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Determination and prediction of transfer ratios for anions in capillary zone electrophoresis with indirect UV detection

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Abstract

Transfer ratios (i.e. the number of moles of the UV-absorbing probe anion displaced by one mole of analyte anion) were determined for the separation of inorganic and organic anions by capillary zone electrophoresis using indirect UV absorbance detection. When the electrolyte was buffered and contained only the probe anion and a single counter-cation, transfer ratios calculated from Kohlrausch theory were found to agree well with values obtained experimentally from accurately determined mobility data. However, these electrolyte systems gave long analysis times and were therefore considered impractical. More useful electrolytes were obtained by the addition of surfactants to suppress or reverse the electroosmotic flow but the co-anion introduced with the surfactant can reduce the value of the measured transfer ratio and hence adversely affect sensitivity. This problem was overcome by the use of a surfactant in the hydroxide form such as cetyltrimethylammonium hydroxide combined with a suitable buffering counter-cation such as protonated 1,3-bis[tris(hydroxymethyl)-methylamino]-propane or tris(hydroxymethyl)aminoethane. Four buffered electrolytes consisting of chromate, benzoate, phthalate, or trimellitate as probes and a suitable surfactant were used to determine transfer ratios. These systems were shown to give transfer ratios that were close to those calculated from Kohlrausch theory, thereby enabling prediction of experimental conditions giving maximum transfer ratios.

Keywords: Transfer ratio; Indirect detection; Detection,; Electrophoresis; Anions

1. Introduction

Capillary zone electrophoresis (CZE) has been used as an alternative to ion chromatography for the analysis of inorganic and low molecular-mass anions [1]. Ions in this class of molecules often have poor chromophoric properties, so direct UV detection is applicable in only a limited number of cases. Indirect detection has been used to overcome this limitation. In this technique, a UV absorbing species (known as the probe) having the same charge as the analyte of interest is added to the electrolyte. Sample ions introduced into the capillary will displace a certain amount of the probe, causing a quantifiable decrease in the background absorbance and hence a measurable detection signal.

The ability of the analyte to displace the probe can be measured using the transfer ratio (TR), which is defined as the number of moles of the probe displaced by one mole of sample ions. It is desirable to know the value of TR for any given system since higher values result in larger peak areas and thus improved sensitivity. On an intuitive level one would expect displacement on an equivalent-per-equivalent basis so that, for example, the TR between a singly charged solute and a singly charged probe would be expected to be unity. However, this behaviour occurs only when the mobilities of the solute and probe are the same [2]. TR values greater than unity occur when the mobility of the probe ion exceeds that of

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the analyte ion, whereas TR values less than unity occur when the reverse is true.

This effect has been explained by Nielen [2], who showed from Kohlrausch theory that the change in concentration (d[A]) of a probe ion (A) caused by an analyte B at concentration [B] is given by:

$$\frac{d[A]}{[B]} = -\frac{z_B}{z_A} \cdot \frac{\mu_A}{\mu_B} \frac{[\mu_B + \mu_C]}{[\mu_A + \mu_C]} = TR \quad (1)$$

where z_A and z_B are the charges on the probe and analyte ions, respectively, and μ_A, μ_B and μ_C are the effective electrophoretic mobilities of the probe, analyte and counter ions, respectively. Bruin et al. [3] have specified that Eq. (1) is valid only for a simplistic electrophoretic system containing three components. Cousins et al. [4] have experimentally determined the TR values for a series of anions using a number of different probes. The experimental values of TR followed the general trend predicted by Eq. (1), but the fit to Eq. (1) was poor. However, their work showed that detection sensitivity could be optimised by consideration of the measured values of TR in conjunction with the molar absorptivity of the probe.

The purpose of this work was to demonstrate the conditions under which TR values calculated using Eq. (1) are in agreement with those determined from experiment, and to provide explanations for non-ideal behaviour. Further, it was envisaged that a more complete understanding of the factors which influence transfer ratios would lead to the design of electrolyte systems which give maximum transfer ratios and hence the greatest detection sensitivity.

2. Experimental

2.1. Instrumentation

The CZE instrument used was a Quanta 4000 (Waters, Milford, MA, USA) interfaced to a Maxima 820 data station (Waters). Separations were performed using a fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) 60 cm×75 μm I.D., effective length 50 cm. Injections were performed hydrostatically by elevating the sample at 10 cm for 10 s.

2.2. Reagents and procedures

All carrier electrolytes and standards were prepared in water treated with a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus. Electrolytes were degassed using vacuum sonication and were filtered through a 0.45 μm syringe filter (Activon Thornleigh, Australia) prior to use.

Chemicals used were of analytical reagent grade. 1, 3-bis[tris(hydroxymethyl) methylamino]-propane (Bis–Tris), tris(hydroxymethyl)aminoethane (Tris), octanesulfonate, heptanesulfonate, hexanesulfonate, pentanesulfonate, butanesulfonate, propanesulfonate, ethanesulfonate, methanesulfonate, tetradecyltrimethylammonium bromide (TTAB), cetyltrimethylammonium hydroxide (CTAOH), mesityl oxide and sodium cyanate were obtained from Aldrich (Milwaukee, WI, USA). Nitric acid, perchloric acid, sulfuric acid, sodium formate, sodium dihydrogen phosphate, sodium sulfate, sodium nitrate, and sodium carbonate were obtained from Ajax (NSW, Australia). Sodium chloride, hydrochloric acid, sodium chromate, chromium trioxide, benzoic acid and phthalic acid were obtained from BDH (Victoria, Australia). Trimellitic acid was obtained from Sigma (St. Louis, MO, USA) and diethanolamine (DEA) was obtained from Fluka (Switzerland).

2.3. Experimental determination of transfer ratios

Transfer ratios were determined using the following steps [4]. First, a calibration plot was constructed of corrected peak area versus molar concentration of the solute using a suitable probe as the carrier electrolyte and indirect UV detection. The correction made to peak area was to account for the different migration velocities of the sample bands and was achieved by dividing the peak area of each solute by its migration time. Second, a calibration plot for the probe was constructed using a suitable UV-transparent electrolyte and the same detection wavelength employed for the solute calibration curve. Finally, TR values were calculated for each solute ion by determining the quotient of the slope of the solute ion calibration plot and the slope of the probe calibration plot.

Details of the experimental conditions used are included in the figure captions.

2.4. Calculation electrophoretic mobilities

Electrophoretic mobilities were calculated using equations in [5] using mesityl oxide as the neutral marker for direct UV and water for indirect UV measurements to determine the rate of electroosmotic flow (EOF).

3. Results and discussion

3.1. Modification of the detector

Since the TR is a quotient of the slopes of two calibration curves determined at different background absorbances, it is imperative that the response of the detector is linear over the absorbance range within which measurements are made or significant error will result. Foret et al. [6] states that a UV detector in CZE is linear up to ~0.1 A.U., with the upper limit being determined mainly by the shape of the capillary detection cell. In our case, measurements made using a zinc lamp at 214 nm exhibited poor linearity up to 0.1 A.U. This was caused by stray light and poor alignment of the source. The lamp in the detector was designed with a broad window to maximise the light intensity, however this arrangement allowed an unacceptable level of stray light.

The detector optics were modified to reduce the stray light by wrapping aluminium foil around the lamp and two slits approximately 4 mm apart and 1 mm in width were cut in it. This arrangement prevented light straying from the reference side of the detector to the sample side. The lamp was then moved approximately 1 cm away from the capillary and aligned so that the beam shone directly through the capillary. After these modifications the stray light was reduced to less than 4% and detector linearity was maintained up to ~0.4 A.U. However, the lamp energy was diminished by a factor of five, which resulted in an increase in noise and therefore reduced sensitivity. Nevertheless, the improved linearity of the detector was essential to the determination of TR values so the modified optics were used for all experiments. When detection was performed at 254 nm, a deuterium lamp was modified in the same manner as described above.

3.2. Model electrolyte–analyte systems

Several constraints must be considered in designing a simple electrolyte–analyte system that might show agreement between experimentally determined and calculated TR values. First, the background electrolyte must consist of two components only: the probe anion and its corresponding counter-cation. Second, the solute anion must have the same counter-cation as the background electrolyte. Third, the background electrolyte should have sufficient buffering capacity to prevent variations in EOF due to changes in electrolyte pH [7]. However, addition of buffers adversely affects the sensitivity of detection since displacement of the buffer anion (rather than the probe anion) by the solute anion can occur [8]. Finally, the calibration plots necessary to calculate TR values must be performed within the linear range of the detector.

Initial experiments were conducted using two systems that satisfied these constraints. The first used chloride, sulfate, cyanate and formate as the solutes, with indirect detection at 214 nm in an electrolyte comprising 2.1 m*M* nitric acid, titrated with Bis–Tris to pH 6.9 (which is the pK_a value of the buffer). This provided a buffered electrolyte that consisted of two components, namely the nitrate ion as the probe anion and the protonated Bis–Tris as the counter-cation. The power supply was set up so that the detector was at the anodic end of the capillary. A counter-EOF separation was used in which the anionic solutes migrated in a direction opposite to the EOF, but with sufficient effective velocity to reach the detector cell. Fig. 1 shows the separation obtained under these conditions. The EOF velocity was determined for subsequent mobility calculations by switching the polarity of the power supply and injecting water. Calibration curves were constructed using sodium salts of the analytes dissolved in the electrolyte to negate the effect of the sodium ions. That is, calculation of the TR value was performed by considering the counter-cation of the analyte to be the Bis–Tris cation.

The second electrolyte–analyte system used a series of aliphatic sulfonates (C_3–C_8) as analytes and an electrolyte comprising 2.0 m*M* chromic acid titrated to pH 8 with Tris buffer, with detection at 254 nm using a deuterium lamp modified as de-

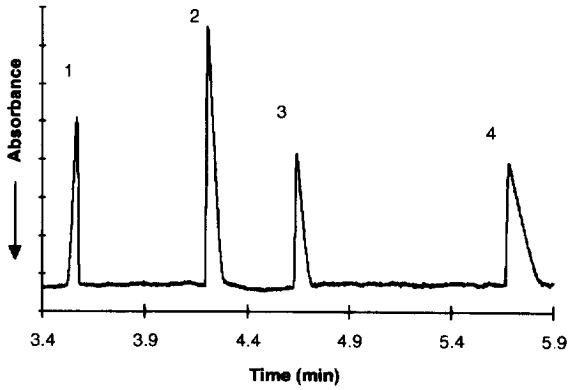

Fig. 1. Separation of chloride, sulfate, cyanate and formate using 2.1 m*M* HNO_3, buffered to pH 6.8 with Bis–Tris. Conditions: separation voltage −25 kV, indirect detection at 214 nm, hydrostatic injection at 10 cm for 10 s, temperature 25°C, background absorbance 0.109 A.U., sample 0.2 m*M* of each anion. Key: 1 = chloride, 2 = sulfate, 3 = cyanate, 4 = formate.

scribed earlier. Again, this provided a buffered electrolyte containing only two components, namely chromate (as the probe anion) and protonated Tris as the counter-cation. The aliphatic sulfonates have low electrophoretic mobilities that are less than the EOF, so the power supply was set up with the detector at the cathodic end of the capillary. Under these conditions, a counter-EOF mode was employed in which the effective velocity of the analytes was towards the detector. Fig. 2 shows the separation obtained.

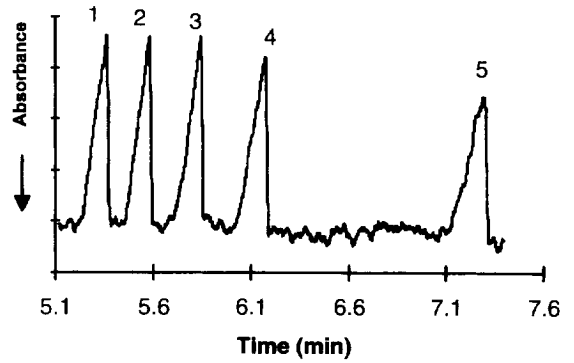

Fig. 2. Separation of aliphatic sulfonates using 2.0 m*M* chromic acid buffered to pH 8.0 with Tris. Conditions: separation voltage 25 kV, indirect detection at 254 nm, hydrostatic injection at 10 cm for 10 s, temperature 25°C, sample 0.3 m*M* of each anion. Key: 1 = octanesulfonate, 2 = heptanesulfonate, 3 = hexanesulfonate, 4 = pentanesulfonate, 5 = propanesulfonate.

3.3. Effects of ionic strength of standards

As described in Section 2.3, the determination of TR involved preparation of calibration plots for both the probe (in the direct mode) and the analyte (in the indirect mode). When the probe calibration was performed for the first electrolyte system, an electrolyte comprising perchloric acid titrated to pH 6.9 with Bis–Tris was used and sodium nitrate was the injected sample. This provided a UV-transparent electrolyte enabling direct detection of nitrate at 214 nm. However, under these conditions it was found that the slope of the calibration plot for nitrate was dependent on the ionic strength of the electrolyte. A more concentrated electrolyte gave a higher slope for the calibration plot. This problem was overcome by dissolution of the analyte standards in electrolyte rather than water.

The same effect occurred also when constructing the calibration plots for the analytes in the indirect mode using nitrate as the probe. Table 1 illustrates the magnitude of the variation in slope when the analytes were dissolved in water or in the electrolyte. Doubling the ionic strength of the electrolyte produced increases in slope of up to 21.6% compared to the standards dissolved in water, whereas agreement was within 6% for the standards dissolved in the electrolyte and the variation could be attributed to random errors. For all subsequent TR determinations the standards were dissolved in the electrolyte.

3.4. Experimental transfer ratios for model electrolyte–analyte systems

Transfer ratios for the analytes in both model electrolyte systems were determined experimentally and theoretical values were calculated using Eq. (1). To permit the calculation, it was necessary to measure the mobilities of the Bis–Tris and Tris cations. These mobilities were determined using 10 m*M* dihydrogen phosphate adjusted to pH 6.8 (for Bis–Tris) or pH 8.0 (for Tris) with NaOH as the electrolyte, with detection at 185 nm.

The experimentally determined values of TR for the analytes are shown in Table 2, together with the calculated values. For both electrolytes agreement between experiment and theory is good, unlike earlier results [4] obtained using electrolyte systems

Table 1
Slopes of calibration curves for different concentration of background electrolyte using standards dissolved in water or the electrolyte

Analyte	Electrolyte	
	4 m*M* HNO_3, 3.6 m*M* Bis–Tris, pH 6.8 Slope±S.D.	2.1 m*M* HNO_3, 1.8 m*M* Bis–Tris, pH 6.8 Slope±S.D.
Dissolved in water		
Chloride	20 900±400	18 900±500
Sulfate	42 600±600	39 000±400
Cyanate	22 400±200	21 500±200
Formate	26 100±700	21 400±600
Dissolved in electrolyte		
Chloride	21 000±700	20 700±100
Sulfate	39 300±500	40 400±300
Cyanate	19 200±400	20 300±400
Formate	24 300±1100	23 000±1500

Conditions: Voltage −25 kV, Injection 10×10 cm hydrostatic, wavelength 214 nm, Temperature 25 °C

and detectors that had not been optimised for the determination of TR values. In the case of the nitrate electrolyte the mobility of the probe is similar to that of the analytes so the experimental TR values show that displacement of the probe by the analyte occurs almost on an equivalent-per-equivalent basis. This displacement ratio is exceeded when the mobility of the probe is greater than that of the analyte, for example in the case of formate, and the reverse situation is also true using chloride as an example. As a further observation, the migration times of the analytes were very reproducible (relative standard deviations around 0.1%), which demonstrates the desirability of buffered electrolytes.

Table 2
Experimental and theoretical transfer ratios

Analyte	Experimental TR±S.D.	Experimental[a] TR±S.D.	Theoretical TR (Eq. (1))	Mobility 10^{-9} m^2 V^{-1} s^{-1}±S.D.
Electrolyte 1[b]				
Chloride	0.97±0.02	–	0.98	−77.1±0.1
Sulfate	1.89±0.04	1.67±0.04	2.05	−68.1±0.1
Cyanate	0.95±0.03	0.75±0.02	1.02	−63.7±0.1
Formate	1.07±0.08	–	1.03	−55.7±0.1
Nitrate	–	–	–	−71.5±0.1
Bis–Tris	–	–	–	19±1
Electrolyte 2[c]				
Octanesulfonate	17.73±0.02	–	0.69	−25.0±0.3
Heptanesulfonate	17.70±0.03	–	0.68	−26.4±0.3
Hexanesulfonate	0.69±0.02	–	0.66	−27.9±0.4
Pentanesulfonate	0.64±0.02	–	0.65	−29.6±0.4
Propanesulfonate	0.64±0.02	–	0.62	−34.1±0.3
Chromate	–	–	–	−77.2±0.1
Tris	–	–	–	17±1

[a] Same conditions as for electrolyte 1 except that 0.5 m*M* TTAB was added to the electrolyte.
[b] Electrolyte 1 conditions: as for Fig. 1. The calibration plot of the probe for electrolyte 1, (NO_3^-) was constructed with 2.0 m*M* $HClO_4$ buffered to pH 6.8 with Bis–Tris.
[c] Electrolyte 2 conditions: as for Fig. 2. The calibration plot of the probe for electrolyte 2, ($CrO4^{2-}$) was constructed with 2.0 m*M* H_2SO_4 buffered to pH 8 with Tris as the electrolyte. Chromate migrated counter to the flow of the EOF.

With the second electrolyte system the mobilities of the analytes were significantly less than that of the chromate probe and the equivalent-per-equivalent displacement (which would give a TR value of 0.5) was exceeded. The peak areas for the aliphatic sulfonates were 25–40% greater than would be expected for a one-to-one displacement based on the rules of electroneutrality. However, this does not necessarily improve the limit of detection by an equivalent amount, due to the asymmetrical character of the peaks resulting from the disparity between the mobilities of the probe and analyte. This suggests that a compromise between high TR and optimal peak shape must be attained in order to maximise sensitivity.

3.5. Practical electrolyte–analyte systems with EOF modifiers

In the analysis of small anions, surfactants are frequently added to reverse the direction of the EOF and thereby to enable the analysis to be conducted in a co-EOF mode with the analytes and the EOF moving towards the detector [9–11]. The surfactants used commonly are bromide salts of quaternary ammonium compounds, for example tetradecyltrimethylammonium bromide (TTAB) and cetyltrimethylammonium bromide (CTAB). Although these EOF modifiers are added typically in concentrations as low as 0.5 m*M*, the presence of the co-anion bromide provides potential competition with the probe in the detection displacement reaction and thereby a reduction in the value of TR. When 0.5 m*M* TTAB was added to the nitrate electrolyte the experimentally determined TR values for sulfate and cyanate were decreased by 12% and 21% respectively (Table 2).

Wang and Hartwick [8] showed that in electrolytes containing binary buffers, analytes will predominantly displace the buffer species having a mobility closest to that of the analyte. Addition of a surfactant to the electrolyte can be considered to be analogous to a binary buffer situation. Since the mobilities of bromide, nitrate, sulfate and cyanate are all similar it would be expected that sulfate and cyanate (as analytes) would displace equally nitrate and bromide (as probes). Given that bromide comprises about 20% of the total concentration of the electrolyte, it would be expected that the TR value should decrease by about 20%. This prediction is in approximate agreement with the decrease in experimentally determined transfer ratios and could partially explain the poor agreement of experimental and theoretical TR values obtained earlier [4], especially for high mobility analytes such as chloride, fluoride and sulfate when analysed with low mobility probes such as benzoate and phthalate. In these cases the bromide ion would be displaced preferentially, thereby reducing the transfer ratio significantly.

Whilst the ideal electrolyte–analyte systems used in this study show that the theoretical TR values can be obtained experimentally when using simple two-component buffered electrolytes, the practicalities of these separation systems are limited. The nitrate electrolyte gave long migration times for slower solutes, and the chromate electrolyte produced poor peak symmetries. To make these systems more practical, an EOF modifier is required to reduce the analysis times by either suppressing or reversing the EOF. Surfactants that introduce co-anions are undesirable due to the reasons discussed previously. Consequently, CTAOH was investigated as a possible EOF modifier since its co-anion is consumed largely by the buffer and any residual hydroxide can be expected to exert little competition with the probe because of its low concentration and the fact that its mobility is much higher than that of the analytes.

Four different probes were investigated, namely benzoic acid, phthalic acid, trimellitic acid and chromic acid, each titrated with diethanolamine (DEA) to pH 9.1 (the pK_a of DEA) and with 0.5 m*M* CTAOH added. This arrangement provided buffered electrolytes and a single co-anion (i.e. the probe ion). Each of the electrolytes was examined with a range of solutes having different mobilities, with TR values being calculated from Eq. (1) and determined experimentally. Figs. 3–6 show the electropherograms obtained for each separation, together with plots of TR versus analyte mobility for each electrolyte. In each plot the solid line indicates the theoretical TR and the points show experimentally determined values, whilst the arrow on the mobility axis marks the mobility of the probe. When the mobility of the analyte is close to that of the probe, the agreement between theory and experiment is good, but agreement diminishes as the difference in mobility be-

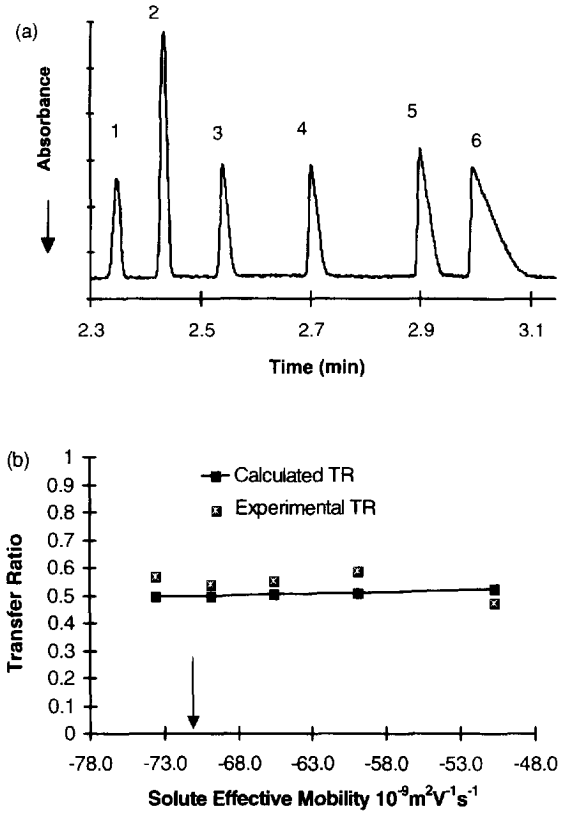

Fig. 3. Electropherogram (a) and plot of calculated and experimentally determined transfer ratios (b) using chromate as probe and CTAOH as EOF modifier. Conditions: electrolyte 5 m*M* chromic acid, 20 m*M* DEA, 0.5 m*M* CTAOH, pH 9.1, separation voltage −25 kV, hydrostatic injection at 10 cm for 10 s, detection wavelength 254 nm, temperature 25°C, sample 0.2 m*M* of each ion. Key: 1 = chloride, 2 = sulfate, 3 = nitrate, 4 = cyanate, 5 = formate, 6 = citrate. The calibration plot for the probe was prepared using 5 m*M* H_2SO_4, 20 m*M*: DEA, 0.5 m*M* TTAB at pH 9.2 as electrolyte.

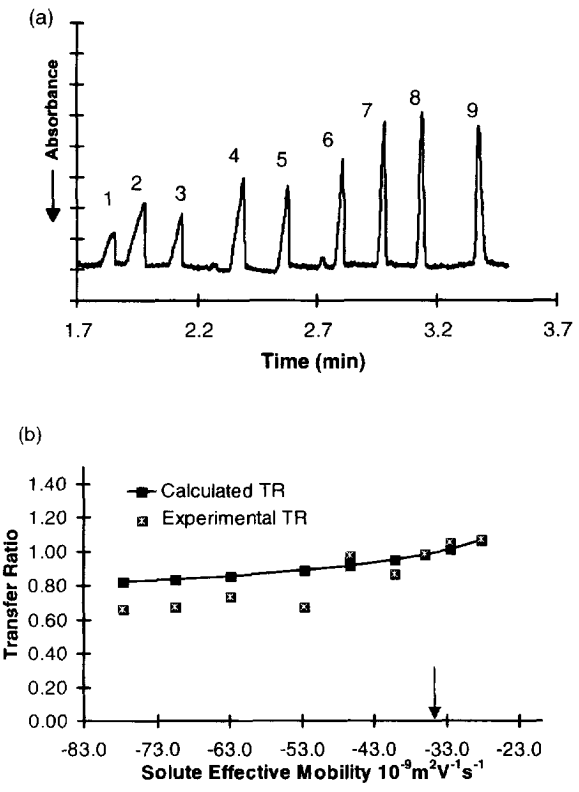

Fig. 4. Electropherogram (a) and plot of calculated and experimentally determined transfer ratios (b) using a benzoate probe and CTAOH as EOF modifier. Conditions: electrolyte 10 m*M* benzoic acid, 20 m*M* DEA, 0.5 m*M* CTAOH, pH 9.1, separation voltage: −25 kV, hydrostatic injection at 10 cm for 10 s, detection wavelength 254 nm, temperature 25°C, sample 0.4 m*M* of each ion. Key: 1 = chloride, 2 = sulfate, 3 = chlorate, 4 = phosphate, 5 = carbonate, 6 = ethanesulfonate, 7 = propanesulfonate, 8 = butanesulfonate, 9 = pentanesulfonate. The calibration plot for the probe was prepared using 10 m*M* methanesulfonic acid, 20 m*M* DEA, 0.5 m*M* TTAB at pH 9.1 as electrolyte.

tween the analyte and probe increases. A strong contributor to this latter trend is peak asymmetry since TR values were determined from corrected peak areas (i.e. peak area divided by the migration time) and this correction becomes less effective as peaks asymmetry increases. Figs. 3–6 show clearly the large changes in peak symmetry that occur when different electrolytes are used.

It is also evident from Figs. 3–6 that when there is a difference between the theoretical and observed TR values, this difference is such that the experimental value is generally greater. Remembering that the chief potential drawback of using an EOF modifier was diminution of the TR value due to competition from the added co-anion, it can be seen that the strategy of using hydroxide as the co-anion introduced with the surfactant eliminates this drawback. Maximum detection sensitivity can therefore be achieved. A further advantage exists in that system peaks caused by the introduction of co-anions are eliminated. These system peaks often interfere with solute quantification and to illustrate this point, the same nine analyte anions were separated with the benzoate probe using identical conditions to those

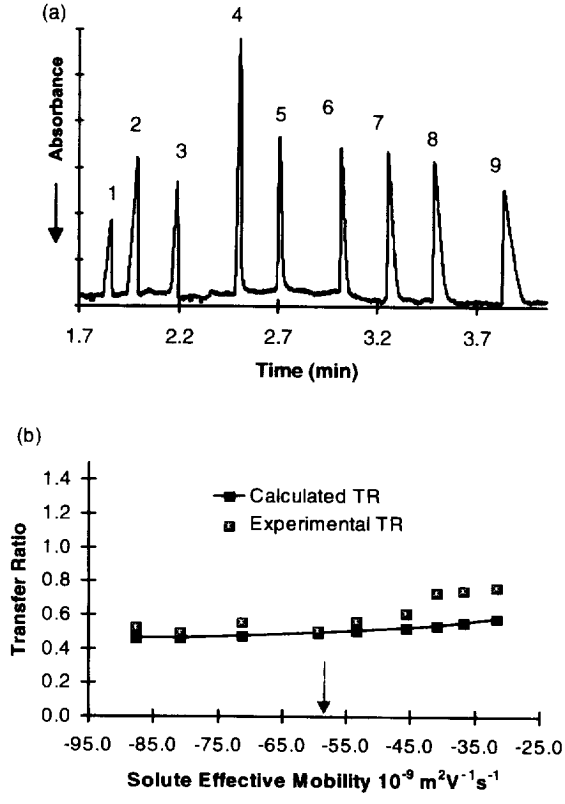

Fig. 5. Electropherogram (a) and plot of calculated and experimentally determined transfer ratios (b) using a phthalate probe and CTAOH as EOF modifier. Conditions: electrolyte 10 m*M* phthalic acid, 40 m*M* DEA, 0.5 m*M* CTAOH, pH 9.3, separation voltage −25 kV, hydrostatic injection at 10 cm for 10 s, detection wavelength 254 nm, temperature 25°C, sample 0.6 m*M* of each anion. Key: 1 = chloride, 2 = sulfate, 3 = chlorate, 4 = phosphate, 5 = carbonate, 6 = ethanesulfonate, 7 = propanesulfonate, 8 = butane sulfonate, 9 = pentanesulfonate. The calibration plot for the probe was prepared using the same electrolyte as for Fig. 4.

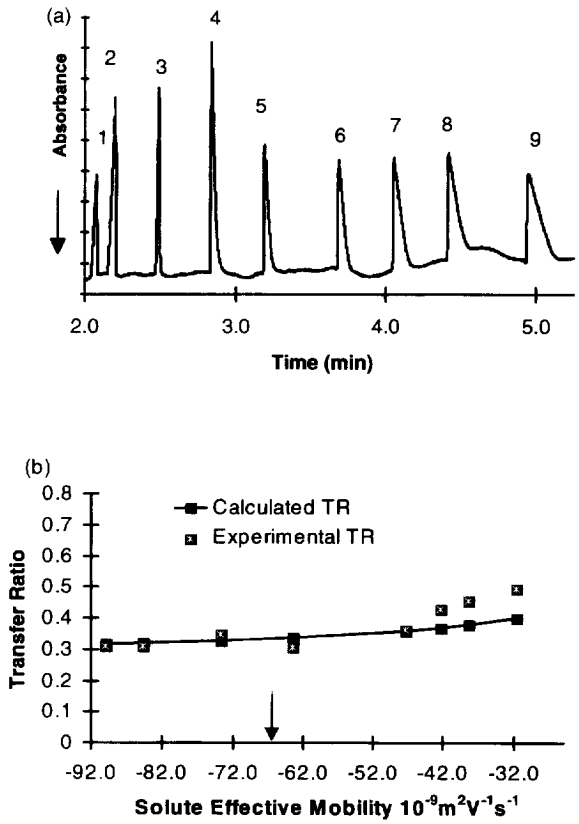

Fig. 6. Electropherogram (a) and plot of calculated and experimentally determined transfer ratios (b) using a trimellitate probe and CTAOH as EOF modifier. Conditions: electrolyte 10 m*M* trimellitic acid, 60 m*M* DEA, 0.5 m*M* CTAOH, pH 9.3, separation voltage −25 k:V, hydrostatic injection at 10 cm for 10 s, detection wavelength 254 nm, temperature 25°C, sample 0.5 m*M* of each ion. Key: 1 = chloride, 2 = sulfate, 3 = chlorate, 4 = phosphate, 5 = carbonate, 6 = ethanesulfonate, 7 = propane sulfonate, 8 = butanesulfonate, 9 = pentanesulfonate. The calibration plot for the probe was prepared using the same electrolyte as for Fig. 4.

employed previously but with TTAB as the EOF modifier (Fig. 7). The bromide co-anion gave a negative system peak (at the migration time of bromide) which completely swamped the peaks of chloride and sulfate. In contrast, chloride and sulfate were easily quantifiable when CTAOH was used as the EOF modifier.

3.6. *Effects of counter-cations on TR*

As the TR value is also governed by the mobility of the counter-cation (see Eq. (1)), this factor must also be considered if TR is to be maximised. Fig. 8 shows the effect on TR of chlorate (of mobility $-66 \cdot 10^{-9}$ $m^2 V^{-1} s^{-1}$) resulting from the use a series of probes with counter-cations of differing mobility, calculated using Eq. (1). A number of general interpretations can be drawn from Fig. 8. First, for a given counter-cation, TR for an analyte shows a general increase as the mobility of the probe approaches and then exceeds that of the analyte and this effect is most pronounced with potassium as counter-cation. Second, the TR value is unaffected by the mobility of the counter-cation when the mobilities of the probe and analyte are the same.

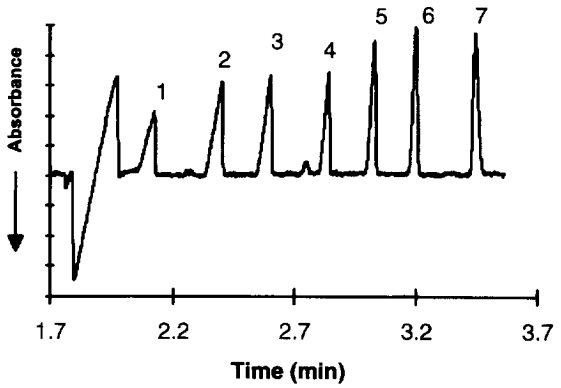

Fig. 7. Electropherogram obtained using a benzoate probe and TTAB as EOF modifier. Conditions: electrolyte 10 m*M* benzoic acid, 20 m*M* DEA, 0.5 m*M* TTAB, pH 9.1, separation voltage −25 kV, hydrostatic injection at 10 cm for 10 s, detection wavelength 254 nm, temperature 25°C, sample 0.4 m*M* of each anion. Key: 1=chlorate, 2=phosphate, 3=carbonate, 4=ethanesulfonate, 5=propanesulfonate, 6=butanesulfonate, 7=pentanesulfonate.

Third, when the mobility of the analyte is less than that of the probe, the TR value is highest with more mobile counter-cations and lowest with less mobile counter-cations. Fourth, when the mobility of the analyte is greater than that of the probe the TR value is highest with less mobile counter-cations and lowest with more mobile counter-cations. Moreover, the effects of the counter-cation increase as the difference in mobility between the analyte and probe become greater. For example the TR for the chlorate anion shown in Fig. 8 is increased by 29% when benzoate ($\mu = -32.9 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}) is used as the probe and the counter-cation is changed from potassium to protonated Tris. The TR is increased by only 8% when phthalate ($\mu = -52.7 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}) is used as the probe and the same change in counter-cation is made.

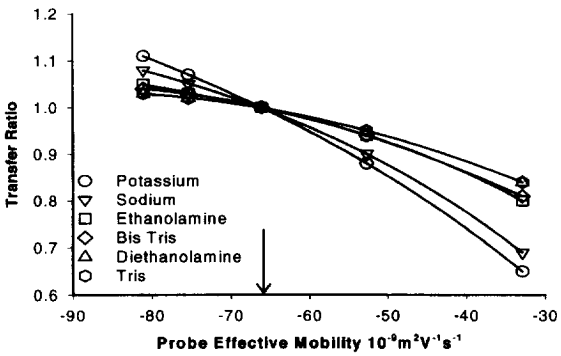

Fig. 8. Theoretical plot of transfer ratio for chlorate versus probe mobility for a series of counter-cations, calculated using Eq. (1). The following mobilities were taken from [12]: chlorate $-66.6 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, potassium $76.0 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, sodium $51.9 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, ethanolamine $= 44.3 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, protonated Tris $14.8 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. The mobility of protonated Bis–Tris, $19 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, was determined experimentally. See text for details.

It is also evident from Fig. 8 that a counter-cation producing the largest enhancement of TR for an analyte of greater mobility than the probe will exert the opposite effect for an analyte of lower mobility than the probe. The best compromise for counter-cation effects on TR (and also for the production of optimal peak shapes) is therefore to ensure that the mobility of the probe is in the middle of the range of mobilities encompassed by the analytes in the particular mixture being separated. A slow counter cation is also desirable to minimise the decrease in TR for a given set of solutes.

4. Conclusions

The sensitivity of indirect detection can be optimised by consideration of the factors that affect TR. Maximum values of TR are obtained when the electrolyte contains a single co-anion and reproducibility is enhanced when the electrolyte is buffered. Eq. (1) was shown to be valid for simple electrolyte systems that contain two components, namely the UV absorbing probe anion and its corresponding counter-cation. Buffering of electrolytes within the above constraints can be achieved by titration of the acid form of the probe to the pK_a of a basic buffer such as Bis–Tris, Tris or DEA.

The addition of a surfactant to reverse the flow of the EOF is desirable to diminish the analysis times. A surfactant that does not introduce a competing co-anion is beneficial in that sensitivity is not diminished for analytes as a result of competitive displacement and also because of the elimination of system peaks that can interfere with the quantification of analytes. This can be achieved by using a surfactant in the hydroxide form such as CTAOH. Electrolyte systems of this type show generally good

agreement with Eq. (1) which allows TR values to be predicted.

Finally, the effects on TR of the counter-cation in the electrolyte are dependent on the relationship between the mobilities of the analyte and the probe. The best way to minimise these effects is to ensure that the mobility of the probe is in the middle of the range covered by the mobilities of the analytes, and to use a counter-cation of low mobility (such as protonated Tris or Bis–Tris).

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