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Miniaturisation of a capillary electrophoresis microchip for the sensing of endocrine disruptors

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Abstract: Although capillary electrophoresis amperometric detector (CE-AD) involving double-T microchannel configuration is a powerful analytical tool in terms of sensitivity and selectivity, its long microchannel configuration hinders further miniaturisation. Therefore a twisted CE microchannel configuration was used in the present study to fabricate CE-AD devices for detection of endocrine disruptors. The analyte separation time varied slightly for the twisted microchannel structure, whereas the detector sensitivities were similar for the two configurations. The conventional indium tin oxide amperometric detector in the device with twisted microchannel configuration was later modified with Prussian blue to enhance the sensitivity of detection.

1 Introduction

Endocrine disruptors are a class of environmental hormones, which have deadly effect on the human body if its exposure extends beyond a threshold. The effects may vary from birth defects, spontaneous abortions and live births, germ cell aneuploidy [1], carcinogenesis through the activation of the N-ras family of oncogenes [2] and eventual manifestation of prostate cancer [3], somatic or germ-cell mutations [4] and even allergic contact dermatitis [5]. One such chemicals, bisphenol A (BPA), whose estrogenic and other endocrine disrupting activities are well established [6], is primarily used as a monomer in the production of polycarbonate and epoxy resins used in manufacturing containers for packaged food and beverages. The unreacted monomers on the containers pose occupational and health hazards to the workforce in the manufacturing industry apart from causing serious health concerns to individuals using the food containers.

The exposure to these chemicals from packaged foods or to the workforce in manufacturing industry can be completely preventable by amending rapid analytical methods for detection of the unreacted monomers. The existing analytical methods include bioaffinity [7] or electrochemical [8] biosensors and high-performance liquid chromatography (HPLC) [9–11]. Although bioaffinity biosensors are unstable for long-term and large-scale use, techniques such as HPLC cannot handle a large number of samples and require sample pre-treatment and other manual time-consuming steps along with a high setup cost. Therefore a more advanced but simple method is desired to analyse the bioavailability of these chemicals.

Capillary electrophoresis (CE) in conjugation with electrochemical detection (ECD) is fast becoming an indispensable tool for the separation and analysis of hazardous chemicals [12, 13]. The samples can be separated through application of a high electric field (100–500 V/cm) while avoiding Joule heating because of a high capillary resistance, thereby shortening the response time. This method has less sample requirements and can be miniaturised to microscale lab-on-a-chips (LOC) using common microfabrication techniques. The common problems with CE-ECD systems include poor detector selectivity and unsuitable microchannel conformation, leading to unsatisfactory separation of the analytes at the CE interface. Although the sensitivity of electrochemical

detectors can be enhanced using redox mediators on the electrode [14], the selectivity and separation efficiency of CE-AD are interlinked and is dependent on microchannel configuration. The double-T configuration is commonly used in the CE microchannel fabrication [15–18]. However, the effective length of the CE microchannel may not be sufficient to resolve a mixture of samples. It necessitates longer microchannel length and so, defies the goal of miniaturisation. Therefore changing the microchannel configuration is a simple way to resolve the problems associated with the analyte separation [19]. Keeping this in view, a twisted or serpentine CE microchannel configuration was investigated in the present study. An LOC device was fabricated with twisted microchannels that had an equivalent length to the previously reported double-T configuration [20] so as to reduce the overall size of the device. The separation efficiency and performance of the devices fabricated with either microchannel configurations were compared so as to deduce whether spiral microchannel influences the separation process.

This study utilised polydimethylsiloxane (PDMS) as a substrate to formulate the twisted CE microchannel for the CE-AD microchip device. It was the choice for polymeric material because of its good optical transparency and low adsorption rate of analyte on its surface. It can be cured at low temperatures, easily replicates moulding and can be adhered on glass surface covalently [13, 21–23]. At the sensing interface, a three-microelectrode system was fabricated on the glass substrate with bare indium tin oxide (ITO) and Prussian blue (PB) modified ITO as the working electrode. The devices were ultimately tested for detection of endocrine disruptors in synthetic samples.

2 Experimental

2.1 Materials and chemicals

Analytical reagent grade ferric chloride hexahydrate, potassium ferricyanide, potassium chloride and hydrochloric acid (32%, W/V) were used for the PB electroplating solution. The HPLC grade testing analyte BPA was supplied by Wako. The reagents used for the microchannels included Sylgard 184 PDMS from Dow Corning Corp. (Midland, MI, USA). A negative photoresist SU-8 and an XP SU-8 developer from MicroChem Company were used to mould the PDMS microchannels. Deionised water (DIW) was used throughout the study.

2.2 Microchip fabrication

The CE-ECD microchip was constructed using a simple fabrication process outlined in the process flow diagram (Fig. 1). Approximately, a 3400 Å ITO electrode layer was deposited on a glass substrate using an R.F. magnetron sputtering system. Subsequently, a 1.8 μm thick photoresist (AZ1512) was spin coated on the ITO-coated glass and patterned for the ITO electrodes. The sputter-deposited ITO layer was etched with a $FeCl_3$/HCl solution.

The PB film was electrodeposited onto the working electrode using a mixture of 20 mM $FeCl_3$, 20 mM $K_3[Fe(CN)_6]$, 0.2 M KCl and 0.1 M HCl. The PB electrode surface was cleaned with acetone and dried with N_2 gas. To fabricate the microchannels, 40 μm thick photoresist (SU-8) was spin coated and patterned on the silicon wafer. The height of the positive patterns on the master moulds was 40 μm when measured with a surface profiler, which was equal to the channel depth created on the PDMS layer. The PDMS layers were prepared by pouring a degassed mixture of a Sylgard 184 silicone elastomer and curing agent (10:1) onto a master mould, and then the PDMS was cured for at least 1 h at 72°C. The cured PDMS was separated from the mould and reservoirs were formed at the end of each channel using a 3 mm circular punch. Subsequently, the PDMS layer was bonded to the ITO-coated glass substrate containing the electrodes by UV–ozone bonding method [24].

2.3 Microchip configuration

The microchip consisted of four reservoirs and the microchannels engraved in PDMS (Fig. 2*a*). Apart from it, three in-channel ITO/PB modified ITO electrodes, two decoupler electrodes and additional electrodes for applying injection/separation electric field were laid on the glass substrate (Fig. 2*b*). The counter-electrode was placed at the end of capillary microchannel to avoid formation of air bubble inside the separation microchannel. The decoupling-ground electrode was positioned ahead of three-electrode electrochemical system (Fig. 2*a*) to minimise interference of high separation field on the electrochemical detection.

The devices were prepared with either the straight channel (Fig. 3*b*) or twisted microchannel configurations (Fig. 3*a*). The microchannel within these devices had an offset [25] of 170 μm with 5 cm long (effective) separation channel. The width and the length of microchannel were 80 μm and 1 cm, respectively. The width of the working electrode (W) was 100 μm, whereas 50 μm for reference electrode (R) and 200 μm for counter-electrode (C) (Fig. 2*b*).

3 CE-ECD procedure

The microchannel was preconditioned prior to use in CE-AD by first flushing acetone for about 40 min and then DIW and then running 10 mM MES buffer (pH 6.5, adjusted using 10 N NaOH) for 1 h in the microchannel with the help of a precision pump (KD Scientific, USA). As a result of this preconditioning, the entire microchannel was filled with buffer solution and no air bubbles were allowed in the capillary. Thereafter, testing analyte (50 μl) was injected in the sample reservoir using a micropipette and an electric field of + 50 V/cm across sample and sample

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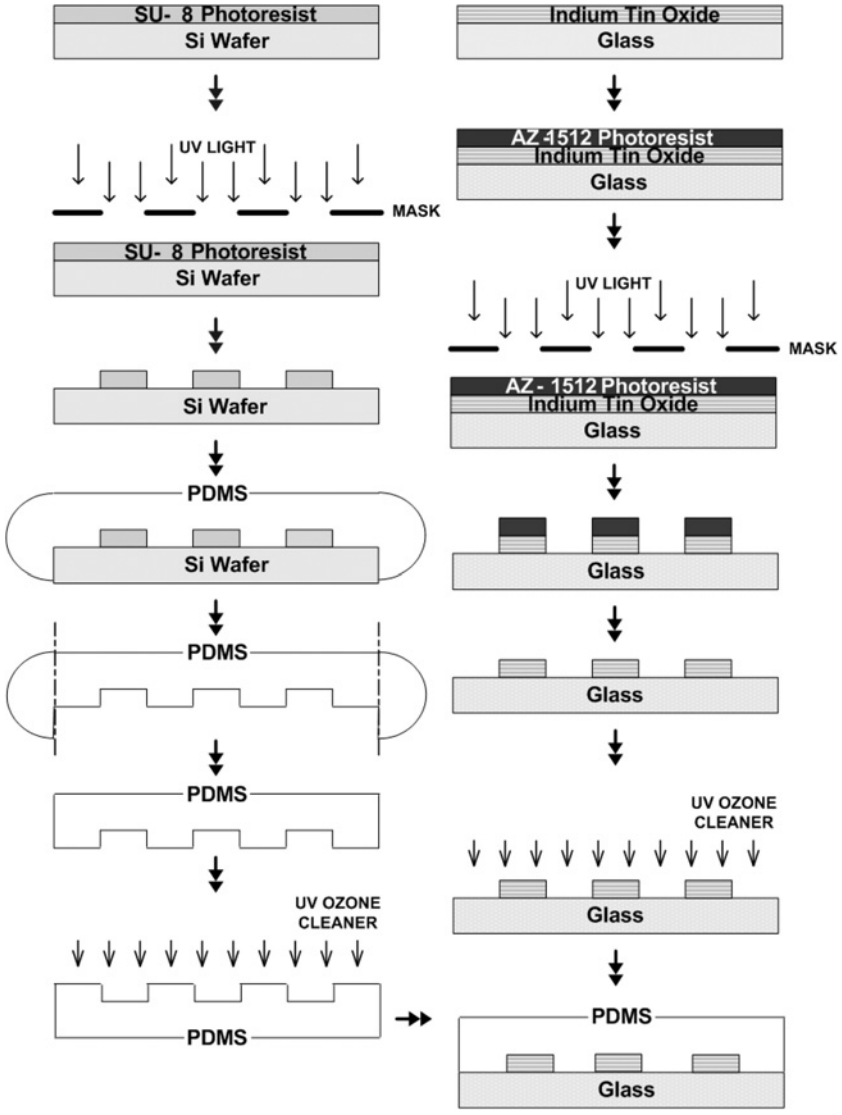

Figure 1 *Process flow for the fabrication of the CE-ECD microchip*

waste reservoir was applied. With this process, the testing analytes could be placed in the separation channel after 7 s of sample injection. Immediately then, the potential was switched in the direction of separation electrodes placed between buffer and waste reservoirs. The separation voltage was +60 V/cm. The amperometric detection on migrating ions was performed with three-electrode configuration placed inside the separation microchannel. The potential between working and reference electrode was +700 mV DC in case of ECD with PB-ITO electrode and +600 mV DC in case of bare ITO electrode. Redox reaction of testing analytes on the working electrode generated peak currents which was detected, recorded and stored directly on a computer. The electronic interface for the electrodes was a Keithley 236 Source-Measure meter, which was interfaced with a computer using General Purpose Interface Bus (GPIB) connection and Labview software. The acquisition rate of this connection was 45 data points/s. To smoothen the acquired signal and hence to enhance signal-to-noise ratio, adjacent figure data averaging logic was used in the Labview program.

4 Results and discussion

The fabrication of twisted microchannel structure was performed with the aim to reduce overall dimensions of CE-AD device. As seen in Fig. 3, the twisted microchannel configuration led to about 40% reduction in the device area compared to the device having straight microchannel configuration. This means more separation field can be applied in small area simply by introducing more turns in the microchannel. The curved structure also had no impact on bonding strength of PDMS to the glass surface. The bonding strength of similar PDMS-based CE-AD device is usually measured qualitatively by observing leakage of liquid during flow. An obstructed microchannel will show fluid leakage or back-pressure. But in the present case, the liquid flow rate in the twisted

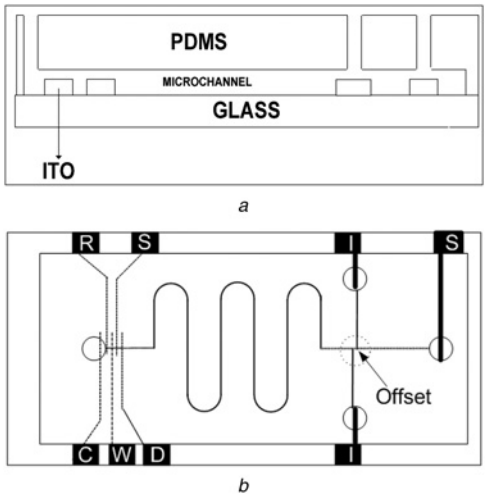

Figure 2 *Configuration of electrodes on the glass substrate of microchip showing (I) sample injection, (S) separation, (W) working, (R) reference, (C) counter and (D) decoupler electrodes*

a Side view
b Top view

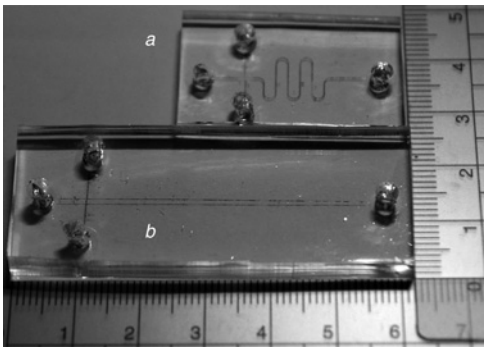

Figure 3 *Photograph of CE-ECD device*

a Twisted microchannel structure
b Straight microchannel structure (not showing the electrodes)
Colour ink was injected in the microchannel to enhance visibility

microchannel devices remained unaltered compared with straight microchannel devices. A comparative investigation of the sample separation pattern using both devices was possible because the fabrication method for both devices was same.

Apart from the miniaturisation and separation analysis, another goal of this study was to enhance the sensitivity of detection. For this purpose, we compared ITO working electrode commonly used for CE-AD device fabrication [26] with the detection sensitivity of PB-modified ITO electrode (the working ITO electrode with PB deposition) and found almost ten-fold (factor 10.03, SD 0.01, $n = 5$) increase in response current using the modification (Fig. 4). In this analysis, BPA solution (1 mM) was injected in the CE-ECD with bare ITO or PB modified working electrode in a twisted channel configuration. This was quite

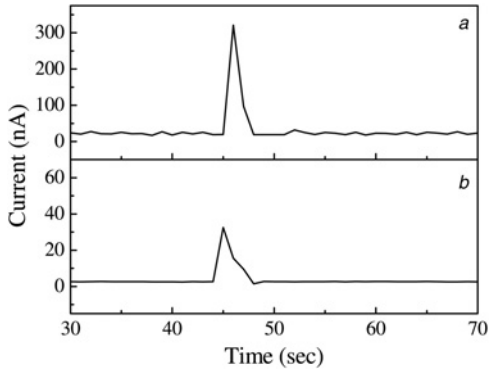

Figure 4 *Electropherogram of 1 mM BPA using twisted channel*

a PB-ITO electrode
b Bare ITO electrode
Conditions: separation voltage 300 V; injection time 7 s; injection voltage 60 V; detection voltage 0.7 V, MES buffer (pH. 6.5)

in accordance with a previous work [14], as the PB or ferric hexacyanoferrate acts as a redox mediator for oxidation of –OH group in phenolic BPA molecule, thereby enhancing the sensitivity of detector. Therefore in further studies, we used PB-modified electrode to compare the effectiveness of straight against twisted microchannel configurations for analyte separation.

In terms of relative separation time, when 1 mM BPA analyte was injected in both the devices, it was found that although, an effective separation was achieved; the separation time was marginally higher in case of twisted channel (45.4 s, SD = 3.3, $n = 7$) compared to straight channel configuration (44.0 s, SD = 1.0, $n = 3$) (Fig. 5). This was obvious for reason that straight channel structure has no corners and so, confers less flow-hindrance than curvilinear structure. On the positive side of this study, the magnitude of response current from PB-ITO detector was similar for both the devices using PB-ITO working electrode (Fig. 5).

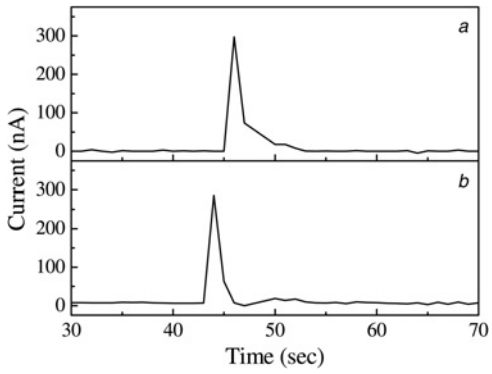

Figure 5 *Electropherogram of 1 mM BPA*

a Twisted microchannel and PB-ITO electrode
b Straight microchannel and PB-ITO electrode
Conditions: separation voltage 300 V; injection time 7 s; injection voltage 60 V; detection voltage 0.7 V

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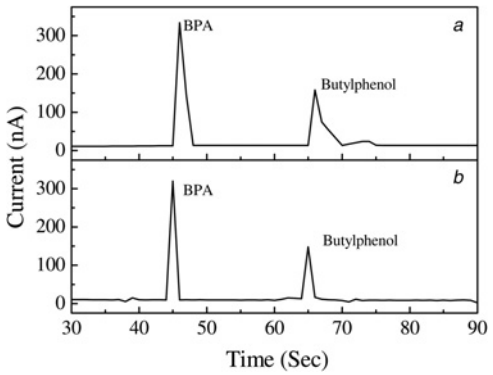

Figure 6 *Electropherogram of a mixture of 1 mM BPA and 1 mM butylphenol*

a Twisted microchannel and PB-ITO electrode
b Straight microchannel and PB-ITO electrode
Conditions: separation voltage 300 V; injection time 7 s; injection voltage 60 V; detection voltage 0.7 V

The final aim of this study was to use the twisted microchannel in separation analysis of realistic samples, where multiple analytes were needed to be separated effectively prior to detection. Therefore we used a synthetic sample containing equimolar mixture (1 mM) of BPA and butylphenol for CE-AD analysis using PB-ITO electrode to verify its resolving power with multiple target analytes. The analytes could be resolved with ease using either channel configurations (Fig. 6). The separation time was again marginally higher in case of twisted channel configuration, with detector peak heights similar in both the cases.

Therefore to our conclusion, the twisted microchannel structure used in this study has its advantages in terms of miniaturisation, negligible flow-hindrance and minute difference in separation time and can replace straight channel configuration, which is still adopted for CE microchip fabrication. This in conjugation with a mediated amperometric detector, such as PB-modified ITO shall make it a powerful electroanalytical apparatus.

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