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A miniaturized analysis system composed of monolith-based microchip electrochromatography coupled with on-line chemiluminescence detection for amino acids

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ABSTRACT

Miniaturized chemical analysis instruments using micromachining techniques are receiving increasing attention. We presented a compact microchip-based electrochromatography (μCEC)-chemiluminescence (CL) system for the detection of amino acids. Monoliths prepared by in situ photopolymerization in the separation channel of the microchip served as the stationary phase of the μCEC coupled with CL detection. Negative pressure combined with electrokinetic force injection method was utilized for sampling and transporting CL reagents in this system. The amino acids were directly analyzed by enhanced Cu^{2+} catalyzed activity for luminol-hydrogen peroxide CL reaction as the formation of Cu^{2+}-biomolecule complexes online. Glycine (Gly), Glutamic acid (Glu), Arginine (Arg) and Aspartic acid (Asp) were chosen as model compounds to evaluate the performance of the μCEC-CL system. The results demonstrated that the proposed system offered a number of benefits including miniaturization, significant simplification in operation and instrumentation; what is more, amino acids were directly analyzed without complex labeling procedure.

1. Introduction

Miniaturization is generally recognized as one of the most important trends in the development of analytical instrumentation [1–4]. Over the past decades, miniaturized total analysis system (μTAS) has experienced the phenomenal success and displayed enormous practical potentials [5–8]. Since the first microchip-based electrochromatography (μCEC) work was published by Ramsey and co-workers in 1994 [9], it has received considerable attention [10,11]. The μCEC is a hybrid method of microchip zone electrophoresis (μCZE) and chip-based liquid chromatography (μLC) which can separate both ionic and neutral compounds. Furthermore, the plug-like electroosmotic flow (EOF) profile results in reduced dispersion of the analytes zone, which increases column efficiency. The μCEC obtains a wide range of applications in biology, medicine, environment and food analysis in recent years [12–14]. Several micromachining technologies have been applied to fabricate the separation column in the channel of the chip [15–17]. For example, Harrison et al. [17] developed an integrated solid-phase extraction bed using a double weir design to construct a cavity in which beads coated with a stationary phase could be trapped. The 30-μm-wide side channel forming part of a three-way junction was designed to feed beads into or out of the chamber created by the two weirs on either side of the side channel. Although these approaches have been proven successful, it is still a crucial challenge to achieve uniform packing micro-scale channels with the stationary phase particles [18]. So the packed bed is replaced by a continuous bed polymerized in situ in the chip channels [19–21]. Thermal polymerization or UV-initiated polymerization is often used for the preparation of monolithic materials in the capillary tubes or microchannels [22–24]. The UV-initiated free-radical polymerization, which enables positioning of the monolith in a specified part of the microchannel through a mask, has been proven suitable for microfluidic devices [25–27]. The UV-initiated polymerization was carried out at room temperature using even low molecular weight alcohols and other low boiling point solvents. These solvents can safely be used to create a versatile series of binary porogenic mixtures which mainly control the porous properties of the monoliths [28].

Amino acids are essential building blocks of biological molecules and they have a close relationship to life activities of biology. The development of new technologies for sensitive and quantitative determination of amino acids has thereby been considered as a very important issue in many chemical and biological researches. It is not

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simple to determinate them by traditional photometric and flurometric detection, since these compounds lack natural chronopher or fluorephore. In order to solve these problems, pre- and post-column derivatization reactions have been developed to convert non-fluorescent amino acids into highly fluorescent derivatives. However, derivative reactions are intricate and time consuming [29]. Chemiluminescence (CL) detection does not need any light source which provides low background with excellent sensitivity and simple instruments [30,31]. It should be an attractive option for detection toward miniaturization. Lin et al. [32] developed a new method for pressurized capillary electrochromatography coupling with CL detection using a modified on-column coaxial flow detection interface for separation and detection of the underivatized amino acids. The amino acids were detected by enhanced Cu^{2+} catalytic activity for luminol–hydrogen peroxide CL reaction when Cu^{2+} interacted with biomolecules to form Cu^{2+}-biomolecule complexes [33]. However, complex interface design was not easy in this assay system. In previous study [34], we had established a miniaturized analysis system composed of microchip electrophoresis and on-line CL for detection of metal ions in aqueous solution by creating a porous monolithic plug in the separation channel as a select valve. But low separation efficiency resulted from asymmetrical EOF in the separation channel.

Inspired by the aforementioned developments, herein we presented a compact μCEC-CL system in which negative pressure combined with electrokinetic force injection method was utilized for analyzing the underivatized amino acids based on the principle of the enhanced effect of Cu^{2+}-amino acid complexes on the luminol–H_2O_2 CL reaction. To our best knowledge, the determination of underivatized amino acids with μCEC-CL system has not been reported. Glycine (Gly), Glutamic acid (Glu), Arginine (Arg) and Aspartic acid (Asp) were chosen as model analytes to evaluate the performance of the μCEC-CL system. Several factors that affected separation and CL intensity of amino acids, such as copper ions concentration, organic modifier content, mobile phase pH and applied voltage were discussed in detail. The assay system we proposed here has some unique features: (1) manipulation of both sample and CL reagents is realized by a simple and low-cost subatmospheric pressure fluid-driven device. Negative pressure combined with electrokinetic force injection method is utilized to achieve variable-volume sample loading; (2) the suggested fluid-manipulation device is rather simple, only consisting of a vacuum pump, a 3-way electromagnetic valve, a needle valve, a vacuum vessel and a single high voltage supply. What's more, all miniaturized components are commercially available and the power consumption of the total μCEC-CL system is much low; (3) copper ions and the amino acids are mixed online in the separation channel to avoid the saturated complex formation, thus enhancing the catalytic ability to achieve direct determination of unlabelled amino acids. The experimental results proved that the proposed μCEC-CL system has the advantages of simplicity, sensitivity and superior separation performance.

2. Experimental

2.1. Reagents and materials

Luminol (3-aminophthalhydrazide), ethylene dimethacrylate (EDMA), hydroxyethyl methylacrylate (HEMA) and 3-methacryloxypropyltris-(trimethylsiloxy)-silane (γ-MAPS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and dimethylamino-pyridine (DMAP) were purchased from Acros Organics (New Jersey, USA). Hydrogen peroxide solution (30%) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycine (Gly), Glutamic acid (Glu), Arginine (Arg) and Aspartic acid (Asp) were purchased from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). All other chemicals were of analytical grade and used without further purification. Ultrapure water (18.2 MΩ cm) used in the experiment was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA). Before use, all the utensils were soaked in 10% HNO_3 for 24 h, rinsed thoroughly in Millipore purified water, and all solutions were filtered through 0.45 μm membrane filters.

2.2. Instrumentation

The schematic diagram of the μCEC-CL system is shown in Fig. 1(a). It consists of a microchip, a photomultiplier tube (PMT, Hamamatsu, Japan) used for CL detection and a subatmospheric pressure fluid-driven device which is described detailedly elsewhere [34,35].

2.3. Microchip fabrication

The microchip was fabricated on soda-lime glass (Shaoguang Microelectronics Co., China) using standard photolithography, wet chemical etching and heat bonding techniques described elsewhere [34]. The channel design of the microchip device used for this work is shown in Fig. 1(b). The channels were etched to a depth of 30 μm and a width of 100 μm. Access holes were drilled into the etched plate with a 3.0 mm diameter diamond tipped drill bit at the terminals of the channels. After permanent bonding by a thermal bonding procedure, four micropipet tips with 4 mm inner diameter and 6 mm tall were joined by epoxy resin on the chip surrounding the holes B, C, S and SW, and three micropipet tips with 7 mm inner diameter and 25 mm tall covered the holes H, L and W serving as reservoirs.

Reservoirs S, SW, B, C, L, H and W were for sample, sample waste, buffer, copper ions solution, luminol, hydrogen peroxide and waste, respectively. The channels between sample reservoir (S), copper solution reservoir (C) and sample waste reservoir (SW) were used for sampling and the channel between the intersection points P_1 and P_2 was used for analytes separation. The spiral channel between P_2 and W was the detection channel, where the sample, luminol and hydrogen peroxide streams met together and mixed, and the chemiluminescent reaction occurred. The spiral detection cell was composed of six arcs, R_1, R_2 and R_3, each in duplicate, which are arrayed symmetrically as shown in Fig. 1(c). Radius of curvature for R_1, R_2 and R_3 were 2.8 mm, 2.2 mm and 0.6 mm, respectively [34].

A macroporous polymer monolith was prepared using a photoinitiated free-radical polymerization technique within the separation channel (P_1–P_2) according to the literature with a minor modification [28]. The polymerization mixture was composed of the monomer (0.40 g EDMA, 0.594 g HEMA), porogenic solvent (40:50:10 wt% methanol/hexane/water, 1.50 g) and photoinitiator (15 mg DMAP). In addition, ionizable monomers, AMPS (0.1% w/v) was also used in the polymerization mixtures to provide negatively charged functionalities. Scanning electron microscope (SEM) micrograph of the macroporous polymer is shown in Fig. 1(b).

2.4. Experimental procedure

For a new microchip, channels were washed sequentially using 1 M NaOH solution and water. Then they were rinsed and conditioned with buffer solution for 1 h. Then, 250 μL and 50 μL running buffer were added into the reservoirs B and SW. 150 μL of sample solution and 150 μL copper ions solution were pipetted into the reservoir S and reservoir C, respectively. Heights of liquid levels of the reservoirs can meet the requirements of $H_B > H_C = H_S > H_{SW}$.