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Indirect amperometric measurement of electroosmotic flow rates and effective mobilities in microchip capillary electrophoresis

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Abstract

End-channel indirect amperometry is based on the principle of Kohlrausch regulating function (KRF). A dilute electroactive ionic species is added to the background electrolyte as a continuously eluting electrophore, which is used as probe. The probe concentration variation with the ω value of KRF in the sampling zone was described schematically in this report. Either cathodic or anodic electroosmotic flow (EOF) rates were monitored in microchip. There was no significant difference between the values of EOF rates measured by present method and current-monitoring method. Detection of electroactive and nonelectroactive analytes can also be accomplished by indirect amperometric method. Hence, the effective mobility of analytes can be accurately calculated. And the response mechanism of nonelectroactive analytes K^+, Na^+ and Li^+ in the indirect method was speculated.
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Keywords: Microchip capillary electrophoresis; Electroosmotic flow (EOF); Effective mobility; Indirect amperometric method

1. Introduction

Electroosmotic flow (EOF) is a convenient mechanism for transporting fluid in microchip capillary electrophoresis (CE). The migration times and quantitation of analytes are greatly affected by changes in EOF [1–6]. As microfluidic technologies mature, increasingly diverse materials and complex solutions are employed, accurate measurement of electroosmotic flow rates is becoming increasingly important. Clearly, for the operation of microfluidic devices, it is highly desirable to characterize and determine the EOF rates during the course of the experiment.

Several methods have been developed to measure EOF rates in CE. The common frequently used methods are neutral molecular markers [7] and current-monitoring measurement [8,9]. The use of uncharged molecules is not as versatile as the latter, since it requires molecules that are truly neutral and at the same time detectable, the condition is not entirely met. The methods described in literature to measure the EOF based on current monitoring assume 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 [1,2,10,11]. Several groups investigated alternative EOF rates determination methods [12–18].

In our previous report, we described a simple method for EOF measurement with detection of sampling zone in microchip capillary electrophoresis [19]. This method is based on the principle of Kohlrausch regulating function (KRF) [20]. A dilute detectable ionic species is added to the background electrolyte (BGE) as a continuously eluting electrophore, which is used as probe. When BGE-like sample at a different concentration is injected, a peak of sampling zone appears and the migration time is corresponding to EOF. No neutral marker was required and a single concentration running buffer was employed.

In this report, either cathodic or anodic EOF rates were monitored in microchip by detecting sampling zone with indirect amperometric method, the RSD was less than 2.8%. There was no significant difference between the values of EOF rates measured by present method and current-monitoring method [8]. Detection of electroactive and nonelectroactive analytes was also accomplished by indirect amperometric method. Hence, the effective mobilities of analytes were accurately calculated.

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And the response mechanism of nonelectroactive analytes K^+, Na^+ and Li^+ in the indirect method was speculated.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade. Sylgard 184, poly(dimethylsiloxane) (PDMS) was from Dow Corning (Midland, MI, USA). 2-(*N*-morpholino)ethanesulfonic acid (MES), 3,4-dihydroxybenzylamine (DHBA) and 4-aminophenol (AP) were purchased from Sigma (St. Louis, MO, USA). Histidine (His), NaCl, LiCl, KCl, $Na_2B_4O_7$, Na_2HPO_4 and KH_2PO_4 were purchased from Nanjing Chemical Reagents Factory (Nanjing, China). Cetyltrimethylammonium bromide (CTAB) was purchased from Shanghai Lingfeng Chemical Reagents (Shanghai, China). Phosphate buffer saline (PBS) solution was prepared with Na_2HPO_4 and KH_2PO_4, MES/His was with MES and His. All solutions were prepared with doubly distilled water and passed through a 0.22 μm cellulose acetate filter (Shanghai Bandao Factory, Shanghai, China). The pH values and the composition of the BGEs used in the indirect method are described in Table 1.

2.2. Apparatus

The cross-type channel of PDMS/PDMS and hybrid PDMS/glass chips for CE with a 3.62 cm long separation channel (effective separation length, 3.30 cm) and 1.0 cm long injection channel were made from PDMS by using a master. Details were described previously in reference [19]. 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 70 °C. After curing, a soft elastomer copy is peeled from the master and 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. The sampling channel was 30 μm width and 18 μm depth and the separation channel was 50 μm width and 18 μm depth.

The glass chip was from Zhejiang University. The sampling channel had the size 60 μm width and 20 μm depth and the separation channel was 60 μm width and 20 μm depth. The total length of the separation channel and injection channel was 4.60 cm (effective separation length, 4.20 cm) and 1.0 cm, respectively.

Table 1
Overview of the running buffer used in indirect amperometric method

Running buffer	Composition	pH
Direct PBS	20 mM Na_2HPO_4, 20 mM KH_2PO_4	6.95
Indirect PBS	20 mM Na_2HPO_4, 20 mM KH_2PO_4, 0.1 mM DHBA	6.95
Direct borate	15 mM $Na_2B_4O_7$, 0.3 mM CTAB	9.19
Indirect borate	15 mM $Na_2B_4O_7$, 0.3 mM CTAB, 0.1 mM DHBA	9.19
Indirect MES/His	20 mM MES, 20 mM His, 0.1 mM DHBA	6.00

The fabrication of equipment setup was described in reference [21]. The microchip was fixed on a Plexiglass holder that integrated a precisely three-dimensional system (Shanghai Lianyi Instrument Factory of Optical Fiber and Laser, Shanghai, China) with the precision of 1 μm in each direction. A clip of optical fiber that can be fastened in the three-dimensional system was used to clip the working electrode. The amperometric detector was located in the detection reservoir (at the channel outlet side) and consisted of an Ag/AgCl wire reference electrode, a Pt wire counter electrode and a laboratory-made carbon disk working electrode (I.D. 300 μm). The working electrode was placed at the channel outlet, the distance between its surface and the channel outlet (10 μm) was controlled by a stereoscopic microscope with micro-ruler (XTB-1; Jiangnan Optical Instrument Factory, Nanjing, China). Electrical contact with the solutions was achieved by placing platinum wires into each of the reservoirs.

The laboratory-made power supply had a voltage ranging from 0 to 5000 and 0 to −5000 V. Parameters, such as sampling voltage, sampling time, separation voltage and separation time can be set up and automatically switched by personal computer. The separation current can be monitored graphically in real time.

End-channel amperometric detection was performed with a CHI 832b electrochemical workstation (CHI, Shanghai, China).

2.3. Electrophoresis procedures

Measurements were performed with the following procedures. In all cases, degassed buffer was introduced into the reservoirs and flushed through the channel via vacuum. The circulation conditions of the microchannel can be judged by the separation or sampling current displayed on the computer screen. A laboratory-made computer program for the power supply was used to control the voltage switching from sampling to separation. Sampling mode was simple crossing without pinch. Before the analysis of analytes, the sampling and wasting buffer cells were filled with running buffer solution. During the separation procedure, the sampling and wasting buffer cells were kept floating. In a routine CE procedure, separation voltage was 1000 V, sampling voltage was 800 V and sampling time was 2 s, detection voltage +1.2 V (versus Ag/AgCl wire). All experiments were performed at room temperature.

Prior to measurement of EOF rates, the current at different voltages was measured to determine whether the relationship between the current and voltage was linear (to identify significant Joule heating). For all the solutions in the assay, the current was proportional to the voltage in the range of 600–2000 V. Therefore, all the experiments were performed at 1000 V.

3. Results and discussion

3.1. System peaks in indirect method

The KRF [20] 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 formulation of the KRF as in Eq. (1) has a restricted use: (1) all constituents must be univalent and (2) the concentration of H^+ ions and OH^- ions must be negligible compared to the concentration of the 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.

If a sample solution is introduced with a ω value different from that of the BGE, this deviating ω 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 electroosmosis, the above zone is driven by EOF, and may be detected and serve as a marker for measuring the EOF.

Amperometric detection of sampling zone involves the addition of a detectable ionic species DHBA to BGE. Provided that a higher concentration BGE-like solution without DHBA was injected as sample, the probe DHBA will be at different concentrations in different zones by adapting the initial ω value during the electrophoretic process. A schematic diagram outlining this process is shown in Fig. 1.

Although the process has been simplified and it is likely that many processes are occurring simultaneously, the detectability is based on the properties of detector-active component which results in a universal detection scheme. 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 versus the Ag/AgCl wire, 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 will appear.

3.2. EOF measurements

3.2.1. Cathodic EOF measurement

Fig. 2 displays a typical indirect detection plot, two peaks are observed, the first peak represents a zone in which the DHBA is virtually absent (assuming diffusion is neglectable) and therefore is positive. This vacancy peak is present because the sample was the BGE-like solution with higher concentration PBS without DHBA. The second peak represents the sampling zone that migrates with the EOF and the migration time corresponds to the electroosmotic flow. Since the concentration of PBS in the sample solution is higher than that in running buffer. In this zone, DHBA is concentrated by adapting the ω value of KRF, a negative peak appears.

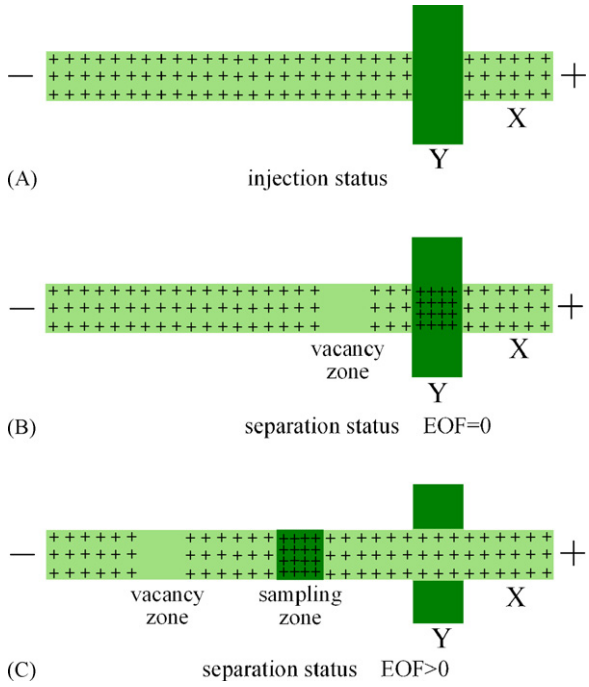

Fig. 1. Schematic diagram of distribution of detector-active species ions in the channel. X, separation channel; Y, injection channel. Symbol "+" represents cation of detector-active species, chroma of the colour represents different concentration of electrolyte. (A) prior to separation voltage applied, a higher concentration buffer filled in the sample channel, detector-active species and lower concentration buffer filled in the separation channel. (B) After a short time of separation voltage applied, assuming that EOF = 0, cationic ions of detector-active species migrate in electrophoretic way, a blank zone denotes that detector-active species is absent. The deviating ω value remains valid at the spot of injection, cationic ions of detector-active species were concentrated in this region. (C) After a short time of separation voltage applied, when EOF > 0, the injection spot migrates with the velocity of the EOF through the capillary.

3.2.2. Anodic EOF measurement

A reversed EOF was also measured by this sampling zone detection amperometric method in glass microchip. 0.3 mM of CTAB was added to BGE as modifier to change EOF direction and a reversed EOF was measured (Fig. 3). Peak c represents vacancy zone, peak d represents sampling zone and peaks a and b represent the negative coions in the sample.

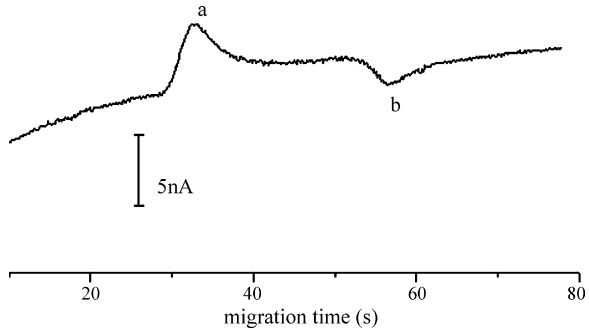

Fig. 2. Electrophoregram in hybrid PDMS/glass channel for cathodic EOF measurement. Peak a, vacancy zone of DHBA; peak b, sampling zone corresponding to EOF. Experimental parameters: sample 30 mM PBS; running buffer 20 mM PBS + 0.1 mM DHBA pH 6.95; sampling voltage 800 V; sampling time 2 s; separation voltage 1000 V; detection potential +1.2 V.

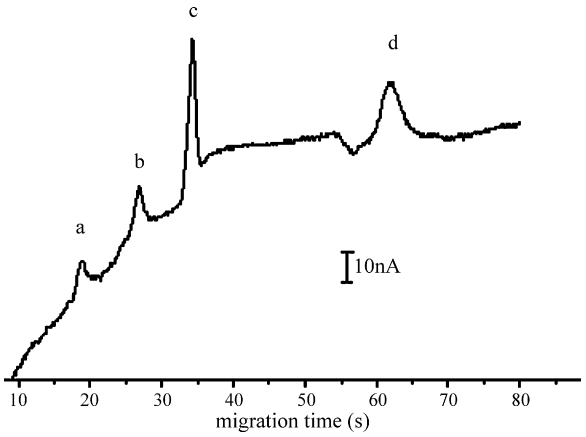

Fig. 3. Electrophoregram for anodic EOF measurement in glass channel. Peaks a and b, coions in the sample; peak c, vacancy zone of DHBA; peak d, sampling zone corresponding to EOF. Experimental parameters: sample 12 mM borate + 0.2 mM CTAB pH 9.19; running buffer 15 mM borate + 0.3 mM CTAB + 0.1 mM DHBA pH 9.19; sampling voltage −800 V; sampling time 2 s; separation voltage −1000 V; detection potential +1.2 V.

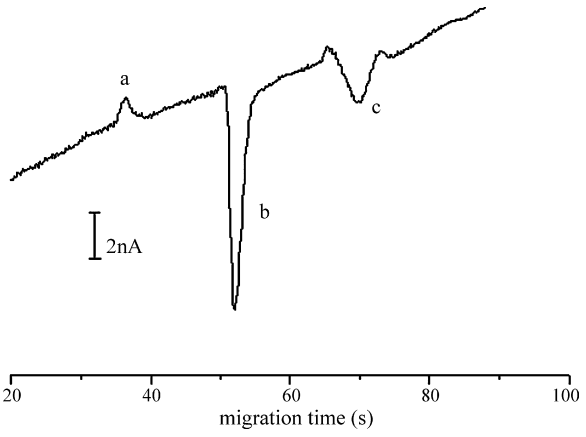

Fig. 4. Electrophoregram in glass channel for effective mobilities determination of electroactive analytes. Peak a, vacancy zone of DHBA; peak b, AP zone; peak c, sampling zone corresponding to EOF. Experimental parameters: sample 30 mM MES/His + 0.7 mM AP; running buffer 20 mM MES/His + 0.1 mM DHBA pH 6.00; sampling voltage 800 V; sampling time 2 s; separation voltage 1000 V; detection potential +1.2 V.

Cathodic and anodic EOF rates were measured by present indirect amperometry and current-monitoring method [8]. In indirect amperometric method, the BGE with 0.1 mM of DHBA was used as running buffer, dilute BGE-like solution at the concentration $c' = 0.75c$ (c: concentration of BGE) was injected as sample. In current-monitoring method, the BGE at the concentration c in the capillary, BGE-like solution at a different concentration $c' = 19/20c$ was used to displace previous solution. Table 2 shows the m_{EOF} values measured by indirect amperometry and current-monitoring method.

The present method provides precise values for EOF. The standard deviation of six flow measurements in different channels was less than 2.8%. The accuracy of present method was assessed by current-monitoring method. The average relative deviation of EOF rates with regard to the conventional method was 2.1%.

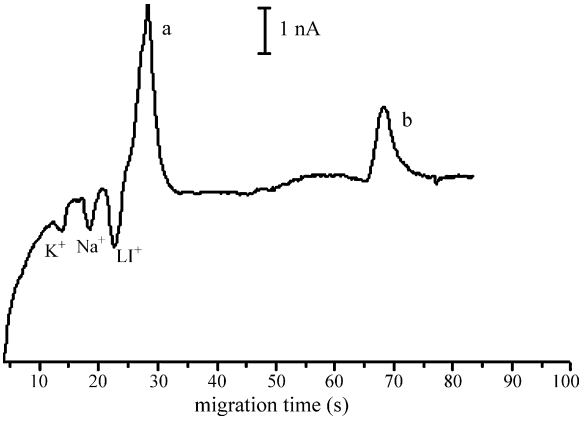

Fig. 5. Electrophoregram in glass channel of 200 μM K^+, Na^+ and Li^+. Peak a, vacancy zone of DHBA; peak b, sampling zone corresponding to EOF. Experimental parameters: running buffer 20 mM MES/His + 0.1 mM DHBA pH 6.00; sampling voltage 800 V; sampling time 2 s; separation voltage 1000 V; detection potential +1.2 V.

3.3. Effective mobilities determination

3.3.1. Effective mobilities of electroactive analytes

Fig. 4 displays an electrpherogram in indirect CE for a sample of detector-active species AP. There are three peaks in the plot, peaks a and c represent vacancy signal of DHBA and sampling zone, respectively, just like that in Fig. 2. The peak b is the signal of AP oxidation.

3.3.2. Effective mobilities of nonelectroactive analytes

In addition, K^+, Na^+ and Li^+ were successfully separated in a PDMS microchip and detected by the present indirect amperometric method as shown in Fig. 5.

According to the migration times of analytes and EOF, effective mobilities of analytes were accurately calculated in Table 3.

Table 2
Indirect amperometric method for EOF measurement

	Indirect method (10^{-4} cm^2/V s)	Current-monitoring method (10^{-4} cm^2/V s)	Relative deviation[a] (%)
Cathodic EOF[b]	1.42 ± 0.04	1.45 ± 0.08	2.1
Anodic EOF[c]	−3.07 ± 0.08	−3.11 ± 0.09	1.3

Six parallel measurements.
[a] With respect to current-monitoring method.
[b] Cathodic EOF: PDMS channel, 20 mM PBS pH 6.95 as running buffer.
[c] Anodic EOF: glass channel, 15 mM borate + 0.3 mM CTAB pH 9.19 as running buffer.

Table 3
Effective mobilities of analytes

Analyte	Effective mobility, m_i (10^{-4} cm^2/V s)
AP	0.81 ± 0.04
K^+	6.95 ± 0.13
Na^+	4.83 ± 0.09
Li^+	3.51 ± 0.07
DHBA	2.47 ± 0.11

Six parallel measurements.

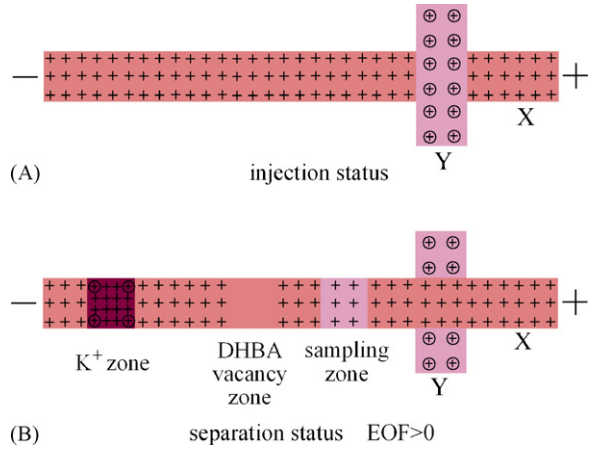

Fig. 6. Schematic diagram of K^+ zone migration in the channel. X, separation channel; Y, injection channel. Symbol "+" represents cation of detector-active species DHBA, "⊕" represents K^+. Chroma of the colour represents different concentration of electrolyte. (A) Prior to separation voltage applied, a lower concentration BGE-like solution containing K^+ filled in the sampling channel, and higher concentration buffer containing DHBA filled in the separation channel. (B) After a short time of separation voltage applied, when EOF > 0, in K^+ zone, cationic ions of DHBA was concentrated and a blank zone denotes that DHBA is absent. The deviating ω value remains valid in the sampling zone, cationic ions of DHBA were diluted in this region.

An uncommonly understood phenomenon in Fig. 5 is that the sign (positive or negative) of the peaks corresponding to K^+, Na^+ and Li^+ is negative. According to the response mechanism of analytes in indirect method, the sign and the magnitude of the peaks of nonelectroactive analytes correspond to the adjustment of the BGE concentration to the KRF of the sample. The sign of the peaks of K^+, Na^+ and Li^+ in the measurement represents that the concentration of DHBA in these zones is increased, but not the expectant result of being diluted according to the principle of coions displacement [22]. The phenomenon was also reported in CE by Desiderio et al. before [23].

The response mode was proposed with K^+ as model analyte. We speculated that K^+ migration velocity was faster than the coions in the electrolyte, then the ability of electronic transfer in the zone that K^+ penetrated was enhanced. Because the electric current in the channel was constant, the pressure distributed in the K^+ zone was decreased. The cations in the electrolyte in K^+ zone migrated slower than that in other zones. The concentration of coions in K^+ zone was increased as a result due to migrating slowly. In indirect amperometry, the concentration of DHBA in K^+ zone was increased deservedly. Schematic state was shown in Fig. 6.

4. Conclusions

The mechanism of EOF measurement with detection of sampling zone was schematically described. Either cathodic or anionic EOF could be measured accurately. The KRF difference between sampling zone and BGE can also be used in other detection methods for measuring EOF. Electroactive and nonelectroactive analytes were detected in the indirect method, and accurate effective mobilities could be calculated, system error is eliminated because the migration times of analytes and EOF is obtained in the same assay. The response mechanism of nonelectroactive analytes was speculated.

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