Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1095 (2005) 193–196
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

# Simple method for the separation and detection of native amino acids and the identification of electroactive and non-electroactive analytes

Jing-Juan Xu, Ying Peng, Ning Bao, Xing-Hua Xia, Hong-Yuan Chen *

*The Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093, China*

Received 14 September 2005; received in revised form 28 September 2005; accepted 29 September 2005
Available online 18 October 2005

**Abstract**

Detection of native amino acids was accomplished using a capillary electrophoresis (CE)–amperometric detection system, in which a single carbon fiber cylinder (CFC) working electrode instead of a carbon fiber disc (CFD) electrode was mounted in the end part of a poly(dimethylsiloxane) (PDMS)/glass hybrid microchannel. Similar to that using CFD working electrode, here, the electrochemical reduction reaction at the working electrode is also coupled from the separation high voltage system, the coupling degree is related to the in-channel length of the CFC. This property simplifies the fabrication procedure of the working electrode and also provides a convenient and sensitive means for the determination of non-electroactive ions by amperometry. The present detection mode is successfully used to electrochemically detect non-electroactive arginine (Arg), threonine (Thr), glutamic acid (Glu) and electroactive cysteine (Cys). Furthermore, by simply changing the detection potential, we can easily distinguish peak mobilities of electroactive amino acids from that of non-electroactive amino acids.


*Keywords:* Indirect amperometric detection; Microchip electrophoresis; Amino acids

## 1. Introduction

Amino acids are a kind of greatly important compounds in clinical and biological areas. Significant amount of researches have been devoted to the development of efficient analytical tools for detecting, identifying, and quantitating amino acids [1–3]. Capillary electrophoresis (CE) has been widely applied to the separation and determination of amino acids [4–6]. The detection methods usually used in combination with CE separation technique were ultraviolet light (UV), fluorescence or laser-induced fluorescence (LIF) detection. The lack of a strong chromophore for aliphatic amino acids has certainly been one of the limitations in the analysis of most native amino acids by these methods. Electrochemical detection (ED) offers high sensitivity and selectivity for analytes that are easily oxidized. However, most underivatized amino acids are not electroactive under typical separation conditions (buffer pH) employed in CE. For efficient separation and sensitive detection using these methods, amino acids generally require derivatization prior to their analysis. This is a labor-intensive and time-consuming process.

Separation of amino acids using microfluidic chips has attracted significant attention, as microchips offer a number of advantages including speed of analysis, portability, ability to multiplex, and compatibility with integration allowing development of "micro-total-analysis systems (μTAS)" [7–17]. The detection methods are mainly focused on LIF and ED and also require derivatization prior to their analysis. Recently we designed a versatile analytical device based on the combination of a carbon fiber disc (CFD) electrode as in-channel detector with a poly(dimethylsiloxane) (PDMS)/PDMS microchip CE system [18]. The potential difference induced by the CE separation electric field between the working electrode and the reference electrode brings about the change of the reduction potential of dissolved oxygen, which can be used to determine non-electroactive analytes by indirect amperometry. In other researches, in order to enhance analysis capacity by providing additional information, multiple detection strategies with somewhat complicated structures such as dual detection electrodes held at different potentials [19,20] and dual fluorescence and electrochemical detection [8] have also been coupled for simultaneous detection on microchips.

* Corresponding author. Tel.: +86 25 83594862; fax: +86 25 83594862.
*E-mail address:* hychen@nju.edu.cn (H.-Y. Chen).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.09.077

In this work, detection of native amino acids was accomplished using a microchip capillary electrophoresis-amperometric detection system, in which a single carbon fiber cylinder (CFC) working electrode instead of the single CFD electrode was mounted in the end part of a PDMS/glass hybrid microchannel. Similar to that using CFD working electrode described in our previous work [18], here, the electrochemical reduction reaction at the working electrode is also coupled from the separation high voltage system, the coupling degree is only related to the in-channel length of the CFC. This approach simplifies the fabrication of the working electrode and also provides a convenient and sensitive means for the determination of non-electroactive ions by amperometry. Furthermore, by simply changing the detection potential, we can easily identify peak positions of electroactive amino acids and non-electroactive amino acids on one detector. It may provide a good method for the analysis of complex system simultaneously containing electroactive and non-electroactive analytes.

## 2. Experimental

### 2.1. Materials and reagents

Sylgard 184 (PDMS) was from Dow Corning (Midland, MI, USA). Arginine (Arg), threonine (Thr), cysteine (Cys), and glutamic acid (Glu) were purchased from Sigma–Aldrich (St. Louis, MO, USA). NaOH, Sodium tetraborate, were purchased from Nanjing Chemical Reagents Factory (Nanjing, China). Sodium tetraborate buffers were prepared and then filtered with 0.22 μm cellulose acetate filter (Xinya Purification Factory, Shanghai, China). The stock solutions of samples (30 mM) were prepared by dissolving analytes in doubly distilled water. Before use, they were diluted with corresponding running buffer. All other chemicals were of analytical grade and used without further purification. All the solutions were prepared with doubly distilled water.

### 2.2. Apparatus

The integrated CE–ED PDMS chip microsystem was similar to that described previously [18]. A laboratory high-voltage power supply has an adjustable voltage range between 0 and 5000 V. A simple-cross single-separation channel PDMS/glass microchip was made based on the combination of a PDMS slice with microchannels and a glass flat (see Fig. 1). The master composed of a positive relief structure of GaAs for the channels was made in No. 55 Electronic Institute (Nanjing, China) by using standard micro photolithographic technology and reaction ion etching. The chip had a 40-mm-long separation channel (from injection cross to the channel outlet) and a 5-mm-long injection channel (between the sample reservoir and injection cross). The channels had a maximum depth of 18 μm and a width of 50 μm. The microchip had a four-way injection cross that was connected to three reservoirs and separation channel. A Plexiglas holder that integrated a precisely three-dimensional system (Shanghai Lian Yi Instrument Factory of Optical Fibre and Laser, China) with the precision of ±1 μm in each direction was fabricated for housing the separation chip and the detector and allowing their convenient replacement and reproducible positioning. A clip of optical fiber that can be fastened in the three-dimensional system, was used to closely clip the CFC electrode. Platinum wires, inserted into the individual reservoirs on the holder, served as contacts to the high voltage power supply.

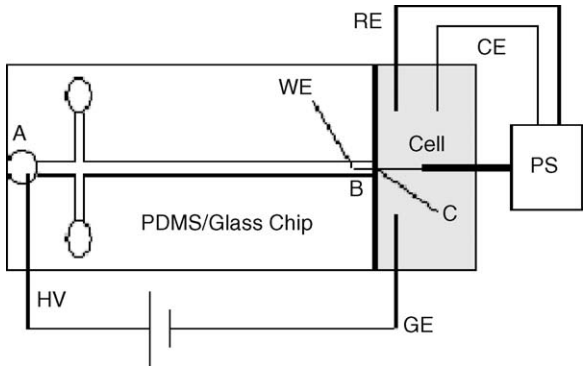


Fig. 1. Schematic diagram of the experimental setup. WE: working electrode, CE: counter electrode, RE: reference electrode, GE: ground electrode, PS: potentiostat, HV: high voltage. (A) Running buffer reservoir; (B) position of the WE in channel; and (C) channel outlet. The length (40 μm) of WE in the separation channel (BC). WE was a carbon fiber cylinder with diameter of 8 μm and length of 1.5 mm; CE, GE were platinum wires; RE was Ag/AgCl.

### 2.3. Electrode fabrication

Single CFC microelectrode was prepared as follows. A glass capillary with inner diameter of 0.5 mm was pulled under a multifunctional glass microelectrode puller (Shanghai Biological Institute, China) to form a fine tip. Then a single carbon fiber with diameter of 8 μm was carefully mounted into the tip and fixed with epoxy. A copper wire was connected with the carbon fiber through carbon powder on the other end of capillary and then fastened with epoxy. Before use, the microelectrode was cut with a clean scalpel to form a 1.5 mm length micro-cylinder electrode under a microscope. Part of the working electrode (40 μm) was placed into the end part of the separation channel. Linear sweep voltammograms and amperometric detection were performed on a CHI 660 Electrochemical workstation (CHI Co., USA). The electropherograms were recorded at a fixed detection potential. The working electrode was treated at +1.5 V for 200 s and then −1.0 V for 200 s before use and once the baseline current obviously changed. All experiments were performed at room temperature.

### 2.4. Electrophoretic procedure

Before use, the PDMS layer with microchannel was ultrasonically cleaned subsequently with water, methanol and water for 10 min each and then dried under infrared lamp. The glass slice was treated by following procedures: dipped in a solution of $H_2O_2$:$H_2SO_4$ (v/v = 1:2) for 30 min, cleaned with doubly-distilled water, dipped in 0.1M NaOH for 20 h, cleaned with water and then dried under infrared lamp. Then the PDMS layer and the glass slice were sealed together to form a reversible

hybrid microchip. After the microchip was held on the holder, a working electrode was inserted into the electrode hole on the platform. Silicon grease was used to prevent leaking of the detection cell. The "running buffer" reservoir was filled with a running buffer and the "sample" reservoir with the sample mixture. The injections were performed by applying a desired potential for 5 s to the sample reservoir with the "detection" reservoir grounded while all other reservoirs floating. Separations were performed by switching the high-voltage contacts and applying the corresponding separation voltages to the running buffer reservoir with the detection reservoir grounded and all other reservoirs floating.

## 3. Results and discussion

In order to characterize the electrochemical behaviour of the CFC electrode with a part in the channel in separation electric field, we investigated the linear sweep voltammograms under the different separation electric fields. The electrochemical behaviour (not shown) is similar to that on the CFD electrode as our previous report [18]. The separation voltage greatly influences the voltammetric performance of the detector. Without separation electric field, the reduction of oxygen at the carbon fibre electrode started at −0.5 V, while the oxidation current occurred at potentials more positive than +1 V. In the presence of separation electric field, the onset potential for oxygen reduction at the CFC electrode shifted to a positive value, while the onset potential for the oxidation of the CFC electrode was not changed. Higher voltage induces a more positive onset reduction potential. Although the exact reason for this phenomenon on CFC is not clear now, it can be inferred that the CFC electrode can also be used to detect analytes using oxygen as electroactive indicator at lower potential and simultaneously detect electroactive analytes at high potential. This property simplifies the fabrication process and facilitates the wide application of this detection mode.

Fig. 2 shows the typical electropherograms of four unlabeled amino acids Arg, Thr, Cys, and Glu at different detection potential with a separation voltage of 1200 V. Baseline separation of these anions was obtained. The peak currents significantly decreased with the increase of detection potential. At 0.6 V, a negative peak was observed on the migration time of Cys and the peaks of other three amino acids disappeared. As we know, among these amino acids, only Cys is electroactive on carbon fibre electrode at 0.6 V. Thus, this negative peak is the oxidation peak of Cys. This phenomenon can be used to distinguish peak positions of electroactive amino acids from that of non-electroactive amino acids by simply changing the detection potential.

Since the electrochemical detection principle is based on the coupling of separation electric field on the electrochemical detector, the influence of separation voltage on detection is a significant concern with respect to improving detection sensitivity. Fig. 3 exhibits the influence of separation voltage on the detection of 2.0 mM Arg, 5.0 mM Thr, 7.0 mM Cys and 7.0 mM Glu. By enhancing the separation electric field, the migration times of the four amino acids decrease with the increase of separation

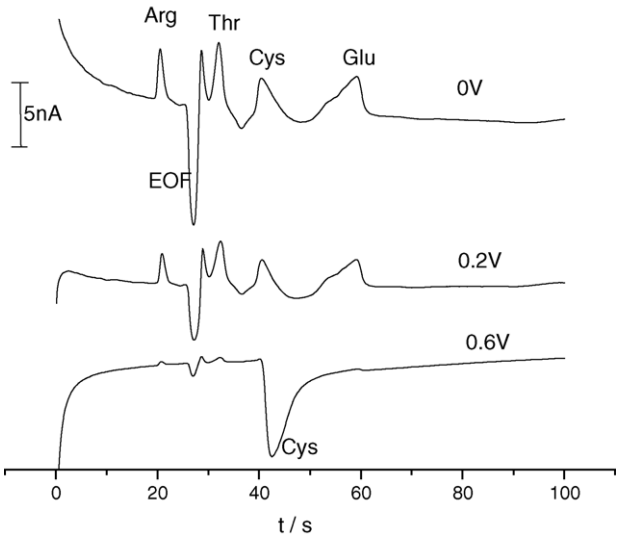


Fig. 2. Electropherograms of: 2.0 mM Arg; 7.0 mM Thr; 5.0 mM Cys; and 7.0 mM Glu with different detection potentials. Experimental parameters: running buffer 5.0 mM pH 9.3 $Na_2B_4O_7$ buffer; sampling voltage 600 V; sampling time 3 s; separation voltage 1200 V. (EOF represents the electroosmtic flow).

electric field. They can be separated in less than 60 s when the separation potential is larger than 1200 V. Meanwhile, a relatively fast ascent of the amperometric signal with the increase of voltage was observed, because the coupling effect on amperometric detection potential is also enhanced with the increase of the separation electric field and, thus, resulting in the larger peak current corresponding to accelerated electrochemical oxidation of oxygen. The separation voltage has a negligible effect on the peak-to-peak background noise level for voltages ranging from 800 to 1600 V. Separation electric fields higher than 1600 V result in higher background and noise levels (attributed to Joule heating effects). Moreover, too high separation electric

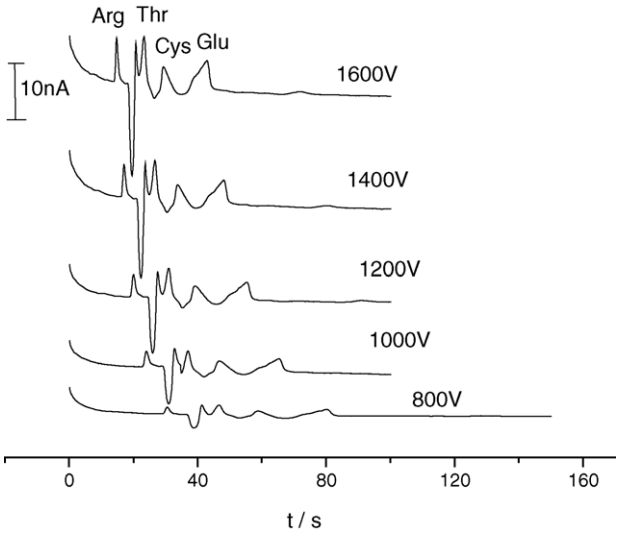


Fig. 3. Electropherograms of: 2.0 mM Arg; 7.0 mM Thr; 5.0 mM Cys; and 7.0 mM Glu with different separation voltages. Experimental parameters: running buffer 5.0 mM pH 9.3 $Na_2B_4O_7$ buffer; sampling voltage 600 V; sampling time 3 s; detection potential 0 V.

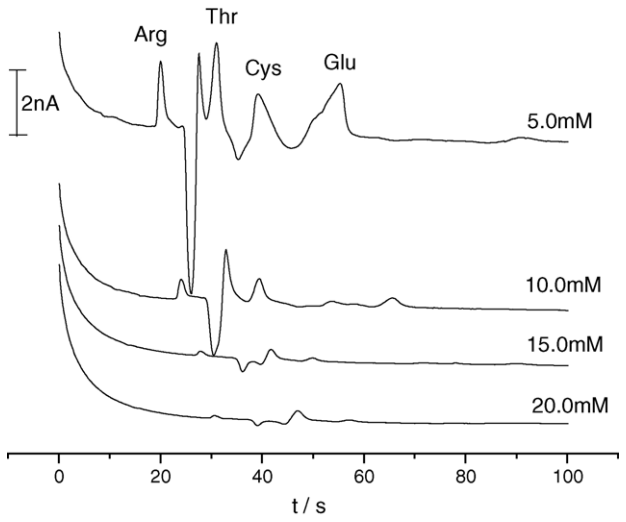


Fig. 4. Electropherograms of: 2.0 mM Arg; 7.0 mM Thr; 5.0 mM Cys; and 7.0 mM Glu in pH 9.3 $Na_2B_4O_7$ buffer with different concentrations. Experimental parameters: sampling voltage 600 V; sampling time 3 s; detection potential 0 V, separation potential: 1200 V.

field will induce the formation of hydrogen bubble at the CFC electrode, then, resulting in a large noise level or clogging of the channel.

The effect of ionic strength on the separation of amino acids was investigated. Fig. 4 shows the effect of changing the buffer concentration on the amino acid separation. With the buffer concentration decreasing from 20.0 to 5.0 mM, the migration times of the four amino acids decrease because the decreased concentration of running buffer would increase the rate of electroosmtic flow (EOF). Meanwhile the peak currents largely increased with the decrease of the running buffer. Further decreasing the buffer concentration, Cys and Glu cannot be separated well (not shown). We also investigated the effect of buffer pH on the separation efficiency and found pH 9.3 $Na_2B_4O_7$ buffer has optimal response.

Preliminary results showed that the in-channel indirect amperometric microchip detector displays well-defined concentration dependence for the four amino acids in the detection range of 500 μM–5.0 mM. When the separation voltage of 1600 V was used, the detection limit of 10 μM for Arg can be obtained.

In conclusion, a simple method for the separation and detection of native amino acids have been established in a PDMS/glass hybrid microchip capillary electrophoresis-amperometric detection system. The successful use of a single carbon fiber cylinder working electrode greatly simplify the electrode fabrication procedures. Meanwhile, the identification of the positions of electroactive analytes and that of non-electroactive samples can also be carried out by simply changing the detection potential. It may provide a good approach for the analysis of complex system simultaneously containing electroactive and non-electroactive analytes. Such a CE–ED microsystem may have wide applications.

## Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20575029, 20475025, 20205007) and the Natural Science Foundation of Jiangsu (No. BK 2004210).

## References

[1] R.W. Zumwalt, K.C. Kuo, C.W. Gehrke (Eds.), Amino Acid Analysis by Gas Chromatography, vol. I–III, CRC Press, Boca Raton, FL, 1987.
[2] M.W. Duncan, A. Poljak, Anal. Chem. 70 (1998) 890.
[3] C. Prata, P. Bonnafous, N. Fraysse, M. Treilhou, V. Poinsot, F. Couderc, Electrophoresis 22 (2001) 4129.
[4] J.T. Smith, Electrophoresis 20 (1999) 3078.
[5] C. Prata, P. Bonnafous, N. Fraysse, M. Treilhou, V. Poinsot, F. Couderc, Electrophoresis 22 (2001) 4129.
[6] P. Véréna, B. Christophe, C. François, Electrophoresis 24 (2003) 4047.
[7] N.J. Munro, Z. Huang, D.N. Finegold, J.P. Landers, Anal. Chem. 72 (2000) 2765.
[8] J.A. Lapos, D.P. Manica, A.G. Ewing, Anal. Chem. 74 (2002) 3348.
[9] K. Tsukagoshi, K. Nakahama, R. Nakajima, Anal. Chem. 76 (2004) 4410.
[10] C.D. Garcıa, C.S. Henry, Anal. Chem. 75 (2003) 4778.
[11] C.L. Schultz, M. Moini, Anal. Chem. 75 (2003) 1508.
[12] L.D. Hutt, D.P. Glavin, J.L. Bada, R.A. Mathies, Anal. Chem. 71 (1999) 4000.
[13] J. Wang, M.P. Chatrathi, B. Tian, Anal. Chem. 72 (2000) 5774.
[14] M.A. Schwarz, B. Galliker, K. Fluri, T. Kappes, P.C. Hauser, Analyst 126 (2001) 147.
[15] R.S. Martin, A.J. Gawron, B.A. Fogarty, F.B. Regan, E. Dempsey, S.M. Lunte, Analyst 726 (2001) 277.
[16] Y.H. Dou, N. Bao, J.J. Xu, H.Y. Chen, Electrophoresis 23 (2002) 3558.
[17] T. Pan, R.T. Kelly, M.C. Asplund, A.T. Woolley, J. Chromatogr A. 1027 (2004) 231.
[18] J.J. Xu, N. Bao, X.H. Xia, Y. Peng, H.Y. Chen, Anal. Chem. 76 (2004) 6902.
[19] R.S. Martin, A.J. Gawron, S.M. Lunte, Anal. Chem. 72 (2000) 3196.
[20] A.J. Gawron, R.S. Martin, S.M. Lunte, Electrophoresis 22 (2001) 242.