

# A New Method to Measure Electroosmotic Flow Mobility of Capillary Electrophoresis by Abrupt Change of Current De-noising via Wavelet Transform

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The electroosmotic flow mobility has been measured by the combination of monitoring the change in electric current during electrophoretic run and operating the wavelet transform. Once the sample solvent zone with different ionic strength from background electrolyte migrated from the capillary, a sudden change in current could be observed from the electric current record of time history. The exact time (in the middle of abrupt range) corresponding to the abrupt change in current was determined by wavelet transform. This work showed that the electroosmotic flow mobility determined by the proposed method was in a good agreement with the neutral marker method commonly used.

**Keywords** Capillary electrophoresis, wavelet transform, electroosmosis measurement, abrupt change of current

## Introduction

Capillary electrophoresis (CE) is attracting much attention as a new separation technique.<sup>1-3</sup> In CE, the application of an electric field to a capillary filled with an electrolyte solution may generate an electroosmotic flow. A precise characterization of the electroosmotic flow is highly desirable not only for a through characterization of an electrophoretic system, but also for optimizing the operation of CE in analyzing a given sample. Upon application of an electric field, a neutral compound dragged with the electrolyte solution has been traditionally used to measure electroosmosis.<sup>4-7</sup> Stringent require-

ments for true neutrality, detectability, and inertness toward the capillary wall are among the major limitations associated with this method. Recently many approaches<sup>8-22</sup> have been proposed as alternatives to the neutral marker method to estimate electroosmosis in capillary. All of these procedures have been demonstrated to give the reliable measurements of electroosmotic flow rate, provided that some cares are taken.

In this paper, the performance of a new method capable of measuring the electroosmotic flow in CE based on the abrupt change of current by wavelet transform of de-noising was described. In electrophoresis experiments, under the action of the applied electric field, the electrolyte moves toward one end of the capillary, and this movement is referred to as the electroosmotic flow. In addition to the bulk flow of the electrolyte, electrophoresis also takes place, that is, the applied electric field exerts a force on charged species. As a result, the injected sample with multi-component is separated into distinct zones corresponding to their different mobilities. Because the neutral solvent zone is only under the influence of the bulk flow of the electrolyte, its running rate is equal to the electroosmotic flow rate. When the neutral solvent zone migrates out of capillary, the whole average conductivity in capillary would have a large degree of change. Accompanied by the change of conductivity, the current has an immediate visible change because there is a direct proportion upon the current and the con-

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ductivity of the CE circuit. It seems easy to acquire the relevant information ( $\Delta I$ ,  $t_i$ ) of the current change from the record of time history of the electric current. Unfortunately, experimental spectra are often complicated by noise, which may come from interfering physical or chemical processes, imperfections in the experimental apparatus, or maybe originate from random, spurious fluctuation signal received by the detector. In common, the signal-to-noise ratio of the current signal obtained by a commercial instrument is so small that the relevant information ( $\Delta I$ ,  $t_i$ ) can not be easily obtained. In order to surmount this drawback and widen the application of this new method, an up-to-date wavelet transform is used in this paper. Wavelets<sup>23,24</sup> are mathematical functions that cut up data into different frequency components, and then study each component with a resolution matched to its scale. They have advantages over traditional Fourier methods in analyzing physical situations where the signal contains discontinuities and sharp spikes. Unlike the Fourier transform, the wavelet transform has dual localization both in frequency and in time. In this paper, the wavelet transform is successfully applied to filter high frequency noise in CE current signal by means of Haar wavelet function. The effects of sample concentration, applied voltage and injection time on the magnitude of sudden change in current were investigated in detail. The experiment data obtained by wavelet transform were used directly without introducing artificial error factors in recognition. By de-noising, the time-point for measurement of electroosmotic flow was performed automatically and very satisfactory calculation results were obtained. In real sample analysis, this method did not require addition of neutral marker to sample solution, and it eliminated the possibility of reaction between the used electrolyte and the neutral marker, so the method was more accurate than the neural marker method.

## Experimental

### Apparatus

A Waters Quanta 4000 CE system (Waters chromatography Division of Milford, MA, USA) was used in the present work. All experiments were performed in uncoated fused silica capillaries manufactured by Yongnian Photoconductive Fibre Factory (Hebei, China). Sam-

ples were introduced from the anodic end of the capillary by hydrostatic injection where the sample vial was raised by 10.0 cm. Data acquisition was carried out with a Maxima 820 chromatography workstation.

### Reagents

All chemicals employed were of analytical grade. All solutions were prepared with distilled and deionized water.

Sodium tetraborate, sodium hydrogenphosphate, sodium dihydrogenphosphate, Tris, and sodium dodecyl sulfate (SDS) were prepared as 0.1 M stocking solutions. The final solutions were prepared by dilution and mixing from the stock solutions. The pH of the electrophoretic buffer was adjusted by 0.1 M HCl or NaOH to the desired values.

### Procedure

Electrophoresis was carried out in a bare capillary after preconditioning with 1 M NaOH solution for 20 min followed by rinsing with water (5 min) and electrolyte (5 min). This treatment was routinely applied at the beginning of every working day. Other conditions were specified as required.

The computer program of wavelet transform was written in MATLAB, and all analyses were performed on a Pentium II 400 MHz computer.

## Results and discussion

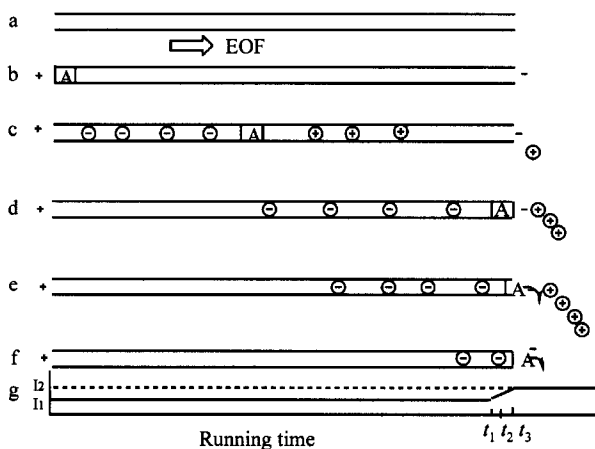
### Origins of the abrupt change of current

The schematic representation of origins of the abrupt change of current during the electrophoretic separation process is shown in Fig. 1. Once the sample solvent zone with different ionic strength from background electrolyte migrates from the capillary, a sudden change in current can be observed from the record of time history of the electric current during CE operation.

If the zone broadening is assumed negligible, the current between  $t_0$  and  $t_1$  (step (b) to (d) in Fig. 1), can be approximately expressed:

$$I_1 = \frac{V}{R_{\text{total}}} = \frac{V}{\rho_b(t) \frac{L - L_s}{S} + \rho_s(t) \frac{L_s}{S}} \quad (1)$$

where  $V$  is the applied voltage,  $R_{\text{total}}$  the average electric resistant,  $\rho_b(t)$  and  $\rho_s(t)$  are the resistivity functions depending upon the running time for the background electrolyte (BGE) and the sample zone,  $L$  and  $L_s$  the total length of the capillary and the injection sample zone length, and  $S$  is the section area of the capillary.



**Fig. 1** Schematic representation of origins of the abrupt change of current during the electrophoretic separation process. Step (a): the capillary tube was rinsed with electrolyte; Step (b): a sample injection was made and electric field was applied. Step (c): the charged species and the sample solvent were running in the capillary tube; Step (e) to step (f): the short process of the sample solvent migrated out of capillary tube, (g) was the schematic current signal in CE, where  $A$  is the representation of the solvent of sample,  $t_1$ ,  $t_2$  and  $t_3$  are corresponding to the start, middle and end point time in the current change profile.

When the sample zone migrates completely out of the capillary ( $t_3 \rightarrow$ ), the current ( $I_2$ ) through the capillary can be expressed as follows:

$$I_2 = \frac{V}{\rho_b(t) \frac{L}{S}} \quad (2)$$

Combining Eq. (1) and (2), the degree of the current change ( $\Delta I$ ) can be expressed:

$$\Delta I = I_2 - I_1 = \frac{VS}{L\rho_b(t)} \frac{\rho_s(t) - \rho_b(t)}{\rho_b(t) \frac{L}{L_s} - \rho_b(t) + \rho_s(t)} \quad (3)$$

### Relevant information extraction by wavelet transform

The wavelet transform is a versatility tool of scientific research. Any process that involves the analysis of a time series is a good candidate for the wavelet treatment. An insight into chemical systems or phenomena via instrumental signals measured can be significantly improved by signal de-noising and extraction of its relevant features.

The purpose of the wavelet transform procedure is to decompose a signal into localized contributions that are characterized by a so-called scale parameter. The mathematical techniques to perform this decomposition have been described in many papers.<sup>25,26</sup> The wavelet transform realizes a correspondence between time domain  $t$  and frequency domain  $\omega$ , by convoluting the signal  $S(t)$  to be analyzed with a given wavelet function  $\psi(t)$  defined in the two-dimensional space  $[\omega, t]$ .

A common algorithm for calculating discrete wavelet coefficients is the so-called Mallat pyramid algorithm.<sup>27</sup> The discrete signal  $S(t)$  can be represented with  $\{C_n^0\}$ ,  $n \in Z$ . The wavelet function can also be represented by high-pass filter (H) and low-pass filter (L). At each scale high (H) and low (L) pass filters are applied to the input signal. The actual shapes of these filters are determined by the kind of wavelet function used. The output from the high-pass filter at each scale is recorded as the wavelet coefficients. The low-pass filter extracts the low frequency components for the next scale where another set of high and low-pass filters is employed.

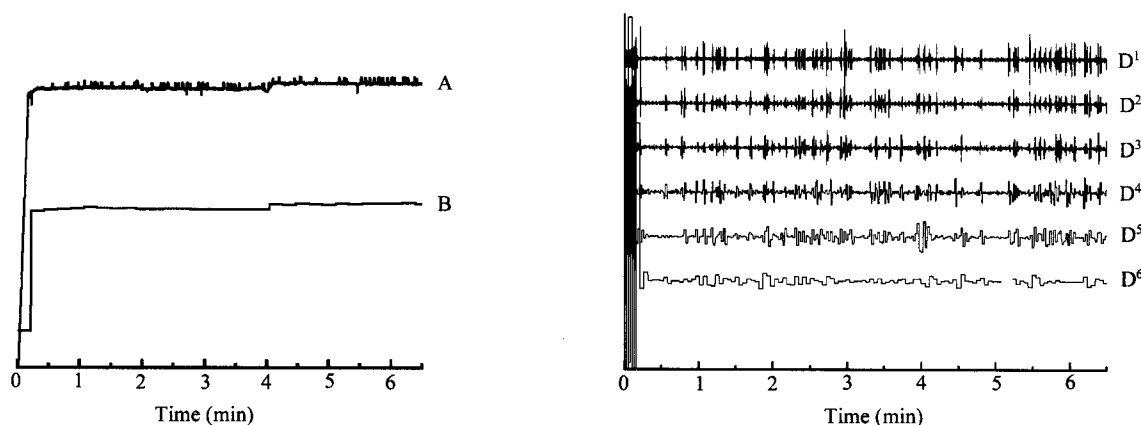
In the present paper, in order to acquire information (such as  $t_i$  and  $\Delta I$ ) from current signals in time domain, a Haar mother wavelet function was used. These approximations and details are shown in Fig. 2 (Low-pass coefficients represented the approximation of original signal and the high-pass coefficients represented noise).

### Effect of the sample concentration, applied voltage and injection time on current increment

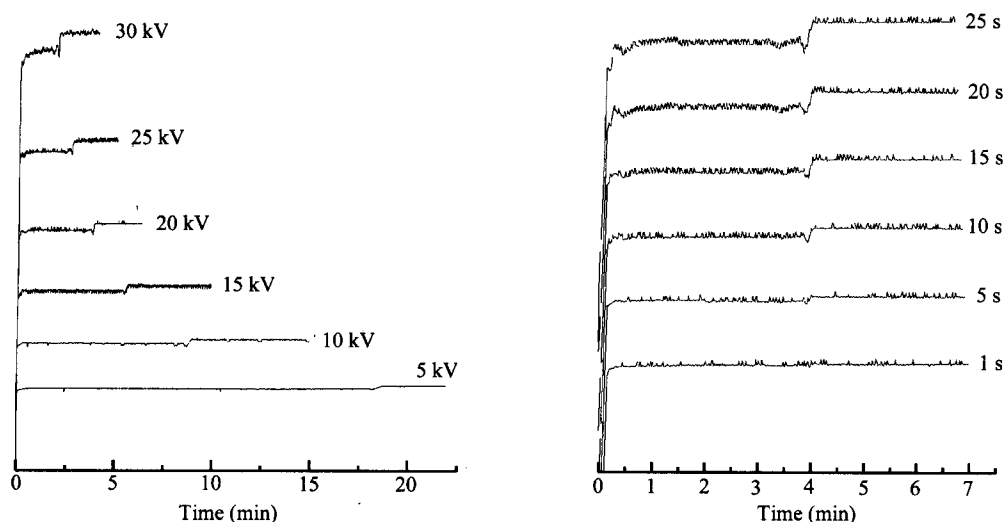
The influences of the sample concentration, applied voltage and injection time on the magnitude of current change were studied in detail using different electrolytes. When the sample concentration was higher than the buffer concentration, the current increment was nega-

tive. Contrarily, the current increment was positive. If the concentration of sample and buffer was equal, the current kept constant in the whole running procedure. In order to find more information about the influence of the sample concentration on the magnitude of current change, we made a non-linear regression between the current change ( $\Delta I$ ) and the buffer concentration ( $C_s$ ) used as the solvent for samples (CE conditions; applied voltage, 20.0 kV, injection time, 10 s, electric buffer, 15 mM  $\text{Na}_2\text{B}_4\text{O}_7$ ):

$$\Delta I = 0.0011 - \frac{0.0013}{\sqrt{\pi/2}} \exp \frac{-(C_s - 16.04)^2}{235.69} \quad (4)$$



**Fig. 2** Scale decomposition of current signal (the scale factor is 7). Left; Original signal (A) and signal treated by wavelet transform (B); Right; High frequency parts (corresponding to the noise signal). Condition: electrolyte, 18 mM  $\text{Na}_2\text{B}_4\text{O}_7$ ; Capillary;  $75 \mu\text{m} \times 48.35 \text{ cm}$  (total length 56 cm); applied voltage: 20 kV; sample injection time; 10 s; sample: 4 mM  $\text{Na}_2\text{B}_4\text{O}_7$ .



**Fig. 3** Relationship between the current and the applied voltage (left) and the sample injection time (right). Conditions: capillary,  $75 \mu\text{m} \times 48.35 \text{ cm}$  (total length 56.0 cm); sample,  $\text{C}_2\text{H}_5\text{OH}:\text{H}_2\text{O}$  (1:1), buffer, 15 mM  $\text{Na}_2\text{B}_4\text{O}_7$ , temperature,  $23.0 \pm 1^\circ\text{C}$ .

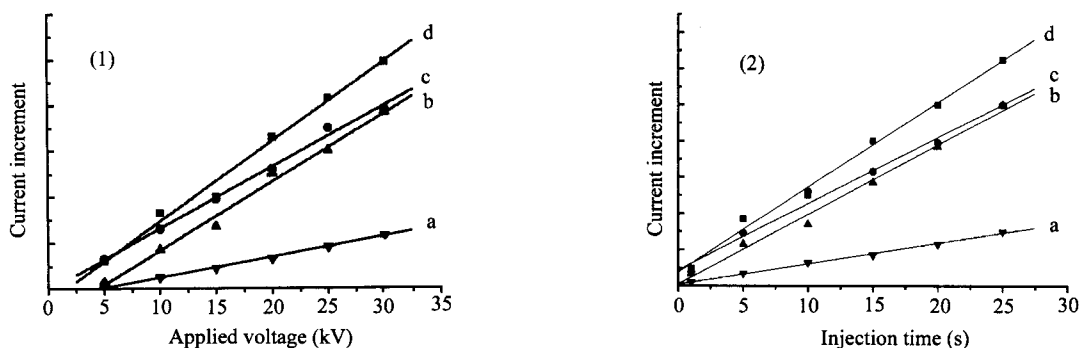
The influences of the applied voltage and injection time are shown in Fig. 3. As shown in Fig. 3, the current increment increased with applied voltage (left) and the injection time (right). In the following study, the linear regressions for four different buffer conditions were performed, and the results are shown in Fig. 4 (all coefficients were over 0.99). These results supported the conclusion from Eq. (3) that the increment of current ( $\Delta I$ ) was proportional to the applied voltage ( $V$ ) and the sample zone length ( $L_s$ ) dependent on the injection time ( $t_{\text{inj}}$ ). From Fig. 4, another important result could also be found easily, *i. e.*, the slopes of the current increment in different buffers varied seriously.

## EOF mobility calculation

To calculate EOF mobility from the running time corresponding to the sudden change in current, it was needed that the formula commonly used for the calculating EOF mobility changed into Eq. (5).

$$\mu_{\text{EOF}} = \frac{V_{\text{EOF}}}{E} = \frac{L^2}{t_i V} \quad (5)$$

where  $L$  is the total length of the capillary,  $t_i$  (corresponding to  $t_1$ ,  $t_2 = (t_1 + t_3)/2$  and  $t_3$ ) is the migration time, and  $V$  is the applied voltage.



**Fig. 4** Dependence of the current increment on the applied voltage (1) and the sample injection time (2). Conditions; capillary,  $75 \mu\text{m} \times 48.35 \text{ cm}$  (total length  $56.0 \text{ cm}$ ); sample,  $\text{C}_2\text{H}_5\text{OH}:\text{H}_2\text{O}$  (1:1), temperature,  $23.0 \pm 1^\circ\text{C}$ ; injection time (1)  $10 \text{ s}$ ; applied voltage (2)  $20 \text{ kV}$ ; electrolytes; a,  $15 \text{ mM Na}_2\text{B}_4\text{O}_7$ ; b,  $40 \text{ mM Na}_2\text{B}_4\text{O}_7$ ; c,  $20 \text{ mM Na}_2\text{HPO}_4 + 15 \text{ mM SDS}$ ; d,  $20 \text{ mM Na}_2\text{HPO}_4 + 25 \text{ mM SDS}$ .

To correctly calculate the EOF by means of Eq. (5), we must find out which is the most proper variable for  $t_i$  in these three variables,  $t_1$ ,  $t_2$  and  $t_3$ . The results by Eq. (5) using  $t_2$  as  $t_i$  sounded reasonable (correlation coefficient  $r = 0.999$ ) because the slope and intercept of the linear regression were  $1.0$  and  $-0.009$ , respectively (Ideally the slope should be  $1.00$  and the intercept should be  $0.00$ ). When using  $t_1$  or  $t_3$  as the calculation time, there was a significant difference between the proposed method and the neutral marker method (their intercepts were  $0.12948$  and  $-0.081$ ). So  $t_2$  should be selected as the calculation time. With the knowledge of the total  $L$  length of the capillary, applied voltage and the middle time  $t_2$  of the sudden change of current, the EOF mobility could be calculated.

*Evaluation of the new method under different conditions and measurement of electroosmotic flow in real sample separation analysis by the new method*

In order to justify the method for the calculation of EOF mobility established in the present work, a comparison between the proposed method and the conventional method was made in forty different conditions, and some results are listed in Table 1. To check whether there was

a significant difference between the two methods, a 't' test was used in all conditions. 't' values were in the range  $0$  and  $9.651 E - 2$  (at the level  $0.05$ ), indicating that there was no significant difference between the new and conventional approaches.

In order to further assess the method, the EOF mobilities were determined using the proposed method during real sample separation analysis. The results obtained are listed in Table 2.

The proposed method has a general validity under various conditions. However, in case the conductivity of sample is close to that of the background electrolyte, the change of current will be too weak to be detected by the commercial capillary electrophoresis system, and thus the method is not applicable under these conditions.

## Conclusion

The utilization of the time-point of the abrupt change of current de-noised through wavelet transform has been proved to be a correct, simple way to measure EOF mobility in CE. In real sample analysis, because the neutral marker was not necessary, which could eliminate the possibilities of reactions of the system. Therefore the stringent requirements for true neutrality, de-

tectability, and inertness toward the capillary wall for marker were not necessary to be considered. Moreover, this on-line simultaneous method could maximally avoid all the experimental and instrumental errors. Therefore,

it resembled the experimental conditions most closely, and could be more precise for the characterization of the electroosmotic flow in CE analyses.

**Table 1** Comparison between the conventional neutral maker method and the proposed method

Conditions		EOF mobility ( $10^{-4}$ cm <sup>2</sup> /(V·s))			
Background electrolyte (mM)	Applied voltage (kV)	Neutral maker method	RSD (%)	Proposed method	RSD (%)
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> :30	20	6.203	1.0	6.205	0.54
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> :20	20	6.465	0.86	6.470	0.33
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> :5	20	7.371	0.62	7.372	0.26
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> :15	25	8.963	0.88	8.961	0.37
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> :15	15	4.655	1.17	4.652	0.58
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> :15	10	2.957	1.07	2.961	0.35
NaH <sub>2</sub> PO <sub>4</sub> :20	20	6.920	0.63	6.907	0.72
Tris-HCl:20 (pH 8.16)	30	9.081	0.65	9.081	0.56
NaAc-HAc:20 (pH 4.81)	30	6.211	0.97	6.203	0.78
NaAc-HAc:40 (pH 5.20)	30	7.125	0.45	7.141	0.32
NaAc-HAc:40 (pH 5.20)	15	2.043	1.04	2.032	0.42
NaH <sub>2</sub> PO <sub>4</sub> :20-SDS:5	20	6.721	0.98	6.723	0.54
NaH <sub>2</sub> PO <sub>4</sub> :20-SDS:10	20	6.607	0.87	6.599	0.32
NaH <sub>2</sub> PO <sub>4</sub> :20-SDS:15	20	6.632	1.23	6.631	0.21
NaH <sub>2</sub> PO <sub>4</sub> :20-SDS:20	20	6.912	1.45	6.914	0.12
NaH <sub>2</sub> PO <sub>4</sub> :20-SDS:25	20	7.003	1.32	7.007	0.42

Five replicates for each method. Conditions: capillary, 75  $\mu$ m  $\times$  56.0 cm (48.35 cm effective length); electrolyte and applied voltage detailed on this Table; the neutral makers, methanol, ethanol and benzene (only in CZE); detected wavelength, 254 nm; injection, the sample vial raised 10.0 cm for 10 s; temperature, 23.0  $\pm$  1  $^{\circ}$ C. The time-point ( $t_2$ ) of the abrupt change of current gained automatically by wavelet transform.

**Table 2** Calculated electroosmotic flow mobility of real samples by the proposed method and the neutral marker method

No.	EOF mobility ( $10^{-4}$ cm <sup>2</sup> /(V·s))			
	Neutral marker method	RSD (%)	Proposed method	RSD (%)
1 #	3.561	0.52	3.573	0.23
2 #	7.182	0.43	7.184	0.15

Eight replicates for each method.

No. 1 #: Sample, trimethoprim (0.04 mg/mL)-sulfamethoxazole (0.08 mg/mL), solvent, 70% ethanol; fused silica capillary, 75  $\mu$ m  $\times$  56.0 cm (48.5 cm effective length); background electrolyte, 30 mM pH 5.55 HOAc-NaOAc; UV detection wavelength, 254 nm; applied voltage 20.0 kV; neutral marker, toluene; temperature, 20.0  $\pm$  1  $^{\circ}$ C.

No. 2 #: Sample, Vitamin B1, Vitamin B6, nicotinic acid; electrolyte, 5 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>; applied voltage, 14 kV; capillary, 75  $\mu$ m  $\times$  56.0 cm (48.5 cm effective length); injection, 20 s; detection, UV 254 nm.

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