



Improved Sensitivity of a Glucose Sensor by Encapsulation of Free GOx in Conducting Polymer Micropillar Structure

Shinhwan Jung^a, Youngkwan Lee^b, and Yongkeun Son^{a,†}

^aDepartment of Chemistry and BK21 School of Chemical Materials Science

^bDepartment of Chemical Engineering, Sungkyunkwan University, Suwon 440-746, Korea

ABSTRACT:

A simple process of fabricating micropillar structure and its influence upon enhancing electrochemical biosensor response were studied in this work. Conducting polymer PEDOT was used as a base material in formulating a composite with PVA. Micro porous PC membrane filter was used as a template for the micropillar of the composite on ITO electrode. This structure could provide plenty of encapsulating space for enzyme species. After dosing enzyme solution into this space, Nafion film tent was cast over the pillar structure to complete the micropillar cavity structure. In this way, the encapsulation of enzyme could be accomplished without any chemical modification. The amount of enzyme species was easily controllable by varying the concentration of the dosing solution. The more amount of enzyme is stored in the sensor, the higher the electrochemical response is produced. One more reason for the sensitivity improvement comes from the large surface area of the micropillar structure. Application of 0.7 V produced the best current response under the condition of pH 7.4. This biosensor showed linear response to the glucose in 0.1~1 mM range with the average sensitivity of 14.06 $\mu\text{A}/\text{mMcm}^2$. Detection limit was 0.01 mM based on $S/N = 3$.

Keywords: Glucose biosensor, Micropillar structure, Conducting polymer composite, PEDOT, Capillary action

Received June 13, 2011 : Accepted June 27, 2011

1. Introduction

Glucose is a fundamental carbohydrate in biology. It is one of the main products of photosynthesis and serves as the primary source of energy in human body. When the glucose level in the bloodstream is not properly regulated, diseases such as diabetes can develop.¹⁾ The glucose biosensor, one of the most commonly used biosensors, have been widely studied and developed in the field of clinical detection, biological analysis, environmental monitoring, and food processing.^{2,3)}

Glucose oxidase (GOx) is a homodimer with a molecular weight of about 150~180 kDa containing two tightly

bound flavine adenine dinucleotide (FAD) cofactors and catalyzes the electron transfer from glucose to oxygen accompanying the production of gluconic acid and hydrogen peroxide. The electrochemical biosensing has its origins in a report in 1962 by Clark and Lyons that describes a potentiometric measurement coupled with the enzyme glucose oxidase to determine glucose in blood plasma. This report also introduced the concept of coupling an enzyme with the signal transduction in the creation of a sensor.⁴⁾ Since the first glucose biosensor reported, much effort has given to improve the sensitivity and the stability of such enzyme-based sensors. The immobilization of enzymes on electrodes and the optimization of biosensors have attracted great interests. The techniques for the enzyme immobilization include covalent attachment to the electrode surfaces,^{5,6)} entrapment by ion-exchange

[†]Corresponding author. Tel.: +82-31-290-7068
E-mail address: ykon@skku.edu

polymers,^{7,8)} conducting polymers, and nonconducting polymers,⁹⁻¹³⁾ sol-gels^{14,15)} and cross-linking in bovine serum albumin-glutaraldehyde.^{16,17)} These reports show that the most important thing in fabricating an electrochemical biosensor is the enzyme encapsulation method which can produce the best signal transducing. From this idea, a good encapsulation should be a method can provide sufficient amount of free enzyme without any modification near the good electrochemical signal transducing electrode. The active electrode area for amperometric detection should be large.

We have considered that conducting polymer micropillar structure can provide the ideal encapsulating structure for the biosensor systems. Because it offers ample space for free enzyme after pitching a covering film and behaves as an electrochemical signal transducer simultaneously. In this work, we tried to show a simple enzyme encapsulating method which confers reasonable sensitivity. The microstructure could be fabricated simply by using capillary action of PEDOT composite. After dosing enzyme into the space between micropillars, the space was masked with Nafion tent to encapsulate the enzyme. To improve the sensitivity, platinum sputtering was done on the pillars structure. The schematic diagram of glucose sensing in this system is shown in Fig. 1. This system can modulate the quantity of enzyme just by loading different concentration of enzyme solution before Nafion coating on the pillars.

2. Experimental

2.1. Materials & equipments

Aqueous dispersion of Poly(ethylenedioxythiophene)/Poly(styrene sulfonate) (PEDOT/PSS, Baytron P 4083)

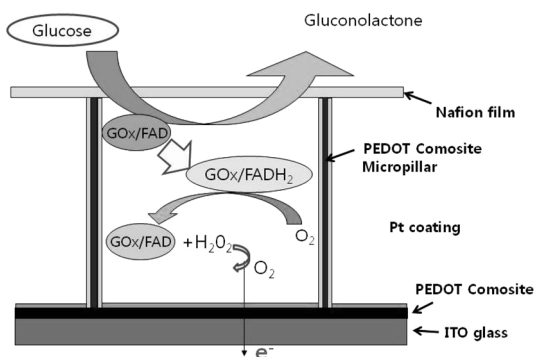


Fig. 1. Schematic diagram of the glucose sensor reaction in Nafion coated micropillar cavity.

was purchased from Bayer and used as a basic material for the conducting polymer composite. PVA (polyvinyl alcohol) was gratefully donated by OCI(Korea). Glucose oxidase (type II-S from *Aspergillus Niger*; ~20% protein; balance primarily potassium gluconate, 21200units/g), D-(+)-glucose, L-ascorbic acid(99%), uric acid(99%) and acetaminophen(98%) were obtained from Sigma-Aldrich. Glucose oxidase solutions were freshly prepared with a PBS solution every 2 weeks. Phosphate buffered saline (PBS) solution consisted of 0.1 M Na_2HPO_4 , 0.1 M NaH_2PO_4 and 0.15 M NaCl and was adjusted to pH 7.4 with 3 M NaOH. Nafion perfluorinated ion-exchange resin(5 wt% solution in a mixture of lower aliphatic alcohols and water) was purchased from Aldrich also, and used as effect cover for the enzyme immobilization. The other chemicals used in this study were mostly of ACS grade. Polycarbonate membrane filters (Isopore) of 10 μm thick having 1.2 μm pores diameter came from Millipore and used as templates. The conducting Indium tin oxide(ITO) glass (Samsung Corning, Korea) was immersed in acetone for a day and then rinsed with distilled water immediately before use.

PEDOT composite were spin-coated onto a piece of ITO glass with a spin-coater (EC101DT, Headway Research, Inc., USA) equipped with a rotary vacuum pump. Pt was applied with a sputter Crossington model 108. All electrochemical potentials in this study were based on an Ag/AgCl(Sat'd KCl) reference electrode. Pt sputtered micro-tubules structure was examined using a field emission scanning electrode microscopy (FESEM, JSM 6700F, JEOL, Japan). The electrochemical measurements were performed using a potentiostat/galvanostat (EG&G M263A).

2.2. Fabrication of enzyme sensor electrode

The sensor preparing scheme is shown in Fig. 2. The conducting polymer composite was prepared by mixing with PEDOT/PSS and PVA. This homemade conducting composite (PEDOT/PSS + PVA) was spin coated onto an ITO glass with spinning rate of 6000 rpm. The conducting composite has two roles, providing space for storing enzyme and making the good electrical connection between the ITO electrode and the sensing system. Later, a piece of PC membrane was fixed on the PEDOT composite film coated onto ITO glass before the composite dried out. The pores of the PC template were filled with PEDOT composite by the capillary action. When the composite had completely dried, the PC template was removed by treating with methylene chloride. Formation of the

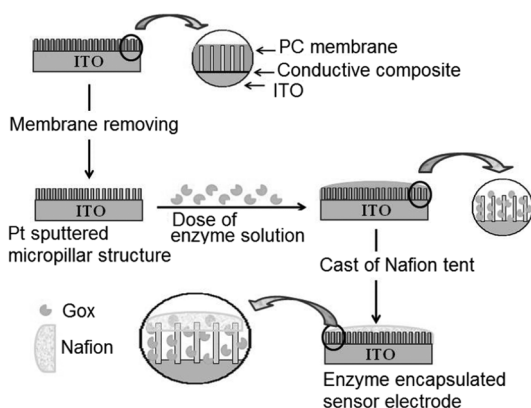


Fig. 2. Schematic illustration of fabricating a glucose sensor adopting micropillar structure to immobilize enzyme species.

composite tubule was examined by using a SEM. Glucose oxidase solution (2000 unit/ml) was loaded onto the conducting polymer composite pillar structure by using a 10 μ l microsyringe. The active area of the enzyme electrode was determined by an O-ring (4 mm diameter). The electrode was placed in a refrigerator to dry for 12 h. After this process, 0.5 wt% Nafion was coated by dropping 10 μ l directly onto the enzyme electrode.

2.3. Electrochemical measurements

Voltammetric measurements were performed by using a one-compartment cell. The cell consisted of a Teflon cell holding an O-ring joint at one end. The enzyme loaded ITO was served as the working electrode and its active area was confined with the O-ring. The cell was described elsewhere.¹⁸⁾ Measurements were carried out in a 10 mL PBS solution (pH 7.4) under steady-state condition. After the background current was stabilized, 0.1 M glucose stock solution was injected into the cell to increase the glucose concentration by 0.1 mM gradually. All amperometric measurements were done at room temperature.

3. Results and Discussion

The commercial track-etched membrane filter show two different sides, a shiny side and a rough (flat). Pores are distributed in random fashion but each pore has two different diameters. The one appeared on shiny side is smaller than the one came out on dull side. The rough side was attached to the ITO using PEDOT composite. SEM image of the resulting micro structure is appeared in Fig. 3. The SEM image was taken after removing the polycarbonate template membrane. The microstructure

was formed with PEDOT composite by the capillary action through the pores in template. One can easily recognize plenty of free space between pillar structures. The 0.5 wt% Nafion solution was formed by the Nafion film on top of the conducting polymer micropillars. SEM image of the final Nafion covered sensor electrode is in Fig. 4. It shows the cross-sectional image of Nafion film on top of the PEDOT microstructure. The space created by the micropillar and the cover up Nafion was used as the enzyme encapsulating space.

The effect of applied potential on the amperometric response to the final glucose biosensor was studied. Fig. 5 shows changes in current response after consecutive addition of glucose to the testing solutions under different

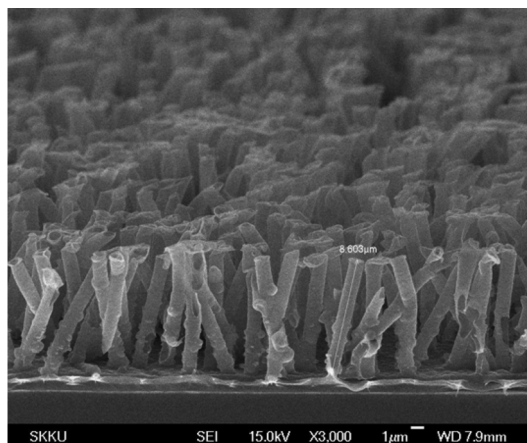


Fig. 3. SEM images of the template micropillar structure after removing the template.

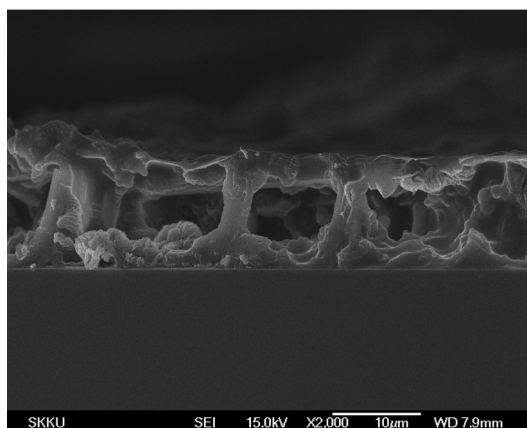


Fig. 4. Cross sectional SEM images of the micropillar cavity structure after casting of Nafion film. Enzyme species is encapsulated in this space.

applied potentials. Each responding current curve shows the current value increases as a function of the glucose concentration in the range from 0.1 mM to 1 mM. The maximum response was observed when the applied potential was 0.7 V as in other studies.^{19,20} This response is due to the oxidation of hydrogen peroxide generated from enzymatic reaction.

The electrochemical response of the biosensor is also dependent upon the amount of loaded enzyme species. Fig. 6 shows the response variation of the sensor. Enzyme loading solutions of different concentration 500, 1000, 1500, 2000 and 4000 unit/ml were used to fill up the microstructure. When the sensor electrodes were filled with solutions of 500, 1000, 1500 unit/mL their response curves are not linear and show inflections at concentrations lower than 0.4 mM. This could be explained as an early saturation effect at the concentration lower than 0.4 mM. Because the amount of the enzyme loaded was not sufficient to react all of the glucose substrate diffused into the reaction space.

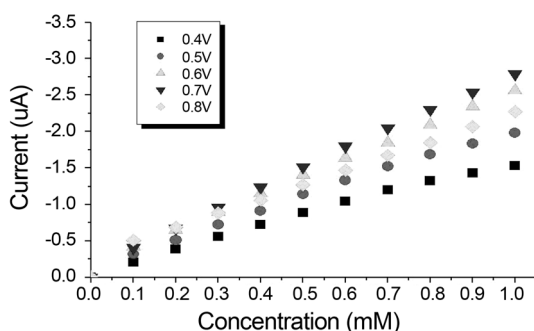


Fig. 5. Amperometric current responses of the biosensor to a successive addition of 0.1 mM glucose from 0.4V to 0.8V applied potential.

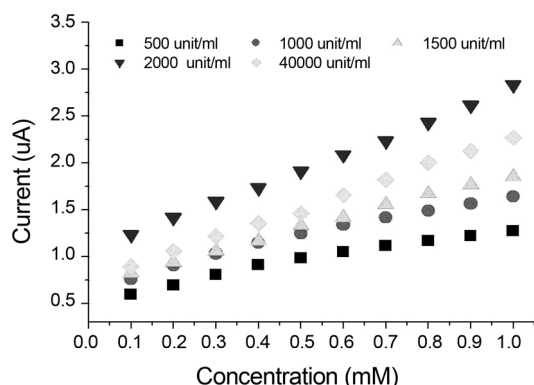


Fig. 6. Responses of the sensor electrodes prepared with different concentration of enzyme solution.

As the enzyme concentration increase, the sensor filled with 2000 unit/mL glucose oxidase solution provided the highest most detection signals in the range of 0–1.0 mM. On the other hand the sensor filled with the enzyme solution of 4000 unit/ml was giving lower signal response. This behavior may be due to the retardation of charge transport by the adsorbed enzyme species on the sensing electrode surface.

The amperometric responses of the biosensor to the random injection of glucose are presented in Fig. 7 to obtain a calibration curve. In this test, several solutions of different concentration were injected to the test solution of PBS. Total of ten times injection were done to test the responding activity of the sensor. The changes in responding current was so fast and stay constant after responding appeared in (a). Calibration curve was obtained as in (b). The calibration curve shows good linear relationship between substrate concentration and sensor response in spite of the random injection. The calibration curve implies this biosensor has linear response to the glucose in 0.11 mM range with the average sensitivity of $14.06 \mu\text{A}/\text{mMcm}^2$. The detection limit was 0.01 mM considering signal to noise ratio was three. This sensitivity

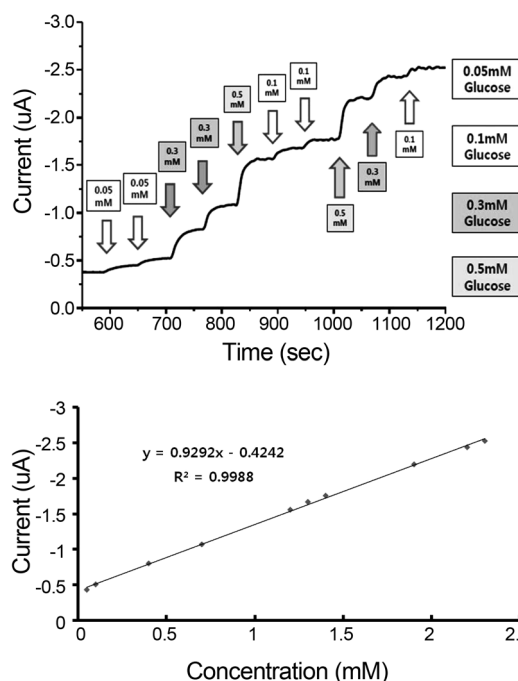


Fig. 7. Amperometric response of the prepared electrode to random injection of glucose concentration.

Table 1. Comparison of glucose sensors based on different immobilization and sensitivity

Fabrication method	Sensitivity	Reference
This work	14.06 $\mu\text{A}/\text{mM}\cdot\text{cm}^2$	
Covalent attachment of glucose oxidase to an Au electrode modified with gold nanoparticles	8.8 $\mu\text{A}/\text{mM}\cdot\text{cm}^2$	[24]
In situ immobilization of glucose oxidase in chitosan–gold nanoparticle hybrid film on Prussian Blue modified electrode	69.26 $\mu\text{A}/\text{mM}\cdot\text{cm}^2$	[21]
Enhanced Sensitivity of a Glucose Sensor Adopting Polymer Microtubule	9.33 $\mu\text{A}/\text{mM}\cdot\text{cm}^2$	[19]
Multilayered construction of glucose oxidase and gold nanoparticles on Au electrodes based on layer-by-layer covalent attachment	5.72 $\mu\text{A}/\text{mM}\cdot\text{cm}^2$	[25]
Controlled multilayer films of sulfonate-capped gold nanoparticles/thionine used for construction of a reagentless bienzymatic glucose biosensor	3.8 $\mu\text{A}/\text{mM}\cdot\text{cm}^2$	[26]

is compared to those of other methods produced in Table 1. The sensitivity appeared in this work shows 1.5~3.7 times greater than other work produced except the one provided by Xue *et al.*²¹⁾ The highest sensitivity in the table came from a sensor fabricated via complicated method. In this work we have introduced a possibility of building a simple glucose sensor with reasonably high sensitivity by using a one-step fabrication of conducting polymer micropillar structure.

In real blood, there are many of interference species and their concentrations are to be below 1/50 of glucose. But they usually produce large current compare to the glucose because their charge transport speed was higher than that of glucose.^{22,23)} In this study, the Nafion solution used as encapsulation effect was prepared by dropping on the surface of conducting polymer micropillar structure to remove interference effects. Fig. 8 shows the amperometric response of the electrode to the consecutive addition of (a) 5 mM glucose, (b) 0.1 mM ascorbic acid, (c) 0.1 mM uric acid and (d) 0.1 mM acetaminophen to the measuring cell. These results indicate that the Nafion film can efficiently avoid the interferences of the ascorbic acid, uric acid, acetaminophen.

4. Conclusions

An attractive amperometric biosensor has been introduced, based on the immobilization of glucose oxidase into the encapsulating micropillar structure on ITO base electrode. As we have shown, this glucose biosensor electrode is very easy to fabricate, producing high sensitivity and performing good selectivity. The conducting polymer micropillar structure has roles of increasing the electrode surface area, and providing ample space for encapsulating free enzyme species. A thin layer of Nafion used as encapsu-

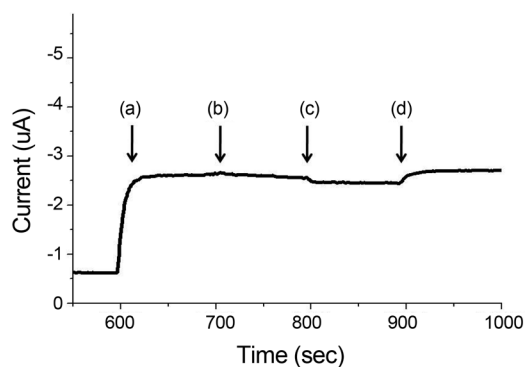


Fig. 8. Current response of interference tests in the prepared biosensor. (a) 5 mM glucose, (b) 0.1 mM ascorbic acid, (c) 0.1 mM uric acid and (d) 0.1 mM acetaminophen.

lation was providing the anti-interferent ability. This biosensor has a wide applicability by just changing the enzyme to be loaded.

Acknowledgements

This work was supported by the Energy & Resource Recycling of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Ministry of Knowledge Economy, Republic of Korea (No. 2010501010002B).

References

1. M. H. Asif, S. M. Usman Ali, O. Nur, M. Willander, C. Brnnmark, P. Strlfors, U. H. Englund, F. Elinder and B. Danielsson, *Biosens. bioelectron.*, **25**, 2205 (2010).
2. N. A. Rakow and K. S. Suslick, *Nature*, **406**, 710 (2000).
3. R. W. Keay and C. J. McNeil, *Biosens. Bioelectron.*, **13**, 963 (1998).

4. L. C. Clark and C. Lyons, *Annals of the New York Academy of Sciences*, **102**, 29 (1962).
5. K. Narasimham and L.B. Wingard, *Anal. Chem.*, **58**, 2984 (1986).
6. Y. Degani and A. Heller, *J. Am. Chem. Soc.*, **110**, 2615 (1988).
7. G. Firtier, M. Vaillancourt and D. Belanger, *Electroanalysis*, **4**, 275 (1992).
8. F. Mizutani, S. Yabuki and T. Katsura, *Anal. Chim. Acta*, **274**, 201 (1993).
9. G. F. Khan, E. Kobatake, Y. Ikariyama and M. Aizawa, *Anal. Chim. Acta*, **281**, 527 (1993).
10. M. Shaolin, *J. Electroanal. Chem.*, **370**, 135 (1994).
11. Z. Zhang, W. Bao and C. Liu, *Talanta*, **41**, 875 (1994).
12. J. Q. Kan, F. Zhou, S. L. Mu AND Y. J. Shi, *Sens. Actuators B*, **30**, 7 (1996).
13. M. Castillo-Ortega, D. Rodriguez, J. Encinas, M. Plascencia, F. A. Mendez-Velarde and R. Olayo, *Sensors and Actuators B*, **85**, 19 (2002).
14. Z. J. Liu, B. H. Liu, J. L. Kong and J. Q. Deng, *Anal. Chem.*, **72**, 4707 (2000).
15. B. Q. Wang, B. Li, Z. X. Wang, G. B. Xu, Q. Wang and S. J. Dong, *Anal. Chem.*, **71**, 1935 (1999).
16. M. Mascini, M. Iannello and G. Palleschi, *Anal. Chim. Acta*, **146**, 135 (1983).
17. L. T. Jin, J. S. Ye, W. Tong and Y. Z. Fang, *Mikrochim. Acta*, **112**, 71 (1993).
18. J. Park, H. Kim and Y. Son, *Sens. Actuators B*, **133**, 244 (2008).
19. M. Lee, Y. Son, J. Park and Y. Lee, *Mole. Cryst. Liq. Cryst.*, **492**, 155/[519] (2008).
20. Y. -Q and K. -K. Shiu, *Electroanalysis*, **16**, 1806 (2004).
21. M. H. Xue, Q. Xu, M. Zhou and J. J. Zhu, *Electrochem. Commun.*, **8**, 1468 (2006).
22. T. Chung, S. Park and H. Kim, *Phys. & High Tech.* **24**, Oct (2004).
23. C. Chen, K. Ishihara, N. Nakabayashi, E. Tamiya and I. Karube, *J. Biomed. Microdevices* **1:2**, 155 (1999).
24. S. Zhang, N. Wang, H. Yu, Y. Niu and C. Sun, *Bioelectrochemistry*, **67**, 15 (2005).
25. W. Yang, J. Wang, S. Zhao, Y. Sun and C. Sun, *Electrochem. Commun.*, **8**, 665 (2006).
26. Y. Sun, Y. Bai, W. Yang and C. Sun, *Electrochim. Acta*, **52**, 7352 (2007).