**Wenrui Jin**
**Daiqing Yu**
**Qian Dong**
**Xiaoying Ye**

Laboratory of Analytical Science, School of Chemistry, Shandong University, Jinan, China

# A new method of quantification of pipemidic acid by capillary zone electrophoresis with end-column amperometric detection

Capillary zone electrophoresis was employed for the determination of pipemidic acid using an end-column amperometric detection with a carbon fiber microdisk array electrode, at a constant potential of –1.10 V *vs.* saturated calomel electrode. The optimum conditions of separation and detection were $1.2 \times 10^{-4}$ mol/LNaOAc – $8.8 \times 10^{-4}$ mol/LHOAc for the buffer solution, 20 kV for the separation voltage, 5 kV and 10 s for the injection voltage and the injection time. The limit of detection was $1.05 \times 10^{-6}$ mol/L or 189 amol (S/N=3). The relative standard deviation was 0.31% for the migration time and 2.0% for the electrophoretic peak current. The method was applied to determining pipemidic acid in human serum.

**Keywords:** Electrophoresis / Electrochemical detection / Detection / Electrodes / Pipemidic acid / Drug / Medicine EL 3683

## 1 Introduction

Pipemidic acid, 8-ethyl-5,8-dihydro-5-oxo-2-(1-pipezazinyl)pyrido[2,3-d] pyrimidine-6-carboxylic acid, is known to be a synthetic antibacterial agent [1]. The use of high performance liquid chromatography has been reported for drug determination [2–5]. Nevertheless, there are no reports on the determination of pipemidic acid by capillary zone electrophoresis (CZE). Recently, CZE has become an important instrumental technique suitable for rapid separation and detection of complex mixtures [6–8]. The primary strength of CZE is its ability to provide extremely high separation efficiencies in short times and to do so with relatively simple instrumentation. Amperometric detection provides excellent sensitivity for the small dimensions associated with CZE, while offering a high degree of selectivity towards electroactive species and low cost [9]. In our laboratory this technique has been applied to cysteine [10], glutathione [11], purine bases [12–14], bovine serum albumin [15], and cytochrome *c* [16]. The theory concerning the current for the end-column amperometric detector in CE has been investigated [17, 18].

In this article we developed a method for detection of pipemidic acid with the end-column amperometric detection at a carbon fiber microdisk array electrode. The separation was performed in a 25 μm ID fused-silica capillary. The detection was carried out by using potentiostatic control of the electrode potential by means of a three-electrode system. The method has been used to determine pipemidic acid in human serum.

**Correspondence:** Professor Wenrui Jin, Laboratory of Analytical Science, School of Chemistry, Shandong University, Jinan, 250100 China
**E-mail:** wenrujin@jn-public.sd.cninfo.net
**Fax:** +86-531-8565167

**Abbreviation: SCE**, saturated calomel electrode

## 2 Materials and methods

### 2.1 Apparatus

#### 2.1.1 Linear sweep cyclic voltammetry

A commercial polarograph (Model 83-2.5; Ningde Analytical Instruments, Ningde, China) coupled with an X-Y recorder (Model 3086-11; Yokogawa Hokuskin, Tokyo, Japan) was used in connection with a cell using potentiostatic control of the electrode potential by means of a three-electrode system, which consisted of a carbon fiber array electrode as the working electrode, a Pt wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. The reference electrode connected to the analyte *via* a salt bridge was filled with the same supporting electrolyte as the cell.

#### 2.1.2 CZE

A reversible high-voltage power supply (Model GDY, Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, China) provided a variable voltage of 0–30 kV across the capillary with an outlet of the capillary at ground potential. Fused-silica capillaries (360 μm OD, 25 μm ID) were purchased from Yongnian Optical Conductive Fiber Plant, Yongnian, China. They were cut to a length of 35 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential.



 0173-0835/00/0505-0925 $17.50+.50/0

Separations were carried out at an applied voltage of 20 kV. The electrochemical detection at a constant potential with CZE was performed using the end-column amperometric approach with a voltammetric analyzer (Model JF-01; Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, Jinan). The detection cell and detector were housed in a faradaic cage in order to minimize the interference from external sources of noise. Electrochemical detection was carried out with a three-electrode system. It consisted of a carbon fiber microdisk array electrode as the working electrode, a coiled Pt wire as the auxiliary electrode, which also served as the ground for the high potential drop across the capillary mentioned above, and an SCE as the reference electrode. The arrangement of the electrochemical detection cell has been illustrated in detail [15].

### 2.1.3 Carbon fiber electrodes

In linear-sweep cyclic voltammetry the carbon fiber array electrodes were used. A small amount of mercury was drawn into the glass capillary (*ca.* 0.5 mm ID, 1 mm OD, 5 cm length). About 60 carbon fibers soaking up acetone with 6 μm diameter were carefully inserted into the glass capillary at the other end. The carbon fiber array was connected to a copper wire (0.4 mm diameter, 12 cm length) *via* the mercury junction by pushing a copper wire down. The other end of the copper wire and the carbon fiber array after drying were bonded to the glass capillary using a low viscosity ethyl α-cyanoacrylate adhesive. The carbon fibers and the adhesive were mixed by lightly touching with a glass bar. A glass tube (1.5 mm ID, 8 mm OD, 6 cm length) was placed outside the glass capillary in order to protect the glass capillary. The copper wire was bonded to the glass tube using epoxy. The carbon fiber array was bonded at the other end of the glass tube and protruded approximately 1 cm from the end. Then the carbon fibers were cut to 4 mm.

At CZE the carbon fiber microdisk array electrodes were constructed using 6 μm carbon fibers. The manufacturing process is similar to the carbon fiber array electrode described above. About 30 carbon fibers with acetone were inserted into a fused-silica capillary (*ca.* 250 μm ID, 375 μm OD, 1.5 cm length). Next the carbon fiber array was immersed into the ethyl α-cyanoacrylate adhesive and the adhesive was passed through the whole carbon fiber array in the fused-silica capillary. Then, the fused-silica capillary with the carbon fiber array was inserted into a glass capillary (*ca.* 0.5 mm ID, 1 mm OD, 2.5 cm length) and was bonded together. Finally, the carbon fiber array protruding from the fused-silica capillary was cut. The carbon fiber microdisk array electrode is illustrated in

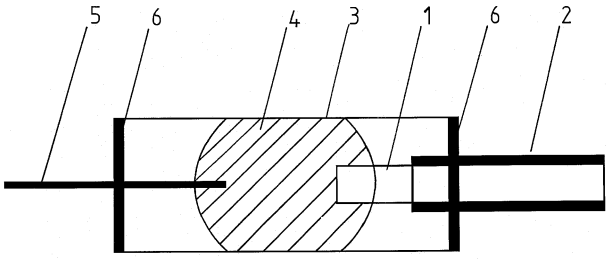


**Figure 1.** A top view of the carbon fiber microdisk array electrode. 1, Carbon fiber microdisk array with ethyl α-cyanoacrylate adhesive; 2, fused-silica capillary; 3, glass capillary; 4, mercury; 5, copper wire; 6, epoxy resin.

Fig. 1. Before use all carbon fiber microdisk array electrodes were cleaned in alcohol and washed with double distilled water for 5 min with a supersonic wave cleaner. During electrophoresis, the electrodes can be directly washed with alcohol and water in the detection cell.

### 2.2 Reagents and solutions

A $2.00 \times 10^{-4}$ mol/L stock solution of pipemidic acid was prepared by dissolving an appropriate amount of pipemidic acid in water and stored at 4°C in a refrigerator. Dilute solutions were obtained by serial dilution of the stock solution with water. All reagents were of analytical grade. All solutions were prepared with double distilled water.

### 2.3 Procedure

For linear-sweep cyclic voltammetry the carbon fiber array electrode was directly inserted in the experimental solution containing pipemidic acid and a linear sweep cyclic voltammogram was recorded. In CZE, the carbon fiber microdisk array electrode was cemented onto a microscope slide, which was placed over a homemade XYZ micromanipulator and glued in place. The position of the carbon fiber microdisk array electrode was adjusted (under a microscope) at the end of the capillary so that the electrode and the capillary were in contact. This arrangement allowed easy removal and realignment of both the capillary and the electrode. The other end of the capillary was inserted into a plastic syringe tip (the metal needle was removed before) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with double distilled water, 0.1 mol/L NaOH, double distilled water and the corresponding separation electrolyte, respectively, by means of a syringe. In addition, the electrolyte solution at the electrochemical cell was also replaced before each run. During the experiments the separation voltage was applied across the capillary and the detection potential was applied at the working electrode. After the electroosmotic current reached a

constant value (after 20 min), the electromigration injection was carried out and the electropherogram was recorded. The separation electrolyte in the capillary was replaced after five or six runs. All potentials were measured *vs.* SCE.

## 3 Results and discussion

### 3.1 Linear sweep cyclic voltammograms of pipemidic acid

The voltammetric characteristic of pipemidic acid has been reported at the hanging mercury drop electrode [19] and glassy carbon electrode [20]. We found that pipemidic acid can also be reduced at the carbon fiber array electrode in NaOAc-HOAc buffer (pH 3.8). Figure 2 shows its typical linear sweep cyclic voltammogram in the solution. There are two reduction peaks of pipemidic acid. Their peak potentials are –0.40 V and –1.00 V, respectively. No anodic peak is observed on the cyclic voltammogram.

### 3.2 Optimum conditions of CZE with end-column amperometric detection

At pH 3.8 there are two reduction peaks of pipemidic acid on the linear-sweep cyclic voltammogram shown in Fig. 2. Therefore, the electrophoretic behavior of pipemidic acid in five solutions at different pH around pH 3.8 was researched. The peak current, $i_p$, the migration time, $t_m$, the width at the half-peak, $W_{1/2}$, on the electropherograms, and the number of theoretical plates, $N$, at different pH are listed in Table 1. $N$ was calculated according to the following equation:

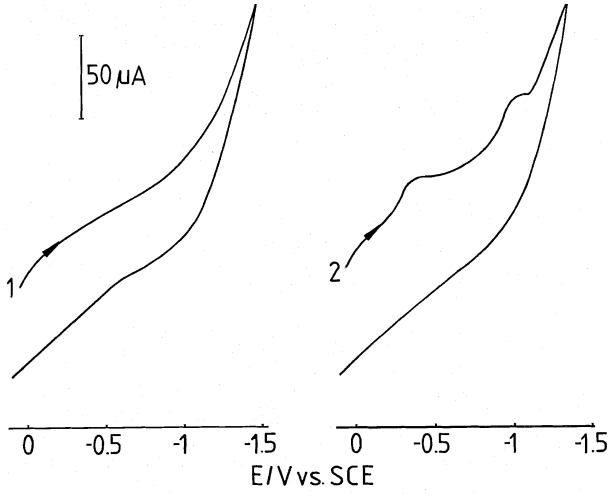


**Figure 2.** Typical linear sweep cyclic voltammogram of pipemidic acid at the carbon fiber array electrode in NaOAc-HOAc, pH 3.8. 1, $4.8 \times 10^{-3}$ mol/L NaOAc-3.5 $\times 10^{-2}$ mol/L HOAc; 2, (1) + $1.00 \times 10^{-4}$ mol/L pipemidic acid. $\nu$ = 100 mV/s.

**Table 1.** The values of $t_m$, $i_p$, $W_{1/2}$ and $N$ in NaOAc-HOAc at different pH

| Buffer | pH | $t_m$ (S) | $i_p$ (nA) | $W_{1/2}$ (S) | $N \times 10^{-3}$ |
|---|---|---|---|---|---|
| $7.5 \times 10^{-5}$ mol/L NaOAc –$9.3 \times 10^{-4}$ mol/L HOAc | 3.6 | 243.4 | 14.9 | 2.4 | 5.7 |
| $1.2 \times 10^{-4}$ mol/L NaOAc –$8.8 \times 10^{-4}$ mol/L HOAc | 3.8 | 238.4 | 18.4 | 2.4 | 5.5 |
| $1.8 \times 10^{-4}$ mol/L NaOAc –$8.2 \times 10^{-4}$ mol/L HOAc | 4.0 | 233.5 | 13.7 | 2.4 | 5.2 |
| $2.7 \times 10^{-4}$ mol/L NaOAc –$7.3 \times 10^{-4}$ mol/L HOAc | 4.2 | 231.9 | 5.10 | 2.4 | 5.2 |
| $3.7 \times 10^{-4}$ mol/L NaOAc –$6.3 \times 10^{-4}$ mol/L HOAc | 4.4 | 230.8 | 4.80 | 2.4 | 5.1 |

Conditions: $5.00 \times 10^{-4}$ mol/L pipemidic acid; capillary, 35 cm length, 25 μm ID; injection, 5 kV for 10 s; separation voltage, 20 kV; detection potential, – 1.20 V

$$N = 5.54 \left( \frac{t_m}{W_{1/2}} \right)^2 \quad (1)$$

$t_m$ decreases with increasing pH, but $W_{1/2}$ was constant; $N$ thus decreased with increasing pH; $i_p$ was the highest at pH 3.8. Therefore pH 3.8 was selected.

The effect of the buffer concentration, $C_B$, on $t_m$, $i_p$, $W_{1/2}$, and $N$ in NaOAc-HOAc of pH 3.8 is listed in Table 2. $C_B$ only indicates the value of the concentration of NaOAc. The ratio of the concentration of NaOAc to the concentration of HOAc is 1:7.33. $t_m$, $N$, and $i_p$ increase with increasing $C_B$. The migration velocity of the substance depends mainly on the electroosmotic velocity, $\nu_{eo}$, of buffer, which is proportional to $\zeta$ potential [21]. With increasing buffer concentration, the thickness of the electric double layer becomes thinner and the $\zeta$ potential becomes smaller. Therefore, $\nu_{eo}$ decreases and $t_m$ increases. $N$ increases because $t_m$ increases and $W_{1/2}$ is constant with increasing buffer concentration (see Eq. 1). In addition, when $\nu_{eo}$ decreases, pipemidic acid has a longer time to be in contact with the surface of the working electrode, which

**Table 2.** The values of $t_m$, $i_p$, $W_{1/2}$ and $N$ at different concentrations of $C_B$ in NaOAc-HOAc, pH 3.8

| $C_B \times 10^5$ (mol/L) | $t_m$ (S) | $i_p$ (nA) | $W_{1/2}$ (S) | $N \times 10^{-3}$ |
|---|---|---|---|---|
| 7.2 | 208.0 | 12.0 | 2.4 | 4.2 |
| 9.6 | 223.5 | 18.0 | 2.4 | 4.8 |
| 12.0 | 238.4 | 18.4 | 2.4 | 5.5 |
| 24.0 | 248.6 | 20.4 | 2.4 | 5.9 |
| 48.0 | 285.9 | 28.7 | 2.4 | 7.9 |

Other conditions as in Table 1

makes more pipemidic acid molecules be oxidized on the surface of the electrode. Therefore, $i_p$ increases with increasing $C_B$. In our experiments $1.2 \times 10^{-4}$ mol/L NaOAc – $8.8 \times 10^{-4}$ mol/L HOAc was used because of higher $i_p$, larger $N$, and lower noise. In addition, in the concentration well reproducibility, a wider linear range and lower limit of detection can be obtained.

Figure 3 shows the relationship between the detected peak current, $i_p$, and the applied potential, $E_d$. When $E_d$ is between –0.95 to –1.05 V, $i_p$ increases with decreasing $E_d$. When $E_d$ is between –1.05 and –1.10 V, $i_p$ is almost constant. When $E_d$ <–1.10 V is applied, $i_p$ decreases rapidly with decreasing $E_d$. Therefore, $E_d$ of –1.10 V was chosen in subsequent experiments. The separation voltage, $V_s$, exerts an influence on $t_m$ and $N$ [22]. Figure 4 shows the dependence of $1/t_m$, $i_p$, $W_{1/2}$, and $N$ on $V_s$; $1/t_m$ is proportional to $V_s$. There is a linear relationship between $N$ and $V_s$; $i_p$ increases with increasing $V_s$. Nevertheless, the noise increases with increasing $V_s$. Therefore, 20 kV for $V_s$ was chosen because of the larger $i_p$ and $N$ and the lower noise. Figure 5 shows the typical electropherograms of $5.00 \times 10^{-5}$ mol/L and $2.00 \times 10^{-6}$ mol/L pipemidic acid at optimum conditions. The shape of the electrophoretic peak is symmetric. Small peak width and little tailing of the peak were obtained.

### 3.3 Reproducibility, limit of detection and linear range

The response for a series of six injections of $5.00 \times 10^{-5}$ mol/L pipemidic acid resulted in a relative standard deviation of 0.31% for $t_m$ and 2.0% for $i_p$, respectively. The limit of detection is $1.05 \times 10^{-6}$ mol/L (according to the ratio of

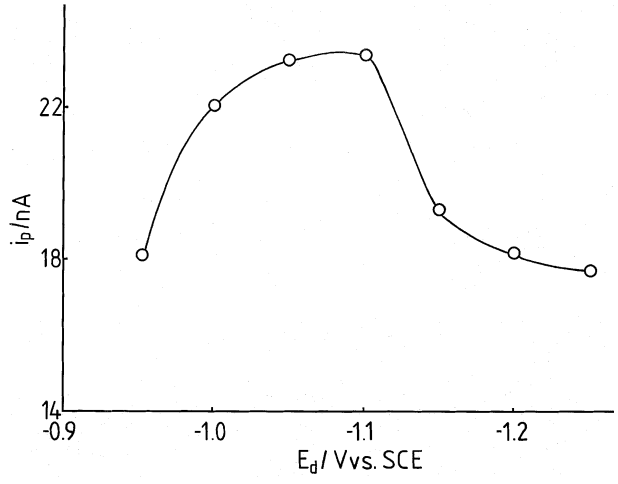


**Figure 3.** Relationship between detected peak current and detection potential. $1.2 \times 10^{-4}$ mol/L NaOAc-$8.8 \times 10^{-4}$ mol/L HOAc. Other conditions as in Table 1.

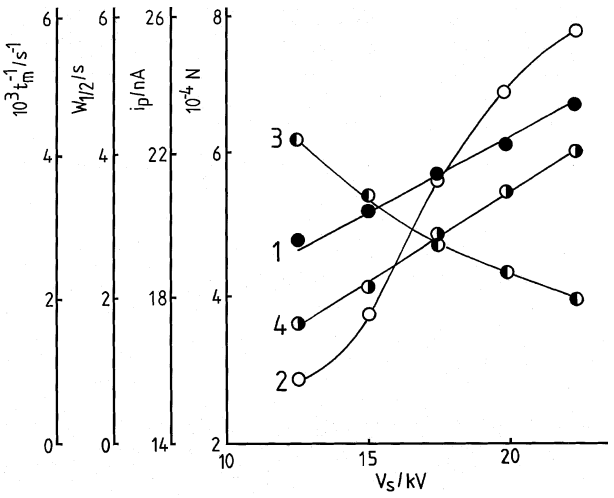


**Figure 4.** 1, Dependence of the reciprocal migration time; 2, peak current detected; 3, width at half-peak and 4, number of theoretical plates on the separation voltage. Detection potential is –1.10 V; other conditions as in Fig. 3.

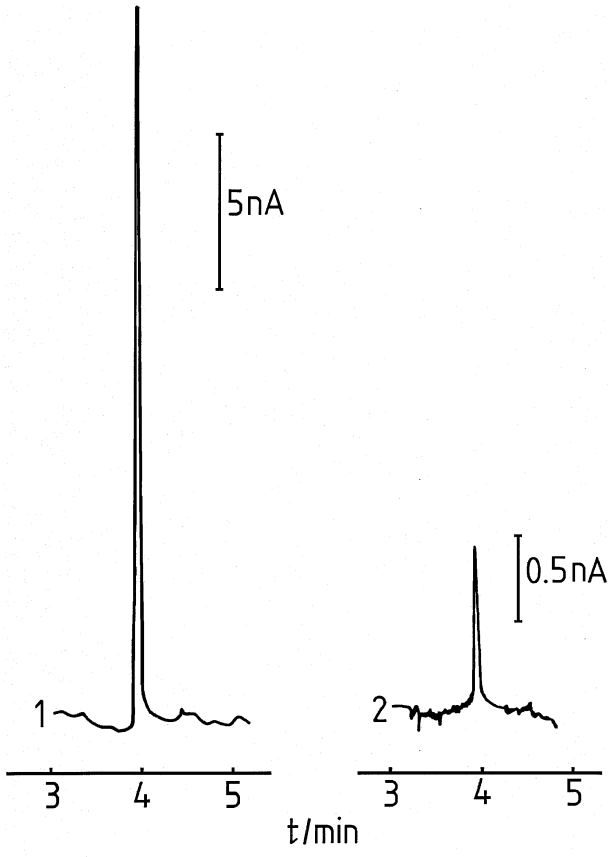


**Figure 5.** Typical electropherograms of pipemidic acid. Concentration of pipemidic acid: 1, $5.00 \times 10^{-5}$ mol/L, 2, $2.00 \times 10^{-6}$ mol/L. Detection potential is –1.10 V; other conditions as in Fig. 3.

signal to noise of 3), which was estimated from the electropherograms obtained for $2.00 \times 10^{-6}$ mol/L pipemidic acid (see Fig. 5, curve 2), or 189 amol for the injected volume calculated. A linear relationship holds between the peak current detected and concentration in the range of $2.00 \times 10^{-6}$ to $1.00 \times 10^{-4}$ mol/L. Least-squares treatment of these data yielded a slope of 486 pA $\mu mol^{-1}$ L with a correlation coefficient of 0.9997.

### 3.4 Determination of pipemidic acid in human serum

A synthetic human serum sample containing $1.00 \times 10^{-4}$ mol/L pipemidic acid was used to verify the possibility of the standard addition method. After the sample solution of 200 μL was diluted to 1 mL with the buffer solution, it was injected into the CZE electrochemical system. The electropherograms of human serum sample without and with the standard solution of pipemidic acid are shown in Fig. 6; only one peak appears on the electropherogram of the synthetic human serum sample (curve 1). This indicates that electrochemical detection has excellent selectivity for determination of pipemidic acid in human serum samples. Figure 6 shows that the peak current detected in the human serum sample increases linearly with increasing standard pipemidic acid, which means that the linear relationship between the peak current and the concentration of pipemidic acid is present for the human serum samples. The concentrations of pipemidic acid in two human serum samples obtained by standard addition method are $9.8 \times 10^{-5}$ and $1.05 \times 10^{-4}$ mol/L, respectively, which agree with the value in the human serum sample. The recovery is between 98% and 102%.

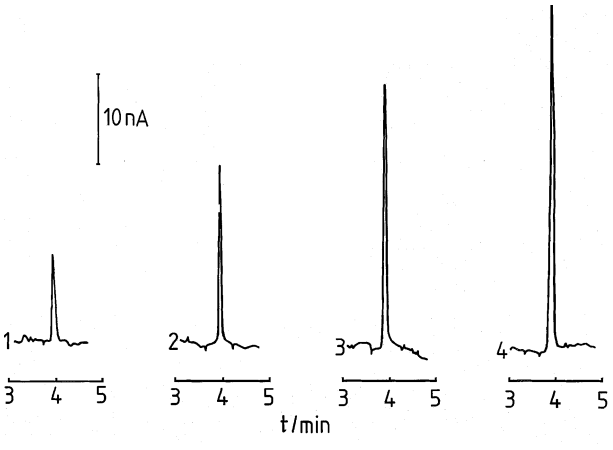


**Figure 6.** Electropherograms of pipemidic acid in the sample of human serum. The concentration of pipemidic acid (mol/L): 1, sample; 2, (1) + 2.00 $10^{-5}$; 3, (1) + $4.00 \times 10^{-5}$; 4, (1) + $6.00 \times 10^{-5}$. Conditions as in Fig. 3.

*This project was supported by the National Science Foundation of China, the Science Foundation of Shandong Province and Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.*

Received April 6, 1999

## 4 References

[1] *The Pharmacopoeia of The People's Republic of China*, Vol. 2, Chemical Industry Press, Beijing 1990, p. 221.

[2] Smethurst, A. M., Mann, W. C., *J. Chromatogr.* 1983, *274*, 421–427.

[3] Fukuhara, K., Matsuki, Y., *J. Chromatogr.* 1987, *416*, 409–413.

[4] Li, S., Cao, Y., Liu, Y., *Chin. J. Pharm. Anal.* 1995, *15(4)*, 36–37.

[5] Cheng, Z., Wang, X., Guo, H., Yang, Y., *Chin. J. Pharm. Anal.* 1996, *16*, 264–265.

[6] Weinberger, R., *Practical Capillary Electrophoresis*, Academic Press, San Diego, CA 1993.

[7] St. Claire III, R. L., *Anal. Chem.* 1996, *68*, 569R–586R.

[8] Shintani, H., Polonsky, J., (Eds.), *Handbook of Capillary Electrophoresis Applications*, Blackie Academic & Professional, London 1997.

[9] Voegel, P. D., Baldwin, R. P., *Electrophoresis* 1997, *18*, 2267–2278.

[10] Jin, W., Wang, Y., *J. Chromatogr. A* 1997, *769*, 307–314.

[11] Jin, W., Wang, Y., *Anal. Chim. Acta* 1997, *343*, 231–239.

[12] Jin, W., Wei, H., Zhao, X., *Anal. Chim. Acta* 1997, *347*, 269–274.

[13] Jin, W., Wei, H., Li, W., Zhao, X., *Anal. Lett.* 1997, *30*, 771–785.

[14] Jin, W., Wei, H., Zhao, X., *Electroanalysis* 1997, *9*, 770–774.

[15] Jin, W., Weng, Q., Wu, J., *Anal. Chim. Acta* 1997, *342*, 67–74.

[16] Jin, W., Weng, Q., Wu, J., *Anal. Lett.* 1997, *30*, 753–769.

[17] Jin, W., Chen, H., *J. Electroanal. Chem.* 1997, *425*, 155–160.

[18] Jin, W., Chen, H., *J. Chromatogr. A* 1997, *765*, 307–314.

[19] Telting-Diaz, M., Ordieres, A. J. M., Garcia, A. C., Blanco, P. T., Diamond, D., Smyth, M. R., *Analyst* 1990, *115*, 1215–1217.

[20] Yang, Y, Ruan, Z., *Fenxi Huaxue (Chin. Anal. Chem.)* 1994, *22*, 823–825.

[21] Towns, J. K., Regnier, F. E., *Anal. Chem.* 1992, *64*, 2473–2478.

[22] Jorgenson, J. W., Lukacs, K. D., *J. Chromatogr.* 1981, *218*, 209–216.