ELSEVIER

Journal of Chromatography A, 735 (1996) 209–220

JOURNAL OF CHROMATOGRAPHY A

Improved reproducibility in capillary electrophoresis through the use of mobility and migration time ratios

Ju Yang, Sahana Bose, David S. Hage*

Department of Chemistry, University of Nebraska, Lincoln, NE 68588-0304, USA

Abstract

Fluctuations in solute migration times are an important source of non-reproducibility in the use of capillary electrophoresis (CE) for the analysis of drugs or biological compounds. This work examined the use of mobility ratios (M) and migration time ratios (R_t) as a means for obtaining a more reliable description of analyte migration in CE. Replicate injections of amino acids or *o*-nitrophenol gave a typical within-day precision of ±1% for R_t and M (range, 0.5 to 1.5%), but a within-day precision of up to 5% (range, 1.0% to 5.5%) for other measures of solute migration, such as migration times and electrophoretic mobilities. Similar results were noted for the day-to-day variations in these parameters. By using R_t values to plot or characterize CE data, it was possible to identify analytes that could not be distinguished from one another based on the use of migration times. Altering the voltage, temperature or current of the CE system produced only small, random variations in M and R_t (±1.0% or less), but much larger and systematic changes in the more standard measures of migration. Similar results were obtained when the capillary length or diameter was changed and when various buffer additives were used.

Keywords: Capillary electrophoresis; Mobility ratio; Migration time ratio; Reproducibility; Amino acids; *o*-Nitrophenol

1. Introduction

The resolving power, speed and versatility of capillary electrophoresis (CE) have made this technique an area of great interest for the analysis of drugs and biological compounds [1–4]. Because of these characteristics, it has been predicted that this technique will soon become an effective complement to current separation methods, such as HPLC and GC, that are now common in the pharmaceutical industry [4]. However, one problem that must first be addressed is the relatively low run-to-run reproducibility of CE separations. In a recent survey of pharmaceutical companies in the USA and UK, increased precision was the most frequently mentioned need for improvement in CE systems [4].

Fluctuations in the migration times of solutes is one of the major reasons for the lack of reproducibility in CE [5–12]. This is particularly important when using relatively non-selective detectors, such as UV–Vis absorbance monitors, in which case the identification of an analyte is generally based on the analyte's measured migration time or electrophoretic mobility. Many of the changes observed in solute migration times can be related to variations in the electroosmotic flow [8–15]. Electroosmotic flow in CE can be affected by a number of different parameters

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00849-7

including temperature, running buffer composition, the applied voltage or current, and the chemical nature of the capillary's internal surface [8–15]. Small changes in any one of these items can potentially result in large shifts in solute migration times. For example, in a routine laboratory setting, 10 to 25% variation in CE migration times has been noted in a number of studies [6–8].

A great deal of past effort has been aimed at improving CE reproducibility through better temperature control, optimization of buffer conditions, and/or the development of improved capillaries or capillary modification methods (e.g., see Refs. [5], [9] and [16]). Another approach for obtaining improved precision in CE is to add various markers to the sample as aids for monitoring shifts in the electroosmotic flow or electrophoretic mobilities of solutes [10–12,14,15]. One advantage of this latter approach is that it can be performed with any type of CE system or separation mode. This approach also has the potential for allowing corrections to be made for any run-to-run variations that are observed in the migration times.

This work will examine the use of neutral markers along with mobility or migration time ratios (M and R_t) as a means for obtaining a more reproducible description of solute migration in CE. The run-to-run precision of these parameters will be examined by making replicate injections of standard amino acid or nitrophenol solutions. The changes in M and R_t as a result of systematic variations in voltage, temperature and current will be determined in a similar fashion. The effect of changes in capillary geometry (e.g., length and diameter) and the use of running buffer additives will also be examined. The results obtained in this work will then be compared with those observed for more standard measures of analyte migration, such as migration times and electrophoretic mobilities.

2. Theory

2.1. Mobility ratio, M

In this work, the mobility ratio (M) for each injected solute was defined as follows when using a neutral marker as the reference:

$$M = \mu_{Net}/\mu f_{eo} \quad (1)$$

$$= (\mu + \mu_{eo})/\mu_{eo} \quad (2)$$

In Eqs. 1 and 2, μ_{eo} is the mobility due to electroosmotic flow, μ is the inherent mobility of the solute and μ_{Net} is the net mobility measured for the solute, or $(\mu + \mu_{eo})$.

The advantage of using mobility ratios to describe analyte migration can be seen by relating M, μ and μ_{eo} to fundamental constants of the CE system. For example, μ and μ_{eo} can be related to these constants through the expressions given below [17]:

$$\mu = z/(6\pi r\eta) \quad (3)$$

$$\mu_{eo} = \epsilon\zeta/(4\pi\eta) \quad (4)$$

In these equations, η represents the solvent viscosity, z is the charge of the solute, r is the solute's effective radius, ϵ is the dielectric constant of the solvent and ζ is the zeta potential at the capillary surface. When Eqs. 3 and 4 are substituted into Eq. 2, this gives the following result for M:

$$M = 2z/(3\epsilon\zeta r) + 1 \quad (5)$$

Eq. 5 shows that, like electrophoretic mobilities, the value of M should be independent of such items as the operating voltage and length of capillary in the CE system. However, unlike electrophoretic mobilities, Eq. 5 indicates that M should also be independent of the viscosity of the CE running buffer (η). This occurs since both μ_{eo} and μ have the same dependence on η (see Eqs. 3 and 4). As a result, taking the ratio of these parameters gives a term that is no longer a function of solvent viscosity. This feature is attractive since many of the items that change electroosmotic flow and electrophoretic mobilities in CE (e.g., temperature and buffer additives) do so by altering running buffer viscosity [11,13–15].

2.2. Migration time ratio, R_t

The migration time ratio (R_t) is another term

that can be used to describe the relative migration of analytes in a CE capillary. In this work, the value of R_t for an injected solute will be defined as follows:

$$R_t = t_{Net}/t_{eo} \quad (6)$$

where t_{Net} is the net migration time measured for the solute and t_{eo} is the migration time measured for a neutral marker during the same run. The same type of ratio has been used by Chen, Wang and Zhang to compare the migration times of injected analytes with those for neutral or charged electrophoretic markers [10,11].

At first glance, R_t and M might appear to be entirely different descriptions of solute migration. However, these two parameters are actually quite closely related. This can be seen by considering the equations that are used to calculate μ_{Net} and μ_{eo} from experimental measurements of t_{Net} and t_{eo}:

$$\mu_{Net} = (L_{eff}L_{tot})/(t_{Net}V) \quad (7)$$

$$\mu_{eo} = (L_{eff}L_{tot})/(t_{eo}V) \quad (8)$$

In these expressions, L_{eff} is the effective length of capillary from the injection end to the detector, L_{tot} is the total length of the capillary, and V is the applied voltage. Because the values of L_{eff}, L_{tot} and V are the same for measurements of t_{Net} and t_{eo} (or μ_{Net} and μ_{eo}) made during the same run, substituting Eqs. 7 and 8 into Eq. 1 results in the following relationship between M and R_t:

$$M = t_{eo}/t_{Net} \quad (9)$$

$$= 1/R_t \quad (10)$$

This relationship indicates a number of important features about both M and R_t. First, since R_t and M are reciprocals of one another, R_t should be independent of the same parameters as already noted for M (e.g., solvent viscosity, electric field, etc.). Furthermore, this type of relationship will give R_t and M an identical relative precision (i.e., R.S.D. values). The fact that M can be determined directly from R_t through the use of only migration times is also convenient since it eliminates the need to know the actual length of the capillary and distance to the detection window (i.e., L_{tot} and L_{eff}) or even the operating voltage (V), a situation that does not occur in normal mobility measurements.

Although R_t and M are related, they are complementary in terms of many of their potential applications. For example, R_t can be used in the same fashion as migration times as a simple index of solute migration or as a means for presenting the results of CE experiments (e.g., see Fig. 1b in Results and Discussion). Like μ_{Net} or μ, M can be used as an index of solute migration in situations where it is desirable to relate this migration to fundamental parameters of the system, such as the solute's charge and mass or its binding to agents that have been added to the running buffer.

The definitions of M and R_t in Eqs. 1 and 6 are based on the use of a neutral marker as the reference; however, charged solutes can also be used as reference compounds for this purpose. This second case might be particularly useful when the electroosmotic flow is small or if there is a large difference in the migration times of the analyte and neutral marker, thus exposing these two compounds to different within-run variations during the CE separation. For this situation, a charged marker that more closely resembles the analyte in its migration time or mobility can instead be used. In this case, M and R_t can be redefined by using the following general equations:

$$M = \mu_{Net}/\mu_{Net,r} \quad (11)$$

$$R_t = t_{Net}/t_{Net,r} \quad (12)$$

where $\mu_{Net,r}$ and $t_{Net,r}$ represent the net electrophoretic mobility and migration time for the reference compound. As before, these expressions will result in an inverse relationship between M and R_t. According to Eq. 3, both the analyte and reference compound will have the same dependence on η, so taking the ratio of their mobilities or migration times should again give values for M and R_t that are independent of shifts in solvent viscosity. As was demonstrated in Eqs. 7–10, these terms should also continue to be independent of such parameters as the capillary length or operating voltage.

3. Experimental

3.1. Reagents

The amino acids and α-cyclodextrin were purchased from Sigma (St. Louis, MO, USA). The *o*-nitrophenol, pyronine Y and mesityl oxide were purchased from Aldrich (Milwaukee, WI, USA). Other chemicals were from Fisher Scientific (Fair Lawn, NJ, USA). All buffers and solutions were prepared using deionized water from a Nanopure water system (Barnstead, Dubuque, IA, USA).

3.2. Apparatus

Experiments performed at room temperature were done on an ISCO Model 3850 capillary electrophoresis system (Lincoln, NE, USA). Except where otherwise stated, the CE capillaries used with this system were 75 cm × 50 μm I.D. columns (45 cm effective length) made from untreated fused-silica that was obtained from Polymicro Technologies (Phoenix, AZ, USA). Data from the Model 3850 system were collected using a Chromlink interface and LCAdvantage software (LDC/Milton Roy, Riviera Beach, FL, USA).

Work examining the effect of CE system temperature on migration times and mobilities was performed on an ISCO Model 3140 capillary electrophoresis system. The CE capillaries for this system were 75 cm × 50 μm I.D. untreated fused-silica columns (45 cm effective length), also made out of materials obtained from Polymicro Technologies. Data from the Model 3140 system were collected using a ChemWindows interface and software from ISCO. Electropherograms generated by both the Model 3850 and 3140 systems were analyzed using in-house programs written in Microsoft QuickBASIC (Redmond, WA, USA).

3.3. Methods

All samples were applied to the CE capillaries by using the vacuum injection mode supplied with the Model 3850 and 3140 systems. The typical injection time was 8 s, which corresponded to an injection volume of about 12 nl. Each capillary column was cleaned with 0.025 mol/l sodium borate buffer (pH 10.0) before use with any running buffers or samples. All running buffers were filtered and then degassed and sonicated for at least 10 min before application onto the CE systems. The temperature of the Model 3850 CE system was determined to be 25 ± 0.4°C throughout the described work. The Model 3140 system was held at 16 to 44 ± 0.5°C during the CE temperature studies.

The *o*-nitrophenol and pyronine Y solutions were prepared in a filtered and degassed 0.0125 mol/l potassium phosphate running buffer (pH 11.0). These samples contained 10–100 μmol/l *o*-nitrophenol or 100 μmol/l pyronine Y along with 0.5% (v/v) acetone as a marker for electroosmotic flow. The amino acid solutions were prepared fresh daily in a filtered and degassed 0.0125 mol/l potassium phosphate running buffer (pH 7.4). These samples contained 100 μmol/l of each amino acid and 0.5% (v/v) of mesityl oxide as a marker for electroosmotic flow. All samples were injected in duplicate or triplicate under each set of experimental conditions tested.

Elution of the amino acids and mesityl oxide was detected on-line at 215 nm. The *o*-nitrophenol and pyronine Y samples were monitored at 295 nm and 230 nm, respectively. The electric field strength used in these studies varied from 16 to 26 kV (i.e., 210 to 350 V/cm). The typical operating current at 20 kV was 23 μA for the pH 11.0 phosphate buffer and 25 μA for the pH 7.4 phosphate buffer. The retention time of each peak was calculated by using its first statistical moment [18]. The values of R_t and M were calculated according to Eqs. 6 and 9, respectively. The values of μ_{Net} and μ_{eo} were determined using Eqs. 7 and 8. The inherent mobility of the solute (μ) was determined by calculating the difference between the μ_{Net} and μ_{eo} values.

4. Results and discussion

4.1. Run-to-run variation in migration times and migration time ratios

The basic problem associated with the reproducibility of CE migration times is illustrated in

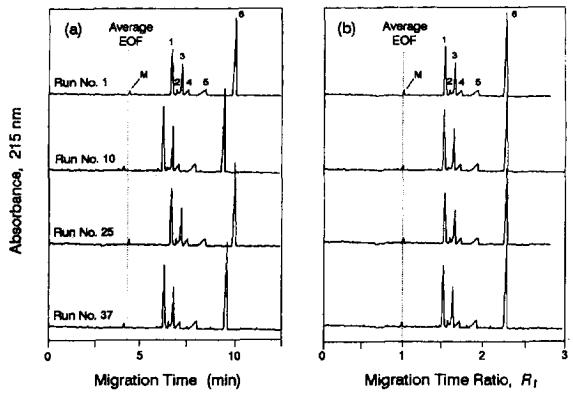

Fig. 1. Variation in the migration times (a) and the migration time ratios (b) for several injections of an amino acid mixture onto a CE system. The injected solutes were the L-enantiomers of: 1, tryptophan; 2, phenylalanine; 3, lysine; 4, leucine; 5, alanine; 6, tyrosine. Mesityl oxide (M) was also added to all samples as a marker for electroosmotic flow (EOF) and for use in the calculation of migration time ratios. The dashed vertical line indicates the average position of the mesityl oxide peak in both the migration time and migration time ratio studies. The operating voltage in these experiments was 22 kV. All other conditions were as described in the text.

Fig. 1a. In this example, a sample containing a mixture of six amino acids and a neutral marker was injected onto a CE system 60 times over two consecutive days. Constant temperature and electric field conditions were used during these studies. The four electropherograms shown in Fig. 1a are representative of the results obtained during the 60 injections. As can be seen in this figure, all of the electropherograms had the same elution pattern but differed significantly in the exact migration times that were noted for each amino acid. Similar variations over replicate injections have been noted in sequential CE analyses of human growth hormone tryptic digests [6] and of peptide mixtures [7].

A summary of the measured variation in the amino acid migration times is given in Table 1. During the course of the 60 injections, the relative standard deviation (R.S.D.) for the migration time of the neutral marker was ±5.0%, while the R.S.D. values for the amino acids ranged from ±2.1% to ±4.6% (mean, ±3.3%). These R.S.D. values are similar to those observed in other studies that have examined the changes in migration times for replicate injections of dipeptides [10] or various organic anions [12]. These types of fluctuations are probably the result of small changes within the capillary over time, such as analyte adsorption to the capillary wall or variations in temperature and current.

One result of these fluctuations was that the migration times observed for some of the amino acids overlapped with the range of migration times noted for neighboring analytes (e.g., tryptophan and phenylalanine, phenylalanine and lysine, or lysine and leucine). In these cases, the migration time was not a reliable indicator for the identification of a particular compound.

Table 1
Variation in the migration time and migration time ratio for replicate injections of an amino acid mixture[a]

Solute	Migration time (min)			Migration time ratio, R_t		
	Range	Average	R.S.D. (%)	Range	Average	R.S.D. (%)
Mesityl oxide	3.9–4.2	4.0 ± 0.2	5.0	n/a	1.00	n/a
Tryptophan	6.0–6.4	6.3 ± 0.2	3.2	1.49–1.52	1.50 ± 0.01	0.8
Phenylalanine	6.2–6.7	6.5 ± 0.3	4.6	1.60–1.65	1.63 ± 0.02	1.1
Lysine	6.4–6.9	6.7 ± 0.2	3.0	1.67–1.69	1.68 ± 0.01	0.6
Leucine	6.8–7.3	7.1 ± 0.2	2.8	1.76–1.79	1.78 ± 0.01	0.6
Alanine	7.7–8.2	8.0 ± 0.3	3.8	1.99–2.02	2.00 ± 0.02	0.8
Tyrosine	9.2–9.7	9.5 ± 0.2	2.1	2.35–2.40	2.38 ± 0.02	0.7

[a] The results shown are for 60 measurements obtained over the course of two days. The number that follows each average value represents a range of one standard deviation. The values in the R.S.D. column represent one relative standard deviation. The experimental conditions were the same as in Fig. 1.

Instead, a comparison of migration time patterns also had to be performed. However, such an approach would not be possible for unknown samples, since these samples may or may not contain all of the analytes used in the pattern comparison.

To help correct for the variability in the migration times, a migration time ratio (R_t) was calculated for all analytes during each run, based on the use of mesityl oxide as an indicator of electroosmotic flow. The values that were observed for the migration time ratios are included in Table 1. The relative precision of the migration time ratios ranged from ±0.6% to ±1.1% (mean, ±0.7%) for all of the amino acids tested. These R.S.D. values were much smaller than those seen for the migration times. In addition, it was found that none of the amino acids gave R_t values that showed any significant overlap with those for neighboring solute peaks.

One advantage of using R_t to describe analyte migration is that this parameter can be used in the same fashion as migration times in plotting electropherograms. An example of this is shown in Fig. 1b. When this was done in this study, it not only gave a solute migration pattern that was similar from one injection to the next, but the positions of the peaks in the pattern were also consistent. These results indicate that R_t is a more reliable and reproducible description of solute movement than ordinary migration times. This also shows that R_t is a superior index for the visual examination and comparison of individual CE runs.

4.2. Run-to-run variation in electrophoretic mobilities and mobility ratios

The run-to-run reproducibility of electrophoretic mobilities and the mobility ratio (M) was examined by making 240 injections of *o*-nitrophenol and acetone (i.e., the neutral marker) over the course of twelve days. During this period of time, twenty injections of this mixture were made daily onto the same CE column under identical operating conditions, including temperature, voltage and running buffer composition. Over the twelve days, the within-day precision for μ_{Net} was between ±1.7% and ±5.4% (mean, ±3.8%); the precision for μ_{eo} ranged from ±1.4 to ±5.5% (mean, ±3.4%). The same R.S.D. values were obtained for the migration times of *o*-nitrophenol and acetone. Values determined for the inherent mobility of *o*-nitrophenol gave only a slightly better within-day precision, with a range of ±1.0 to ±4.7% (mean, ±2.6%). For the same set of data, the R.S.D.'s for the within-day precision of M (and R_t) were ±0.5% to ±1.5% (mean, ±0.9%). These results are consistent with those of the amino acid studies and again illustrate the improved precision that is gained by using mobility or migration time ratios to describe solute movement in CE.

The day-to-day variation in these migration indexes was studied by comparing the average values of μ_{Net}, μ_{eo} and M that were determined during each day of the twelve-day study. The results are shown in Fig. 2. As indicated by Fig. 2a, the average net mobilities of *o*-nitrophenol and acetone gave a similar pattern in their fluctuations over the course of this work. The day-to-day fluctuations in these values were ±3.7% and ±2.8%, respectively. The day-to-day variation in the average inherent mobility of *o*-nitrophenol was ±3.5%. However, the day-to-day variation in the average value of M was ±1.0% during the same experiments (Fig. 2b). Equivalent results were obtained when a comparison of the average migration times and migration time ratios were made. From these data, it was determined that M and R_t were more consistent measures of solute mobility than electrophoretic mobilities or migration times during long-term CE studies.

4.3. Effect of changes in operating voltage

The applied voltage (or electric field strength) is one of the most important parameters in the optimization of a CE separation. However, by varying the operating voltage there will also be a change in the electroosmotic flow of the system and in the migration times for each of the injected analytes [17]. This is demonstrated in Fig. 3a for injections of the same amino acid mixture as used in Fig. 1. In this example, an

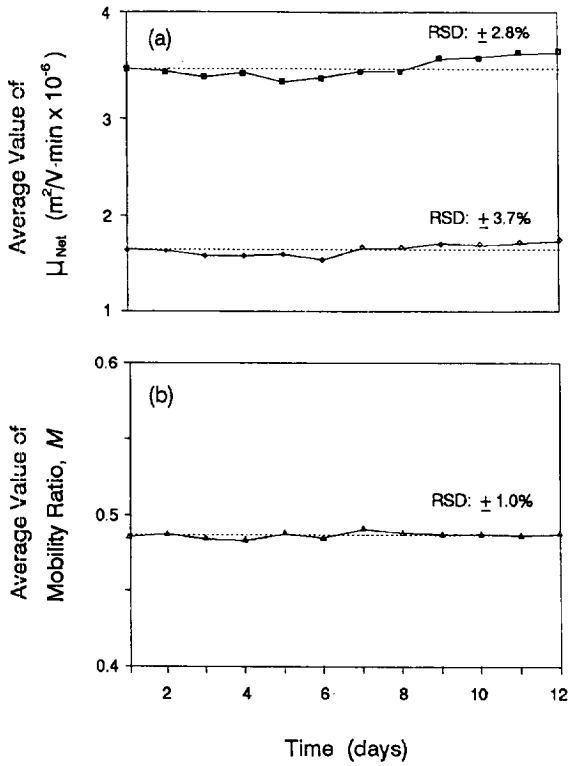

Fig. 2. Variation in the daily average of the net electrophoretic mobilities (a) and mobility ratios (b) for replicate injections of *o*-nitrophenol and acetone. In the top portion of the graph, both the average electrophoretic mobilities of *o*-nitrophenol (◇) and acetone (■) are given for a total of twenty injections on each day of the study. The lower graph (△) shows the average values of the mobility ratio for *o*-nitrophenol versus acetone. The operating voltage was 18 kV throughout this study. All other conditions were the same as described in the text.

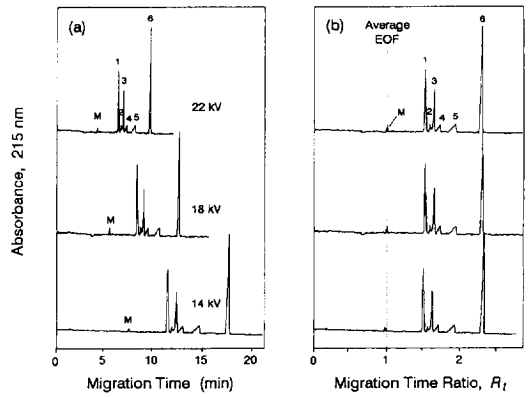

Fig. 3. Effect of varying the applied voltage on the migration times (a) and migration time ratios (b) measured for an amino acid mixture. The injected solutes were the same as in Fig. 1. The dashed vertical line in (b) indicates the average position of the mesityl oxide peak in the migration time ratio studies. All experimental conditions were as described in the text.

increase in the applied voltage from 14 to 22 kV produced roughly a two-fold decrease in the migration times for all of the amino acids and the neutral marker. However, when the same results were plotted versus the migration time ratio (Fig. 3b), no noticeable change in peak positions was noted (i.e., a random variation in R_t of ±0.5 to ±1.0% for each of the six amino acids and three voltages tested). As explained earlier in the Theory section, this behavior would be expected since the value of R_t should be independent of the applied voltage and electric field strength.

Although the net or inherent electrophoretic mobility of a solute should be independent of voltage, this is not necessarily true if other system parameters (i.e., capillary temperature) are affected by the change in voltage. This effect is shown in Fig. 4 for the injection of *o*-nitrophenol and acetone. In going from an applied voltage of 14 to 25 kV, the net electrophoretic mobilities for the analyte and the neutral marker increased in a parallel fashion, with a total observed change of 25% and 24%, respectively. The corresponding shift in the migration times was 53% for both compounds; the change in the inherent mobility of *o*-nitrophenol was 23%. This effect can be explained by the change in running buffer viscosity that results from the increase in joule heating at the higher voltages [11,19]. Under the same experimental conditions, the mobility ratios (and values of R_t) showed only random variations, with an R.S.D. of ±0.5%. This type of behavior was expected, since Eqs. 5 and 10 show that both M and R_t are independent of voltage and solvent viscosity. These results, along with the data in Fig. 3, indicate that mobility and migration time ratios should be more stable than migration times or electrophoretic mobilities in identifying analytes in situations where there are changes in the operating voltage between runs.

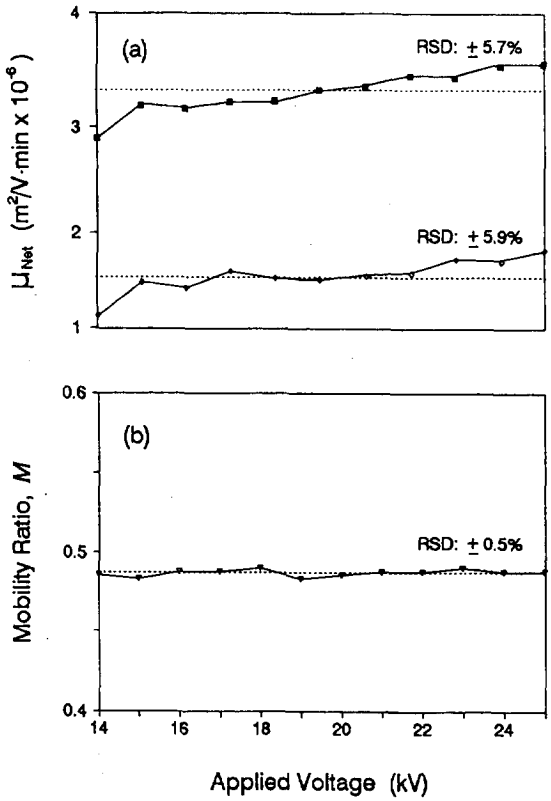

Fig. 4. Effect of varying the applied voltage on the net electrophoretic mobilities (a) and mobility ratios (b) for replicate injections of *o*-nitrophenol and acetone. In the top portion of the graph, both the electrophoretic mobilities of *o*-nitrophenol (◇) and acetone (■) are given. The values in the lower graph (▽) represent the ratio of the electrophoretic mobilities for *o*-nitrophenol versus acetone. The conditions were as described in the text.

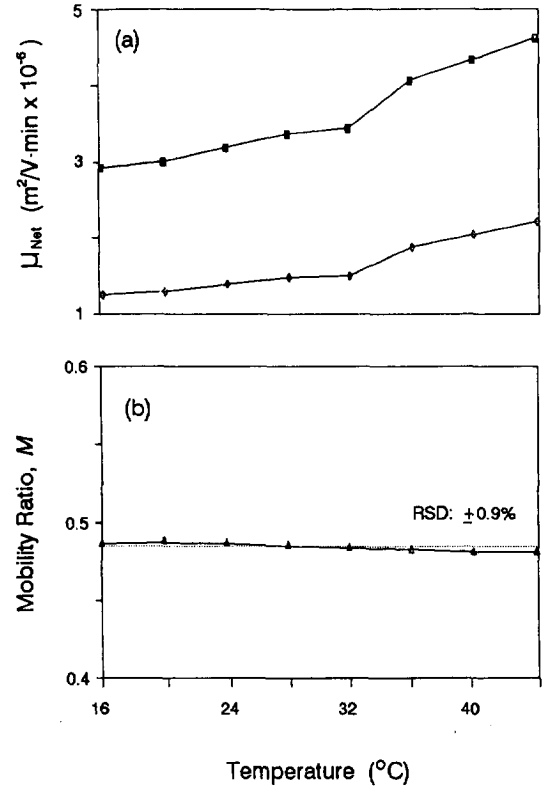

Fig. 5. Effect of varying the temperature of the CE system on the net electrophoretic mobilities (a) and mobility ratios (b) for replicate injections of *o*-nitrophenol and acetone. In the top portion of the graph, both the electrophoretic mobilities of *o*-nitrophenol (◇) and acetone (■) are given. The values in the lower graph (△) represent the ratio of the electrophoretic mobilities for *o*-nitrophenol versus acetone. The applied voltage in this study was 18 kV. All other conditions were as described in the text.

4.4. Effect of changes in temperature and current

The effect of temperature on the reproducibility of CE peak position was examined by varying the temperature of the CE capillary chamber. This was done at temperatures between 16 and 44°C for a series of injections of *o*-nitrophenol and acetone. The results are shown in Fig. 5. An increase in system temperature produced an increase in the net electrophoretic mobilities for both *o*-nitrophenol and acetone, with a pattern that was similar to that observed in Fig. 4 for an increase in applied voltage. The total observed increase in these mobilities was 52 to 58%. This was accompanied by a decrease of 36 to 37% in the migration times of these solutes and a change of 64% in the inherent mobility of *o*-nitrophenol. Similar shifts in the migration times of various dipeptides have been reported in other work performed over this temperature range [10,11]. As stated in the voltage studies, these shifts in migration times and electrophoretic mobilities can be explained by the changes that are produced in the running buffer viscosity as the temperature of the CE capillary is raised.

Under the temperature conditions given in Fig.

5, the mobility ratio for *o*-nitrophenol showed only a small level of random variation in its value (R.S.D., ±0.9%). The same behavior was seen for plots made with migration time ratios. As in the voltage studies, the fact that no apparent shift in these values occurred with an increase in temperature simply reflects the fact that both the mobility ratio and migration time ratio are independent of solvent viscosity. These data suggest that M and R_t are more reproducible than migration times and electrophoretic mobilities as indicators of analyte migration in cases where there are temperature fluctuations.

During these studies, there was an increase in current on the CE system as the temperature was raised. The current went from 20 μA at 16°C to 48 μA at 44°C. This followed a trend that was parallel to that noted in Fig. 5 for the change in electrophoretic mobilities with temperature. This observation probably reflects a decrease in resistance of the running buffer at the higher temperatures. The fact that the values of M and R_t did not change significantly under these conditions indicates that these parameters are also independent of current. The same conclusion for migration time ratios has been reached in studies by Chen et al. [10].

4.5. Effect of changes in capillary geometry

If the CE capillary is changed between runs, then differences in the diameter, length or positioning of the old and new capillaries can be yet another source of variation in the migration times. The effect of varying such factors is shown in Table 2. In this table only data for the migration times and R_t are shown, since the electrophoretic mobilities were automatically corrected for any changes in column length during the calculation of these parameters (see Eqs. 7 and 8).

Increasing the length of capillary from the injection end to the detector (L_{eff}) by 0.5 to 1.0 cm or increasing both L_{eff} and the total length of the capillary (L_{tot}) by 0.5 to 1.0 cm resulted in a small but consistent increase in the values of t_{eo} and t_{Nel}. In this case, the higher values measured for t_{eo} and t_{Nel} probably resulted from the greater distance that analytes had to travel to reach the detector flow-cell. A smaller increase

Table 2
Effect of changes in capillary length and diameter on various absolute and relative migration parameters

Change in capillary conditions[a]	Observed change in migration parameters (%)		
	t_{eo}	t_{Nel}	R_t
Increase in L_{eff} (L_{tot} constant):			
+0.5 cm	+1.2	+1.5	+0.4
+1.0 cm	+3.8	+4.9	+0.7
Increase in L_{tot} (L_{eff} constant):			
+0.5 cm	+0.4	+0.5	0.1
+1.0 cm	+0.8	+0.6	−0.1
Increase in both L_{eff} and L_{tot}:			
+0.5 cm	+1.0	+1.0	+0.6
+1.0 cm	+3.2	+3.5	+0.3
I.D. change from 75 to 50 μm	+32.9	+33.5	+0.4

The t_{eo} and t_{Nel} values are for acetone and *o*-nitrophenol, respectively. The R_t results represent the migration of *o*-nitrophenol versus that of acetone.

[a] The initial capillary in all studies was 70 cm × 75 μm I.D. total length, with an effective length of 50 cm from the injection end to the detector.

in migration times was seen when only L_{tot} was increased. In this latter situation, the migration times were altered by the corresponding decrease in electric field strength that occurred as the same voltage was applied across a greater length of capillary.

Changing the diameter of the capillary had the largest effect on migration times, with the values of t_{eo} and t_{Net} both increasing by 33% as the I.D. of the capillary was decreased from 75 to 50 μm. This increase in t_{eo} and t_{Net} was identical to the relative decrease in the radius (and internal surface area) of the capillary. This indicated that the higher values of t_{Net} and t_{eo} were simply due to a decrease in the electroosmotic flow.

In all of these situations, the change seen in R_t was small (−0.1% to +0.7%) and generally appeared to be random in nature. This small variation, even in the presence of large variations in migration times, arose from the similarity of the change in the values for the neutral marker and charged solute. As a result, it was concluded that R_t (and therefore also M) was not sensitive to these types of alterations in capillary geometry.

4.6. Effect of running buffer additives

Many current CE techniques use some type of running buffer additive to enhance the separating ability of the CE system. Examples include methods based on the addition of surfactants, binding agents or organic solvents to the running buffer [e.g., see Refs. 13–15 and 20–23]. Fig. 6 shows the effect on mobility ratios and electrophoretic mobilities when using α-cyclodextrin as a binding agent. This was examined since it is known that varying the concentration of this type of agent will alter the running buffer viscosity, thus leading to a change in electrophoretic mobilities and migration times [13–15]. In Fig. 6 it was found that an increase in α-cyclodextrin levels from 0 to 15 mmol/l produced a shift in the net electrophoretic mobilities of 12 and 11% for acetone (the neutral marker) and pyronine Y (a charged solute that does not bind to α-cyclodextrin), as well as a change of 14% in the inherent mobility of pyronine Y. However, since

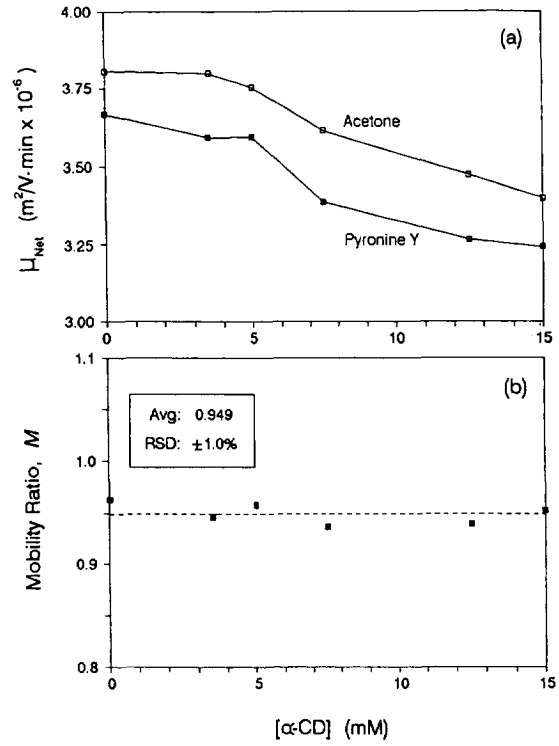

Fig. 6. Effect of running buffer concentration of α-cyclodextrin (α-CD) on (a) the net electrophoretic mobilities for acetone and pyronine Y and on (b) the mobility ratio for pyronine Y. The applied voltage was 20 kV and the total capillary length was 100 cm (65 cm effective length). All other conditions were the same as given in the text. The dashed horizontal line in the lower graph shows the average mobility ratio that was obtained for pyronine Y. The inset to the lower graph gives the value of the average mobility ratio and its relative standard deviation.

these two compounds had similar changes in their net mobilities, the resulting mobility ratio for pyronine Y versus acetone changed by only ±1.0%. The same type of results were observed when using migration time ratios. Similar behavior would be expected when using mobility ratios or migration time ratios with CE systems that contain other buffer additives, such as surfactants or proteins.

The effect of using a mixture of organic and aqueous solvents in the running buffer was examined for solutions containing 0 to 5% (v/v) methanol in the pH 11.0 phosphate buffer. As the level of methanol was increased to 5%, the

net mobilities for *o*-nitrophenol and acetone decreased by 25% and 18% versus the results obtained when no methanol was present. The migration times for these compounds increased by 34% and 23% under the same conditions, and the inherent mobility of *o*-nitrophenol shifted by 11%. A much smaller change was noted in the migration time ratio and mobility ratio for *o*-nitrophenol (4.5% and 7.9%, respectively).

Although the change that was observed for R_t and M in the presence of increasing methanol levels was smaller than that seen for the standard migration times and mobilities, the variation in R_t and M was larger than that seen in any of the previous experiments. This can be explained by the fact that multiple factors were being affected by the addition of organic solvent to the running buffer. One of these items was the solvent viscosity; however, it has already been shown that R_t and M should be independent of this factor. Two other items that would change with the methanol content would be the dielectric constant of the running buffer and the zeta potential of the CE capillary [23]. As shown in Eqs. 5 and 10, R_t and M are related to both these factors when a neutral marker is used as the reference. This explains why some variation was noted in R_t and M with methanol content, but the fact that R_t and M are independent of viscosity also indicates why the change in these parameters was smaller than that observed in the migration times and electrophoretic mobilities.

5. Conclusion

This work examined the use of mobility ratios (M) and migration time ratios (R_t) as a means for providing a more reliable description of analyte migration in CE. The relationship between these two ratios was examined and it was demonstrated theoretically that each should be independent of such factors as voltage and solvent viscosity. Both M and R_t gave good within-day precision in work with model solutes, with an R.S.D. of ±0.6% to ±1.1% for 60 replicate injections of six amino acids and ±0.5% to ±1.5% for twenty daily injections of *o*-nitrophenol over twelve days. By comparison, the within-day precision for more typical indicators of migration, such as migration times and electrophoretic mobilities, varied by ±1.0 to ±5.5%, respectively, under the same conditions. Similar results were obtained when comparing the day-to-day variation in these parameters. The better reproducibility of the M and R_t values makes these ratios potentially useful in following the migration of solutes or in determining the identity of unknown compounds during long-term CE studies. This latter point was demonstrated by the fact that the use of R_t values in the analysis of amino acid separations made it possible to identify some solutes that could not be distinguished based on the use of only standard migration time data.

Further studies were carried out to examine the effects on M and R_t of changing several CE operating conditions. Altering the voltage, temperature or current of the CE system produced only small, random variations in M and R_t (i.e., ±1.0% or less), while much larger and systematic changes were noted in the corresponding migration times and electrophoretic mobilities. Similar results were obtained when the capillary length or diameter was changed and when buffer additives, such as cyclodextrins, were used. Some change in M and R_t was noted in the presence of organic solvents when using a neutral marker as the reference; however, even in this case the effect was much less than that observed for migration times or electrophoretic mobilities. Based on this work, it was concluded that M and R_t should provide an improved means of describing analyte migration during routine CE assays.

Acknowledgements

This work was supported by the Van Slyke Society of the American Association for Clinical Chemistry. The use of the Model 3850 and 3140 CE systems was generously provided by ISCO, Inc. (Lincoln, NE, USA).

References

[1] G.M. McLaughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Morris, J.A. Morrison and T.J. Bronzert, J. Liq. Chromatogr., 15 (1992) 961.
[2] C. Silverman and C. Shaw, in J.P. Landers (Editor), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1994, Ch. 10.
[3] K.D. Altria and S.D. Filbey, J. Liq. Chromatogr., 16 (1993) 2281.
[4] K.D. Altria and M.T. Kersey, LC–GC, 13 (1995) 40.
[5] M.A. Strege and A.L. Lagu, J. Liq. Chromatogr., 16 (1993) 51.
[6] J. Frenz, S.-L. Wu and W.S. Hancock, J. Chromatogr., 480 (1989) 379.
[7] T. Dülfer, R. Herb, H. Herrmann and U. Kobold, Chromatographia, 30 (1990) 675.
[8] B.J. Wanders, T.A.A.M. van de Goor and F.M. Everaerts, J. Chromatogr. A, 652 (1993) 291.
[9] S.E. Moring, J.C. Colburn, P.D. Grossman and H.K. Lauer, LC–GC, 8 (1990) 34.
[10] N. Chen, L. Wang and R. Zhang, J. Liq. Chromatogr., 16 (1993) 3609.
[11] Y.K. Zhang, N. Chen and L. Wang, J. Liq. Chromatogr., 16 (1993) 3689.
[12] J.H. Jumppanen and M.-L. Riekkola, Anal. Chem., 67 (1995) 1060.
[13] S. Honda, A. Taga, K. Susuki, S. Susuki and K. Kakehi, J. Chromatogr., 597 (1992) 377.
[14] S.G. Penn, D.M. Goodall and J.S. Loran, J. Chromatogr., 636 (1993) 149.
[15] F.A. Gomez, L.Z. Avila, Y.-H. Chu and G.M. Whitesides, Anal. Chem., 66 (1994) 1785.
[16] D.N. Heiger and R.E. Majors, LC–GC, 13 (1995) 12.
[17] P. Camilleri (Editor), Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1993.
[18] E. Grushka, M.N. Myers, P.D. Schettler and J.C. Giddings, Anal. Chem., 41 (1969) 889.
[19 D.S. Burgi, K. Salomon and R.-L. Chien, J. Liq. Chromatogr., 14 (1991) 847.
[20] T.J. Ward, Anal. Chem., 66 (1994) 633A.
[21] S. Terabe, K. Otsuka and T. Ando, Anal. Chem., 57 (1985) 834.
[22] H. Salimi-Moosavi and R.M. Cassidy, Anal. Chem., 67 (1995) 1067.
[23] C. Schwer and E. Kenndler, Anal. Chem., 63 (1991) 1801.