02 *M*, respectively.

displacement. For cationic analytes with mobilities close to that of potassium and histidine the TR values do not change so much and, although, a large profit in TR value can be gained choosing a BGE with such a composition that the m_{SP} is close to the mobility of the cationic analyte, it must be remembered that in that case the separation can be disturbed by the presence of the system peak. To demonstrate some of the described effects, in Fig. 4 the measured electropherograms are shown for 5-s pressure injections of a sample solution of 0.0005 *M* of (1) potassium, (2) sodium, (3) TMA, (4) TEA and (5) TBA applying BGEs consisting of mixtures of potassium, histidine and acetic acid at a pH of 4.7 with concentrations of respectively (a) 0.0, 0.01 and 0.02 *M* (a single co-ion histidine), (b) 0.002, 0.008 and 0.02 *M* and (c) 0.008, 0.002 and 0.02 *M* (applied voltage = 10 kV). In Fig. 4a all analytes are dips according to the relationship 0.01 in Fig. 3A. According to the relationship 0.008 in Fig. 3A, potassium is not visible in Fig. 4b, and a system peak is visible with a mobility higher than that of sodium and the analytes are dips. In Fig. 4c potassium is not visible, sodium and TMA are small peaks and there is a huge system peak with a mobility close to the dip of TEA, just according the relationship 0.002 in Fig. 3A. All these peaks/dips are in agreement with the relationships of Fig. 3A, where the mobilities of the analytes used are indicated with the numbered arrows. A disadvantage in the use of BGEs with more than one co-ions as can be seen in Fig. 4c, is the presence of system peaks [16–19], which can disturb the separation especially if the mobility of the analyte m_S approach the mobility of the system peak m_{SP}, although just in that case larger TR values can be obtained.

3.5. TR values for BGEs at low and high pH values

If BGEs are applied consisting of a single cationic and anionic species at respectively high or low pH, the question is whether the OH^- and H^+ ions can act as a second anion or cation respectively, and it is of interest to know whether the TR values are affected. In the foregoing Sections 3.3 and 3.4, we saw that the presence of system peaks [besides the electroosmotic flow (EOF) dip] was observable in the TR versus m_S relationships bij the presence of discontinuities. Therefore, calculations are carried out for BGEs consisting of histidine as a UV absorbing cation and several acids as counterions at low pHs, and for BGEs consisting of ethanolamine as a cation and benzoic acid at high pHs. In Fig. 5A, the calculated relationships between cationic TR_{Hist} values for the co-ion histidine versus the mobilities m_S at infinite dilution of cationic analytes are given, applying BGEs consisting of 0.01 *M* histidine and, respectively, (○) 0.02 *M* acetic acid at pH 4.7, (▲) 0.02 *M* formic acid at pH 3.7 and (●) 0.02 *M* chloroacetic acid at pH 2.9. The numbered arrows indicate the mobilities of the analytes separated in the electropherograms given in Figs. 5B and C. At the pH of 4.7, the obtained curve is as expected. A continuous relationship, with all negative TR values and a TR value of -1 for $m_S = m_{Hist}$ (selective displacement). In fact there is a system peak at pH 4.7, but the m_{SP} is very small (ca. 1×10^{-9} m^2/Vs).

Table 2
Calculated and experimentally determined mobilities for the system peaks in the diverse BGEs

Composition BGE	Effective mobility $m \times 10^9$ (m^2/Vs)	
	Calculated	Experimentally determined[a]
Fig. 2B		
0.005 *M* histidine + 0.005 *M* potassium + 0.02 *M* acetic acid at pH 4.7	42.1	41.3
Fig. 3A		
0.000 *M* histidine + 0.010 *M* potassium	—	—
0.002 *M* histidine + 0.008 *M* potassium	31.0	30.4
0.004 *M* histidine + 0.006 *M* potassium	38.0	40.8
0.006 *M* histidine + 0.004 *M* potassium	46.6	43.9
0.008 *M* histidine + 0.002 *M* potassium	57.4	55.9
0.010 *M* histidine + 0.000 *M* potassium all mixed with 0.02 *M* acetic acid at pH 4.7	—	—
Fig. 5A and B		
0.01 *M* histidine + 0.02 *M* acetic acid at pH 4.7	1.16	0.8
0.01 *M* histidine + 0.02 *M* formic acid at pH 3.7	10.9	12.0
0.01 *M* histidine + 0.02 *M* monochloroacetic acid at pH 2.9	41.3	44.0

[a] For the experimentally determined values migration times are measured from the diffuse edge of the peaks.

The mobilities of the system peaks can be deduced from the place of the discontinuities but can also be calculated with the procedure described in [16]. In Table 2 all given values are mobilities at the ionic strength of ca. 0.01. At lower pHs the mobility of the system peak increases and is about 11×10^{-9} m^2/Vs for the BGE at pH 3.7. Of course the relationships intersect each other at $m_S = m_{Hist}$. The TR values at pH 3.7 are less favourable for $m_S > m_{Hist}$, and they increase, in an absolute way, for $m_S < m_{Hist}$ approaching the mobility of the system peak. The effect of the presence of high concentrations of H^+ is much clearer applying the BGE at pH 2.9 where the calculated m_{SP} is ca. 46×10^{-9} m^2/Vs at infinite dilution, as can be concluded from the position of the discontinuity in the TR relationship. For a recalculation of the mobilities of the system peak from values at infinite dilution to values of finite dilution the system peak can be considered as to be a monovalent cationic species. For $m_S < m_{SP}$ the analytes are always dips in the electropherograms (negative cationic TR values of histidine), positive for $m_{SP} < m_S <$ ca. 65×10^{-9} m^2/Vs and dips again for higher values of m_S. The TR value of zero at a mobility of m_S of ca. 65×10^{-9} m^2/Vs seems to indicate that this is the mobility of the second co-ion H^+ (compare Fig. 2B). To check these relationships and the effect of low pH values on the values of the transfer ratios electropherograms are measured for the separation of 5-s pressure injections of equimolar solutions of 5×10^{-4} *M* of potassium nitrate, sodium nitrate and lithium nitrate applying the BGEs of Fig. 5A. In Fig. 5B, the measured electropherograms are given for the BGEs (a) histidine acetate at pH 4.7, (b) histidine formate at pH 3.7 and (c) histidine monochlororacetate at pH 2.9. The numbered peaks/dips refer to the ions of (1) potassium, (2) sodium and (3) lithium. The system peaks and EOF dips are indicated with SP and EOF. Clearly it can be seen that the mobility of the system peak increases with lower pH values, whereas the mobility of the EOF decreases. The remark by Lu and Westerlund that they could not observe two system peaks (including the EOF peak) as described by Beckers is true, because these peaks are only observable at low pH values and not at the pH values of 6.1 and 6.8 applied by them [13]. At the pH values (a) 4.7 and (b) 3.7, all sample cations are present as dips in the electropherograms. At a pH

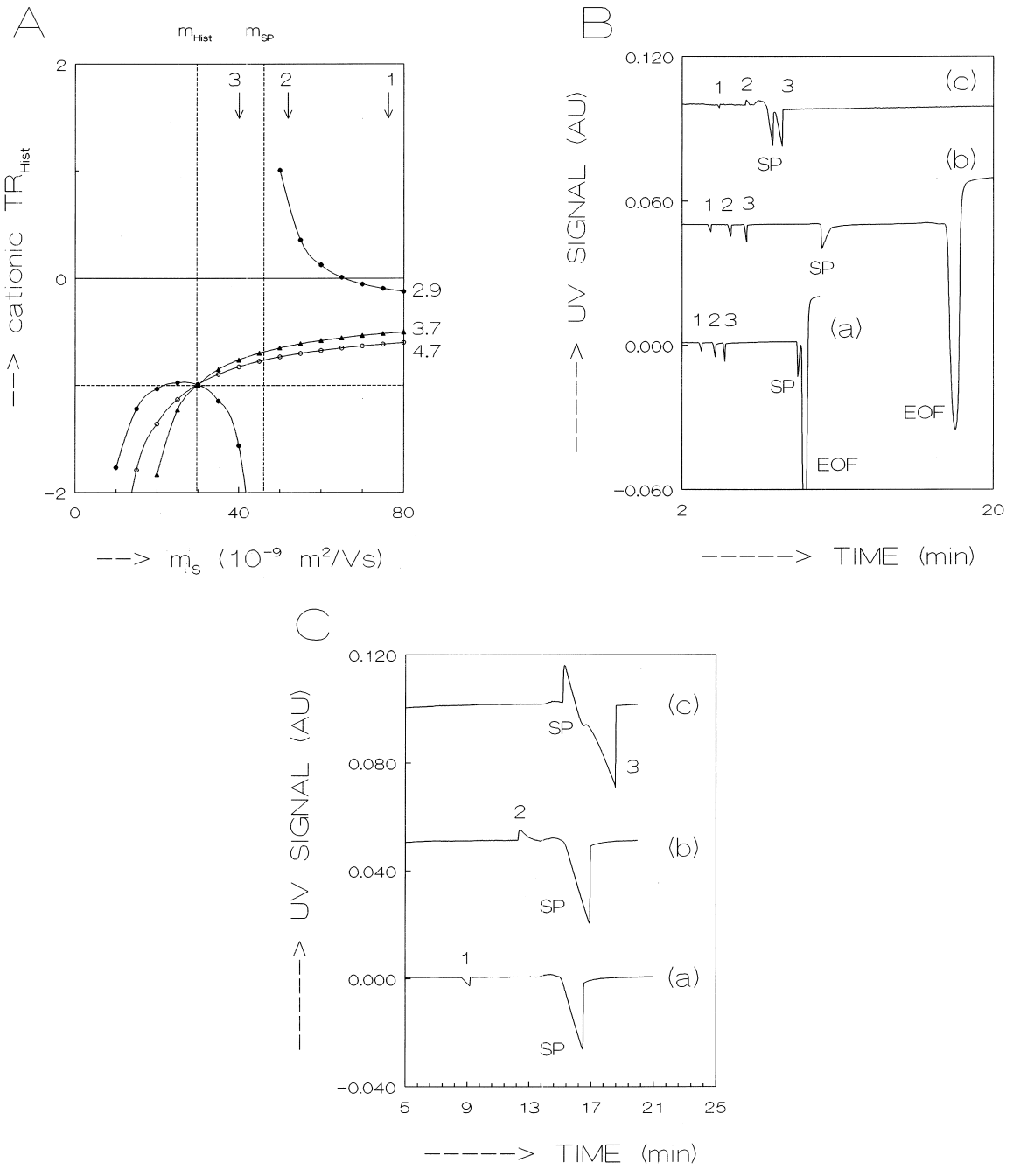

Fig. 5. (A) Calculated relationships between cationic TR values for the co-ion histidine versus the mobilities m_s at infinite dilution of cationic analytes applying BGEs consisting of 0.01 *M* histidine and respectively (○) 0.02 *M* acetic acid at a pH 4.7, (▲) 0.02 *M* formic acid at pH 3.7 and (●) 0.02 *M* chloroacetic acid at pH 2.9. The numbered arrows indicate the mobilities of the analytes separated in the electropherogram of (B). For further information see text. (B) Measured electropherograms for 5-s pressure injections of equimolar sample solutions of 5×10^{-4} *M* of (1) potassium nitrate, (2) sodium nitrate and (3) lithium nitrate applying the BGEs of (A) at a pH of (a) 4.7, (b) 3.7 and (c) 2.9. The mobility of the system peak (SP) increases for lower pH values. Applied voltage = 10 kV. (C) Measured electropherograms for 10-s pressure injections of 5×10^{-3} *M* sample solutions of (a) potassium nitrate, (b) sodium nitrate and (c) lithium nitrate applying a BGE of histidine monochloroacetate at a pH of 2.9 (applied voltage = 5 kV).

of 2.9, potassium is a small dip, sodium is a small peak, whereas lithium is a large dip, just in accordance with the calculated relationships given in Fig. 5A. The shape of the system peak SP in electropherogram (c) is rather confusing. For that reason the electropherograms for the separation of 10-s injections of sample solutions of 0.005 *M* of the single sample analytes are given in Fig. 5C, measured with an applied voltage of 5 kV. For the electropherograms of (a) potassium and (b) sodium, the SP is a dip. For the electropherogram of (c) lithium, the SP is a peak. In the electropherogram of the separation of the mixture the system peak is a dip again, showing the complex behaviour of the SP depends on the amount and mobility of the sample analyte.

For a discussion of the TR values for BGEs with a single (negative) co-ion at high pH values, calculations are carried out with BGEs consisting of ethanolamine (p*K* value ca. 9.5) and benzoic acid as a chromophore). In Fig. 6 the calculated relationships between anionic TR values of the co-ion benzoate versus the mobilities m_S at infinite dilution of anionic analytes (absolute values) are given applying BGEs consisting of 0.02 *M* ethanolamine and, respectively, (○) 0.01 *M* benzoic acid at pH 9.5, (▲) 0.005 *M* benzoic acid at pH 10.0 and (●) 0.002 *M* benzoic acid at pH 10.4. Analogue with the calculated cationic TR values at low pH values, a continuous relationship is obtained for a pH of 9.5, whereas at higher pH values a discontinuity appears. To see whether the mobility of this discontinuity increases for higher pH values the relationship is calculated for a BGE consisting of a solution of 0.01 *M* of a hypothetical cation with a mobility of 30×10^{-9} m^2/Vs and a p*K* value of 11 (buffering counterion) and 0.005 *M* benzoic acid at a pH of 10.9. In Fig. 6 the relationship is given (▼) and it clearly can be seen that the mobility of the discontinuity increases comparably with the behaviour of hydrogen ions at low pH values. Experiments carried out at high pH values showed that indeed system peaks are present in the electropherograms. At high pH values the base lines of the UV signals are often not stable due to the fact that for BGEs at high pH no suitable buffering ionic species can be found and often reproducible electrophoretic phenomena can be observed applying not buffering BGEs.

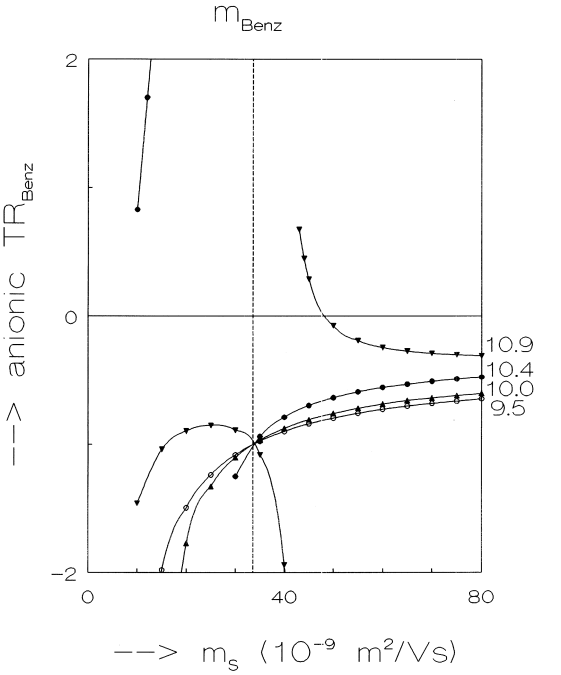

Fig. 6. Calculated relationships between anionic TR values of the co-ion benzoate versus the mobilities m_S at infinite dilution of anionic analytes (absolute values) applying BGEs consisting of 0.02 *M* ethanolamine and, respectively, (○) 0.01 *M* benzoic acid at pH 9.5, (▲) 0.005 *M* benzoic acid at pH 10.0 and (●) 0.002 *M* benzoic acid at pH 10.4 and (▼) a buffering cation with p$K=11$ and 0.005 *M* benzoic acid at pH 10.9. The numbers refer to the pH of the BGEs. For further information see text.

4. Conclusion

The use of transfer ratios and calculations of the TR values applying Eq. (1) based on the KRF is not useful when applying BGEs with more than one co-ion, applying BGEs with absorbing counterions and applying BGEs at very low and high pH values. In these cases it is advisable to use the concept of Eq. (2) and to calculate the TR values according to a mathematical model including the effect of H^+ and OH^- ions. In the calculations values for anionic and cationic TR values have to be distinguished. Calculated relationships between the TR values versus the mobilities at infinite dilution of the sample analytes indicate, moreover, the existence of system peaks in the electropherograms by the presence of discontinuities in the relationships. This is the case applying BGEs with two of more co-ions, but also

applying BGEs with a single co-ion at low or high pH values because the H^+ or OH^- ions act as a second co-ion. All these phenomena are experimentally verified, although application of UV absorbing BGEs at high pH values is often troublesome by lack of buffering ionic species at high pH, whereas the use of non-buffered BGEs results in unstable base lines of the UV signals and a lot of often reproducible electrophoretic phenomena. Some further remarks have to be made about the applicability of the calculated TR values. Although the TR values often increase if the mobilities of the sample ions differ much from those of the co-ions of the BGE, the gain in UV signal is counteracted by an increasing electrodispersion. The influence of electrodispersion is already described in an elegant way, although neglecting the effect of H^+ and OH^- ions, by Gebauer and Bocek introducing the concept of the velocity slope S_x [20,21]. These effects can also be simulated by a recently published simulation program in spreadsheet [22]. Although in this paper the TR values are discussed in relation with the analysis of UV transparent ionic species (indirect UV mode), these values are also valid and applicable in case of UV absorbing analytes, whereby the UV signal is determined by the concentrations of all ionic species present in a sample zone and taking into account their molar absorption coefficients [8].

References

[1] F. Foret, S. Fanali, L. Ossicini, P. Bocek, J. Chromatogr. 470 (1989) 299.
[2] H. Small, T.E. Miller, Anal. Chem. 54 (1982) 462.
[3] W.G. Kuhr, E.S. Yeung, Anal. Chem. 60 (1988) 2642.
[4] E.S. Yeung, W.G. Kuhr, Anal. Chem. 63 (1991) 275 A.
[5] F. Kohlrausch, Ann.Phys. (Leipzig) 62 (1897) 209.
[6] FEP. Mikkers, University of Technology, Eindhoven, 1980, Thesis.
[7] M.W.F. Nielen, J. Chromatogr. 588 (1991) 321.
[8] J.L. Beckers, J. Chromatogr. 549 (1991) 345.
[9] T. Wang, R.A. Hartwick, J. Chromatogr. 589 (1992) 307.
[10] J.L. Beckers, J. Chromatogr. A 679 (1994) 153.
[11] J. Collet, P. Gareil, J. Chromatogr. A 716 (1995) 115.
[12] P. Doble, P. Anderson, P.R. Haddad, J. Chromatogr. A 770 (1997) 291.
[13] B. Lu, D. Westerlund, Electrophoresis 19 (1998) 1683.
[14] M.G. Cikalo, D.M. Goodall, W. Matthews, J. Chromatogr. A 745 (1996) 189.
[15] J.L. Beckers, J. Chromatogr. A 764 (1997) 111.
[16] J.L. Beckers, F.M. Everaerts, J. Chromatogr. A 787 (1997) 235.
[17] P. Gebauer, P. Bocek, J. Chromatogr. A 772 (1997) 73.
[18] H. Poppe, Anal. Chem. 64 (1992) 1908.
[19] F.E.P. Mikkers, Anal. Chem. 69 (1997) 333.
[20] P. Gebauer, P. Bocek, Anal. Chem. 69 (1997) 1557.
[21] P. Gebauer, P. Bocek, Anal. Chem. 70 (1998) 3397.
[22] F.E.P. Mikkers, Anal. Chem. 71 (1999) 522.