

Conductivity detection for monitoring mixing reactions in microfluidic devices

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A conductivity detector was coupled to poly(dimethylsiloxane)–glass capillary electrophoresis microchips to monitor microfluidic flow. Electroosmotic flow was investigated with both conductivity detection (CD) and the current monitoring method. No significant variation was observed between these methods, but CD showed a lower relative standard deviation. Gradient mixing experiments were employed to investigate the relationship between the electrolyte conductivity and the electrolyte concentration. A good linear response of conductivity to concentration was obtained for solutions whose difference in concentrations were less than 27 mM. The new system holds great promise for precision mixing in microfluidic devices using electrically driven flows.

Introduction

Capillary electrophoresis (CE) on microchips is an emerging technology that has potential to revolutionize chemical, biological and clinical analysis.^{1–5} Miniaturized analytical systems, particularly ‘lab-on-a-chip’, decrease fabrication cost, time of analysis and reagent consumption.^{6–8} The success of microchip CE has led researchers to develop additional fluidic applications on a microchip (microfluidics), including chemical synthesis and sample preparation.^{9–11} Many microfluidic applications use electrically driven flows (termed electroosmotic flow, EOF) owing to its mechanical simplicity.^{12,13} EOF arises when the negatively charged inner wall of fused silica capillaries contacts an electrolyte solution.¹⁴ Hydrated cations in buffer are attracted to the negatively charged inner wall and become arranged into a compact layer, tightly bound to the wall, and a loosely bound diffuse layer. The electrical potential between the compact layer and diffuse layer, the zeta potential, depends on the nature of the capillary surface and the ionic strength of the electrolyte solution. When an external electric field is applied, the diffuse layer moves toward the cathode, resulting in a bulk solution flow (EOF). However, EOF will not behave as ideally as described, as ionic strength, analyte adsorption on the walls and Joule heating can all affect the zeta potential¹⁴ and hydrodynamic flow can, sometimes, have an influence on the bulk solution flow.

Several methods have been reported for determining EOF.^{15–19} The most widely used method relies on the injection of an electrically neutral compound followed by recording its migration time through the capillary.¹⁵ The neutral marker method requires that the molecule stay uncharged during the process and have no interaction with the electrolyte or the capillary wall. Another method measures the mass change in one reservoir over a timed interval when high voltage is applied across the capillary.^{16,17} A potential difficulty with this method is mass loss by evaporation. The fluorescent marker method is an alternative method to measure the EOF.¹⁸ This involves the introduction of a fluorescent agent downstream in the EOF direction and monitoring its movement at the end of the capillary. However, this method requires the introduction of a fluorescent reagent which could contaminate the sample or buffer. The method also needs a second set of fluorescent instrumentation to monitor the fluorescent agent. The current monitoring method was first reported by Huang *et al.*¹⁹ to

monitor the EOF. This method is based on the current change, which follows Ohm’s law, in the channel when an electrolyte of different ionic strength fills the channel. The time required for the separation current to reach a steady state value can be used to calculate the EOF.

Conductivity detection (CD) has been coupled with both conventional and microchip CE.^{20,21} CD is based on the change in bulk solution conductivity between two electrodes when an analyte band passes through the electrode gap. Any molecule can be detected if it causes a change in the conductivity between electrodes. The sensitivity of conductivity techniques is not strongly dependent on the detector (electrode) size. As a result, there is little loss of sensitivity when the electrode size scales down, which should couple well with microelectrode and microchip CE.

We report a simple method to monitor the EOF *via* conductivity detection. This method involved the fabrication of the microelectrodes which were in direct contact with solution. The whole system is easily miniaturized and relatively inexpensive. We compared CD with the current monitoring method for measuring EOF. The results showed that CD (RSD 1.9%) was more reproducible than current monitoring (RSD 5.9%). We also investigated the response of conductivity to buffers of different concentration. CD showed a good response for gradient mixing and a linear relationship between the conductivity and the relative buffer concentration. This new system offers great promise for monitoring precision mixing in microfluidic systems using electrically driven flows.

Experimental

Reagents

All chemicals were used as received from the manufacturer. Sylgard 184 poly(dimethylsiloxane) (PDMS) elastomer knit was purchased from Dow Corning (Midland, MI, USA). Methanol, propan-2-ol, hydrofluoric acid, hydrogen peroxide and nitric acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Pt wire (diameter 0.5 mm) was supplied by Goodfellow (Huntingdon, UK). SU-8 50 negative photoresist and XP SU-8 developer were obtained from Microchem (Newton, MA, USA). SC 1827 positive photoresist and 351

developer were obtained from Shipley (Marlborough, MA, USA). Hydrochloric acid was purchased from EM Science (Gibbstown, NJ, USA) and sulfuric acid from LabChem (Pittsburgh, PA, USA). *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) was obtained from Sigma Chemical (St. Louis, MO, USA).

Chip fabrication

A 2.5 in square glass plate was cleaned with piranha solution [$\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ (2 + 1)] (**Caution:** *Piranha solution should be handled with extreme care owing to its strong oxidizing ability*). The plate was then placed in a propan-2-ol bath for 2 min followed by rinsing thoroughly with de-ionized water, dried with N_2 gas and baked at 105 °C for 2 min. Sequential deposition of 150 Å of Ti and 1000 Å of Au (99.99% pure, Kurt Lesker, Clairton, PA, USA) on the glass plate was accomplished using an electron beam evaporator. After cleaning with piranha solution, the deposited plate was coated with positive photoresist using a spin coater (Laurell Technologies, North Wales, PA, USA) at 2200 rpm for 30 s. A digitally produced mask containing the electrode pattern was then placed on the coated plate and exposed to near-UV radiation for 15 s. The photoresist was developed and cured by post-exposure baking. The Au and Ti were etched using *aqua regia* [HCl-HNO_3 (3 + 1)] and Ti etch (2.0% HF -0.5% HNO_3), respectively. Finally, acetone was used to remove the photoresist from the plate. Prior to use, the electrode plates were cleaned with piranha solution for 10 min. The width of the working electrode in the detection zone was 54 μm. The process of deposition and patterning is shown in Fig. 1.

A 3 in silicon wafer was preconditioned as described above for the glass plate. The wafer was coated with SU-8 50 negative photoresist. The coated wafer was exposed to near-UV radiation for 5 min through a digitally produced mask. A positive relief of the intended channel pattern was formed on the wafer after the unexposed photoresist had been removed in developer. Once completed, a single molding master could be used to produce hundreds of PDMS replicas. A degassed mixture of Sylgard 184 silicone (PDMS) elastomer and curing agent (10 + 1) was poured on to a silicon master that had been cleaned sequentially with water and methanol and dried with a stream of nitrogen. After 3 h of curing at 65 °C, the PDMS replica was peeled from the mold, resulting in a pattern of negative relief channels and reservoirs in the PDMS. Buffer reservoirs were then opened with a hole punch and the PDMS was trimmed to size with a scalpel.

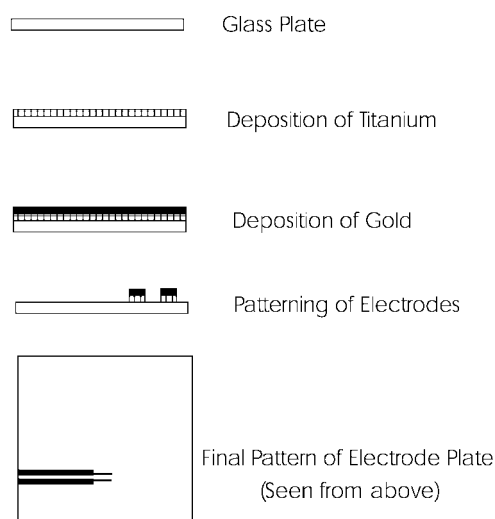


Fig. 1 Evaporation and patterning process of the electrode plate (not to scale).

Reversible sealing as reported by several groups was employed.^{22,23} A PDMS replica and an electrode plate were rinsed with methanol and brought into contact with one another prior to drying. Alignment of the working electrodes relative to the microfluidic channel was accomplished at the same time using a stereomicroscope. The assembled microchip was then dried in an oven at 65 °C for 5 min. This method of reversible sealing gave a consistent sealing for the reported experiments. The use of reversible sealing also allowed the continued use of electrode plate.

A schematic diagram of the microchip used for CD of EOF is shown in Fig. 2.

Conductivity measurements

The buffer was introduced into reservoirs and flushed through the microfluidic channel using a vacuum aspirator until no air bubbles were observed with a microscope. The buffer solution in one of the buffer reservoirs was then replaced by a different concentration buffer solution. A potential was applied to both buffer reservoirs while the waste reservoir was grounded. After 15 min of conditioning, the potential between one buffer reservoir and the waste reservoir was removed. This allowed only one buffer to flow down the microfluidic channel. Buffer switching was accomplished by moving the potential to the second buffer reservoir. The time required for the conductivity to plateau was measured for each run and was indicative of the different concentration buffer filling the microfluidic channel. Six consecutive measurements were made for each experiment. The time required for the conductivity to reach this plateau was used as the migration rate of a neutral marker. The EOF was determined by

$$\mu_{\text{EOF}} = L_t L_d / Vt \quad (1)$$

where L_t is the length of the total microfluidic channel (4.2 cm), L_d is the length of the microfluidic channel from T-intersection to the waste reservoir (3.4 cm), V is the total applied voltage (840 V) and t is the time required to reach the new conductivity plateau.

Results and discussion

The conductivity detector design was driven by considering the size of the in-channel electrodes and their separation. A simple electrical model for ac conductivity detection consists of the solution resistance in series with the double-layer capacitance of the detection electrodes. For these experiments, the solution

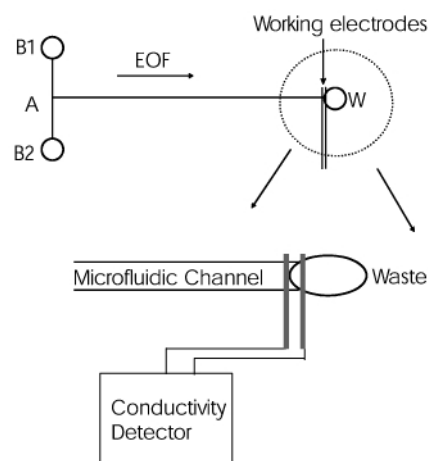


Fig. 2 Schematic diagram of microchip used for conductivity detection of electroosmotic flow (not to scale).

resistance is of the order of 10 M Ω per cm of channel. Thus, an estimate for the baseline resistance between the in-channel detection electrode and the out-of-channel electrode is about 100 k Ω with a detection electrode positioned 100 μm from the channel exit (the resistance outside the channel is assumed to be negligible). The most linear behavior for conductivity detection will occur when the impedance of the circuit is dominated by the resistance of the solution. That is, the capacitive reactance of the circuit should be much smaller than the resistance. For an electrode in solution, the capacitive reactance is modeled by

$$X_C = 1/(2\pi f C_D) \quad (2)$$

where f is the excitation frequency and C_D is the double-layer capacitance. In aqueous solution, C_D is of the order of 20 $\mu\text{F cm}^2$. The detection electrode area is fixed by two parameters. For an electrode on the floor of the channel, the down-channel width cannot increase arbitrarily since a separation voltage of 250 V cm^{-1} will produce a 1.25 V drop across a 50 μm wide electrode. This voltage drop is sufficient to cause opposite edges of the electrode to act as local cathodes and anodes for solvent reduction/oxidation, which can lead to bubble formation. The breadth is fixed by the separation channel width. Thus, the design is constrained to an area of $4 \times 10^{-5} \text{ cm}^2$ and a C_D of 800 pF. Given this constraint, an excitation frequency of 30 kHz was chosen to produce a capacitive reactance value of 5.3 k Ω , comfortably smaller than the expected resistance.

The detector circuitry consists of a CMOS-inverter oscillator which produces a 0–5 V square wave at 27 kHz. The square wave is filtered by a single-pole low-pass RC filter to produce a 100 mV_{pp} triangle wave, which is applied to the in-channel detection electrode. For safety, and to provide electrical isolation from the separation voltage, the excitation and output signals are both passed through two 0.1 μF series capacitors. The capacitors attached to the detection electrodes are rated at 1.2 kV dc. The resulting ac current signal is transduced using an operational amplifier (OA) current follower to a voltage with an overall gain of 10^{-7} A V^{-1} . In addition, the amplifier's gain was shaped to provide a bandpass filter with a center frequency of 30 kHz. After amplification, the ac signal is rectified using a precision OA rectifier and filtered. A signal offset is provided at the output to offset the baseline conductance signal. The circuit was tested using an RC substitution box and (using a 1 nF series capacitor) was found give linear behavior between R values of 10–100 k Ω and had a dynamic range of 1000 (*e.g.*, a 100 Ω change on a 100 k Ω offset).

Fig. 3 presents an electropherogram of conductivity as a function of time. At the beginning, the microfluidic channel was filled with high concentration buffer with high voltage applied

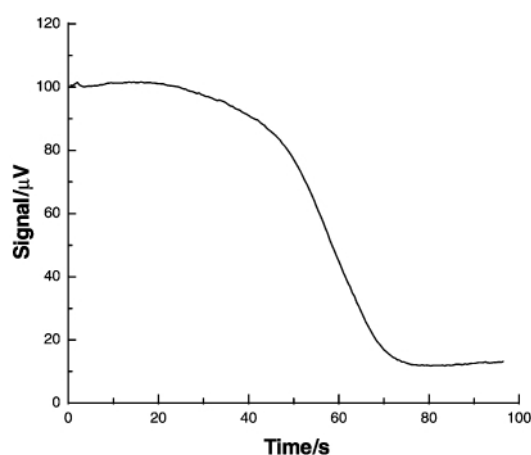


Fig. 3 Electropherogram of solution flow from high to low ionic strength. Conditions: applied voltage, 200 V cm^{-1} ; run buffers, 15 and 30 mM TES (pH 7.0).

from B1 to W. When the high voltage was switched to B2 to W, the low concentration buffer replaced the high concentration buffer. The detected conductivity decreased continuously with time. A conductivity plateau was obtained when the microfluidic channel was completely filled with the low concentration buffer. Because the microfluidic channel from B1 to A or from B2 to A could not be replaced with any other concentration buffer, even with high voltage on, the time required to reach a conductivity plateau was the time required to replace the buffer in the microfluidic channel from A to W. The EOF values were detected in six consecutive runs, *i.e.*, we recorded the time required to obtain a conductivity plateau from high concentration to low concentration and the time required to obtain a conductivity plateau from low concentration to high concentration. The respective EOF value was given by eqn. (1), where L_t is the distance from B to W and L_d is the distance from A to W. The results showed that the RSD of each six runs was 1.9%. The possible influence of different chips, different times, and resealing after a day's run was investigated. The difference among all the recorded times was less than 2%. The results proved that EOF detection *via* CD is reliable for microfluidic devices.

Huang *et al.*¹⁹ first reported the current monitoring method for measuring the EOF in capillary zone electrophoresis. Our CD was compared with current monitoring for the determination of EOF and the results are presented in Table 1. The EOF value *via* the current monitoring method was $2.63 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ and *via* CD was $2.69 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. No significant difference in absolute values was observed between these two methods; however, CD showed greater accuracy than the current monitoring method as the RSD for CD and current monitoring were 1.9 and 5.9%, respectively. This again verifies the ability of CD to measure fluid flow in microfluidic devices accurately and precisely.

Owing to the lower RSD of CD, we investigated the possibility of accurate control of microfluidic mixing *via* conductivity monitoring. It should be noted that this type of measurement is not possible using the current monitoring method. First, we applied high voltage to both buffer reservoirs (B1 and B2) which were filled with different concentrations of buffer. As a result, both buffers flowed to the T intersection and mixed in the microfluidic channel. The high voltage to reservoir B2 was then removed. Consequently, buffer in B1 flowed through the microfluidic channel to the conductivity detector. After the conductivity plateau was obtained, high voltage was reapplied to both buffer reservoirs. Buffer mixture flowed in the microfluidic channel again until signal plateau occurred. Buffer from reservoir B2 was used to fill the microfluidic channel until a conductivity plateau was obtained. The electropherogram for the entire process is presented as steps a, b, c and d in Fig. 4. In step a, high voltage was applied to both reservoirs. As a result, both high and low concentration buffers flowed down the microfluidic channel mixing at the T intersection. The resulting buffer concentration in the microfluidic channel should be between the high and the low concentrations. Because the conductivity of a solution is dependent on its concentration, the conductivity of such a buffer mixture should be greater than that of the low concentration buffer and less than that of the high concentration buffer. This trend has been proved in Fig. 4. Steps a and c represent the conductivity of the mixture and steps b and d represent the conductivity of low and high concentration buffers, respectively.

Table 1 Comparison of EOF results for CD and current monitoring

Parameter	CD	Current monitoring
EOF average ($\text{cm}^2 \text{ V}^{-1} \text{ s}^{-1}$)	2.63×10^{-4}	2.69×10^{-4}
RSD (%) ($n = 6$)	1.9	5.9

According to gradient mixing experiments, the relative conductivity difference was measured between two different concentration buffers. It was difficult to tell the absolute value of a certain buffer concentration as the baseline detector response varied from day to day. However, we found a good linear relationship between the conductivity difference and concentration difference between two buffer solutions. We employed the gradient mixing method to measure the conductivity difference between 3 and 30 mM, 6 and 27 mM, 9 and 24 mM, 12 and 21 mM and 15 and 18 mM TES solutions. We plotted the relative conductivity *versus* relative concentration and the results are shown in Fig. 5. A good linear relationship was obtained between concentration difference and con-

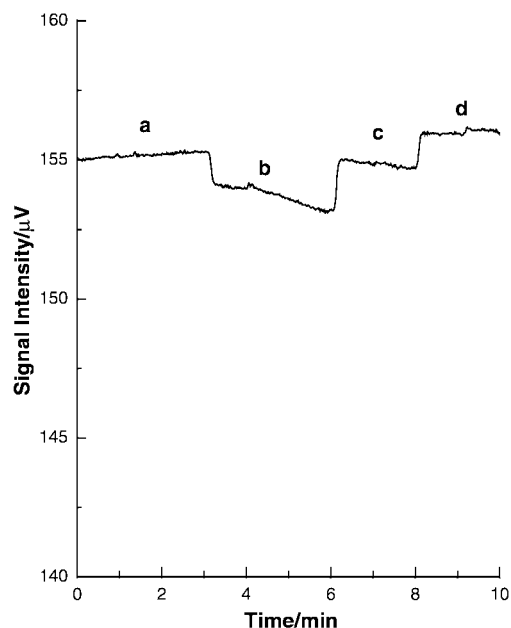


Fig. 4 Electropherogram of gradient mixing. Conditions as in Fig. 3. Step a, high voltage was applied to B1 and B2; step b, high voltage was applied to B1; step c, high voltage was applied to B1 and B2; step d, high voltage was applied to B2.

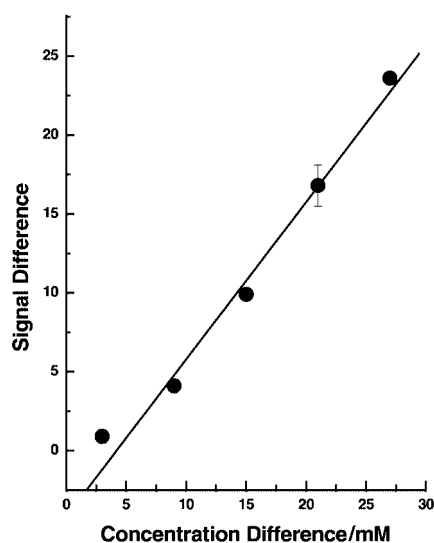


Fig. 5 Linear relationship of relative conductivity and relative concentration. CE conditions: applied voltage, 200 V cm^{-1} ; run buffers, 3 and 30 mM, 6 and 27 mM, 9 and 24 mM, 12 and 21 mM, 15 and 18 mM TES (pH 7.0). The error bars represent one standard deviation and are contained within the points for the points not showing error bars.

ductivity difference. The correlation coefficient was 0.991 and the sensitivity was 0.997 signal mM^{-1} . This will allow accurate control of mixing to be achieved by conductivity control, given an initial calibration step.

Conclusions

Conductivity detection can be used to measure EOF for microchip CE. The gradient mixing experiments showed a promising way to control accurately the mixing of different concentration buffers. This new method of monitoring flow has potential for use in a wide range of applications including chemical analysis and synthesis on a chip. CD flow monitoring is much simpler than previous methods and can be implemented without the need for expensive, off-chip instrumentation configurations. Future work will be focused on the accurate control of mixing different solutions *via* conductivity detection on a microchip.

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