Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 833 (2006) 129–134
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of excitatory amino acids in biological fluids by capillary electrophoresis with optical fiber light-emitting diode induced fluorescence detection

Chunling Wang [a], Shulin Zhao [b], Hongyan Yuan [b], Dan Xiao [a,b,*]

[a] College of Chemistry, Sichuan University, Chengdu 610065, China
[b] College of Chemical Engineering, Sichuan University, Chengdu 610065, China

Received 23 October 2005; accepted 12 January 2006
Available online 24 February 2006

Abstract

The capillary electrophoresis (CE) system with optical fiber light-emitting diode (optical fiber LED) induced fluorescence detector was developed for the analysis of the excitatory amino acids (EAAs) tagged with naphthalene-2,3-dicarboxaldehyde (NDA). The separation of EAAs was carried out in an uncoated fused-silica capillary (50 cm × 75 μm i.d.) with a buffer of 10 mM borate at pH 9.3 and an applied voltage of 20 kV. High sensitivity was obtained by the use of optical fiber LED induced fluorescence detector with a violet LED as the excitation light source. The limits of detection (S/N = 3) for glutamic acid (Glu) and aspartic acid (Asp) were 2.1×10^{-8} and 2.3×10^{-8} M, respectively. The detection approach was successfully applied to the analysis of Glu and Asp in biological fluids including human serum, rabbit serum and human cerebrospinal fluid (CSF) with satisfactory results.

Keywords: Capillary electrophoresis; Optical fiber; Light-emitting diode; Excitatory amino acids; Biological fluids

1. Introduction

Glutamic acid (Glu) and aspartic acid (Asp) are major excitatory amino acids (EAAs) in the nervous system. Glu, the brain's main excitatory neurotransmitter, is toxic in excess, overexciting neurons to the point of killing them. It causes much of the damage that occurs after a stroke and is also probably the chief neuron-killing villain in neurodegenerative diseases [1]. After traumatic or ischemic damage to the central nervous system, there is a pathological release of EAAs, particularly Glu and Asp in the extracellular fluid. Increase of extracellular EAAs is believed to be partially responsible for the brain damage resulting from a variety of neurological conditions including hypoxia/ischemic, stroke, epilepsy, and head injuries [2–3]. The functions of excitatory amino acids in various neurological processes can be realized by monitoring changes of their levels. Therefore, it is important to develop analytical techniques for the determination of EAAs in biological samples such as cerebrospinal fluid (CSF), serum and neural tissue.

Analytical methods based on high-performance liquid chromatography (HPLC) are so far commonly used for the determination of EAAs in biological samples [4–8]. However, HPLC method suffers from high cost and complicated operations. Meanwhile, the high resolution, short analysis time, low sample and reagent consumption make capillary electrophoresis (CE) become a rapidly growing separation technique. It has become one of the most powerful tools for the analysis of a wide variety of species, including inorganic compounds, proteins, organic acids, amino acids, and neurotransmitters [9,10]. In spite of its powerful separation ability, CE faces the challenge of improving the detection sensitivity required by the small injection volume and amount. So electrochemical [11], chemiluminescence (CL) [12] and laser induced fluorescence (LIF) detectors [13] stand out for their perfect sensitivity. In the electrochemical detector system, the high separation voltage could interfere with detection of the electrochemical signal, and the contamination of electrode surfaces might also pose problems in the analysis of

* Corresponding author. Tel.: +86 28 85407958; fax: +86 28 85407958.
E-mail address: xiaodan@scu.edu.cn (D. Xiao).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.01.013

real sample [14]. The CL detector still suffers from the limited application due to lack of CL reaction for many compounds. CE with laser induced fluorescence detection is the most sensitive detection scheme and has also been used to analyze amino acid neurotransmitters in microdialysis samples [15], the dorsal root ganglion of the rat [16], and cerebral cortex of rat [17]. However, there are many limitations of laser source, such as large volume, high cost, high power consumption and limited lifetime.

Recently, LED induced fluorescence detector has aroused much interest [18–22] because of its low cost, simplicity and flexibility. The LED induced fluorescence detection systems in the previous CE studies [23,24] suffer from the light reflecting and scattering on capillary surface, for which that the excitation light excited analytes from outside of the capillary at the detection window, which could result in high background noise and low detection sensitivity. Instead, in the new home-built optical fiber light-emitting diode (optical fiber LED) induced fluorescence CE detection system, an optical fiber was inserted directly into the tail end of capillary and was settled right at the detection window. The excitation light was transmitted just to the detection window by the optical fiber to excite analytes, which avoided the light reflecting and scattering on capillary surface [25]. The excitation light was guided into the capillary directly and could be utilized completely to excite analytes. In this study, a simple, sensitive and reproducible detection system has been developed for the analysis of the excitatory amino acids in human serum, rabbit serum and human cerebrospinal fluid from healthy subjects.

2. Experimental

2.1. Chemical

Glu, Asp and other amino acids were purchased from Shanghai Biochemical Reagents Company (Shanghai, China). Naphthalene-2,3-dicarboxaldehyde (NDA) was obtained from Fluka (Buchs, Switzerland). All the other chemicals and organic solvents used in this work were of analytical grade.

Individual standard stock solution of amino acid (1 mM) was prepared in 0.1 M HCl solution and stored at 4 °C [26]. A mixture of standard amino acids or individual standard amino acids were further diluted as required with 0.1 M HCl solution and kept at 4 °C before using. NDA solution (2 mM) was prepared weekly in methanol and kept in dark at 4 °C. KCN solution was prepared (20 mM) in water. Glu and Asp were dissolved in 0.1 M HCl solution. The running buffer solution was prepared by dissolving 0.3814 g $Na_2B_4O_7{\cdot}10\ H_20$ in 100 ml water (10 mM borate) and adjusted to pH 9.3 with 0.1 M NaOH solution or 0.1 M HCl solution. Milli-Q water was used throughout the work. All solutions were filtered through a 0.45 μm membrane filter.

2.2. Apparatus

Fig. 1 illustrates the basic design of the CE with optical fiber LED (CE–optical fiber LED) induced fluorescence detection system. A high-voltage supply (0–30 kv, Beijing Cailu Science Instrument Company, Beijing, China) was used to drive the electrophoresis and a 50 cm × 75 μm i.d. uncoated fused-silica capillaries (Hebei Optical Fiber, China) was used for the separation. The detection window was made by removing off a 5 mm section of polyimide coating on the capillary with about 2.0 cm distance to the tail end of the capillary. A solid bare optical fiber with a diameter of 40 μm (Beijing Glass Institute, Beijing, China) was used as excitation light transmission. The violet LED (Shifeng Optic and Electronics Ltd., Shenzheng, China; applied voltage, +3.5 V; intensity, ∼3 mW; peak wavelength 425 nm, spectral half width ∼25 nm) was used as excitation light source. The light emanating from the LED was focused by a 100× microscope objective (Olympus, Japan) before coupling into the light-guiding optical fiber. The upper end of optical fiber (about 2.0 cm) was inserted into the tail end of separation capillary and introducing exciting light to reach the detection window (Fig. 1, insert A). Fluorescence emission from analyte was collected by

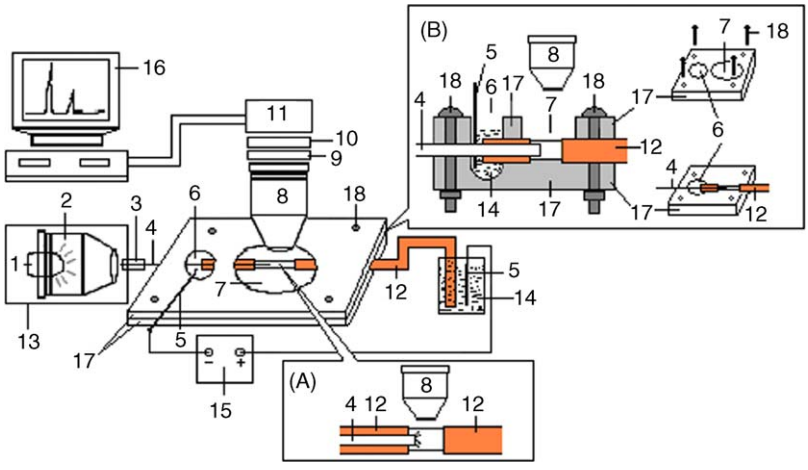

Fig. 1. Schematic diagram of the capillary electrophoresis instrument with the optical fiber LED induce fluorescence detector. (1) LED; (2) 100× microscope objective; (3) capillary for fixing optical fiber; (4) optical fiber; (5) Pt electrodes; (6) buffer reservoir; (7) detection window; (8) 40× microscope objective; (9) spatial filter; (10) cut-off filter; (11) PMT; (12) electrophoretic capillary; (13) darkbox; (14) electrolyte buffer; (15) high-voltage power supply; (16) computer. (17) organic glass chip; and (18) bolt.

a 40× microscope objective (Olympus, Japan) and focused on a spatial filter (0.3 mm) and passed through a yellow cut-off filter (maximum wavelength at 455 nm) before reaching the PMT (CR105 equipped with a socket assembly, Beijing Hamamatsu Photon Techniques Inc. Beijing, China). The output signal was recorded and processed with a computer using in-house written software. The insert B shows the design of detection cell. The upper end of optical fiber was inserted into the tail end of the electrophoretic capillary to take excitation light just at the detection window. The inserted optical fiber did not effect the separation of the species that had been separated before. The capillary, optical fiber and a Pt wire electrode were fixed on an organic glass chip, and a buffer reservoir was made in the chip.

2.3. Biological samples preparation

The blood samples were obtained from healthy subjects and kept on ice until being centrifuged at 6000 × *g* for 15 min at 4 °C. The serum layer was carefully collected and stored at −80 °C. Before assay, 0.5 ml of serum sample was diluted with 0.5 ml of acetonitrile and shaken vigorously for 15 min to deposit proteins, then left on ice for 1 h. After centrifuging at 10,000 × *g* for 15 min, the deproteinized serum was used as analyte in the experiment [27].

The CSF samples were obtained from healthy subjects and stored at −80 °C before assay. The process of CSF samples preparation was the same as the process described above for blood samples.

2.4. Precolumn derivatization

Glu and Asp show neither native UV absorption nor fluorescence. Therefore, a chemical derivatization with NDA was necessary for detection. A 10 μl of standard amino acid solution, 10 μl deproteinized serum, and 30 μl of deproteinized CSF analyte solution were allowed to react with 50 μl of NDA (2 mM in methanol), respectively in the presence of 50 μl of potassium cyanide (20 mM in water) [28,29] and 150 μl of 10 mM borate buffer solution (pH 9.3). The reaction mixture was vortexed and kept at room temperature for 30 min. Then, the derivative sample solution was injected for separation without additional purification.

2.5. CE procedure

The new capillary was preconditioned by flushing with 1 M NaOH for 30 min before the first use. Between two consecutive injections, the capillary was rinsed sequentially with 0.1 M NaOH, water and running buffer for 3 min each. Following precolumn derivatization with NDA, the sample solution was injected into the capillary by hydrodynamic flow at a height differential of 20 cm for 10 s. After the introduction of derivative sample solution, 20 kV were applied across the capillary. All separations were performed on an uncoated fused-silica capillary (50 cm × 75 μm i.d.) with a buffer of 10 mM borate at pH 9.3. Fluorescence was excited by a violet LED with a maximum wavelength at 425 nm and detected after passing through a 0.3 mm spatial filter and a 455 nm yellow cut-off filter. The electropherogram was recorded and EAAs quantification was achieved by measuring the CE peak height. All CE procedures were conducted at 25 °C.

3. Results and discussion

3.1. Choice of LED

In order to obtain high excitation efficiency, the emission wavelength of LED had to match the excitation wavelength of NDA derivative. Therefore, the excitation wavelength of LED should be chosen carefully. Fig. 2 shows the excitation (spectrum1) and fluorescence (spectrum 2) spectra of NDA-labeled EAAs. As can be seen, NDA-labeled EAAs produced high fluorescence with a maximum excitation at 445 nm and a maximum emission at 486 nm. Because the fluorescence compounds had little wavelength difference ($\lambda_{em} - \lambda_{ex} = 41$ nm), if a LED with maximum wavelength at 445 nm had been used, it would have overlapped for approximately 35% with the fluorescence emission of NDA-labeled EAAs, which would result in high background. It could also be seen from Fig. 2, a shoulder peak was situated at 425 nm in the excitation spectrum of the NDA-labeled EAAs. When a violet LED with a maximum wavelength at 425 nm and a full-width at half-maximum of ∼25 nm was used, there would be only slight overlapping with a fluorescence emission of NDA-labeled EAAs. It appeared that this LED with a maximum wavelength at 425 nm was optimum for exciting the NDA-labeled EAAs. So a LED with a maximum wavelength of 425 nm was chosen for this experiment.

3.2. Optimization of separation conditions

Many endogenous amino acids in biological fluids reacted with NDA forming fluorescent derivatives, which might interfere with the determination of Glu and Asp. Therefore, to achieve an efficient separation between Asp and Glu as well as other endogenous amino acids, the separation conditions such as pH of running buffer and the applied voltage were investigated.

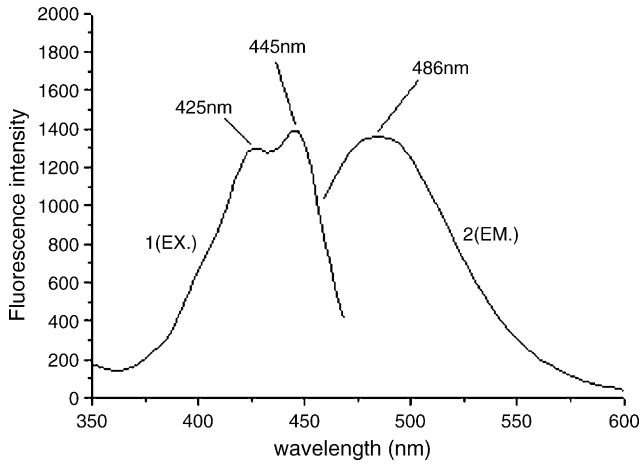

Fig. 2. Typical spectra: (1) excitation spectrum of the NDA-labeled EAAs; (2) emission spectrum of the NDA-labeled EAAs.

3.2.1. Effect of pH of running buffer

In CE separation, changes in pH of running buffer may cause changes in the charges of analytes, which would lead to changes in separation selectivity. In this separation system, pH affected directly the ionization of NDA-labeled EAAs. In weak basic running buffer, NDA-labeled EAAs remain negatively charged. Therefore, the weak basic borate buffers in the pH at range of 8.7–9.8 were used to study the effects of separation. In fused-silica capillaries, with the increase of the pH of the buffer, the charges on the capillaries wall are increased and the electroosmotic flow is also increased, which can be ascribed to the dissociation of surface silanol groups. It is now generally accepted that the electroosmotic mobility strongly up to pH 7–8. At higher pH, the mobility increases very slowly because the dissociation of surface silanol groups gradually reaches saturation. In the range of pH 8–9, the ionic strength effect is not strong, which result in a predominant dependence of electroosmotic mobility on the pH of buffer. And the effect of buffer pH is so moderate that the electroosmotic mobility remained nearly constant [30]. So the migration times for Glu and Asp at pH 8.7 are close to that at pH 9.0. When pH value is higher than 9.0, the ionic strength effect is stronger than the "pure" pH effect. The borax–NaOH system can be considered as weak base-strong acid-type buffer. Increasing the pH will result in an increase in the ionic strength and a decrease in electroosmotic flow [30]. So the migration times for EAAs were increased gradually with increasing pH values of buffer. As shown in Fig. 3, other amino acids very likely migrate faster than Glu and Asp in all investigated pH values. They may not affect the separation of Glu and Asp. Glu and Asp could be well separated under the conditions. As higher fluorescence intensity, well-shape peak and optimum migration time were obtained at pH 9.3. So, the buffer of pH 9.3 was used as the running buffer.

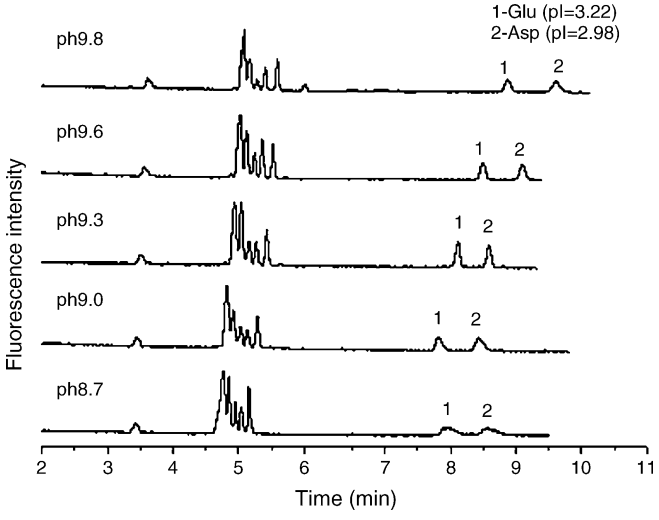

Fig. 3. Electropherograms of standard solution containing 20 NDA-labeled amino acids at a concentration of 7.7×10^{-7} M. Electrolyte composition was 10 mM borate buffer (at pH 8.7, 9.0, 9.3, 9.6 and 9.8). Capillary was 75 μm i.d., 50 cm in length. Temperature 25 °C; Applied voltage was 20 kV; LED maximum wavelength was 425 nm.

3.2.2. Effect of applied voltage

Applied voltage affects directly the migration time and peak shape of analytes. Increasing operating voltage may increase electroosmotic flow and decrease the time of migration, however, may result in excessive Joule heating that ruins the resolution and repeatability. Generally, higher resolution should be achieved when more time is available for the separation of Asp and Glu, while longer migration time should also cause peak broadening. Thus, a 20 kV of applied voltage is optimum considering the appropriate migration time and acceptable Joule heating.

3.3. Linearity, limit of detection, reproducibility

In the present work, optical fiber LED induced fluorescence detection CE method for simultaneous determination of Glu and Asp in biological fluids was examined by determining their performance characteristics regarding linearity, limit of detection and reproducibility (precision). The results were summarized in Table 1.

In order to test the fluorescence response linearity, the EAAs standard solutions at a series of concentration were determined. As can be seen, the linear ranges were from 5.2×10^{-8} to 2.6×10^{-5} M for NDA-labeled Glu (NDA–Glu) and from 5.8×10^{-8} to 2.9×10^{-5} M for NDA-labeled Asp (NDA–Asp), respectively, which were better than 1×10^{-6}–1×10^{-4} M for NDA–Glu and NDA–Asp by electrochemical detector [31] and were similar to 10^{-8}–10^{-5} M for NDA–Glu and NDA–Asp by LIF detector [32]. The correlation coefficients were higher than 0.999. The optical fiber LED induced fluorescence detector offered better limits of detection (LODs) (2.09×10^{-8} M for NDA–Glu and 2.31×10^{-8} M for NDA–Asp) than that obtained by UV detector (10^{-5}–10^{-6} M) [33] and electrochemical detector (1.2×10^{-6} M for NDA–Glu and 1.1×10^{-6} M for NDA–Asp) [31], and similar LODs to that obtained by CL detector (2×10^{-8} M with luminol reaction reagent and 5×10^{-9} M with ATP reaction reagent) [34] and LIF detector (3.7×10^{-9} M for NDA–Glu and 1.7×10^{-8} M for NDA–Asp) [32]. The reproducibility of the method was investigated by analyzing stand EAAs 5 times, and was demonstrated by means of relative standard deviation (R.S.D.). Satisfactory reproducibilities were obtained and the R.S.D.s of peak height were less than 5% for standard Glu and Asp.

Table 1
Linear ranges, regression equations, correlation coefficients, detection limits and relation standard deviations of peak height for standard Glu and Asp

Amino acid	Glu	Asp
Linear range (M)	5.2×10^{-8}–2.6×10^{-5}	5.8×10^{-8}–2.9×10^{-5}
Regression equation[a]	$y = 24.5x + 4.44$	$y = 20.1x + 4.03$
Correlation coefficients	0.9999	0.9996
LOD (M)	2.09×10^{-8}	2.31×10^{-8}
R.S.D. (%)[b]	2.2	2.8

See Section 2.5 for CE condition.
[a] x: NDA–AAs concentration (μM); y: fluorescent intensity (mV).
[b] $n = 5$.

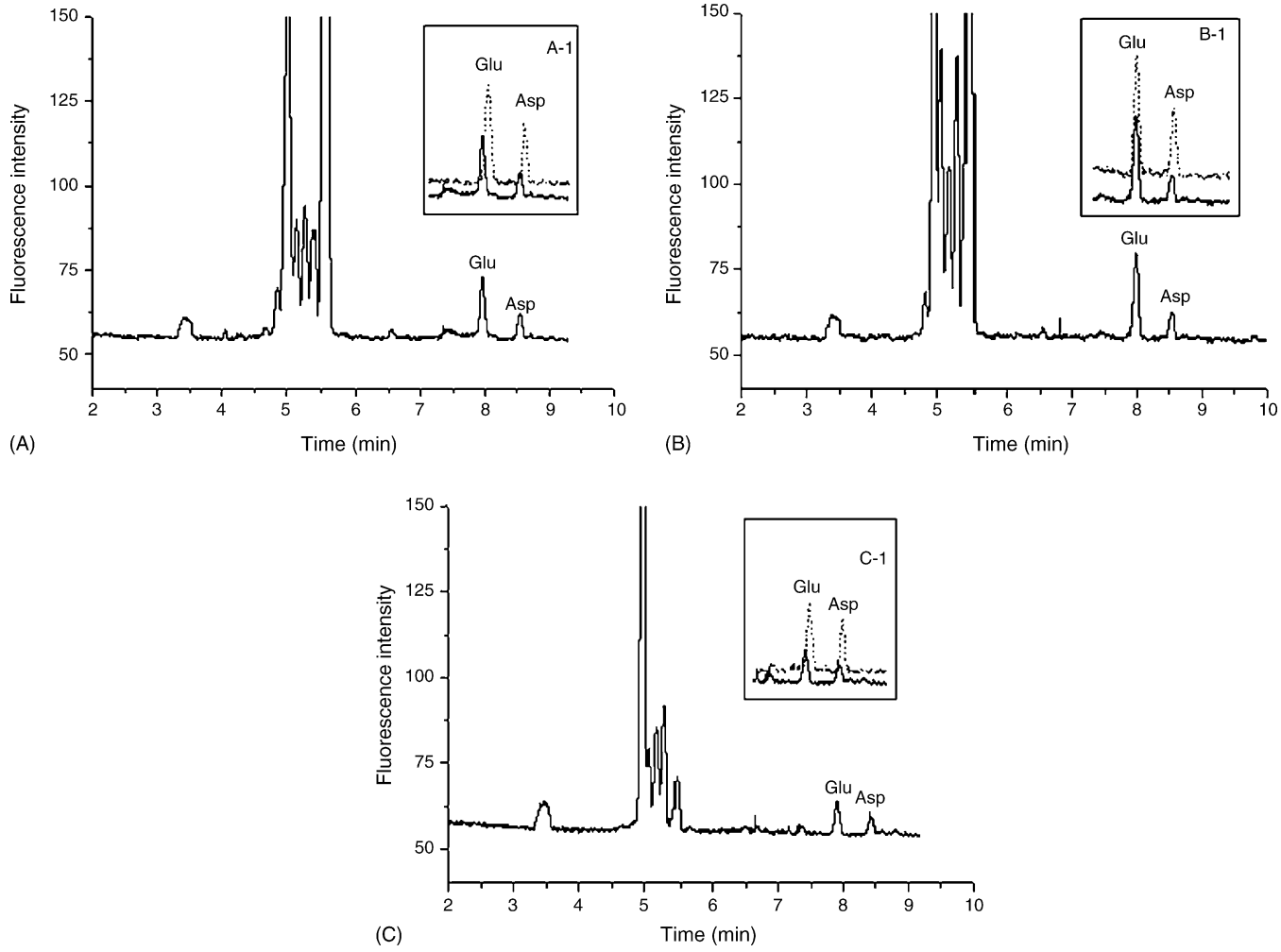

Fig. 4. Electropherograms obtained from the separation of: rabbit serum (A); human serum (B); and human CSF sample (C). Solid trace of insert was obtained from sample analysis and the dotted trace was obtained from sample spiked with 4.6×10^{-7} M NDA–Glu and NDA–Asp. Electrolyte composition was 10 mM borate buffer (pH 9.3). Other CE condition was as in Fig. 3.

3.4. Samples analysis

Rabbit serum, human serum and human CSF samples from healthy subjects were analysed, respectively. After preparation and precolumn derivatization as described in Sections 2.3 and 2.4, respectively, the samples were measured by CE-optical fiber LED induced fluorescence detection using an uncoated fused-silica capillary. The CE condition was described in Section 2.5. Under this CE condition, other amino acids or biogenic amines migrate faster than the EAAs, and the derivatives of Glu and Asp were completely resolved as single peaks within 10 min. Glu and Asp were detected in all the samples tested. The concentration of Asp and Glu in the serum appears a higher level than that in the CSF, and the ratio of Glu to Asp (Glu/Asp) in the serum is also higher than that in the CSF. The typical electropherograms obtained from these samples analysis were

Table 2
Determination results of Glu and Asp in biologic fluids

Sample	Glu concentration[a] (μM)	R.S.D. (%)	Recovery[b] (%)	Asp concentration[a] (μM)	R.S.D. (%)	Recovery[b] (%)
Rabbit serum-1	29.34	2.1	90.3	10.08	2.2	91.3
Rabbit serum-2	22.07	2.4		4.71	1.1	
Human serum-1	40.94	2.8	93.4	9.86	4.3	94.5
Human serum-2	34.86	3.5		7.12	2.8	
Human CSF-1	1.94	3.1	99.2	1.44	3.1	98.8
Human CSF-2	2.18	2.8		1.74	3.6	

See Section 2.5 for CE condition.
[a] Mean ($n = 5$).
[b] Mean ($n = 3$).

shown in Fig. 4 and the insert of Fig. 4 (solid trace). The peaks corresponding to Asp and Glu were well identified. To verify the peak identification, 5 μl of 25 μM standard Glu and Asp solutions were added to the sample solution, and the sample solution was derivatized and separated again. The electropherograms obtained were also shown in the insert of Fig. 4 (dotted trace). The recovery data were obtained by spiking biological fluids samples with known quantities of standard solutions of Glu and Asp. The analytical results were summarized in Table 2. As can be seen, the levels of Glu and Asp determined by this method were within the range of the values reported in the literatures [35–38] and the R.S.D.s of the concentration for both Glu and Asp were less than 5%. The recoveries for Glu and Asp were found to be 90.3–99.2 and 91.3–98.8%, respectively, which were close to 89–105% for NDA–Glu and 90–107% for NDA–Asp by electrochemical detector [39] and were close to $99.2 \pm 7.8\%$ for NDA–Glu and $99.4 \pm 6.0\%$ for NDA–Asp by LIF detector [40].

4. Conclusions

A CE-optical fiber LED induced fluorescence detector was developed for the analysis of real biological samples. Quantitative measurements of free Asp and Glu in rabbit serum, human serum and human CSF had been demonstrated. The present detector was a sensitive, accurate, simple and economic detection scheme, which can be easily set up in laboratory, since an inexpensive LED was used as excitation source, and a cheap optical fiber for guiding the excitation light was utilized. Although we primarily focused on the analysis of EAAs in biological fluids, the home-built detection system could also be expected to apply to the determination of EAAs in neural tissue. The present detection method represented a new approach for EAAs investigations and leaded to further potential uses in the future.

Acknowledgment

This work was supported by a grant from the Innovation Foundation of Sichuan University.

References

[1] C. Holden, Science 300 (2003) 1866.
[2] S.A. Lipton, P.A. Rosenberg, N. Engl. J. Med. 330 (1994) 613.
[3] M.F. Ritz, P. Schmidt, A. Mendelowitsch, Neurochem. Res. 27 (2002) 1677.
[4] B.D. Sloley, O. Kah, V.L. Trudeau, J.G. Dulka, R.E. Peter, J. Neurochem. 58 (1992) 2254.
[5] M.E. Bovingdon, R.A. Webster, J. Chromatogr. B 659 (1994) 157.
[6] Y.V. Tcherkas, L.A. Kartsova, I.N. Krasnova, J. Chromatogr. A 913 (2001) 303.
[7] R.F. Goldsmith, J.W. Earl, A.M. Cunnigham, Clin. Chem. 33 (1987) 1736.
[8] D.J. Begley, A. Reichel, A. Ermisch, J. Chromatogr. B 657 (1994) 185.
[9] T.M. Olefirowicz, A.G. Ewing, Anal. Chem. 62 (1990) 1872.
[10] K. Vuorensola, H. Sirén, R.A. Ketola, Electrophoresis 22 (2001) 4347.
[11] Z.H. He, W.R. Jin, Anal. Biochem. 313 (2003) 34.
[12] X.J. Huang, Z.L. Fang, Anal. Chim. Acta 414 (2000) 1.
[13] D.M. Pinto, E.A. Arriaga, D. Craig, J. Angelova, N. Sharma, H. Ahmadzadeh, N.J. Dovichi, C.A. Boulet, Anal. Chem. 69 (1997) 3015.
[14] F. Li, C.H. Zhang, X.J. Guo, W.Y. Feng, Biomed. Chromatogr. 17 (2003) 96.
[15] D.M. Zhang, J.M. Zhang, W.Y. Ma, D.Y. Chen, H.W. Han, H.J. Shu, G.Q. Liu, J. Chromatogr. B 758 (2001) 277.
[16] L. Zhang, H. Chen, S. Hu, J.K. Cheng, Z.W. Li, M. Shao, J. Chromatogr. B 707 (1998) 59.
[17] H. Li, H. Wang, J.H. Chen, L.H. Wang, H.S. Zhang, Y. Fan, J. Chromatogr. B 788 (2003) 93.
[18] A.K. Su, Y.S. Chang, C.H. Lin, Talanta 64 (2004) 970.
[19] B.C. Yang, F. Tan, Y.F. Guan, Talanta 65 (2005) 1303.
[20] M.J. Lu, T.C. Chiu, P.L. Chang, H.T. Ho, H.T. Chuang, Anal. Chim. Acta 538 (2005) 143.
[21] H.M. Huang, C.H. Lin, J. Chromatogr. B 816 (2005) 113.
[22] G. Zunic, Z. Jelic-lvanovic, M. Colic, S. SpasiC, J. Chromatogr. B 772 (2002) 19.
[23] B.C. Yang, Y.F. Guan, Talanta 59 (2003) 509.
[24] S.J. Chen, M.J. Chen, H.T. Chang, J. Chromatogr. A 1017 (2003) 215.
[25] S.L. Zhao, H.Y. Yuan, D. Xiao, Electrophoresis 27 (2006) 461.
[26] G. Aliqureshi, L. Fohlin, J. Bergstrom, J. Chromatogr. 297 (1984) 91.
[27] Z.J. Shen, Z.M. Sun, L. Wu, K. Wu, S.Q. Sun, Z.B. Huang, J. Chromatogr. A 979 (2002) 227.
[28] Z. Quan, Y.M. Lin, Electrophoresis 24 (2003) 1092.
[29] T.B. Polak, M. Kassai, K.B. Grant, Anal. Biochem. 297 (2001) 128.
[30] J. Vindevogel, P. Sandra, J. Chromatogr. 541 (1991) 483.
[31] Q. Dong, W.R. Jin, J.H. Shan, Electrophoresis 23 (2002) 559.
[32] S. Parrot, V. Sauvinet, J.M. Xavier, D. Chavagnac, L.M. Badina, L.G. Larrea, P. Mertens, B. Renaud, Electrophoresis 25 (2004) 1511.
[33] C. Kuyper, R. Milofsky, Trends Anal. Chem. 20 (2001) 232–240.
[34] R. Dadoo, A.G. Seto, L.A. Colon, R.N. Zare, Anal. Chem. 66 (1994) 303.
[35] R.F. Adams, J. Chromatogr. 95 (1974) 189.
[36] S. Matsumura, H. Kataoka, M. Makita, J. Chromatogr. B 681 (1996) 375.
[37] F.J.J. Jimenez, J.A. Molina, C. Vargas, M.O. Pareja, T. Gasalla, E. Cisneros, E. Cisneros, J. Arenas, J. Neurol. Sci. 141 (1996) 39.
[38] G. Nouadje, H. Rubie, E. Chatelut, P. Canal, M. Nertz, P. Puig, F. Couderc, J. Chromatogr. A 717 (1995) 293.
[39] Q.F. Wang, W.R. Jin, Electrophoresis 22 (2001) 2797.
[40] H. Zhang, W.R. Jin, Electrophoresis 25 (2004) 480.