Chinese Chemical Letters Vol. 16, No. 2, pp 225-228, 2005 225
http://www.imm.ac.cn/journal/ccl.html

Analysis of Glutamic Acid in Cerebrospinal Fluid by Capillary Electrophoresis with High Frequency Conductivity Detection

Hai Yun ZHAI[1], Jun Mei WANG[2], Xiao Li YAO[3], Xue Cai TAN[1], Pei Xiang CAI[1], Zuan Guang CHEN[2]*

[1] College of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275
[2] Department of Chemistry, Medical College, Sun Yat-sen University, Guangzhou 510089
[3] The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080

Abstract: A rapid method to determine glutamic acid (Glu) in cerebrospinal fluid (CSF) by capillary electrophoresis with high frequency conductivity detection (contactless conductivity detection) was described. The CSF sample was pretreated with silver cation resin to remove high concentration of Cl^- ions in CSF. The separation was achieved in the buffer solution of 10 mmol/L Tris and 8 mmol/L boric acid at the separation voltage of 20.0 kV. Glu showed linear response in the range of 5.0×10^{-6} to 6.0×10^{-3} mol/L, the limit of detection was 1.0×10^{-6} mol/L. The method was used for analysis Glu in CSF satisfactorily with a recovery of 97.8—98.8%.

Keywords: Capillary electrophoresis, high frequency conductivity detection, contactless conductivity detection, glutamic acid, cerebrospinal fluid.

Glutamic acid (Glu) is the most important amino acid in the nerve center system[1]. It is essential to detect Glu in cerebrospinal fluids (CSF), because the level of Glu in CSF has implicated several diseases such as metabolic diseases or central nervous system disorders[2]. The measurement of Glu in CSF had been performed by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) with induced fluorescence detection[3-7]. Since Glu shows no fluorescence, different reagents had been used for Glu derivatization. The major drawback of the detection is the instability of derivatized Glu and the time-consuming derivatization process.

In this study, a self-made high frequency conductivity detector[8] was used to detect Glu without any derivatization. This contactless conductivity detection is more convenient, and durable, since the detector electrodes are not in contact with the electrolyte. Analysis of Glu in CSF with conductimetric detection has been hampered by the large amount of Cl^- (about 130 mmol/L), which resulted in poor resolution for Glu. The interference due to the presence of high levels of Cl^- could be eliminated by silver cation resin. After simple and rapid treatment, Glu in CSF could be detected successfully by the high frequency conductivity detector.

* E-mail: chenzg@gzsums.edu.cn

Experimental

A high-voltage power source made by piezoelectric ceramics [9] was used to provide a separation voltage of 0—30 kV over a 150 μm-id fused silica capillary (Yongnian Fiber-optics Factory, Hebei Province). A high frequency conductivity detector[8] was used for detection.

L-Glu was of biochemical grade, and other chemical reagents were of analytical grade. All of them were obtained from Dongzheng Chemical Co. (Guangzhou, China). All solutions were prepared with redistilled water. Stock solutions of boric acid (H_3BO_3) and tris-(hydroxymethyl)-aminomethane(Tris) were prepared in the concentr- ation of 0.1 mol/L, then appropriate quantities of H_3BO_3 and Tris were diluted to Tris-H_3BO_3 buffer system of desired concentrations and ratios. Silver cation resin CHROMAFIX Ag^+ cartridges were purchased from Water Co. (Water Taunton, MA, USA).

All cerebrospinal fluids samples were obtained from the Department of Neurology, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou. CSF samples were stored at -20 ℃ before analysis (no more than 8 weeks). The proteins and chloride ions in the sample were removed. 0.50 mL of CSF plus 0.50 mL of CH_3OH, was centrifuged at 3800 rpm for 20 min. Supernatants (0.40 mL) were injected into CHROMAFIX Ag^+ cartridge (the cartridge was conditioned with 1.0 mL CH_3OH followed by 1.0 mL redistilled water at a flow-rate of 1.0 mL/min) to remove Cl^- ions. The cartridge was then washed with 0.20 mL redistilled water at a flow-rate of 0.5 mL/min, the first 0.20 mL of sample effluent was discarded to avoid on-column dilution, and the later sample effluences were collected for analysis.

A fused-silica capillary of 60 cm×150 μm-id with effective length of 53 cm was used in the experiments. Before using, the capillary was rinsed with 0.1 mol/L NaOH for 15 min, with water for 5 min, and then with separation buffer for 10 min. The treated samples were introduced into the capillary by a gravity injection method with 20 cm hydrostatic pressure for 10 s. The separation was achieved in the buffer solution of 10 mmol/L of Tris and 8 mmol/L of boric acid at the separation voltage of 20.0 kV. The experiment was carried out at a constant temperature of 25℃.

Identification of the peaks for components was carried out by injection of individual standards and/or by spiking samples with known standards. Migration time and peak areas were recorded. Linearity was assessed from the analysis of standard Glu solution at concentrations ranging from 5.0×10^{-6} mol/L to 6.0×10^{-3} mol/L. Linear regression coefficients (r) and slopes, as well as intercepts for the calibration curves were determined.

Results and Discussion

In order to obtain a satisfied separation and detection of Glu in CSF, various analytical conditions have been researched. Different buffer solutions including the systems of Tris-H_3BO_3, ethylenediamine-H_3BO_3, NaH_2PO_4-Na_2HPO_4 were tested. The experimental results showed that the current in the buffer of NaH_2PO_4-Na_2HPO_4 was too high to separate well, and ethylenediamine-H_3BO_3 solution could not give a high detection sensitivity of Glu, it took longer time to detect Glu in the medium. Therefore, the

Figure 1 Calibration graph of Glu

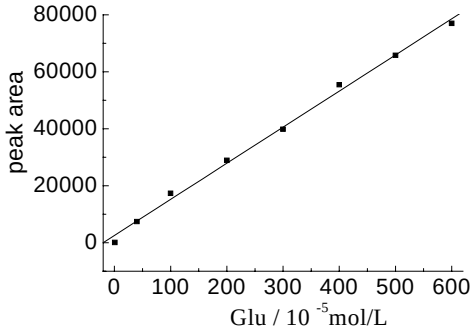
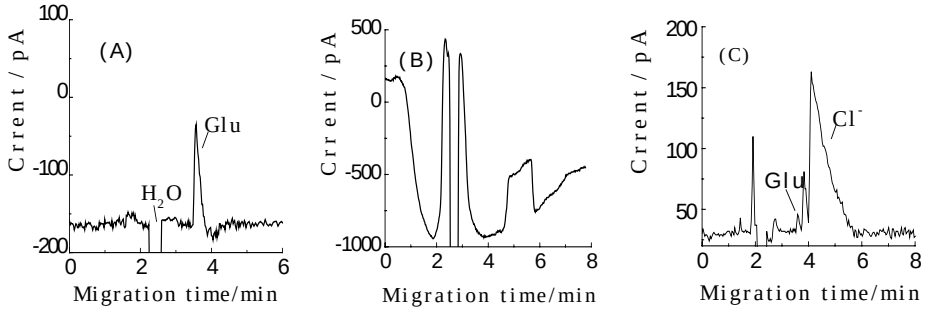


Figure 2 Electropherogram of Glu and CSF sample



Voltage 20 kV; Temperature 25℃; Buffer 10 mmol/L Tris + 8 mmol/L H_3BO_3; (A) Glu standard solution, 1.0×10^{-4} mol/L; (B) CSF before treated; (C) treated CSF

Table 1 Determination result and spiked recovery for CSF

Sample No.	Concentrations of Glu (μmol/L)	Added (μmol/L)	Found (μmol/L)	Recovery %	RSD %
1	21.2	40	60.3	97.8	3.1
2	18.4	40	57.7	98.3	2.7
3	15.5	40	55.0	98.8	2.8

Tris-boric acid system was chosen to be the buffer solution in this experiment. Besides, the influences of running electrolyte composition (pH, ratios and concentrations), as well as the separation voltage were researched and the best analytical conditions were obtained. As shown in **Figure 2** (A), Glu could be separated and detected rapidly and sensitively in the buffer solution of 10 mmol/L of Tris and 8 mmol/L of boric acid at the separation voltage of 20.0 kV. Under the chosen condition, there was a linear relationship between the Glu concentration and the peak area in the range of 5.0×10^{-6}—6.0×10^{-3} mol/L (linear equation Y = 133 X - 39.9, r = 0.998, X/10^{-5}mol/L), as shown in **Figure 1**. The limit of detection was 1.0×10^{-6} mol/L (S/N=3), and RSD was 2.2% (n=6).

Direct determination of Glu in CSF samples by using conductivity detector is impossible because of the large amount of Cl^- ions, as shown in **Figure 2** (B). In order to

decrease the high concentration of Cl^- in CSF, the samples were treated with high capacity cation resin in Ag^+-form to remove Cl^- ions. It was proved that most of Cl^- in the samples could be retained in CHROMAFIX Ag^+ cartridge, a kind of cation resin in Ag^+-form. On the other hand, no other amino acids could be found after pretreatments of samples, it seemed that, under the chosen conditions, the cartridge was useful for retention of other amino acids with higher isoelectric point, therefore, the presence of other amino acids could not made a hamper of determination of Glu in CSF. Using this approach, the quantity of Glu in CSF that contained initially about 130 mmol/L of Cl^- was determined successfully. **Figure 2** (C) was the electropherogram of CSF sample after pretreatment. It was shown that the Cl^- peak was sufficiently reduced to permit the determination of Glu in CSF. This result indicated that the Ag^+ cartridges could selectively retain chloride ions in the sample. Additional results were listed in **Table 1**.

Conclusion

A new method was developed for the determination of Glu in CSF by capillary electrophoresis with high frequency conductivity detection. Large amount of Cl^- ions in CSF could be removed efficiently by using CHROMAFIX Ag^+ cartridge. The method is rapid, easy and economic.

Acknowledgment

This project was supported by the Guangdong Provincial Foundation of Natural Science (021808)

References

1. A. John, M. D. Pietropaoli, B. Frederick, *J. Trauma*, **1992**, *33*, 403.
2. T. L. Perry, C. Krieger, S. Hansen , *et al.*, *Ann. Neurol.*, **1990**, *28*, 12.
3. H. L Rowley, K. F. Martin, C.A. Marsden, *J. Neurosci. Methods*, **1995**, *57*, 93.
4. Qu Y Arckens L., E. Vandenbussche, S. Geeraerts, *et al.*, *J. Chromatogr. A*, **1998**, *798*, 19.
5. D. J. Begley, A. Reichel, A. Ermisch, *J. Chromatogr. B Biomed. Appl.*, **1994**, *657*, 185.
6. Y. M. Li, Y. Qu, E. Vandenbussche, *et al.*, *J. Neurosci. Methods*, **2001**, *105*, 211.
7. G. Nouadje, H. Rubie., E. Chatlut, P. Canal, *et al*., *J. Chromatogr. A*, **1995**, *717*, 293.
8. Z. G. Chen, J. Y. Mo, *Chem. J. Chin. Univ.*, **2002**, *23*, 801.
9. Z. G. Chen, J. Y. Mo, X. Y. Yang, *et al.*, *Chin. Chem. Lett.*, **1999**, *10*, 231.

Received 30 December, 2003