CAPILLARY ELECTROPHORESIS ELECTROCHEMICAL DETECTOR WITH NOBLE MICROCHANNEL STRUCTURE FOR MINIATURIZATION

Development of a Capillary Electrophoresis Microchip Format Electrochemical Detector for Endocrine Disruptors Sensing

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Abstract: Numerous researches have been focused on capillary electrophoresis (CE) and amperometric detection (AD) using a double-T micro-channel configuration. The combination of these two techniques becomes a powerful analytical tool due to enhanced features in terms of sensitivity and selectivity. The developed CE-AD chip is low cost and requires less power consumption. Its high compatibility with micro-fabrication technology has made it popular for analysis of different compounds. However, due to the need to further miniaturize the CE-AD device, a twisted CE micro-channel configuration is fabricated and tested in this study. Furthermore, enhanced analyte separation due to delayed response time can be achieved using a serpentine CE separation micro-channel. Phenolic compounds were used as testing analytes to confirm the results using different types of running buffers. Also, the data gathered from the new micro-channel configuration is compared with the previously gathered results obtained from double-T separation micro-channels.

1 INTRODUCTION

Established analytical methods require sophisticated equipment in order to obtain accurate and reliable results. However, available apparatuses are bulky, expensive and require sample pre-treatment steps in order to minimize interferences. In lieu of these conventional techniques, the micro-scale lab-on-a-chip (LOC) devices could provide better performances and other benefits. One in particular is analytical cost reduction due to LOC cheaper production, lower reagent volume requirement and shorter analytical time. Though micro-fabrication has become successful to miniaturize capillary electrophoresis amperometric detector (CE-AD) LOC devices, numerous problems are yet to be solved. One of the common predicaments is the poor selectivity of the detector which is related to the unsatisfactory separation at the CE component. Double T configuration has been commonly used in CE microchannels. However, the effective length of a CE microchannel may not be sufficient to complete the separation process or may require longer channel in order to attain an effective separation, which defies the goal of miniaturization. Other ways include proper tuning of separation fields in order to provide an appropriate migration time for different analytes in the sample but this technique could not aid in further miniaturization of the device. Changing the microchannel configuration is another way to resolve separation problem. In this study, a twisted or serpentine CE microchannel configuration is investigated. An LOC device is fabricated with twisted microchannels with equivalent length of the previously reported double T configuration. In a twisted configuration, the size of the device can be further reduced. The influence of the separation field is much less at curved channel regions hence it is expected to have a more effective separation as it slightly increases migration time of different analytes. Interest in the use of polymeric materials such as poly-dimethylsilioxane (PDMS)

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and poly-methylmethacrylate (PMMA) has increased over the past few years. PDMS has been widely discussed due to good optical transparency for detection, to cure at low temperatures, easily replicates molding and good adhesion. Many PDMS applications in CE microchip and microfluidic channel design have been reported. This study utilized PDMS as a substrate for formulating the twisted CE microchannel for CE-AD microchip device. As previously reported, a 3-microelectrode system was fabricated on a glass substrate using bare indium tin oxide (ITO) and Prussian blue (PB) as catalyst on the working electrode.

2 EXPERIMENTAL

2.1 Materials and Chemicals

The chemicals used for PB electroplating solution were composed of ferric chloride hexahydrate (97%), potassium ferricyanide (99%), potassium chloride, and hydrochloride acid (32%). The testing analyte bisphenol A (BPA) was supplied by Wako. All of which were diluted to get the desired concentrations. Reagents used for microchannels include Sylgard 184 PDMS from Dow Corning Corp. (Midland, MI, USA). In order to mold PDMS microchannels, negative photoresist SU-8 and XP SU-8 developer from MicroChem Company were used. Throughout the study, deionized water (DIW) was used.

2.2 Microchip Fabrication

Figure 1 shows the simple process flow diagram for the fabrication of the CE/ECD microchip. For the electrodes in CE/ECD system, the ITO layer was deposited on a glass substrate by R.F. magnetron sputtering system. The thickness of the ITO film is 3400 Å. The 1.8 µm thick photoresist (AZ1512) was spin-coated on the ITO-coated glass and patterned for ITO electrodes. The sputter deposited ITO layer was etched with $FeCl_3$/HCl solution. In order to avoid the interference of a high electric field with EC detection, one decoupling-ground electrode as the cathode of CE electric field was positioned in front of the three-electrode electrochemical system.
The PB film was electrodeposited on the working electrode. Electroplating solution is consisted of 20 mM FeCl3, 20 mM $K_3[Fe(CN)_6]$, 0.2 M KCl and 0.1 M HCl. All the PB electrode surfaces were cleaned with acetone and dried with N_2 gas. In order to fabricate microchannels, 40 µm thick photoresist (SU-8) was spin-coated and patterned on the silicon wafer. The height of the positive patterns on the moulding masters, which is equal to the channels depth created on the PDMS layer, was 40 µm when measured with a surface profiler. The PDMS layers were fabricated by pouring a degassed mixture of Sylgard 184 silicone elastomer and curing agent (10:1) onto a molding master, followed by curing for at least 1 hour at 72 °C. The cured PDMS was separated from the mold, and reservoirs were made at the end of each channel using a 3 mm circular punch. The channel width is 80 µm. The double-T injector channel had an offset of 170 µm with 5 cm long separation channels and 1 cm long injection channel. Also, the channel width is 80 µm. The twisted injector channel had an offset of 170 µm with 7 cm long separation channels and 1 cm long injection channel. Before bonding the PDMS layer to ITO-coated glass substrate containing the electrodes, the two components were UV-Ozone exposed to improve their bonding strengths.

2.3 Microchip Configuration

The microchip consists of four reservoirs and micro-channels from PDMS (Fig. 2(a)), three electrodes and electrodes for applying injection / separation electric field on the glass substrate (Fig. 2(b)). The width of the working electrode (W) is 100 µm, 50 µm for reference electrode (R) and 200 µm counter electrode (C). In order to lower noise level, the working electrode is surrounded by the reference electrode.

2.4 CE/ECD Procedure

Each microchannel was preconditioned prior to use. Acetone solution was flushed through the microchannel for about 40 min to clean the microchannels. Next, DIW and buffer solution were flushed through the capillary for an hour respectively. The running buffer solution was 10 mM 2-(N- morpholino) ethanesulfonic acid (MES) fixed to pH 6.5 using 10 N NaOH. After preconditioned, the entire microchannels would be full of buffer solution. For CE process, no bubbles in the capillary were permitted. The testing analytes were injected by applying electric field of + 50 V/cm from sample reservoir to sample waste reservoir. With this process, the testing analytes are placed at the head of the separation channel after about 7 sec and buffer solution is still inside the microchannels. Separation of the analytes was performed by applying electric field of + 60 V/cm between buffer

and detection reservoir. Amperometric detection was performed in a three-electrode configuration. The potential between working and reference electrode was +700 mV DC in case of ECD for PB/ITO electrode and +600 mV DC for bare ITO and Au electrodes. When redox reaction between electrodes and testing analytes happened, peak currents were detected, recorded and stored directly on a computer.

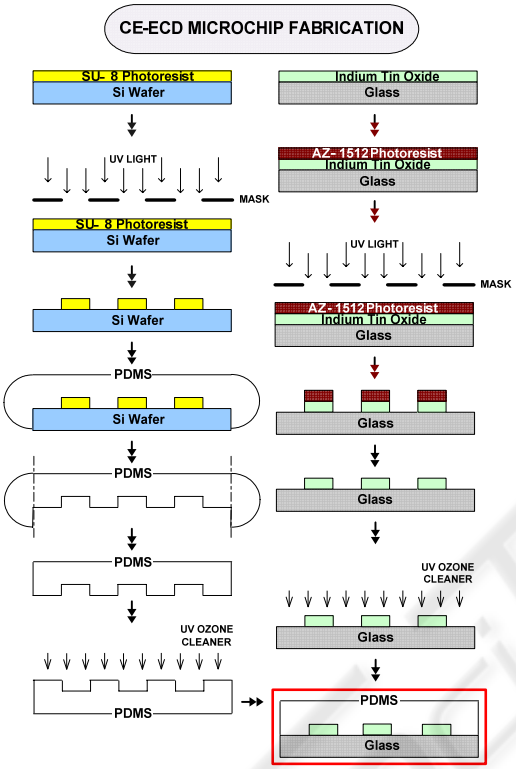

Figure 1: Process flow for the fabrication of the CE/ECD microchip.

3 RESULTS AND DISCUSSION

BPA was used to demonstrate the performance of CE-AD microchip. Measurements were repeated for several times. Figure 3 shows electropherogram of 1 mM BPA and butylphenol mixture by PB/ITO electrode using double-T channel. Figure 4 shows electropherogram of 1 mM BPA by PB/ITO electrode using twisted channel. Especially figure 4 shows reproducibility of BPA detection. At the same flow rates, the transport of ionic species to the working electrode by double-T channel is slightly faster than the twisted channel. Due to the curved corners in twisted channels, the ionic transport rate is slightly decreased. These zones are generally not

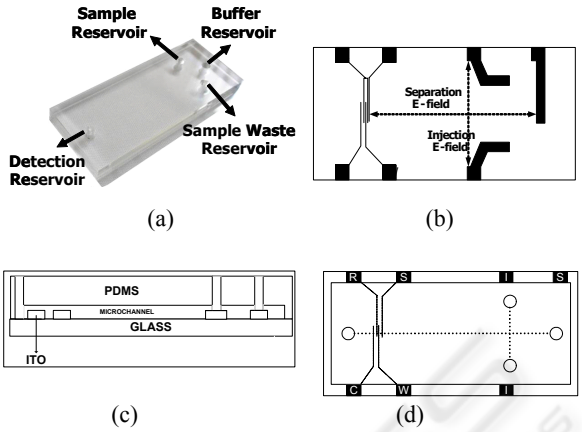

Figure 2: Configuration of (a) PDMS molding containing microchannels and reservoirs and (b) electrodes on the glass substrate. (c) Microchip side view and (d) Microchip top view.

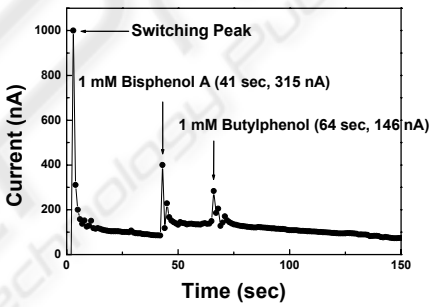

Figure 3: Electropherogram of ECD using double-T channel. Analytes are 1 mM BPA and 1 mM butylphenol mixture. (PB/ITO electrode); Condition: Separation voltage, 300 V; injection time, 7 sec; injection voltage, 60 V; detection voltage, 0.7 V

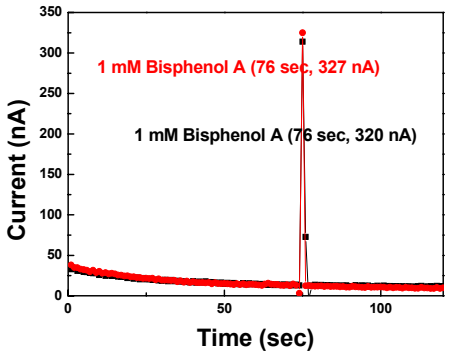

Figure 4: Electropherogram of ECD using twisted channel. Analytes are 1 mM BPA. (PB/ITO electrode); Condition: Separation voltage, 300 V; injection time, 7 sec; injection voltage, 60 V; detection voltage, 0.7 V.

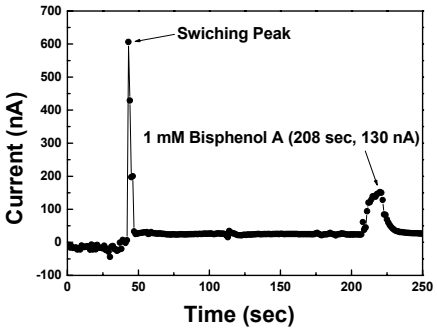

Figure 5: Electropherogram of ECD using sodium-borate buffer (pH 8.5). Analytes are 1 mM BPA. (bare ITO electrode); Condition: Separation voltage, 300 V; injection time, 40 sec; injection voltage, 60 V; detection voltage, 0.6 V.

affected by the separation electric field. The new CE-AD chip is a fused PDMS-glass substrate. With the CE-AD chip using twisted channel, 1 mM BPA was detected. Results revealed that twisted channels are effective to further miniaturize the exiting double-T channel CE-AD chip; it also has a more distinguished selectivity or analyte separation. After a sample injection, the EC detector current rapidly reached its steady-state background level with the pH 6.5 buffer solutions and the BPA was easily separated. The flat baseline and low noise level indicate an effective isolation from the driving voltage. Figure 5 show electropherogram of 1 mM BPA by bare ITO electrode using sodium-borate buffer. CE-AD using basic buffer is slower to flow than CE-AD using acid buffer. Even if working electrode is different, amperometric detector has same channel structure.

4 CONCLUSIONS

The performance of our CE-AD microchip using twisted channel was compared with those of conventional CE-AD microchip using double-T channel. Moreover, our device has several benefits such as fast separation, high sensitivity, and simple fabrication, which makes our CE-AD microchip a good candidate to substitute the conventional CE-AD devices. Our results demonstrate that twisted channel is an effective technique to further reduce the size of the current CE-AD LOC devices.

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