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Monitoring of endocrine disruptors by capillary electrophoresis amperometric detector

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ABSTRACT

A capillary electrophoresis amperometric detector was fabricated using Prussian blue (PB) modified indium tin oxide (ITO) electrode and twisted microchannel configuration. These devices were used for detection of endocrine disruptors, such as bisphenol A (BPA) and butylphenol after their effective separation in microchannel. The microchip had a limit of detection (LOD) as low as 59 nM for analytes. This approach was further applied in detection of these contaminants in Styrofoam food container used in many East Asian countries, such as Korea and China, for packaging of instant noodle, a popular fast food.

1. Introduction

Endocrine disruptors are exogenous chemicals that interfere with human endocrine hormonal system and produce adverse effects such as birth defects [1], carcinogenesis [2], mutations [3] and skin diseases [4]. Bisphenol A (BPA) is one such chemical [5] and is used mainly as monomer during production of polycarbonate and epoxy resins. The epoxy is used as coating material inside almost all food cans and beverage bottles, from which it can easily leach out into food [6,7]. For these reasons, there have been widespread food safety and health concerns, although the safe oral intake limit for BPA is 0.0000125 mg per kg body weight per day [8]. Therefore, exposure to these chemicals through packaged foods can be prevented via quality control during manufacturing stage of cans and bottles, where excess of BPA can be selectively degraded [9]. For this purpose, rapid and reliable analytical methods are in demand. Established analytical methods, such as high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometer (GC–MS) cannot handle large number of samples and also require sample pre-treatment [10–12]. Capillary electrophoresis (CE) in conjugation with amperometric detection (AD) is a growing choice for detection of such chemicals. Using this technique, samples can be separated in a rapid way by applying high electric field (100–500 V/cm), therefore allowing to analyze individual components of the sample. The method has low sample requirement and can be miniaturized to lab-on-a-chip (LOC) devices using microfabrication techniques.

Therefore, in the present study, we developed a CE–AD device having serpentine microchannel configuration. This device was built on glass substrate with PDMS mold for making microfluidic channel. The amperometric detection system contained Prussian blue (PB) modified ITO microelectrodes patterned on glass surface. These CE–AD devices were used for detection of endocrine disruptors in spiked as well as real samples.

2. Experimental

2.1. Materials and chemicals

The HPLC grade testing analyte bisphenol A (BPA) was supplied by Wako. 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer, butylphenol, penylphenol and nonylphenol were purchased from Sigma–Aldrich, USA. The chemicals: ferric chloride hexahydrate, potassium ferricyanide, potassium chloride and hydrochloric acid (32%, W/V) were used for PB electroplating and were of analytical reagent grade. Sylgard 184 PDMS (Polydimethylsiloxane) from Dow Corning Corp. (Midland, MI, USA) was used for microchannel fabrication. Negative photoresist SU-8 and XP SU-8 developer were used for molding of PDMS microchannels and were purchased

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from MicroChem Corp. Deionized water (DIW) was used throughout the experiments.

2.2. Microchip fabrication

The CE–AD microchip was fabricated as per process flow diagram shown in Fig. 1. At first, approximately 3400 Å ITO electrode layer was deposited on a clean glass substrate by R.F. magnetron sputtering. Subsequently, 1.8 μm thick photoresist (AZ1512) was spin-coated on the ITO-coated glass for fabrication of ITO electrodes. The sputter deposited ITO layer was etched with $FeCl_3$/HCl solution. Prussian blue (PB) film was electrodeposited on 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 using N_2 gas. For fabrication of microchannels, 40 μm thick photoresist (SU-8) was patterned on the silicon wafer. Subsequently, a PDMS layer was obtained by pouring a degassed mixture of Sylgard 184 silicone elastomer along with curing agent (10:1) on this molding master. It was cured for at least 1 h at 72 °C and then separated from the mold. The reservoirs were made at the end of each channel using a 3 mm circular punch (Fig. 2a). The PDMS mold and ITO-coated glass substrate were subjected to UV–Ozone treatment and were bonded together.

2.3. Microchip configuration

The microchip consisted of two reservoirs for sample and buffer inlet and two waste reservoirs at the end of microchannel in the PDMS mold (Fig. 2a and b). The amperometric detector consisted of three-electrode system. The width of working (W), reference (R) and counter (C) electrodes were 100, 50 and 200 μm respectively. Additional electrodes were laid on the glass substrate for applying injection (I) and separation (S) electric fields (Fig. 2b). The twisted microchannel had 1 cm long injection and 5 cm long separation channels with a width of 80 μm and 170 μm offset (Fig. 2b). The working electrode was kept close to the reference electrode to lower electrical noise (Fig. 2b).

2.4. CE/ECD procedure

The running buffer for CE–AD consisted of 30 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) (pH 6.5). Each microchannel

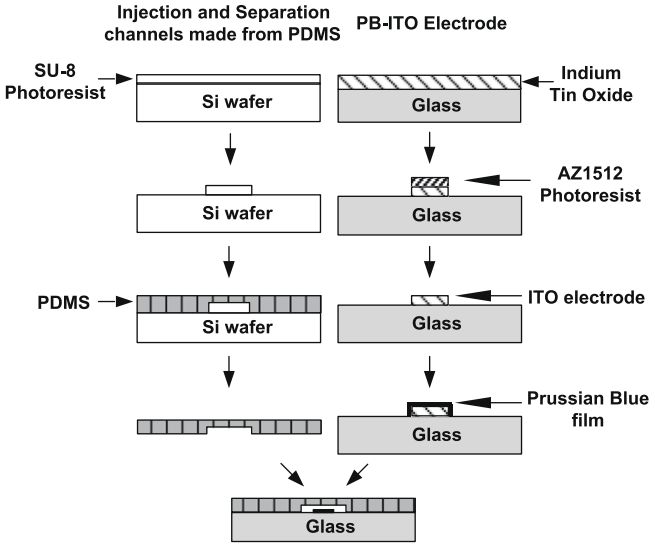

Fig. 1. Process flow for the fabrication of the CE/ECD microchip.

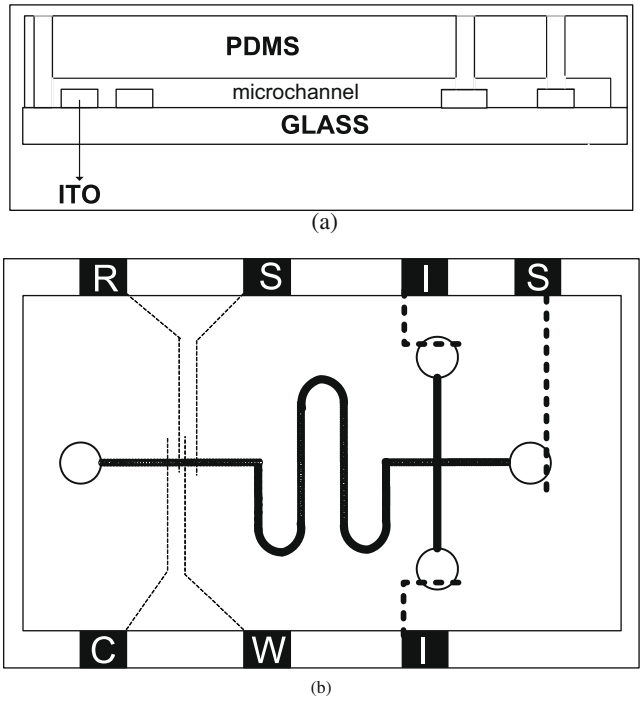

Fig. 2. Configuration of PDMS mold containing microchannels: side (a) and top view (b).

was preconditioned before measurement of analyte. At first, acetone was flushed through the microchannel for 5 min followed by deionized water (DIW) and MES buffer for 15 min each using a precision pump (KD Scientific, USA). As a result of this preconditioning, the entire microchannel was filled with buffer and at the same time devoid of air bubbles. The testing analyte (10 μl) was injected into sample reservoir and an electric field (I) of +30 V/cm was applied across sample and waste reservoirs. With this process, the testing analytes could be placed at the start of separation channel after 7 sec of injection. Separation of the analytes was performed by applying electric field (S) of +60 V/cm between buffer and detection reservoir. Amperometric detection was performed with three-electrode configuration placed in the path of buffer flow. The potential between working and reference electrode was +700 mV DC. Redox reaction of testing analytes on the working electrode generated current peaks, which was detected, recorded and stored directly on a notebook computer using a Kiethly 236 Source–Measure meter with GPIB connection and Labview software interface. The acquisition rate of this connection was 45 data points per second.

For analysis of target analytes in Styrofoam plastic containers, 30 g of it was cut into smaller pieces and soaked in 300 ml water in a clean glass beaker. It was warmed at 75 °C in an oven for 30 min to extract adsorbed chemicals. The extract was cooled down to room temperature before injecting appropriate amount in the CE–AD microchip. Further, in another set of reaction, 1 μM BPA and butylphenol to final concentration were added to this extract to augment the sensor output.

3. Results and discussion

CE–AD is a powerful analytical tool in terms of sensitivity, selectivity and low sample requirement. The biggest advantage of this methodology is to achieve selectivity to target analytes due to their separation prior to detection. While double-T microchannel configuration is more commonly used for fabrication of CE–AD devices [13,14], twisted microchannel configuration brings advantage of

further miniaturization. Therefore, in our present study, we fabricated CE–AD devices using twisted microchannels engraved in PDMS mold. Samples were added in the injection well and after their separation in the microchannel, individual compounds could be detected by amperometric method on PB-modified ITO electrode.

The purpose of using PB-modified ITO electrode in this study was that ITO offers low cost and ease in fabrication. On the other hand Prussian blue is a known redox mediator that enhances the sensitivity of detection [15]. However, to our experience, one disadvantage of using PB–ITO electrode was that only a low separation voltage (60 V/cm) could be applied because of electrode burning beyond 70–100 V/cm. Yet, to reduce power consumption and minimize Joule heat, present approach of keeping low separation voltage could be considered an advantage over faster separation time.

The fabricated CE–AD device was first used for preparation of a standard curve of various concentrations of BPA (Fig. 3). The hyperbolic nature of this curve indicated wider detection range for bisphenol A which could be useful in detection of BPA in real samples where its concentration may vary as much as 40–100 nM (human urine samples) [16,17] to even mM range (industrial samples). In order to deduce BPA concentration from sensor response, a normalized equation was obtained upon curve fitting of this data set. The software Origin (version 7.5) from Origin Labs Inc. was used for this purpose and the fit function used was Langmuir extended model (Langmuir EXT1) (Eq. (1)), which closely matched the data range. Upon substituting the coefficients obtained upon curve fitting, the normalized equation (Eq. (2)) could be used for deducing BPA concentration from sensor response.

$$Y = abx^{1-c} \Big/ \left(1 + bx^{1-c}\right) \tag{1}$$

where, a, b and c are coefficients of equation and Y and X are dependent and independent variables, respectively.

$$X = \left[273.224Y \Big/ \left(1.2838 \times 10^{-6} - Y\right)\right]^{1.7328} \tag{2}$$

where, X is BPA concentration (μM) and Y is the sensor response (A).

The background noise for the device was recorded using plain buffer in the microchannel and found to be as 0.487 nA (SD = 0.08 nA, $n = 5$). Therefore, at acceptable signal to noise ratio (S/N = 3), the limit of detection (LOD) for the device was calculated using Eq. (2) as 59 nM of BPA. The LOD for our in-channel PB-modified ITO detector was comparable to an end-channel CE–AD device (50 nM for Propranolol detection) [18] and better than an in-channel device from other group (460 nM for dopamine and 940 nM for catechol) [19]. A better LOD for our devices can be attributed to the use of PB redox mediator.

The total run time was less than 3 min for each sample. The CE–AD device was further tested against a mixture containing 1 mM each of BPA, butylphenol, penylphenol and nonylphenol. Each of these compounds generated amperometric peaks (Fig. 4). The position of these peaks on this electropherogram corresponded to target analyte.

This CE–AD system was also tested for analysis of endocrine disruptors in real samples. Styrofoam plastic containers are generally used as packaging material for fast food in many East Asian countries. These containers are often contaminated with endocrine disruptors [9]. For detection of these compounds, adsorbed chemicals in vessel were extracted with water, which was then injected on CE–AD microchip to record amperometric peaks (Fig. 5A) which corresponded to BPA and butylphenol. The intensity of these peaks

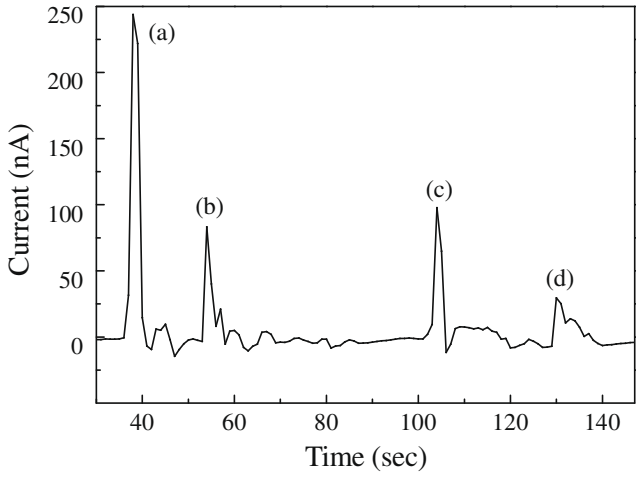

Fig. 4. Electropherogram of a mixture of 1 mM each of (a) BPA, (b) butylphenol, (c) penylphenol, and (d) nonylphenol with PB/ITO electrode.

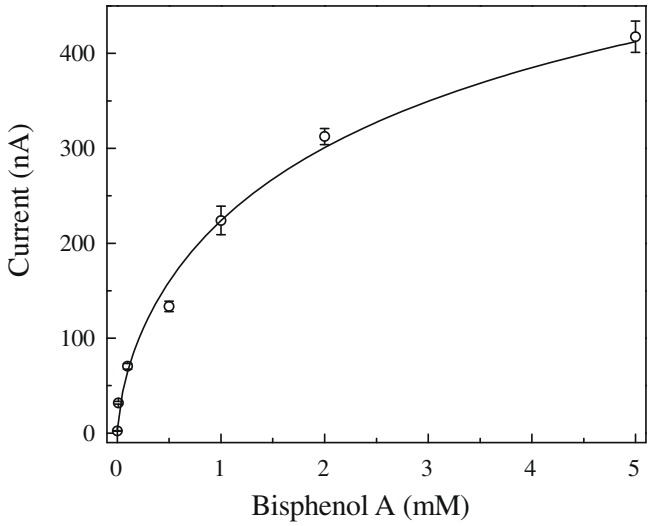

Fig. 3. Concentration dependent response current for analyte bisphenol A. Error bars represent standard deviation of triplicate measurements.

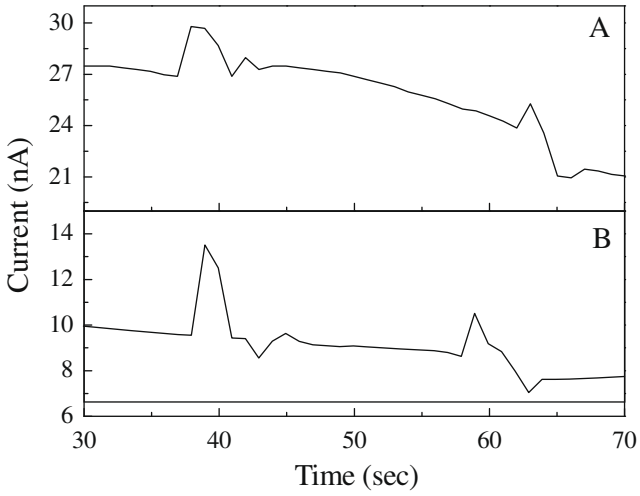

Fig. 5. Electropherogram of Styrofoam water extract (A) and of Styrofoam water extract spiked with 1 μM BPA and butylphenol (B) using PB/ITO electrode.

could be augmented by addition of 1 μM each of BPA and butylphenol to the water extract (Fig. 5B). This confirmed the presence of two different endocrine disruptors in the plastic container.

4. Conclusion

A twisted microchannel capillary electrophoresis amperometric detector was fabricated in the present study. It was used for detection of a group of phenolic compounds, considered endocrine disruptors or structurally similar to it. The microchip had a LOD as low as 59 nM for analyte such as bisphenol A (BPA) and butylphenol after their effective separation in microchannel. This approach was further applied in detection of these contaminants in Styrofoam food container. This preliminary work can therefore, be extended for in-depth study of endocrine disruptors in a variety of other contaminated samples.

Acknowledgments

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