

Detection of Bisphenol A Using Layer-by-Layer Covalent Anchoring of Polyamidoamine Dendrimer to Gold Electrodes

Pi-Guey Su* and Chia-Wei Tseng

Department of Chemistry, Chinese Culture University, Taipei 111, Taiwan

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Novel amperometric bisphenol A (BPA) sensors were fabricated by covalently bonding polyamidoamine (PAMAM) dendrimers layer-by-layer onto a gold electrode on quartz crystal substrate using a peptide chemical protocol. Au electrodes was pretreated with 3-mercaptopropionic acid (MPA) and further reacted with generation 5 amine-terminated PAMAM dendrimer (G5-NH₂) using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) as the peptide coupling reagent. The BPA-sensing film thus prepared was characterized by atomic force microscopy (AFM) and electrochemical impedance spectroscopy (EIS). The effects of the intermediate layer, the generations (sizes) of PAMAM dendrimer and the concentration of PAMAM dendrimer on the BPA sensing properties (response) of the sensors were investigated to optimize the fabrication of the modified electrodes. Furthermore, factors that affected the detection processes were optimized. The BPA sensor that was based on the G5-NH₂-MPA/Au electrode exhibited a high response, satisfactory linearity, a low detection limit and favorable long-term stability.

Keywords: Bisphenol A, PAMAM Dendrimer, Gold Electrode, Covalently Bonding, Long-Term Stability.

1. INTRODUCTION

Bisphenol A (BPA) is a chemical that is produced in large amounts mainly for use in the production of plastic container, dishes, and milk bottles. However, it is an endocrine-disrupting chemical whose toxicity and biological effects may cause cancer. An association between BPA and breast cancer has been made.^{1,2} Therefore, the detection and removal of BPA are very important. Several analytical methods, including high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), gas chromatography–mass spectrometry (GC–MS) and molecular imprinting have been used to detect and quantify BPA.^{3–6} However, these methods have been found to have practical limitations of cost, complexity, the need for highly expert handling and lack of applicability to on-site monitoring. Therefore, a new method for detecting BPA is required.

An electrochemical sensor is a simple and inexpensive device that is used to monitor pollutant. Furthermore, it has the further advantages of high sensitivity, simplicity

of operation, the potential for miniaturization and the ability to be used in *in-situ* analysis.⁷ BPA is electrochemically active but the direct determination of BPA using a bare electrode is impossible because the responses of BPA as a traditional electrochemical sensor are very poor.⁸ Therefore, to increase the sensitivity and selectivity of such a sensor for detecting BPA, many modified electrodes have been used. These include tyrosinase,⁹ cobalt phthalocyanine (CoPc)¹⁰ and MCM-41¹¹-modified carbon paste electrodes (CPE), tyrosinase-single-walled carbon nanotubes (SWCNTs) paste electrodes,¹² tyrosinase-silk fibroin-multi-walled carbon nanotubes (MWCNTs)-CoPc,¹³ MWCNT-gold nanoparticles (GNPs)¹⁴ and chitosan-Fe₃O₄¹⁵-modified glassy carbon electrodes (GCE).

Polyamidoamine (PAMAM) dendrimers are highly branched well-defined three-dimensional macromolecules with hydrophilic terminal functional groups (–NH₂).¹⁶ They have attracted considerable attention recently because of their high geometric symmetry, easily controlled nanosize, controllable surface functionality, film-forming ability and chemical stability, which have led to

*Corresponding author; E-mail: spg@faculty.pccu.edu.tw

in the extension of their use to biosensors, chemical sensors and modified electrode surfaces.^{17–20} Recently, many modified electrodes that are based on PAMAM have been used to detect BPA.^{8,21,22} Zhu et al. fabricated amperometric sensors by dropping gold nanoparticles-silk fibroin-PAMAM onto GCE,⁸ CoTe quantum dots (CoTe QDs) and PAMAM onto GCE²¹ and PAMAM-Fe₃O₄ onto GCE.²² However, the performance of the amperometric sensors is worsened during long-term use because the sensing films are not anchored to the GCE.

Layer-by-layer (LBL) self-assembly method is based on sequential adsorptions of ionized polyelectrolytes and oppositely charged materials in aqueous solutions. LBL self-assembly has many advantages over other methods, including simplicity, low-cost, low temperature of deposition, controllable thickness (from nanometers to micrometers) and the need for no complex equipment.^{23–25} In recent years, since new peptide coupling reagents have been developed for use in organic synthesis, peptide coupling reactions have been widely studied. In a typical peptide coupling reaction, a carboxyl group of a compound is initially activated by a suitable peptide coupling reagent, and it is subsequently reacted with an amine group in another compound.²⁶ Kwak et al. fabricated a hydrogen peroxide sensor that was made by bonding PAMAM onto thiol-modified gold electrodes on an Si wafer using *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) as a peptide coupling reagent.²⁷ However, no attempt has been made to anchor the PAMAM dendrimer onto a gold electrode in the fabrication of amperometric BPA sensors by the combination of LBL with a peptide chemical protocol. In this study, the preparation and performance of covalently modified gold electrodes are compared with those of bare gold electrodes that are coated electrostatically with PAMAM dendrimers. Two generations (sizes) of amine-terminated PAMAM dendrimers (G1-NH₂ and G5-NH₂) were anchored onto the 3-mercaptopropionic acid (MPA)- or 11-mercaptoundecanoic acid (MUA)-modified gold electrode on a quartz crystal substrate using EDC as the peptide coupling reagent. The covalently modified electrode greatly improved the response, the repeatability and the long-term stability of BPA sensing. The films were characterized by atomic force microscopy (AFM) and the electrochemical method of impedance spectroscopy (EIS). The effects of the intermediate layer, the generations (sizes) of PAMAM dendrimers and the concentration of PAMAM dendrimers on the BPA sensing properties (response) of the sensors were investigated. The optimal conditions for determining BPA were also investigated.

2. EXPERIMENTAL DETAILS

2.1. Materials

Generations 1 and 5 amine-terminated PAMAM dendrimers (G1-NH₂ and G5-NH₂; 5 wt% in methanol

solution, Aldrich) were used without further purification. 3-Mercaptopropionic acid (95%; MPA), 11-Mercaptoundecanoic acid (95%; MUA), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (95%; EDC) and BPA were obtained from Aldrich. 0.1 M BPA stock solution was prepared with anhydrous ethanol and kept in a refrigerator at 4 °C. Working solutions were freshly prepared before use by diluting the stock solution. The pH of the solutions was adjusted using the following buffers: sodium acetate/hydrochloric acid for pH 2–3.5, sodium acetate/acetic acid for pH 4–6.5 and KH₂PO₄/NaOH for pH 7–8. All reagents used were analytical grade. All used deionized water (DIW) was prepared using a Milli-Q Millipore (Bedford, MA, USA) purification system, and the resistivity of water was above 18.0 MΩ cm⁻¹.

2.2. Fabrication of PAMAM-Anchored on Au Electrodes on Quartz Crystal Substrate

The quartz crystal substrates were purchased from ANT Technologies Corp., Taiwan. The surface area of the Au electrode on a quartz crystal substrate was 0.102 cm². The substrates were firstly immersed for 3 min in a bath that contained a solution of H₂O₂/H₂SO₄ (1:2 volume ratio). They were thoroughly rinsed with DIW following each step. This process made them hydrophilic. The pretreated substrate was immersed for 24 h in a 2.0 mM solution of MPA (75/25% ethanol/DIW) or MUA (75/25% ethanol/DIW) in ethanol at room temperature and then washed thoroughly in 75/25% ethanol/DIW to remove the non-chemisorbed materials to form an MPA- or MUA-modified electrode (MPA/Au or MUA/Au). Then, the MPA/Au or MUA/Au was immersed for 12 h in 0.5 mL PAMAM solution that contained 200 mM EDC at room temperature and then washed thoroughly in DIW to remove any non-anchored PAMAM dendrimers. The PAMAM-MPA/Au and PAMAM-MUA/Au electrodes were then obtained.

2.3. Instruments and Analysis

The surface microstructure of the thin films that were coated on a substrate was investigated using an atomic force microscope (AFM, Ben-Yuan, CSPM 4000) in tapping mode which the horizontal and vertical resolution are 0.26 and 0.10 nm, respectively. A standard three-electrode cell, connected to an electrochemical workstation (ZAHNER ZENNIUM, Germany), was used to make the electrochemical measurements. All electrochemical measurements were made in the presence of a 10 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (1:1) mixture (with 0.2 M NaCl as the supporting electrolyte). Cyclic voltammetry (CV) was performed in a potential range from 0.2 to 1.0 V with a scan rate of 100 mV s⁻¹. All the measurements were made at 25 ± 1 °C.

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of G5-NH₂-MPA/Au and G5-NH₂/Au Electrodes

Figure 1(a) shows the layer-by-layer procedure for anchoring PAMAM dendrimers onto the thiol-modified gold electrode using conventional peptide chemical protocols. Firstly, the MPA monolayer was chemisorbed onto the gold electrode surface with the formation of a gold–thiolate bond. Then, G5-NH₂ was anchored to the surface of the MPA-modified gold electrodes by forming a peptidic bond: the carboxylic acid groups in the MPA were activated by EDC (peptide coupling reagent), which reacted with amine groups in the G5-NH₂ to form chemical amide bonds (shown as G5-NH₂-MPA/Au electrode). To provide reference cases and to study the response, the repeatability and the stability of the BPA sensing using amperometric sensor with covalently surface-confined of PAMAM dendrimer, bare gold electrodes were electrostatically modified directly

without thiol by exposure to solutions of G5-NH₂ to form the G5-NH₂/Au electrode, as shown in Figure 1(b).

3.1.1. Electrochemical Behavior of G5-NH₂-MPA/Au Electrode

Electrochemical impedance spectra (EIS) were obtained to characterize the interface properties of the modified electrodes. In EIS, the diameter of the semicircle at higher frequencies in the Nyquist diagram that was obtained by impedance spectroscopy yields the interfacial electron transfer resistance (Ret), which determines the electron transfer kinetics of the redox probe at the electrode surface. Figure 2 presents the Nyquist diagrams of that were obtained from the EIS of the bare Au electrode, the MPA/Au electrode and the G5-NH₂-MPA/Au electrode. The bare Au electrode exhibited a high Ret about 1.2 kΩ (curve a). As the MPA was chemisorbed on the Au surface, the Ret increased to a high value about 20.3 kΩ

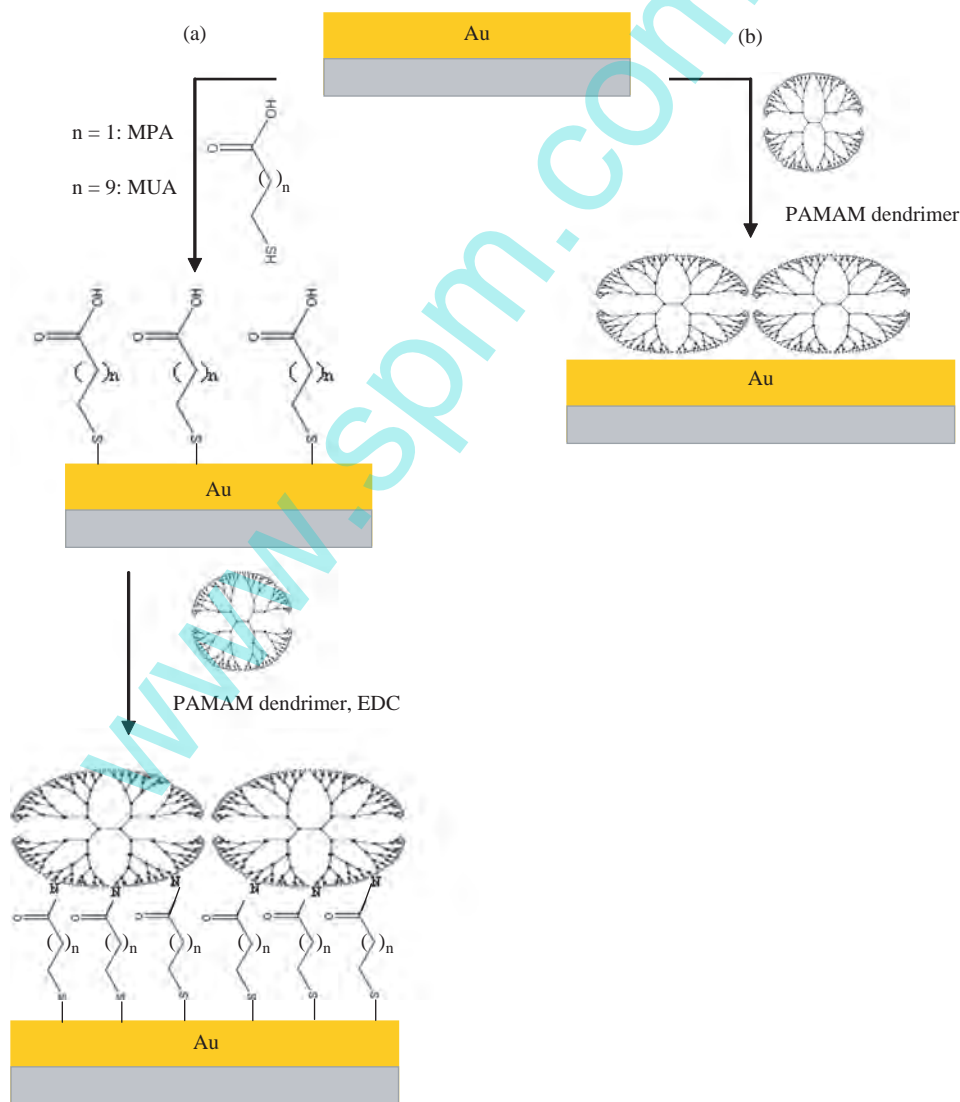


Fig. 1. Two procedures for preparing PAMAM-modified electrodes: (a) covalent bonding and (b) electrostatic modification.

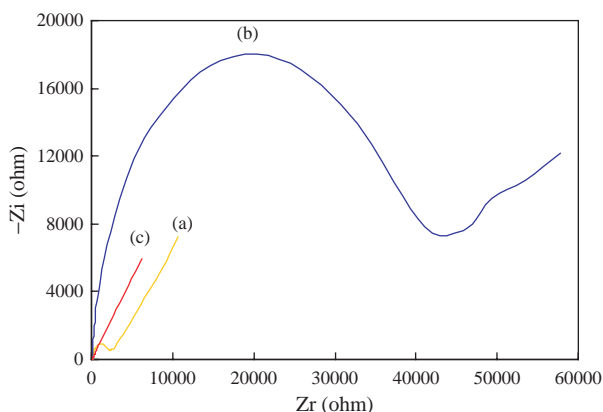


Fig. 2. Electrochemical impedance spectra of (a) bare Au electrode, (b) MPA/Au electrode and (c) G5-NH₂-MPA/Au electrode.

(curve b) because many negative charges from the $-\text{COO}^-$ groups on the MPA film perturbed the interfacial electron transfer between the electrode and the electrolyte solution. When G5-NH₂ was further anchored onto the surface of the MPA/Au electrode, the curve was an almost straight line (curve c), revealing a very low interfacial electron transfer resistance. The positively charged amino groups of G5-NH₂ had a three dimensional structure and a large specific surface area, which greatly increased the electrode surface area, effectively attracting negatively charged $[\text{Fe}(\text{CN})_6]^{3-/4-}$, improving the electron transfer rate. This phenomenon also clearly suggests that the G5-NH₂ dendrimers were successfully anchored onto the MPA/Au surface.

3.1.2. Cyclic Voltammetric Behaviors of BPA of G5-NH₂-MPA/Au Electrode

Figure 3 plots the cyclic voltammograms of bare Au (curve a), MPA/Au (curve b) and G5-NH₂-MPA/Au

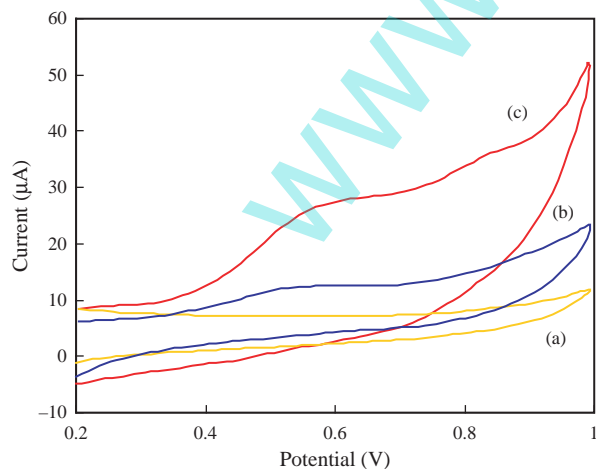


Fig. 3. Cyclic voltammograms of 0.25 mM BPA at (a) bare Au electrode, (b) MPA/Au electrode and (c) G5-NH₂-MPA/Au electrode, measured at 0.1 M PBS (pH 8) with scan rate of 100 mVs⁻¹ and an accumulation time of 240 s.

(curve c) electrodes, measured at 0.25 mM BPA in 0.1 M PBS (pH 8). Every electrode yielded only an irreversible oxidation peak during the sweep from 0.2 V to 1.00 V, indicating that the electrode response of BPA was typical of a totally irreversible electrode reaction, consistent with the literature.^{10,11} When G5-NH₂ dendrimer was anchored on the MPA/Au electrode, a higher oxidation current response was observed (curve c) than that of either the bare Au (curve a) or the MPA/Au (curve b) electrode. At pH 8.0, the external amino-groups and the internal tertiary amine of G5-NH₂ dendrimer was protonated²⁸ and then the positively charged of G5-NH₂ dendrimer effectively adsorbed the negatively charged BPA, enhancing the current response. Additionally, the oxidation potential of BPA at the G5-NH₂-MPA/Au electrode (0.562 V) was lower than that at the CPE (0.89 V, vs. SCE),²⁹ Pt electrode (0.74 V, vs. Ag/AgCl)³⁰ and GCE (0.65 V, vs. Ag/AgCl).³¹ However, the oxidation potential of BPA herein was slightly higher than that at PAMAM-gold nanoparticles-silk fibroin/GCE (0.456 V),⁸ PAMAM/CoTe/GCE (0.490 V)²¹ and PAMAM/Fe₂O₃/GCE (0.541 V).²² The variation in the oxidation potential of BPA may be attributed to the matrix electrode, the fabrication procedure and the materials in the modified electrode.²¹

3.1.3. Microstructure Characteristics of Surface and Response Properties of G5-NH₂/Au and G5-NH₂-MPA/Au Electrodes

Figures 4(a) and (b) present the surface morphology of the G5-NH₂/Au and G5-NH₂-MPA/Au thin films on a quartz crystal substrate, obtained using tapping-mode AFM, respectively. The size of each image is 5 μm × 5 μm. Table I shows the roughness of, and size of the spheres in, G5-NH₂/Au and G5-NH₂-MPA/Au thin films. The surface of G5-NH₂-MPA/Au thin film (12.7 nm) was rougher than that of G5-NH₂/Au thin film (5.5 nm). The spheres in G5-NH₂/Au were larger than those in G5-NH₂-MPA/Au thin films. Figure 4(c) presents the surface morphology of the G5-NH₂-MPA/Au thin film with 3.2 μM G5-NH₂. The size cluster circular (Φ) of the G5-NH₂-MPA/Au thin film with 3.2 μM G5-NH₂ was 46 nm. The G5-NH₂-MPA/Au thin film with 3.2 μM G5-NH₂ had a larger clusters than the film with 2.2 μM G5-NH₂ (40 nm), perhaps because the G5-NH₂ dendrimers aggregate and interpenetrate at high concentration of added G5-NH₂ dendrimer. The difference between the surface morphologies of G5-NH₂/Au and G5-NH₂-MPA/Au thin films arose from the difference between the interactions that developed between the dendrimers and the Au electrode and those in thiol-modified electrode.

Table I also compares the responses of the G5-NH₂/Au and G5-NH₂-MPA/Au electrodes and their repeatabilities and long-term stability. The sensor response was expressed as $\Delta I = I - I^0$, where I and I^0 are the current of the

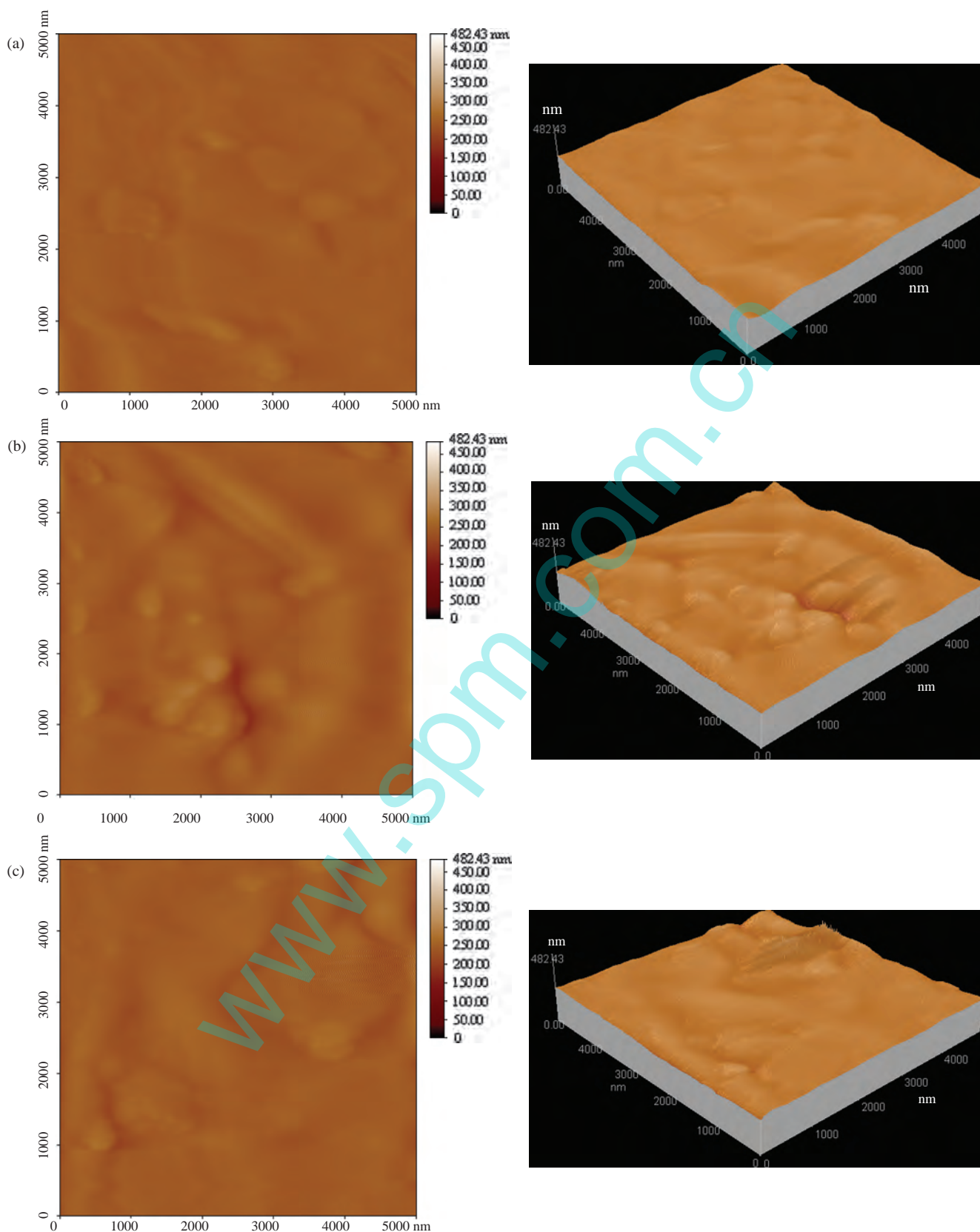


Fig. 4. AFM micrographs of (a) G5-NH₂/Au thin film, (b) G5-NH₂-MPA/Au thin film using 2.2 μM G5-NH₂ and (c) G5-NH₂-MPA/Au thin film using 3.2 μM G5-NH₂.

Table I. Roughness, sphere size and sensing characteristics (response, repeatability and long-term stability) of G5-NH₂/Au and G5-NH₂-MPA/Au thin films on quartz crystal substrate.

	Electrode	
	G5-NH ₂ /Au	G5-NH ₂ -MPA/Au
RMS (nm)	5.5	12.7
Size cluster circular (Φ) (nm)	69 \pm 40	40 \pm 25
Response ^a (μ A)	8.59	16.79
Repeatability ^b (%)	23.29	4.85
Stability (days, retained response) ^c (%)	28, 55	117, 82

Notes: ^aThe sensor response was given by $\Delta I = I - I^0$, where I and I^0 are the current of the sensor in tested solution with and without BPA, respectively, measured at 0.1 M PBS (pH 8), scan rate: 100 mV s⁻¹ and accumulation time: 240 s; ^bThe repeatability of sensor was given as relative standard deviation (R.S.D.); ^cThe response of G5-NH₂-MPA/Au electrode in 0.25 mM BPA.

sensor in the tested solution with and without BPA, respectively. The repeatability of the response of the sensor was presented as a relative standard deviation (R.S.D.) of the sensors within a batch. The G5-NH₂-MPA/Au thin film exhibited a stronger response with better repeatability than did the G5-NH₂/Au thin film because the G5-NH₂-MPA/Au thin film had a higher effective surface area than the G5-NH₂/Au thin film and a stable configuration (Table I). Moreover, the long-term stability of the G5-NH₂-MPA/Au thin film was better than that of G5-NH₂/Au thin film. Therefore, the method of fabrication of G5-NH₂-MPA/Au

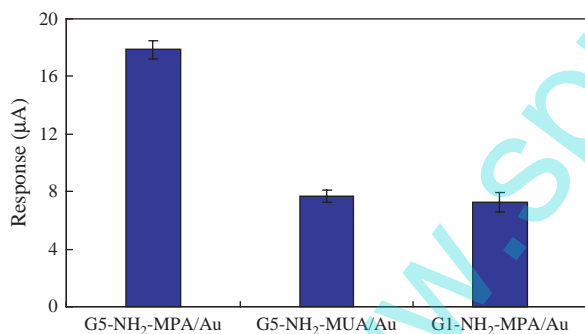


Fig. 5. Response for 0.25 mM BPA to various PAMAM-thiol-modified electrodes measurement conditions are as in Figure 3.

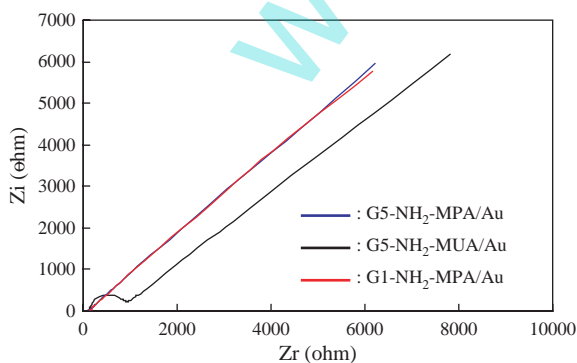


Fig. 6. Electrochemical impedance spectra of various PAMAM-thiol-modified electrodes.

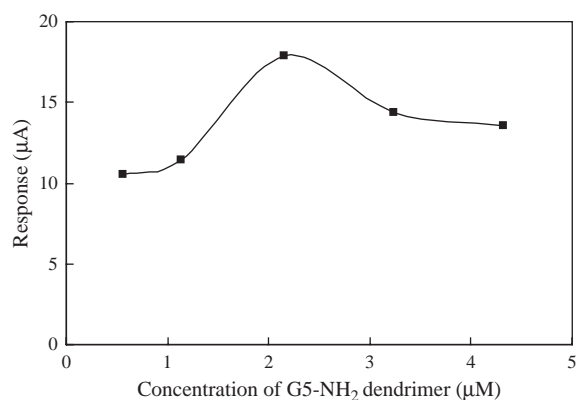


Fig. 7. Effect of concentration of G5-NH₂ on response of G5-NH₂-MPA/Au electrode in 0.25 mM BPA measurement conditions are as in Figure 3.

thin film that was based on the covalently PAMAM-thiol-modified electrode was further tested to evaluate the BPA-sensing characteristics.

3.2. Response of PAMAM-Thiol-Modified Electrodes

3.2.1. Effects of Intermediate Layer (MPA and MUA) and Generation of PAMAM Dendrimer (G1 and G5) on Response of BPA

Figure 5 plots the effects of the intermediate layer (MPA and MUA) and the generation of PAMAM dendrimer (G1 and G5) in the PAMAM-thiol-modified electrode on its response to BPA. The G5-NH₂-MPA/Au electrode exhibited a stronger response than did the G5-NH₂-MUA/Au or G1-NH₂-MPA/Au electrodes. EIS analysis was performed to determine the possible reasons for the changes in the response characteristics of the PAMAM-thiol-modified electrode. Figure 6 presents the EIS of G1-NH₂-MPA/Au, G5-NH₂-MPA/Au and G5-NH₂-MUA/Au electrodes. A comparison between the EIS of the G5-NH₂-MPA/Au electrode and that of the G5-NH₂-MUA/Au electrode based on the same generation of PAMAM dendrimer

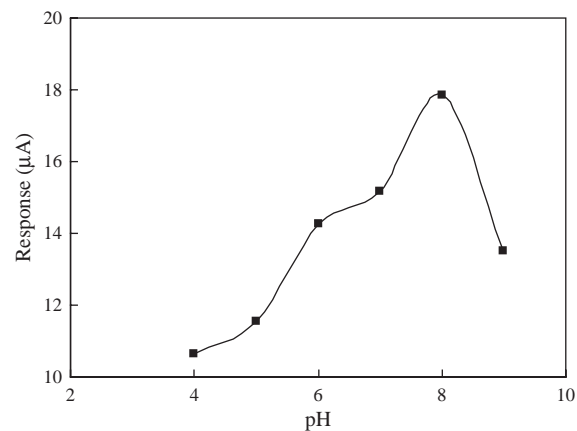


Fig. 8. Effect of pH on response of G5-NH₂-MPA/Au electrode in 0.25 mM BPA, measurement conditions are the same as in Figure 3.

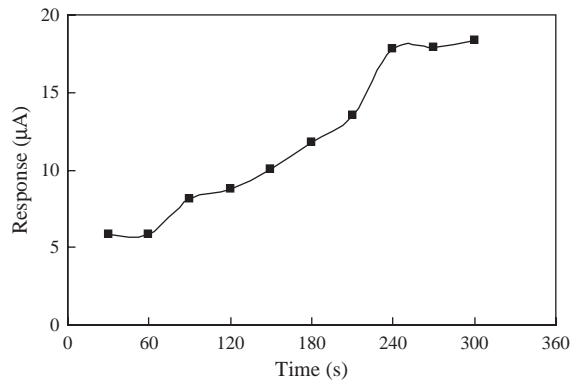


Fig. 9. Effect of accumulation time on response of G5-NH₂-MPA/Au electrode in 0.25 mM BPA measurement conditions are as in Figure 3.

(G5-NH₂) revealed that the G5-NH₂-MUA/Au electrode exhibited a semicircular with a large diameter suggesting that it exhibited weaker interfacial electron transfer and therefore a weaker response to BPA determination.^{8,21,22} A comparison between the EIS of the G1-NH₂-MPA/Au electrode and that of G5-NH₂-MPA/Au electrode based on the same intermediate layer (MPA) revealed no difference in EIS. Therefore, the response of the G5-NH₂-MPA/Au electrode to BPA increased in proportion to the size of the molecules and the number of functional groups of the dendrimer. The number of terminal groups increased exponentially as function of dendrimer size or generations: a G1-NH₂ dendrimer has 8 terminal groups and this number increased to 128 for G5-NH₂ dendrimer. With increased generations of dendrimer also number of adsorption sites for BPA increased. Therefore, the response of the G5-NH₂-MPA/Au electrode to BPA was higher than that of the G1-NH₂-MPA/Au electrode.

3.2.2. Effect of Concentration of G5-NH₂ Dendrimer on Response of BPA

Figure 7 plots the effect of the concentration of G5-NH₂ dendrimer in the G5-NH₂-MPA/Au electrode on the characteristics of its response to BPA. When the

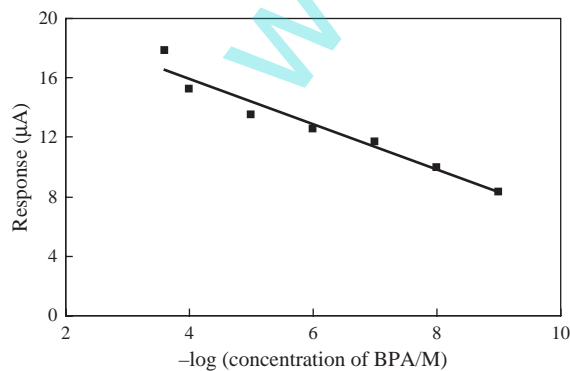


Fig. 10. Linear dependence of response of G5-NH₂-MPA/Au electrode on concentration of BPA measurement conditions are as in Figure 3.

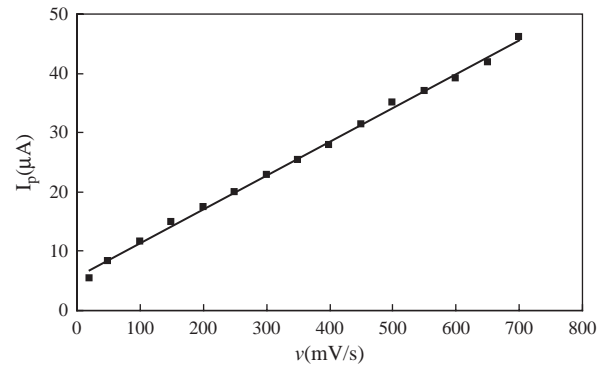


Fig. 11. Plot for the dependence of peak current on scan rate based on G5-NH₂-MPA/Au electrode with different scan rates by cyclic voltammetry, measurement conditions are the same as in Figure 3.

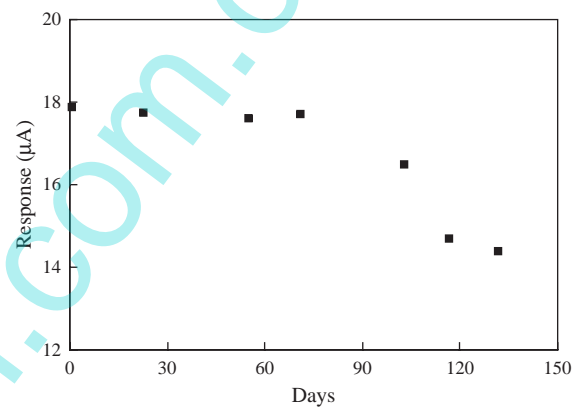


Fig. 12. Long-term stability of response of G5-NH₂-MPA/Au electrode in 0.25 mM BPA measurement conditions are as in Figure 3.

concentration of G5-NH₂ dendrimer that was added to the G5-NH₂-MPA/Au electrode was less than 2.2 μM , the response of the G5-NH₂-MPA/Au electrode increased with the concentration of the G5-NH₂ dendrimer, because the effective covalent dendrimer surface coverage increased. However, when the concentration of added G5-NH₂ dendrimer increased over 2.2 μM , the response of the G5-NH₂-MPA/Au electrode decreased because the dendrimers may aggregate and interpenetrate, reducing the effective area that is accessible to the BPA. Therefore, the G5-NH₂-MPA/Au electrode with 2.2 μM G5-NH₂ dendrimer was further tested to evaluate its BPA-sensing characteristics.

Table II. Recovery test of G5-NH₂-MPA/Au electrode.

BPA concentration (μM)		
Added	Found	Recovery (%)
1	0.879	87.9
0.1	0.0865	86.5
0.01	0.00882	88.2
0.001	0.000872	87.2
0.0001	0.0000911	91.1

Table III. Comparison of performance of BPA sensor developed herein with those of sensors that are based on PAMAM dendrimer-modified electrodes.

Sensor	Fabrication method	Linear range (μM)	Detection limit (μM)	Stability (days, retained response) (%)	References
G5-NH ₂ -MPA/Au	Covalently bonding	0.001–2.5	0.0002	117, 82	This work
G4-NH ₂ -AuNPs-SF/GCE	Physical adsorption	0.001–1.33	0.0005	14, 91	[8]
G4-NH ₂ -CoTe/GCE	Physical adsorption	0.013–9.89	0.001	35, 72	[21]
G4-NH ₂ -Fe ₃ O ₄ /GCE	Physical adsorption	0.01–3.07	0.005	30, 86	[23]

3.3. Response Characteristics of G5-NH₂-MPA/Au Electrode

3.3.1. Optimization Conditions for Determining BAP of G5-NH₂-MPA/Au Electrode

Figure 8 plots the effect of solution pH between 4 and 9 on the response of a G5-NH₂-MPA/Au electrode in 0.25 mM BPA, determined using cyclic voltammetry. The response current of BPA gradually increased with increasing pH from 4.0 to 8.0, and decreased as the pH increased further. The amine groups in G5-NH₂ were completely protonated at about pH 8.0,²⁸ and could then adsorb the negatively charged BPA to maximize the current response. Then, when the pH exceeded 8.0, the protonation level of G5-NH₂ decreased, reducing the BPA adsorbance, reducing the current response. Therefore, pH 8.0 was used in this experiment. Figure 9 plots the effect of the accumulation time on the response of the G5-NH₂-MPA/Au electrode in 0.25 mM BPA, as determined by cyclic voltammetry. The response increased gradually with the accumulation time up to 240 s, after which the peak current hardly changed perhaps because of the saturation of the adsorption of BPA at the electrode surface. To balance sensitivity and working efficiency, an accumulation time of 240 s was used. Figure 10 plots the response current of the G5-NH₂-MPA/Au electrode as a function of the negative logarithm of the concentration of BPA. The measurements were made at pH 8, an oxidation potential of 0.562 V, a scan rate of 100 mV s⁻¹ and an accumulation time of 240 s. This curve was the acceptably linear ($Y = -1.528X + 22.02$; $R^2 = 0.9455$, where Y is the response current; X is the negative logarithm of the concentration, and R^2 is the correlation coefficient) in the range of 0.001 to 250 μM . Figure 11 plots the effect of scan rate (v) on the oxidation of BPA was investigated by cyclic voltammetry. The oxidation peak currents increased linearly with scan rate in the range of 20–700 mVs⁻¹. Therefore, according to the results of Figures 10 and 11, the oxidation of BPA at G5-NH₂-MPA/Au electrode was a typical Freundlich adsorption-controlled electrode process. Detection limits (D.L.) were calculated as three times the standard deviation of seven replicate measurement of the blank sample. The D. L. was 0.0002 μM . Figure 12 plots the long-term stability. The electrode retained 82% of its original response even after 117 days. The G5-NH₂-MPA/Au electrode was then applied for the analysis the spiked river water sample. The results of the recovery studies are listed in Table II. The recoveries of the

methods were in the range of 86–91% for the spiked river water sample, indicating that the proposed procedure was very sensitive, selective and accurate enough for practical application. Table III compares the BPA sensing properties of the G5-NH₂-MPA/Au electrode with those obtained in previous reports^{8,21,22} based on PAMAM dendrimer-modified electrodes, as determined by electrochemically. The long-term stability and sensitivity of the BPA sensor herein exceeded those of the BPA sensors that were made from G4-NH₂-CoTe/GCE, G4-NH₂-AuNPs-SF/GCE and G4-NH₂-Fe₃O₄/GCE electrodes. The BPA sensor herein also had a reasonable linear range and a low detection limit.

4. CONCLUSIONS

A BPA sensor was successfully fabricated by layer-by-layer anchored PAMAM dendrimer onto the thiol-modified gold electrode on a quartz crystal substrate using conventional peptide chemistry protocols, revealing that the covalently modified electrode greatly increased the stability of the BPA sensing. The intermediate layer and generation of the PAMAM dendrimer dominated the BPA sensing properties (response). The G5-NH₂-MPA/Au electrode exhibited good response, acceptable linearity ($Y = -1.528X + 22.02$; $R^2 = 0.9455$) between the response and the negative logarithm of the concentration from 0.001 to 250 μM , a low detection limit (0.0002 μM) and good long-term stability (as it retained 82% of its original response even after 117 days) measurement were made in 0.1 M PBS (pH 8), at a scan rate of 100 mV s⁻¹ and an accumulation time of 240 s.

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References and Notes

1. A. Krishnan, P. Stathis, S. Permeth, L. Tokes, and D. Feldman, *Endocrinology* 132, 2279 (1993).
2. M. Munoz-de-Toro, C. Markey, P. Wadia, E. Luque, B. Rubin, C. Sonnenschein, and A. Soto, *Endocrinology* 146, 4138 (2005).
3. J. Sajiki, K. Takahashi, and J. Yonekubo, *J. Chromatogr. B* 736, 255 (1999).
4. H. Sambe, K. Hoshina, K. Hosoya, and J. Haginaka, *Analyst* 130, 38 (2005).
5. J. Zhang, G. M. Cooke, I. H. A. Curran, C. G. Goodyer, and X. Cao, *J. Chromatogr. B* 879, 209 (2011).

6. Y. Wang, Y. Yang, L. Xu, and J. Zhang, *Electrochimica Acta* 56, 2105 (2011).
7. K. R. Rogers, J. Y. Becker, J. Wang, and F. Lu, *Field Anal. Chem. Technol.* 3, 161 (1999).
8. H. S. Yin, Y. L. Zhou, S. Y. Ai, R. X. Han, T. T. Tang, and L. S. Zhu, *Microchim. Acta* 170, 99 (2010).
9. S. Andreescu and O. Sadik, *Anal. Chem.* 76, 552 (2004).
10. H. S. Yin, Y. L. Zhou, and S. Y. Ai, *J. Electroanal. Chem.* 626, 80 (2009).
11. F. Wang, J. Yang, and K. Wu, *Anal. Chim. Acta* 638, 23 (2009).
12. D. G. Mita, A. Attanasio, F. Arduini, N. Diano, V. Grano, U. Ben-civenga, S. Rossi, A. Amine, and D. Moscone, *Biosens. Bioelectron.* 23, 60 (2007).
13. H. Yin, Y. Zhou, J. Xu, S. Ai, L. Cui, and L. Zhu, *Anal. Chim. Acta* 659, 144 (2010).
14. X. M. Tu, L. S. Yan, X. B. Luo, S. L. Luo, and Q. J. Xie, *Electro-analysis* 21, 2491 (2009).
15. C. Yu, L. Gou, X. Zhou, N. Bao, and H. Gu, *Electrochimica Acta* 56, 9056 (2011).
16. S. M. Grayson and J. M. J. Frechet, *Chem. Rev.* 101, 3819 (2001).
17. I. Grabchev, X. Qian, V. Bojinov, Y. Xiao, and W. Zhang, *Polymer* 43, 5731 (2002).
18. N. B. Li, J. H. Park, K. Park, S. J. Kwon, H. Shin, and J. Kwak, *Biosens. Bioelectron.* 23, 1519 (2008).
19. Z. Zhang, W. Yang, J. Wang, C. Yang, F. Yang, and X. Yang, *Talanta* 78, 1240 (2009).
20. Y. Qu, Q. Sun, F. Xiao, G. Shi, and L. Jin, *Bioelectrochemistry* 77, 139 (2010).
21. H. Yin, Y. Zhou, S. Ai, Q. Chen, X. Zhu, X. Liu, and L. Zhu, *J. Hazard. Mater.* 174, 236 (2010).
22. H. Yin, L. Cui, Q. Chen, W. Shi, S. Ai, L. Zhu, and L. Lu, *Food Chem.* 125, 1097 (2011).
23. G. Decher, J. D. Hong, and J. Schmitt, *Thin Solid Films* 210, 831 (1992).
24. G. Decher, *Science* 277, 1232 (1997).
25. A. A. Mamedov, N. A. Kotov, M. Prato, D. M. Guldi, J. P. Wicksted, and A. Hirsch, *Nat. Mater.* 1, 190 (2001).
26. S. Y. Han and Y. A. Kim, *Tetrahedron* 60, 2447 (2004).
27. N. B. Li, J. H. Park, K. Park, S. J. Kwon, H. Shin, and J. Kwak, *Biosens. Bioelectron.* 23, 1519 (2008).
28. W. Chen, D. Tomalia, and J. Thomas, *Macromolecules* 33, 9169 (2000).
29. W. Huang, *Bull. Korean Chem. Soc.* 26, 1560 (2005).
30. S. Tanaka, Y. Nakata, T. Kimura, M. Kawasaki, and H. Kuramitz, *J. Appl. Electrochem.* 32, 197 (2002).
31. H. Kuramitz, M. Matsushita, and S. Tanaka, *Water Res.* 38, 2331 (2004).