Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 23, No. 2, 165-171, 2010

A glucose biosensor based on deposition of glucose oxidase onto Au nanoparticles poly(maleic anhydride)-grafted multiwalled carbon nanotube electrode

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(Received January 25, 2010; Accepted February 11, 2010)

금 나노입자/폴리(maleic anhydride) 그래프트 탄소나노튜브에 글루코스 옥시다아제 담지를 기반으로 한 글루코스 바이오센서

박명화 · 손평수 · 장주환★ · 최성호★

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(2010. 1. 25. 접수, 2010. 2. 11. 승인)

Abstract: Glucose oxidase (GOD_{ox}) immobilized biosensor was fabricated by two methods. In one of the methods, gold nanoparticles (Au-NPs) prepared by γ-irradiation were loaded into the poly(maleic anhydride)-grafted multi-walled carbon nanotube, PMAn-*g*-MWCNT electrode via physical entrapment. In the other method, the Au-NPs were prepared by electrochemical reduction of Au ions on the surface of PMAn-*g*-MWCNT electrode and then GOD_{ox} was immobilized into the Au-NPs. The GOD_{ox} immobilized biosensors were tested for electrocatalytic activities to sense glucose. The sensing range of the biosensor based on the Au-NPs physically modified PMAn-*g*-MWCNT electrode was from 30 μM to 100 μM for the glucose concentration, and the detection limit was 15 μM. Interferences of ascorbic acid and uric acid were below 7.6%. The physically Au deposited PMAn-*g*-MWCNT paste electrodes appear to be good sensor in detecting glucose.

요 약: 글루코스 옥시다아(GOx)제 고정화 바이오센서를 두 가지 방법으로 제조 하였다. 첫 번째 방법은 폴리(maleic anhydride) 그래프트 탄소나노튜브(PMAn-g-MWCNT) 전극에 감마선 조사법으로 제조 된 Au 나노입자를 물리적으로 흡착시킨 후, GOx을 고정화 시켜 바이오센서를 제조한 경우이고, 다른 하나는 PMAn-g-MWCNT 전극에서 Au 이온을 전기화학적으로 환원시켜 Au 나노입자를 코팅 시키고, 그 위에 GOx을 고정화 시켜 바이오센서를 제조 한 경우이두. 제조된 바이오센서에 대해 효율 평가를 수행 하였는데, 물리적 흡착법으로 제조된 전극의 경우 검출 범위는 30 μM~100 μM이었으며, 검출한계는 15 μM이었다. 또한 ascorbic acid와 uric acid에 대한 검출한계는 7.6%이었다. 물리적으로 Au 나노입자가 흡착된 전극의 경우가 글루코스 측정에 매우 우수한 전극임을 확인 하였다.

Key words: maleic anhydride, MWCNT, gold nanoparticles, glucose oxidase, radiation-induced graft polymerization

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1. Introduction

Owing to the unique properties of nanomaterials, direct electrochemistry and the catalytic activity of many enzymes has been observed at electrodes modified with various nanomaterials such as metal oxide nanoparticles, metal nanoparticles, carbon nanotubes, and others.[1-5] The sensitivity and performance of biosensors are being improved using nanomaterials in their construction. Various nanostructures have been examined as hosts for enzyme immobilization via approaches including protein adsorption, covalent attachment, enzyme encapsulation, and sophisticated combinations of methods. Nanomaterials can not only provide a support for the assembly of enzyme molecules, but can also enhance the electrical-transfer process between enzyme molecules and the electrode.

Many enzymes have been employed to prepare various kinds of biosensors using carbon nanotubes (CNTs).[6-10] Usually, enzymes are immobilized onto CNTs for physical adsorption[11] and covalent bonding.[12,13] In order to immobilize enzymes onto CNTs, a functional group is required because of the interaction between enzymes and the surface of CNTs. Radiation-induced graft polymerization (RIGP) is a beneficial method for introduction of functional groups into various polymer materials using specially selected monomers. There have been several reports about RIGP of polar monomers onto polymer subtracts to obtain hydrophilic properties for versatile applications.[14-16] The RIGP method can easily functionalize the surface of CNTs as desired. However, little has been reported about the functionalization of CNTs by RIGP.

The glucose sensor prepared using multiwalled carbon nanotubes (MWCNT) was prepared by the thin film method.[17,18] In order to prepare a thin film electrode, the MWCNT must be well dispersed in a polymer solution as the binder. However, MWCNT do not disperse well in polymer solution and therefore, many researchers have failed in preparing the thin film electrode using MWCNT. The MWCNT paste electrode can solve this problem for preparing biosensors.

It has been reported that a nanometer-sized colloidal gold particle can adsorb redox enzymes and proteins without any loss of their biological activity.[19-22] In addition, colloidal gold nanoparticles (Au-NPs) are widely used as a model system because of their ease of synthesis and surface modification,[20] good biocompatibility,[23] as well as their ability to act as tiny conduction centers which facilitate electron transfer.[24] Au-NPs have been produced in solution by radiation-induced reduction of Au ions as precursors without chemical reducing agents.[25,26] The species arising from the radiolysis of water, solvated electrons, e_{aq}^-, and H· atoms are the strongest reducing agents. They easily reduce Au ions producing Au nanoparticles.

In this study, we prepared the PMAn-*g*-MWCNT by radiation-induced graft polymerization (RIGP) of maleic anhydride (MAn) vinyl monomer in the presence of MWCNT in aqueous solution. The prepared PMAn-*g*-MWCNT were evaluated by TEM, and the amounts of carboxylic acid were also calculated by titration method. Then, the PMAn-*g*-MWCNT paste electrode was prepared by mixing PMAn-*g*-MWCNT and mineral oil, and packed in an acetal group connected to copper wire. The Au-NPs prepared by g-irradiation were loaded onto the PMAn-*g*-MWCNT electrode via physical entrapment in order to immobilize the enzyme. In the other method, the Au-NPs were prepared by electrochemical reduction of Au ions on the surface of the PMAn-g-MWCNT electrode and then glucose oxidase (GOD_{ox}) was immobilized onto the Au-NPs. Finally, the sensing efficiency of the biosensor based on the physical deposition of Au-NPs and electrochemical deposition of Au-NPs observed glucose by cyclic voltammetry. Interference effects of additive compounds for the assay of glucose were observed.

2. Experimental

2.1. Reagents

Maleic anhydride (MAn), glucose oxidase (GOD_{ox}), and glucose were purchased from Aldrich. MWCNT (95% pure, 10 nm in diameter, and 10 um in length)

were obtained from Hanwha Nanotech Co., Ltd (Korea). Mineral oil was obtained from Sigma-Aldrich Chemical Co., hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot nH_2O$) was obtained form Kojima Chemical Co., Ltd (Japan). Solutions for the experiments were prepared with water purified in a Milli-Q puls water purification system (Millipore Co. Ltd., the final resistance of water was 18.2 $M\Omega cm^{-1}$) and degassed prior to each measurement.

2.2. Preparation of the PMAn-g-MWCNT electrode

MWCNTs were purified to remove the catalyst and non-crystallized carbon impurities. MWCNTs were treated with a mixture, H_2SO_4/HNO_3=3/1(vol-%), and in the process, they were cut into shorter segments.[27] The purified and cut MWCNTs were used as the supporting materials for graft polymerization of MAn. The MWCNTs (0.3 g) and MAn (0.6 g) were mixed in an aqueous solution (250 mL). Nitrogen gas was bubbled through the solution for 30 min to remove oxygen gas, and the solution was irradiated by γ-ray from a Co-60 source under atmospheric pressure and ambient temperature. A total irradiation dose of 30 kGy (dose rate =1.0×10^4 Gy/h) was used. To the 20 mg of PMAn-*g*-MWCNT, 0.1 M NaOH 5 mL was added, and then stirred at room temperature for 5 h. The products were washed with HCl and then dried in vacuum in order to introduce of carboxylic acid group.

2.3. Fabrication of glucose sensor

Fig. 1 shows the preparation procedure of the glucose sensor by physical and electrochemical deposition. The 30 mg of the PMAn-*g*-MWCNT and 20 μL of mineral oil were mixed, and then packed in an acetal group tightly connected to copper wire. The electrochemically Au deposited PMAn-*g*-MWCNT paste electrode was obtained by electrochemical reduction of 1 mM $HAuCl_4$ with 500 s at -1000 mV. In another method, the Au-NPs prepared by γ-irradiation were deposited onto the PMAn-*g*-MWCNT paste electrode for 12 h.[28] Finally, the biosensors were prepared by adsorption of 3.0 mg. mL^{-1} GOD_{ox} solution onto the Au-NPs PMAn-*g*-MWCNT paste electrode for 12 h at 4 °C. The prepared GOD_{ox} biosensor was then refrigerated until use.

2.4. Instrumentation

Cyclic voltammetric and chronoamperometric experiments were performed with a Potentiostat/ Gavanostat model 283 (Ametek PAR, U.S.A). All

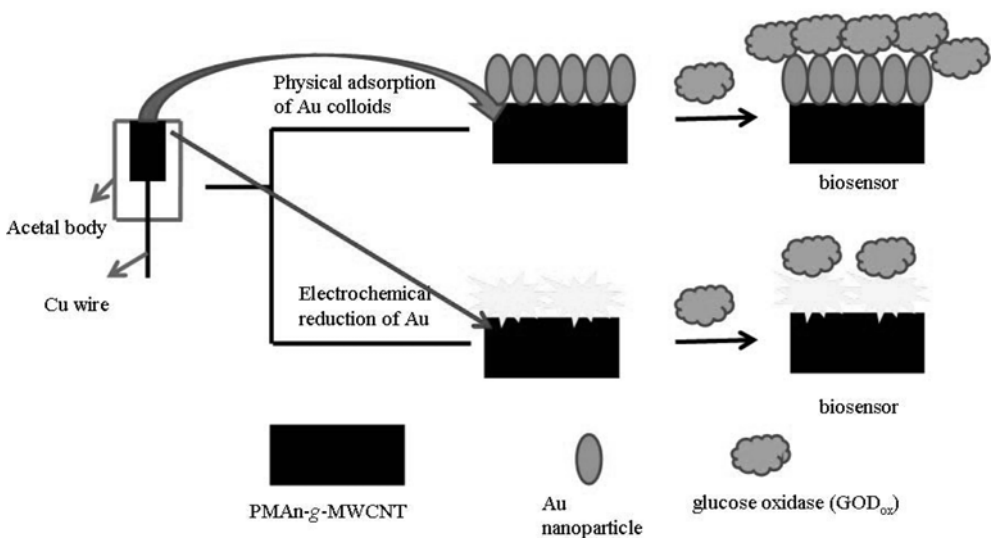

Fig. 1. Schematic fabrication pprocedure of glucose biosensor by physically and electrochemically deposition.

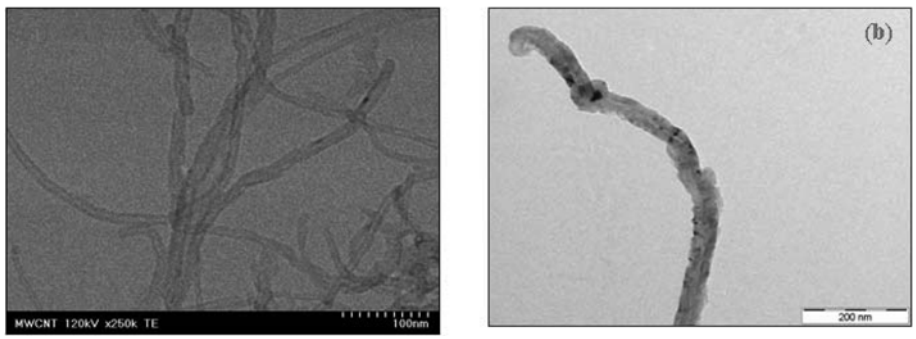

Fig. 2. TEM images of the purified MWCNT (a), and PMAn-*g*-MWCNT (b) prepared by γ-irradiation.

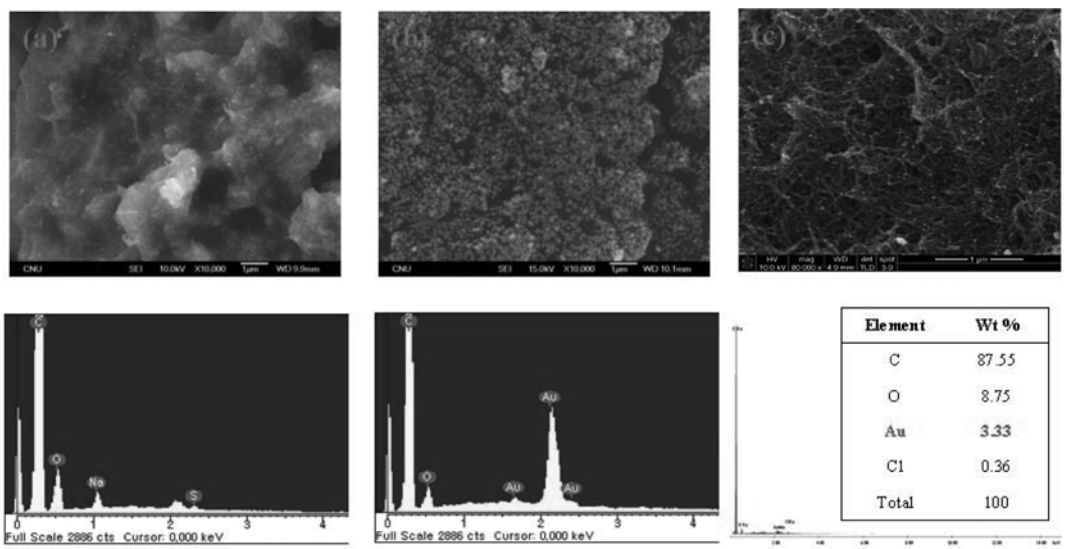

Fig. 3. SEM images of the PMAn-g-MWCNT paste electrode without Au-NPs (b), based on the electrochemically deposition of Au-NPs (b), and physically deposition of Au-NPs (c).

experiments were carried out with a conventional three-electrode system. The working electrode was the PMAn-*g*-MWCNT paste electrode (diameter: 2 mm). The counter electrode was platinum wire, and the reference electrode was Ag/AgCl (saturated KCl). The surface of the Au-NPs PMAn-*g*-MWCNT paste electrode was studied by scanning electron microscopy (SEM) (FE-SEM, JSM-7000F, JEOL Ltd., Japan). The Au-NPs PMAn-*g*-MWCNT was also evaluated by transmission electron microscopy (TEM) (Tecnai G2 Spirit, FEI Company, USA). Electron transfer resistance was also obtained by Impedance (IM6ex, PP240, ZAHNER electric, German).

3. Results and Discussion

Radiation-induced graft polymerization is a good method for introduction of functional groups onto various polymer materials using specially selected monomers. There have been several reports about RIGP of polar monomers onto polymer subtracts to obtain hydrophilic properties for versatile application.[16-18] The RIGP method can easily functionalize the surface of MWCNT. We performed the RIGP of MAn on the purified MWCNT in aqueous solution. We selected the MAn vinyl monomer because they could be mixed well with

mineral oil, and also they could be changed into the carboxylic acid group from the anhydride group by reaction with NaOH. In the TEM images (*Fig.* 2), we confirmed that PMAn was successfully grafted on the MWCNT. We reacted the 20 mg of PMAn-*g*-MWCNT and 5.0 mL of 0.1 M NaOH at room temperature for 5 h. The products were immersed in 0.01 M NaOH solution, and then the solution was titrated using 0.001 M HCl solution. The amount of carboxylic acid group of PMAn-*g*-MWCNT was 19.6 mg/g. The introduction of the carboxylic acid group onto PMAn-*g*-MWCNT was a very important factor in preparing the paste electrode because it induces hydrogen bonding with mineral oil, and as a result, the PMAn-*g*-MWCNT electrode was prepared.

In order to immobilize GOD_{ox} on the PMAn-*g*-MWCNT paste electrode, Au-NPs were deposited,as described in *Fig.* 1. *Fig.* 3 shows the SEM images of the without Au-NPs (a), with electrochemically deposited Au-NPs (b), and physically deposited Au-NPs onto PMAn-*g*-MWCNT (c). As shown in *Fig.* 3 (b), Au-NPs by electrochemical deposition on the PMAn-*g*-MWCNT paste electrode had a large particle size and uniform morphology. In *Fig.* 3-c, the morphology of Au-NPs prepared by physical deposition onto the PMAn-*g*-MWCNT paste electrode were small with uniform morphology. However, the amounts of Au-NPs on the PMAn-*g*-MWCNT electrode prepared by physical deposition were much lower as shown, in *Fig.* 3-c. This result was shown in similar

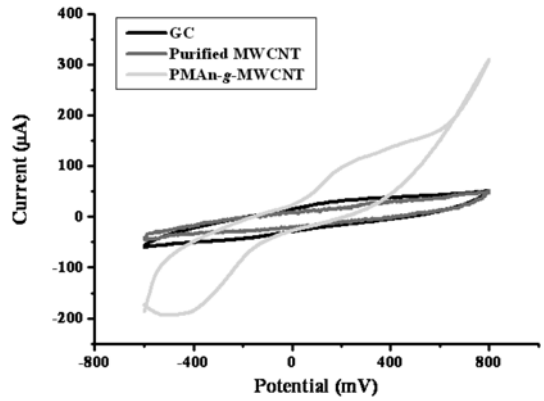

Fig. 4. Cyclic voltammograms of the paste electrodes in the presence of 5 mM $Fe(CN)_4^{3-/4-}$ in 0.5 M KCl solution with scan rate of 100 mV/s.

results with our previous work.[29]

Electrolytes influence the electrochemical behavior of the prepared electrode. *Fig.* 4 shows the cyclic voltammograms for GC, purified MWCNT electrode and PMAn-*g*-MWCNT paste electrode in 0.5 M KCl in the presence of 5.0 mM $K_3Fe(CN)_6$ solution with a scan rate of 100 mV/s. As shown in *Fig.* 4, the small redox peaks for $Fe(CN)_6^{3-/4-}$ on the GC electrode were exhibited in 0.5M KCl solution. The redox peaks for the purified MWCNT electrode indicate similar patterns for the GC electrode. However, the redox peaks and capacity for the PMAn-*g*-MWCNT paste electrode appeared larger than that of the GC and the purified MWCNT electrode for $Fe(CN)_6^{3-/4-}$ in 0.5M KCl electrolytes. This result means that the PMAn-*g*-MWCNT paste electrode

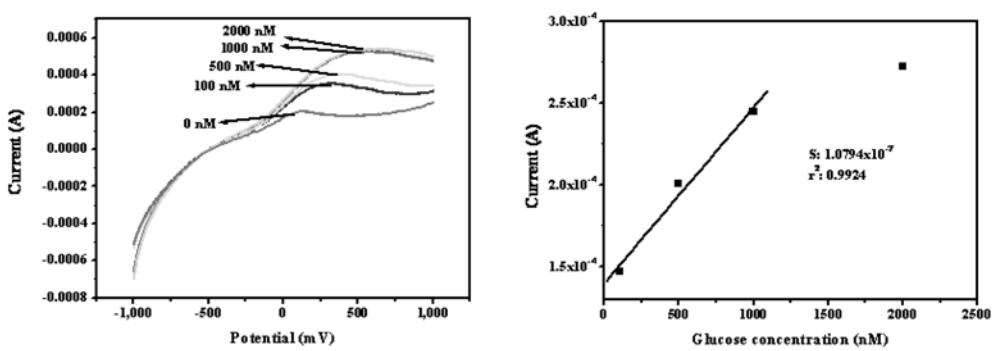

Fig. 5. Cyclic voltammograms (left) and calibration plot (right) of the physically Au deposited PMAn-g-MWCNT paste electrode according to the glucose concentration.

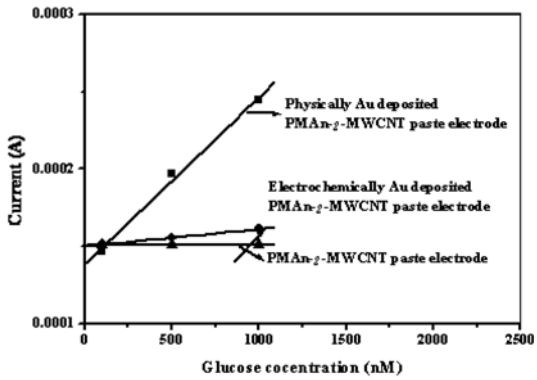

Fig. 6. Calibration plots of various PMAn-g-MWCNT paste electrodes according to the glucose concentration.

allows the transfer of the electron from electrolyte where the carboxylic acid group is acting as an ion-exchange group. The electron transfer resistance of the reaction was measured using Impedance. From the results, the electron transfer resistance of the PMAn-*g*-MWCNT paste electrode obtained a smaller value than that of the GC and purified MWCNT electrode in 0.5M KCl solution with 5 mM $Fe(CN)_6^{3-/4-}$.

Fig. 5 shows the cyclic voltammograms (left) and calibration plot (right) of the enzyme biosensor based on physical deposition of Au-NPs onto the PMAn-g-MWCNT paste electrode according to the glucose concentration. As shown in this figure, it could be observed that the peak currents of the biosensor increased with an increasing glucose concentration. Furthermore, the wide sensing range was observed from the calibration plot of the enzyme biosensor from 100 nM to 1000 nM of glucose concentration.

Fig. 6 shows the comparison of the enzyme-modified biosensor prepared by PMAn-g-MWCNT without Au-NPs, the electrochemical Au-NPs deposition, and the physical Au-NPs deposition. As shown in comparison data, the sensing range of the biosensor based on the physical deposition of Au-NPs is higher than that of the biosensor based on the electrochemicaldeposition of Au-NPs and without Au-NPs. The order of the sensing range of the enzyme biosensor was as follows: the biosensor based on physical deposition of Au-NPs > the biosensor based on electrochemical deposition of Au-NPs > the biosensor without Au-NPS.

Table 1. Interference effect of 5.0 μM the various compounds on the assay of 5.0 mM glucose on the biosensor based on Au-NPs modified PMAn-*g*-MWCNT electrded by physical deposition (n=3)

Interferent	Relative reponse to the glucose in the presence of the interferent (%)
Ascorbic acid	101.8
Uric acid	107.6

Relative response (%)=

$$\frac{\text{CA current in the mixture of glucose and interferent}}{\text{CA current in the glucose}} \times 100\%$$

Interference effects of ascorbic acid and uric acid on the assay of glucose at physically Au deposited PMAn-*g*-MWCNT paste electrodes were observed. All of the compounds tested were presented at a concentration of 50 μM with a glucose concentration of 500 μM. As shown in Table 1, the interferences were small and below 7.6%. Thus, physically Au deposited PMAn-*g*-MWCNT paste electrodes appear to be good sensors in detecting glucose.

4. Conclusions

The electron transfer resistance of the PMAn-*g*-MWCNT paste electrode was less than the GC or purified MWCNT paste electrode. The paste-type biosensor based on PMAn-*g*-MWCNT was successfully prepared for the detection of glucose. The order of the sensing range of the enzyme biosensor was: the biosensor based on the physicall deposition of Au-NPs > biosensor based on the electrochemical deposition of Au-NPs > biosensor without Au-NPs. The sensing range of the biosensor based the physical deposition of Au-NPs is from 30 μM to 100 μM. The interferences of ascorbic acid and uric acid on the biosensor based on the physical deposition of Au-NPs were below 7.6%. The physically Au deposited PMAn-g-MWCNT paste electrodes appear to be good sensor in detecting glucose.

Acknowledgments

This study was supported by the Hannam University

Research Fund (2009).

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