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Short Communication

Low EOF rate measurement based on constant effective mobility in microchip CE

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A new method for quickly determining low EOF rates (μ_{EOF}) in microchip CE is described. The measurement is based on the notion that the effective mobility (μ_{eff}) of an analyte is a constant in a certain BGE. The μ_{eff} of an analyte is determined in a reference fast-electroosmosis microchip, and the apparent mobility (μ_{app}) of the analyte can be determined in the microchip with unknown low electroosmosis, and then μ_{EOF} in the low-electroosmosis microchip can be calculated according to the equation $\mu_{\text{EOF}} = \mu_{\text{app}} - \mu_{\text{eff}}$. By an indirect method or other conventional methods, μ_{eff} can be easily measured in the reference microchip. The proposed method is particularly useful for low-electroosmosis measurements in wall-modified microchannels.

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Lab-on-a-chip technology has been greatly developed since the 1990s [1]. It offers researchers many advantages, such as high sample throughput, minimized consumption of sample and reagents, easy integration of sample manipulation, and reduced analytical time and costs. Many microchips have been fabricated, such as silicon, fused silica, borosilicate glass, elastomers, resins, and an 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 usually 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 μ_{EOF} . Several conventional representative methods employed on microchip are as follows. A neutral compound that, upon application of an electric field, is dragged with the electrolyte has been traditionally used to measure electroosmosis [2]. In another approach, Huang *et al.* [3] measured electroosmosis by measuring the current-time profile generated as the buffer filling the channel is replaced with another buffer of a

slightly different ionic strength. The current-monitoring procedure is a true on-line method, the device (an ammeter) used is usually part of the CE system.

In our previous report, we described a simple method for EOF measurement by detecting the sampling zone in microchip CE [4]. This method is based on the principle of the Kohlrausch regulating function (KRF). The migration time corresponding to EOF is measured by detecting the sampling zone. A dilute electroactive species is added to the BGE as a probe; when the BGE without probe at different concentrations is injected as the sample, the sampling zone can be detected due to changes in the probe concentration in it.

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 the neutral marker and sampling zone methods, the signal is inconspicuous 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 inferred EOF values from indirect phenomena instead of obtained data [5, 6]. For very low electroosmosis measurement in CE, there are two methods developed [7, 8]. These approaches were combined with pressure-driven mobilization to achieve accelerated EOF measurements. In most current microchip CE applications, electroosmotic pumping with applied voltages is the only

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Abbreviations: 4-AP, 4-aminophenol; DHBA, 3,4-dihydroxybenzylamine

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driving mechanism used to control microfluidic flow. To the best of our knowledge, no practical method was reported for very low or zero electroosmosis measurement in microchip CE.

The importance of mobility determinations and the experimental difficulty involved prompted us to develop a fast method to detect low or zero EOF. In this paper, we propose a method to calculate EOF by detecting the μ_{app} of a fast migrating analyte. The mechanism of this measurement is that the μ_{eff} of an analyte is a constant independent of the microchannel property in a given BGE. The μ_{app} of an analyte can be determined in the microchip with low electroosmosis, and μ_{eff} can be determined in a reference fast-electroosmosis microchip, then the μ_{EOF} in the unknown microchip can be calculated according to the following equation:

$$\mu_{\text{EOF}} = \mu_{\text{app}} - \mu_{\text{eff}} \quad (1)$$

The results obtained from the described method agree well with those from conventional methods. The constant μ_{eff} method is particularly useful for low-electroosmosis measurements in wall-modified microchannels.

All experiments were performed with an assembled microchip CE. A microchip with cross-type channel was combined with an end-channel amperometric detector. The amperometric detector was located in the detection reservoir and consisted of an Ag/AgCl wire reference electrode, a Pt wire counter electrode, and a home-made carbon disk working electrode (300 μm id) [9]. Amperometric detection was performed with a CHI 832b electrochemical workstation (CHI, 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. The parameters and treatments of microchips used in this research are listed in Table 1. All reagents were of analytical grade. Sylgard 184 (PDMS) was from Dow Corning (Midland, MI). MES, 3,4-dihydroxybenzylamine (DHBA), 4-aminophenol (4-AP), poly(vinyl alcohol) (PVA), average M_r 85 000–124 000 powder 87–89% hydrolyzed and Brij56 were purchased from Sigma–Aldrich (St. Louis, MO, USA). PBS solutions were prepared from Na_2HPO_4 and KH_2PO_4 (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).

Electrophoresis was performed following the procedures below. Sampling mode was simple crossing without pinch. Before injecting a sample, the sampling and wasting buffer reservoirs were filled with running buffer solution. During the separation stage, the sampling and waste buffer voltages were kept floating. In a routine CE procedure, the separation voltage was 800 V to avoid Joule heating. All experiments were performed at room temperature ($20 \pm 5^\circ\text{C}$).

The protocol for low electroosmosis measurement is depicted as follows. First a sampling zone detection method for EOF measurement is performed in a fast-electroosmosis microchip [4]. Briefly, a fast migrating analyte is added to running buffer as a continuously eluting electrophore, then a BGE-like sample at a different concentration is injected, and the schematic electropherogram obtained under these conditions is shown in Fig. 1A. Secondly, a normal CE is performed in the unknown EOF microchip with the same running buffer, when the analyte is sampled and a normal

Table 1. Parameters and treatments of microchips used in research

Microchip	Length (effective length) ^{a)} (cm)	Depth (μm)	Width (μm)	Fabrication and treatment
Glass	4.60 (4.20)	20	60	Fabricated by Zhejiang University using standard photolithography and chemical wet etching techniques
PDMS	3.62 (3.30)	20	50	Fabricated using a GaAs master [10]
Brij56 channel	3.62 (3.30)	20	50	PDMS channel treated with Brij56 [10]
PVA channel	3.62 (3.30)	20	50	PDMS channel treated with PVA [6]

a) Length: total length of channel (from running buffer reservoir to channel end); effective length: the length of effective separation channel (from crosspoint to channel end).

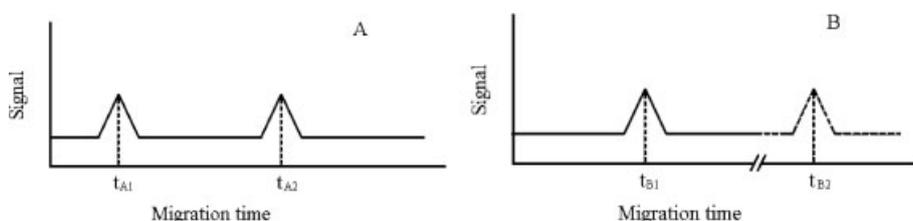


Figure 1. Schematic electropherograms obtained by (A) an indirect method in a fast-EOF microchannel and (B) a normal method in a low-EOF microchannel.

electropherogram obtained under these conditions is shown in Fig. 1B, however, the peak of dash line is a suppositional signal of EOF. t_{A1} and t_{A2} refer to the migration time of the analyte and EOF in channel A, while t_{B1} and t_{B2} refer to that in channel B.

According to the theory that the μ_{eff} of an analyte is a constant in a given BGE, a detectable probe has the same μ_{eff} in channel A and channel B (Fig. 1).

$$\mu_{\text{eff}A1} = \mu_{\text{eff}B1} \quad (2)$$

where $\mu_{\text{eff}A1}$ and $\mu_{\text{eff}B1}$ are the effective mobilities in channel A and channel B, respectively. The μ_{eff} of the analyte is calculated with the μ_{app} of the analyte and the coefficient of EOF as:

$$\mu_{\text{eff}A1} = \mu_{\text{app}A1} - \mu_{\text{EOFA}} = \frac{L_A L_{A\text{eff}}}{t_{A1} V_A} - \frac{L_A L_{A\text{eff}}}{t_{A2} V_A} \quad (3)$$

$$\mu_{\text{eff}B1} = \mu_{\text{app}B1} - \mu_{\text{EOFB}} = \frac{L_B L_{B\text{eff}}}{t_{B1} V_B} - \frac{L_B L_{B\text{eff}}}{t_{B2} V_B} \quad (4)$$

where $\mu_{\text{app}A1}$, μ_{EOFA} , and V_A are the μ_{app} of analyte, EOF mobility, and separation voltage applied in channel A, respectively. $\mu_{\text{app}B1}$, μ_{EOFB} , and V_B are those in channel B. L_A , $L_{A\text{eff}}$, L_B , and $L_{B\text{eff}}$ are the length and effective length of channel A and channel B. Thus, μ_{EOFB} can be expressed as:

$$\mu_{\text{EOFB}} = \mu_{\text{app}B1} - \mu_{\text{app}A1} + \mu_{\text{EOFA}} = \frac{L_B L_{B\text{eff}}}{t_{B1} V_B} - \frac{L_A L_{A\text{eff}}}{t_{A1} V_A} + \frac{L_A L_{A\text{eff}}}{t_{A2} V_A} \quad (5)$$

assuming that $L_A = L_B = L$, $L_{A\text{eff}} = L_{B\text{eff}} = L_{\text{eff}}$ and $V_A = V_B = V$, μ_{EOFB} can be calculated as:

$$\mu_{\text{EOFB}} = \frac{L L_{\text{eff}}}{V} \left(\frac{1}{t_{B1}} - \frac{1}{t_{A1}} + \frac{1}{t_{A2}} \right) \quad (6)$$

The described method is based on constant effective mobility of an analyte in different microchannels with the same running buffer. The selected flow marker in this technique cannot interact with either unknown chip walls or reference chip walls, since it is the basic premise of the method for measuring EOF. Accurate EOF values rely on the accurate determination of analyte mobilities in two microchannels. There are three possible sources of systematic error in EOF measurements: (i) measurement of channel length, (ii) composition of BGE and sample solutions, and (iii) experimental conditions such as separation voltage. One way to minimize

these sources of error in a modified microchannel is to use the native microchip before modification for the reference measurements. We must point out here that the constant μ_{eff} method for EOF measurement highly relies on the accuracy and repeatability of the data from the reference chips.

In order to test the invariance of analyte effective mobilities in different microchips, DHBA and 4-AP were employed as probes, and effective mobilities of the analytes in glass and PDMS microchips were measured. We decreased the difference in experimental conditions in the two electrophoretic performances to the best of our abilities. The same BGEs were employed as running buffer. Six replicates of the measurements were made, and the results are listed in Table 2. Effective mobilities of analytes in different microchannels were consistent with each other.

The main purpose of the proposed constant μ_{eff} method is to measure low and zero EOF values, which is difficult to measure by conventional methods. PDMS channels modified with either Brij 56 or PVA were employed as model microchips and native PDMS channels were employed as the reference microchips. In both the modified and reference channels, measurements were made with either PBS running buffer (pH 7.00 20 mM) or MES (pH 6.00 20 mM). The electropherograms for EOF measurements in the PVA channel with PBS running buffer are shown in Fig. 2. In Fig. 2A, peak a represents DHBA absence in the zone, and peak b represents sampling zone [4]. In Fig. 2B, peak a represents DHBA oxidation. According to the data in Fig. 2, EOF values in the PVA channel with PBS running buffer can be calculated. The sampling zone method and current-monitoring method were employed for comparison. Data in Table 3 show that average EOF values obtained by the constant μ_{eff} method and reference methods agree well with each other.

As far as the current monitoring method is concerned, with MES running buffer, EOF in either the Brij56 channel or PVA microchannel could not be detected, because a small current change occurred when a diluted electrolyte solution was electroosmotically pumped to the whole channel. With PBS running buffer, EOF could not be detected in the PVA microchannel. The reason is that the BGE component in the small reservoirs varied under the high electric field for more than 600 s, with the result that no stable gradient plot was obtained. As far as the sampling zone method is concerned, with MES running buffer, EOF could not be detected because no significant signal of the sampling zone was observed due to

Table 2. Invariance of effective mobilities of analytes in different microchannels^{a)}

Running buffer	DHBA μ_{eff} (10^{-4} cm ² /(Vs))		4-AP μ_{eff} (10^{-4} cm ² /(Vs))	
	Glass microchip	PDMS microchip	Glass microchip	PDMS microchip
PBS (pH 7.00 20 mM)	1.27 ± 0.02	1.28 ± 0.03	0.55 ± 0.02	0.54 ± 0.03
MES (pH 6.00 20 mM)	2.51 ± 0.05	2.48 ± 0.07	0.84 ± 0.03	0.86 ± 0.04

a) Six parallel measurements.

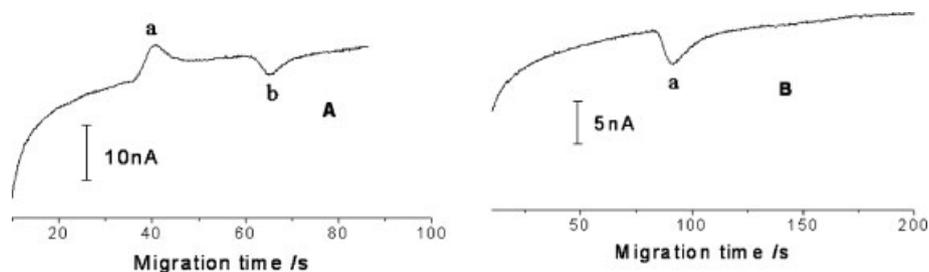


Figure 2. Electropherograms obtained by (A) the sampling zone method in a native PDMS microchannel, peak a: DHBA absence in the zone, peak b: sampling zone, sample: 25 mM PBS, running buffer: 20 mM PBS + 0.1 mM DHBA pH 7.00. (B) Normal method in a PVA-coated PDMS microchannel, peak a: DHBA oxidation, sample: 25 mM PBS + 0.1 mM DHBA, running buffer: 20 mM PBS pH 7.00. Experimental parameters: sampling voltage 600 V, sampling time 2 s, separation voltage 800 V, detection potential +1.2 V.

Table 3. Comparison of EOF measurements by the proposed constant μ_{eff} method and reference methods^{a)}

Running buffer	μ_{EOF} of Brij 56 channel (10^{-4} cm ² /(Vs))			μ_{EOF} of PVA channel (10^{-4} cm ² /(Vs))		
	Constant μ_{eff} method	Sampling zone method	Current monitoring method	Constant μ_{eff} method	Sampling zone method	Current monitoring method
PBS (pH 7.00 20 mM)	1.40 ± 0.04	1.42 ± 0.05	1.47 ± 0.05	0.22 ± 0.02	0.23 ± 0.04	
MES (pH 6.00 20 mM)	1.35 ± 0.03	1.33 ± 0.03		0.20 ± 0.02		

a) Six parallel measurements.

band broadening for more than 600 s migration. By using the proposed constant μ_{eff} method for low-EOF measurement, a significant signal could be observed and considerable time is saved. Only 50 and 100 s were needed in PVA microchannel with MES and PBS running buffer systems, respectively. However, migration time of the zone corresponding to EOF is more than 600 s.

In conclusion, we have proposed a constant μ_{eff} method for fast measurement of low electroosmosis in microchip CE. The method can be performed in common microchip CE devices. Considerable time is saved for low electroosmosis measurement compared to conventional methods, and a stable direct signal was observed for very low or zero electroosmosis measurement. The constant μ_{eff} method eliminates most of the experimental difficulties of the conventional EOF determination methods, such as a BGE with low concentration introduced in the current-monitoring method and detectable analyte without charges in the neutral marker method.

For μ_{eff} measurements in the reference microchip, we employed the sampling zone method in this paper, and other methods, such as the carbon fiber in-channel method [11], can also be employed. Effective mobilities can also be calculated from apparent mobilities by traditional normal methods and EOF mobilities by the current-monitoring method. If the μ_{eff} of an analyte could be obtained from a database, we could also calculate μ_{EOF} of an unknown microchip according to the μ_{app} of the analyte detected in a single run.

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