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A strategy to modulate the electrophoretic behavior in plastic microchips using sodium polystyrene sulfonate

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ABSTRACT

Plastic microchips have been broadly used as disposable microfluidic devices, but the poorly defined surface properties limit their application. Herein, we proved that an anionic polymer could be used as the background electrolyte (BGE) to provide a strong and stable cathodic electroosmotic flow (EOF) and modulate the electrophoretic behavior for efficient separation in relative thicker microchannels (~75 μm id). A cathodic EOF of $\sim 3.3 \times 10^{-4}$ cm^2 V^{-1} s^{-1} was maintained using sodium polystyrene sulfonate (PSSNa) with a molecular weight of 5×10^5 as the BGE, which ensured fluorescein isothiocyanate labeled biogenic amines (BAs) appeared ahead of other components in the electropherograms obtained with microchips of cyclic olefin copolymer. Four selected BAs appeared within 50 s and theoretical plate numbers of 8.0×10^5/m were achieved. The role of PSSNa was evaluated with streaming potential, dynamic light scattering, contact angle and atomic force microscopy. Its functionalities as surface modifier, viscosity regulator and pseudostationary phase were also confirmed. The proposed electrophoretic method was applied in the fast determination of BAs in fish meat samples.

1. Introduction

As a compact version of capillary electrophoresis (CE), microchip electrophoresis (MCE) inherits critical advantages such as high efficiency, less reagent and sample consumption, while its great potential for miniaturization and integration makes it very suitable for fast and real-time analysis [1,2]. However, the application of MCE in the real world is still limited [3–5], far from that was expected at its early stage of the development. The cost of microchips has been taken as a main concern and various materials have been reported for the fabrication of cheaper microchips [6]. Plastics has been proved to be promising for commercial mass production of disposable microfluidic devices due to its attractive advantages such as reduced cost, simplified manufacturing procedures and the broad range of available polymeric materials that may satisfy the diverse requirements of various applications [7]. Plastic materials can effectively reduce the cost of microchips themselves, but their hydrophobic surfaces and poorly defined electroosmotic flow (EOF) limit their wide application in electrophoresis. Several approaches and techniques have been used for the surface modification but most of them, except dynamic coating [8–10], may significantly complicate the analysis procedure and compromise the advantages of MCE. Another issue that affects the robustness of MCE is the clogging of the microchannels by airborne particles. To reduce the chance of clogging, thicker microchannels should be used but that can impact the performance of the separation due to the shorter microchannels on the microchips and Joule heating. Our previous works indicated that polymer additives with proper viscosity in the background electrolyte (BGE) can ensure high separation performance in thicker microchannels of plastic microchips [11–13].

MCE can be an ideal tool for rapid screening of hazardous substances in food or agriculture products [4,14]. The food safety issue that is indicated by the growing number of food safety incidents around the world is becoming a serious concern. The World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and other related agencies are reinforcing the food safety supervision and management [15], which will significantly increase the requirement of rapid analysis technologies. To develop robust and easy-to-use MCE for the food analysis is therefore highly necessary. Biogenic amines (BAs) are nitrogenous organic compounds with low molecular weight commonly found

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in animals, plants and microorganisms. Their concentration can be used as an indicator of freshness and hygiene conditions of food production [16]. High concentrations of BAs may be an indicator of poor food quality and may cause serious health problems [17]. Some studies have shown that histamine (HIS) may give rise to anaphylactoid food poisoning and tryptamine (TRY) can raise blood pressure [17,18]. The diamines such as putrescine (PUT) and cadaverine (CAD) can enhance the toxicity of HIS and react with nitrite to generate potentially carcinogenic nitrosamines [19].

We have developed an effective MCE protocols for rapid BAs determination using hydroxypropyl cellulose (HPC) as the viscosity regulator and surface modifier [20], but with HPC the EOF was significantly suppressed and all analytes migrated after the derivartization reagent fluorescein isothiocyanate (FITC) and other matrix components. Exploring methods to actively control the EOF and the migration order of the analytes is apparently helpful to increase the analysis speed and reduce the influence of the sample matrices. Amplifying the surface charge may be a potential way to reach this goal.

Polyelectrolytes, which have ionizable groups in the main skeleton or side chains, are extensively used in industry and scientific research [21]. The modification of surfaces through layer-by-layer assembling of polyelectrolytes has been widely used in either CE [22] or MCE [23]. Henry's group adopted a polymer bilayer consisting of a cationic layer of polybrene (PB) and an anionic layer of dextran sulfate (DS) to get stable EOF in PDMS microchips, the direction of EOF can be changed [24]. Use of a single ionic polymer in MCE as the additive of the BGE has also been reported [25,26].

Sodium polystyrene sulfonate (PSSNa) is a widely used anionic polyelectrolyte [27–29]. Previous studies indicated that the surface activity and conformation of PSSNa could be influenced by its concentration, degree of sulfonation, co-existing salt etc [28,30,31]. In addition, PSSNa was used to modify the membrane materials to enhance separation performance [32] and as a drug for hyperkalaemia [33]. Its application in CE and MCE was also reported. Pranaitytė et al. [34] used PSSNa as a pseudostationary phase to separate anionic, cationic and neutral compounds in capillary electrokinetic chromatography. It was also investigated as an additive with the interaction with quaternary ammonium compounds in CE [35]. Chen's group [36] adopted PSSNa and poly(dially dimethyl ammonium chloride) to form polyelectrolyte multilayers in PDMS microchips for aminophenol isomers separation. Wu et al. [26] reported PSSNa as a pseudostationary phase to provide higher stability and efficiency for the separation of antibiotics on glass microchips than that with surfactant sodium dodecyl sulfate (SDS).

In this work, high molecular weight (MW 500000) PSSNa was utilized as the BGE to cope with both surface and clogging problems mentioned previously. The functions of PSSNa also include the surface charge modulation and viscosity regulation in addition to the pseudostationary phase. A stable cathodic EOF was implemented to cyclic olefin copolymer (COC) microchannels and highly efficient separation of four selected BAs in relatively larger microchannel (~75 μm id) was achieved. The effect of PSSNa on the surface properties of COC and the hydrodynamic radius of PSSNa molecules at different pHs were evaluated. A robust MCE protocol was established and its applicability was demonstrated by the determination of BAs in fish meat.

2. Experimental

2.1. Apparatus

The MCE device including a computer controlled high voltage power supply, a LIF detector and a negative pressure switching valve was same as that reported previously [13]. The excitation source of the detector was a 473 nm DPSS laser (MBL-III-473, Changchun New Industries Optoelectronics Tech Co. Ltd., Changchun, China). The laser beam was focused onto the microchannel through a microscope objective (20×, Beijing 7-Star Optical Instruments Co. Ltd., Beijing, China). A dichroic mirror (505 nm, DM505) and a long-pass filter (520 nm, BP520), both from Shenyang HB Optical Technology Co. Ltd., Shenyang, China, were used to reflect the laser and get rid of the laser scattering. The fluorescence was collected by an avalanche photodiode (APD, AD500-8-TO52S2, Silicon Sensor, German) and fed to a NI USB-6009 data acquisition card (National Instruments, Austin, TX, USA). The viscosity of the solution was measured using an Ubbelohde viscometer in a super thermostatic water bath set at 25 °C (DF-02, Nanjing Fangao Technology Co., Ltd., Nanjing, China). The conductivity of solution was obtained using a conductivity meter (DDS-307, Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China). The microchips with cross-type microchannel configuration (~75 μm id) were used. The detailed fabrication of the microchannel was same as described previously [37] except slight difference in the temperature due to the different batch of COC plates. Both wire-embossing (127 °C) and microchannel sealing (117 °C) were performed by sandwiching the COC substrates between two glass microscope slides with binder clips.

2.2. Chemicals and solution preparation

Histamine dihydrochloride (98%), tryptamine hydrochloride (98+%), cadaverine hydrochloride (98%) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China), putrescine hydrochloride (98%) was from Beijing BioDee Biotechnology Co., Ltd. (Beijing, China). FITC was obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium poly(styrene sulfonate) (PSSNa, MW 500000) was purchased from J&K Chemical Ltd. (Beijing, China). Sodium tetraborate and pyridine were products of Tianjin Guangfu Science and Technology Development Co., Ltd. (Tianjin, China). SDS was a product of Chengdu Kelong Chemical Reagent Company (Chengdu, China). HPC was a product of Sangon Biotech Co., Ltd. (Shanghai, China). Anhydrous ethanol, anhydrous methanol, acetonitrile and acetone were obtained from Rionlon Bohua Pharmaceutical & Chemical Co., Ltd. (Tianjin, China). Trichloroacetic acid (TCA) was provided by Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All reagents used in this experiment were analytical grade or above. The hairtail and grass carp samples used for determination of BAs were bought from a local supermarket.

Four BAs stock solutions (40 mmol/L) were prepared in distilled water. Fresh FITC solution in acetone was prepared daily by dissolving 1.5 mg of FITC in 200 μL acetone containing 1% (v/v) pyridine (redistilled) and stored in the dark at 4 °C. The BGE used for separation of BAs was prepared by diluting PSSNa stock solutions (2%, w/v) with distilled water. The desired pH values of the PSSNa solutions were adjusted by addition of NaOH (1.0 mol/L) under the monitoring of a pH meter. All solutions were filtered with 0.22 μm cellulose acetate filtrate membranes (Shanghai Xingya Purification Materials Factory, Shanghai, China) before use.

2.3. Derivatization

The derivatization of BAs was performed as follows: 2.0 μL of FITC solution and 28.0 μL of 10 mmol/L sodium tetraborate buffer (pH 9.2, 25 °C) were added in 10.0 μL of BA standard or sample solution in a 0.5 mL micro-centrifuge tube. The mixture was vortex-mixed for 10 s and heated at 65 °C in a water bath for 15 min. The derivative was diluted to the desired concentration with BGE and used for MCE analysis immediately.