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Method for the Accelerated Measurement of Electroosmosis in Chemically Modified Tubes for Capillary Electrophoresis

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A traditional neutral marker approach is combined with fast pressure-driven mobilization to achieve a method that provides accelerated electroosmosis measurements in capillary electrophoresis systems. A marker injection is made prior to and following timed high-voltage application. The marker-sandwiched electrolyte plug is then pressure-driven out of the capillary tube under closely controlled conditions, while the signal is monitored in the usual way. Substantial reduction in measurement time is achieved by allowing the marker to travel only a fraction of the effective length (the injection to detection distance) over a shorter period of time, compared to the traditional method where voltage is applied until the neutral marker shows up at the detector window. One simple equation was derived to express the electroosmotic mobility of the electrolyte (μ_{eo}) in terms of common operational variables. The method is particularly useful for low-electroosmosis measurements in wall-modified capillary tubes. The scope and limitations of the method are discussed.

Chemical or physical modification of capillaries for electrophoretic separations is a common approach to minimize unwanted solute interactions with the tube walls. Such modifications are normally accompanied by a drastic reduction of electroosmosis; the bulk electrolyte flow driven by the electrokinetic force originated at the wall/solution interface. Since electroosmosis often has a strong impact on capillary electrophoresis (CE) separations, its measurement is important for a thorough characterization of an electrophoretic system. A neutral compound that, upon application of an electric field, is dragged with the electrolyte has been traditionally used to measure electroosmosis.[1] Stringent requirements for true neutrality, detectability, and inertness toward the capillary wall are among the major limitations associated with this method. A few approaches have been proposed as alternatives to the neutral marker method to estimate electroosmosis in capillary tubes. Altria and Simpson measured electroosmosis by weighing the amount of the electrolyte that leaves the capillary upon the application of voltage for a known period of time.[2] While the procedure per se does not interfere with the electrophoretic process, the gravimetric method is an intrinsically off-line one which requires the use of a device (a balance) that is completely alien to most CE systems. Additionally, special precautions must be taken to minimize solvent evaporation. In another approach, Huang et al. measured electroosmosis by monitoring the current−time profile generated upon electroosmotically pumping a slightly diluted electrolyte solution (with respect to that in the reservoirs) to fill the whole capillary length.[3] The current-monitoring procedure is a truly on-line method that uses devices (an ammeter or, alternatively, a conventional detector) that, in contrast to a balance, are usually part of the CE system. On the other hand, because an ionic strength gradient must be created in order to detect a gradual current change, and since the electroosmosis itself is a function of the electrolyte's ionic strength, the method does interfere with the property it attempts to measure. The extent of such an interference should be a function of the concentration gradient used. Unfortunately, the proponents of the current-monitoring method did not evaluate thoroughly the extent of this potential bias in their electroosmosis measurement. An additional disadvantage of the method is the necessity of some sort of regression procedure to obtain the time at which the diluted electrolyte has been completely displaced in the capillary. Recently, Lee et al. attached a sheath flow cuvette to the grounded end of a capillary to continuously measure electroosmosis by monitoring the fluorescence from a dye solution.[4] While this approach provides real-time, on-line measurements, its suitability under low electroosmosis conditions is yet to be demonstrated.

In spite of the well-known difficulties of the traditional neutral marker method, this approach seems to surpass in popularity all other methods simply because in routine work the marker is just one more sample to inject. Nevertheless, this method also suffers from prohibitively long measurement times when low-electroosmosis systems such as modified capillaries are under test. In fact, the CE literature often describes the virtual elimination of electroosmosis after capillary modification by pointing out that a neutral marker does not appear at the detector after a certain number of hours. The method presented here eliminates such a limitation from the traditional marker method by providing procedure acceleration while maintaining its simplicity and on-line fashion.

(1) Jorgenson, J. W.; Lukacs, K. D. *Anal. Chem.* **1981**, *53*, 1298−1302.
(2) Altria, K. D.; Simpson, C. F. *Anal. Proc.* **1986**, *23*, 453−454.
(3) Huang, X.; Gordon, M. J.; Zare, R. N. *Anal. Chem.* **1988**, *60*, 1837−1838.
(4) Lee, T. T.; Dadoo, R.; Zare, R. N. *Anal. Chem.* **1994**, *66*, 2694−2700.

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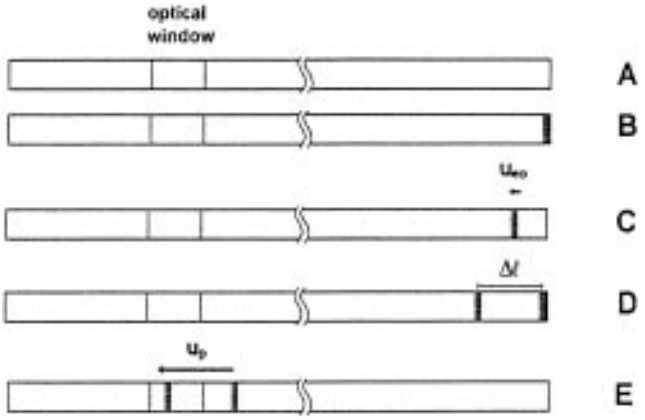

Figure 1. Protocol utilized to measure electroosmosis by the present method: (A) The capillary tube is initially rinsed with electrolyte. (B) A marker injection is made. (C) Electric field is applied for a fixed time (t_v) to electroosmotically move the marker band. At the end of this period the marker band along with a dragged plug of electrolyte have traveled a distance Δl. (D) A second marker is injected to demark the end of the electrolyte plug. (E) Upon application of a pressure differential between inlet and outlet ends (positive pressure or vacuum) the marker-sandwiched electrolyte plug reaches the detection window and the traveling times for the two marker bands are recorded in the usual manner.

EXPERIMENTAL SECTION

Apparatus. An Applied Biosystems (Division of Perkin-Elmer Corp., Foster City, CA) Model 270HT instrument was used for capillary electrophoresis. Capillary tubes were purchased from Polymicro Technologies (Phoenix, AZ).

Procedure. The protocol for the new approach is depicted in Figure 1. Briefly, a marker injection is made prior to and after applying high voltage (V) for a given time (t_v). The marker-sandwiched electrolyte plug is then pressure-driven out of the capillary tube, while the signal is monitored in the usual way. Electrophoresis was carried out in bare capillaries after preconditioning with 1 M NaOH solution for 20 min followed by rinsings with water (5 min) and electrolyte (5 min). This treatment was routinely applied at the beginning of every working day. Other conditions are specified as required. 5 mM dimethyl sulfoxide (DMSO) or 10 mM mesityl oxide solutions were used as the neutral marker. A typical electropherogram obtained under these conditions is shown in Figure 2.

RESULTS AND DISCUSSION

Derivation. During the purely electroosmotic displacement (step C in Figure 1), the electroosmotic velocity of the electrolyte, u_{eo}, can be approximated to

$$u_{eo} \cong \Delta l/\Delta t \tag{1}$$

where Δl is the distance traveled by the electrolyte and Δt is the travel time, i.e., the period during which the voltage is applied (t_v). Assuming that the flow velocity, u_p, is maintained constant during the final pressure-driven mobilization (step E in Figure 1), the second marker travels over the entire effective length, l, while the first one does so over a shorter distance $l - \Delta l$. Therefore,

$$u_p = \frac{l}{t_2} = \frac{l - \Delta l}{t_1} \tag{2}$$

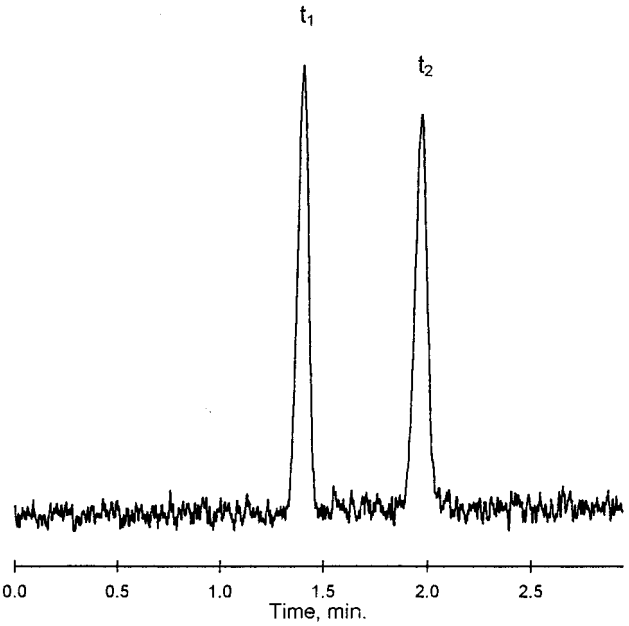

Figure 2. Typical electropherogram obtained upon application of the accelerated neutral marker approach. Conditions: capillary, 50 μm × 50.0 cm (30.0 cm effective length); temperature, 30 °C; neutral marker, mesityl oxide detected at 254 nm; injection, 0.8 s vacuum at 5 in.Hg (17 kPa); electric field strength, 30 kV/m (7 μA). Electrolyte, 20 mM citric acid/sodium citrate buffer, pH 3.0.

where t_1 and t_2 are the pressure-driven mobilization times for the first and second marker bands, respectively. Solving for Δl one obtains

$$\Delta l = l\left(1 - \frac{t_1}{t_2}\right) \tag{3}$$

Thus, u_{eo} can be expressed as

$$u_{eo} = \frac{l}{t_v}\left(1 - \frac{t_1}{t_2}\right) \tag{4}$$

Finally, from the definition of electroosmotic mobility, μ_{eo} ($= u_{eo}$-(L/V), L being the total tube length) one obtains

$$\mu_{eo} = \frac{Ll}{Vt_v}\left(1 - \frac{t_1}{t_2}\right) \tag{5}$$

Accelerated vs Conventional Neutral Marker Method. The central idea of the new method is to speed up the electroosmotic velocity determination by measuring only a short fraction Δl of the effective length l over a similarly short time interval t_V. The two marker injections are simply used to determine the ends of the electrolyte plug dragged by the electric field. In contrast, the conventional method involves a single marker injection followed by voltage application *until* the neutral compound reaches the optical window, that is,

$$u_{eo} \cong l/t_m \tag{6}$$

where t_m is the marker's migration time. The advantage of applying the new approach should be obvious.

Consider for instance a typical polyacrylamide-modified tube exhibiting a μ_{eo} value in the vicinity of 4×10^{-10} m^2 V^{-1} s^{-1} at 60 kV/m.[5] For the new method this represents a readily determined travel distance Δl of about 2 cm in less than 15 min. In contrast, with the conventional method the marker would take 5 h to travel a typical l distance of 40 cm under the same conditions. Clearly, this is a prohibitively long run for most practical purposes. Additionally, prolonged exposure of the electrolyte to high electric fields may cause extensive buffer depletion which, in turn, can lead to unwanted electroosmosis variations. Excessive band broadening due to axial diffusion is another unattractive feature associated with prolonged runs.

In the past, a different approach to shorten the neutral marker traveling distance involved the addition of this compound to the electrolyte at the outlet reservoir and use reversed electroosmotic flow by reversing polarity.[5] The time (t_m) required by the marker front to appear at the detector window is readily determined from the electropherogram. In this particular case, eq 6 becomes $u_{eo} \cong (L - l)/t_m$. While this approach can provide fast electroosmosis measurements, the short traveling distance of the neutral marker is fixed by the tube dimensions L and l, resulting in a limited method versatility. In contrast, in the new approach the marker traveling distance can be varied as needed by changing t_v and/or V. Furthermore, there is no need to add the marker to the electrolyte at the outlet reservior.

Several important observations can be made with respect to the equations above. First, it should be pointed out that the validity of eq 5 lies in the ability of the CE system to maintain a *constant* pressure differential across the capillary ends, that is, on how accurately the system satisfies eq 2. Most commercial CE instruments possess such a capability as part of their hydrodynamic injection systems. The instrument used in this work has a microprocessor-controlled vacuum system which provides for pressure variations of 1% or less. In practice, this can be readily evaluated from replicate t_2 measurements. It seems evident that this is the main source of variability of the method as a whole.

Second, notice that the term in parenthesis in eqs 3−5 is dimensionless and hence independent of the actual hydrodynamic mobilization velocity. The magnitude of the latter, however, is important for reasonable electroosmosis measurement times.

Third, in principle, eq 4 suggests that the shorter the t_v, the more closely the measured u_{eo} approaches its true value (strictly, dl/dt). Accordingly and, since—in virtue of eq 3—Δl is normally shorter than l, the new method should approach the true electroosmotic velocity better than the conventional one. There must be, however, a practical limit to this, mainly imposed by the time required for the CE assembly to reach thermal equilibrium upon application and/or removal of the electric field. To examine this issue more closely, we plotted the measured electroosmotic mobility as a function of voltage duration, t_v. Clearly, as shown in Figure 3 for two electric field strengths, consistently higher electroosmotic mobility values are found for voltages applied for less than 10 min under the conditions utilized. Upon application/removal of the electric field, the current takes a finite time (about 2 s) to reach/abandon its steady-state value, as evidenced by a round-cornered current−time profile, as opposed to a square wave form. This is shown in Figure 4A. Likely, the current delay when the voltage is turned on is at least partially offset by a similar delay when turned off, and as a consequence, little deviation from

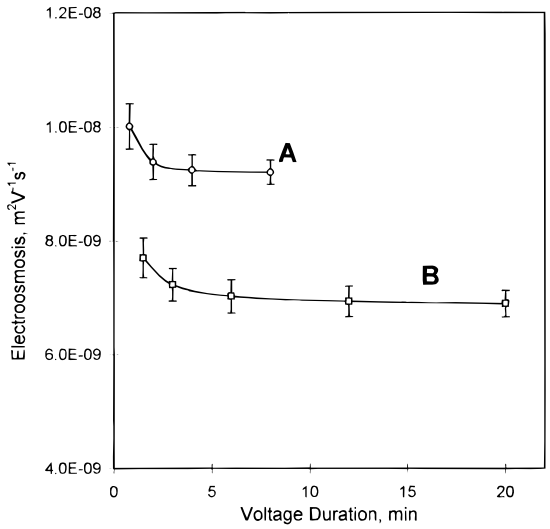

Figure 3. Dependence of measured electroosmotic mobility on voltage duration at two different electric field strengths. Conditions: capillary, 52 μm × 50.0 cm (30.0 cm effective length); temperature, 30 °C; DMSO neutral marker detected at 210 nm; injection, 0.8 s vacuum at 5 in.Hg (17 kPa); electric field strength, (A) 30 kV/m (7 μA), (B) 50 kV/m (11 μA). Electrolyte, 20 mM citric acid/sodium citrate buffer, pH 3.0.

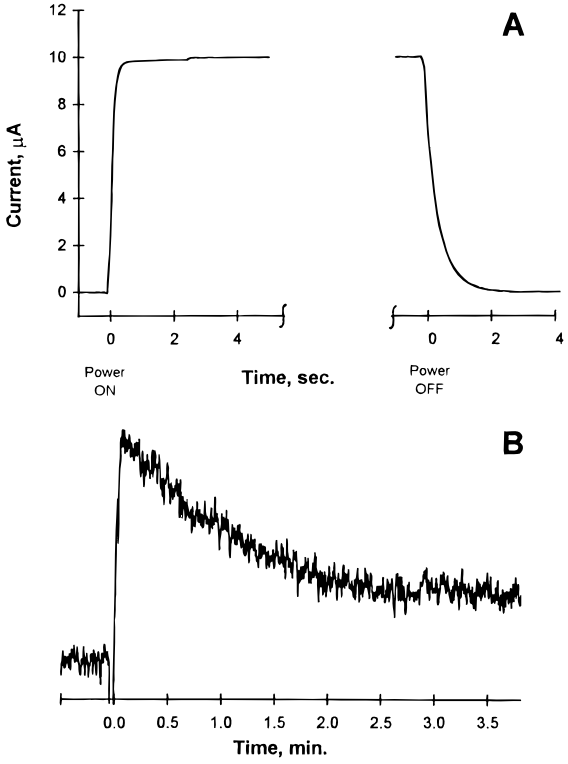

Figure 4. Current (A) and detector signal (B) transients occurring upon application and removal of a constant electric field (50 kV/m, 10-μA steady-state current). Neutral marker, mesityl oxide detected at 254 nm. Other conditions as in Figure 2. Notice the different time scale used in A and B. Effect of voltage removal is not shown in curve B.

(5) Rodriguez, R.; Wehr, T.; Zhu, M.-D.; Siebert, C. J. Poster presented at the 13th International Symposium of Proteins, Peptides and Polynucleotides (ISPPP), San Francisco, CA, Dec 1993.

(6) Chiari, M.; Nesi, M.; Sandoval, J. E.; Pesek, J. J. *J. Chromatogr., A* **1995**, *717*, 1−13.

the nominal t_v value in eq 5 can be expected. The temperature rise immediately following the application of the electric field results in a concomitant increase in μ_{eo}, as well as other temperature-dependent properties such as electrolyte viscosity, conductivity, optical density, refractive index, etc. In contrast to a fast current steady state and assuming that temperature is the single most important factor affecting μ_{eo}, one can expect the latter to reach steady state about 20−30 s after power-on. Evidence for temperature transients of this duration has been recently reported in Raman microthermometry studies at comparable power densities.[7] Additionally, real-time measurements of initial fluorescence decay in a sheath flow cuvette (Figures 6 and 7 of ref 4) are consistent with a similar delay to reach steady state. Every CE practitioner is familiar with the increase and subsequent decay of the UV detector signal at power-on. A typical example is depicted in Figure 4B. While the magnitude of the detector's signal transient is wavelength-dependent, its duration appears to be consistent with a slow attainment of thermal steady state. As an inference from the observations above, the voltage duration, t_v, must be chosen to be long enough as to render the effect of the initial temperature transient negligible. The extent of the bias associated with this effect depends primarily on the effectiveness of the cooling system to rapidly dissipate Joule heat. It must be pointed out that this initial transient is inevitable regardless of the method of electroosmosis measurement. In other words, μ_{eo} values derived from the conventional neutral marker method as well as the gravimetric method are also affected by a similar bias.

Comparison of the μ_{eo} values obtained by the conventional and accelerated neutral marker methods reveals a slightly but consistently higher result (0.5−1.0%) for the latter. A typical set of data is shown in Table 1. Although within the overall precision of the new method (roughly 1%), such an apparent discrepancy deserves further comment. The experimental difference can be accounted for if one considers that eqs 2 and 6 were derived under the tacit assumption that the length of the neutral marker injection plug, l_{inj}, is negligible in comparison to the effective length l. Application of the Hagen−Poiseuille equation to the conditions shown in Table 1 furnishes an estimated l_{inj} of about 3 mm, that is, about 0.75% of l. A detailed re-examination of the geometries of capillary tube and sample plug leads to a more accurate expression for eq 2:

$$u_p = \frac{l - \frac{1}{2}l_{inj}}{t_2} = \frac{l - \frac{3}{2}l_{inj} - \Delta l}{t_1} \qquad (2')$$

which in turn leads to a new eq 3:

$$\Delta l = \left(l - \frac{1}{2}l_{inj}\right)\left(1 - \frac{t_1}{t_2}\right) - l_{inj} \qquad (3')$$

Similarly, a more accurate electroosmotic velocity equation for the conventional method will be

(7) Liu, K.-L. K.; Davis, K. L.; Morris, M. D. *Anal. Chem.* **1994**, *66*, 3744−3750.

Table 1. Comparison of Electroosmotic Mobility Measurements ($m^2\ V^{-1}\ s^{-1}$, $\times 10^{-9}$) by the Conventional and Accelerated Neutral Marker Methods[a]

	uncorrected			corrected		
	conv	accel	% rel dev[b]	conv	accel	% rel dev[b]
	7.05	7.06	0.23	7.02	6.98	−0.61
	6.96	7.00	0.55	6.93	6.91	−0.31
	6.86	6.91	0.72	6.84	6.83	−0.15
	6.92	7.00	1.24	6.89	6.92	0.38
	6.89	6.97	1.08	6.87	6.88	0.22
	6.96	7.04	1.07	6.94	6.95	0.21
	6.91	6.97	0.87	6.88	6.89	0.01
	6.92	6.99	0.98	6.89	6.90	0.12
	6.84	6.90	0.83	6.82	6.82	−0.04
	6.88	6.95	1.08	6.85	6.87	0.21
	6.83	6.87	0.59	6.80	6.78	−0.28
	6.82	6.91	1.30	6.79	6.82	0.42
	6.81	6.86	0.83	6.78	6.78	−0.05
	6.86	6.95	1.39	6.83	6.87	0.52
	6.86	6.94	1.26	6.83	6.86	0.39
mean	6.89	6.96	0.94	6.86	6.87	0.07
% rsd	0.93	0.83		0.93	0.84	
t-value	2.88			0.21		
t-critical[c]	2.05			2.05		

[a] Fifteen alternating replicates for each method. Conditions: capillary, 50 μm × 60.0 cm (40.0 cm effective length); electrolyte, 20 mM citric acid/sodium citrate buffer, pH 3.0; mesityl oxide neutral marker detected at 254 nm; injection, 1 s at 5 in.Hg (17 kPa) vacuum; electric field strength, 30 kV/m (7 μA); temperature, 30 °C (viscosity of water at this temperature, 7.0×10^{-7} Pa s). A long voltage duration for the accelerated method (27 min) was used to minimize any difference due to thermal equilibrium effect (comparable to a mean t_m of 32.25 min for the conventional method). [b] With respect to the previous conventional measurement. [c] At the 5% significance level.

$$u_{eo} \cong \frac{l - \frac{1}{2}l_{inj}}{t_m} \qquad (6')$$

As shown in Table 1, when these corrections are applied to the corresponding expressions for μ_{eo}, a *t*-test indicates that no statistically significant difference exists between the two approaches.

Finally, it should be noted that application of the new method results in a more extensive band broadening in relation to that of the conventional approach with a comparable measurement time. This is due to the parabolic velocity profile associated with hydrodynamic pumping in contrast to the flat profile of electroosmosis alone. This extensive band broadening becomes evident when one compares the shape of the first and second peaks, particularly when $t_1/t_2 \ll 1$ and despite using identical marker injections (results not shown). Nevertheless, the extra band broadening associated with the hydrodynamic mobilization used in the new approach does not represent a major obstacle for a reliable electroosmotic flow determination, since peak resolutions as low as 0.6 are sufficient to obtain two distinctive maxima.

CONCLUSIONS

We have developed a method well suited for accelerated measurement of electroosmosis in surface-modified capillaries. In most commercial instruments, closely controlled vacuum or pressure sample injection systems are provided, and therefore, implementation of the method described here does not require any modification of the instrumental setup.

While the utilization of short power duration is suggested by strict velocity (dl/dt) considerations, to obtain accurate electroos-

mosis measurements a long enough power application should be used to minimize the positive bias associated with the unavoidable temperature transient immediately following power application. It should be emphasized that such effect is also operating in the gravimetric, current-monitoring, and conventional neutral marker methods.

An important application of the new method includes the evaluation of long-term hydrolytic stability of chemically modified tubes by monitoring changes in electroosmosis upon prolonged exposure to high electric fields and extreme electrolyte pH's.

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