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Low electroosmotic flow measurement by tilting microchip

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ABSTRACT

A novel method for low electroosmotic flow (EOF) rates measurement by tilting microchip which based upon the hydrostatic pressure conception and sampling zone method is described. Sampling zone could be detected in the tilting microchip but not in non-tilting one due to the hydrostatic pressure driven. The method is fulfilled to calculate low EOF rates by detecting the liquid flow velocity driven by hydrostatic pressure, and difference between the apparent mobility of the migrating analyte in two modes is caused by the effect of hydrostatic pressure. And then the EOF rates in unknown low EOF microchip can be calculated. Different microchannels modified with bovine serum albumin (BSA), myoglobin (MB) and polyvinyl alcohol (PVA) were used to verify the method, the EOF rate value was 1.73 ± 0.03, 1.21 ± 0.05, $0.34 \pm 0.04 \times 10^{-4}$ cm^2 V^{-1} s^{-1}, respectively. The results obtained by the proposed method were agreed well with conventional methods.

1. Introduction

Nowadays, more and more researchers have devoted to the works on micro total analysis system (μ-TAS) as its rapid development since 1990s [1]. Owing to its excellent advantages including high sample throughput, minimized consumption of sample and reagents, easy integration of sample manipulation, and reduced analytical time and costs, lab-on-a-chip technology has been widely applied in a lot of domains, such as cell handling and analysis, biomimetic and biopowered systems, clinical diagnosis, immunoassays, DNA, proteins, other bioassays, environmental concerns and gas analysis [2].

Electroosmotic flow (EOF) is a convenient mechanism for transporting fluid in microchip capillary electrophoresis (CE). The migration times and quantity of analytes are greatly affected by changes in EOF [3–8]. As microfluidic technologies mature, increasingly diverse materials and complex solutions are employed, accurate measurement of EOF rates is becoming increasingly important. Clearly, for the operation of microfluidic devices, it is highly desirable to characterize and determine EOF rates during the course of experiments.

Many approaches have been proposed to determine EOF rates [9]. Neutral molecular markers approach [10] and the current-monitoring measurement [11] are common frequently used methods. Lee et al. described the design and performance of a method capable of measuring the EOF rate in capillary zone electrophoresis (CZE) by monitoring the fluorescence from a dye solution [12]. This approach provides real-time, on-line measurements.

Practical difficulties arise when very slow or zero EOF is to be determined because the bands may take a long time to migrate past the detector in the methods mentioned above. In neutral marker approach and detection of sampling zone methods, the signal is inconspicuous for a long migration time more than 600 s. In the current-monitoring method, no good reproducible gradient plots could be obtained, because the composition of background electrolyte in the reservoirs varied under high electric field for a long time due to the limited volume of BGE employed. Researchers always speculated the EOF values without obtained data according to indirect phenomena [13,14]. And real-time measurement also has the problem that its suitability under low electroosmosis conditions is yet to be demonstrated.

For very low electroosmosis measurement in CE, there are two methods developed in virtue of pressure-driven [15,16]. In current microchip devices, the driven force is always high electric field only. A low EOF rate measurement approach based on constant effective mobility was proposed in our group before [17], in which, a reference fast-electroosmosis microchip was used. The properties of channel wall, channel length, composition of BGE and experimental parameters were all the causes of systematic error.

The importance of mobility determinations and the experimental difficulties involved prompted us to develop a fast method for exactly determining low or zero EOF. Herein, we propose a method for low EOF rates measurement by tilting the microchip which

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was based upon the hydrostatic pressure conception and sampling zone method in the same microchip. The hydrostatic pressure was generated simply by adjusting the liquid level in different reservoir [16,18–20], and widely studies have shown the feasibility of using the hydrostatic pressure injection method by combining it with electrokinetic forces on a microfluidic chip.

We proposed a method which was fulfilled by detecting the sampling zone in tilting microchip, and the liquid flow driven by hydrostatic pressure was calculated with a migrating analyte, and difference between the apparent mobilities of the migrating analyte in two modes is absolutely caused by the effect of hydrostatic pressure. The proposed method is particularly useful for low-electroosmosis measurements in wall-modified microchannels.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade. Sylgard 184 Polydimethylsiloxane (PDMS) was from Dow Corning (Midland, MI, USA). 3,4-Dihydroxybenzylamine (DHBA), bovine serum albumin (BSA), myoglobin (MB), polyvinyl alcohol (PVA), average Mr 85,000–124,000 powder 87–89% hydrolyzed 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).

2.2. Apparatus

A microchip with cross-type channel combined with end-channel amperometric detector. The amperometric detector was located in the detection reservoir and consisted of a Ag/AgCl wire reference electrode, a Pt wire counter electrode and a homemade carbon fibre working electrode (I.D. 7 μm) [21]. Amperometric detection was performed with a CHI 832b electrochemical workstation (CHI Co., Shanghai, China).

The laboratory-made power supply had a voltage ranging from 0 to 5000 V and 0 to −5000 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.

2.3. Preparation of microchips

2.3.1. Fabrication of PDMS microchip

The master with a positive relief structure of GaAs for the channels was made using microphotolithographic technique. A cross-type channel of PDMS chip with a 3.2 cm long separation channel (effective separation length, 3.0 cm) and 1.0 cm long injection channel and a flat substrate were fabricated from PDMS as the described procedure elsewhere [22]. Briefly, A mixture of elastomer precursor and its curing agent (ratio of 10:1) (Sylgard 184) were degassed, poured over the GaAs master, and cured for 150 min at 80 °C. After the replica was peeled from the mold, holes (3 mm diameter) were punched. A flat PDMS substrate (0.3 mm thick) was obtained via casting and curing the prepolymer mixture in a large flat glass box (5 cm × 5 cm). The PDMS layer with microchannels and the PDMS flat were ultrasonically cleaned subsequently with acetone, ethanol, and water, and then dried under infrared lamp. Finally, they were sealed together to form a reversible PDMS microchip.

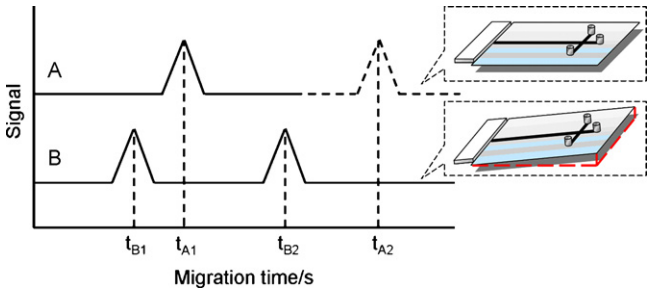

Fig. 1. Schematic electrophoretograms and diagrams of non-tilting microchip (A) and tilting microchip (B), and define them as pattern A and pattern B, respectively.

2.3.2. PDMS channel coated with BSA

Referring to ref. [23], for preparing BSA-coated microchannels, PDMS sheet with microchannel and flat substrate were treated by oxygen plasma. The two parts sealed immediately and irreversibly after they joined together [14]. A phosphate-buffered solution (PB, pH 6.98) of BSA (10 mg/mL) was added into a reservoir, and then the microchip was placed for 30 min at room temperature. After this incubation period, the inlet and outlet reservoir solutions were replaced with the standard phosphate buffer. A voltage of 250 V/cm was then applied to the channels for 10 min in order to wash them by electroosmotic pumping.

2.3.3. PDMS channel coated with MB

The method to prepare MB-coated microchannels was similar with the BSA-coated method just using MB (10 mg/mL) instead of BSA.

2.3.4. PDMS channel coated with PVA

As mentioned in ref. [14], PDMS sheet with microchannel and flat substrate were treated by oxygen plasma, and then were joined together, after that 1% PVA aqueous solution was added into the reservoirs. The chip was incubated for 10 min at 25 °C, and then the reservoirs and channels were emptied by a vacuum pump. Adsorbed PVA coating was dried by heating. The above procedure was repeated as needed, resulting in multilayer PVA coating. In our experiment, microchannel was coated for one time. Finally, the microchip was thermally immobilized in a vacuum oven.

2.4. Electrophoresis procedures

Electrophoresis procedures can be described briefly that in the initial state, the microchip was leveled, the sample was fully filled in sample reservoir and running buffer was fully filled in the sample waste reservoir, buffer reservoir and buffer waste reservoir to keep the liquid level in a plane (Fig. 1A). After 2 s sample loading, the microchip was tilted for separation. A wedge with defined height was used to tilt the microchip for desired angle and electrophoresis was implemented under high separation voltage simultaneously (Fig. 1B).

EOF measurement was performed with sampling zone method [24], which is based on the principle of the Kohlrausch regulating function (KRF). A dilute electroactive ionic species is added to the BGE as a probe. When a BGE solution at different concentration without probe is injected as sample, a peak of sampling zone appears and the migration time is related to EOF. According to the reference, 30 mM PB, 20 mM PB + 0.1 mM DHBA (pH 6.98) was used as sample and running buffer in our research, respectively.

3. Results and discussion

3.1. Derivation

The hydrostatic pressure was generated simply by adjusting the liquid level in different reservoirs with titling microchip. It had an effect on quickening liquid velocity. The effect of hydrostatic pressure generated to liquid in channel can be calculated by comparing the signal of tilting microchip with that of non-tilting microchip.

Liquid flow driven by hydrostatic pressure (v_{hydro}) can be expressed as follows:

$$v_{hydro} = v_{appB2} - v_{appA2} \quad (1)$$

where v_{appA2} is the EOF velocity in pattern A and v_{appB2} is the EOF velocity combined with liquid flow driven by hydrostatic pressure in pattern B. Similarly, the following equation can be deduced.

$$v_{hydro} = v_{appB1} - v_{appA1} \quad (2)$$

so, we can get that

$$v_{appA2} = v_{appB2} + v_{appA1} - v_{appB1} \quad (3)$$

Since the relationship between electric driven liquid flow velocity v and mobility is

$$v = \frac{\mu V}{L} \quad (4)$$

where V and L are the separation voltage and length, respectively.

Thus, v_{appA2} can be expressed as

$$\mu_{appA2} = \frac{LL_{eff}}{V}\left(\frac{1}{t_{B2}} + \frac{1}{t_{A1}} - \frac{1}{t_{B1}}\right) \quad (5)$$

where v_{appA2} and L_{eff} are the EOF mobility in pattern A and effect length, respectively. Furthermore, v_{appA2} is exactly the value we need, namely μ_{EOFA}, the low EOF mobility in pattern A.

3.2. Effect of tilted angle on measurement

Hydrostatic pressure was generated by liquid level difference between buffer reservoir and buffer waste reservoir after tilting the microchip in this method. In order to obtain an optimized hydrostatic pressure, different tilted angles such as 30°, 15° and 7° were investigated for separation procedure.

The effect of tilted angle on liquid flow was discussed in our previous report [20], that increase of titled angle will accelerate the liquid flow rate and eliminate measurement time. Fig. 2 shows the electrophoretograms we performed. As the increasing of tilted angle, liquid level difference increased which induced the enhancement of hydrostatic pressure. For that, migration time decreased with the increasing of tilted angle. Furthermore, the three electrodes were not stable in the solution with the excessive tilted angle because of little liquid volume, so that the three-electrode system could not perform normally. And 15° tilted angle was selected as the optimum titled angle in our research. According to our previous research [20], when 15° tilted angle was employed, the theoretical flow rate is about 72 μm/s. The liquid flow rate we measured in this research is 58 μm/s, it is agreed well with theoretical result considering approximation in channel inner diameter, solution viscosity and measurement errors.

3.3. Measurement of low EOF

The major purpose of proposed method is to measure very low and zero EOF values, which could not be measured by conventional methods.

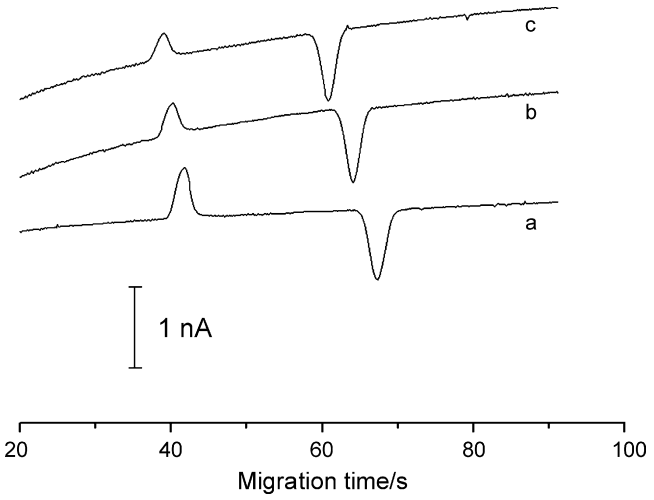

Fig. 2. Electrophoretograms illustrating the effect of different tilted angle on liquid flow in native PDMS channel. (a) 0° tilted angle, (b) 7° tilted angle and (c) 15° tilted angle. Sample, 30 mM PB; running buffer, 20 mM PB + 0.1 mM DHBA; pH 6.98. Experimental parameters: sampling voltage, 600 V; sampling time, 2 s; separation voltage, 600 V; detection potential, +1.4 V.

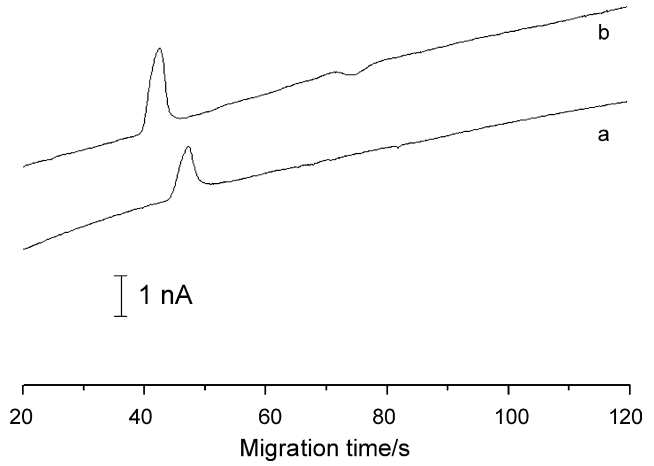

Fig. 3. Electrophoretograms for low EOF measurement with sampling zone detection in BSA coated PDMS channels. (a) In non-tilting microchip and (b) in tilting microchip with 15° tilted angle. Sample, 30 mM PB; running buffer, 20 mM PB + 0.1 mM DHBA; pH 6.98. Experimental parameters: sampling voltage, 600 V; sampling time, 2 s; separation voltage, 600 V; detection potential, +1.4 V.

Three modified PDMS channels with BSA, MB and PVA were employed for EOF measurement. In our experiment, 30 mM PB, 20 mM PB + 0.1 mM DHBA (pH 6.98) was used as sample and running buffer, respectively. In a routine CE procedure, sampling voltage was 600 V; sampling time was 2 s; separation voltage was 600 V (we used 800 V in PVA-coated microchannel), detection voltage was +1.4 V (vs. Ag/AgCl wire). All experiments were performed at room temperature. The CE with electrokinetic injection was performed with the procedure reported previously [25]. The electrophoretograms for EOF measurement in BSA channel are shown in Fig. 3.

According to electrophoretograms in Fig. 3 and data in Table 1, the outstanding qualities of the method we proposed were that, not only low EOF values could be obtained conveniently but also a significant signal could be observed and considerable time is saved. The EOF in modified channels was measured with previous reported constant effective mobility method [17] to verify the proposed method, the results were agreed well with each other.

Table 1
Comparison of low EOF measurements of three modified PDMS channels ($n = 6$)

	t_{A1} (s)	t_{A2}[a] (s)	t_{B1} (s)	t_{B2} (s)	EOF with proposed method (10^{-4} cm^2 V^{-1} s^{-1})	EOF with constant effective mobility [17] (10^{-4} cm^2 V^{-1} s^{-1})
Modified with BSA	47.6	92.6	43.3	77.6	1.73 ± 0.03	1.76 ± 0.03
Modified with MB	56.4	132.0	50.7	104.5	1.21 ± 0.05	1.17 ± 0.04
Modified with PVA	53.2	358.2	47.3	194.7	0.34 ± 0.04	0.30 ± 0.04

[a] t_{A2} is calculated by the value of EOF.

The flow profile at the tilting mode (parabolic flow) is different from that at normal mode (plug flow), it may affect the accuracy of EOF measurement. We observed that the effect is limited from the comparison with conventional method with data in Table 1. We consider that effect of flow profile variation on definition of migration time (peak maximum) of peaks is limited, and at the tilting mode, the hydrostatic pressure accelerates the liquid flow velocity and therefore the peak broadening is eliminated.

Cationic DHBA was used as a reference marker in the proposed method, it may be retained by anionic BSA immobilized on the PDMS channel surface at pH 6.98. During electrophoresis procedure, the interaction is low because of the presence of high concentration Na^+ and K^+, we could observe that no significant tailing in the peak in Fig. 3. In the proposed method, all parameters that affect the reference marker DHBA migration rate are the same at the tilting mode and normal mode except liquid flow profile, so the systematic errors were eliminated mostly, this advantage assures the good accuracy of EOF measurement.

The proposed method is particularly useful for low or zero electroosmosis measurements in wall-modified microchannels with its wonderful stability and reproducibility. Besides, the present method has its applicability on the measurement of the faster EOF rate on bare quartz or oxidized PDMS chips.

4. Conclusion

We have developed a method well suited for accelerated measurement of low EOF in wall-modified microchip. The method can be performed in common microchip CE devices. Considerable time is saved for low electroosmosis measurement compared with conventional methods, and a stable direct signal was observed for very low or zero electroosmosis measurement. Good reproducibility was achieved. The EOF rate values of BSA-coated microchannels, MB-coated microchannels and PVA-coated microchannels according to the proposed method were 1.73 ± 0.03, 1.21 ± 0.05, $0.34 \pm 0.04 \times 10^{-4}$ cm^2 V^{-1} s^{-1}, respectively. The operational procedure of the approach is also considerably simplified, on the same microchip, using the same solution, with only two continuous CE procedures performed first on the normal microchip and next tilted microchip that eliminates the systematic error to the lowest extent.

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