Int. J. Electrochem. Sci., 7 **(2012)** 7927 - 7934

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Short Communication

Fabrication and Modeling of Ultrasensitive Label Free Impedimetric Immunosensor for IgG based on Poly(o-phenylenediamine) Film Modified Gold Electrode

Minghao Wang[1], *Lixin Cao*[2,*], *Peisheng Yan*[2], *Ningning Wu*[2]

[1] Department of Applied Chemistry, School of Chemical Engineering & Technology, Harbin Institute of Technology, China
[2] School of Ocean Science and Technology, Harbin Institute of Technology (Weihai Campus), Weihai, China
[*]E-mail: caolixin668@yahoo.com.cn

Received: 9 July 2012 / *Accepted:* 5 August 2012 / *Published:* 1 September 2012

A label free impedimetric immunosensor for IgG with high sensitivity, fine reproducibility and wider linear range was fabricated via immobilizing goat anti-rabbit Immunoglobulin G (IgG) onto Poly(*o*-phenylenediamine) (P*o*PD) electropolymerized film modified gold electrode by glutaraldehyde cross-linking. An electrochemical interfacial modeling of biomolecular recognition was constructed and reasonably interpretated. The linear detection concentration ranges of IgG were 0.1~10 ng/mL and 10~100 ng/mL. The detection limit was 0.05 ng/mL(s/n=3). The immunosensor could be reused more than 25 times when renewed by HCl-Glycine buffer solution (pH 2.80).

Keywords: Immunosensor; poly-o-phenylenediamine (P*o*PD); Label-free; Electrochemical Impedance Spectroscopy (EIS); Immunoglobulin (IgG)

1. INTRODUCTION

Recent years, electrochemical immunosensors have attracted much interest in many scientific fields such as clinical analysis [1-2], food detection [5-6] and environmental monitoring [3-4] by taking the combined advantages of electroanalysis and immunoreaction. Compared with other sensors, impedimetric immunosensor is characterized by label-free, simple, time saving, requiring no special reagents, and driving at lower voltage [7-9]. However, some further research work should be done to improve the sensitivity and understanding of the interfacial model of biomolecular recognition for its meaningful application.

The stable immobilization of antibodies or antigens on the electrode with complete retention of their biological recognition properties is a crucial step for successful construction of immunosensors [10-11]. One effective solution is using electropolymerized film based immobilization technique, which allows direct deposition of a polymer film over the electrode surface with different sizes and geometries followed by biomolecules immobilization [12-13]. P*o*PD (poly-o-phenylenediamine) ultrathin layer of self-limiting thickness could easily be obtained on different conducting substrates, which is helpful for the amino and imino groups to protrude to bind antibody or antigen more stably, and benefical for the regeneration of biosensor [14-16].

In this article, an ultrasensitive, impedimetric immunosensor with good reproducibility for Immunoglobulin G (IgG) based on electropolymerization P*o*PD film modified gold electrode was developed. Our work focused on the development of an antigen-antibody reaction based EIS ultrasensitive quantitative detection system, including not only the fabrication of an electrochemical immunosensor but also and a reasonable electrochemical interfacial model of biomolecular recognition.

2. EXPERIMENTAL PART

2.1. Apparatus and Reagents

Rabbit IgG (dry power) and goat anti-rabbit IgG (purification) were obtained from Beijing Solarbio Science & Technology Co., Ltd.. Bovine serum albumin (BSA), Glutaraldehyde (GA) (50%) aqueous solution and o-phenylenediamine were purchased from Sangon Biotech Co. (Shanghai), Ltd. Piranha solution (a mixture of H_2SO_4 98% and H_2O_2 30% , V/V=3:1) was used to clean the surface of gold electrode. All the reagents used were analytical Reagents. Double-distilled water was used throughout this study.

Electrochemical measurements were performed with IM6eX (ZANHER-Elektrik GmbH & Co.KG, Germany) and Epsilon2000 (BAS Company, USA) electrochemical workstation. P200 adjustable pipette (Dragon Company, Finland) and Pipetman P2 adjustable pipette (Gilson Company, France) were used to transfer trace solution.

2.2. The Development of PoPD Modified Electrode

The working electrode which was polycrystalline gold disk of 3 mm in diameter was polished successively with wet alumina powder (0.3 and 0.05 μm) to mirror-like, followed by cleaning in a piranha solution for 10 min, then rinsing with ethanol and water in an ultrasonic bath for 5 min in sequence. The polished electrode then was cleaned by voltammetrically cycling, between -0.1 and 1.2 V *vs.* SSE at a scan rate of 50 mV/s in 0.5 mol/L H_2SO_4 until a stable cyclic votammogram was obtained.

The electropolimization of P*o*PD film on gold electrode was carried out in a nitrogen-purged aqueous electrolyte solution containing 0.05 mol/L monomer and 1 M H_2SO_4 by cycling voltammetry, the potential between -600 and 1000 mV versus SSE, scan rate 50 mV/s. A thin film with 30 deposition cycles was used in all experiments.

2.3. Preparation of IgG Immunosensor

Immunosensor was made by optimized steps and parameters in following sequences: activation of P*o*PD/Au for 60 min, immobilization of IgG antibody for 40 min and bloking non-specific sites for 30 min at 33.0℃. After every step, the electrode was washed with water and then aired at room temperature.

2.4. IgG Detection

The immunosensor was incubated in IgG diluent with different concentration for 30 min, washed with and then put into PBS solution. EIS was carried out with conventional three-electrode systems, BSA/anti-IgG/P*o*PD/Au as working electrode, platinum as the counter and Ag/AgCl electrode as the reference. The DC potential was -200 mV (*vs*. Ag/AgCl), the AC potential 5 mV, and frequencies ranging from 100 kHz to 0.1 Hz.

3. RESULTS AND DISCUSSION

3.1. Evaluation of Immunosensor

Fig. 1A shows cyclic voltammograms of Au and PoPD/Au electrode in $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ solution. There was no red-ox peaks appeared at P*o*PD/Au electrode, indicating P*o*PD hindered the electron transfer of the red-ox couple $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ at P*o*PD/Au electrode. This result was in coincidence with the results got from EIS, shown in Fig. 1B. The Nyquist plots shows that the electrode processes were controlled by charge transfer at higher frequencies and diffusion dominant at lower frequencies at both the Au and P*o*PD/Au electrodes. Electron transfer resistance R_{ct} at P*o*PD/Au electrode was much larger, indicating the charge transfer was more difficult at P*o*PD/Au than that at Au electrode.

Fig. 2 shows the Nyquist plots of the assembling of immunosensor at different stage in PBS solution. The electrochemical impedances increased continually. This could be ascribed to the blocking layer coating on electrode surface, rowing thicker and thicker with the assembly procedure. The access of H^+ was hindered and the charge transfer resistance increased accordingly. In addition, we observed that the result was not stable if the redox couple $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ was used. This might be ascribed to the fact that the redox couple could reduce the activity of the protein molecules.

Jun Yano [17] proposed that the backbone of elctropolymerized P*o*PD obtained in sulfuric acid solution was 1,4-substituted benzenoid-quinoid structure through the study of ^{1}H-FTNMR, FTIR spectra and the elemental analysis. In acid solution, one amino-group of OPD can be protonized to $-NH_3^+$. The possible reaction mechanism of assembly procedure was suggested, shown in Fig. 3.

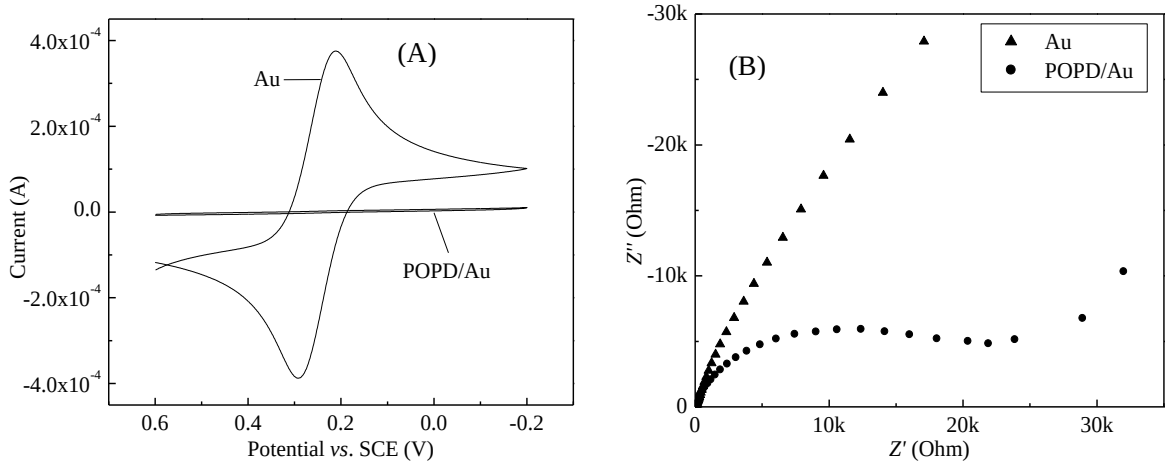

Figure 1. (**A**) Cyclic voltammograms of Au and PoPD/Au electrodes in 20 mmol/L $K_3Fe(CN)_6$, 20 mmol/L $K_4Fe(CN)_6$ + 0.5 mol/L KCl solution at scan rate 50 mV/s. (**B**) Nyquist plots for Au, PoPD/Au electrode in the same solution as above measured at fixed DC potential 0V (*vs*. SCE) with amplitude 5 mV. The frequency range is 100 KHz ~ 10 mHz.

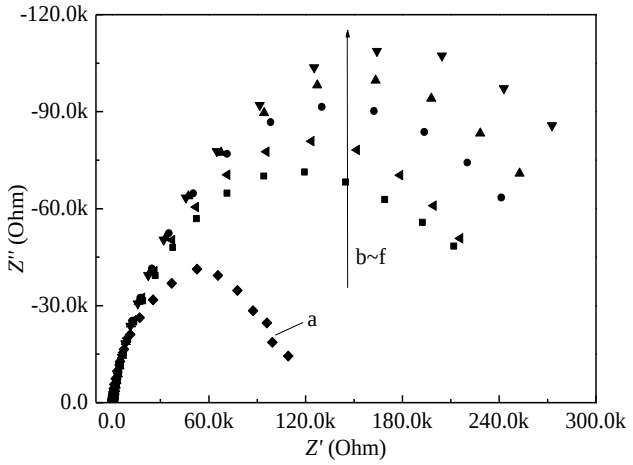

Figure 2. Nyquist plots of immunosensor at different processes, (a)Au, (b)P*o*PD/Au, (c)GA-P*o*PD/Au, (d)Ab/GA-P*o*PD/Au, (e)BSA-Ab/GA-P*o*PD/Au, (f) Ag/BSA-Ab/GA-P*o*PD/ Au.

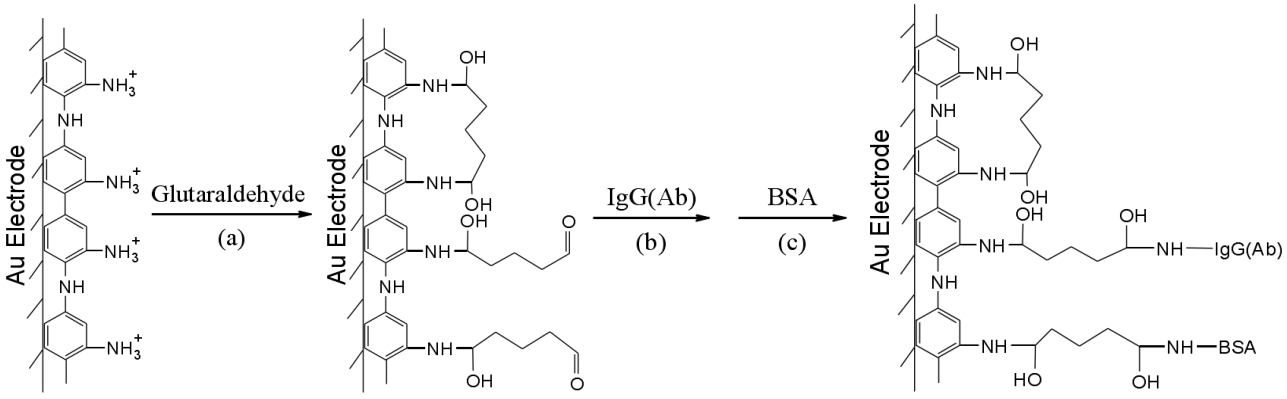

Figure 3. Schematic diagram for reaction mechanism during immunosensor assembly procedure.

3.2. Study of Interfacial Modeling of Biomolecular Recognition

The software ZsimpWin was employed for simulation of the EIS response. Combined with the possibly reaction mechanism and simulation results, we inferred that the possible equivalent circuit would be R(C(R(QR$_p$)))(CR), shown in Fig. 4. The fitting effect of this equivalent circuit was shown in Fig. 5A Nyquist plots and Fig. 5B Bode plots.

As shown in Fig. 4, C_1 stood for the interfacial capacity element between electrode surface and solution; R_1 was the resistance caused by H^+ moving from solution to electrode surface; Q was a constant phase angle element related to capacitance (for the calculated results "n" was nearly 1) between P*o*PD polymer film and solution; R_p represented electron transfer impedance of H^+ reacting on the polymer film surface; C_{PoPD} and R_{PoPD} were capacitance and resistance element that related to the P*o*PD film.

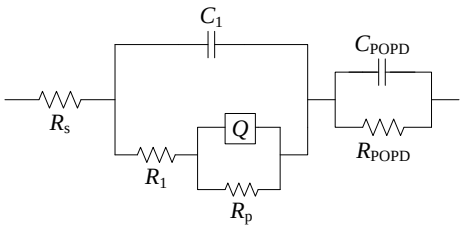

Figure 4. Equivalent circuit of interface modeling of biomolecular recognition for the immunosensor.

3.3. Quantitative Detection of IgG

The Nyquist plots for different IgG concentrations ranging from 0 ng/mL to 1000 ng/mL was presented in Fig. 5. The EIS results could be quantitatively analysed by using the software ZsimpWin according to the equivalent circuit $R(C(R(QR_p)))(CR)$.

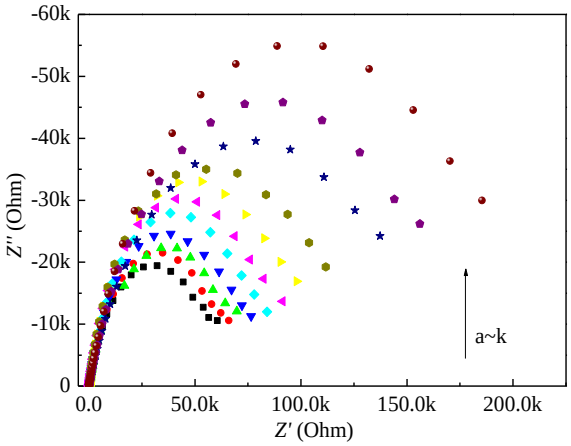

Figure 5. Nyquist plots for Ag/BSA-Ab/GA-P*o*PD/Au electrode after incubating the immunosensor in IgG solution with concentration of 0 (a), 0.1 (b), 1 (c), 3 (d), 5 (e), 7 (f), 10 (g), 60 (h), 450 (i), 750 (j), 1000 (k) ng/mL, respectively.

The calibration plots (Fig. 6) were got from the relationship between the IgG concentrations and ΔR_p, the difference values of electrochemical reaction impedances (R_p) before and after immunoreaction. The piecewise linear relationship can be seen from Fig. 6A and B.

As shown in Fig. 6A and B, there were two good linear relationships between ΔR_p and IgG concentration in the ranges of 0.1~10 ng/mL (correlation coefficient r = 0.9933) and 10~1000 ng/mL (correlation coefficient r = 0.9975), respectively. The detection limit was 0.05 ng/mL (s/n = 3). It was much lower than those of related studies which were mostly around 1 ng/mL [18-19].

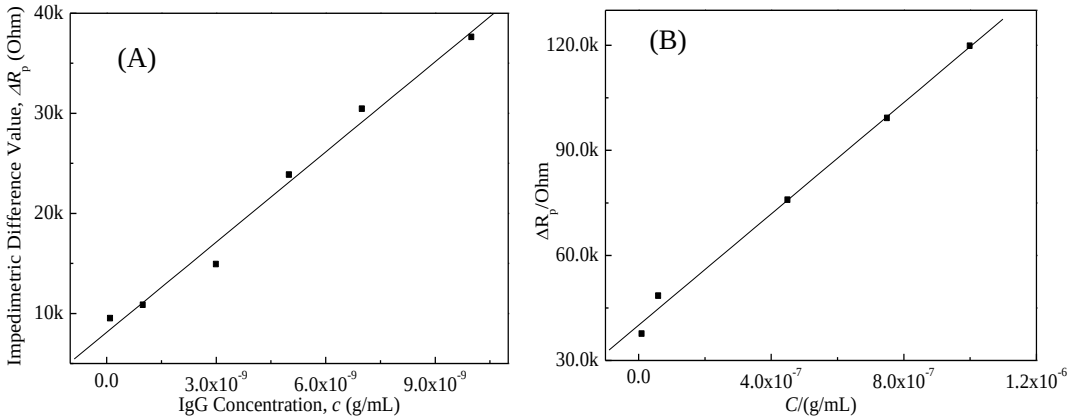

Figure 6. (A) Calibration plot for IgG concentration ranging from 0.1 to 10 ng/mL. (B) Calibration plot for IgG concentration ranging from 10 to 1000 ng/mL.

3.4. Capabilities of Immunosensor

In immunochemistry, acid, alkali and some highly ionic buffers with certain concentration can be used to dissociate the antigen-antibody complex to make immunosensors reusable. In this study, 0.1 mol/L H_3PO_4, 0.1 mol/L NaOH, 4 mol/L Urea, and 0.2 mol/L HCl + 0.2 mol/L glycine buffer solution (pH 2.80) were tried for dissociation agents. Urea could not dissociate antigen completely, strong acid, strong alkali made protein molecules inactive and denatured.

Table1. Reproducibility of The Immunosensor

IgG Concentration (ng/mL)	Number of Detection					RSD (%)
	1	2	3	4	5	
10	105.8	105.6	103.0	106.9	105.1	1.36
100	113.5	115.6	114.5	115.7	112.8	1.11
1000	121.9	120.6	124.4	124.5	122.3	1.37

While HCl+Glycine buffer solution was found to have best dissociation effect and could make the immunosensor be reused for more than 25 times.

Reproducibility of the immunosensor was tested by measuring IgG standard solution with different concentration 10, 100 and 1000 ng/mL for 5 times respectively. Impedimetric response values and RSDs of normalized signals have been shown in Table 1. It can be seen from Tab. 1 that the maximum RSD (n=5) of impedimetric response was less than 1.37%. This indicated that the label-free impedimetric immunosensor constructed in this study has good reproducibility.

4. CONCLUSIONS

A label free ultrasensitive impedimetric immunosensor for IgG was fabricated by immobilizing goat anti-rabbit IgG onto P*o*PD electropolymerized film modified gold electrode by glutaraldehyde cross-linking. PBS (pH 7.20) solution without additional redox couple was satisfactory for the probing. The work on the impedimetic immunosensor will not only determine its application for IgG detection, but also will aid in understanding the mechanism and electrochemical interfacial modeling of biomolecular recognition of electrochemical immunosensor. Our further work will concentrate on its application in environmental monitoring and food detection.

ACKNOWLEDGEMENTS
This work was supported financially by National Natural Science Foundation of China(21273056), Innovative Scientific Research Group of Harbin Institute of Technology and Weihai Science and Technology Plan (2008-81).

References

1. Z. Y. Zhong, W. Wu, and D. Wang, et al., *Biosensors and Bioelectronics*, 25(2010), 2379.
2. K. Kerman, N. Nagatani, M. Chikae, T. Yuhi, Y. Takamura, and E. Tamiya. *Anal. Chem.*, 78 (2006) 5612.
3. O. A. Sadik, M. Jeanette, and Van Emon, *Biosensors and Bioelectronics*, 11 (1996) 1.
4. J. Parellada, A. Narvaez, M. A. Lopez, E. Dominguez, J. J. Fernandez, V. Pavlov, and I. Katakis, *Analytica Chimica Acta*, 362 (1998) 47.
5. A. G. Mantzila, V. Maipa, and M. I. Prodromidis, *Anal. Chem.*, 80 (2008) 1169.
6. L. Micheli, R. Grecco, R. Badea, D. Moscone and G. Palleschi, *Biosensors and Bioelectronics*, 21 (2005) 588.
7. J. S. Daniels, and N. Pourmand, *Electroanalysis*, 19 (2007) 1239.
8. I Ciani, H Schulze, D K. Corrigan, G Henihan, A R Mount, et al., *Biosensors and Bioelectronics*, 31(2012)413
9. A Anita, O Kobra, S Hanieh, et al., *Clinical Biochemistry*, 44(2011)S16
10. R. E. Ionescu, N. Jaffrezic-Renault, L. Bouffier, C. Gondran, S. Cosnier, D. Pinacho, M. Marco, F. J. Sanchez-Baeza, T. Healy, and C. Martelet, *Biosensors and Bioelectronics*, 23 (2007) 549.
11. B. Corrya, J. Uilkb, and C. Crawley, *Analytica Chimica Acta*, 496 (2003) 103.
12. J. M. Fowler, M. C. Stuart, and D. K. Y. Wong, *Anal. Chem.*, 79 (2007) 350.
13. S. Cosnier, *Biosensors and Bioelectronics*, 14 (2009) 443.
14. B. Liu, L. S. Lu, Q. Li, et al., *Microchimica Acta*, 173(2011)513
15. Z. Liu, S. Huang, D. C. Jiang, B. H. Liu, and J. L. Kong, *Analytical Letters*, 37 (2004) 2283.

16. R. Mazeikiene, and A. Malinauskas. *Synthetic Metals*, 128 (2002) 121.
17. X. G. Li, X. L. Ma, and M. R. Huang. *Chemical sensors*, 28 (2008) 15.
18. Yano J, *Chem. Soc. Faraday Trans*, 33 (1995) 2435.
19. J. H. O. Owino, A. Ignaszak, A. AI-Ahmed, P. G. L. Baker, H. Alemu, J. C. Ngila, and E. I. Iwuoha, *Anal. Bioanal. Chem.*, 388 (2007) 1069.
20. R. Khan, and M. Dhayal, *Biosensors and Bioelectronics*, 24 (2009) 1700.