Fabrication of molecularly imprinted uric acid biosensors based on a novel amine-imide type conducting polymer

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ABSTRACT

In this study, a molecularly imprinted polymer (MIP) based uric acid sensor was fabricated using a novel amine-imide type conducting polymer. Calibration curves of the sensor were depicted based on the amperometric steady-state current in different concentrations of UA ranging from 0 to 1.125 mM. The sensitivities of MIP and non-MIP electrodes were calculated to 24.72 and 6.63 μA $mM^{-1}cm^{-2}$, respectively. The imprinting efficiency, defined as the ratio of the sensitivity of MIP to that of NMIP electrode, was 3.7 in this work. The limit of detection (LOD) for the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode was calculated to be 2.4×10^{-3} mM on the basis of signal to noise ratio (S/N) equaling to 3. It was found that the MIP sensor showed relatively good sensitivity, large linear range, and high imprinting efficiency with a low LOD for sensing UA. The MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode was also tested for ascorbic acid (AA), and the current response of 0.04 mM AA is less than 7 % of the current response of 0.4 mM UA.

Keywords: amperometric, molecularly imprinted polymers, uric acid, uric acid biosensors

1 INTRODUCTION

Uric acid (UA) is the principle final product of purine metabolism in human body **[1]** and is related to many disorders such as gout, hyperuricemia and Lesch-Nyhan Syndrome **[2]**. Besides, uric acid is also one of the most important kidney calculus indices in human plasma. According to the above descriptions, monitoring the concentration of UA in human blood and urine may prevent and thus control the corresponding diseases. Electrochemical methods were commonly accepted as the fastest analytical assay compared with traditional spectroscopy methods **[3]**. The most well developed electrochemical method for detecting UA is based on the enzymatic approach. Uricase enzyme is reacted with UA and hydrogen peroxide produced is detected electrochemically. However, this method still has some fatal problems, such as the high cost and the low stability of enzymes, and the detection is indirect (detecting the reaction products). Several studies based on the non-enzymatic methods for detecting UA have been proposed. These include modified electrodes using multiwall carbon nanotube (MWCNT) **[4]**, activated glassy carbon **[5, 6]** and redox mediator **[7]**.

Molecularly imprinted polymers (MIPs), polymers derived from artificial antibody possess versatile polymerization methods for fabricating synthesis materials to mimic biological molecules. During the last decade, the molecular imprinting technique has been developed as analytical tools **[8, 9]**. MIPs can be used as artificial enzymes with the advantages of low price and higher thermal and chemical stability than that of enzyme. The applications of MIPs have been proposed in recent research fields, such as chromatography, sensors and drug delivery. In this study, we report an uric acid electrochemical sensor using the features of a molecularly imprinted polymer.

2 EXPERIMENTAL

2.1 Chemicals and apparatus

The conducting polymer used in this research is synthesized from N,N-bis(4-aminophenyl)-N',N'-diphenyl-1,4-phenylenediamine and 3,3',4,4'-benzo-phenonetetra carboxylic dianhydride, abbreviated as Poly(PD-BCD). The structure is shown in **Fig. 1**. This polymer was provided by Professor G. S. Liou's group **[10]**. Uric acid (UA, 98%), ascorbic acid (AA, 99%), phosphate buffer saline tablet (PBS) and potassium chloride (KCl) were purchased from Aldrich (USA). 1-methyl-2-pyrrolidinone (NMP, 99%) was purchased form Sigma (USA). Deionized water (DIW, >18 MΩ) was produced by Purelab Maximum (ELGA, UK). Indium-tin oxide (ITO, 10 Ω/□) glass was supplied by RiTdisplay Corporation (Hsinchu Industrial Park, Taiwan). All chemicals were used as received.

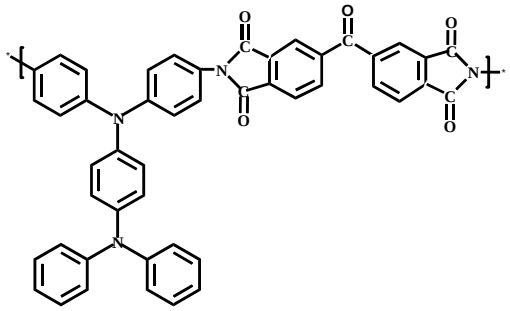

Fig. 1. Structure of Poly(PD-BCD).

Amperometric measurements were carried out using potentiostat/galvanostat model CHI 440 (CH Instruments) and compatible software. All electrochemical experiments were carried out at room temperature with a three-electrode system containing a 50 mL glass cell, Ag/AgCl sat's KCl electrode as the reference electrode, platinum plate as counter electrode and MIP-ITO/Poly(PD-BCD)$_{UA}$ or NMIP-ITO/Poly(PD-BCD) as working electrode.

2.2 MIP and non-MIP electrodes preparation

The ITO glass was used as the substrate, and was ultrasonically bathed in a 0.1 M HCl for 5 minutes before using. The substrate was rinsed with DIW and dried in air. After cleaning, the epoxy tape was applied on the ITO-coated surface to fix the surface area at 1.0 cm × 1.0 cm. The MIP sensing electrode, designated as MIP-ITO/Poly(PD-BCD)$_{UA}$, was made by mixing 0.6 wt% Poly(PD-BCD) with 1.5 mM UA in 1-methyl-2-pyrrolidinone (NMP) and dip-coated the resultant solution on the ITO glass. A programmed temperature variation was employed to remove the solvent from the electrodes under vacuum. The temperature was kept at 60 °C for 4 hours and rose to 80 °C for 2 hours, finally the temperature was increased to 180 °C for 2 hours. After removing solvent, Cu tape (3M Company) was pasted on the conductive surface of the electrode as a bus bar. The MIP electrodes were washed by deionized water in order to wash out UA template and dried under nitrogen blow. The non-MIP electrode, designated as NMIP-ITO/Poly(PD-BCD), was obtained by the same method except without adding UA for comparison purpose.

2.3 Electrochemical measurement

Cyclic voltammetry (CV) was applied to ensure that templates were totally removed from the polymer surface by DIW extraction and the potential was swept from 0.1 to 0.9 V at a scan rate of 0.1 V/s. Furthermore, the film's charge capacities were also checked by using CV.

UA was detected at the MIP-ITO/Poly(PD-BCD)$_{UA}$ and NMIP-ITO/Poly(PD-BCD) electrodes using an amperometric method. The linear sweep voltammetry (LSV) for the MIP electrode was done in a potential window between 0.5 and 1.0 V. The background current was recorded in 0.02 M phosphate buffer saline (PBS, pH=7.4) containing supporting electrolyte of 0.1 M KCl, and the total current was detected in the background solution with 1.5 mM UA. The net current was obtained by subtracting the background current from the total current. A proper operating potential was determined from the plateau part of the LSV curve. The calibration curve was obtained by calculating the net steady-state current densities at various concentrations of UA from 0 to 1.125 mM from which the sensitivity and the detection limit can be estimated. With respect to the interference experiment, the steady-state current under the coexistence of UA and ascorbic acid (AA) with corresponding concentration was detected by an amperometric method to determine the selectivity.

3 RESULTS AND DISCUSSION

3.1 Amperometric detection of UA

For evaluating the performance of a sensor, the most important factors are sensitivity and selectivity. In the following sections, these two critical factors will be discussed. In **Fig. 2**, UA oxidation peak can be easily observed on the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode before extracting the template. After template extraction, the oxidation peak of UA disappeared. To ensure the same amount of poly(PD-BCD) was coated on the MIP and non-MIP electrodes, the charge capacities of both electrodes were checked using the CV method, as seen in **Fig. 3**. Calculation on the charge capacities was based on the integration area of each CV curves. In order to verify that the UA template have formed the active sites on the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode, scanning electron microscope (SEM) was used to observe the surface morphology. **Fig. 4** is the SEM photos of MIP and non-MIP electrodes and these photos reveal that the MIP electrode possesses porous surface formed by the imprint of UA.

Before amperometric detection, the potential of detection must be determined using a polarization curve. **Fig. 5** is the LSV of the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode at a sweeping rate of 0.1 mV/s. As a result, a plateau between 0.8 and 1.0 V was identified as the limiting current zone, which is resulted from the mass transfer control. By applying the potential within the limiting current zone, the current is proportional to the concentration of UA. The sensing potential was set at 0.85 V to obtain a steady-state current response.

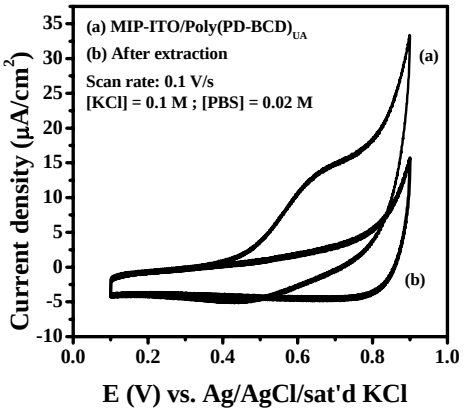

Fig. 2. Cvs for the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode in 0.1 M KCl solution (a) before extraction of UA template and (b) after extraction.

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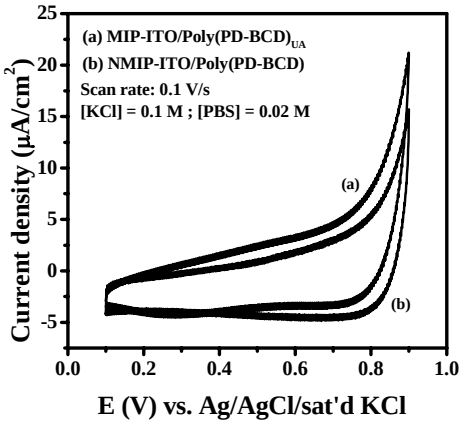
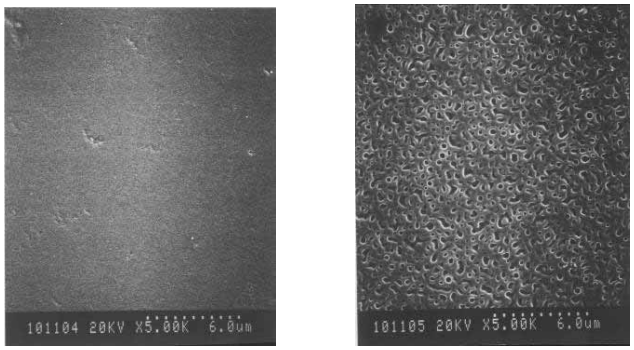


Fig. 3. Cvs for (a) MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode and (b) NMIP-ITO/Poly(PD-BCD).

Amperometric experiments were carried out to examine the current responses for the MIP and non-MIP modified electrodes. A step-wise increase of the UA concentration was changed from 0 to 1.125 mM and the potential was hold at 0.85 V. In **Fig. 6**, for both MIP and non-MIP electrodes, good linear relationships between the current density and the concentrations of UA were found for all whole concentration range of UA with the correlation coefficients of greater than 0.995. The calibration curves show that the sensitivities of MIP-ITO/Poly(PD-BCD)$_{UA}$ and NMIP-ITO/Poly(PD-BCD) are 24.72 and 6.63 μA $mM^{-1}cm^{-2}$, respectively. The current density responses of the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode were higher than those of the NMIP-ITO/Poly(PD-BCD) electrode.



(a) (b)

Fig. 4. SEM images of (a) non-MIP and (b) MIP electrodes.

This result reveals that the imprinted sites were formed at the surface of the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode. The imprinting efficiency, defined as the ratio of the sensitivity of the MIP electrode to that of the NMIP one, was 3.7 in this work. The limit of detection (LOD) for the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode was calculated to be 2.4×10^{-3} mM on the basis of signal to noise ratio (S/N) equaling to 3.

In real applications, it is proposed that the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode acts as a sensing electrode and the NMIP-ITO/Poly(PD-BCD) acts as a reference one.

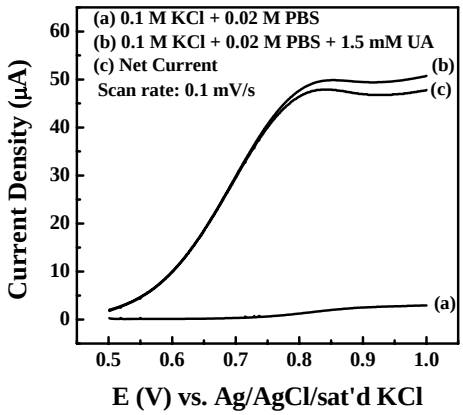


Fig. 5. LSV for MIP-ITO/Poly(PD-BCD)$_{UA}$, including (a) background current, (b) total current, and (c) net current.

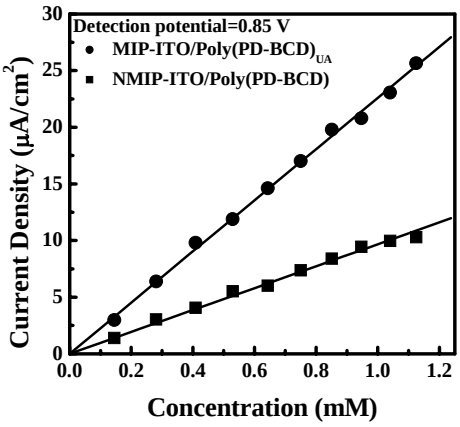


Fig. 6. Calibration curves for MIP and NMIP electrodes.

3.2 Selectivity of the MIP modified electrode

MIP sensors specificity was considered by comparing various current density responses for chemicals which are coexisted in human serum. The most important interference in electrochemical routine analysis of UA is ascorbic acid. The normal concentration of AA in human serum is about 45.8±16.2 μM and is less influenced by dietary or smoke behavior [11]. In this study, the interference test was done with concentration of AA set at 0.04 mM. **Fig. 7** shows the individual and the coexisted current density responses of the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode in the normal concentration range of UA and AA. It indicated that the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode detects negligible current densities for AA in normal serum level. The current

response of 0.04 mM AA is less than 7 % of that collected from 0.4 mM UA. This shows that AA has a low interference on the MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode in sensing UA.

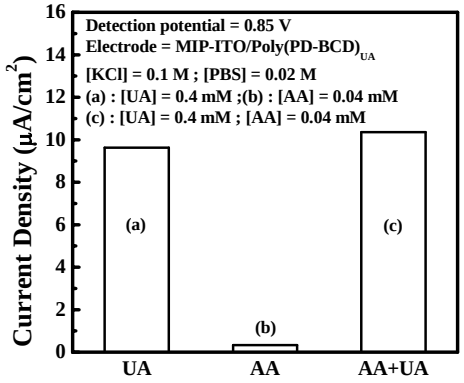


Fig. 7. The interference effect of ascorbic acid.

4 CONCLUSIONS

A novel amine-imide type of conducting polymer, Poly(PD-BCD), was used for fabricating a molecularly imprinted uric acid biosensor. From the CV response of the MIP-ITO/Poly(PD-BCD)$_{UA}$, the oxidation peak of UA disappeared after the extraction process. This result reveals that UA molecules were totally removed from the polymer surface by extraction. In other words, UA has imprinted on the polymer surface successfully. Besides, the SEM image of the MIP electrode provided another evidence that UA molecules created specific sites on the MIP electrode. By applying a constant potential at 0.85 V, a good linear relationship between the current density and the UA concentration (from 0 to 1.125 mM) was achieved, from which a sensitivity of 22.59 μA $mM^{-1}cm^{-2}$ can be obtained. The limit of detection was calculated to be 3×10^{-4} mM on the basis of signal to noise ratio (S/N) equaling to 3. The MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode can discriminate UA form AA, which is a major interference of UA using electrochemical methods. The coexistence of AA does not show significant interference effect on the UA sensing. The MIP-ITO/Poly(PD-BCD)$_{UA}$ electrode possesses good sensitivity and selectivity for UA detection and exhibits good detection limit. The present investigation has a potential to become practical and to be used as a portable and digital UA sensor in the future.

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