J. Sep. Sci. 2001, *24*, 658–662

Rongying Wang,
Xiaoning Lu,
Mingjia Wu

Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, National Analysis and Research Center of Electrochemistry and Spectroscopy, Changchun, Jilin 130022, China

Chiral separation of promethazine by capillary electrophoresis with end-column amperometric detection

Sensitive end-column amperometric detection has been successfully coupled to capillary electrophoresis for chiral separation of promethazine, with a carbon fiber microdisk electrode as working electrode. Baseline separation and sensitive detection were achieved under optimum conditions: 0.030 M Na_2HPO_4 and 0.015 M citric acid at pH = 2.50, 1.0 mM β-CD, 10 kV separation voltage, and detection potential 1.10 V (vs Ag/AgCl). The numbers of theoretical plates were higher than 700 000, and the detection limit was 5×10^{-8} M. On-line treatment of the electrode has also been studied and discussed.

Key Words: Capillary electrophoresis; Amperometric detection; Chiral separation; Promethazine

Ms received: March 16, 2000; revised: July 2, 2001; accepted: July 16, 2001

1 Introduction

In recent years, there has been an unprecedented growth of interest in the separation of enantiomers of pharmaceutically and clinically important compounds. Apart from their optical characteristics, the physical and chemical properties of enantiomers are exactly the same so their separation is a challenge for analysts. Many methods such as GC and HPLC have been used for enantiomer separation. Recently, capillary electrophoresis (CE) has been found to be an ideal supplement to classical chromatography for the separation of enantiomers because of its high efficiency, short analysis time, flexibility of rapid incorporation of various selectors, and feasibility of method development [1]. A number of papers on enantiomers separation by CE have appeared, in which a wide variety of chiral selectors have been employed, including proteins, modified crown ethers, antibiotics, chiral micelles, and cyclodextrins (CDs), or their derivatives. Among them CDs and their derivatives are the most popular [2–5].

In most commercially available instruments, UV absorbance is used for detection and it is the dominant detection technique for chiral separation. Although the UV detector is reliable and easy to handle, it has an inherent disadvantage, i.e. a relatively high detection limit due to its short optical path length. Compared to the UV detector, the laser-induced fluorescence (LIF) detector has a higher sensitivity and seems to be the ideal solution for this problem. However, the applicability of LIF is limited to compounds that fluoresce or compounds that can be derivatized with a fluorescent tag. Moreover, instruments with LIF detectors are rather expensive. Electrochemical detection (ED), especially amperometric detection with a microelectrode, which has a sufficient detection limit, good selectivity, and low instrument cost, is a promising approach to detection. Much work has been done on the determination of a variety of compounds by CE with ED [6–8]; however, very little [9, 10] has been done on detection of enantiomers. Coupling electrochemical detection to chiral separation is a fairly new research field, and a good extension of CE–ED.

This article is concerned with the chiral separation of promethazine followed by end-column amperometric detection. A carbon fiber microdisk electrode, which is commonly used in CE–ED because of its wide potential window, easy treatment, and low price, was used as a working electrode. A newly designed, home-made amperometric detection cell was used which was convenient for proper alignment of the capillary with the working electrode. Detection limit and column efficiency improved greatly compared to UV detection. The parameters that would affect separation and detection such as β-cyclodextrin concentration, buffer pH and concentration, applied voltage and detection potential were studied and optimized. Treatment of the electrode on-line was also studied and discussed.

Correspondence: Dr. Mingjia Wu, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, National Analysis and Research Center of Electrochemistry and Spectroscopy, Changchun, Jilin 130022, China.

1615-9306/2001/0808–0658$17.50+.50/0

2 Experimental

2.1 Instruments

2.1.1 Cyclic voltammetry

A model 832 electrochemical analyzer (CH Instrument, TN, USA) connected to a 486 computer was used for all cyclic voltammetry studies. A three-electrode system was used with a carbon fiber microdisk electrode as working electrode, a Pt wire as the auxiliary electrode, and a Ag/AgCl (in saturated KCl solution) as the reference electrode.

2.1.2 Capillary electrophoresis

A high-voltage power supply (Beijing Institute of New Technology, China) provided a variable voltage of 0–30 kV. The outlet of the capillary was at ground potential with Pt wire. Fused-silica capillary (360-μm OD, 25-μm ID, 35-cm length.) from Heibei Yongnian Optical Fiber Plant, China. Amperometric detection at a constant potential coupled with CZE was performed by end-column approach using a voltammeter (Jinan Fourth Radio Factory, China) and a microamperometer (model 902-pA, Ningde Analytical Instrument, China). A recorder (JASCO, JAPAN) recorded the electropherograms. The detection cell and microamperometer were housed in a faradic cage in order to minimize the interference from external sources of noise. Electrochemical detection with CZE was carried out with three-electrode system, which consisted of a carbon fiber microdisk electrode, a Ag/AgCl (in saturated KCl solution) electrode, and Pt wire as described above. The working electrode was placed at the outlet of the separation capillary.

2.1.3 Electrode and capillary

The construction of a 30-μm carbon fiber microdisk electrode has already been described [11]. A new capillary was filled with 0.1 M NaOH solution for 24 h and then flushed with doubly distilled water and the corresponding separation electrolyte for 3–5 min before use. To achieve good reproducibility, the capillary was rinsed with H_2O, NaOH, H_2O, and the running buffer for 3 min, respectively, between two injections.

2.2 Reagents and chemicals:

Racemic promethazine hydrochloride was obtained from Aldrich (USA) and was dissolved in doubly distilled water at concentration of 1.0 mM as stock solution. Before use, it was diluted to the desired concentration with doubly distilled water. β-CD was purchased from Sigma (St. Louis, Mo, USA); urea, NaOH, Na_2HPO4 from the Institute of Analysis and Testing for Drugs and Biological Reagent of China; citric acid, from Tianjin Chemical Factory (Tianjin, China). All reagents and chemicals were of analytical reagent grade and were used as received without further purification. Solutions and samples were prepared from doubly distilled water and were filtered through 0.45-μm cellulose acetate filters (Jiangsu Qilin Medical Instrument Factory, China) before use.

3 Results and discussion

3.1 Electrochemistry

3.1.1 Detection potential

Cyclic voltammetry of 10^{-4} M promethazine was carried out in a solution of 0.030 M Na_2HPO4, 0.015 M citric acid and 1.0 mM β-CD at pH = 3.0 in the potential range of –0.2 to 1.20 V. An irreversible oxidation peak was observed at 0.8–1.0V, which indicated that promethazine could be detected electrochemically. The typical cyclic voltammogram is shown in **Figure 1**. Because the mobile system was always different from the static system, hydrodynamic voltammetry of promethazine was carried

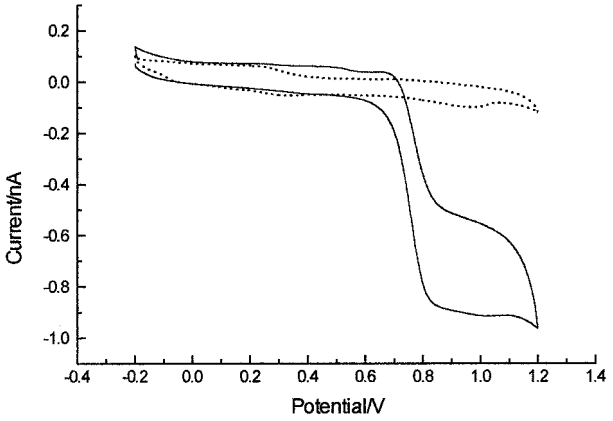

Figure 1. Cyclic voltammograms of promethazine. Conditions: 10^{-4} M promethazine. Buffer: 0.030 M Na_2HPO4 and 0.015 M citric acid, 1.0 mM β-CD at pH = 3.0. Scan rate: 50 mV/s.

out in the potential range of 0.4–1.3 V, and the hydrodynamic voltammogram obtained is shown in **Figure 2**. When the potential was less than 1.10 V, the response of promethazine increased with increasing potential; after that the response decreased with increasing potential, so 1.10 V was selected as detection potential.

3.1.2 Treatment of electrode on line

The adsorption of both promethazine and β-CD on the carbon fiber electrode caused the peak current to decrease if the electrode was not treated regularly. Regular electrode treatment was therefore required to obtain reproducible results. Ultrasonic cleaning has proved to be

Original Paper

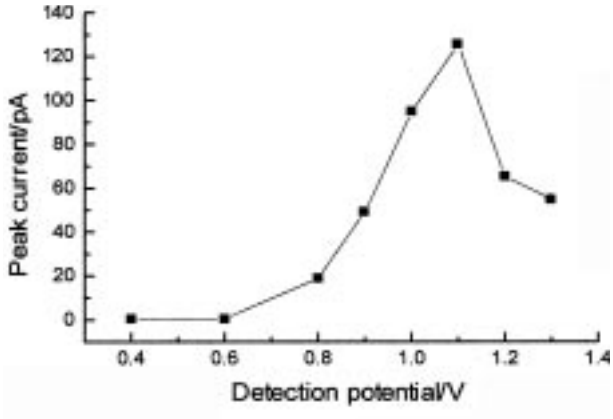

Figure 2. Hydrodynamic voltammogram of promethazine. Conditions: 3.0×10^{-6} M promethazine. Buffer: 0.030 M Na_2HPO4 and 0.015 M citric acid, 1.0 mM β-CD at pH = 3.0. Capillary: 25-μm ID, 360-μm OD, and 33-cm length. Injection by electromigration: 10 kV, 3 s. Separation voltage: 10 kV.

an effective method for renewing the surface of the electrode. However, disassembling and remounting the electrode was a time-consuming procedure, and since the electrode seldom stayed in exactly the same position the reproducibility of the peak high became poor. Thus an online method of treating the electrode had to be found. Several treatment methods were carried out, such as (1) cyclic voltammetry from −1.5 to 2.0 V for one, two, or five times; (2) anodic polarization at 2.0 V for 120 s, cathodic polarization at −2.0 V for 10 s, stabilization at 0.0 V for 120 s for one or three times; (3) anodic polarization at 2.0 V for 5 min, 10 min, 15 min respectively. It was observed that anodic polarization at 2.0 V for 10 min was the effective treatment method and after treating, the reproducibility of the peak current was satisfactory. The results are shown in **Figure 3**.

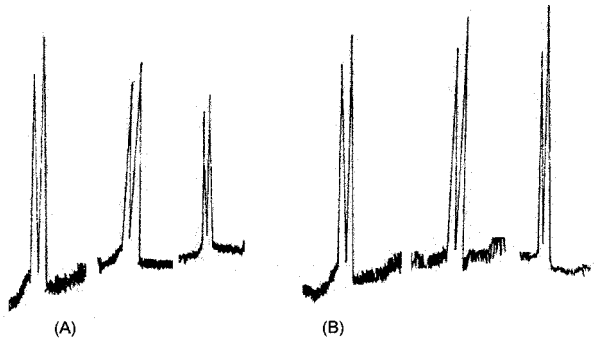

Figure 3. The influence of electrode treatment on response. (A) without treatment and (B) after treatment. Conditions: 10^{-6} M promethazine. Detection potential: 1.10 V. Other conditions are the same as in Figure 2.

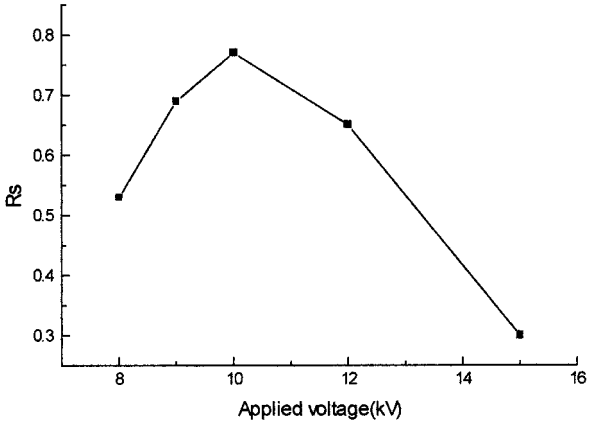

Figure 4. The influence of applied voltage on resolution. Conditions: 10^{-6} M promethazine. Buffer: 0.030 M Na_2HPO4 and 0.015 M citric acid, 1.8 mM β-CD at pH = 3.0. Other conditions are the same as in Figure 2.

3.2 Optimization of separation conditions

3.2.1 Applied voltage

The impact of the applied field strength on resolution was studied under otherwise unchanged conditions, the voltage was increased from 8.0 to 9.0, 10.0, 12.0, 15.0 kV, corresponding to field strengths of 229, 257, 286, 343, and 429 V/cm, respectively. The resolution was calculated using the equation $R_s = 1.18\ (t_2 - t_1)/(w_{0.5\,(1)} + w_{0.5\,(2)})$ [12], where t_1, t_2 are the migration time of two enantiomers ($t_2 > t_1$), and $w_{0.5\,(1)}$, $w_{0.5\,(2)}$ is the peak width at half height. The optimum resolution was obtained at 10 kV (**Figure 4**), with the resolution decreased rapidly at higher voltages. High voltage leads to good resolution in theory; however, it also results in greater Joule heating and therefore decreases the efficiency and enhances band broadening. While high voltage shortens the separation time, interaction of the analytes with the CDs and the separation require sufficient time. Hence too high a migration velocity will decrease the resolution.

3.2.2 β-CD concentration

β-CD concentration is always the most important factor influencing resolution in chiral separation. β-CD was added to the buffer at different concentrations in the range of 0.0−1.8 mM. Increasing chiral selector concentration prolonged migration time, due to the complexation of promethazine with β-CD and high viscosity. Increasing the β-CD concentration improved the resolution when the concentration was lower than 1.0 mM; after that, the resolution decreased with increasing β-CD concentration (**Figure 5**). The results are in accordance with theoretical studies on the existence of a maximum resolution between two extreme concentrations of β-CD [13].

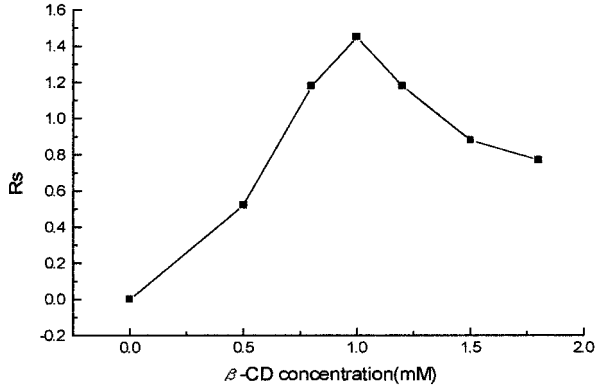

Figure 5. The influence of β-CD concentration on resolution. 10^{-6} M promethazine. Buffer: 0.030 M Na_2HPO4 and 0.015 M citric acid at different β-CD concentration, pH = 2.50. Other conditions are the same as in Figure 2.

3.2.3 pH value

The pH value of the electrolyte is another important parameter influencing separation, so the effect of the run buffer pH on the separation of promethazine enantiomers was also evaluated. Under otherwise constant conditions, the buffer pH was changed from 2.50–3.50 in 0.50 pH value increments. Promethazine was always charged as a cation in the pH range studied; its effective electrophoretic mobility was almost constant, while the electroosmosis increased with the increasing pH. High EOF typically results in poor resolution in chiral CE, which can be explained as follows: the separation is dependent upon the interaction between cyclodextrin and the analytes, and, as stated above, this interaction requires sufficient time; if the time is too short, enantiomers cannot be separated. Hence, a low run buffer pH resulted in slow electroosmosis and favorable resolution of the promethazine enantiomers. pH = 2.50 was selected.

3.2.4 Buffer concentration

The run buffer concentration affects resolution in chiral CE by intensifying the hydrophobic interaction of the analyte with the cyclodextrin and improving the enantioselectivity [14]. In addition, run buffer concentration also affects the separation efficiency in CE. Too low a concentration of run buffer leads to both low efficiency and poor resolution [11]. Otherwise high concentration causes high separation current, which will interfere with electrochemical detection. In off-column amperometric detection, the separation current is isolated from sensing electrode with a decoupler. Even so, for the large diameter capillary (>25 μm) the concentration and compositions should be selected carefully to eliminate the separation current [11]. On the other hand, for a 25 μm capillary, the separation current is small and its effect on detection can be ignored. Thus a buffer consisting of 0.030 M Na_2HPO_4 and 0.015 M citric acid was chosen. A typical electropherogram recorded optimum conditions is shown in **Figure 6**.

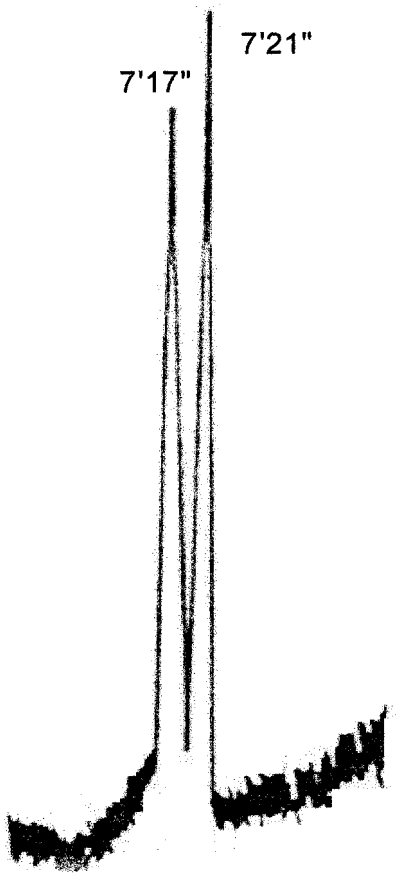

Figure 6. Typical electropherograms of promethazine enantiomers. 10^{-6} M promethazine. 0.030 M Na_2HPO4 and 0.015 M citric acid at pH = 2.50, β-CD concentration is 1.0 mM. Other conditions are the same as in Figure 2.

3.3 Comparison between UV and electrochemical detection

Promethazine enantiomers were detected by a simple electrochemical detector, e.g. end-column amperometric detection. The results are compared to UV detection (see **Table 1**). Both the resolution and the column efficiencies have been improved in end-column amperometric detection scheme, which can be explained as following:

In CE, the aim of chiral separation is to obtain the highest efficiencies and best resolution (the most satisfactory performance) within shortest analysis time. All factors should be considered when separation performance was discussed. Because of the analyte and the buffer used in UV and ED detection technique are the same, only several parameters such as field strength, capillary dimension, temperature and Joule heating, and dead detection volume were selected and discussed in detail.

Table 1. Comparison of separation conditions and results in UV and amperometric detection.

	CE-UV	CE–ED
Capillary length (cm)	64.5	35
Capillary inside diameter (μm)	75	25
Applied field strength (V/cm)	264	287
Concentration of ß-CD (mM)	1.5	1.0
Migration time	13 min 45 s, 14 min 01 s	7 min 17 s, 7 min 21 s
Resolution	1.60	1.65
Plate number per meter	4.8×10^5, 4.9×10^5	7.6×10^5, 7.7×10^5
Detection limit (mol/L)	3×10^{-6}	5×10^{-8}

According to Jorgenson [15], the separation efficiency, in term of the total numbers of the theoretical plates, *N*, is calculated as:

$$N = \mu V/2D \quad (1)$$

And the migration time is:

$$t = L^2/\mu V \quad (2)$$

Where μ is the apparent electrophoretic mobility of analyte, *V* the applied voltage, *L* total length of capillary, *D* the solute's diffusion coefficient.

From above equations, high efficiencies are best achieved through the use of high voltage, and the analysis time also decreases at high voltage. Although the capillary length plays no role in separation efficiencies, it has a profound influence on migration time.

The influence of temperature within a tube caused by Joule heating can contribute significantly to the plate height and seriously reduce the efficiency of the system. Joule heating is closely related to the current in the capillary, which is proportional to the square of capillary radius [16]. Thus the capillary radius has a decisive influence on Joule heating. Heat generation in a narrow bore capillary is smaller than in a larger one; moreover, heat dissipation is more effective in narrow capillary owing to the higher surface-to-volume ratio. In our work, the capillary inside diameter (25 μm) was much smaller than it was in UV detection (75 μm), whereas the field strength was higher in the former. Both of these factors tend to improve separation performance. If no other influences played a role, the efficiencies should be greatly improved in an ED detection set-up. However, it came as no surprise the efficiencies were not improved very much: compared to the UV detector, the dead detection volume cannot be ignored in end-column amperometric detection. This led to some loss of efficiency; however, the loss was not serious because of the properties of our ED detection cell. As a final result, the efficiencies and resolution were better in ED. Additionally the inherent sensitivity of ED lowered the detection limit by 2 orders of magnitude to 5×10^{-8} M and the analysis time was shortened.

4 Conclusion

This article reports a successful example of chiral separation by CE with an end-column amperometric detector. A low detection limit was obtained. In theory, any electroactive enantiomer can be detected by electrochemical detection, making this an extensive research field open to further development in the areas of quality control of enantiomers synthesis and determination of enantiomer purity. Further studies will be devoted to the separation of other clinically interested enantiomers.

Acknowledgment

This project is supported by the National Natural Science Foundation of China (29475211).

Reference

[1] S. Fanali, *J. Chromatogr. A* **1996**, *735*, 77.

[2] A.-E.F. Nassar, F.J. Guarco, D.E. Gran, J.D. Stuart, W.M. Reuter, *J. Chromatogr. Sci.* **1998**, *36*, 19.

[3] B.C. Lin, X.F. Zhu, U. Epperlein, M. Schurierskott, R. Schlunk, B. Koppenhoefer, *J. High Resol. Chromatogr.* **1998**, *21*, 215.

[4] S. Fanali, S. Furlanetto, Z. Aturki, S. Pinzanti, *Chromatographia* **1998**, *48*, 395.

[5] M. Fillet, L. Fotsing, J. Crommen, *J. Chromatogr.A* **1998**, *817*, 113.

[6] C.G. Fu, L.N. Song, Y.Z. Fang, *Anal. Chim. Acta* **1998**, *371*, 81.

[7] T.D. Boer, K. Ensing, *J. Chromatogr. A* **1997**, *788*, 212.

[8] F.M. Matysik, *Electrochim. Acta* **1998**, *43*, 3475.

[9] M.E. Hadwiger, S.R Torchia, S. Park, M.E. Biggin, C.E. Lunte, *J. Chromatogr. A* **1996**, *681*, 241.

[10] X.M. Fang, F.Y. Gong, Y.Z. Fang, *Anal. Chem.* **1998**, *70*, 4030.

[11] R.Y. Wang, X.N. Lu, M.J. Wu, Er.K. Wang, *J. Chromatogr. B* **1999**, *721*, 327.

[12] I.E. Valkó, H. Sirén, M.L. Riekkola, *J. Chromatogr. A* **1996**, *737*, 263.

[13] S.A.C. Wren, R.C. Rome, *J. Chromatogr.* **1992**, *603*, 235.

[14] R. Kuhn, F. Stoeck, F. Ernj, *Chromatographia* **1992,** *33*, 32.

[15] J.M. Jorgenson, K.D. Lukacs, *Science* **1983**, *222*, 226.

[16] J.E. Valkó, S.P. Porras, M.L. Riekkola, *J. Chromatogr A* **1998**, *813*, 179.