Short Communication

**EOF measurement by detection of a sampling zone with end-channel amperometry in microchip CE**

A simple method for EOF measurement by detection of sampling zones with end-channel amperometry in microchip CE is developed. This method is based on the principle of the Kohlrausch regulating function (KRF). A dilute electroactive ionic species is added to the BGE as a continuously eluting electrophore which is used as a probe. When a BGE-like sample at a different concentration is injected, a peak of sampling zone appears and the migration time is related to EOF. In a microchip CE with hybrid PDMS/glass channel, a cathodic EOF of the hybrid glass/PDMS microchip was measured by end-channel amperometry; the effects of sample concentration and different probes on EOF rate were discussed. The present method was applied to monitor EOF rates in glass and in PDMS microchips. There was no significant difference between the values of EOF rates measured by the present method and the current-monitoring method. Detection of nonelectroactive analytes K⁺, Na⁺, and Li⁺ can also be accomplished by the indirect amperometric method. Hence, the effective mobility of analyte can be accurately obtained.

**Keywords:** EOF measurement / End-channel amperometry / Microchip CE

**DOI 10.1002/elps.200600110**

EOF plays a major role in a variety of microfluidic applications, such as lab-on-a-chip devices. EOF is well known to be dependent on the surface charge of the capillary wall, the electric field, and the parameters of the conducting medium. In addition, the injection of a sample zone and separation of its components will alter EOF in CE and microchip CE [1–4]. As microfluidic technology matures, increasingly diverse materials and complex solutions are employed, and accurate methods for measurement of EOF rates are becoming increasingly important.

Several methods have been developed to measure EOF rates in CE. The commonly used methods are neutral molecular markers [5] and current-monitoring measurement [6]. The use of uncharged molecules is not as versatile as the latter, since it requires the molecules to be truly neutral and at the same time detectable, and the condition does not entirely meet. Current-monitoring measurement assumes a constant wall surface potential and electrical double layer thickness when an electrolyte solution is replaced with another solution of the same electrolyte with slightly different ionic concentration. In fact, it is not always the case. Several groups have investigated alternative EOF rate determination methods [7–12] including the measurement of the mass change in the electrolyte vials before and after the electrophoretic separation in CE [7], the introduction of a fluorescent agent and the monitoring of its movement with a separate detector [8, 9], and imaging EOF using fluorescence and NMR [10–12].

System zones in CZE have been widely studied [13–18]. The Kohlrausch regulating function (KRF) [19] prescribes that its numerical value is locally invariant in time. If an EOF is present, these zones with different values migrate with the velocity of EOF through the capillary. By using the most common UV detector, these zones are
visible in electropherograms only if at least one of the ionic species of the BGE is UV-absorbing. The contactless conductivity detector [20] is also used to be an alternative detector for system zones both in aqueous [21] and nonaqueous [22] media. Amperometry is the most widely reported electrochemical detection method for chip-based separations [23–26]. In the present work, we developed a method for direct monitoring of EOF rates in microchip CE with end-channel amperometry. Our approach is based on the detection of a sampling zone. A dilute electroactive species was added to the BGE as a probe; when the BGE without probe at different concentrations was injected as the sample, the sampling zone would be detected due to changes in the probe concentration in it. The changes of EOF with the concentration of sample and the length of sampling zone were studied theoretically and experimentally. The present detection method has been successfully used to measure the EOF rates in hybrid PDMS/glass, glass, and PDMS microchips. Anodic EOF in glass chip modified with CTAB was also measured. All experimental results showed there was no significant difference between the values obtained by the present method and current-monitoring method. The indirect amperometry could also be applied for detecting nonelectroactive analytes such as K\(^+\), Na\(^+\), and Li\(^+\) in aqueous solution, and accurate effective mobilities of analytes could be obtained.

All experiments were performed with an assembled microchip CE. The cross-type channels of PDMS with a 3.62 cm long separation channel (effective separation length, 3.30 cm) and 1.0 cm long injection channel were made from Sylgard 184 (Dow Corning, Midland, MI, USA) by using a GaAs master (No. 55 Electronic Institute, Nanjing, China). The sampling channel was 30 \(\mu\)m wide and 18 \(\mu\)m deep and the separation channel was 50 \(\mu\)m wide and 18 \(\mu\)m deep. The structured piece was sealed to the planar surface of a nonstructured PDMS plate or glass wafer (microscope slides 7101, Sheyang Huida Medical Products, China) after rigorous cleaning and drying to form PDMS/PDMS chip or hybrid PDMS/glass chip. The glass chip was fabricated by Zhejiang University based on custom design; the sampling channel had the size 60 \(\mu\)m wide and 20 \(\mu\)m deep, and the separation channel had 60 \(\mu\)m wide and 20 \(\mu\)m deep. The total length of separation channel and injection channel was 4.60 cm (effective separation length, 4.20 cm) and 1.0 cm, respectively. The amperometric detector consisted of an Ag/AgCl wire reference electrode, a Pt wire counter-electrode and a home-made carbon disc working electrode (id 300 \(\mu\)m), with the working electrode placed at the channel outlet. The laboratory-made power supply had a voltage ranging from 0 to 5000 V and 0 to −5000 V; separation current can be monitored graphically in real time. End-channel amperometric detection was performed with a CHI 832b electrochemical workstation (CHI, Shanghai, China). All chemicals were of analytical-reagent grade. MES, 3,4-dihydroxybenzylamine (DHBA), and 4-aminophenol (AP) were from Sigma (St. Louis, MO, USA). CTAB was from Shanghai Lingfeng Chemical Reagents (Shanghai, China). PBS solutions were prepared from Na\(_2\)HPO\(_4\) and KH\(_2\)PO\(_4\) (Nanjing Chemical Reagents Factory, Nanjing, China). All solutions were prepared with double-distilled water and passed through a 0.22 \(\mu\)m cellulose acetate filter (Shanghai Bandao Factory, Shanghai, China).

According to the KRF, when a sample solution is introduced with a certain value \(\omega\), this deviating \(\omega\) value remains valid at the spot of injection. Provided that there is no EOF, this sampling zone is stationary and the migrating sample ions are replaced by those of the BGE at a concentration adjusted to the KRF of the sample. In the presence of EOF, the above zone is driven by EOF, and may be detected and serve as a marker for measuring EOF rates. Indirect amperometric detection has been accomplished by the addition of a cationic electrophore, DHBA, to the electrophoretic buffer. When the working electrode is held at a constant potential of \(+1.2\) V, DHBA is oxidized. Continuous oxidization of the species, as it passes through the detector region, produces a constant background current. The zone in which DHBA is absent or diluted appears to be reverse peak. If the cationic DHBA is concentrated in a zone, an enhanced peak appears. A series of experiments were performed and the electropherograms are shown in Fig. 1.

Plot 1 in Fig. 1A displays an electropherogram in normal direct CE for a sample of detector-active species DHBA. It is observed that DHBA is oxidized and results in a negative peak. Plot 2 displays a typical indirect detection electropherogram, where two peaks are observed, the first peak representing a zone in which the DHBA is virtually absent and therefore is positive; we also observe that the migration time is coincident with that of DHBA in direct mode (Plot 1). This vacancy peak is present because the sample was a BGE-like solution without DHBA. The second peak represents the sampling zone that migrates with the EOF and the migration time corresponds to the EOF. Since the concentration of the sample is higher than that of BGE, in this zone, DHBA is concentrated by adapting the \(\omega\) value of the sample, and a negative peak appears. When an assay was performed with the sample which was the same as BGE in 20mM PBS + 0.1mM DHBA, no peak was observed in Plot 3.

The KRF \(\omega\) value of the sampling zone is determined by the property of sample solution: if the \(\omega\) value is higher than that of the BGE, an enhanced peak (negative signal)
Figure 1. Electropherograms in hybrid PDMS/glass chip for (A) different mode analysis (1) direct mode, sample: 20 mM PBS + 0.1 mM DHBA, running buffer: 20 mM PBS, pH 6.95. (2) Indirect mode, sample: 30 mM PBS, running buffer: 20 mM PBS + 0.1 mM DHBA, pH 6.95. (3) Indirect mode, sample is the same as running buffer: 20 mM PBS + 0.1 mM DHBA, pH 6.95. Experimental parameters: sampling voltage, 800 V; sampling time, 2 s; separation voltage, 1000 V; detection potential, +1.2 V.

is visible; if the η value is lower, a reversed peak (positive signal) can be observed as shown in Fig. 1B. When the concentration of sample is equal to that of BGE, we cannot observe the peak of sampling zone, only the vacancy peak is observed. Both negative and positive peak height increases with the increase in concentration difference between the sample and the BGE.

Another interesting phenomenon could be observed in Fig. 1B: the migration time of the sampling zone was found to increase with the increase in sample concentration. A semiquantitative formula (9) is deduced. The bulk EOF (νb) in the capillary for sample volume injected into the capillary is calculated using the following equation [2, 3]:

\[
ν_b = \frac{[γ_1 ν_{EOF1} + (1 - γ_1) ν_{EOF2}]}{[γ_1 + (1 - γ_1)]}
\]

where \((1-γ_1)\) is the percentage of the capillary filled with the sample buffer plug, \(ν_{EOF1}\) and \(ν_{EOF2}\) are the EOF values for a capillary filled only with BGE and sample buffers, respectively, and \(γ\) is the ratio of the resistivity of the BGE to the sample (\(γ = ρ_1/ρ_2\)). According to the Helmholtz-Smoluchowski (HS) formula [27–29], the absolute value \(ν_{EOF}\) is proportional to the zeta-potential (ζ) at the electrolyte/substrate interface

\[
m_{EOF} = -\frac{Z\zeta}{4πε}
\]

and the zeta-potential (ζ) and the thickness of the diffusion double layer (δ) have the following relationship [29, 30]:

\[
ζ = -\frac{4πδε}{ε}
\]

where ε is the dielectric constant of the fluid, E is the applied electric field, η is the fluid viscosity, and ε is the amount of charge per unit surface area. When the BGE is certain, the concentration of BGE determines the thickness of the diffusion double layer (δ):

\[
δ = \left[3 \times 10^7 |Z| c^{1/2}\right]^{-1}
\]

Z and c are the number of valence electrons and the concentration of the electrolyte. According to Eqs. (2–4), assuming \(k_c = c_2/c_1\), we can deduce the following approximate equation:

\[
\frac{ν_{EOF1}}{ν_{EOF2}} = \frac{m_{EOF1}}{m_{EOF2}} = \frac{c_2}{c_1} = \sqrt{k_c}
\]

In addition, according to the modified Ohm's law

\[
Eσ = j
\]

where σ is the specific conductivity and j is the current density. For a separation capillary with a constant open hole cross-section, the current density j is constant for the whole electrophoretic system at each moment. For a
specific zone conductivity, $\sigma$ can be expressed by the equation

$$\sigma = \sum_i c_i |m_i| F$$  \hspace{1cm} (7)

where $c_i$ and $|m_i|$ refer to the concentrations and absolute values of the mobilities of all ionic species and $F$ is the Faraday constant. Based on the Eqs. (6) and (7), we can conclude the following equation: $\frac{\rho_1}{\rho_2} = \frac{c_2}{c_1}$, namely

$$\gamma = k_c$$  \hspace{1cm} (8)

Applying Eqs. (5) and (8) to Eq. (1), the following formula is deduced

$$v_b = \frac{k_c x + \frac{1-x}{\sqrt{k_c}} V_{EOF}}{k_c x + (1-x) V_{EOF}}$$  \hspace{1cm} (9)

Namely, the bulk EOF ($v_b$) is a function of the ratio of sample concentration to BGE concentration ($k_c$) and the length of sampling zone ($1-x$). According to the formula (9), when $1-x$ is constant, $v_b$ decreases with the increase of sample concentration, and then the migration time of the sampling zone increases. The function exactly explains the experimental phenomenon in Fig. 1B.

In cross-type channels with floating injection mode, the length of sampling zone increases with the increase of sampling time, because of diffusion and some incursion of sample into the separation channel due to the applied injection voltage [31, 32]. According to the formula (9), the migration time of sampling zone decreases with increase of injection time for a more dilute sample than BGE, but the migration time increases with the increase of injection time for a more concentrated sample.

Because the migration time of sampling zone is dependent on the concentration of sample, a question arises regarding the concentration of the sample to be employed in the EOF measurements. To investigate this question, the PBS samples with different concentrations were employed to measure EOF. When the concentration of BGE is at $c$ and the concentration of sample is at $c'$, we observed no significant difference between the values when $c'$ ranges from 0.75 to 1.25 $c$. The relative error was less than 2.6%. The experimental results also agreed very well with the theoretical results. Therefore, we conclude that the EOF rates can be accurately measured by this simple indirect amperometry.

We also demonstrated the migration time of sampling zone was not dependent on the property of the additive probe, and that an alternative electroactive species AP was used. The peaks of sampling zones with DHBA (Fig. 2A-2b) and AP (Fig. 2A-4b) as additive detector-active species appeared at the same migration time but the sample voids (Figs. 2A-2a and 2A-4a) were different.

**Figure 2.** Electropherograms for influence of probe property on EOF measurement. (A) different electroactive species as probe in hybrid PDMS/glass chip. (1) Sample: 20 mM PBS + 0.1 mM DHBA, running buffer: 20 mM PBS, pH 5.70. (2) Sample: 25 mM PBS, running buffer: 20 mM PBS + 0.1 mM DHBA, pH 5.70. (3) Sample: 20 mM PBS + 0.1 mM AP, running buffer: 20 mM PBS, pH 5.70. (4) Sample: 25 mM PBS, running buffer: 20 mM PBS + 0.1 mM AP, pH 5.70. (B) negative charged DHBA as probe in glass chip. Sample: 12 mM borate, running buffer: 15 mM borate + 0.1 mM DHBA, pH 9.19. Experimental parameters are the same as in Fig. 1.
Table 1. Application of the indirect amperometric method of EOF measurement

<table>
<thead>
<tr>
<th>Channel/substrate composition</th>
<th>BGE</th>
<th>(m_{\text{EOF}}/(10^{-4} \text{ cm}^2/(\text{Vs})))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indirect amperometry(^b)</td>
<td>Current-monitoring method(^c)</td>
</tr>
<tr>
<td>PDMS/glass PBS (20 mM, pH 6.95)</td>
<td>1.79 ± 0.03</td>
<td>1.81 ± 0.07(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.82 ± 0.09(^e)</td>
</tr>
<tr>
<td>PDMS/PDMS PBS (20 mM, pH 6.95)</td>
<td>1.44 ± 0.04</td>
<td>1.48 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>1.76 ± 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.54 ± 0.02</td>
<td>2.64 ± 0.12</td>
</tr>
<tr>
<td>Glass PBS (20 mM, pH 6.95)</td>
<td>2.03 ± 0.07</td>
<td>2.00 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>3.58 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.92 ± 0.06</td>
<td>4.05 ± 0.16</td>
</tr>
<tr>
<td>Borate (15 mM, pH 9.19)</td>
<td>−3.12 ± 0.08</td>
<td>−3.16 ± 0.09</td>
</tr>
</tbody>
</table>

\(^a\) Five parallel measurements.
\(^b\) BGE at concentration \(c\), the same BGE at a different concentration \(c' = 0.75c\) was injected as sample.
\(^c\) BGE at the concentration \(c\) in the capillary, the same BGE at a different concentration \(c' = 19/20c\) was used to displace previous solution.
\(^d\) BGE: 20 mM PBS + 0.1 mM DHBA, pH 6.95.
\(^e\) BGE: 20 mM PBS, pH 6.95.
\(^f\) CTAB was added to BGE as modifier to change the EOF direction.

The influence of charged sign of probe on EOF measurement was also investigated (Fig. 2B). In glass microchip, borate buffer (pH 9.19) served as BGE, and 0.1 mM DHBA as probe, which is negatively charged in the BGE. Dilute borate buffer (pH 9.19) without DHBA was injected as sample. Only a sampling zone peak appeared in the electropherogram and no vacancy peak appeared. The migration time of sampling zone only depends on EOF and is independent on the charged sign of additive. The migration time of vacancy peak depends on the effective mobility of the probe and EOF. When the probe is neutral, it migrates with EOF, and the vacancy peak and sampling zone are the same; a single peak was formed by detectable component vacancy and not by the KRF, because the concentration of neutral component does not vary with the \(\omega\) value.

EOF rates of hybrid PDMS/glass, PDMS/PDMS, and glass microchip were measured by the present indirect amperometry. Table 1 shows the EOF mobility \(m_{\text{EOF}}\) values measured by indirect amperometry and current-monitoring method. The data indicate the present method provides precise values for EOF. The SD of five flow measurements in different channels ranges from 0.8 to 2.2%. The average relative error of EOF rates measured using the two methods is 2.1%. In addition, 200 \(\mu\)M of K\(^+\), Na\(^+\), and Li\(^+\) were successfully separated in a PDMS microchip and detected by the present indirect amperometric method. Running buffer was 20 mM MES + 0.1 mM DHBA. According to the migration times of analytes and EOF, effective mobilities of K\(^+\), Na\(^+\), Li\(^+\), and DHBA are 6.90 ± 0.10, 4.88 ± 0.07, 3.44 ± 0.08, and 2.51 ± 0.11 \(\times 10^{-4}\) cm\(^2\)V\(^{-1}\)s\(^{-1}\), respectively.

In conclusion, a simple, easy operating method has been developed for measuring EOF rates by detection of sampling zone with end-channel amperometry. EOF can be measured irrespective of whether the probe is positively or negatively charged. Both cathodic and anodic EOF were measured successfully. A semiquantitative formula was deduced to illuminate the influence of concentration of sample and length of sampling zone on EOF rates. When analytes are detected by this indirect method, accurate effective mobilities of analytes can be calculated. System error is eliminated because the migration times of analytes and EOF are obtained in the same assay.

This work was supported by the National Natural Science Foundation of China (20325516, 20635020, 20521503)

References