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ARTICLE *in* ANALYTICAL CHEMISTRY · APRIL 1996

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Fast, Accurate Mobility Determination Method for Capillary Electrophoresis

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A new method for accurately determining effective mobilities and electroosmotic flow rates for capillary electrophoresis is described. The proposed method can be performed using most commercial capillary electrophoresis instruments. Problems inherent to the conventional mobility determination method such as a variable electroosmotic flow during the run and migration through unthermostated regions of the capillary are eliminated with the use of the proposed method. In addition, very low effective mobilities and electroosmotic flow rates can be measured quickly and reproducibly. Also, cation mobilities and anion mobilities can be measured in a single run regardless of the magnitude or direction of the electroosmotic flow.

Over the last decade, capillary electrophoresis (CE) has become an important separation tool because it permits the separation of anionic, cationic, and neutral analytes in a single run and can result in separation efficiencies as high as 500 000 theoretical plates.[1] Though separation efficiencies are high in CE, selectivity optimization is still important if one wants to combine fast separations with good peak resolution. Improved commercial instrumentation, with adequate thermostation and programmable methods, permits the rapid, rational development and optimization of CE separation methods. Optimization methods that rely on physical models require the use of solute mobilities rather than separation times.[2,3]

The observed velocity of an analyte, v_{obs}, is calculated as

$$v_{obs} = \frac{L_d}{t_{migr} - t_{ramp}/2} \tag{1}$$

where L_d is the length of the capillary from the injection point to the detector, t_{migr} is the migration time of the analyte (or the migration time of a neutral marker used for the determination of the velocity of the electroosmotic flow, v_{eo}), and t_{ramp} is the time during which the potential increases linearly from zero to the programmed value at the beginning of the run.[4] If the total length of the capillary is L_t, and the applied potential is V_{prog}, the observed mobility of the analyte, μ_{obs}, is calculated by normalizing the observed velocity of the analyte with the electric field strength, E, as

$$\mu_{obs} = \frac{L_t L_d}{V_{prog}(t_{migr} - t_{ramp}/2)} \tag{2}$$

The effective mobility of the analyte, μ_{eff}, is calculated from the observed mobility of the analyte and the coefficient of the electroosmotic (EO) flow, μ_{eo}, as

$$\mu_{obs} = \mu_{eff} + \mu_{eo} \tag{3}$$

Since the value of μ_{eff} depends on the composition (e.g., pH, ionic strength, and additive concentration) and the temperature of the background electrolyte (BGE), this is the primary parameter in thermodynamic studies and resolution optimization schemes.[2,3] Therefore, the accurate determination of this value is important.

There are several factors that influence the accuracy of mobility measurements in CE. While μ_{eff} is constant in a given BGE, μ_{eo} (and consequently μ_{obs}) can vary from run to run. When the neutral marker migrates past the detector before the analytes of interest, any change that occurs in the EO flow after the neutral marker passes the detector window remains undetected. Likewise, when the analyte migrates past the detector before the neutral marker, it does not experience any late changes that the EO marker does, resulting again in a biased μ_{eff} value. Also, due to design constraints, in most instruments the entire length of the capillary (separation chamber) cannot be thermostated at the programmed temperature. Therefore, the electrophoretic velocities through the unthermostated regions of the capillary (the inlet and the outlet sections of the capillary) will be different from the electrophoretic velocities through the thermostated region. This causes two problems: (1) μ_{eff} will change when the ambient temperature changes between runs, and (2) μ_{eff} will change when the length of the capillary is altered because the percentage of the unthermostated segment of the capillary will be different. Some of these problems can be eliminated, as suggested recently, by establishing two detection windows on the same capillary.[5] The main drawback of this approach is that it cannot be used with most commercial instruments, and it does not eliminate the problem of a changing EO flow during the run.

Practical difficulties arise when very slow electrophoretic and electroosmotic mobilities are to be determined because the bands may take several hours to migrate past the detector. Additionally, depending on the electrode polarity used, ions that migrate against the EO flow may not be observed when their velocity is faster

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(3) Rawjee, Y. Y.; Vigh, Gy. *Anal Chem.* **1994**, *66*, 619.
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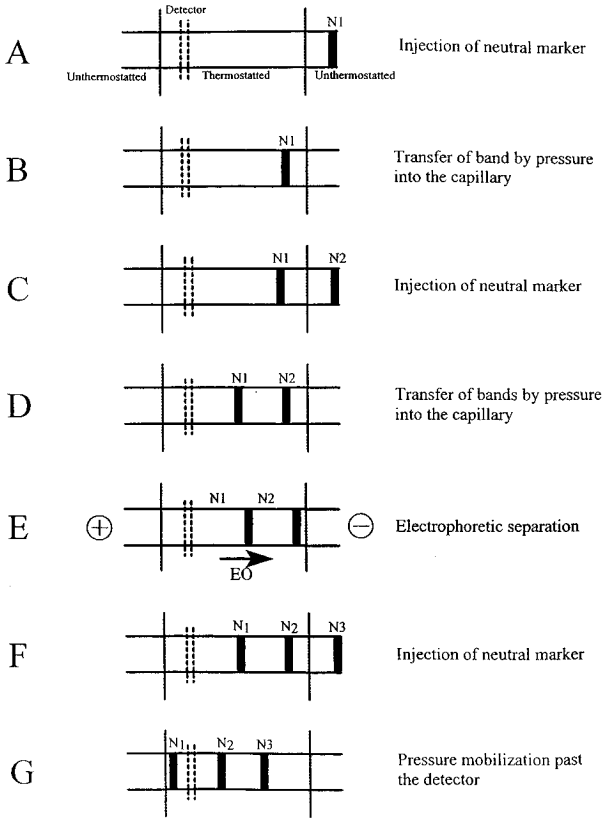

Figure 1. Steps involved in the determination of μ_{eo}.

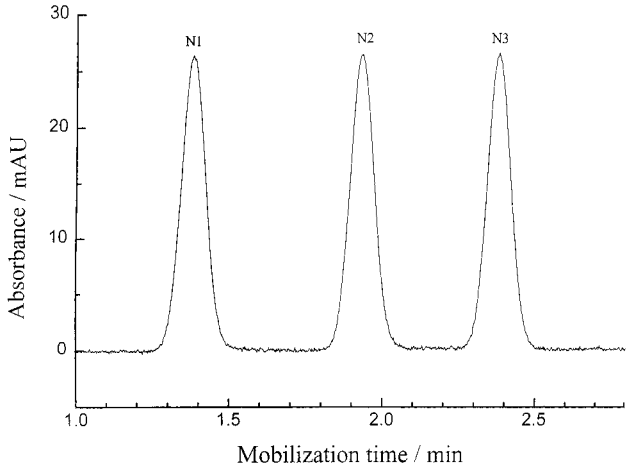

Figure 2. Detector trace for μ_{eo} determination. Run conditions: MES BGE, pH 6.5, eCAP Neutral coated capillary, 50 μm i.d., L_T = 66.35 cm, L_d = 59.75 cm, T = 25 °C, V_{prog} = 15 kV, and t_{migr} = 5 min. Peaks N_1, N_2, and N_3 are benzyl alcohol.

than that of the EO flow. Consequently, complex samples that contain analytes of widely different mobilities cannot be characterized by a single separation.

The importance of mobility determinations and the experimental difficulties involved prompted us to develop a fast and accurate method that requires, regardless of the magnitude or direction of the EO flow, only a single CE run for the determination of both the effective mobilities of cationic and anionic analytes and the coefficient of the electroosmotic flow.

THEORY

Fast Determination of μ_{eo}. The proposed method relies on precise pressure rinses available with most commercial CE instruments. Figure 1 shows the sequence of steps involved in the determination of μ_{eo}. First, the capillary is filled with the BGE to be used. Next, a solution of a neutral marker, prepared with the same BGE, is injected into the capillary for time t_{inj} as shown in step A of Figure 1 (band N_1). Then, in step B, band N_1 is transferred a distance into the capillary by applying, for time t_{tr}, the injection pressure upon the vial that contains the pure BGE. Third, in step C, another band of the neutral marker solution is injected (band N_2), again for time t_{inj}. Then, in step D, band N_2 is transferred a distance into the capillary by applying the same injection pressure, for the same time t_{tr}, upon the vial that contains the pure BGE. Next, in step E, the run potential, V_{prog}, is applied for time t_{migr}. During this time both bands, N_1 and N_2, move toward the cathode (for a cathodic EO flow), with mobilities equal to μ_{eo}. Then, after the potential returns to zero, in step F, a third band of the neutral marker solution is injected into the capillary (band N_3), for time t_{inj}. Finally, in step G, the injection pressure is applied again onto the pure BGE vial and data acquisition is initiated simultaneously to record the passage of all three bands by the detector. The detector trace obtained during this pressure mobilization step is shown in Figure 2.

The final pressure mobilization velocity, v_m, with which all three bands move by the detector is calculated by

$$v_m = \frac{L_d}{t_{N_3} + t_{inj}/2 - t_d} \quad (4)$$

where t_{N_3} is the time required to push N_3 past the detector. The second term in the denominator, $t_{inj}/2$ corrects for the movement of the centroid of the last neutral marker band (N_3) during its injection into the capillary. The third term in the denominator, t_d, is added to account for the delay, if any (see Results and Discussion) between the beginning of the final pressure mobilization step and the start of the data acquisition process.

The difference between the recorded mobilization times for band N_2 (t_{N_2}) and band N_1 (t_{N_1}) is used to determine the starting position of band N_2 in the capillary at the beginning of the electrophoresis step (step E) as

$$L_{init} = (t_{N_2} - t_{N_1} - t_{inj}/2)v_m \quad (5)$$

The difference between the recorded mobilization times for band N_3 (t_{N_3}) and band N_2 (t_{N_2}) is used to determine the final position of band N_2 in the capillary before the injection of band N_3 (step F) as

$$L_{final} = (t_{N_3} - t_{N_2} - t_{inj}/2)v_m \quad (6)$$

The distance traveled by the neutral markers during electrophoresis (L_{eo}) is therefore calculated by

$$L_{eo} = L_{final} - L_{init} \quad (7)$$

that is

$$L_{eo} = [(t_{N_3} - t_{N_2}) - (t_{N_2} - t_{N_1})]v_m \quad (8)$$

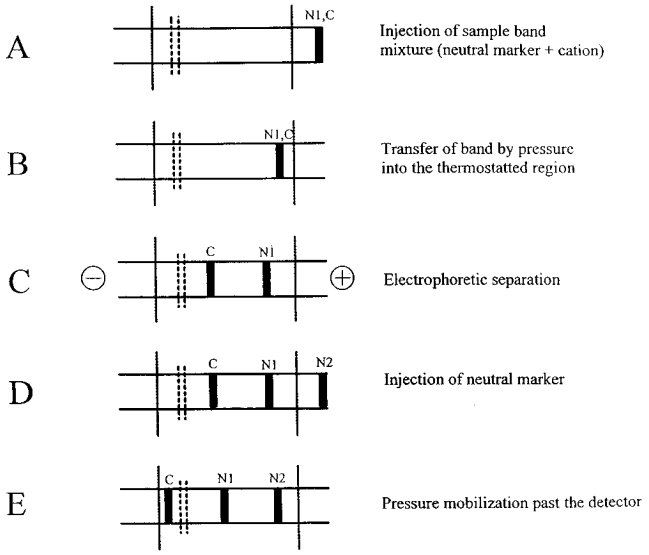

Figure 3. Steps involved in the determination of μ_{eff} for a cation with a cathodic eo flow.

Then, μ_{eo} can be calculated as

$$\mu_{eo} = \frac{L_{eo}L_t}{V_{prog}(t_{migr} - t_{ramp-up}/2 - t_{ramp-down}/2)} \quad (9)$$

where $t_{ramp\text{-}up}$ and $t_{ramp\text{-}down}$ are the times it takes to linearly change the applied potential between 0 and V_{prog}.[4]

Fast Determination of μ_{eff} for Cations and Anions. Figure 3 shows the sequence of steps used to determine μ_{eff} for a cation. First, in step A, the sample, which contains a neutral marker and the cation dissolved in the BGE, is injected into the BGE-filled capillary. Then, in step B, the sample band is transferred into the thermostated segment of the capillary by applying, for time t_{tr}, the injection pressure onto the vial that contains the pure BGE. Next, in step C, the separation voltage, V_{prog}, is applied for time t_{migr}, during which time the neutral marker moves toward the cathode (for a cathodic EO flow) with a mobility μ_{eo}, while the cation moves toward the cathode with a combination of its electrophoretic mobility, μ_{eff}, and μ_{eo}. After the programmed potential, V_{prog}, returns to zero, in step D, a neutral marker band, or N_2, is injected for time t_{inj}. Finally, in step E, the injection pressure is applied again to the pure BGE vial and data acquisition is initiated simultaneously to record the passage of all three bands by the detector (the detector trace is shown in Figure 4).

The final pressure mobilization velocity, v_m, is again calculated by eq 4, except that t_{N_2} is used for t_{N_3}. During the electrophoresis step (step C), the cation (band C) and the neutral marker (band N_1), which were introduced in the first injection, separate. The distance between these peaks, L_C, can be calculated as

$$L_C = (t_{N_1} - t_C)v_m \quad (10)$$

where t_{N_1} is the recorded mobilization time for the neutral marker from the first injection (band N_1) and t_C is the recorded mobilization time for the cation from the first injection (band C). Thus, μ_{eff} for the cation can be calculated as

$$\mu_{eff}^{C} = \frac{L_C L_t}{V_{prog}\,(t_{migr} - t_{ramp-up}/2 - t_{ramp-down}/2)} \quad (11)$$

A similar equation can be derived for anionic analytes, except that

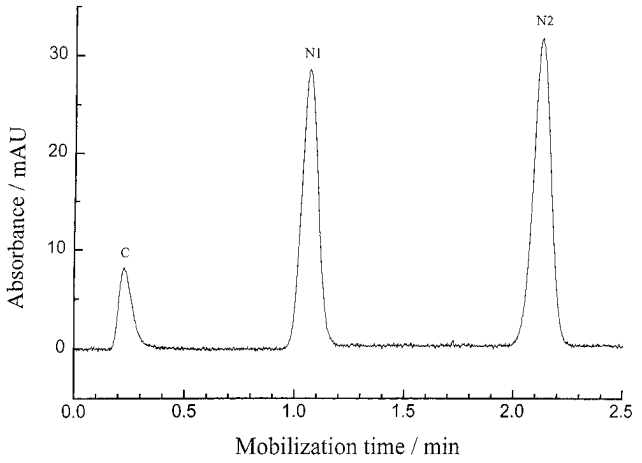

Figure 4. Detector trace for μ_{eff} determination of a cation. Run conditions: MES BGE, pH 6.5, eCAP Neutral coated capillary, 50 μm i.d., L_T = 66.35 cm, L_d = 59.75 cm, T = 25 °C, V_{prog} = 15 kV, and t_{migr} = 5 min. Peak C is BTM and peaks N_1 and N_2 are benzyl alcohol.

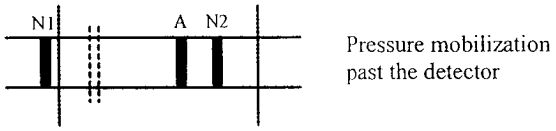

Figure 5. Band pattern in the capillary for the separation of a neutral marker and an anion.

because the migration order of the bands (neutral marker and anion, both from the first injection) is switched (Figure 5), the distance between the peaks is

$$L_A = (t_A - t_{N_1})v_m \quad (12)$$

and μ_{eff} for the anion is

$$\mu_{eff}^{A} = \frac{L_A L_t}{V_{prog}\,(t_{migr} - t_{ramp-up}/2 - t_{ramp-down}/2)} \quad (13)$$

The same equations (eqs 11 and 13) apply when the sample contains both a cation and an anion, a case for which the recorded detector trace is shown in Figure 6.

EXPERIMENTAL SECTION

Experiments were performed on a P/ACE 2100 CE unit (Beckman Instruments, Fullerton, CA) using 50 μm i.d. eCAP Neutral coated capillaries (Beckman P/N 477441) and 25 μm i.d. untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ). The test components, naphthalenesulfonic acid sodium salt (NSA), *p*-toluenesulfonic acid (PTSA), benzyltrimethylammonium bromide (BTM), and benzyl alcohol (the neutral marker), as well as the BGE components, morpholinoethanesulfonic acid (MES) and lithium hydroxide monohydrate, were purchased from Aldrich Chemical Co. (Milwaukee, WI). Water used for all solutions was obtained from a Milli-Q unit (Millipore, Milford, MA).

The BGEs were prepared by diluting 3 mL of a MES buffer stock solution (pH 6.5) to 25 mL with Milli-Q water. The buffer stock solution contained 5.35 g of MES and 0.83 g of $LiOH{\cdot}H_2O$ in 100 mL of Milli-Q water. During the mobility determinations,

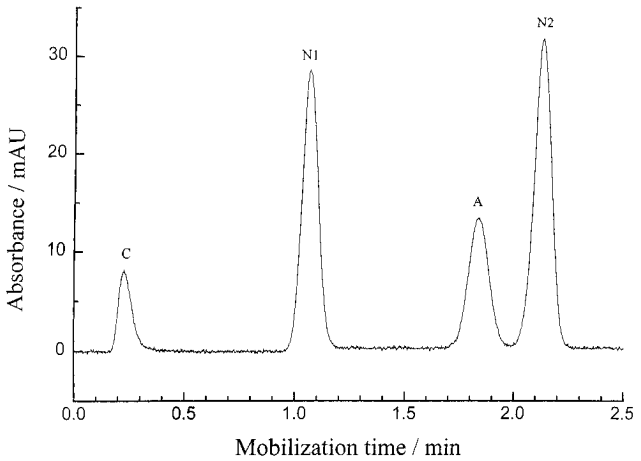

Figure 6. Detector trace for μ_{eff} determination of a cation and an anion in a single separation. Run conditions: MES BGE, pH 6.5, eCAP Neutral coated capillary, 50 μm i.d., L_T = 66.35 cm, L_d = 59.75 cm, T = 25 °C, V_{prog} = 15 kV, and t_{migr} = 5 min. Peak A is NSA, peak C is BTM, and peaks N_1 and N_2 are benzyl alcohol.

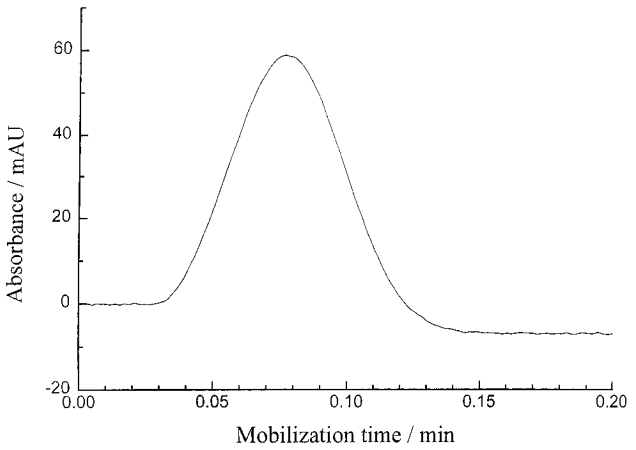

Figure 7. Detector trace for a pressure mobilization experiment in which the front of the analyte band was in the detector window at the application of pressure (t = 0.00 min on the time axis). Run conditions: MES BGE, pH 6.5, untreated fused-silica capillary, 25 μm i.d., L_T = 65.69 cm, L_d = 58.99 cm, and T = 25 °C. Analyte is benzyl alcohol.

the eCAP Neutral capillary was rinsed between each run for 1 min with Milli-Q water and 2 min with the fresh run buffer. Rinse times for the untreated capillaries were 5 and 10 min with Milli-Q water and the fresh run buffer, respectively.

The programmed potential, V_{prog}, was set at 15 kV and electrophoretic separation times (t_{migr}) were set for 5 min, unless otherwise stated, with a linear voltage ramp-up and ramp-down time of 0.17 min.[4] The thermostated portion of the capillary was maintained at 25 °C, unless otherwise noted The pressure mobilization velocities (v_m) were varied from 0.25 to 0.92 cm/s. Injection times (t_{inj}) were 1 s, and transfer times (t_{tr}) were set for 30 s unless otherwise stated.

(The actual methods and sample tables in the GOLD software required for the μ_{eo} and μ_{eff} determinations are posted on the Worldwide Web site www.beckman.com in the P/ACE product section.

RESULTS AND DISCUSSION

Accurate Determination of the Mobilization Velocity. The proposed method relies on the accurate determination of both the differential spacing of the solute bands and the mobilization velocity, v_m. The differential band spacings are influenced only by random errors, which cause precision problems but not accuracy problems. The calculated mobilization velocities, on the other hand, are influenced by both systematic errors and random errors, leading to both accuracy and precision problems. If there is a systematic error associated with the determination of time t_{N_3}, its effects will be most severe for the shortest mobilization times (highest mobilization velocities).

There are two likely contributors to a systematic error in the v_m determinations: (1) a pressure ramp at the beginning of the push which results in a lower velocity at the start of the mobilization step and (2) a delay between the start of the mobilization and the start of the data acquisition process. In order to test whether either of these events occurs, a simple experiment was designed. A sample band was injected into the BGE-filled capillary. Next, the band was transferred into the capillary by applying pressure to the pure BGE vial until the front of the sample band appeared in the detector window and the absorbance signal reached 10% of the peak maximum level. Then, the pressure was released, the sample band came to a halt, and the UV absorbance signal became constant. Subsequently, the next line of the sample table in the GOLD software was executed where pressure was again applied onto the BGE vial and the detector signal shown in Figure 7 was recorded. Since the initial autozero command was disabled, there should have been an immediate, significant change in the absorbance signal as soon as the command line in the method was executed. However, the absorbance profile in Figure 7 indicates that the absorbance change began only at approximately 2 s into the run, signifying the existence of a time delay, t_d.

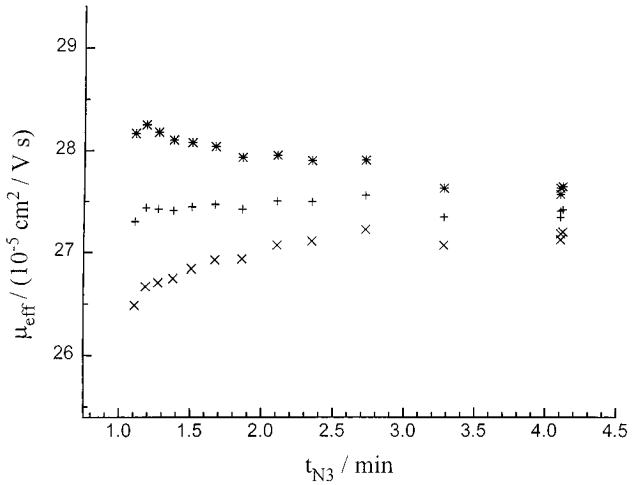

Figure 8. Effect of the delay time (t_d) on the calculated μ_{eff} of NSA as a function of t_{N3}. Symbol ⋆ represents a t_d = 4 s in eq 4. Symbol × represents a t_d = 0 s in eq 4. Symbol + represents a t_d = 2 s (determined from Figure 7) in eq 4. Run conditions: MES BGE, pH 6.5, eCAP Neutral coated capillary, 50 μm i.d., L_T = 66.35 cm, L_d = 59.75 cm, T = 25 °C, V_{prog} = 15 kV, and t_{migr} = 5 min for all runs.

The effects of such a time delay are shown in Figure 8, where μ_{eff} is plotted for NSA as a function of t_{N_3} (corresponding to different mobilization velocities). Symbol × shows the μ_{eff} values that are obtained when the delay time is not taken into consideration in the calculation of v_m (eq 4, t_d = 0). The plot indicates that, without a delay time correction, the effective mobilities are

Table 1. Consistency of Pressure Mobilization Velocity (v_m)[a]

run	$\Delta t_{A_1,N_1}$ (min)	$\Delta t_{A_2,N_2}$ (min)	$\Delta t_{A_3,N_3}$ (min)	$\Delta t_{N_1,N_2}$ (min)	$\Delta t_{N_2,N_3}$ (min)	av $\Delta t_{A,N}$ (min)	RSD $\Delta t_{A,N}$ (%)	av $\Delta t_{N,N}$ (min)	RSD $\Delta t_{N,N}$ (%)
1	0.3445	0.345	0.346	0.9705	0.971	0.3452	0.2	0.9708	0.04
2	0.350	0.3485	0.3505	0.9685	0.970	0.350	0.3	0.969	0.1
3	0.3495	0.350	0.350	0.9705	0.972	0.3498	0.08	0.971	0.1
4	0.349	0.3495	0.3495	0.9705	0.971	0.3493	0.08	0.9708	0.04
						12 data pair av $\Delta t_{A,N}$ (min)	12 data pair RSD $\Delta t_{A,N}$ (%)	8 data pair av $\Delta t_{N,N}$ (min)	8 data pair RSD $\Delta t_{N,N}$ (%)
						0.349	0.6	0.971	0.1

[a] Values calculated from sequential injections, four parallel runs. The capillary cartridge was thermostated at 25 °C. All run conditions as in Figure 9.

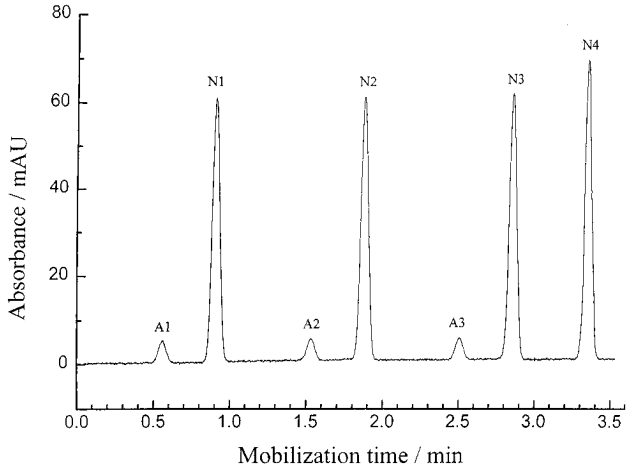

Figure 9. Detector trace for three successive injections of a benzyl alcohol/NSA sample. Run conditions: untreated fused-silica capillary, 25 μm i.d., L_T = 65.69 cm, L_d = 58.99 cm, T = 25 °C, V_{prog} = −30 kV, t_{migr} = 1 min, and t_{tr} = 58 s. A_1, A_2, and A_3 are NSA bands from injections 1, 2, and 3, respectively. N_1, N_2, and N_3 are benzyl alcohol bands from injections 1, 2, and 3, respectively.

underestimated at high mobilization velocities (low t_{N_3} values). Symbol * shows the μ_{eff} values that are obtained when t_d = 4 s is used in eq 4. This plot indicates that, with such a high delay time correction, the effective mobilities are overestimated when the mobilization velocity is high (t_{N_3} is low). Finally, when t_d = 2 s (estimated from the first baseline section in Figure 7) is used in eq 4, the plot shown by symbol + is obtained. Clearly, the μ_{eff} values are constant, regardless of the mobilization times (mobilization velocities), indicating that the correct delay time has been used.

Consistency of the Mobilization Velocity. The calculated μ_{eff} values will be correct only if the mobilization velocity, v_m, remains constant during the mobilization step, from start to finish. In order to test the invariance of v_m throughout the capillary, three sample bands (benzyl alcohol and NSA dissolved in BGE), separated from each other by the same transfer time, were injected successively (Figure 9). Since all three sample bands were inside the thermostated section, and the applied voltage was well within the linear region of the Ohm plot, the migration time differences between the benzyl alcohol and the NSA peaks should remain constant for the successive injections, unless v_m changed. Four replicates of the same measurements were made, and the results are listed in Table 1. Columns 2−4 represent the peak time differences for the three successive injections in any given run, the four rows represent the four replicate runs. It can be seen that there is no gradual change in the time differences listed in any given line (no bias in mobilization velocity as a function of distance in the capillary), and the relative standard deviations are less than 0.3%. There is no gradual change in the time differences from line to line either (run-to-run reproducibility); the overall relative standard deviation for the 12 data points is 0.6%.

The next two columns in Table 1 indicate the time differences between the successive neutral analyte peaks. Again there is no significant gradual change either from injection to injection (no difference in neutral band spacing as a function of successive pressure transfers) or from run to run, indicating that μ_{eo} can be obtained reproducibly with the proposed method.

Since temperature is a significant optimization variable in CE, it was important to test how much the mobilization velocity varied when the temperature in the unthermostated and the thermostated sections of the capillary were different. The same experiments were repeated as above, except that the cartridge temperature was programmed to 40 °C. According to Table 2, there is no significant gradual change either from injection to injection (no bias in mobilization velocity as a function of push distance into the capillary) or from run to run, indicating that the BGE viscosity must remain essentially constant throughout the pressure mobilization step; i.e., the temperature of the BGE must reach the programmed value very rapidly.

Determination of Very Small μ_{eo} Values. The proposed method can be used for the determination of very low μ_{eff} and μ_{eo} values. Seven consecutive runs were executed, alternating between the proposed mobility determination method (duration, 5 min) and the conventional mobility determination method (duration, 104 min). In the conventional method, the neutral marker was injected at the detector side (short side) of the capillary and V_{prog} was applied until the neutral marker band migrated past the detector. The average μ_{eo} values obtained by the two methods agree well: $(0.47 \pm 0.03) \times 10^{-5}$ cm^2/(V s) for the conventional method, $(0.49 \pm 0.03) \times 10^{-5}$ cm^2/(V s) for the proposed method. Considerable time is saved if the proposed method is used (5 min separation vs 104 min separation).

Elimination of the Effects of Initial Transient Isotachophoretic Phenomena (Initial Ionic Composition of the Sample) on Analyte Mobilities. When the ionic strength of the initial sample zone is greatly different from the ionic strength of the BGE, the measured analyte mobilities will be influenced by transient isotachophoretic phenomena. This effect is most severe when the programmed migration time is short. Analyte mobilities free of transient isotachophoretic effects might be obtained by

Table 2. Consistency of Pressure Mobilization Velocity (v_m)[a]

run	$\Delta t_{A_1,N_1}$ (min)	$\Delta t_{A_2,N_2}$ (min)	$\Delta t_{A_3,N_3}$ (min)	$\Delta t_{N_1,N_2}$ (min)	$\Delta t_{N_2,N_3}$ (min)	av $\Delta t_{A,N}$ (min)	RSD $\Delta t_{A,N}$ (%)	av $\Delta t_{N,N}$ (min)	RSD $\Delta t_{N,N}$ (%)
1	0.349	0.3505	0.3515	0.7835	0.784	0.350	0.4	0.7838	0.04
2	0.351	0.350	0.3505	0.778	0.7785	0.3505	0.1	0.7783	0.04
						6 data pair av $\Delta t_{A,N}$ (min)	6 data pair RSD $\Delta t_{A,N}$ (%)	4 data pair av $\Delta t_{N,N}$ (min)	4 data pair RSD $\Delta t_{N,N}$ (%)
						0.3504	0.2	0.781	0.4

[a] Values calculated from sequential injections, two parallel runs. The capillary cartridge was thermostated at 40 °C. Run conditions as in Figure 9 except $T = 40$ °C and $t_{tr} = 45$ s.

the double detector method,[5] but, again, the scheme cannot be used with most commercial instruments. The method proposed here can eliminate the untoward effects of the initial transient isotachophoretic phenomena. The sample mixture contains the ions of interest and a neutral marker in a matrix that has an ionic strength significantly different from that of the BGE. The sample is injected into a BGE filled capillary and transferred to the thermostated region as before. Then a separation is performed at the programmed potential $V_{prog,}$ for a time t_{migr}. After the potential returns to zero, a second sample injection is performed for the same injection time, t_{inj}, and this band is also transferred into the thermostated region of the capillary. The second separation is then performed at the programmed potential V_{prog} for a time t_{migr}. The time t_{migr} and V_{prog} are selected such that the ions from the first injected band begin their migration during the second electrophoretic separation in a section of the capillary that is filled with the pure BGE. By subtracting the effective distance migrated by the analyte present in the second sample from the effective distance migrated by the analyte present in the first sample, the migration distance of the analyte, L_{migr}, free of initial transient isotachophoretic effects, can be calculated as

$$L^{A}_{migr} = [(t_{N_1} - t_{A_1}) - (t_{N_2} - t_{A_2})]v_m \qquad (14)$$

Then, μ_{eff} for the anionic analyte can be calculated as

$$\mu^{A}_{eff} = \frac{L^{A}_{migr} L_T}{V_{prog}\,(t_{migr} - t_{ramp-up}/2 - t_{ramp-down}/2)} \qquad (15)$$

The detector trace for such a procedure is shown in Figure 10. The sample was dissolved in a solution that contained, in addition to the 28 mM MES BGE (pH 6.5), 27 mM LiCl. When the mobility for NSA is calculated from the peaks obtained from the second injection band (N_2 and A_2 in Figure 10) according to eq 13 as usual, the value obtained is $(26.80 \pm 0.06) \times 10^{-5}$ cm^2/(V s). When eq 15 is used for the mobility determination, μ_{eff} for NSA is $(27.2 \pm 0.2) \times 10^{-5}$ cm^2/(V s). For runs where the sample contained only NSA dissolved in the MES BGE (with no additional LiCl salt present), μ_{eff} for NSA was $(27.18 \pm 0.02) \times 10^{-5}$ cm^2/(V s). As expected, the mobility value for NSA calculated from the second injection band is lower than the value obtained from eq 15 due to the initial transitional isotachophoretic effects caused by the additional LiCl content of the sample. Thus, by selecting the appropriate operating conditions, the proposed method can be used to obtain effective mobility values that are not tainted by initial transient isotachophoretic effects.

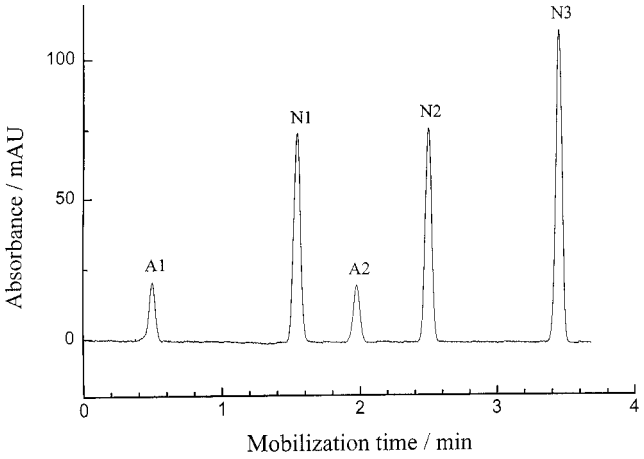

Figure 10. Detector trace for a double separation. A_1 and A_2 are NSA from injections 1 and 2, respectively. N_1 and N_2 are benzyl alcohol (neutral marker) from injections 1 and 2, respectively. N_3 is benzyl alcohol for the determination of v_m. Run conditions: 25 μm i.d. untreated fused-silica capillary, $L_T = 65.74$, 28 mM MES BGE, pH 6.5, $V_{prog} = -20$ kV, $t_{migr} = 2$ min, $T = 25$ °C, and 27 mM LiCl in sample vial.

Precision of Effective Mobilities Determined by the Proposed Method. In order to characterize the long-term precision of the proposed mobility determination method, four runs were performed on successive days under identical run conditions (eCAP Neutral coated capillary, 50 μm i.d., $L_T = 66.35$ cm, $L_d = 59.75$ cm, $T = 20$ °C, $V_{prog} = 15$ kV, and $t_{migr} = 5$ min), yielding an NSA mobility of $(24.5 \pm 0.1) \times 10^{-5}$ cm^2/(V s).

CONCLUSIONS

It has been shown that the proposed method yields precise and accurate effective mobilities for both anions and cations, from a single run, regardless of the magnitude of the μ_{eo}. The method can also be used to quickly determine μ_{eo} values as low as 0.5×10^{-5} cm^2/(V s). It eliminates most of the experimental errors inherent to the conventional CE mobility determination methods, such as migration through the unthermostated region of the capillary and variations of the EO flow that do not actually influence the migration of the analyte bands. In addition, the proposed method is considerably faster than the conventional CE separations. The proposed method has one known drawback: due to the use of pressure-induced flow during the transfer and final mobilization of the bands, additional (hydrodynamic) band broadening occurs which might result in poorer peak resolution for closely spaced peak pairs.

ACKNOWLEDGMENT

Partial financial support of this project by the Texas Coordination Board of Higher Education ARP program (Project 010366-016) and Beckman Instruments (Fullerton, CA) is greatfully acknowledged. We are also indebted to Beckman Instruments for the loan of the P/ACE 2100 instrument as well as Dr. Bart Wanders from Beckman Instruments for his advice concerning pressure control and calibration on the P/ACE instrument.

Received for review September 27, 1995. Accepted January 15, 1996.[⊗]

AC950968R

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1996.