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Technical Notes

Electroosmotic Flow Control of Fluids on a Capillary Electrophoresis Microdevice Using an Applied External Voltage

Nolan A. Polson and Mark A. Hayes*

Department of Chemistry and Biochemistry, Arizona State University, P.O. Box 871604, Tempe, Arizona 85287-1604

Independent control of electroosmosis is important for separation science techniques such as capillary zone electrophoresis and for the movement of fluids on microdevices. A capillary electrophoresis microdevice is demonstrated which provides more efficient control of electroosmosis with an applied external voltage field. The device is fabricated in a glass substrate where a 5.0 cm separation channel (30 μm wide) is paralleled with two embedded electrodes positioned 50 μm away in the substrate. With this structure, greatly reduced applied external potentials (≤120 V compared to tens of kilovolts) still effectively altered electroosmosis. The efficiency for the control of electroosmosis by the applied external field is improved by approximately 40 times over published values.

Recent advances in the field of microfabricated devices have made remarkable progress toward the miniaturization of separation-based systems.[1,2] On these systems electroosmosis (also termed electroosmotic flow) provides an efficient and simple means of fluid flow control, even in a network of interconnecting channels.[3−5] Electroosmosis generates a flat-flow profile regardless of the shape and dimensions of the channel, thus minimizing dispersion within the system. Since electroosmosis is directly proportional to the applied axial voltage field, the control of flow in each channel can be effected by varying the potential gradient.

While electroosmosis provides a near-ideal flow propulsion mechanism, in practice it has proven difficult to apply reliably. Electroosmosis is generated by a complex interplay between surface composition and buffer characteristics (and external voltage fields).[6−8] Since it is essentially based on surface chemistry, minute amounts of materials depositing on (or leaving) the surface can create dramatic changes in this flow. This has resulted in poor reproducibility for flow control on microdevices.[9]

Electroosmosis is also an important component of capillary zone electrophoresis. Electroosmosis directly influences the efficiency, resolution, and reproducibility of most electrokinetic separation techniques.[10] Capillary electrophoresis and related techniques have been demonstrated for a number of different applications on microdevice formats.[1−3,9,11−17]

Electroosmosis can be altered by a variety of methods. Examples of purposefully altering the electroosmotic flow include buffer additives,[18−22] altering buffer pH,[23−25] altering buffer concentration,[23,26,27] and coating the inner wall of the capillary.[18,19,28] These techniques permanently alter the surface structure or buffer

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composition and result in a static, new electroosmotic mobility which cannot be intentionally altered in response to changing conditions within the channel or capillary tube.

In contrast to these static methods, dynamic control of electroosmosis has been demonstrated by applying an additional perpendicular voltage field across the wall of standard fused silica capillaries used in conventional capillary electrophoresis systems.[29−33] The external voltage flow control technique requires no permanent changes in surface structure nor altered buffers.

External voltage flow control was first demonstrated using resistive solutions or materials covering the majority of the outer surface of the capillary.[29] This design required resistive materials so that the radial potential gradient matched the potential gradient of the buffer on the interior of the capillary. Later work demonstrated that the effect could be generated by conductive materials or ionized gas and that the matching of the interior potential gradient was unimportant in obtaining the effect.[33−35]

This study demonstrates the development of external voltage control of electroosmotic flow for microfabricated fluidic microdevices. The external electrodes were positioned 50 μm away from the inner surface of a 30 μm wide channel. With this structure, a small range of applied radial voltages (120 V) induced significant changes in flow velocity. The absolute magnitude of the applied voltage is 50−80 times less than that required in standard capillary electrophoresis systems.

EXPERIMENTAL SECTION

Reagents. Sodium dihydrogen phosphate (NaH_2PO_4) was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI) and was used as received. *N*-(2-aminoethyl)-4-amino-3,6-disulfo-1,8-naphthalimide, dipotassium salt (lucifer yellow), was obtained from Molecular Probes (Eugene, OR) and was used as received. All NaH_2PO_4 buffers were prepared to a 20 mM concentration and adjusted to pH 3.0 using phosphoric acid (EM Science, Gibbstown, NJ). Lucifer yellow was prepared (1 mg/mL) using NaH_2PO_4 buffer. All buffers and samples were degassed under vacuum for 5 min and were filtered with a Millex-LCR filter unit (0.5 μm pore size; Bedford, MA). Lucifer yellow solutions were filtered with a Nalgene filter (0.2 μm pore size; Fisher Scientific, Pittsburgh, PA). All buffers and samples were prepared with 18 MΩ purified water drawn from a NANOpure UV ultrapure water filtration system (Barnstead, Dubuque, IA).

Planar Microdevice. The capillary electrophoresis microdevice was designed in-house and manufactured by the Alberta Microelectronic Centre (Edmonton, Alberta, Canada). This device consisted of a long separation channel intersected by two off-set side channels (Figure 1a). The substrate was Corning 0211 glass (Precision Glass and Optics, Santa Ana, CA). The device measures 2.54 cm × 7.62 cm. The channel dimensions were 30 μm wide and 10 μm deep. The side channels were off-set by 500 μm (Figure 1b). The separation channel (injection zone to the buffer waste reservoir) was 5.0 cm long. External voltage electrodes were located parallel to the main channel separated by 50 μm of glass substrate (Figure 1c). They extended 6.0 mm total, centered at 9.0 mm from the separation waste reservoir. Other dimensions were as follows: the injection waste port (reservoir 1) to the center channel was 4.5 mm, the sample injection port (reservoir 3) to the center channel was 3.5 mm, and the buffer inlet port to the beginning of the injection loop was 6.0 mm. The total main channel length was 56.5 mm, and the total injection channel length was 8.5 mm. The voltage field strength was determined by calculating the potential of the buffer (assuming a linear potential gradient) immediately adjacent to the center of the external electrode. The effective voltage field was the difference between the calculated buffer potential and the applied electrode potential. Changes in electroosmotic velocity were monitored according to previous studies.[38]

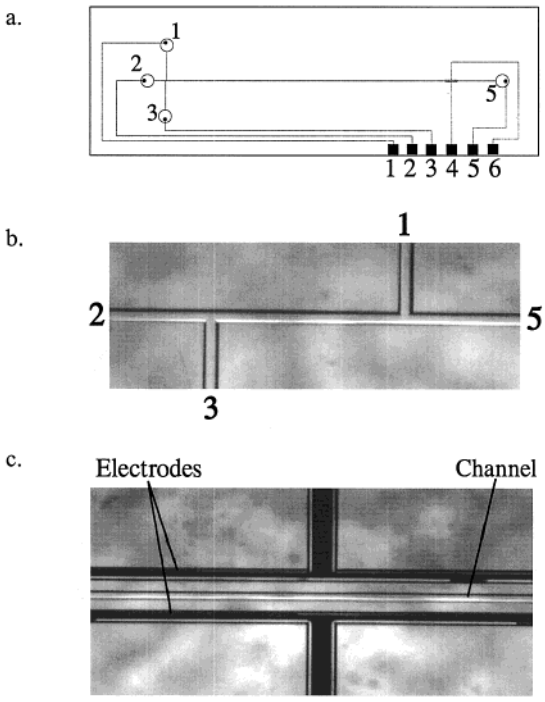

Figure 1. (a) Schematic of the capillary electrophoresis microdevice equipped with external voltage electrodes: 1, injector waste port; 2, separation port inlet; 3, sample inlet port/injector port; 4, external voltage electrode; 5, separation waste port; 6, external voltage electrode. Total size of the structure: 2.54 cm × 7.62 cm. (b) Micrograph of the injection (150 pL) zone within the separation channel. The sample is injected from reservoir 3 to reservoir 1 and separated from reservoir 2 to reservoir 5 using applied voltage fields. (c) Micrograph of external voltage electrodes on the capillary electrophoresis microdevice. Electrodes are placed 50 μm away from the surface of the channel (30 μm wide × 10 μm deep).

Apparatus. Two series 225 high-voltage power supplies were used to apply potential to the reservoir and external electrodes (Bertran, Hickesville, NY). An Olympus Vanox microscope (Tokyo, Japan) and an Olympus IX70 inverted research microscope (Tokyo, Japan) were used for imaging. An Omnichrome model 100 HeCd laser was used as the fluorescence excitation

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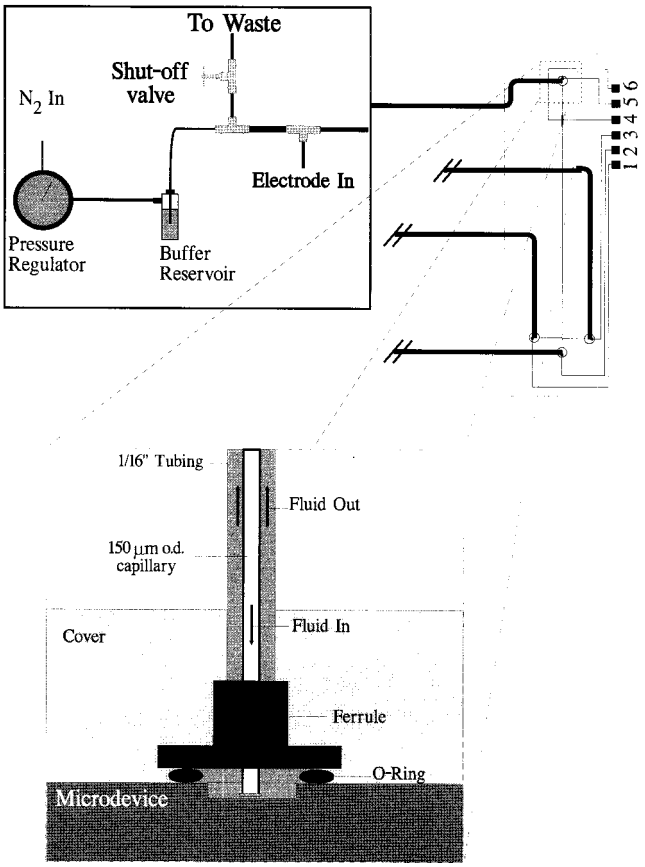

Figure 2. Schematic of the coaxial flushing system for the capillary electrophoresis microdevice.

source (442 nm). Image acquisition was performed with an RS170 CCD camera (CSI Electronics, East Hartford, CT) integrated with National Instruments LabVIEW IMAQ image acquisition software and hardware (National Instruments, Austin, TX), where imaging programs were developed in-house. Data analysis was performed using MathCAD 7.0 (MathSoft, Inc., Cambridge, MA) and Excel (Microsoft Corp., Seattle, WA) programs that also were developed in-house.

A schematic of the experimental system used is shown in Figure 2. Pressure regulators (Granger, Phoenix, AZ) were used to control flow during pressure and rinsing procedures. Buffer and samples were delivered to the channel entrance by a coaxial flushing system. Specifically, a 150 μm outer diameter (o.d.)/50 μm internal diameter (i.d.) capillary tubing (Polymicro Technologies, Phoenix, AZ) was inserted into a 1.59 mm o.d./762 μm i.d. Teflon tube (Upchurch Scientific, Oak Harbor, WA). Electrodes were placed such that fluidic contact was maintained via the solutions within the Teflon tubing to ensure electrical continuity. Each tube of the coaxial system was equipped with a shut-off valve, which allowed the selective control of fluidic movement throughout the system. These shut-off valves remained open and the pressure regulators were turned off throughout the external voltage flow control experiments.

Image Acquisition. Image acquisition was performed by integrating the CCD camera to the microscope and imaging the separation channel immediately after the injection tee. Sample was injected by applying a voltage field (−823.6 V/cm) from reservoir 3 to reservoir 1 (ground) while reservoirs 2 and 5 were floated. Image acquisition was initiated immediately after the switch from injection to separation voltages. Migration was performed by applying a voltage field (−123.9 V/cm) from reservoir 2 to reservoir 5 (ground) while reservoirs 1 and 3 were floated. Images were taken every 0.5 s and were analyzed using the central area of the channel 24.7 μm wide for a length of 1.58 mm. The middle 10 pixels along the length were averaged to form an array. The electropherograms were normalized to peak height. Multiple trials were performed for statistical purposes. Data acquisition was performed 0.5 mm down the separation channel for initial testing of the device. Later experiments acquired images 5.0 mm down the separation channel to simulate a capillary electrophoresis experiment. The fluorescence intensity of lucifer yellow was monitored at these distances over time. Due to slight variations over several weeks, some data sets were normalized to 0 V applied external potential.

RESULTS AND DISCUSSION

Although dynamic control of electroosmosis by an applied external voltage field has been demonstrated and investigated by several groups, this technique has yet to gain widespread acceptance or commercial application. This is despite a recognition that this type of independent control would provide many distinct improvements over present practice.[9,39] The lack of interest stems from the limited range of compatible buffers and pH ranges, and the complications associated with applying several kilovolts of potential to the outer surface of capillaries. The first of these issues has been addressed, where the range of compatible buffers has been extended recently.[38] The present work demonstrates the ability to fabricate a microfluidic channel/electrode system whereby control of electroosmotic flow may be achieved under low-voltage conditions, resulting in high control efficiencies.

Control of electroosmosis can be performed on a microfabricated device where a simple offset-cross-injection design was used (Figure 1a,b).[14,15] The width and depth of the channel dimensions were limited by commercial fabrication restrictions, but the relatively large bore provided a reduced risk of clogging. The electrodes were designed and fabricated as embedded integral parts to the microdevice. Due to poor adhesion of the electrodes in buffer-exposed areas, additional electrodes were placed within the waste lines of each reservoir to maintain electrical continuity of the system. However, the embedded external voltage electrodes remained stable and intact.

The microfluidic fabrication resulted in channels with well-defined structure and smooth, clean walls and intersections. The offset-cross-injection zone (total length 500 μm) constituted a 150 pL injection volume (Figure 1b). To ensure continuity, the current through the channel was monitored throughout the experiments at the ground electrode leads. If widely varying or no current was detected, the continuity was reestablished before further experimentation.

Moving materials to or from the microdevice required a coaxial flow system. Individual pressure regulators and coaxial input/output lines were constructed which were able to manipulate fluids efficiently (Figure 2). This design was used to quickly flush out and change solutions in the reservoirs on the chip. With this

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Table 1. External Voltage Effects on Normalized Observed Mobility[a]

effective external voltage (V)	normalized change in obsd mobility (exptl μ_{obsd}/initial μ_{obsd})	no. of trials	effective external voltage (V)	normalized change in obsd mobility (exptl μ_{obsd}/initial μ_{obsd})	no. of trials
−26	1.25 ± 0.12	10	64	1.00 ± 0.54	41
4	1.17 ± 0.30	13	84	0.99 ± 0.05	4
14	1.19 ± 0.14	9	104	0.98 ± 0.03	4
34	1.26 ± 0.46	21	124	0.94 ± 0.03	4
44	1.11 ± 0.10	5	144	0.93 ± 0.07	4

[a] Linear correlation: $y = -2.13 \times 10^{-3}x + 1.07$ ($R^2 = 0.8356$). Note: a 0 V applied voltage at the external electrodes results in a 64 V effective field (data were normalized to this value) (20 mM NaH_2PO_4, pH 3.0).

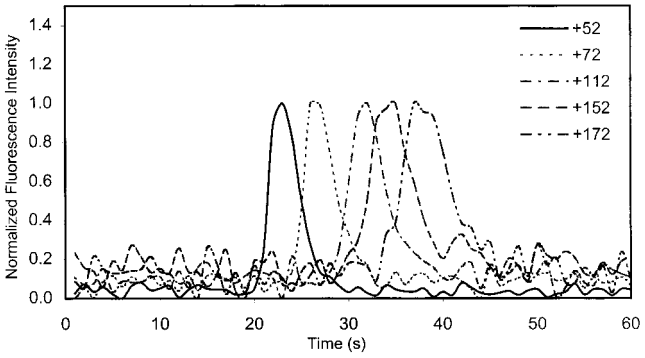

Figure 3. Electropherogram of lucifer yellow, 5 mm away from the injection zone within the separation channel at various effective external voltages. The separation voltage field strength is −123.9 V/cm (pH 3.0, 20 mM NaH_2PO_4). The magnitudes of external voltages are listed in the figure.

system, fresh solution could be pumped directly to the immediate entrance of the reservoir while used buffer and sample were flushed out in a matter of seconds. This system provided an efficient means of moving fluids from sample containers and buffer reservoirs to the microdevice.

Initial experiments were performed over several weeks. Since these data were collected over an extended period of time, some variations in the initial electroosmosis occurred. Therefore, the data were normalized to the initial electroosmosis rate to simplify the comparison between data sets. The results of all preliminary experiments are summarized in Table 1. This table represents collated normalized data sets for several separate experiments (n = 118). The data show a linear correlation between the applied radial voltage and the normalized relative velocity according to the equation $y = -2.13 \times 10^{-3}x + 1.07$ ($R^2 = 0.8356$).

Further studies were performed which did not require normalization by monitoring the fluorescence signal of the injected dye further down the separation channel, thus simulating a capillary electrophoresis experiment. The elution time for the fluorescent dye varied dramatically with a small range of external voltage fields (Figure 3). The peak shapes are not ideal, where increased dispersion is apparent in slower moving peaks due to poorly constrained ("nonpinched") injections. Peak elution times varied by as much as 16.1 ± 3.3 s over the 5.0 mm separation distance (Table 2). These data demonstrate a total change in observed electroosmotic mobility of 7.9×10^{-5} cm^2/V·s with a change in external voltage field of 120 V. Note that if shunting of the potential field at the externally fabricated electrodes were responsible for the change in apparent migration rate of the lucifer yellow, the change in electroosmotic flow would be opposite that

Table 2. Changes in Observed Mobility Caused by External Voltage Effects[a]

effective external voltage (V)	elutn time[b] (s)	obsd mobility (μ_{obsd}) ($\times 10^4$ cm^2/V·s)	no. of trials
52	21.7 ± 0.3	−1.86 ± 0.03	5
72	24.1 ± 3.7	−1.68 ± 0.25	6
112	32.2 ± 2.1	−1.25 ± 0.08	10
152	35.4 ± 2.6	−1.14 ± 0.08	5
172	37.8 ± 4.1	−1.07 ± 0.11	5

[a] Linear correlation: $y = 6.6 \times 10^{-7}x - 2.1 \times 10^{-4}$ ($R^2 = 0.9362$). [b] Data taken 5 mm away from the injection zone (20 mM NaH_2PO_4, pH 3.0).

which was observed. The maximum mobility both in theory and in practice is approximately 8×10^{-4} cm^2/V·s,[3,9−12,21,32,33,38,40] so by changing the external voltage field by only 120 V, approximately 10% of the maximum allowable change was effected. A linear correlation exists between the applied external voltage and the observed mobility according to the following equation: $y = 6.6 \times 10^{-7}x - 2.1 \times 10^{-4}$ ($R^2 = 0.9362$). The slope is significantly different from for that of the data presented in Table 1 because these data have not been normalized.

Studies examining various aspects of external voltage flow control have appeared.[31,33,35,40,41] Within this body of work several inner and outer diameter capillaries were used. This provides a resource to compare the effectiveness of flow control for the microfabricated structure described here versus standard capillary electrophoresis systems using fused silica substrates. This analysis was limited to studies using buffers consistent with those used in this study (pH 3, 1−50 mM phosphate).[31,33,35,40,41] To quantitate the effectiveness of an applied external voltage field for altering the flow velocity, the following analysis was undertaken (Table 3). First, the total positive range of the effective external voltage was cataloged, where the values ranged from +3 to +10 kV. Since the absolute values of the inner and outer radii influence the effectiveness of the applied field, the field strength was multiplied by a cylindrical capacitor factor of $1/(r_i \ln(r_o/r_i))$.[31,32,36,37] This resulted in values of 109−450 V/μm for the capacitor field gradient. The corresponding change in electroosmotic mobility ($\Delta\mu_{eof}$) was cataloged (or calculated from the figures and experimental details) and ranged from 8.0×10^{-5} to 3.1×10^{-4} cm^2/V·s. Next, an efficiency factor (Γ) was calculated, where $\Delta\mu_{eof}$ was divided by the applied capacitor field strength ($\Gamma = \Delta\mu_{eof}/[V_r/$

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Table 3. Literature Survey Indicating Relative Effectiveness of an Applied External Voltage for Altering Electroosmosis

ref (first author)[a]	ionic strength[b] (mM)	capillary i.d./o.d. (μm)	range of radial voltage (V)	effective radial voltage[c] (V/μm)	$\Delta\mu_{eof}$ ($\times 10^4$ cm^2/V·s)	Γ [$\times 10^6$(cm^2/V·s)/(V/μm)]
41 (Wu)	10	50/150	3000	109	1.4	1.3
35 (Wu)	10/20	50/150	6000	218	0.80	0.37
31 (Huang)	10	50/150	8000	291	1.5	0.51
31 (Huang)	1	50/150	8000	291	1.4	0.48
31 (Huang)	10	50/150	10000	364	1.9	0.52
31 (Huang)[d]	10	50/150	5000	182	2.3	1.3
31 (Huang)[e]	10	50/150	6000	218	0.95	0.43
33 (Hayes)	1	25/144	6000	208	3.2	1.5
33 (Hayes)	1	10/144	6000	450	2.2	0.49

[a] Data cataloged or calculated from experimental details or figures and then cataloged. [b] All buffers were at pH 3. [c] According to $V_r/(r_i \ln(r_o/r_i))$; see the text and ref 32 for explanation. [d] Capillary coated with an organic phase containing butyl functional groups. [e] Capillary coated with an organic phase containing amino functional groups.

($r_i \ln(r_o/r_i)$)]). The results varied from $\Gamma = 3.7 \times 10^{-7}$ to 1.5×10^{-6} (cm^2/V·s)/(V/μm). For the experimental data set presented here (Figure 3, Table 2), the range of applied potential was 120 V across a 50 μm wall (field strength of 2.4 V/μm) and the electroosmotic mobility range was 7.9×10^{-5} cm^2/V·s. This results in an efficiency factor (Γ) of 3.3×10^{-5}. When the efficiency of the microfabricated capillary electrophoresis device is compared to that of conventional fused silica systems (literature survey, Table 3), the ability of the microfabricated device to control flow versus the applied external voltage field is approximately 40 times better than the average value.

Dramatically improved efficiency is demonstrated for control of electroosmosis with small applied external potentials (120 V) on a capillary electrophoresis microdevice with embedded electrodes. Quantitative data sets (normalized and simulated capillary zone electrophoresis) indicate that the system is stable and consistent while providing efficient control. When the applied field is corrected for wall thickness and channel width according to a capacitive model, it is an average of 40 times more efficient in changing the electroosmotic flow than in previously published studies using standard fused silica capillaries. Further studies are planned to investigate the source and mechanism of this dramatically improved flow control.

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