Improved Current-Monitoring Method for Low Electroosmotic Flow Measurement in Modified Microchip

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Wei Wang[1,2], Wenya Wu[1], Fang Zhou[1], Jian-Rong Zhang[1], Qiang Miao[3], Jun-Jie Zhu[1,✉]

[1] Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, 210093 Nanjing, People's Republic of China; E-Mail: jjzhu@nju.edu.cn
[2] School of Chemical and Biological Engineering, Yancheng Institute of Technology, 224003 Yancheng, People's Republic of China
[3] Department of Chemical Engineering, School of Chemistry and Chemical Engineering, Nanjing University, 210093 Nanjing, People's Republic of China

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Abstract

Current-monitoring method is a widely used approach to measure electroosmotic flow (EOF) in microchip, but low and zero EOF is difficult to be measured. In this report, the mechanism of current-monitoring method was explained with Kohlraush regulation function principle, and an improved current-monitoring method was developed for low EOF measurement with tilting microchip. Fluid flow in the channel was accelerated with the help of hydrostatic pressure generated by tilting microchip, the time of dilute solution displacing the concentrated one in channel was shortened. EOF could be calculated according to the time difference between twice experiments under two different applied voltages by tilting microchip. Low even zero EOF could be measured by this improved current-monitoring method. Three modified microchips were characterized to verify the method. EOF in microchannels modified with poly(vinyl alcohol), bovine serum albumin and myoglobin were 0.27 ± 0.05, 0.16 ± 0.05 and −0.45 ± 0.04 × 10^{-4} $cm^2\ V^{-1}\ s^{-1}$, respectively.

Keywords

Microchip capillary electrophoresis
Low electroosmotic flow measurement
Tilting microchip
Hydrostatic pressure

Introduction

Manipulation of fluids in channels with dimensions of tens of micrometers, microfluidics, has emerged as a distinct new field [1]. The first applications of microfluidic technologies have been in analysis, for which they offer a number of useful capabilities: the ability to use very small quantities of samples and reagents and to carry out separations and detections with high resolution and sensitivity; low cost; short times for analysis; and small footprints for the analytical devices [2]. Microfluidics exploits its important characteristics of fluids in microchannels, such as small size, laminar flow and electroosmotic flow (EOF).

Electroosmotic flow is a particularly useful characteristic in microsystems [3]. When an ion-containing fluid (for example, water) is placed in a microchannel that has fixed charges on its surface (such as silicon dioxide or surface-oxidized PDMS) and an electrical potential is applied along the channel, the fluid moves as a plug, rather than with the parabolic-flow profile observed when pumping is accomplished by applying pressure to the fluid. EOF minimizes the broadening of plugs of sample that occurs with many pressure-driven systems, and allows very high resolution separations of ionic species. It is a key contributor to electrophoretic separations in microchannels [4].

Many microchips have been fabricated such as silicon, fused silica, borosilicate glass, elastomers, resins, and an

Table 1. Microchips performed in the experiments

Microchips	Length (cm)	Depth (μm)	Width (μm)	Fabrication and treatment
BSA channel	3.50	20	50	PDMS channel fabricated using GaAs master [11] and treated with BSA [15]
Myoglobin channel	3.50	20	50	Treated with myoglobin [15]
PVA channel	3.50	20	50	Treated with PVA [15]

increasing number of thermoplastics. To meet the requirements of microfluidic applications, chemical or physical modification of microchannels is a common approach to minimize unwanted solute interactions with the walls and reduce electroosmosis. Since electroosmosis often has a strong impact on microfluidic systems, its measurement is important for a thorough characterization of a microchannel.

Many approaches have been proposed to determine EOF rates. The average EOF rate could be measured by weighing the effluent from a capillary with an analytical balance [5–7], as a result, the method was called weighing measurement. The neutral marker method is the earliest reported method for measuring EOF in CE [8, 9]. A neutral compound is injected as sample, and its migration time to the detector is used to calculate EOF rate. Sampling zone method [10, 11] was reported to measure EOF rate in microchip capillary electrophoresis. The sampling zone is driven by EOF, and may be detected and serve as a marker for measuring the EOF. Conductivity measurements have been used to measure average EOF and to continuously monitor EOF in capillaries [12, 13]. Zare and co-workers [12] measured average EOF using a conductivity detector at the detection end of a CE capillary. Current-monitoring method was the most widely employed approach for EOF measurement. The method was reported by Huang et al. [12]. For this method, the solution reservoir at the injection end of the capillary has a different ionic strength from the solution filling the capillary. Consequently, the electric current changes when the solution at the injection end of the capillary fills the capillary and changes the total conductivity in the capillary. Average EOF is measured by determining the time it takes for the current to stop changing when the entire capillary has been filled with the solution from the injection reservoir.

Practical difficulties arise when very slow or zero EOF is determined because the bands may take a long time to migrate past the detector in the methods mentioned above. In neutral marker and detection of sampling zone methods, the signal is unconspicuous for a long migration time. In the current-monitoring method, no good reproducible gradient plots could be obtained, because the composition of the BGE in the reservoirs varied under high electric field for a long time due to the limited volume BGE employed in microchip CE. Researchers always speculated the EOF values according to indirect phenomena without obtained data [14, 15].

For very low EOF measurement in CE, there are two methods developed in virtue of pressure-driven with pump [16, 17]. In current microchip devices, the driven force is always high electric field only without pump. We also reported a method for low EOF rates measurement based on constant effective mobility in microchip capillary electrophoresis [18], but a reference microchip was employed. Sampling zone method with titling microchip [19] developed in our group is a good approach for low EOF measurement, but an ionic marker was employed and additional detector was needed.

Current-monitoring method was the most widely employed approach for EOF measurement because of its simpleness and convenience. The method was reported by Huang et al. [12]. But the limitation was obvious that low EOF could not be measured due to long time needed to achieve a current platform, as a result, the mutation of current as a function of time was not obvious. We developed an improved method by tilting microchip to measure low EOF. The principle of the method is that the fluid flow in the channel is accelerated by hydrostatic pressure force. The interface between two solutions passes the channel quickly with the drive of hydrostatic pressure no matter what EOF is. Under the same tilted angle, EOF could be calculated according to the time difference between twice experiments with two different applied voltages. The advantages of current-monitoring method were retained and disadvantages were overcome in the improved method.

Experimental

Chemicals

All reagents were of analytical grade. Sylgard 184 (PDMS) was from Dow Corning (Midland, MI, USA). Poly (vinyl alcohol) (PVA), average Mr 85,000–124,000 powder 87–89% hydrolyzed, bovine serum albumin (BSA) and myoglobin (from horse heart, minimum 90%, pI 7.0) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Na_2HPO_4, and KH_2PO_4 were purchased from Nanjing Chemical Reagents Factory (Nanjing, China). All solutions were prepared with doubly distilled water and passed through a 0.22 μm cellulose acetate filter (Shanghai Bandao Factory, Shanghai, China).

Microfluidic Chip System

The microfluidic chip system was designed as our previous report [11]. The laboratory-made power supply had a voltage ranging from 0 to 5,000 V and 0 to −5,000 V. The current can be monitored graphically in real time. Electrical contact with the solutions was achieved by placing platinum wires into each of the reservoirs. Microchips performed in the research are simple cross-type and the parameters are shown in Table 1.

EOF Measurement Procedures

Schematic of microchip status in the process of measurement is shown in

Fig. 1. The procedure is to fill the channels and reservoir W with electrolyte at a concentration C and to fill reservoir S with the same electrolyte but at a different concentration C'. When an electric field V is applied, while the microchip is tilted at the same time, the current change is recorded. It is not necessary for great difference between concentrations C and C', 5% is sufficient for observing current changing.

In our studies we used an electrolyte consisting of 20 mM sodium phosphate buffer with a pH of about 7.0. This solution is said to have the concentration C. The same electrolyte mixture at concentration C' is prepared by diluting the electrolyte at concentration C with water (19:l) so that $C' = 0.95$ °C. Schematic plots of current versus time are shown in Fig. 2.

Results and Discussion

Mechanism of Current-Monitoring Method

Current-monitoring method is based on Kohlraush regulation function (KRF) [20]. KRF prescribes that its numerical value ω is locally invariant in time and is defined as

$$\omega = \sum_i \frac{c_i}{|m_i|} \quad (1)$$

where c_i and m_i refer to the concentrations and actual mobilities of all ionic species, assuming only the presence of fully ionized monovalent ionic constituents. The attractive aspect of the KRF lies in that it is a conservation law. The value ω, states the existence of a certain function of the constituent's concentrations that is conserved—not dependent on time. It means that electrophoretic processes are regulated by the initial conditions. The KRF has a certain value at a given point along the migration path (along the capillary tube) prior to applying of electric field, the information of the initial conditions is kept in the form of the ω value during the whole electrophoretic process.

When an electrolyte at a different concentration C' with deviating ω value is filled in the solution reservoir S, if there is no EOF, the flux of ions out of the concentration boundary will be exactly equal to the flux toward it. Consequently, this concentration boundary will be stationary, neglecting the effect of diffusion. In other words, the apparent movement of the concentration boundary in channel comes solely from the EOF of the whole bulk solution. In the presence of electroosmosis, the above concentration boundary is moving at EOF velocity.

In current-monitoring method, the electrolyte at concentration C in channel is displaced gradually by the electrolyte with concentration C'. Along with the resistance in channel, the current varies with time.

Principle of Improved Current-Monitoring Method

The principle of the method is that the fluid flow in the channel is accelerated by hydrostatic pressure force. Under different applied electric field with the same microchip tilted angle, the difference of fluid flow velocity in two modes is due to EOF difference under different electric fields.

Fluid flow is contributed jointly by EOF and hydrostatic pressure-driven.

$$V = V_{\mathrm{EOF}} + V_{\mathrm{hydrostatic}} \quad (2)$$

where V is the fluid flow velocity in microchannel, V_{EOF} is EOF velocity, and $V_{\mathrm{hydrostatic}}$ is the solution flow velocity only under the drive of hydrostatic pressure. Thus the velocities of fluid flow in the channels under different electric field $E1$ and $E2$ could be expressed as follows:

$$V_{E1} = V_{\mathrm{EOF1}} + V_{\mathrm{hydrostatic}} \quad (3)$$
$$V_{E2} = V_{\mathrm{EOF2}} + V_{\mathrm{hydrostatic}} \quad (4)$$

where V_{E1}, V_{E2}, V_{EOF1} and V_{EOF2} are the fluid flow velocity and EOF velocity under electric field $E1$ and $E2$, respectively. Flow velocity V_{E1} and V_{E2} are calculated with the channel length L and migration time $t1$ and $t2$.

$$V_{E1} = L/t1 \quad (5)$$
$$V_{E2} = L/t2 \quad (6)$$

and V_{EOF} can be obtained by $\mu_{\mathrm{EOF}}\ E/L$,

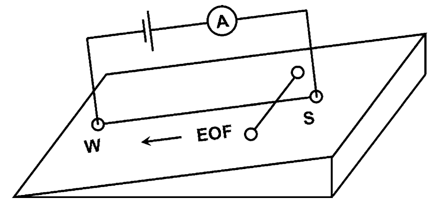

Fig. 1. Schematic setup state for EOF measurement with improved current-monitoring method

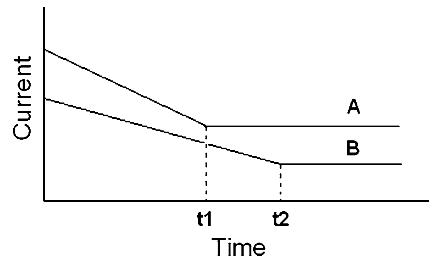

Fig. 2. Schematic traces show the current versus time for C' buffer replacing C buffer in channel under the electric field $E1$/L **a** and $E2$/L **b** in tilted microchip

Then

$$V_{\mathrm{EOF1}} = \mu_{\mathrm{EOF}} E1/L \quad (7)$$
$$V_{\mathrm{EOF2}} = \mu_{\mathrm{EOF}} E2/L \quad (8)$$

Substitution of Eq. (4) into (3) gives

$$V_{E1} - V_{E2} = V_{\mathrm{EOF1}} - V_{\mathrm{EOF2}} \quad (9)$$

Finally, substitution of Eqs. (5–8) into (9) yields

$$L/t1 - L/t2 = \mu_{\mathrm{EOF}} E1/L - \mu_{\mathrm{EOF}} E2/L \quad (10)$$

after rearrangement

$$\mu_{\mathrm{EOF}} = \frac{L^2\left(\frac{1}{t1} - \frac{1}{t2}\right)}{(E1 - E2)} \quad (11)$$

Effect of Tilted Angle and Electric Field on EOF Measurement

Limitation of conventional current-monitoring method was that low EOF could not be measured due to long time needed to achieve a current platform, as a result, the mutation of current trace was not obvious. The improvement of the proposed method is accelerating the fluid flow with help of hydrostatic pressure, consequently, an obvious mutation in the trace of current as a function of time could be observed.

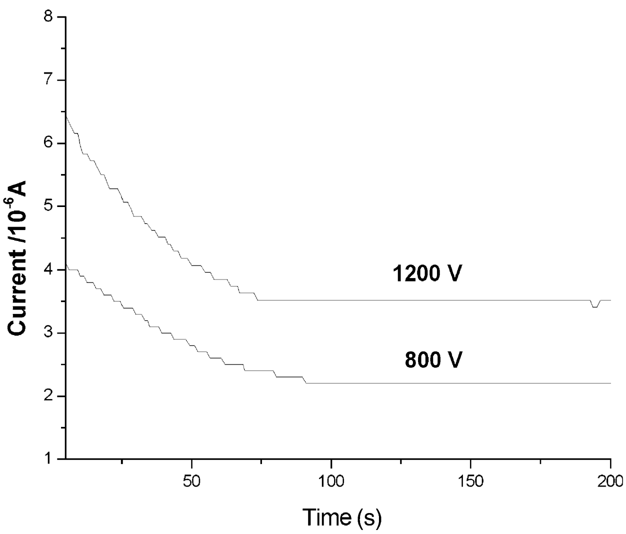

Fig. 3. Experimental plots for EOF measurement in a PVA modified PDMS microchannel under the electric field strength 800 V and 1,200 V with about 30° tilted angle

Table 2. Comparison of EOF measurements by proposed improved current-monitoring method and constant effective mobility method [21][a]

Microchip	Running buffer	$\mu_{EOF}/(10^{-4}\ cm^2\ V^{-1}\ s^{-1})$	
		Improved current-monitoring method	Constant effective mobility method
BSA channel	PBS (pH 6.24 20 mM)	0.16 ± 0.05	0.18 ± 0.04
Myoglobin channel	PBS (pH 5.29 20 mM)	−0.45 ± 0.04	−0.43 ± 0.03
PVA channel	PBS (pH 7.00 20 mM)	0.27 ± 0.05	0.26 ± 0.03

[a] Six parallel measurements

When EOF is low even zero, fluid flow in the channel is mainly driven by hydrostatic pressure by tilting microchip. In our previous report [21], effect of tilted angle on fluid flow in PDMS channel was detailed. For a 1-cm-long, 30-μm-i.d. PDMS channel, with 30° tilted angle, the difference of liquid height level ($\triangle H$) is approximately 0.50 cm, as a result that fluid flow rate is about 170 μm s^{-1}. The data were demonstrated by the following Eq. (12), known as Hagen–Poiseuille's Equation [22, 23].

$$v = \frac{d^2}{32\mu} \bullet \frac{\Delta P}{L} \tag{12}$$

Here v is the maximum average velocity on the capillary, L is the length of the channel. μ is the viscosity of the solution. d is the inner diameter of the channel.

In the experimental procedure, maximum available tilted angle could be used as long as the setup is stable for measurement. In our research, 30° tilted angle was employed. Namely, with PBS running buffer and 50-μm-i.d. channel, the mutation could be observed in less than 200 s even EOF is zero [21]. It is important to notice that the chip-to-chip repeatability of flow rate is poor only with the driven of hydrostatic pressure. The flow velocity in microfluidic flow channels is sensitive to surface properties because of the large surface-to-volume ratio. In the microfluidic device, the flow velocity usually varies under the same pressure. This may be caused by roughness of the PDMS channel walls [24].

In this research, the electric fields applied in the measurements referred to electrophoresis experiments. Two aspects were related to the electric fields employed: (1) The current flow through the channel results from the electric power applied. If a low electric power is applied, the mutation of current–time plot is not obvious when a dilute buffer solution replaces the concentrated one in the channel. (2) According to the Eq. (11), the difference between $t1$ and $t2$ results from the difference between $E1$ and $E2$. Making enough difference between $t1$ and $t2$ gives an assurance avoiding measurement errors. In our research, moderate electric fields of 1,200 and 800 V were applied as $E1$ and $E2$, respectively. EOF measurement plots in PVA modified microchip were shown in Fig. 3. A precise EOF could be calculated based on the data from the figures.

Applications

The main purpose of the proposed improved current-monitoring method is to measure low and zero EOF values, which is difficult to measure by conventional methods. PDMS channels modified with either proteins or PVA were employed as model microchips. Constant effective mobility method was employed for comparison. Data in Table 2 show that average EOF values obtained by improved current-monitoring method and constant effective mobility method agree well with each other.

Conclusions

We have proposed an improved current-monitoring method for low EOF measurement. Modified microchips with protein and PVA were applied to verify the method. The approach has advantages over current-monitoring method for measuring low EOF.

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References

1. Whitesides GM (2006) Nature 442:368–372. doi:10.1038/nature05058
2. Manz A, Harrison DJ, Verpoorte EMJ, Fettinger JC (1992) J Chromatogr 593:253–258. doi:10.1016/0021-9673(92)80293-4
3. Santiago JG (2001) Anal Chem 73:2353–2365. doi:10.1021/ac0101398
4. Znaleziona J, Petr J, Knob R, Maier V, Sevcik J (2008) Chromatographia 67:S5–S12. doi:10.1365/s10337-007-0509-y
5. van de Goor AAAM, Wanders BJ, Everaerts FM (1989) J Chromatogr A 470:95–104. doi:10.1016/S0021-9673(00)94203-1
6. Altria KD, Simpson CF (1986) Anal Proc 23:453–454
7. Altria KD, Simpson CF (1987) Chromatographia 24:527–532. doi:10.1007/BF02688539
8. Jorgenson JW, Lukacs KD (1981) Anal Chem 53:1298–1302. doi:10.1021/ac00231a037
9. Lukacs KD, Jorgenson JW (1985) J High Resolut Chromatogr 8:407–411. doi:10.1002/jhrc.1240080810
10. Wang W, Zhao L, Jiang LP, Zhang JR, Zhu JJ (2006) Electrophoresis 27:5132–5137. doi:10.1002/elps.200600110
11. Wang W, Zhao L, Zhang JR, Zhu JJ (2007) J Chromatogr A 1142:209–213. doi:10.1016/j.chroma.2006.12.092
12. Huang X, Gordon MJ, Zare RN (1988) Anal Chem 60:1837–1838. doi:10.1021/ac00168a040
13. Wanders BJ, van de Goor TAAM, Everaerts FM (1993) J Chromatogr A 652:291–294. doi:10.1016/0021-9673(93)80671-T
14. Hsieh YY, Lin YH, Yang JS, Wei GT (2002) J Chromatogr A 952:255–266. doi:10.1016/S0021-9673(02)00079-1
15. Wu D, Luo Y, Zhou X, Dai Z, Lin BC (2005) Electrophoresis 26:211–218. doi:10.1002/elps.200406157
16. Williams BA, Vigh G (1996) Anal Chem 68:1174–1180. doi:10.1021/ac950968r
17. Sandoval JE, Chen SM (1996) Anal Chem 68:2771–2775. doi:10.1021/ac9511509
18. Wang W, Zhao L, Zhou F, Zhang JR, Zhu JJ (2007) Electrophoresis 28:2893–2896. doi:10.1002/elps.200600781
19. Zhou F, Wang W, Wu WY, Zhang JR, Zhu JJ (2008) J Chromatogr A 1194:221–224. doi:10.1016/j.chroma.2008.03.085
20. Kohlrausch F (1997) Ann Phys 62:209–239
21. Wang W, Zhou F, Zhao L, Zhang JR, Zhu JJ (2008) Electrophoresis 29:561–566. doi:10.1002/elps.200700207
22. Nakayama Y, Boucher RF (1999) Introduction to fluid mechanics. Arnold, London
23. Du WB, Fang Q, Fang ZL (2006) Anal Chem 78:6404–6410. doi:10.1021/ac060714d
24. Luo Y, Yu F, Zare RN (2008) Lab Chip 8:694–700. doi:10.1039/b800606g