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Photo-lithographically impregnated and molecularly imprinted polymer thin film for biosensor applications

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Abstract

A voltammetric sensor for albuterol was investigated where we combined the techniques of microfabrication and molecular imprinting to construct on-chip devices using photoirradiation of cross-linkable polymers. Molecularly imprinted polymer was coated as a thin film onto the gold working electrode on chip and the analyte was directly quantified by differential pulse voltammetric measurements.

Keywords: Biosensors; Molecularly imprinting; Albuterol; Beta-Blockers

1. Introduction

Molecular-imprinting polymerization (MIP) is a technique for creating recognition sites for an analyte molecule in a synthetic polymeric substrate. These artificially-generated recognition sites have their shapes, sizes and functionalities complementary to the analyte, and are capable of rebinding the analyte molecules in preference to other closely related structures [1–3].

Photochemical initiation of the synthesis of thin layer imprinted films on membranes had been proven to be very successful [4,5]. Generally, researches focused on surface functionalization of porous membranes by grafting with MIP. The object of this work was to combine microfabrication with molecular imprinting for the construction of on-chip devices using photoirradiation of cross-linkable polymers. Screen-printed electrode technology is another attraction for the production of disposable sensors [6,7]. It has the advantages such as disposability, high reproducibility and requires no calibration. But it is difficult to minimize sensor via screen-printed electrode technology. Willauer and Collins [8], and Kaniansky et al. [9] have reviewed the miniaturization on capillary electrophoresis chips. The system studied in this article is different from the capillary electrophoresis chip, but it exhibits potentials to minimize sensors. In this article, microfabrication and molecular imprinting were combined to develop on-chip devices which are able to minimize the sensor in any shape and easy to produce multi-array sensor.

The binding of template with the cross-linked polymeric matrix can be showed with the acquisition system, such as voltammetry [10–13], quartz crystal microbalance [14], etc. We have employed the method of differential pulse voltammetry [15,16] after spin coating the MIP solution on a gold electrode which is coated on chip followed by UV curing. In other words, molecularly imprinted polymers were coated as a thin film onto the gold working electrode on chip and the analyte was directly quantified by differential pulse voltammetric measurements.

Albuterol was the monitored species of our choice. It is a β-agonist drug used in the treatment of asthma in human, and promotes muscle growth and reduces body fat in cattle; therefore it can reduce the cost of meat production. However, the danger of residues from the abuse of β-agonists have been underscored by several human poisoning incidences where the consumption of animal food products containing β-agonist residues [17–21].

We have synthesized polynorbornene containing various functional groups by a ring opening metathesis polymerization [22–25] for the purpose of imprinting albuterol. By combining microfabrication and electrochemical techniques, it

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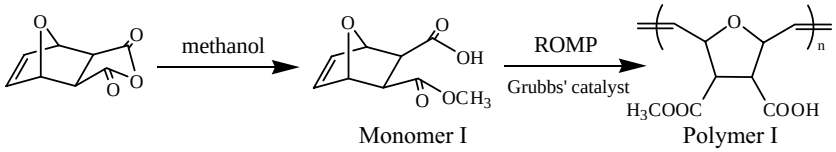

Fig. 1. The preparation of functional polymer (Polymer I).

is aimed to achieve multi-array detection and miniaturized devices for sensor applications.

2. Experimental

2.1. Materials

Tetrahydrofuran (THF; Aldrich) and methanol (EL, Mallinckrodt) were distilled over sodium/benzophenone and magnesium/iodine, respectively to remove trace amounts of water. *exo*-Bicyclo[2,2,1]hept-5-ene-2,3-dicarboxylic anhydride (Aldrich), Grubb's catalyst (Strem), benzoin (TCI), albuterol (Sigma), graphite (Timcal graphite), poly(vinylidene fluoride hexafluopropylene) (ATOFINA chemicals), ethyl acetate (TEDIA), hydrogen chloride (Union Chemical Works), hexane (ACS, Aldrich) were used without further purification. All other chemicals and solvents (HPLC

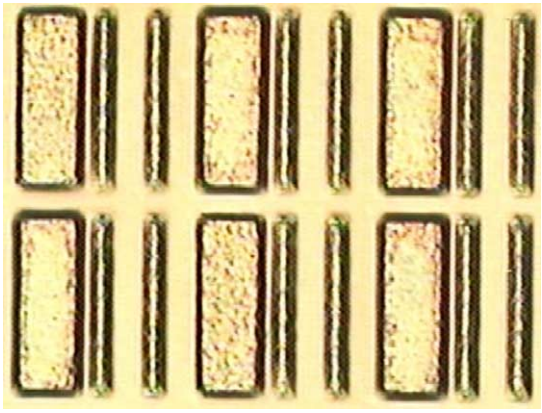

Fig. 3. Representation of patterns developed by lithographically imprinted polymer. The pattern dimension was 50 μm. The homogeneous dispersion of graphite fragments in the pattern section was showed.

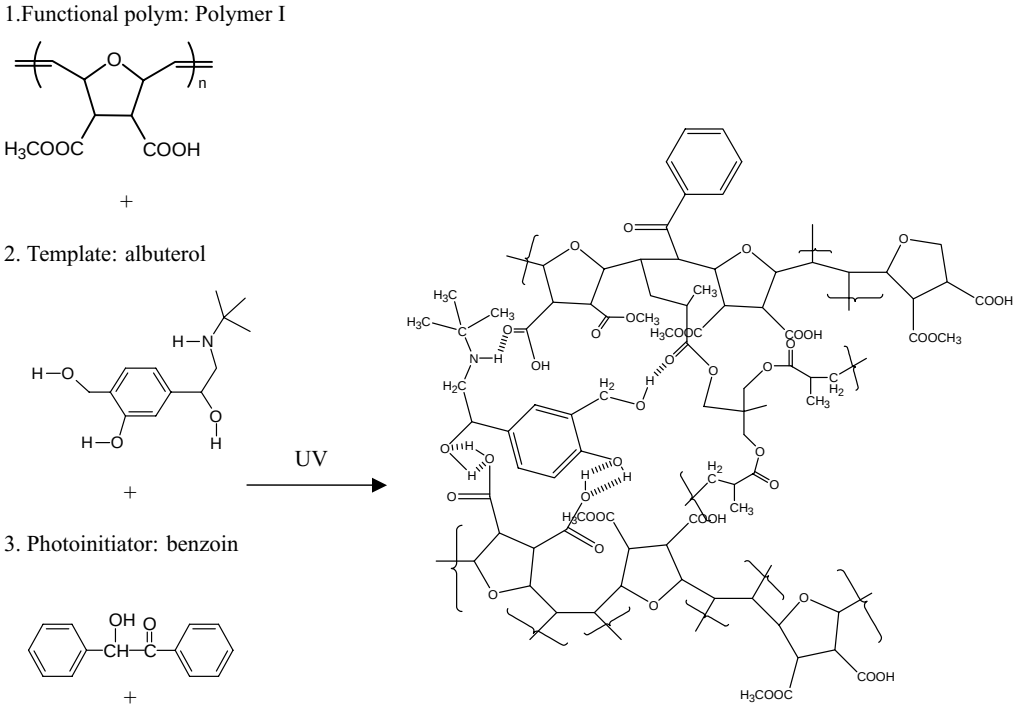

Fig. 2. Probable encapsulation mechanism of template molecule by the cross-linked polymer.

grade) were obtained from commercial sources and used as received.

2.2. Synthesis of functional polymer

Fig. 1 shows the preparation of functional polymer (Polymer I), a precursor to make MIP. *exo*-7-oxabicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic anhydride (5.0 g) in methanol (20 ml) was heated with stirring at 45 °C for 16 h. The mixture was then concentrated under vacuum and recrystallized from diethyl ether–light petroleum (bp 00–00 °C) to get Monomer I [22]. ^{1}H NMR [(500 MHz, C_3OD_6) δ (ppm) 6.471 (s, 2H), 5.140 (s, 2H), 3.585 (s, 3H), 2.786 (s, 2H)]. Monomer I (3.92 g) was dissolved in dry THF. A solution of Grubb's catalyst (0.059 g, monomer/catalyst molar ratio = 259) [23–25] in THF was injected and the reaction mixture was heated to 60 °C with stirring for 15–20 min. When a gel like polymer appeared a few drops of methanol was added to quench the reaction. The polymer was isolated by pouring the reaction mixture into a large

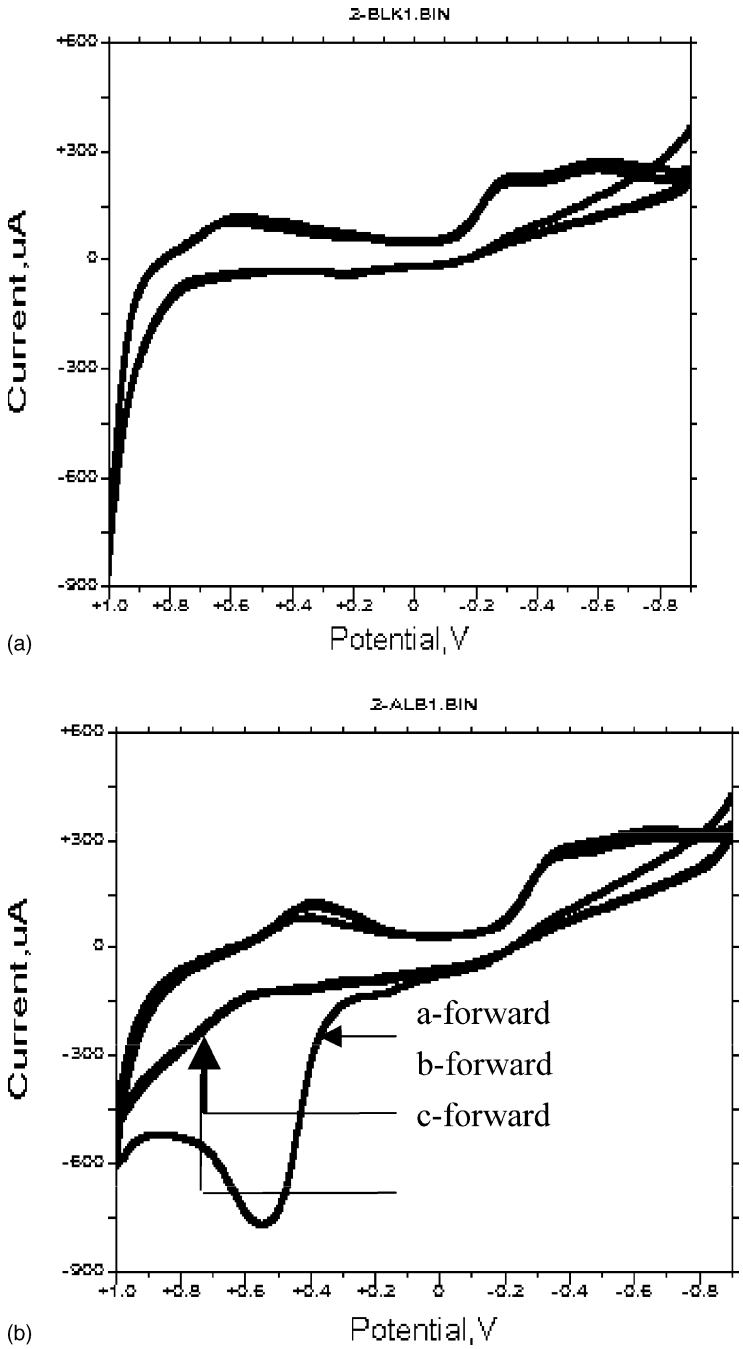

Fig. 4. (a) The current–voltage diagram of cyclic voltammetry shows carbon paste transducer of phosphate buffer solution, (b) the current–voltage diagram of cyclic voltammetry shows carbon paste transducer of albuterol at a concentration 10^{-2} M; a: first cycle, b: second cycle, c: third cycle.

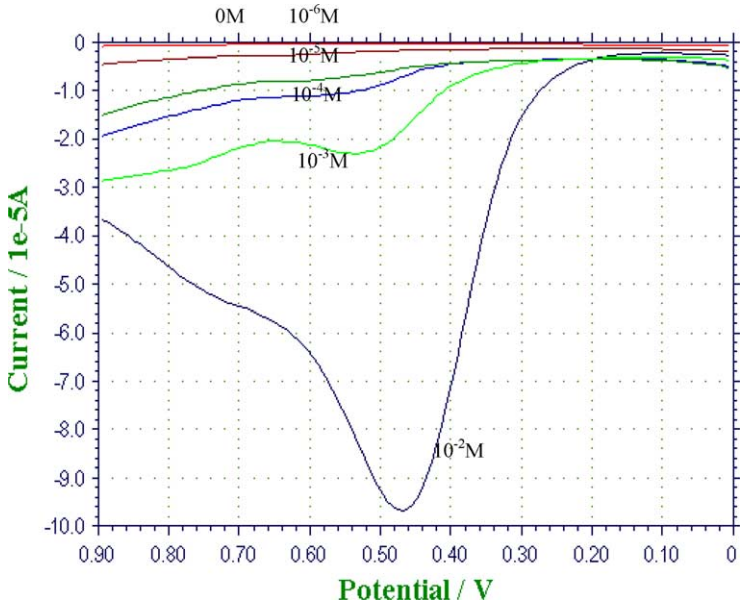

Fig. 5. The current–voltage diagram of differential pulse voltammetry diagrams of various albuterol concentrations for CPT.

excess of methanol and re-dissolved in THF. An off-white rubbery polymer (Polymer I) was re-precipitated by adding a non-solvent like hexane into the THF solution. Molecular weight was determined by gel permeation chromatography. The number–average molecular mass and the polydispersity are 15939 and 1.6735, respectively.

2.3. Preparation of molecular-imprinting photoresist solution

A THF–methanol solution was prepared by mixing Polymer I (0.716 g), benzoin (0.107 g), trimethylolpropane trimethacrylate (0.239 g), albuterol (0.095 g), poly(vinylidene fluoride hexafluopropylene) (0.202 g), graphite (1.818 g) for spin casting.

2.4. Preparation of MIP transducer (MIPT): lithography processes

Solution of molecular-imprinting photoresist solution was spin coated on the gold working electrode on-chip and at 500 rpm in 15 s. The chip was then placed under Photoresist Processing & Mask Aligner System (model: Karl Suss MA-4 Canon PLA-501F) for pattern alignment and broad-band ultraviolet exposure. After developing, clear pattern was obtained on the electrode surface. This pattern was named MIPT and will be applied for multi-array detection. Fig. 2 illustrates the UV-cured structure of molecular-imprinted polymer.

In order to evaluate the performances of MIPT, an additional transducer (carbon paste transducer, CPT) was made by adding poly(vinylidene fluoride hexafluopropylene) and graphite into THF and spread it on a gold electrode at 500 rpm for 15 s. This CPT was subjected to ultraviolet light exposure under Photoresist Processing & Mask Aligner System similarly.

2.5. Electroanalytical detection of analyte

Differential pulse voltammetry analysis of totally irreversible oxidation peak of albuterol was conducted using a CH Instrument Electrochemical Analyzer (CHI 620). Experiments were carried out in a glass cell designed to suit a three-electrode potentiostatic unit.

The MIPT and CPT were used as working electrodes in a three-electrode system. A platinum plate auxiliary electrode and a reference Ag/AgCl were used. Phosphate buffer solution (PBS) was made of sodium chloride (8.0063 g), potassium chloride (0.1998 g), sodium phosphate (1.1502 g), potassium phosphate (0.2001 g), and deionized water to have the final volume of 1 l. All measurements were carried out using 20 ml PBS (pH 7.4) at room temperature without stirring.

Cyclic voltammetry were recorded at 10 mV/s and with a potential window between 0.00 and 0.90 V versus Ag/AgCl. Differential pulse voltammetry were recorded with a potential window between 0.00 and 0.90 V versus Ag/AgCl. Concentrations of albuterol were changed from 10^{-6} to 10^{-2} M.

3. Results and discussion

3.1. Lithography pattern

In order to enhance the conductivity and sensitivity in electroanalytical behavior, graphite was added into the cross-linkable MIP compositions. Fig. 3 shows pattern of

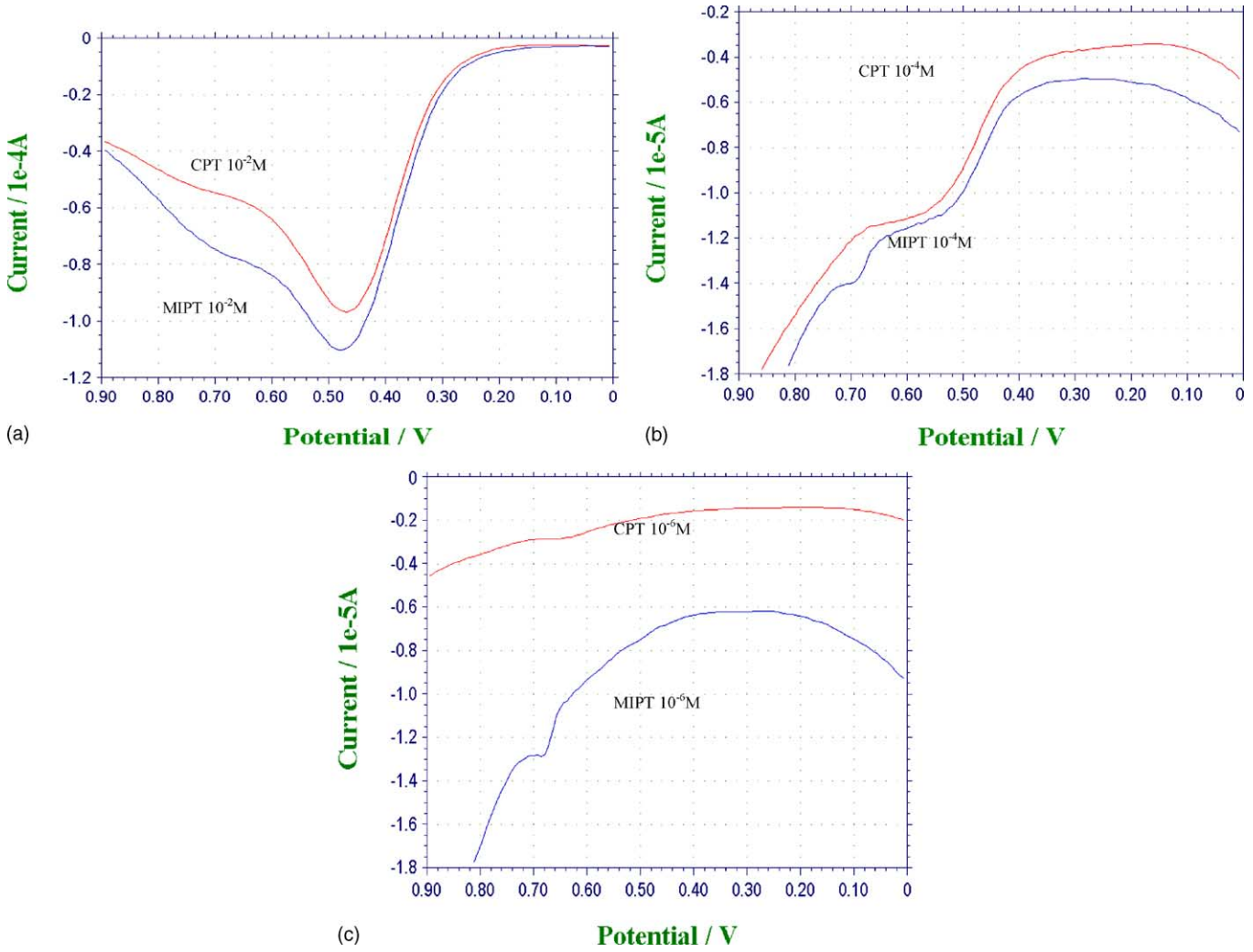

Fig. 6. Comparisons of current–voltage diagram of differential pulse voltammetry diagrams for CPT and MIPT, (a) albuterol concentration 10^{-2} M, (b) albuterol concentration 10^{-4} M (c) albuterol concentration 10^{-6} M.

molecular-imprinting photoresists. The pattern dimension is 50 μm. It can be observed a homogeneous dispersion of graphite fragments as shown.

3.2. Cyclic voltammetry and differential pulse voltammetry measurements of albuterol

The electroanalytical behavior of albuterol at CPT is similar to turbutaline [26]. The drug was irreversibly oxidized at ca. 0.47 V versus Ag/AgCl. Fig. 4a was the current–voltage diagram of cyclic voltammetry that shows carbon paste transducer of phosphate buffer solution. Fig. 4b shows the current–voltage diagram of cyclic voltammetry of albuterol at a concentration 10^{-2} M. In the first forward scan, an oxidation signal is appeared at ca. 0.47 V versus Ag/AgCl and no corresponding reverse reduction signal is noted. During the second and third cycles no oxidation signal is observed.

In Fig. 5, the current–voltage diagram of differential pulse voltammetry, shows the changes of albuterol concentration at CPT. Different albuterol concentrations result in different current intensities of the current–voltage diagram of differential pulse voltammetry. A non-linear relation was found for albuterol concentrations (10^{-2} to 10^{-6} M) versus current intensities of the current–voltage diagrams of differential pulse voltammetry. Fig. 6 illustrates the current–voltage diagram of differential pulse voltammetry for CPT and MIPT at albuterol concentration of 10^{-2} to 10^{-4} and, 10^{-6} M, respectively. Upon comparison, the signal of electrode coated with MIP solution (MIPT) is larger than that of electrode coated with carbon paste (CPT). And there are shoulder peaks for DPV curves of MIPT which implies a clear recognition of the template by the imprinted, photo-cured polymer.

4. Conclusions

A voltammetric sensor for the albuterol was investigated where the techniques of micro-fabrication and molecular imprinting were combined to construct on-chip devices using photoirradiation of cross-linkable polymers. The MIPT thus formed is more sensitive than the traditional CPT transducer and with template recognition.

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