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A glass/PDMS electrophoresis microchip embedded with molecular imprinting SPE monolith for contactless conductivity detection

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ABSTRACT

A simple glass/PDMS microfluidic chip for on-line sample pretreatment and contactless conductivity detection, which consisted of a 0.17 mm-thick glass cover and a PDMS substrate embedded with an SPE monolithic column, was presented in this paper. Using this integrated microchip system, sample extraction, injection, separation and detection were automatically performed in sequence. A sample of auramine O was concentrated by molecularly imprinted SPE (MISPE), eluted to sample reservoir, and injected by electromigration into separation channel for electrophoresis separation and contactless conductivity detection, with the calculated detection limit (S/N = 3) being 2.5 μg mL^{-1}. The monolithic column was utilized to quantitatively extract auramine O, yielding an on-line enrichment factor of about 12. The microchip system is reliable and applicable to the analysis of auramine O in food sample.

1. Introduction

Micro total analysis system (μ-TAS) or Lab on a chip (LOC), which was first proposed by Menz et al. [1], has been highlighted due to remarkable sensitivity, automation, low solvent consumption, short separation time, miniaturization and portability. Nowadays, microfluidic chips, especially microchip capillary electrophoresis (MCE), have been widely applied in biomedical analysis, clinical diagnostics, environment monitoring, drug screening, cell cultivation and food safety. Despite the undeniable success of microfluidic chip technologies, sample pretreatment, especially that with complicated matrix, is a critical operation prerequisite for trace target compounds at concentrations lower than the detection limits of the microfluidic system. Therefore, developing a selective and sensitive method for sample purification and concentration is of great importance. Fortunately, a number of efforts have been successfully miniaturized for sample pretreatment steps, such as analyte extraction [2–4], sample filtration [5,6], sample desalting [7–9] and sample preconcentration [10,11].

SPE, one of the most important and widely used pretreatment techniques [12], permits both the preconcentration of analytes and the reduction of interfering components. Molecular imprinting technique can recognize and capture target molecules selectively, which provides eligible molecularly imprinted polymers (MIPs) for analysis and separation. Synthetic materials with artificially introduced recognition sites have high binding affinity, stability, and specificity toward target molecules, and thus have been applied in chromatography [13–15], sensors [16–18] and catalysis [19]. Their use in SPE, referred to as molecularly imprinted SPE (MISPE) [20–26], is one of the most advanced applications of MIPs. As selective adsorbents, MIPs potentially allow target analytes to be selectively extracted from complex matrices without undesirable components. Therefore, it is of special interest when the sample is complex with interference causing signal overlap in separation and quantification.

In recent years, portability and miniaturization have been focused on [2–4,27–31]. In order to accomplish SPE within a chip, diverse methods, such as coating [32–34], packing [28–30,35,36] and in situ polymerization of a monolithic column [27,31,37,38], have been reported to introduce stationary phase. Comparatively, monolithic column, which is usually prepared by in situ polymerization, has drawn much attention because of facile preparation procedure with flexibly controllable pore size and porosity, fretless construction, various surface properties, high selectivity, physicochemical stability and applicability in harsh chemical media. The merits of MISPE monolithic column and microfluidic chips can be combined when integrated. Recently, fabrication and optimization of chip-formed MISPE have been spotlighted [38–40].

Contactless conductivity detection (CCD), a simple and universal detection technique, has been extensively used in microfluidic systems [41–57]. Contactless conductivity mode is a particular form of conductivity-based detector where the electrodes are entitled to a longer life by being insulated from the electrolyte. Besides, it benefits

Abbreviations: AIBN, azobis (isobutyronitrile); CCD, contactless conductivity detection; EDMA, ethylene dimethacrylate; EOF, electroosmotic flow; LOC, Lab on a chip; MAA, methacrylic acid; MCE, microchip capillary electrophoresis; MIPs, molecularly imprinted polymers; MISPE, molecularly imprinted SPE; NIP, nonimprinted polymer; PMMA, poly(methyl methacrylate); PDMS, poly(dimethylsiloxane); μ-TAS, Micro total analysis system.

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microchip separations in the effective isolation between electrodes and electrolyte, simple construction, convenient manipulation, the elimination of electrodes fouling, and short analysis time. In our previous studies, MCE-CCD devices have been updated and the MCE-CCD method has been applied [43–48]. CCD, which was first introduced to MCE by Guilt et al. [49], has been successfully combined with poly(methyl methacrylate) (PMMA) [42,46,48,50,51,56], glass [45,47,49,52,53], glass/poly(dimethylsiloxane) (PDMS) [44,57], and PDMS [54,55] microchip to determine a variety of samples. Generally speaking, the sensitivity of CCD is enhanced with decreasing thickness of insulating layer. Hence, thinner layer is preferred for CCD microchip. To this end, three main schemes of CCD implementation have been introduced by different groups, including microelectrodes that were covered with insulated layer and incorporated into the separation channel [46,49], buried in the widened part of the separation channel [44,52,54,55], and placed along the separation channel from the outside of microchip [42,45,47,48,50,51,53,56]. The third scheme, being relatively convenient, is most acceptable. We have reported a CCD device [45] with independent and replaceable electrodes and thin cover glass chip. However, how to construct and use this kind of glass-based chip with a very thin cover remains tricky. For example, the bonding of etched substrate glass with a 100 μm-thick cover glass was not always successful, glass chip could only be polished into a thin cover plate by special protocols [52,53], and PMMA chip with thin cover was not applicable in aqueous solution because PMMA was soluble in some organic solvents. Organic solvents were often employed on microchip platforms to elute analytes during on-chip SPE, so glass [28,30] or PDMS [29,35] was usually used to fabricate microchip owing to their special physical and chemical properties. As a universal detector, CCD has been used to analyze all charged analytes, especially small inorganic ions and organic ions which have low electrochemical activities for amperometry detection or weak absorptions for optical determination. Nevertheless, CCD has not been widely applied hitherto owing to the lack of sensitivity and selectivity, particularly in the analysis of trace substance in a complex matrix. Coupling MISPE with CCD is possible to combine the merits of molecular recognition, great ability for enrichment and nice commonality for CCD. After preconcentration is finished by the MISPE column, analysis and detection can be carried out by MCE-CCD, thus overcoming the disadvantages in detection. The new method is advantageous in fast analysis, low consumption of solvent and low cost.

Nowadays, food safety has become a global concern. As a basic industrial dye of aromatic amines, auramine O (Fig. S1) can rapidly dye textiles, leather products, and wood stain products. Besides, long-term intake of auramine O can lead to teratogenesis and cancer. Therefore, it has been prohibited from being added into food in many countries. The European Community has stressed that the food additive legislation excluded the use of colors other than those specifically authorized by Directive 94/36/EC [58]. We previously applied MISPE-HPLC to detect auramine O in shrimp [59]. In order to verify the feasibility of MISPE-MCE-CCD, MCE-CCD was selected to analyze and to detect auramine O that was charged in a weak acidic buffer.

Therefore, in this study, a novel glass/PDMS hybrid microchip embedded with an MISPE capillary monolithic column for CCD was presented to fulfill the abovementioned requirements. A 0.17 mm-thick glass cover was used to elevate the sensitivity of CCD. The durable glass/PDMS chip, being highly cost-effective, is suitable for mass production. The MISPE capillary monolithic column prepared by in-situ polymerization could be easily embedded into the glass/PDMS hybrid chip due to the elasticity of PDMS, and notably, could be conveniently removed or replaced. With a pump-driving and electrokinetic-injection system, on-line SPE-MCE procedures, including sample extraction, injection, separation and detection, were executed automatically. The properties of the microfluidic system were discussed, with its feasibility and performance demonstrated in the determination of nonpermitted additive auramine O in food sample.

2. Experimental

2.1. Materials and equipment

PDMS and Sylgard 184 elastomer curing agent were obtained from Dow Corning (San Diego, CA, USA). Glass cover slips (0.17 mm thick) were obtained from Shanghai Jinglun Industrial Glass Co., Ltd. (Shanghai, China). Auramine O, ethylene dimethacrylate (EDMA), methacrylic acid (MAA) and azobis (isobutyronitrile) (AIBN) were all purchased from Aladdin Reagent Company (Shanghai, China), other analytical-grade solvents were purchased from Guangzhou Reagents Company (Guangzhou, China), and the shrimp were purchased from a local supermarket. Glass capillaries with an inner diameter (i.d.) of 0.5 mm and an outer diameter (o.d.) of 0.6 mm were purchased from West China University of Medical Science (Chengdu, China). All stock and buffer solutions were prepared with redistilled water and stored at 4 °C. Prior to use, all solutions were filtered through a 0.22 μm nylon filter.

The home-made microchip capillary electrophoresis system consisted of a high voltage supplier and a contactless conductivity detector. The high voltage supplier provided potential-constant direct currents of 100–500 V for injection and 500–5000 V for separation. The home-made contactless conductivity detector has been described previously [43]. Four programmable micropumps, which were purchased from Longer Pump Company (LSP01-2A, Baoding, China), were connected to a personal computer with a D/A control card (NI USB-6009, NI, USA).

2.2. Fabrication of the MISPE monolithic capillary column

The monolithic column was fabricated following a process described previously by us [59]. Briefly, the monolithic column was directly prepared by UV-generated in situ polymerization in a glass capillary (10 cm long, 0.5 mm i.d. and 0.6 mm o.d.). The template auramine O and the functional monomer MAA (0.04 mL) were dissolved in porogen comprising methanol (0.5 mL), toluene (0.5 mL) and dodecanol (1.5 mL), to which were then added the cross-linker EDMA (0.4 mL) and the initiator AIBN (15 mg) respectively. After several minutes of stirring until complete dissolution, the mixed solutions were purged with N_2 for 5 min to remove oxygen, and introduced to the glass capillary column immediately, with both ends of the capillary sealed with rubbers. The subsequent polymerization was initiated by UV irradiation for 10 h under UV light (365 nm, VWR). After the polymerization was completed, the glass capillary was first washed using micropump at a flow rate of 0.1 mL min^{-1} with 2 mL of methanol/ammonium hydroxide (9:1, v/v) to remove the unreacted reagents and excess templates, and then washed exhaustively with methanol until it failed to detect auramine O in the eluent under optimized conditions. A corresponding nonimprinted polymer (NIP) monolithic capillary column was prepared identically in the absence of template.

2.3. Fabrication of microfluidic chip

First of all, 3D stereogram layout of the microchannel network was designed by AutoCAD and the copper mold was fabricated according to the setting layout on a commercially available fine carving (Fig. S2(a)–(b)). This fabrication procedure was extremely simple and cheap, allowing the accurate construction of a remarkably robust copper mold. The fabrication of the chip was schematically depicted in Fig. 1(a)–(d). The chip substrate was fabricated from PDMS produced by replica molding. The PDMS elastomer and Sylgard 184 curing agent were mixed (10:1, w/w), and degassed thoroughly by a vacuum pump for 20 min. Subsequently, the mixture was poured into the copper mold which carried a relief of desired microstructure. Then the mold was cured in an 80 °C oven for 30 min to form the PDMS layer that was cut off with a surgical scalpel and carefully peeled off. A part of the SPE channel (about 2.3 cm) was cut off with a surgical scalpel to spare space for the MISPE monolithic capillary column. Followed by the