Journal of Chromatography B, 980 (2015) 20–27

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Dynamic pH junction–sweeping technique for on-line concentration of acidic amino acids in human serum by capillary electrophoresis with indirect UV detection

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ARTICLE INFO

Article history:
Received 9 May 2014
Accepted 11 December 2014
Available online 29 December 2014

Keywords:
Dynamic pH junction–sweeping technique
On-line concentration
Acidic amino acids
Indirect UV detection
Binding constant

ABSTRACT

Glutamic acid (Glu) and aspartic acid (Asp), as two important neurotransmitters, have been the focus of increasingly intense research over the past several years. Glu and Asp are present in biological fluids such as serum at trace levels, but complex components in biological matrices make it difficult to determine them in biological samples. In this paper, a sensitive and simple method coupled with indirect UV detection, using benzoic acid (BA) as the UV-absorbing probe, was developed and validated for the quantitative determination of Glu and Asp in human serum and Compound Amino Acid Injection-18 AA. The method combines a dynamic pH junction with a sweeping technique using β-cyclodextrin (β-CD) as the complexing agent for sweeping. Employing this proposed method, low detection limits of 0.061 μg/mL for Glu and 0.032 μg/mL for Asp were obtained. The sensitivity was improved 30- and 55-fold for Glu and Asp compared to conventional CE method. Standard curves were linear ($r > 0.999$) over the concentration range of 0.1–8.0 μg/mL. To further improve the resolution of Asp from interfering substances in human serum, 6% (v/v) methanol was added to the sample matrix, and resulted in the detection limits of 0.125 μg/mL for Glu and 0.057 μg/mL for Asp. With a simple precipitation of protein, the method has been successfully applied to the analysis of human serum, and the recoveries (82% for Glu and 87% for Asp) were achieved with relative standard deviations of 1.9% and 2.0%, respectively.

1. Introduction

Glu and Asp are very important amino acid neurotransmitters in the mammalian central nervous system [1,2]. In the course of neurological diseases, the blood–brain barrier is damaged. Thus the permeability of the blood–brain barrier rises, which allows the passage of proteins and other substances by diffusion or active transport. Then the level of amino acid neurotransmitters in the serum may vary from the level of these substances in the cerebrospinal fluid and brain tissue. Jakubowska-Solarska et al. [3] observed that the Glu and Asp level in multiple sclerosis patients was significantly higher than in the control group, and revealed a significant positive correlation between multiple sclerosis incidence and the level of Glu and Asp in serum. Therefore, their quantitative analysis of the serum is of great significance to disease diagnosis.

Capillary electrophoresis (CE) has attracted considerable interest in the analysis of amino acids because of its high separation resolution and efficiency, fast analysis, versatility for numerous analytes, and extremely small (nanoliter) sample injection volume compared with other analytical methods [4–8]. UV absorbance detection, fluorescence detection and mass spectrometry are conventionally used for detecting amino acids after CE separation. Among them, UV absorbance detection is more popular because of its low costs and its relatively lax requirements for sample treatment [9]. However, most amino acids such as Glu and Asp have no UV chromophores and need to be derivatized with chromophore groups, which is tedious and time consuming. Indirect UV detection, in which the analyte is detected as a decrease in absorbance when a chromophoric ion is added to the background solution (BGS), is a good choice because it does not require the derivatization step [9]. However, a major disadvantage of this method is its

Abbreviations: Asp, aspartic acid; AFMC, analyte focusing by micelle collapse; BA, benzoic acid; BGE, background solution; CE, capillary electrophoresis; CZE, capillary zone electrophoresis; EOF, eletroosmtic flow; FASS, field-amplified sample stacking; Glu, glutamic acid; LIF, laser induced fluorescence; LVSS, large-volume sample stacking; LOD, limit of detection; LOQ, limit of quantitation; MS, mass spectrometry; MSS, micelle to solvent stacking; NED, *N*-(1-Naphthyl)ethylenediamine dihydrochloride; SEFs, sensitivity enhancement factors.

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http://dx.doi.org/10.1016/j.jchromb.2014.12.009
1570-0232/

low sensitivity, which is caused by the short optical path length for on-line UV detection and the low sample injection volumes.

Improvements in the sensitivity of detection after CE separation continue to be a hot topic, especially for analysis of biological samples in which the analytes are present at trace levels. While some efforts to improve the detection limits have been directed at off-line extraction and cleanup methods (e.g., solid-phase or liquid-phase extraction) [10,11], most investigators have placed emphasis on developing on-line concentration techniques that enable the injection of large volumes of sample without compromising the resolution and efficiency. So far, a number of on-line concentration techniques have been demonstrated, including dynamic pH junction [12], pH-mediated stacking [13], sweeping [14], large-volume sample stacking (LVSS) [15], field-amplified sample stacking (FASS) [16], transient moving chemical reaction boundary [17], analyte focusing by micelle collapse (AFMC) [18], and micelle to solvent stacking (MSS) [19]. It is worth noting that a combination of two on-line sample concentration techniques can further improve sensitivity [20,21]. Among these techniques, dynamic pH junction, pH-mediated stacking, LVSS, and sweeping have been used for on-line concentration of amino acids [8].

In this paper, a combination of dynamic pH junction and sweeping with indirect UV detection is adopted to improve the detection sensitivity for Glu and Asp. It is widely accepted that the foundation for concentration by a dynamic pH junction in the capillary format originated in 1990 from the work of Aebersold and Morrison [22]. Then, Britz-McKibbin et al. [12] described and investigated this technique and introduced the name “dynamic pH junction”. This method is based on the creation of a pH discontinuity that is established by injecting the sample at a different pH than that of the BGS, and it can be used to concentrate weakly ionic analytes. It is also critical to note that there must be a substantial difference in mobility of the analytes at these different pH values, such that when the analyte moves from one pH to the other there is an associated change in the velocity [23]. Thus, concentration is a reasonable result, because the reversal of the migration direction from the sample zone to BGS zone causes the analyte to be concentrated in a small area. Sweeping utilizes the interactions between the sample in the matrix and a pseudostationary phase or a complexing agent that is present only in the BGS [24]. The accumulation is based on chromatographic partitioning, complexation, or interactions between the analytes and the additives during the electrophoretic process [25–27]. The most common sweeping agents used are micelles [20,24], but β-CD and its derivatives also have been adopted [28,29]. In our studies, we applied β-CD as complexing agent for the sweeping technique. The technique, a combination of dynamic pH junction and sweeping with indirect UV detection, integrates the merits of both dynamic pH junction and sweeping and improves separation selectivity and sensitivity. It can improve the focusing efficiency for certain analytes if it is compared to that of either dynamic pH junction or sweeping alone [29,30]. It also extends the usefulness of on-line focusing methods in CE to simultaneously preconcentrate and separate both weakly acidic (hydrophilic) and neutral (hydrophobic) analytes using inexpensive buffers and conventional instrumentation [30].

A simple model based on the study by Britz-McKibbin et al. [30] is shown in Fig. 1. The process of dynamic pH junction–sweeping is as follows. The capillary is filled with BGS (pH 7.5) containing 10 mM Tris, 8 mM BA, and 10 mM β-CD, and a long sample plug prepared in an acidic sample solution (phosphate, pH 2.5) is injected by gravity into the capillary (Fig. 1a). Glu and Asp, with pI values of 3.22 and 2.79, respectively, are negatively charged at pH 7.5 and positively charged at pH 2.5. The analytes migrate to cathode at pH 2.5 and in the reverse direction at pH 7.5. Once the voltage is applied, the hydroxide ions (OH^-) in the BGS (pH 7.5) migrate with rapid mobility into the sample zone, while Glu and Asp migrate

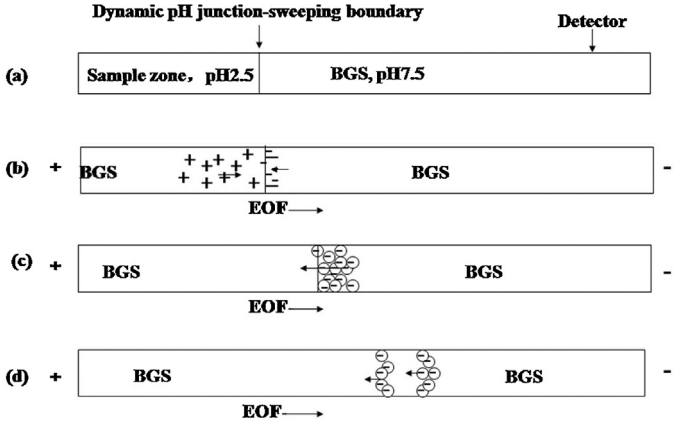

Fig. 1. Schematic diagram of the online concentration of Glu and Asp by CE with a dynamic pH junction–sweeping technique. (a) The capillary is conditioned with BGS, and a large-volume of sample plug is injected by gravity. (b) The dynamic pH junction is initiated. (c) The analytes interact with β-CD, and sweeping is completed. (d) The analytes are separated in the normal CZE mode.

in the opposite direction, leading to an abrupt local pH increase at the front edge of the sample zone and a reverse in the migration direction of the analytes (Fig. 1b). As a result, the mobilities of the analytes in the front edge experience a dramatic drop, so the analytes become stacked. Then, the analytes interact with β-CD and are swept (Fig. 1c). Two main factors are responsible for the interaction. First, the analytes, Glu and Asp, are low molecular mass compounds, which have good access to the cavities of β-CD. Second, hydrogen bonding between the carboxyl groups of the analytes and hydroxyl groups of β-CD enhances the complex formation. Subsequently, the separation of Glu and Asp proceeds according to the normal capillary zone electrophoresis (CZE) mode (Fig. 1d).

2. Experimental

2.1. Apparatus

All CE experiments were performed on a CL1030 high-performance CE apparatus with a UV detector (Beijing Cailu Instrumental Co., Beijing, China). Uncoated fused-silica capillaries purchased from Yongnian Optical Fiber Factory (Hebei, China) were used. The dimensions of the capillary are 50 cm × 50 μm I.D × 375 μm O.D. The effective length of the capillary is 42 cm from the injection end of the capillary. The data acquisition was carried out with an HW-2000 Chromatography Workstation (Shanghai Qianpu Software Company, Shanghai, China). UV detection was carried out at 214 nm. Samples were introduced into the capillary by hydrodynamic injection, where the sample vial was raised by 15.5 cm. A PH-3C acidity meter (Shanghai Hongyi Instrument Co., Ltd., Shanghai, China) was used for the pH measurement.

The new capillary was flushed with 1 M sodium hydroxide for 1 h and then with redistilled water for 10 min before use. At the beginning of each working day, the capillary was flushed sequentially with distilled water (5 min), 100 mM NaOH (5 min), distilled water (5 min), and the running buffer (5 min). Simultaneously the CE instrument was warmed up until a stable baseline was achieved. Between consecutive analyses, the capillary was rinsed with distilled water (2 min), 100 mM NaOH (2 min), distilled water (2 min), and the running buffer (3 min). The capillary washing pressure is 80 kPa. The capillary was left filled with distilled water overnight.

2.2. Chemicals and reagents

Glu and Asp were obtained from J&K China Chemical Ltd (Beijing, China). The standards were used as received. Human serum