

## Research Article

# Characteristics of Molecularly Imprinted Polymer Thin Layer for Bisphenol A and Response of the MIP-Modified Sensor

Izumi Kubo,<sup>1</sup> Nobuyuki Yokota,<sup>1</sup> Yusuke Fuchiwaki,<sup>2</sup> and Yuko Nakane<sup>1,3</sup>

<sup>1</sup> Department of Bioinformatics, Graduate School of Engineering, Soka University, 1-236 Tangi, Hachioji, Tokyo 192-8577, Japan

<sup>2</sup> Health Technology Research Center, National Institute of Advanced Industrial Science and Technology, 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan

<sup>3</sup> RIKEN Quantitative Biology Center, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan

Correspondence should be addressed to Izumi Kubo, kubo@t.soka.ac.jp

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We examine the characteristics of molecularly imprinted polymer (MIP) layers for bisphenol A (BPA) to investigate the effect of their thickness on the performance of the BPA sensor. MIP thin layers for bisphenol A were polymerized on a sputtered gold electrode by UV light irradiation for 2 to 30 min. Their thickness, as determined by a QCM analyzer, was  $3.6 \pm 0.3$  nm after 5 min of irradiation and increased as the irradiation time increased to 30 min. AFM images of the MIP-modified surface suggested that the gold electrode was covered with a smooth MIP layer. The anodic peaks of BPA and ascorbic acid caused by gold electrode and the MIP-modified electrode were compared, and the electrode with MIP polymerized for 5 min showed more selectivity to BPA than that polymerized for 2 min. The MIP thin layer thus has potential as a sensing element of a chemical sensor.

## 1. Introduction

Enormous amounts of bisphenol A (BPA) have been used as a major material to produce polycarbonate, which is widely used in food containers because of its heat durability and solidity. When hot food or drink is placed into the polycarbonate container, BPA remaining in the plastic tends to dissolve into the food or drink. The eluted BPA from the containers might be consumed with food into the human body. Evidence from laboratory studies suggests that BPA may act a toxicant for developing tissues and has stimulated debate regarding the impact of human exposure to BPA [1]. Animal studies suggest that prenatal and early postnatal exposure to BPA may cause serious effects on brain development [2–4], sexual differentiation [5], behavior [2, 6, 7], and immune function [8–10]. There is a fear that contamination of BPA in food or drink may have a broad range of adverse effects on public health [11–14]. In Japan the concentration in food or drink must be lower than 2.5 ppm as established by food hygiene law. Conventionally, BPA concentration has been measured with the use of HPLC [15–18] or GC/MS [19, 20]. These apparatuses are expensive and the methods

need sample pretreatment. Thus, a rapid and easy method for monitoring and analyzing BPA is required.

We have been working on developing a simple and easy monitoring system for BPA based on an electrochemical reaction and artificial receptor of BPA. Since BPA is an electroactive species, it can be oxidized and detected electrochemically at 2.5 ppm [21]. As a promising artificial receptor, we utilized molecularly imprinted polymer (MIP). MIP particles for BPA were thermally synthesized by Sanbe and Haginaka for the application to a column chromatography utilizing it as a specific binding resin [22]. MIP recognizes a template molecule in its recognition site and is expected to be more stable than biological receptor. We are working on utilizing MIPs as a recognition element of chemical sensors. We modified the preparation procedure of MIP for BPA reported by Sanbe and Haginaka and applied the fabricated MIP to detect BPA coupled with an electrochemical detection system. In this system MIP was utilized as small particles packed in a column [23]. We are also working on the establishment of a BPA sensor by fabricating an electrode with a layer of MIP, which has recognition sites for BPA. To detect BPA bound to MIP from a sample solution,

oxidation of BPA is an efficient way, although MIP is itself an insulator and the redox reaction cannot be performed. In previous studies, we developed atrazine [24] and simazine [25, 26] sensors consisting of a gold electrode modified with an MIP layer synthesized by UV irradiation, and the sensor was immersed in LiCl solution to introduce an electrolyte into the MIP layer prior to redox reaction of these herbicides. Although this method was effective to measure redox current, the electrolyte could not be kept for long in the MIP layer [24]. Instead of introducing an electrolyte into the MIP layer, we decided to prepare an MIP layer on the surface of the electrode as thinly as possible by UV illumination to introduce BPA to the surface of the electrode.

A thin MIP layer is a promising element of chemical sensors [27–30] and its binding affinity and selectivity to analytes have been investigated. However, the effect of its thickness on the sensor response has not yet been reported.

In this study, we examine the characteristics of MIP layers to investigate the effect of their thickness on the performance of a BPA sensor. MIP was polymerized by UV irradiation, and we examined its irradiation time, thickness, and appearance, as well as the selectivity of the sensor.

## 2. Experimental

**2.1. Materials and Methods.** BPA, which is a standard chemical for environmental analysis, was obtained from Kanto Chemicals (Tokyo, Japan). 4-vinylpyridine (4-VPY), 2,2'-azobis (2,4 dimethylvaleronitrile), and ethylene dimethacrylate (EDMA) were obtained from WAKO Pure Chemicals. Other reagents were of laboratory grade and used without any purification.

**2.2. Preparation of MIP Receptor for BPA on QCM.** In this study, we used a plate-type gold electrode (QA-A9-Au, Seiko EG&G, Chiba, Japan) for the modification with MIP. As the shape of the electrode is a flat plate, it is suitable for direct polymerization of prepolymer solution on its surface. The size of the electrode is 5 mm in diameter.

Prior to preparing MIP layer on the electrode, a circular opening was fabricated on plate electrode with photosensitive polyimide insulator coating (Photoneece PW-1500 TORAY) to make defined area ( $\phi = 1.5$  mm) of electrochemical detection.

The surface of the gold layer was cleaned for 30–60 sec by sputtering ionized air with an ion coater (IB-2, EIKO Engineering). Immediately after cleaning, the electrode was immersed in allyl mercaptan (53 mM) for 2 h to introduce vinyl groups onto the surface of the electrode for covalent bonding to MIP, after which it was dried with nitrogen gas.

According to the slightly modified procedure reported previously [25], MIP was polymerized using BPA as template and 4-VPY and EDMA as a functional monomer and a cross-linker, respectively. A prepolymer cocktail was prepared by dissolving BPA (128 mg), 4-VPY (0.37 mL), EDMA (2.5 mL), and 2,2'-azobis (2,4 dimethylvaleronitrile) (30 mg) in toluene (5 mL). The prepolymer cocktail was purged with

nitrogen gas for 3 min and spread on the electrode by spin coating at 1000 rpm for 10 sec with a spin-coater (Kyowa Riken, model K-359 S-1). Polymerization was performed by UV light (350 nm) irradiation with a hand-held UV lamp (Model UVGL-58, FUNAKOSHI, Tokyo) at room temperature. After irradiation, the MIP-modified electrode was dried at room temperature overnight. It was then washed in methanol repeatedly to remove unreacted reagents including BPA until no reagents were left in the methanol used in washing, as detected by UV absorption.

**2.3. Measurement of the Thickness Apparatus of QCA.** Quartz Crystal Microbalance (QCM) measurements were carried out with a Quartz Crystal Analyzer QCA 917 (SEIKO EG&G) equipped with a 9 MHz AT-Cut Au-QCM (Figure 1(a)) modified by an MIP thin layer. To determine the thickness of the MIP, before and after the polymerization of the MIP on the QCM electrode, the frequency shift was measured by the QCA. At first, the frequency shift of the naked QCM was measured. After the fabricating the dried MIP layer through the irradiation of UV light on the prepolymer cocktail on the electrode, the frequency shift decreased, and after washing off excess reagents, the frequency shift gradually increased and reached a steady state. The difference in the frequency shift between the naked QCM and the completely washed MIP layer-modified QCM was determined as mass of MIP.

The increase in the mass of 1 ng on the surface of the QCM was determined as a decrease of 1 Hz by the QCA. The density of the MIP layer was estimated to be 1.0. From the density and the mass determined by the QCA, the volume of the MIP layer on the QCM was obtained. The thickness of the MIP was calculated through the division of the volume by the area of QCM electrode.

**2.4. Electrochemical Measurement.** Electrochemical measurements were performed with a voltammetry analyzer (ALS1200, BAS, Tokyo, Japan) and a three-electrode electrochemical cell. The MIP receptor-modified electrodes were used as the working electrode. An Ag/AgCl electrode and a platinum wire electrode were used as a reference and a counter electrode, respectively. All the electrochemical measurements were carried out in a glass vial containing 10 mL of deoxygenated solution (0.1 M phosphate buffer, 50 mM KCl, pH 7.0) at room temperature.

**2.5. AFM Observation.** Topographic images were obtained by AFM to confirm the polymerized MIP on the electrode surface. AFM observations were carried out with a scanning probe microscope SPA-400 and probe station SPI-4000 (SII NanoTechnology Inc.) with a contact mode in the atmosphere.

The intrinsic resonance frequency of the cantilever was 12 kHz, and the spring constant was 0.16 N/m. Images were collected with a scan rate of 6 Hz for a scan area of 200 nm.

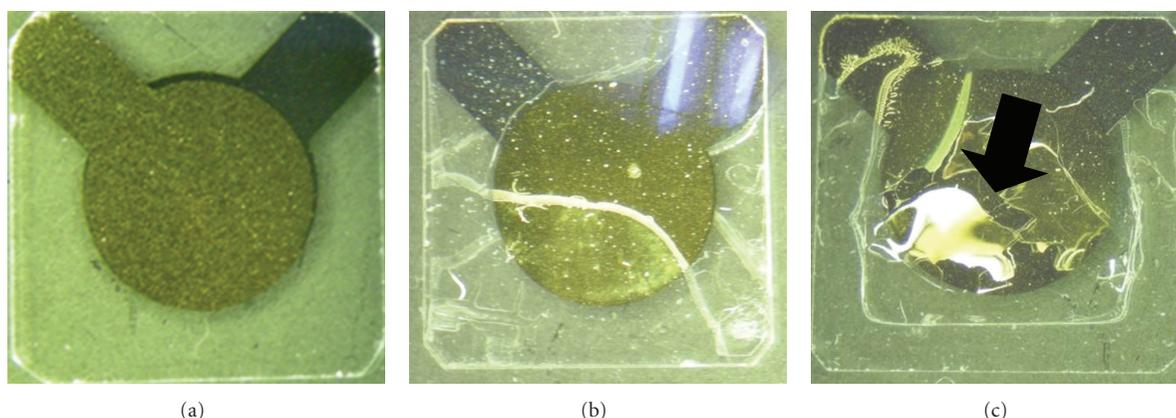


FIGURE 1: Photographs of BPA-MIP modified electrodes. (a) QCM electrode before MIP preparation. (b) The electrode after polymerization of MIP by UV irradiation for 2 min. (c) The electrode after polymerization of MIP by UV irradiation for 30 min. The black arrow indicates a crack in the MIP film.

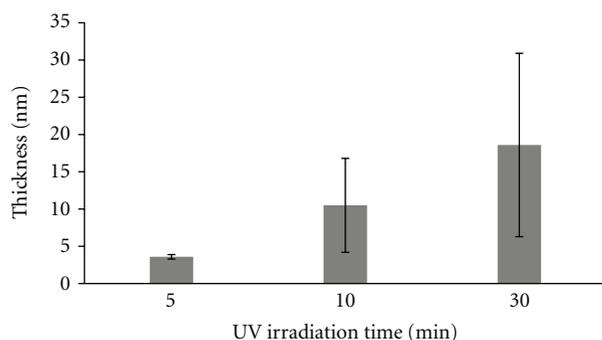


FIGURE 2: Thickness of the MIP film determined by QCM measurement and UV irradiation time.

### 3. Results and Discussion

**3.1. Thickness of MIP.** After irradiation of UV light for 2 to 30 min, an MIP layer formed on the QCM electrode at a glance (Figures 1(b) and 1(c)). The thickness of the MIP layer on the electrode was determined by QCA at an irradiation time of 2, 5, 10, and 30 min. At every irradiation time, three or more samples were prepared and served to QCM determination. Although the appearance of the MIP layer prepared by 2 min irradiation was rougher than the naked electrode, a decrease in frequency shift was not clearly observed. The thickness of the MIP layer increased as the irradiation time increased from 5 to 30 min, and that of 5, 10, and 30 min was  $3.6 \pm 0.3$ ,  $10.5 \pm 6.3$ , and  $18.6 \pm 12.3$  nm, respectively (Figure 2). A linear relationship was observed between irradiation time ( $x$ , min) and the thickness ( $y$ , nm) of the MIP layer:  $y = 0.54x + 2.4$ . The thickness of these layers was in the nm range and the very thin layer of MIP was prepared by a short period of light irradiation. The thickness of the MIP layer polymerized after 5 min irradiation was the most reproducible among all irradiation times. Longer irradiation created a thicker layer and some of the MIP

layer prepared after 30 min had cracks due to its rigidity (Figure 1(c)).

QCA analysis demonstrated that thin MIP layers were prepared through UV irradiation even only after 5 min. However, the thickness is the value over the entire electrode on which MIP layer was prepared.

**3.2. AFM Observation.** AFM observations were performed to explore the finer structure of the MIP on the electrode. The topographic image of the surface of the gold electrode used in this study revealed that the electrode consisted of small gold grains (Figure 3(a)). The cross-sectional profile showed that the height and diameter of the grain was 6–7 nm and 80 nm, respectively. It resembled a considerably flat hill with a slight slope. Then the MIPs prepared for 2, 5, and 10 min UV irradiation were observed. Prior to washes with methanol, the surface of the MIP layer polymerized for 10 min UV irradiation was completely different from the gold grain surfaces. No grain-like structure was observed and the surface was smoother than that of the gold electrode. After the washes with methanol, rougher surfaces appeared. The image before and after the washes was different between the naked gold grain and the smooth surface. As shown in Figure 3(b) the prepared MIP polymer covered the grains of sputtered gold and the surface became smoother. As for the MIPs prepared after 5 and 2 min UV irradiation, characteristic images were almost the same, although a distinct topographic image was not observed. The polymer was not considered to have formed grains but rather a flat layer along the grains (Figure 4). We then estimated the averaged slope angle from the cross-section profile of the AFM images and compared the change of the angles. The slope of the naked gold electrode was  $4^\circ$  and after the polymerization but before washes, the angle decreased  $1.5^\circ$  (5 min) and  $2.0^\circ$  (2 min). Then the slope increased again after washes to  $2.4^\circ$  (5 min) and  $3.0^\circ$  (2 min) (Figure 5). Even after 2 min irradiation, the surface was smoother than that of the naked gold electrode.

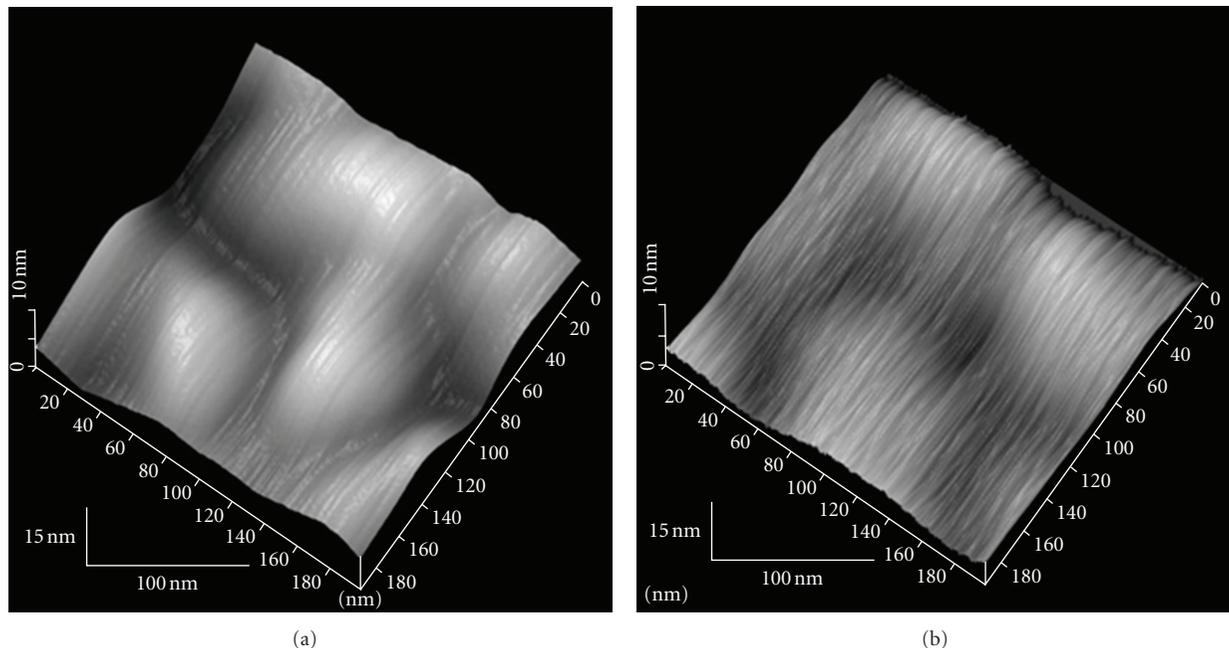


FIGURE 3: AFM images of the surface of bare electrode and MIP-electrode. (a) Bare electrode. (b) MIP-electrode polymerized for 5 min.

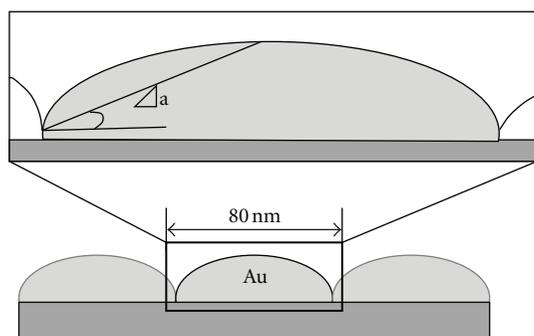


FIGURE 4: The concept of mean slope angle. Mean slope angle (triangle a) was calculated from the highest and lowest surface points in the arbitrary 80 nm area.

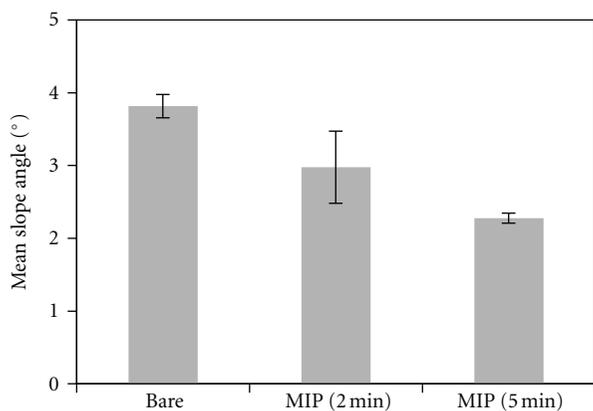


FIGURE 5: Polymerization time and mean slope angle after washes. The mean slope angles of the bare electrode and the MIP-electrode calculated by AFM image,  $n = 3$ .

### 3.3. Electrochemical Response of the MIP-Modified Electrode.

The electrochemical responses to BPA were examined with the use of an MIP-modified electrode. A cyclic voltammogram of ferricyanide (5 mM) was examined to verify the coverage of the gold electrode by MIP. The electrode with MIP showed no redox peak for ferricyanide, irrespective of the irradiation time, while the bare gold electrode clearly showed a redox peak. As for the MIP prepared by 10 min UV irradiation, an electrochemical response to BPA was not observed with the use of the MIP-modified electrode; however, as for the electrodes prepared after 2 and 5 min irradiation, a response was clearly observed. The thickness of the MIP following 10 min UV irradiation might not be thin enough to obtain an electrochemical response. The MIPs prepared after 5 and 2 min UV irradiation were examined in detail and both of the MIP-modified electrodes showed an anodic peak current to BPA (5  $\mu$ M) at 580 mV (Figure 6). The anodic peak potential to BPA observed with the use of the MIP thin-layer-modified electrode was the same as that of the naked gold electrode reported previously [23]. Then we compared the electrochemical response of the MIP thin-layer-modified electrode. An electroactive chemical species other than BPA, ascorbic acid, was examined, because food or biological samples often contain ascorbic acid, which may act as interference to the electrochemical measurement of BPA. Ascorbic acid was oxidized with the use of the naked gold electrode, and the peak potentials were 180 mV. With the use of the MIP-modified electrode, the anodic peak potential of BPA did not change and the same with the MIP layer prepared for 2 min and 5 min UV irradiation, whereas the peak potential of ascorbic acid shifted 20 mV to a positive potential. The thin MIP layer on the electrode may have worked as a blocking layer to the oxidation of

TABLE 1: The response of the MIP-electrode and bare one to BPA and ascorbic acid.

Electrode	Polymerization	BPA		Ascorbic acid	
		Peak potential mV	Peak current nA/ $\mu$ M	Peak potential mV	Peak current nA/ $\mu$ M
Au		580	27.3 (100%)	180	13.6 (100%)
MIP	2 min	580	17.8 (65.0%)	200	7.6 (55.9%)
MIP	5 min	580	12.5 (45.7%)	200	4.5 (32.9%)

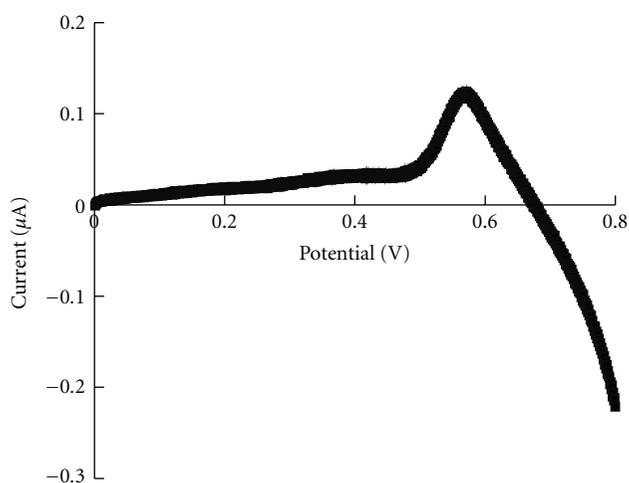


FIGURE 6: Cyclic voltammogram of BPA with MIP-modified electrode. Polymerization time of MIP was 5 min. BPA concentration was 5  $\mu$ M. Scan rate was 0.1 V/s. 0.1 M PBS, pH = 7.0.

ascorbic acid not only during electron transfer but also during the diffusion of ascorbic acid but not as a blocking layer to BPA because there are binding sites of BPA in the MIP layer. When we compared the anodic peak current of ascorbic acid and BPA, the currents to both decreased. The ratio of the current to that of the naked gold electrode, an index of the effect of the MIP layer to electron transfer, was much smaller for ascorbic acid than for BPA (Table 1). These phenomena demonstrated that BPA diffused to the MIP layer more selectively than ascorbic acid. Moreover, the MIP layer prepared after 5 min UV irradiation showed more selectivity than that prepared with 2 min irradiation, although the layer did not completely block the oxidation of ascorbic acid. The MIP layer did not work perfectly to block the diffusion of smaller molecules such as ascorbic acid ( $M_w = 176.12$ ), but its peak potential was small enough not to affect the electrochemical determination of BPA. Larger or hydrophobic molecules did not effect to the electrochemical reaction of BPA ( $M_w = 228.29$ ) when MIP-modified electrode was used.

In conclusion, a very thin layer of MIP was prepared by short UV irradiation to the prepolymer cocktail on the electrode and without introducing electrolyte into the layer, the MIP thin-layer-modified electrode showed an electrochemical response to BPA indicating that it has the potential as a sensing element of a chemical sensor.

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